Fructophilic Yeasts

Consequences of Yeast Strain Selections

Jason Mabbett

Jason Mabbett is a technical applications manager for AB Biotek. He has been employed in this role for 10 years and, prior to moving to and focusing exclusively on North America three years ago, also oversaw South America. Prior to joining AB Biotek, Mabbett studied viticulture and enology at Lincoln University, New Zealand, and completed vintages in Argentina, Australia, New Zealand and the United States.

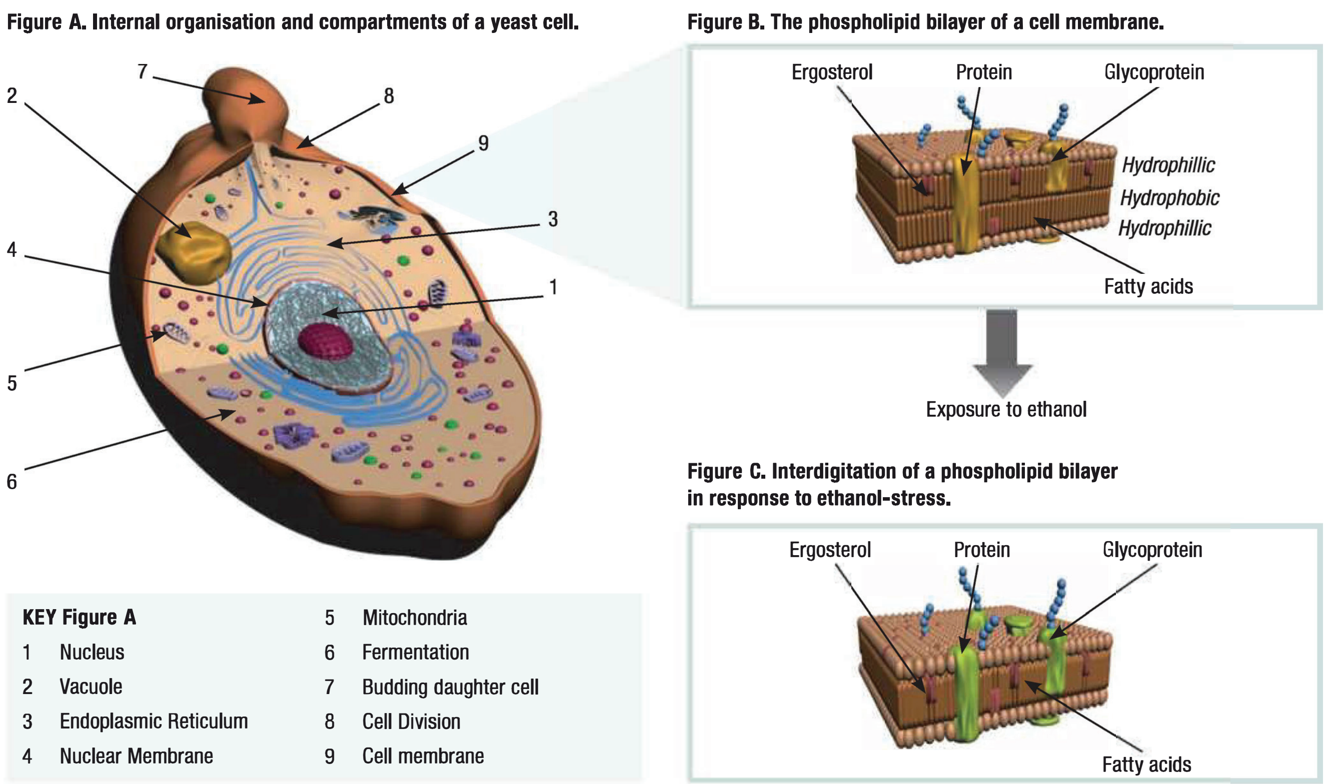
HISTORICALLY, THERE HAVE BEEN many causes attributed to stuck and sluggish ferments. These include such vineyard and viticultural factors (high-harvest Brix, nutrient deficiencies, fungal degradation and agricultural residues, including pesticides, fungicides and herbicides), cellar management (incorrect strain selection, incorrect rehydration procedures, incorrect fermentation temperatures, over-clarification of the must and yeast assimilable nitrogen [YAN] levels), inhibitory substances (ethanol, acetic acid, mid-chain fatty acids and sulfites) and physical factors (pH and temperature extremes).

As wine researchers and yeast manufacturers have achieved greater understanding of wine fermentations some of the above-mentioned issues have largely become redundant. The decrease attributed to these causative factors has seen a concomitant rise in one particular factor which is now viewed as being the predominant issue associated with stuck and sluggish fermentations: the ratio of glucose to fructose.[6] Anecdotal evidence suggests that in more than 90 percent of the cases (and some even characterize the incidence rate as much higher, about 95 percent)[4] where there is a stuck or sluggish fermentation, the glucose:fructose ratio is less than 1.0. That this should be the case is not surprising.

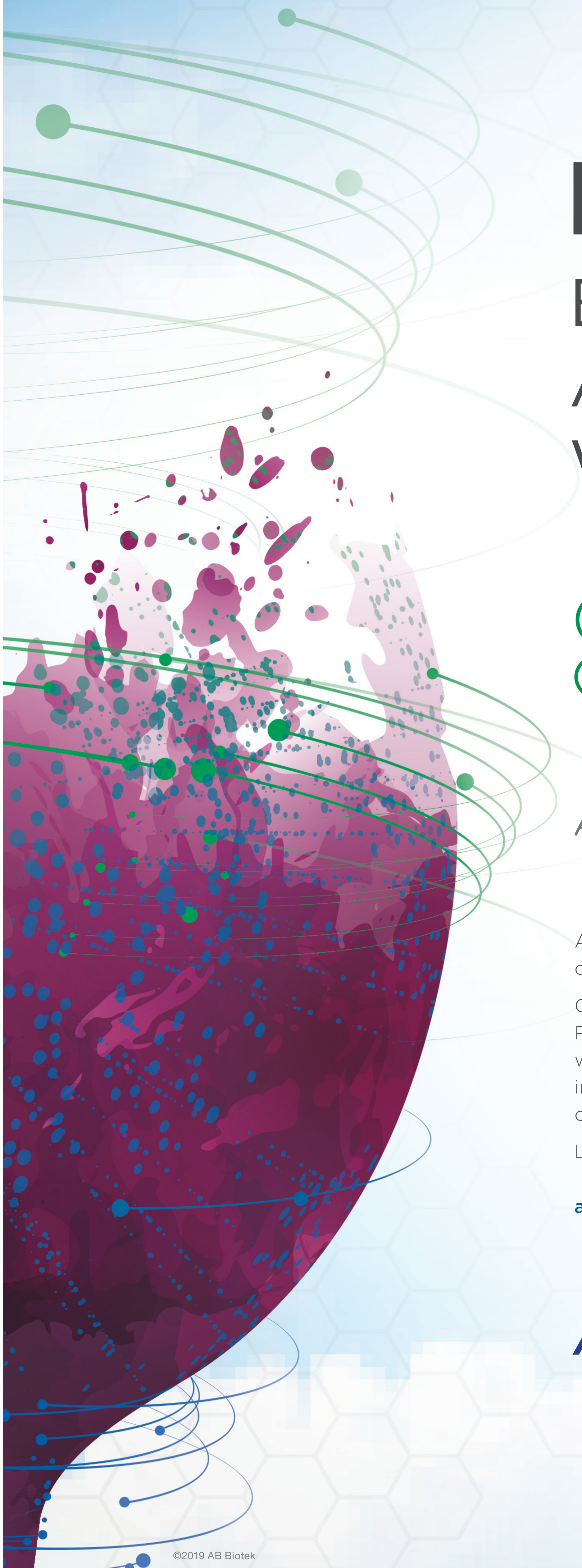
Saccharomyces cerevisiae is generally a glucophilic yeast, meaning it preferentially consumes glucose as opposed to other sugars. Many people believe that the use of a *Saccharomyces bayanus* strain will help avoid these problems; however, this is incorrect

Basically, the old taxonomy of yeast was based on their ability to mate. When geneticists started finding multiple pieces of DNA sequences that arose from the *Saccharomyces* sensu-stricto group, they realized that this was not the best method. With the advent of DNA sequencing, a more accurate methodology could be used. This has shown that many yeast species/strains are a mix of multiple strains with one or two dominant parents.

During this process it was revealed that those strains being used in the wine industry, which were commonly referred to as "Bayanus," were not



Figures. (A) Representation of a yeast cell highlighting key areas of the cell that are affected by ethanol. (B) Diagrammatic representation of a magnified cell membrane illustrating the bilayer organisation of phospholipids and the integration of proteins, some of which acts as gateways for transport of nutrients. Ethanol is soluble in cell membranes, and has dramatic impacts on the integrity of its structure. In fact, the membrane is thought to be one of the principal 'targets' for ethanol. (C) This figure illustrates the impact of ethanol on biological membranes. Fatty acids from opposite sides of the phospholipid bilayer become interdigitated and proteins are thought to be affected (illustrated as a colour change, yellow to green, between Figures A and B) however, the precise affects of ethanol on membrane proteins is not well understood. *Illustrations by Dung Quoc Tran.*



actually *Saccharomyces bayanus* but instead *Saccharomyces cerevisiae* and are mainly from the "Prise de Mousse" family.[7,8,10] Some yeast manufacturers and resellers still label these yeasts as *Saccharomyces bayanus*, which is incorrect. Reinforcing this was the apparent discovery of some true *Saccharomyces bayanus* strains isolated from Patagonia (from non-inoculated fermentation processes).[5] However, after sequencing, these have been reclassified as *Saccharomyces eubayanus*. The defining characteristics of these strains are that they are generally cold-tolerant and not fructophilic. In actuality there are very few *Saccharomyces eubayanus* strains have been isolated so far, and even fewer are commercially available.

During fermentations, glucose is, more often than not, consumed at a higher rate than fructose (the other predominant sugar in wine fermentations). As a consequence, the proportion of fructose increases as the fermentation progresses. When fructose becomes the predominant sugar at the end of fermentation this often leads to sluggish or stuck fermentations. It is important to recognize, however, that *Saccharomyces cerevisiae* yeast strains (the majority used in winemaking) consume certain types of sugar to varying degrees. Some are glucophilic and others fructophilic, and in between exists a continuum. In order to understand why this is important it is necessary to understand the genesis of this problem, the consequences and how this issue may be addressed.

Why is This Occurring?

In part, this may be attributable to an increase in alcohol levels and the desire to produce more fruit-forward wines. As many critics and consumers have noted, table wines used to have significantly lower alcohol levels than they currently do. Wines with 14% to 14.5% ABV are commonly produced today. It is also not uncommon to see wines with more than 15% ABV. But why is this? Leaving aside any debates concerning global warming and the implications of this, two related reasons stand out: the first being related to commercial imperatives and the second is viticultural.

In the last 30 years, key wine critics/reviewers have "pushed" red wines, in particular, towards a certain style where wines have higher levels of ripe fruit and softer tannins (ignoring the influence of oak). This wine style has often received higher scores and higher critical acclaim from influential reviewers, which has subsequently increased sales. Partly as a consequence of this, the distinction between sugar and physiological ripeness has become increasingly important.

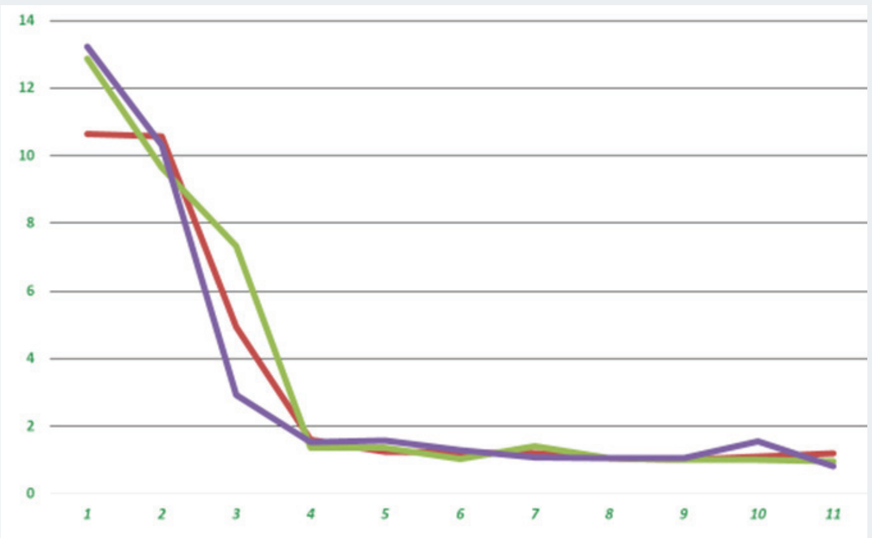
In warmer climates, physiological ripeness commonly trails sugar ripeness. Generally speaking, physiological ripeness is regarded as being more detrimental to wine quality than sugar ripeness. To this end, the notion of "hang time" has become more important—leading to physiological ripeness and, by implication, the wine style likely to garner critical acclaim. To fully understand the implications of this it is important to take a step back to Viticulture 101—in particular, the growth phases of the grapevine.

Three Growth Phases of the Grapevine

In phase one, berry size is set, the berries are green and the respiration rate is fast. Photosynthesis is sufficient to support the berry's nutritional demands. Acid concentration is high, and sugar concentration is low and constant. In this phase, the glucose to fructose ratio is greater than 1.0. In phase two, the berry growth tempo declines, and acids reach their highest levels. Sugars, especially glucose, begin to accumulate. This phase ends with the onset of *veraison*. Phase three sees an increase in the berry mass and volume. The glucose:fructose ratio is now in equilibrium.[7]

Importantly, however, the longer the grapes remain on the grapevine the more fructose accumulates proportionately. While there is actually little data related to the change in sugar composition during these growth phases (and the available data is relatively dated), some evidence to support the above can be seen in two graphs, FIGURES 1 and 2.

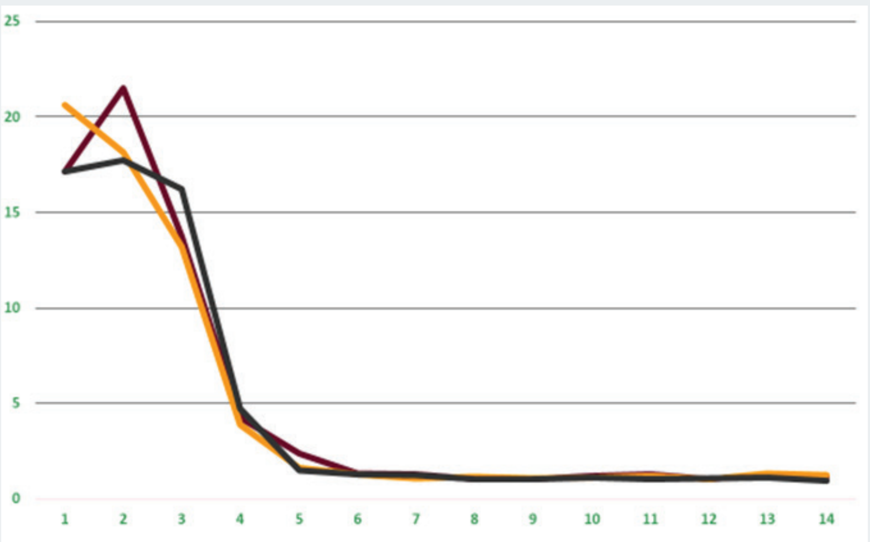
FIGURE 1: Growth Phases of the Berry



AB BIOTEK

FIGURE 1: Change in the ratio of glucose to fructose in Pinot Noir in the Central Coast (2013) prior to harvest

FIGURE 2: Growth Phases of the Berry



AB BIOTEK

FIGURE 2: Change in the ratio of glucose to fructose in Cabernet Sauvignon in the Central Coast (2013) prior to harvest

The consequences of this process and extended "hang time" are pronounced, not only from a wine quality standpoint but also the possible implications: stuck and sluggish fermentations. Bearing in mind that fructose is approximately twice as sweet as glucose, any fructose that is unconsumed can detrimentally affect wine quality due to the fact that wines may be perceived sweeter than they actually are. Additionally, the residual fructose also means a lower ethanol yield and a higher risk of microbial spoilage.[1] In part, these consequences can be mitigated somewhat by using fructophilic yeast strains that have a higher capacity to consume fructose.

The capacity of certain yeast strains to preferentially consume a certain type of sugar is something that is by no means new. In 1932 **Edward Romer Dawson** published a paper: "The Selective Fermentation of Glucose and Fructose by Yeast." However, two important conclusions he arrived at were that the selectivity exhibited by any particular yeast is not constant and this is dependent on cultural conditions to which the yeast has been subjected to during growth. It is not hard to see, therefore, how researchers such as **Linda Bisson** (**University of California, Davis**) have pointed out that the high residual concentration of fructose may be a symptom rather than a cause of a stuck or sluggish fermentation.[2] But why is this?

Yeast performance is determined partly by genotype, or genetic makeup, which is species- and strain-dependent. Wine yeast strains differ in terms of fermentation kinetics, nitrogen requirements, ethanol tolerance, temperature tolerance and also glucose:fructose consumption (to name but a few defining characteristics). These strain differences are more pronounced in stressful conditions, suggesting differences in adaption to the environment.[6] To this end, researchers have found that with respect to the consumption of glucose and fructose, nitrogen supplementation helps strongly stimulate fructose utilization and that, under high ethanol conditions, fructose utilization is inhibited more than glucose utilization.[1] Thus the use of a fructophilic yeast strain will not necessarily ensure a problem-free fermentation in and of itself. It will certainly reduce the likelihood, but it is by no means a "silver bullet."

To further reduce the likelihood of a stuck or sluggish ferment that is a consequence of the imbalance in the glucose: fructose ratio, the differences between grape varieties have to be taken into account (you are more likely to have problems with Chardonnay than Chenin Blanc, for example).[3,7] Vintage also has to be taken into account. In warm, dry vintages there is, generally, a lower glucose:fructose ratio. Yeast strain selection should, in part, be based on composition of the sugars in the must. The use of a fructophilic yeast strain is recommended where there is a higher amount of fructose than glucose when the glucose:fructose ratio is less than 1.0. Probably most important, where there is more fructose than glucose, significant attention should be paid to other additional risk factors, such as insufficient YAN and high potential final alcohol.

Fermentation problems usually arise due to the presence and impact of more than one stress factor. Some research has shown that a high starting YAN might stimulate fructose consumption preferentially, thus suggesting that analysis of the initial YAN level is necessary. When the must was supplemented with nitrogen, strains consumed between circa 6 percent and 9 percent more glucose and between circa 13 percent and 17 percent more fructose. Moreover, supplementation of diammonium phosphate at a late stage of fermentation also enhanced fructose consumption.[1]

Furthermore, researchers have noted that multiple factors (some of which are unavoidable— for example, increasing levels of ethanol) generally have a synergistic effect on each other.[6] This suggests that while the ability to preferentially consume fructose is important, other attributes of fructophilic yeast strains may be just as important: low nitrogen demands and alcohol tolerance for example. Ultimately the one thing a winemaker can most easily control is the selection of the yeast strain to be utilized in the fermentation, taking into account not only whether it is fructophilic but also other attributes of the strain.

In the interim, yeast manufacturers and researchers will continue to assist by attaining a better understanding of the wine microorganism physiology and the impact on its environment. Additionally, through selective breeding, hybridization, adaptive evolution and investigation of other yeast species that might be better suited to the fermentation of fructose, we may be able to arrest issues related to stuck and sluggish fermentations.

Take Away

- Where the ratio of glucose:fructose is less than 1.0, winemakers should consider the use of fructophilic yeast strains to ensure less likelihood of a stuck or sluggish ferment. All yeast manufacturers and resellers have fructophilic strains, and their technical sales representatives will be able to point winemakers in the right direction.
- However, when the glucose:fructose ratio is less than 1.0, the use of a fructophilic yeast strain will not necessarily ensure a successful fermentation all the time. Fermentation management is fundamental.
- Attention has to be paid to other risk factors, such as proper yeast rehydration, YAN level, fermentation temperature and high potential final alcohol, in order to ensure a successful fermentation. **WBM**

References

1. Berthels, N., Cordero-Otero, R., Bauer, F.F., Thevelein, J.M. and Pretorius, I.S. "Discrepancy in glucose and fructose utilization during fermentation by *Saccharomyces cerevisiae* wine yeast strains" in Fems Yeast research 4, 2004, pp. 683 - 689.
2. Bisson, L.F. "Stuck and sluggish fermentations" in Am. J. Enol. & Vit., 150, 1999, pp. 1 - 13.
3. Kliewer, W.M. "Changes in concentration of glucose, fructose, and total soluble solids in flowers and berries of Vitis vinifera" in Am. J. Enol. & Vit., 16, 1999, pp. 101 - 110.
4. Lallemand. "The-Fermentation-of-Fructose-in-winemaking" in *lallemandwine.com/wp-content/uploads/2014/12/Wine-Expert-The-Fermentation-of-Fructose-in-winemaking-2013.pdf*.
5. Libkind, D., Hittinger CT, Valério, E., Gonçalves, C., Dover, J., et al. 2011 Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc Natl Acad Sci USA 108: 14539–14544.
6. Malherbe, S., Bauer, F.F. and Du Toit, M. "Understanding Problem fermentations—A Review" in South African J. of Enol. & Vit., Vol. 28, No. 2, 2007, pp. 169 - 186.
7. Naumova, E.S., Naumov, G.., Michailova, Y.V., Martynenko, N.N. and Masneuf-Pomarede, I 2011 Genetic diversity study of the yeast *Saccharomyces bayanus* var. *uvarum* reveals introgressed subtelomeric *Saccharomyces cerevisiae* genes. Res Microbiol 162: 204 - 213.
8. Nguyen, H.V., Lepingle, A. Gaillardin, C. 2000 Molecular typing demonstrates homogeneity of *Saccharomyces uvarum* strains and reveals the existence of hybrids between *S. uvarum* and S. *cerevisiae* including the *S. bayanus* type strain CBS 380. Syst Appl. Microbiol. 23: 71–85.
9. Snyman, P. The glucose:fructose ratio of wine grapes in wineland.co.za. 2006.
10. Vaughan-Martini, A. Martini, A. 2011 Chapter 61 - *Saccharomyces Meyen* ex Reess (1870). In: The Yeasts (Fifth Edition). pp. 733–746.

Yeast and Nutrition

A Q&A WITH JASON MABBETT

There are many different types of yeast. How do you know which one to select?

Yeast should be selected based on the grape variety and wine style you would like to create. However, there are other key factors to consider: The ethanol tolerance of the yeast strain should exceed the projected final ethanol titer of the fermentation. Nitrogen requirements should match the nutritional conditions of the juice. The temperature tolerance should be considered if uniform temperature control is a concern. Compatibility of the yeast strain with malolactic fermentation (MLF) is an important consideration if MLF is desired.

Production of specific aroma compounds is a consideration, but the ability to produce a spectrum of volatile characters is partially dependent upon composition of the juice. The aromas produced will vary, depending upon the levels of precursors present. The production and quantity of esters and thiols can also be favored through fermentation temperature profiling; this is most obvious and decisive in grapes such as Sauvignon Blanc.

There is no single "right" choice of yeast strain. In fact, for each grape variety there are many possible choices that will all make lovely wine. It is, however, important to understand the attributes of each yeast strain to ensure that you choose the one that will give the optimal fermentation profile and desired sensory characteristics you want.

How important is it to rehydrate yeast?

Extensive research shows that the yeast cell wall is very fragile during the first few minutes of rehydration. When a dessicated yeast cell rehydrates, its cell wall is swelling and the membrane is gaining back its elasticity. If rehydration is not properly carried out, the cell can leak important cellular compounds through the membrane, which is extremely permeable at the time of rehydration. As a consequence, the yeast will lose viability, and the subsequent populations will have reduced capability to undertake grape juice fermentation. Arguably the most important information yeast manufacturers provide is instructions for the correct preparation of active dry wine yeast, which is essential for optimum performance.

Can you add too much or too little yeast? Will it ruin the flavor/taste?

The amount of the inoculum influences the lag phase (the initial growth rate before rapid, exponential growth) and general fermentation speed, as well as, potentially, the flavor of the finished wine.

A small inoculum will result in a longer lag phase and more risk of contamination as the inoculated strain seeks to dominate other yeast that may be present (even after SO_2 additions). While these strains can add aroma and complexity to a wine, they can also negatively influence a wine's aroma. For example, some strains of *Kloeckera apiculata* can potentially produce up to 25 times the amount of acetic acid typically produced by *S. cerevisiae*. In addition, these native strains can also lead to longer fermentation times or, in some cases, to stuck fermentations possibly due to the production of acetic acid, octanoic and decanoic acids, or "killer" factors.

Conversely, too much yeast can speed up fermentation and can lead to early yeast autolysis (yeast death) and hence a yeasty/bread-like flavor added to the wine.

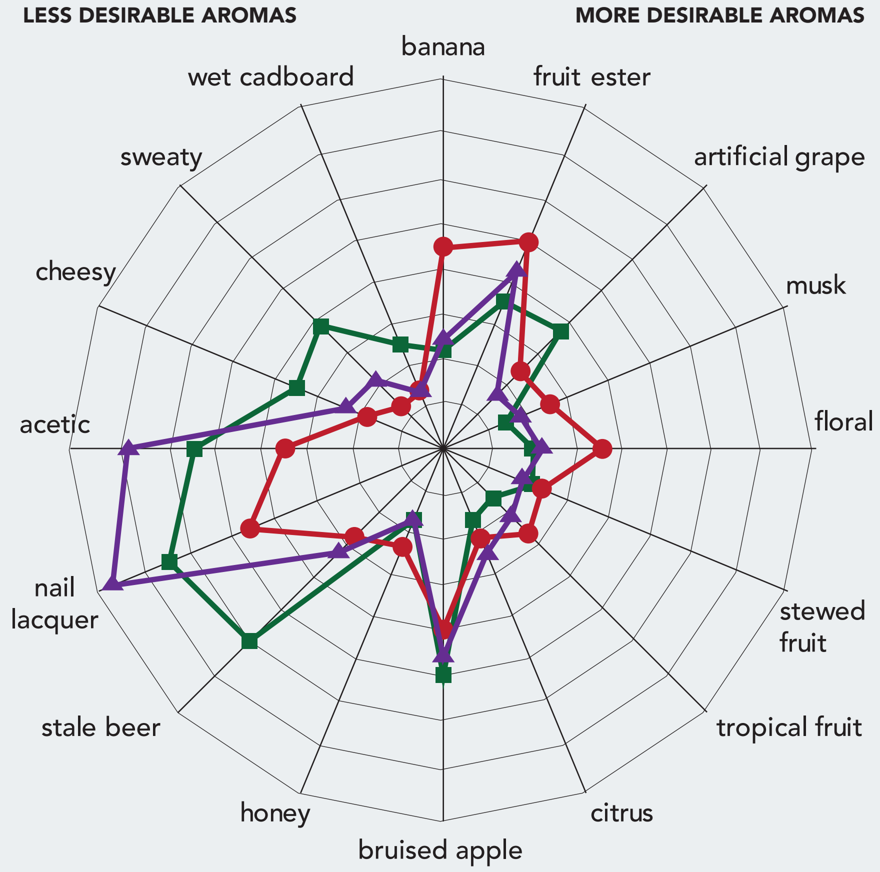
How do different nitrogen sources influence yeast fermentation performance and/or sensory characteristics?

Nitrogen comes in two forms: inorganic nitrogen, such as ammonium salts (DAP) that are added during alcoholic fermentation, and organic nitrogen such as small peptides and free amino acids—all derived from added yeast (inactive or autolysate) and from the grape juice itself. When yeast cells are inactivated, part of the cell protein are hydrolyzed and become available as small peptides and amino acids that live yeast can assimilate during fermentation. Yeast autolysates contain more YAN than inactive yeast. Yeast benefit from a mix of different nitrogen sources; the use of both organic and inorganic nitrogen is important for optimal growth and performance.

The inorganic form of nitrogen is more readily consumed by yeast, and it can be easily absorbed by yeast cells during the growth phase and even as the alcohol concentration rises during primary fermentation. Amino acids, on the other hand, require energy expenditure in order to be brought into the cell through transport proteins located on the cell membrane.

Nitrogen compounds are necessary for complete and clean-smelling ferments. Yeast assimilable nitrogen (YAN) can strongly influence production of some of the volatile metabolites, especially the acetate and ethyl esters, which are known to be positive to wine aroma when in balance. For example, in Chardonnay, the flavor and style of wine is dramatically modulated by the initial YAN concentration of the grape juice. Please see the work done by **Bell and Henscke**: *https://onlinelibrary.wiley.com/toc/17550238/11/3.*

FIGURE 3: Effect of nitrogen addition on wine aroma



Descriptive sensory analysis of Chardonnay wines made by fermentation, with *Saccharomyces cerevisiae* AWRI 796, of a grape juice containing 160 mg N/L (GREEN SQUARE) or 320 (RED DOT) or 480 mg N/L (BLUE TRIANGLE) made by supplementation with ammonium chloride (Torrea and Henschke 2004) in S-J. Bell and P.A. Henschke (2005) *Australian Journal of Grape and Wine Research* 11, 242-295; and reproduced here with permission from the *Australian Society of Viticulture and Oenology*.

Why do some fermentation nutrients have vitamins and trace elements included and how do they assist yeast performance?

ADDITIVE	PURPOSE
Biotin (Vitamin B7)	Increases viable yeast population and fermentation rate.
Magnesium	Magnesium prolongs exponential growth, resulting in increased yeast cell mass. The addition of magnesium also reduces the decline in fermentative activity as it is a critical cofactor in many stress-related transcription factors. Ultimately this results in stress-related proteins being produced, thus protecting the yeast cell and allowing it to ferment more easily.
Niacin (Vitamin B3)	Same as Biotin.
Nicotinamide (Vitamin B3)	Involved in the synthesis of nicotinamide adenine dinucleotide (NAD+), a co-enzyme that is important in maintaining the redox balance of the cell and the process of ethanol fermentation itself.
Pantothenic Acid (Vitamin B5)	Involved in the synthesis of sulfur-amino acids, such as cysteine and methionine, through the sulfate reduction sequence (SRS) pathway, which assists to reduce H_2S and volatile acidity production. Also used in the yeast production process to reduce cell wall adhesion and remove clumpiness.
Pyridoxine hydrochloride (Vitamin B6)	Involved in the synthesis of sulfur-amino acids, such as cysteine and methionine, through the SRS pathway.
Thiamine (Vitamin B1)	Increases yeast biomass and speed of fermentation.
Zinc	Zinc is a co-factor for numerous important biosynthetic and metabolic enzymes including, significantly, various glycolytic enzymes and alcohol dehydrogenase. In addition, it plays critical regulatory roles through the action of Zn finger DNA binding proteins and affects yeast-yeast flocculation. Zinc is also known to modulate yeast stress responses, mainly due to its role as a co-factor for the antioxidant enzyme superoxide dismutase.

When should the nutrient be added?

Yeast metabolize nutrients at different times throughout fermentation. Adding nutrients at the most optimal time can enhance yeast performance. As fermentation progresses and the ethanol level rises, yeast becomes less and less able to assimilate nutrients. Ethanol is inhibitory to key cell wall transporters; and if components are added after inhibition, the substrates will not be taken into the cell.

The most effective time to add key nutrients is once the *Saccharomyces* population has become dominant, generally 24 to 48 hours after the rehydrated yeast inoculum has been added. Generally, manufacturers recommend adding complex nutrients one-third of the way through fermentation in terms of sugar consumption. Inactivated yeast can be added throughout fermentation for various purposes: early additions can be beneficial for detoxification of the grape juice to make it easier for the rehydrated yeast to perform, and late additions can contribute to mouthfeel in the wine.

The goal is to keep yeast healthy and vital, so adding nutrients during the exponential phase before nutrition becomes limiting is preferred. Few nutrients are toxic; but if nutrients are added too early, nutrients could precipitate out or be adsorbed with other organic material in the must.

Can you add too much nutrient? What happens if excessive nutrients are added?

Overfeeding of fermentations can be as problematic as underfeeding as very rapid fermentation rates are likely to lead to overheating of the fermentation and loss of volatile aroma compounds. Adding excess nitrogen may lead to microbiological problems as it becomes fodder for spoilage organisms, such as *Brettanomyces*, *Acetobacter* and Lactic acid bacteria from the *Lactobacillus* and *Pediococcus* genera.

Can yeast nutrients be added for bacteria (MLF)? Why or why not?

Malolactic bacteria cannot utilize inorganic nitrogen sources. Bacteria cannot store nor synthesize all essential amino acids, so complex nutrients must be supplemented.

Newly fermented wine can often be deficient or void of nutrients due to yeast utilization. Nutritional depletion can cause sluggish or even stalled malolactic fermentations. Due to the complex nutritional requirements of malolactic bacteria and the relatively harsh medium for growth, minimizing nutritional stress is important. In addition to amino acids and peptides, which are the most influential nitrogen sources required for malolactic growth, B-complex vitamins and trace minerals are especially important. **WBM**