# Technical notes

## Nutrients to support fermentation: what are they and do they work?

### Introduction

To be competitive in the overcrowded wine market, winemakers require their fermentations to be reliable and efficient, and this applies to both alcoholic and malolactic fermentation (MLF). Sluggish or stuck fermentations cause delays in production and lead to increased risk of spoilage, with associated product downgrade or loss. Products that enhance the performance of wine yeast and bacteria, and take some of the risk out of fermentation management are increasingly used and are a welcome addition to the winemaker's toolkit. This article presents an overview of current knowledge on the efficacy of fermentation nutrients that are available to winemakers.

### Nutrition for alcoholic fermentation

Just as the foundation for good wine is in the vineyard, the foundation for good yeast performance in alcoholic fermentation is in the preparation of the yeast inoculum, and this includes the use of active dry yeast (ADY). An understanding of how ADY is produced and what happens when it is rehydrated can provide insight into the possible benefits of using fermentation nutrients. Practitioners of wild fermentations may also benefit from a greater understanding of how and when to use nutrient additives, but should be aware that almost all of the research conducted in this area has involved fermentations using ADY.

### Active dry yeast production

Manufacturing conditions for ADY are designed to maximise three main factors: biomass production, survival during the drying process, and activity following rehydration. In the manufacturing process, yeast propagation is carried out in multiple stages in a nutrient-supplemented molasses medium. Once propagation is complete, vacuum filtration is used to remove residual medium. The resultant yeast cake is extruded to form small particles which are then dried. Relatively small variations in the drying process can dramatically influence the viability of the final product but, in general, active dry yeast products are produced with between 60 and 90% viability. The loss of viability has been attributed to dehydration of the yeast to a moisture level below 15% (Bayrock and Ingledew 1997) as is required for long-term storage (Ebbutt 1961). Because of the influence of production variables, the fermentation performance of ADY can vary between different suppliers of the same strain.

### Yeast rehydration and the use of rehydration nutrients

Despite their general resilience, the drying process is damaging to wine yeast cells and leads to a dramatic increase in cell membrane permeability (Crowe et al. 1992). When dried yeast cells are placed back into an aqueous environment, much of the cells' content may be lost through damaged membranes, leading to large-scale viability loss. It is this characteristic of the drying process that has driven much of the research in ADY rehydration and the development of rehydration nutrients. Two factors that contribute to a high percentage recovery of viable yeast from dried cultures are the temperature of the rehydration solution (optimally between 35 and 43°C) and the presence of salt in that solution.

It is generally accepted that the cause of leakage from cells during rehydration is the damage caused to the membrane when water is removed during ADY manufacture. Because the membrane is permeable during rehydration, it presents the possibility that if solutes can be lost from the cell, perhaps they can also be added. Dulau et al. (2004) proposed a method for supporting yeast fermentation through the addition of various factors during rehydration. The idea of loading the cells with all they might need to perform reliably during fermentation is a tantalising one. Adding high concentrations of inactive dry yeast (IDY) during rehydration is one method that is widely promoted as achieving this aim.

IDY preparations are produced primarily by thermal inactivation of high density yeast cultures. Addition of these products to a rehydrating live yeast culture should provide all of the components of the original IDY biomass. In theory this should assist with the maintenance of cell viability during rehydration by adding salts or glucose indirectly into the rehydration medium (Beker et al. 1984) and therefore should protect against loss during rehydration. This may complement the benefits of maintaining an optimal temperature during rehydration; however, there is little published data to support this idea.

The main purpose of adding IDY during rehydration is to strengthen the yeast before inoculation into highly clarified or otherwise stressful grape juices. Other benefits have been proposed, such as fortification with vitamins and minerals, but there is no published evidence that any vitamin or mineral content in IDY is taken up by yeast during rehydration. While the vitamin and mineral components will still be present when the rehydration solution is added to the bulk ferment, their concentrations will be relatively low compared to the amounts present in most juices at the start of fermentation and they are therefore unlikely to have a significant effect.

At this time, there is not enough published research to reliably guide the use of IDY. The AWRI advises that it might be beneficial in high stress fermentations, such as in high sugar juices, highly clarified musts, or under highly anaerobic conditions, and particularly when those stress conditions are combined.

## Fermentation additives

Any discussion about nutrient requirements during fermentation begins (and usually ends) with nitrogen. The nitrogen content of a must is a well-known and characterised factor that influences both the performance of fermentation and the aroma profile of resultant wines (AWRI publication #875). Additions of diammonium phosphate (DAP) at the beginning of fermentation are often made as a precautionary measure against potential fermentation problems, especially if the nitrogen status is not known, or reactively in response to signs that a fermentation is becoming reductive. This practice is common despite the ambiguous relationship between DAP supplementation and hydrogen sulfide concentrations in finished wine (AWRI publication #1256).

While grapes typically provide all the essential nutrients required to complete fermentation, cases of nutrient deficiency do sometimes occur. This may be the result of particular vineyard conditions or due to post-harvest nutrient depletion caused by suboptimal processing and storage conditions before fermentation. From a fermentation performance perspective, application of DAP is a suitable treatment for the correction of low YAN juice. Treatment with nitrogen in the form of amino acids does not appear to provide additional benefit (AWRI publication #1277, Gobbi et al. 2013). In any case, products that deliver adequate concentrations of amino acids are not commercially available due to the high cost of such products and regulatory issues.

In addition to its effects on fermentation performance, nitrogen management can be used to influence wine aroma (AWRI publication #1256). However, the impact can vary depending on the nitrogen source. It is in this space that nutrient supplements other than DAP have gained a foothold. DAP has the capacity to drive pH down compared to the use of complex nitrogen supplements; DAP additions can lead to a drop of up to 0.2 pH units, depending on the buffering capacity of the juice (Torija et al. 2003, AWRI publication #1277). This may be a consideration when fermenting low pH white juices. Supplementing juice with high additions of DAP can increase volatile acidity and ethyl acetate in the finished wine; this effect is less pronounced when complex nitrogen supplements are used rather than ammonia alone (AWRI publication #1277, Martinez-Moreno et al. 2014). Furthermore, fermentations supplemented with a high amino acid component produce wines judged to have a more desirable sensory profile with positive 'fruity' and 'floral' attributes compared to DAP addition alone (AWRI publication #1277).

Vitamins are another component of fermentation additives that are often claimed to benefit either fermentation performance or aroma profile, but there is little evidence to support their general use. The work of Monk (AWRI publication #210) found that the addition of vitamins did not stimulate yeast growth or sugar attenuation in fresh grape juices. Some specific conditions, however, can cause nutrient depletions in juice; these include:

- Very high concentrations of $SO_2$ in juice, which can inactivate thiamine
- Transportation of juice over long distances or extended cold soaks/macerations, allowing initiation of fermentation by wild yeasts which can strip some nutritional components, including thiamine (Bataillon et al. 1996).

Grape musts depleted in this way may not support rigorous subsequent fermentation by *S. cerevisiae*. In such scenarios vitamin addition has been shown to help alleviate sluggish fermentations (Medina et al. 2012). Such nutrient depletions are unlikely to be a problem in freshly prepared grape juice.

## Nutritional requirements and commercial nutrients for wine malolactic bacteria

Malolactic fermentation (MLF) is important in the production of most red, some white and some sparkling wines. MLF is conducted by select species of lactic acid bacteria, principally *Oenococcus oeni*. This bacterium has several unique traits that enable it to survive and grow in harsh wine environments, including tolerance to both ethanol and acidic conditions.

While *O. oeni* is naturally present during winemaking, the natural microflora of a wine fermentation is not particularly reliable in delivering efficient and timely MLF. Slow or stuck MLF can lead to economic losses through production inefficiencies and depreciation of wine quality.

This has led to the development of bacterial starter cultures, which enable greater control over MLF with substantial improvements in MLF efficiency and reliability. Nevertheless, even when using starter cultures problematic MLFs can still occur, although at a reduced frequency, and mostly in difficult wine conditions. Other factors, including the interactive effects of wine yeast on malolactic bacteria, can also impact on the success or failure of MLF. For example it is thought that use of wine yeast strains having a high nitrogen demand may diminish the availability of some essential nutrients required by *O. oeni*, particularly in wines prepared from low nitrogen juices (AWRI publications #732, #773, Theodore et al. 2004).

It is well known that *O. oeni* requires a range of nutrients for growth. In this context, the nutritional requirements of malolactic bacteria in conjunction with the nutritional status of the wine have received increased attention as important factors influencing MLF performance. In addition there is notable inter-strain variation in essential amino acid requirements (Remize et al. 2006, Terrade and Mira de Orduña 2009). Unlike yeast, *O. oeni* cannot utilise ammonium as a nitrogen source, but it can utilise nitrogen from several other sources including amino acids and peptides (Remize et al. 2006).

Based on the current understanding of MLF bacteria nutritional requirements, a number of commercial IDY preparations have been developed for use as nutrients to improve MLF performance of starter cultures in wine (reviewed by Pozo-Bayón et al. 2009). Technical information provided by suppliers generally indicates that these products provide a source of bacterial nutrients such as amino acids and polysaccharides. Some brands may also include other specific components such as yeast hulls which may facilitate absorption of toxic medium-chain fatty acids produced by yeast. Proprietary recommendations for use of IDY bacterial nutrients include cases where nutrient status of the wine may be limiting, conditions are difficult for MLF induction and where MLF is slow or stuck.

While there is some evidence provided by suppliers of the effectiveness of these nutrients on MLF strains, there is a lack of published independent data on their composition, modes of action and general efficacy in promoting MLF in different wine conditions.

## Conclusion

Current research at the AWRI has commenced investigating the role(s) of commercially available nutrients on the conduct of alcoholic and malolactic fermentation in wine production. The results of these studies will provide the Australian wine industry with comprehensive data concerning the efficacy of these nutrients, enabling informed decisions to be made.

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