

Nitrogen Plays Many Roles During Fermentation

Uncovering the relationship between nitrogen and aroma development

By Anne Ortiz-Julien, Ann Dumont, Edouard Lordat and Gordon Specht

Nitrogen is a key factor that has a significant impact on wine fermentation. It is the most important yeast nutrient, influencing both fermentation kinetics and wine quality. In this article, we look at the different forms of nitrogen used by yeast and their impact on wine.

Nitrogen is present in grape must in different forms: inorganic (ammonium) and organic (amino acids, peptides and proteins).¹ Nitrogen that can be used by yeast during alcoholic fermentation is called “assimilable nitrogen.” The needs for assimilable nitrogen are different according to the wine yeast used.

Yeast cells also contain nitrogen in the form of proteins, peptides—particularly tripeptides—and amino acids. YAN (yeast assimilable nitrogen) includes free α -amino acids (AA), ammonium and some peptides.⁹ Inactivated yeast-based nutrients can also be used by wine yeast to efficiently carry out alcoholic fermentation.

YAN = free α -amino acids + NH_4^+ + some small peptides

Proline is the only free α -amino acid (AA) not assimilated by yeasts during alcoholic fermentation, even if it is one of the AAs most abundant in grape must.

FAN = Free α -Amino Nitrogen, which is equivalent to the free α -amino acids

The main free AAs (in quantity) in grapes include proline, arginine, glutamate

A study by L. Crépin showed that amino acids could be classi-

fied into three groups based on when they were taken up by the yeast during alcoholic fermentation. Lysine was the first amino acid consumed by the yeast, followed by asparagine, threonine, glutamate, leucine, histidine, methionine, isoleucine, serine, glutamine and phenylalanine. The last amino acids used by the yeast included valine, arginine, alanine, tryptophan and tyrosine.⁵ The initial concentrations of these compounds did not alter the order in which they were consumed, except for arginine and ammonium.

Nitrogen is essential for yeast growth and yeast metabolism. In winemaking, YAN plays a key role at two different levels:

- It represents an important nutritional factor for yeasts during alcoholic fermentation due to its function in protein synthesis and sugar transport.
- It is essential for the biosynthesis of wine quality markers like higher alcohols, thiols and esters by wine yeast.

YAN concentrations in natural grape musts range from about 60 mg/L to 500 mg/L, depending on grape variety, vintage and microclimate. In nitrogen-deficient con-

ditions, yeast growth and fermentation speed are limited. A low initial YAN concentration has been shown to cause slow and sluggish fermentations, which is why nitrogen addition to the must has become a necessary step in wine production.

YAN measurement is a good way for a winery to assess the general state of grapes in terms of nitrogen quantity, but not necessarily nitrogen quality, which is another key parameter from the point of view of fermentation and the sensory quality of the final wine.

Different form of YAN

In order to properly manage alcoholic fermentation, the best fermentation practices recommend both the addition of nitrogen in inorganic (ammonium) and/or an organic form. Organic nitrogen has been shown to be the most efficient and complete nutrient to

complete fermentation and achieve the full aromatic potential of the grape.¹⁰

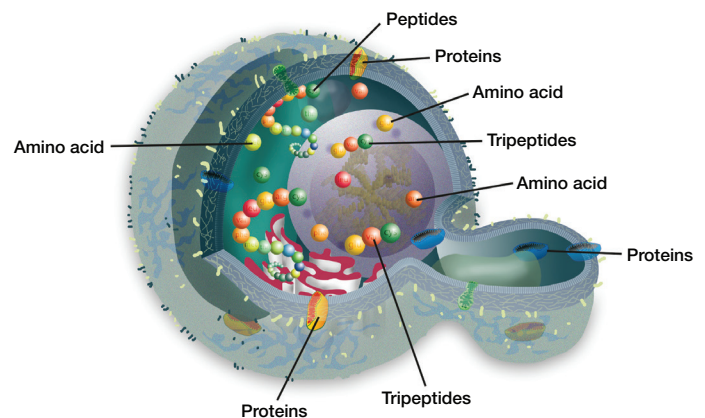
In must, one-third of the nitrogen is found in ammonium form and two-thirds in amino acids, but musts are often deficient in YAN (less than 150 mg/L).⁸

Nitrogen from external sources can be added to the must to assist with fermentation. They come in two forms:

Inorganic nitrogen: ammonium salts such as diammonium phosphate (DAP), which is added during alcoholic fermentation.

Organic nitrogen: in the form of proteins, peptides, tripeptides and free amino acids that are part of the cells from yeast (see “Nitrogen-Containing Compounds in Yeast Cells”). When yeast cells are inactivated through various production processes (inactivated yeast and yeast autolysate), the

NITROGEN-CONTAINING COMPOUNDS IN YEAST CELLS



Nitrogen-containing compounds (amino acids, peptides and proteins) are among the contents of a yeast cell.

nitrogen found in the yeast proteins, peptides, tripeptides and free amino acids is available for the fermenting yeast cells to use during alcoholic fermentation. For example, Fermaid O (100% yeast autolysate derived from enological *S. cerevisiae*) is composed exclusively of organic nitrogen.

When is the best time to add nitrogen?

Extensive studies have determined that the best time to add nitrogen is one-third of the way through fermentation, when the yeast population has reached its peak (see “Timing of Organic Nitrogen Addition”).

A single addition of inorganic nitrogen at the beginning of fermentation is not recommended, as it will lead to a very high yeast population, a sudden increase in fermentation speed that is accompanied by an exothermic reaction (heat production) and high nitrogen depletion. This results in sluggish and stuck fermentation since the yeasts will be left without ad-

The references for this article are available online at winesandvines.com

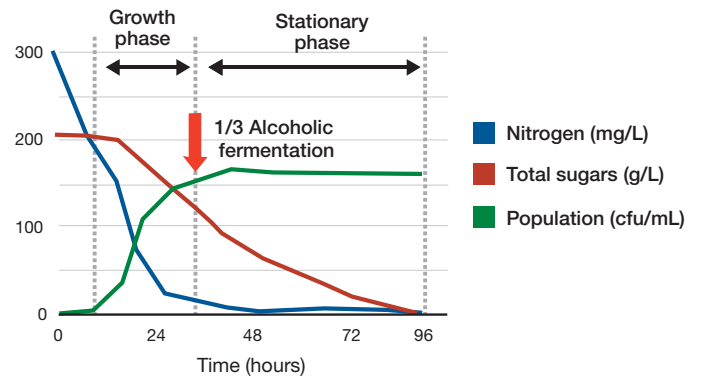
equate nitrogen. Sluggish or stuck fermentations can occur with a single addition of DAP (30 g/hl equivalent to 63 mg/L of YAN) at the onset of the fermentation.

Although it has been shown that the best time to add nitrogen is at about one-third of alcoholic fermentation, when organic nitrogen is added at the beginning of fermentation and at one-third of fermentation, the use of organic nitrogen is slower and more controlled. Consequently, the fermentation is more even—with no heat peaks and better temperature control—and the alcoholic fermentation goes to completion (see “Impact of Organic Nutrition on Alcoholic Fermentation”).

Effect of nitrogen on fermentation kinetics

YAN content is one of the most important regulators of fermentation speed; it impacts yeast

TIMING OF ORGANIC NITROGEN ADDITION



About one-third of the way through fermentation is the optimum time to supplement the fermenting must with appropriate nutrition (YAN, oxygen and sterols sources). Source: A. Julien, 2015

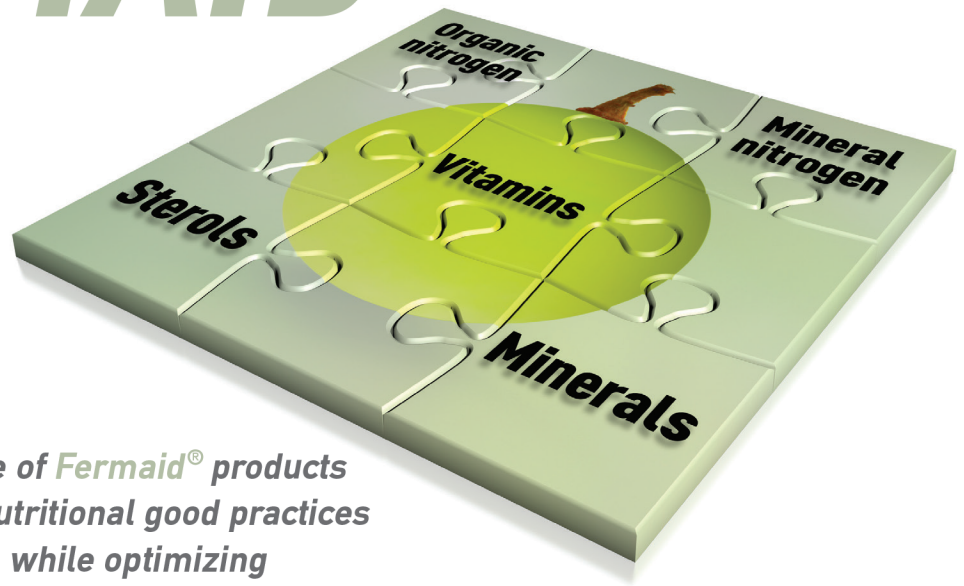
biomass at the beginning of fermentation and sugar transport kinetics during fermentation. As soon as must has a nitrogen deficiency at the end of the growth phase, there is a decrease in protein synthesis and sugar transport activity. YAN addition to nitrogen-deficient must leads to a significant decrease in fermentation length by reactivating protein

synthesis and increasing sugar transport speed, which results in an increase in the fermentation rate.

There are many studies describing the difference in efficiency between organic and inorganic nitrogen additions with respect to kinetics. We compared the efficiency of both sources (DAP for inorganic nitrogen and

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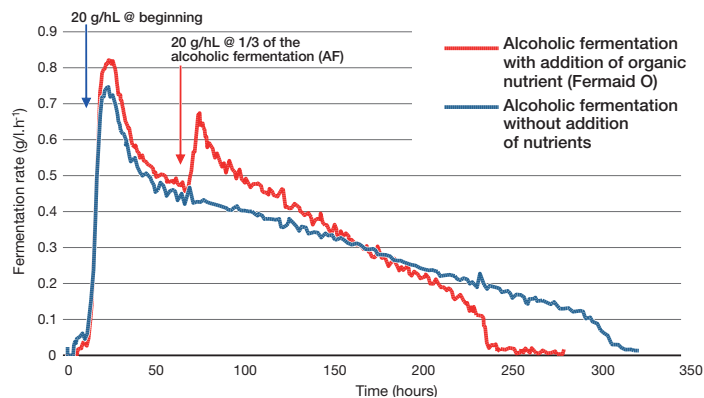
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IMPACT OF ORGANIC NUTRITION ON ALCOHOLIC FERMENTATION



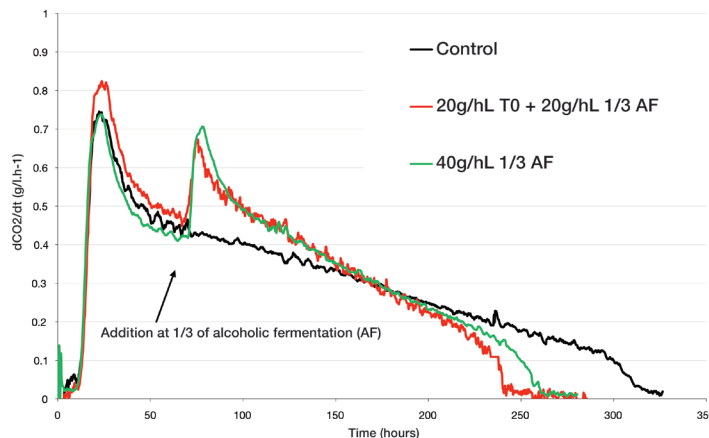
Impact of organic nutrition addition on alcoholic fermentation. 20 g/hl (8 mg/L of YAN) of organic nitrogen added at the beginning of the fermentation, and another 20 g/hl (8 mg/L of YAN) at one-third of the alcoholic fermentation.

Fermaid O for organic nitrogen) and applied two nutrition strategies using the same amount of YAN: 16 mg/L, in organic compared to the inorganic form. A third fermentation was performed without nutrition, as a control.

The graph “Impact of Inorganic and Organic Nutrition on

Alcoholic Fermentation” shows that for an equivalent amount of assimilable nitrogen added, the addition of organic nitrogen effectively enables the fermentation kinetics (green line) to complete fermentation. With the inorganic nitrogen source added (purple line), the fermentation is sluggish

ORGANIC NUTRIENT: ADDITION TIME COMPARISON



Data comparing the two-step additions and a single addition at one-third of the alcoholic fermentation of organic nutrient Fermaid O.

then stuck, as is the case with the control fermentation (blue line) with no nitrogen added. For an equivalent dosage of YAN, an organic nitrogen source is much more efficient than an inorganic nitrogen source.

In a highly nitrogen-deficient must (100 mg/L YAN), an appro-

priate organic nutrition strategy is sufficient enough to complete alcoholic fermentation.

Nitrogen impact on aromatic profile of wine

The metabolism of nitrogen, notably from amino acids, generates the formation of numerous aroma



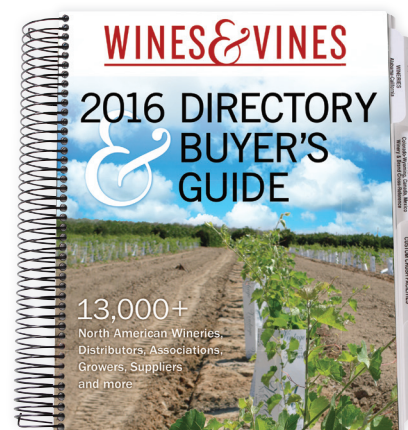
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AVOIDING STUCK FERMENTATION WITH PROPER NUTRITION

Assimilable nitrogen is essential for protein synthesis in yeasts. It is usually the main limiting nutrient and the major reason for slow fermentation in standardized conditions. There is a relationship between the assimilable nitrogen concentration in the must and the maximum fermentation rate. Ammonia nitrogen and free amino acids are the main nitrogen sources even though some small peptides can also be assimilated.

Assimilable nitrogen is considered deficient when it is less than 150 mg/L. In such cases nitrogen supplementation is very efficient at about one-third

of alcoholic fermentation. When nitrogen is added too early, the cell population and the fermentation rate during the first half of fermentation are increased, but the cell viability at the end may be decreased.

To reduce the risk of stuck fermentation, add nitrogen at the beginning of the stationary phase (after 30% to 40% of fermentation) and combine this addition with oxygenation or to add a complex product containing other nutrients, such as lipids. Ammonia nitrogen can now be replaced by organic nitrogen, with a better effect on fermentation kinetics.

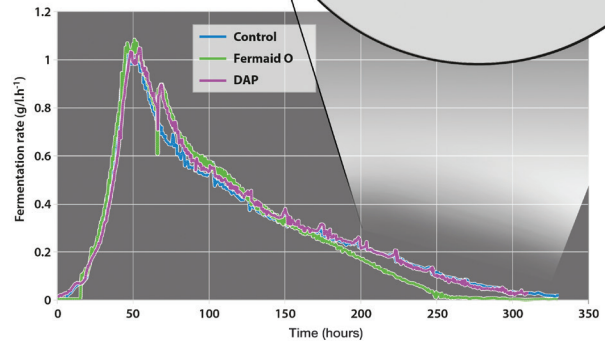
—Dr. Jean-Marie Sablayrolles

compounds involved in the aroma matrix of wine: higher alcohol and acetates. Yeast metabolism also influences the expression/

creation or preservation of certain aroma precursors of an amino nature (cysteinylated precursors or glutathionylated precursors of

IMPACT OF INORGANIC AND ORGANIC NUTRITION ON ALCOHOLIC FERMENTATION

Fermentation kinetics are shown for a Viognier fermented at 18° C with a sugar concentration of 215 g/L and initial YAN less than 100 mg/L. Additions of 16 mg/L of YAN in two different forms—inorganic nitrogen (DAP) and organic nitrogen (organic nutrient)—were made at two stages of fermentation: 8 mg/L of YAN at the beginning of alcoholic fermentation and 8 mg/L of YAN at one-third of alcoholic fermentation. The enlarged section represents the end of the fermentation.



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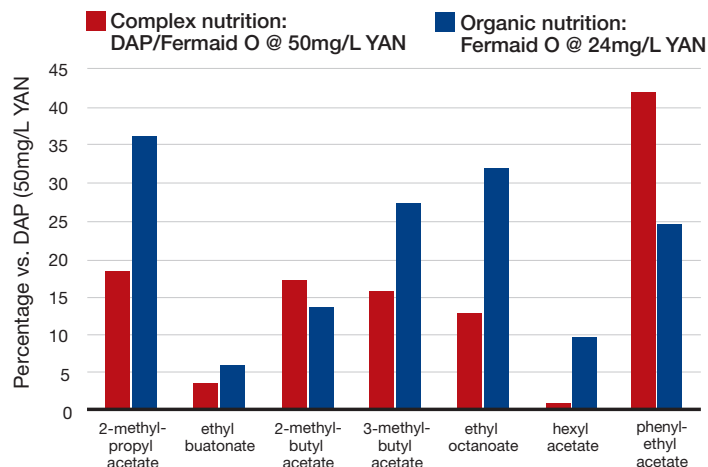
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varietal thiols). As a result, the nitrogen composition of the must can modulate the aroma profile of a wine. Organic nutrients have also been shown to positively influence the formation of aroma compounds when added during alcoholic fermentation.

Impact of different nitrogen sources on sensory profile

Esters: The metabolism of amino acids (anabolism and catabolism) by yeast leads to the formation of higher alcohols, acetate esters and ethyl esters. The ester profile of

ESTER FORMATION WITH ORGANIC NUTRITION



Chardonnay from Yalumba Winery in Australia fermented with two different sources of nitrogen (DAP and Fermaid O).

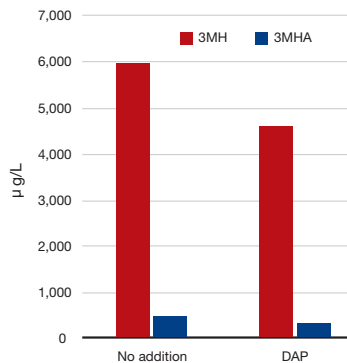
Chardonnay (see “Ester Formation With Organic Nutrition”) was different when different forms of nitrogen were added.

For example, comparing the synthesis of ester compounds with an addition of 50 mg/L of YAN in DAP form compared to 24 mg/L of YAN (Fermaid O) and 50 mg/L of YAN (DAP/Fermaid O) showed a significant increase for all aromatic compounds that are observed with the organic nutrient, demonstrating the impact of organic nitrogen compared to inorganic nitrogen on the formation of esters.

Thiols: During fermentation it has been shown that excessive inorganic ammonium or addition at the beginning of alcoholic fermentation limits the release of varietal thiols by the yeast.¹² Catabolic repression by the inorganic nitrogen in the form of ammonium prevents the synthesis of amino acid transporters in fermenting yeast. It also limits the entry of thiol precursors of the cysteinylated form into the cell and, consequently, their intracellular conversion into volatile thiols (see Transport of Thiol Precursors in Yeast Cells”).

In terms of thiol production (see “Thiol Formation With DAP”), the thiols 3-MH and 3-MHA are lower when DAP is used. This observation, made in the laboratory and on a pilot scale, has led to implementation of a strategy for nitrogen nutrition during alcoholic fermentation, with the addition of organic

THIOL FORMATION WITH DAP



Thiol concentration in Sauvignon Blanc (Gers, France 2004) with DAP addition.¹²

nutrient only, divided between the beginning of the alcoholic fermentation and at one-third of the alcoholic fermentation.

Two different formulas of organic nutrients have been tested on Colombard juice, which is very rich in thiol precursors. Looking at the results on the 3-MH release and its acetate of 3-MH, we confirmed the positive impact of organic nutrition on thiol conversion and expression (See “Thiol Formation With Organic Nutrition”).

Impact of nitrogen source on wine sensory profile

In all trials, we were able to highlight the positive influence of organic nutrient on the wine sensory profile, mainly when compared to inorganic nutrition such as DAP addition. In (See “Sensory Profile With Organic Nutrition”), the dif-

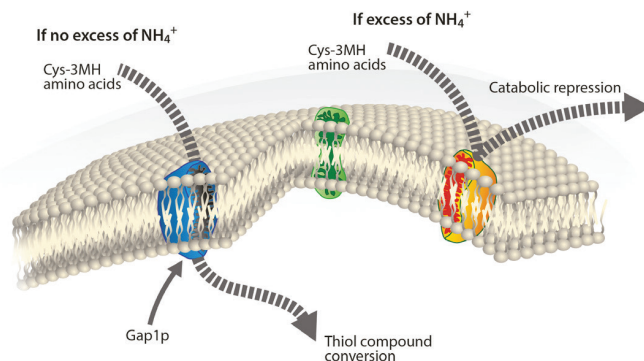
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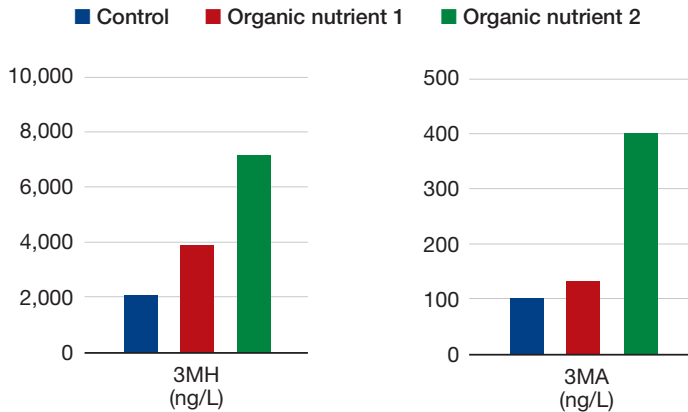
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TRANSPORT OF THIOL PRECURSORS IN YEAST CELLS



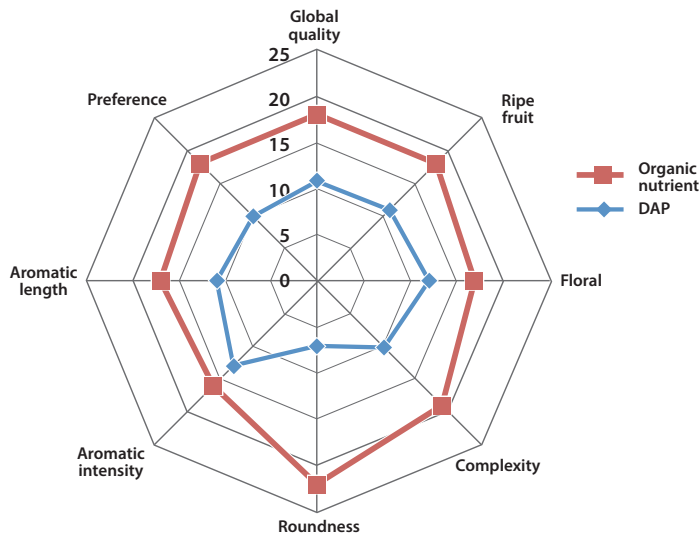
Transport of Cys-3MH amino acids into the yeast cell membrane via the Gap1p transport protein, and catabolic repression in the presence of excessive inorganic nitrogen in the must.

THIOL FORMATION WITH ORGANIC NUTRITION



Thiol concentration (3-MH and 4-MMP) in Colombard (France, 2014) with different organic nutrients compared to a control fermentation (without nutrition strategy).

SENSORY PROFILE WITH ORGANIC NUTRITION



The sensory profile of a 2012 Viognier from Côtes du Rhône (France) compares organic and inorganic nutrition.

ferences are very significant: A professional tasting panel preferred the organic nutrition, which produced a more complex and intense wine with greater aromatic length.

Conclusion

Wine yeast requires certain essential elements to transform must into wine: sugars, vitamins and minerals, some oxygen and—also very important—a sufficient concentration of YAN. This type of nitrogen is composed of amino acids, some small peptides and ammonium that can be used by yeast cells to not only complete

fermentation but also to fully develop the sensory profile of a wine. Many wine musts are deficient in nitrogen, and the type of nutrient used—as well as the timing of addition—is crucial to the development of alcoholic fermentation. 🚫

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