

Nitrogen Metabolism During Fermentation*

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Nitrogenous compounds play an important role in winemaking. They serve as nutrients for the growth and metabolic activity of the yeast during fermentation; and as proteins, they also influence wine stability, particularly in white wine.

Quantitatively, next to sugars, nitrogenous compounds are the most important nutrient substances found in grape must. Ammonia, which exists as ammonium (NH_4^+) ions in must, and amino acids are the predominant nitrogen-containing compounds that are utilized by yeast. On a dry weight basis, about 10% of yeast weight consists of nitrogen. All the nitrogen used in building cellular material (population 10^8 cells/ml) during fermentation is taken up from the must. It is therefore important that the must contain sufficient amounts of nitrogen to support a healthy yeast population during fermentation.

Nitrogen metabolism

The grape must contain many amino acids. The main amino acids include proline, arginine, alanine, glutamate, glutamine, serine, and threonine. Many factors influence the amount of amino acids present in the must. The major factors include variety, soil, climate, fertilization practices, and the degree of ripeness.

During the course of metabolism, the nitrogen-containing compounds are used by yeast in the following ways:

- They are taken up and used directly in the biosynthetic process.
- They are converted to some other form and used in biosynthesis.
- They are degraded to release nitrogen, which is then used to meet the metabolic needs of the cell.

Transport of nitrogenous compounds

Regulation and control of metabolic activity of a compound is generally achieved by controlling the transport of the compound into the cell. There are three ways of transporting materials into the cell.

Diffusion

In the process of diffusion a compound simply passes through the plasma membrane (lipid bilayer) and enters the cell without any assistance. This type of movement occurs when a concentration gradient exists and the compound simply moves from a high concentration gradient to a lower concentration gradient until equilibrium is reached.

Facilitated diffusion

In facilitated diffusion the movement of a substance is assisted by a protein. However this process does not require energy. Must sugars such as glucose and fructose are transported into the cell by facilitated diffusion. It is important to note that neither diffusion nor facilitated diffusion can accumulate a compound against the concentration gradient. In the case of sugar metabolism the sugar is degraded upon entering the cell, and thus does not achieve a high concentration inside the cell. The concentration of sugar is higher in must than inside the cell.

Active transport

Active transport involves protein-mediated transport, which requires energy. With active transport, a substance can be accumulated against the concentration gradient.

Most of the nitrogen-containing compounds are transported into the cell by active transport. Generally the concentration of nitrogenous compounds is higher inside the cell than in an external medium such as must. Several amino acid transport systems have been identified in yeast. General amino acid permease (GAP) transports several amino acids. It is inhibited by the ammonium ions. It is, therefore, active later in the fermentation when the must is depleted of ammonium ions. The yeast also contains specific amino acid transporters, which transport specific or a closely related group of amino acids.

Many amino acid transport systems are proton symports, which means that amino acid transport is linked with proton transport. Thus when an amino acid molecule enters the cell, a proton (H^+) also enters the cell. This incoming proton creates a problem. The difference in the must pH and the cell pH is about 3 pH units. This means the H^+ ion concentration in the must is a thousand-fold greater than the cytoplasm (inside cell). When the H^+ ion enters the cell along with amino acid it lowers cell pH. To prevent acidification of cytoplasm and maintain cell pH, the cell must export the proton into the outside medium. This proton export is achieved by a membrane bound ATPase which serves as a hydrogen ion pump. Since protons are exported against the H^+ ion concentration, it requires energy (active transport). The energy is obtained from hydrolysis of the

ATP molecule. For each hydrogen ion pumped out, one molecule of ATP is degraded. The mechanism of amino acid transport is shown in Figure 1.

Movement of amino acid and proton in the absence of ethanol

Amino acid transport is strongly inhibited by ethanol. When a significant amount of ethanol is produced during fermentation the membrane becomes permeable and allows massive influx of protons by diffusion inside the cell. These excess protons from cytoplasm are removed by using a proton pump. However to prevent overloading the pump's capacity to export protons, the cell shuts down the H⁺ ion coupled amino acid transport. Thus, in order to stop too many protons from entering the cell, the cell stops the uptake of amino acids along with protons. This mechanism helps explain the way ethanol limits nitrogen uptake; and consequently, poor response of nitrogen addition on yeast growth when added later in fermentation. Figure 2 shows the effect of ethanol on H⁺ ion influx.

Generally the yeast take up amino acids early in fermentation when the ethanol concentration is relatively low, accumulates and stores them in vacuoles and uses them later when needed for metabolic activity. This approach also gives the yeast cell a competitive advantage because it depletes nutrients from the medium, and thus deprives other organisms from getting nutrients.

Amino acid synthesis

Ammonium ions and amino acids must be key nitrogen-containing nutrients. Yeast can synthesize most of the amino acids needed to build cellular proteins. Amino acids can be formed by using direct amination or a transamination reaction.

In direct amination the ammonium ion is incorporated into a carbon compound produced during sugar metabolism. For example, NADP⁺ dependent glutamate dehydrogenase enzymes catalyze the reaction, which fixes an ammonium ion in α -ketoglutarate to produce glutamate (Fig. 3.).

In the case of a transamination reaction, the amino group is transferred from a donor molecule to a receptor molecule. Glutamate can serve as an amino group donor to produce different amino acids. For example:

In yeast, in addition to amination or transamination, other (complex) pathways for the synthesis of amino acids also exist.

Amino acid utilization

Yeast can degrade amino acids to obtain ammonium ions, which can be used in building other nitrogenous compounds needed for metabolic activity. NAD dependent glutamate dehydrogenase catalyzes the reaction that converts glutamate to NH₄⁺ ion and α -ketoglutaric acid.

In yeast nitrogen metabolism NH₄⁺ ion and glutamate play a vital role. These compounds are directly used in biosynthesis and are, therefore, preferred sources of nitrogen for yeast growth.

In addition to the compounds mentioned above, other preferred nitrogenous compounds include glutamine, alanine, serine, threonine, aspartate, asparagine, urea, and arginine. Arginine and proline are the major amino acids found in grapes. The utilization pathway for proline requires the presence of oxygen; therefore, in the anaerobic conditions of fermentation, yeast cannot use proline as a nitrogen source. This point is important to remember when analyzing grape juice for amino acid content to determine the available nitrogen level.

Arginine degradation results in the formation of urea and ornithine. Urea is further degraded to ammonia and carbon dioxide. Several enzymes participate in this reaction. Urea can combine with ethanol yielding ethyl carbamate, which is a known carcinogen. This matter should be of some concern and attempts should be made to reduce or eliminate the urea level in wine. The second end product arising from arginine is ornithine, which can be broken down to proline or other polyamines. As stated earlier, proline cannot be degraded under anaerobic conditions and therefore will appear in wine.

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