Yeast Nutrition for Healthy Fermentations

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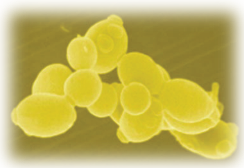
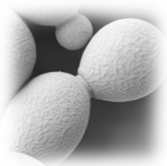
Introduction

Fermentation is a key step in the production of high-quality spirits. Raw material composition, nutrient addition and operating conditions are all factors that directly affect yeast metabolism, growth (biomass generation) and fermentation. Diverse feedstocks differ not only in the type of fermentable sugar but also in the amount of assimilable nitrogen, minerals, salts, and vitamins which affect the metabolic activity of yeast. Therefore, tailored nutritional blends added to the substrate feedstock are required for optimal fermentation performance and the biosynthesis of pleasant volatile aroma and flavor compounds.





Nitrogen

Nitrogen is important in maintaining yeast health because it is required for biomass generation and the synthesis of protein and DNA/RNA. Yeast cannot fix atmospheric nitrogen but needs to assimilate it from the fermentation media. There is a fine balance for nitrogen requirements, and both a deficiency or an excess could negatively affect fermentation and ethanol production.

Saccharomyces cerevisiae can assimilate a variety of organic and inorganic nitrogen sources, which together are generally referred to as yeast assimilable nitrogen (YAN).

Typical inorganic nitrogen sources include liquid ammonia, ammonium hydroxide, ammonium sulphate and phosphate salts. Phosphate salts could be compounds such as monoammonium phosphate (MAP) and diammonium phosphate (DAP).

Ammonium salts provide nitrogen, which is incorporated into intermediates of the central carbon metabolism (α-keto acids) to generate amino acids. Amino acids are not only the building blocks for proteins, but also key anabolic and catabolic nitrogen shuttles in yeast metabolism.

Organic nitrogen includes free amino acids and small peptides, composed of up to 5 amino acids, and it is generally referred to as free amino nitrogen (FAN). While proteins are composed of amino acids, these need to be hydrolyzed into smaller components (peptides and amino acids) during mashing and fermentation to become assimilable by yeast. The enzymes that hydrolyze proteins and peptides are called proteases. Both malted grains and commercial proteases can be added to increase FAN. Organic nitrogen available in the feedstocks can be measured and referred to as FAN.

The utilization of nitrogen sources is highly regulated to allow the cell to respond to nutrient availability. This includes the ability of yeast to monitor and respond to concentrations of nutrients in the extracellular environment and repress assimilation of poor nitrogen source when preferred ones are available. This regulation mechanism is known as nitrogen catabolite repression (NCR). While the rate at which different nitrogen sources are assimilated by *Saccharomyces cerevisiae* is strain dependent, the order in which they are assimilated is generally conserved among strains. Ammonium ions are assimilated rapidly at the beginning of fermentation while amino acids and small peptides are assimilated gradually and in groups, from the most preferred to the least preferred group. This preference is defined based on their ability to sustain growth as sole nitrogen sources.

Organic nitrogen is assimilated gradually and is a long-lasting source of nitrogen which allow for steady cell viability throughout fermentation. (Figure 1).

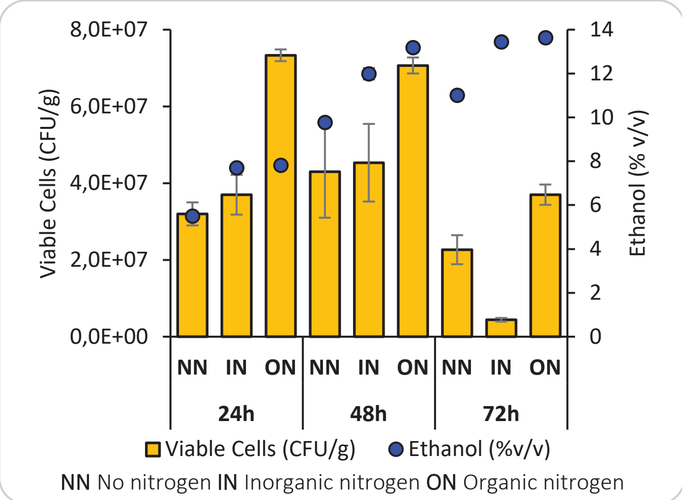


Figure 1: Effect of organic and inorganic nitrogen on yeast viability and fermentation kinetics in grain whiskey fermentation.

How much nitrogen does yeast need?

As a rule of thumb, 200-250 ppm of YAN is required by most strains for growth and efficient fermentation. For low ethanol titers, less nitrogen may suffice depending on operating conditions. YAN corresponds to the nitrogen present in the nitrogen source. In the case of diammonium phosphate (DAP: inorganic nitrogen) nitrogen represents 21.2 % of the molecular weight. In the case of organic nitrogen, glycine is generally used as a reference for amino acids and the nitrogen content represents 18.7 % of the molecular weight of glycine. Most substrates contain insignificant concentrations of inorganic nitrogen (this is added exogenously) and so FAN measurements are generally used to indicate their total nitrogen content.

Figure 2 shows the average FAN content of different feedstocks. All grain feedstocks, with the exception of 100 % malt whisky, generally require nitrogen supplementation to reach optimal fermentation performance.

Nitrogen management is needed to assume consistent kinetics, with higher nitrogen level corresponding to faster kinetics. Excess nitrogen is not economical and could result in a less efficient fermentation.

How is the nitrogen concentration measured?

There are many different methods that require specialized laboratory equipment and are used by analytic laboratories to measure the nitrogen concentration before, during and after fermentation.

Titration is the simplest option as it doesn't require expensive specialized equipment. However, the reagents required are toxic and must be handled with care. Furthermore, titrations can be timely to setup and require large volumes for accuracy. Alternatively, distillers could easily assess the specific nitrogen requirement in a feedstock by performing small-scale fermentation tests and varying the concentration of nutrition. The effect of nitrogen can be easily detected in the form of a kinetic boost (shorter fermentation time) and optimal dosage would help achieve maximum final ethanol concentrations.

Inorganic ions and vitamins

In addition to nitrogen, yeast needs inorganic ions and vitamins for healthy growth and fermentation. These inorganic ions are necessary to the yeast metabolism as enzymatic cofactors (zinc, manganese, magnesium), intracellular messengers (calcium), osmo-regulators (potassium, inositol) and protein components (iron) (Figures 2, 3 and 4). Inorganic ions are generally defined as macro- or micronutrients based on their abundance. Vitamins act as electron and functional group carrier molecules, which are essential to support the metabolic enzymatic activity involved in yeast homeostasis and fermentation.

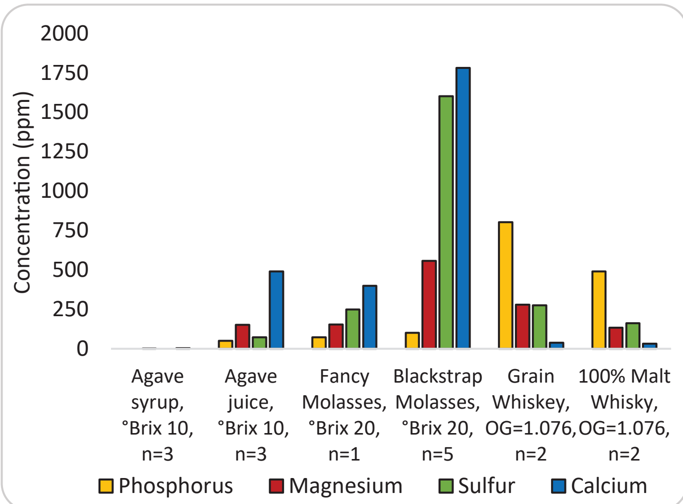


Figure 2: Average macronutrient concentration in different types of feedstocks normalized to typical initial fermentation densities. n= number of substrates analyzed

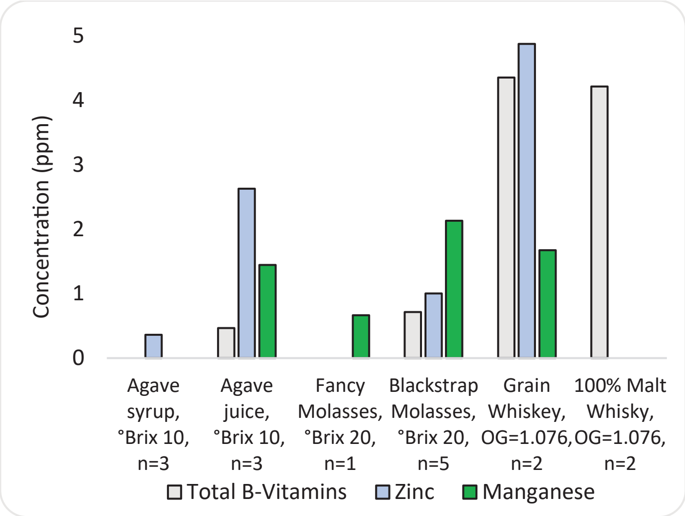


Figure 3: Average micronutrients and B-vitamin concentrations in different types of feedstocks normalized to typical initial fermentation densities. n= number of substrates analyzed

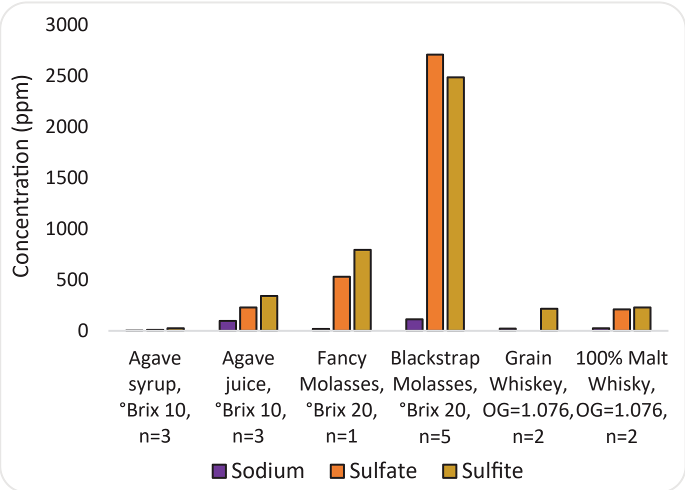


Figure 4: Average concentrations of stressors in different types of feedstocks normalized to typical initial fermentation densities. n= number of substrates analyzed

Starch-based feedstocks

Starch-based feedstocks are used to produce whisk(e)ys and neutral grain spirit (NGS or GNS). The most common grain feedstocks include malted or un-malted varieties of barley, maize (corn), wheat, rye, oat and sorghum. Organic nitrogen is naturally present in starch-based feedstocks, but because they are in the form of proteins and thus not readily available, they need to be broken down by protease.

Proteases are enzymes that cleave proteins and polypeptides that are present in mash to convert to amino acids and smaller peptides. This conversion represents an efficient solution to release assimilable nitrogen from the proteins already present in the feedstock. In addition to releasing assimilable sources of nitrogen throughout the entire fermentation, supporting yeast metabolism, the cleavage of starch-bound proteins can also liberate bound sugars which may results in a yield increase. This is the reason why protease supplementation is recommended rather than DAP addition in starch-based fermentation.

Proteases can be added both by the inclusion of malt and protease-based nutrients, such as DistilaVite® HY. Commercial proteases offer the advantage of consistency over malt inclusion, which is dependent on the malting process, the specific activity of malt derived proteases, mashing profile and inclusion level which can be variable.

Figure 5 shows the effect of organic nitrogen, released by a commercial protease in corn whiskey fermentation. The inclusion of the protease resulted in a faster fermentation and complete utilization of mash sugars.

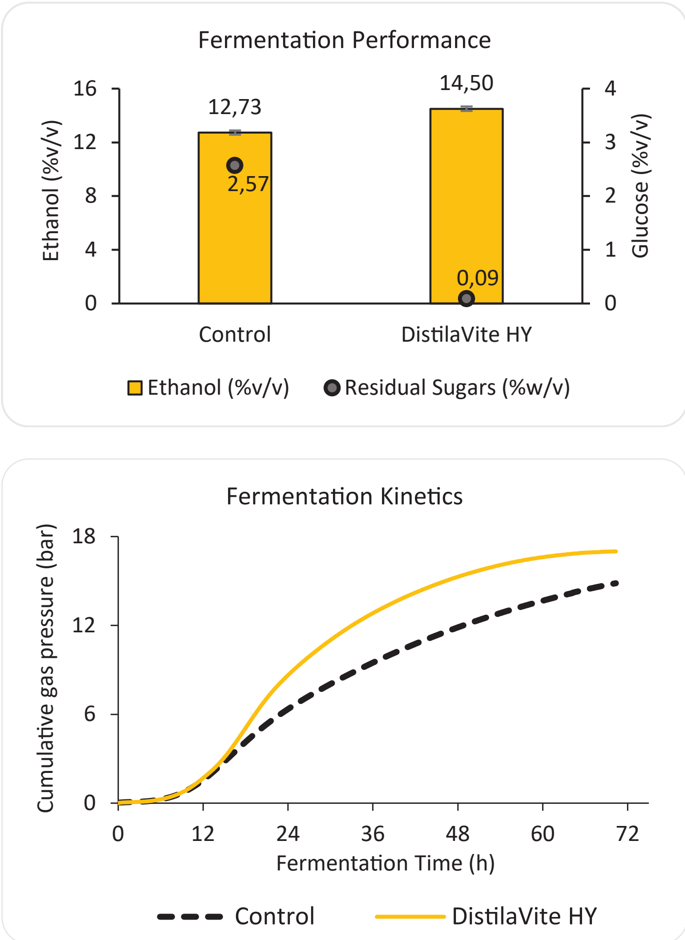


Figure 5: Whiskey corn mash fermentation with and without addition of a protease- based nutrient.

Sugar-based feedstocks

Sugar-based feedstocks generally benefit from complex nutritional supplementation including nitrogen, minerals and vitamins.

Fancy molasses, maple syrup, agave juice and agave syrup contain very low FAN concentrations (Figure 6) and are notably low in various macronutrients, micronutrients, and vitamins (Figure 3 and 4).

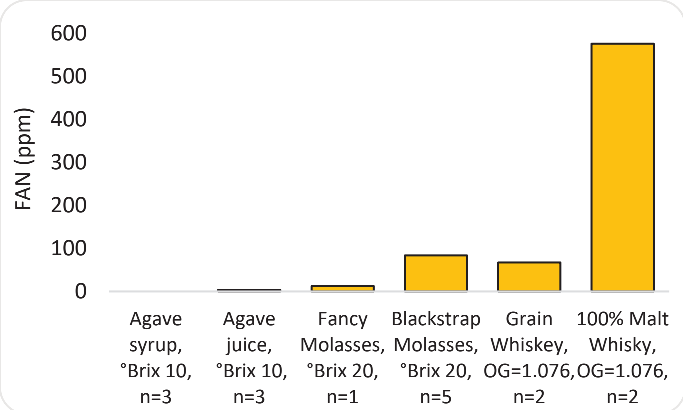


Figure 6: FAN average concentration in different types of feedstocks normalized to typical initial fermentation densities. n = number of substrates analyzed.

Blackstrap molasses contains moderate concentrations of FAN and vitamins and is high in inorganic ions. This substrate however contains the highest concentrations of stressors, such as sodium, sulfate and sulfites, which may impact yeast health during fermentation.

Another major stressor that yeast faces in sugar-based fermentations is osmotic stress due to high dissolved solid content, including non-carbohydrate components and sugar, of which glucose has the greatest impact on yeast.

The use of complex nutrition helps compensate substrate deficiency during fermentation and sustaining yeast metabolism to better resist to mineral and osmotic stress. A positive effect on fermentation has been demonstrated with tailored nutrients, such as DistilaVite® VM and DistilaVite® GN.

Role of nitrogen in congener biosynthesis

Nitrogen also influences the synthesis of volatile aroma and flavor compounds (congeners), both by sustaining yeast homeostasis and more directly by certain amino acids being the precursors of higher alcohols and esters, which are responsible for pleasant fruity and floral notes.

Higher alcohols and esters

Higher alcohols are produced through the Ehrlich pathway, which is used by yeast to degrade amino acids. Acetate esters, such as phenethyl acetate and isoamyl acetate, are produced by the condensation of these higher alcohols and Acetyl CoA.

In addition, enzymes and important coenzymes, such as Coenzyme A (involved in the synthesis of both acetate and ethyl esters), are synthesized from amino acids.

The type of nutrition and the balance between organic and inorganic nitrogen can impact flavor and aroma congener production.

Sulfur-containing compounds

Sulfur containing compounds are typically defined as off-flavours in most distilled spirits categories but could be desirable at low concentrations. Sulfur compounds are derived both from the raw material as well as yeast metabolism.

Hydrogen sulfide and sulfur dioxide are intermediates in the formation of sulfur containing amino acids and coenzymes from sulfate. In absence of proper nitrogen supplementation, the synthesis of sulfur containing amino acids and coenzymes is affected and hydrogen sulfide and sulfur dioxide accumulate as off-notes in the fermentation media.

These can then be further oxidized to produced congeners such as sulfur dioxide or form other defect congeners such as dimethyl sulfide (DMS) or dimethyl disulfide (DMDS), which confer vegetal notes in the final distillate.

Diacetyl

Diacetyl (buttery, popcorn) is a common off-flavor produced from α-acetolactate, an intermediate in the production of valine from pyruvate, resulting from non-enzymatic oxidation. Diacetyl is not detectable when live yeast is present in the fermentation medium and therefore late yeast viability plays a crucial role in the reduction of this congener to 2,3-butanediol.

Increasing late viability of cells helps to reduce overall diacetyl levels in the final beer (wash). Using nitrogen liberating enzymes such as proteases or supplying fermentation with sufficient levels of nitrogen can help to increase late cell viability by supplying amino acids and peptides throughout the entire fermentation process.

Conclusions

Nutrition is essential to sustain yeast growth and metabolism for efficient and consistent fermentation and congener biosynthesis.

This article presents an overview of yeast nutritional requirements in different substrates for distilled spirit production, and how the choice of tailored nutrition can be used to optimize fermentation efficiency, increase yield, and contribute to controlling off-flavour congener synthesis. Our team at Lallemand Biofuels & Distilled Spirits (LBDS) is more than happy to provide further information, troubleshoot and offer technical solutions according to your process.

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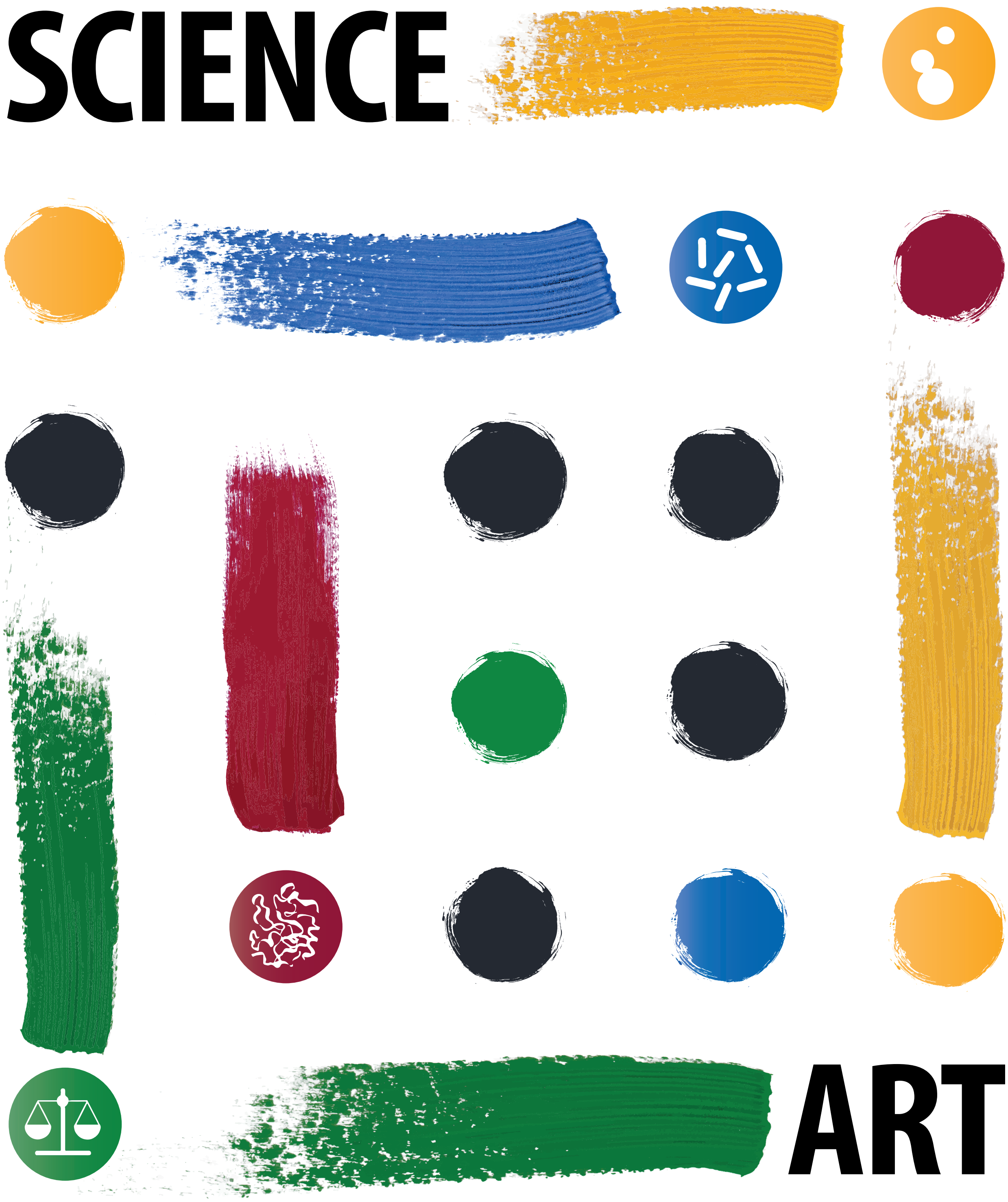

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