Fermentation: Fermenting Flavours with Yeast

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Think about the aroma of coffee in the morning, of freshly baked bread when you walk outside the boulangerie, or of red wine or a foamy beer on a Saturday night. Those are all aromas that we can sense by mentioning them; most of us feel related to them. The interesting part is where these food and beverage flavours and aromas come from. Most people probably do not realise that yeast is responsible for producing most of these during the process of fermentation. These characteristic aromas and flavours come from the action of microbes that, in fact, have the most important role during the fermentation process. In 1986, the well-known author Lalli Nykänen stated that “the formation of the most dominant compounds occurring in beverages depends more on the yeast selected than the raw materials used in fermentation” and that “the body of flavour is formed during fermentation by yeast”. Over the years, many scientific studies have focused on aroma production by yeast, and these observations have been supported.

**Yeast**

The essential ingredient for the production of alcoholic beverages and other fermented foods is yeast, but what is yeast? From the food and beverage field, yeast is seen as a food enhancer. In general, most people have a positive impression of yeast, as it converts cheap materials to expensive goods. From a microbiologist’s point of view, yeasts are unicellular microorganisms that belong to the fungal kingdom. In other words, yeasts are related to organisms such as edible mushrooms and moulds present in some types of cheese. The yeast cell has an egg-like shape and can only be seen under a microscope (Figure 46.1). These microorganisms are normally found in different environments such as soil, plants, skin, leaves, fruits, water and the human intestinal tract.

About 1500 species of yeast have been identified, but fewer than 20 are typically associated with food fermentation. Of these, *Saccharomyces cerevisiae*, which got its name from Greek words meaning “sugar fungus”, is the most important and the most widely studied yeast species. *S. cerevisiae* has desirable characteristics for producing fermented foods and beverages, such as high ethanol production and tolerance, the ability to complete fermentation efficiently, a lack of toxicity for the consumer, and the production of desirable flavour and aroma molecules. The accumulation of these positive characteristics can be explained by the fact that humans have domesticated yeast from wild relatives. Some studies have revealed how industrial traits have been selected over time for the best quality of the final food product. Therefore, yeasts with desirable traits have been selected as starters for other fermentations and slowly adapted to industrial conditions, giving rise to improved yeast strains.

However, *S. cerevisiae* is not the only yeast species present in food fermentations; there is a diverse microbial community found in the raw material of fermented beverages, for example, in the grapes used for winemaking and in the winery environment. This microbial community includes other yeast species, moulds and bacteria. These other yeast species, called non-traditional yeasts, are being explored by the brewing, winemaking and baking industries, as some may have desirable properties, such as resistance to different environmental constraints, production of compounds of interest such as higher alcohols, and the use of different biological mechanisms to survive. Some of the commercial yeasts have been developed purely on the basis of fast growth and effective performance. In contrast, some other yeast strains have been developed to improve traits and increase production levels of desired compounds such as aromas.
Fermentation

Yeast is a microorganism that can convert raw materials such as grape juice and malt into valuable products through fermentation. In principle, during fermentation, the yeast converts sugars to alcohol and carbon dioxide (CO$_2$). In the case of bread production, the yeast consumes the starch in the flour and releases gas that causes the bread dough to rise. Likewise, in an alcoholic beverage process, the yeast converts the sugars present in fruit juice or malt into alcohol, resulting in products with high amounts of ethanol and sometimes bubbles of CO$_2$ and foam. Other important products of fermentation are volatile molecules that give different flavours and aromas to the beverage.

Technically, the process of fermentation by yeast is carried out anaerobically to enable the cell to maximise its fermentative abilities. The main biochemical reaction is the conversion of glucose into pyruvate, followed by the cleavage of pyruvate to produce CO$_2$ and acetaldehyde, which later is converted to ethanol by an alcohol dehydrogenase (Querol and Fleet, 2006). The main reactions taking place during fermentation are shown in Figure 46.2. Furthermore, the general fermentation yield of \textit{S. cerevisiae} in alcoholic beverages is such that one cell is able to ferment its own weight of glucose per hour, producing up to 18% volume of ethanol. This is a valuable fact in the fermentation industry, as cheap substrates are converted into expensive products through a biological process carried out by yeast. There are important factors that influence the fermentation and therefore, the quality of the final product; the main factors are the yeast strain, temperature, sugars, nitrogen source, oxygen availability and pH.

For millennia, yeast has been used to produce alcoholic beverages such as wine, beer, cider and other spirits. Originally, the fermentation of food and beverages was a spontaneous process that, over the years and the development of civilisation, was replaced by a controlled process, in which known cultures of yeasts were used to start the fermentation. Therefore, the fermentation process has been improved in many ways, increasing the quality of the products and the process itself.

Flavour and Aroma Properties of Yeast

When consuming fermented products, we find special flavours and aromas that make the food or drink unique. The presence of those flavours is an important matter, as the organoleptic fingerprint of the product changes depending on the presence or absence of the aroma compounds (Figure 46.3). Some of these come from the ingredients used for fermentation, for instance, the use of sweet or acid grapes in winemaking, or toasted or pale malt in the case of beer; the choice of using one ingredient or the other changes the final aroma profile of the product. Some other aromas come from the ageing process, especially in cases when the beverage is stored in wood barrels, a phase when chemical exchange between the wood and the beverage takes place.

FIGURE 46.2 Overview of metabolic routes during alcoholic fermentation inside the yeast cell. On the left, glucose conversion into ethanol and CO$_2$. On the right, the Ehrlich pathway and main reactions contributing to production of aroma volatiles.
Finally, the part that most affects the flavour and aroma profile is the type of yeast used and the fermentation conditions.

Each yeast has different ways to assimilate substrates and yields different products depending on its genetic make-up or genotype. Thus, depending on its genotype, the yeast reacts differently to the conditions that it finds in its environment. This will cause different enzymes to be produced inside the cell and ultimately, will lead to a distinct flavour and aroma profile. This is the reason why the choice of yeast species to produce fermented beverages is crucial. The fermentation factors with the highest impact on flavour and aroma production are the yeast strain, the temperature and the nitrogen source. Fermentations at low temperature, for instance, result in aroma retention and increased production of aroma compounds. Moreover, although the use of a specific type of nitrogen source increases or decreases the formation of specific aroma compounds, the difference in the aroma production is attributed to the metabolism of each yeast species.

The yeast takes amino acids and sugars to convert them into aroma molecules that give different organoleptic properties to the product: “fruity”, “floral”, “cheesy” or “rancid”, for instance. So, when the label description of some fermented beverages has fruity and flowery tones in the aroma profile, it is not referring to the fact that roses, banana or pineapple were added to the beverage but to the use of particular yeast species that produce those aroma characters. While we depend on yeast to produce most of the great aromas that we associate with our favourite fermented beverages, excessive production of aroma volatiles is not always beneficial; high amounts of these compounds can have the opposite effect on the aroma profile. A specific example is ethyl acetate, an aroma compound produced by winery yeasts, which in concentrations of 25–30 mg/L gives a pleasant fruity aroma to the wine, but in concentrations higher than that is perceived as giving a solvent- or nail-polish-like odour. A case of too much of a good thing!

The flavour and aroma compounds present in fermented food products are volatile molecules with low molecular weight, small enough to reach our sensory receptors and be recognised by the olfactory and gustatory system. Most of these flavour molecules are alcohols, aldehydes, esters and organic acids that are products of fermentation (Dzialo et al., 2017).

How are the aroma molecules produced in yeast? What happens inside the yeast cell is that some components of the raw fermentation material are slowly converted into higher alcohols and esters through a mechanism called the Ehrlich pathway, described by the German biochemist Felix Ehrlich over 100 years ago. The Ehrlich pathway (Hazelwood et al., 2008) is a catabolic route whereby the amino acids available in the media are converted into higher alcohols through three main enzymatic steps, which are illustrated in Figure 46.2. The Ehrlich pathway steps are explained as follows:

- Transamination: The reaction consists of the transfer of amines from the amino acid to another molecule to form the respective α-keto acid; there are four enzymes catalysing the reaction, encoded by the \( BAT1, BAT2, ARO8 \) and \( ARO9 \) genes;
- Decarboxylation: This an irreversible step where the α-keto acids remaining from the transamination step lose a carbonyl group to form the respective aldehyde and release \( CO_2 \). This reaction can be catalysed by five decarboxylases, encoded by the \( PDC1, PDC5, PDC6, ARO10 \) and \( THI3 \) genes;
- Reduction to higher alcohols: This is the last step of the Ehrlich pathway. The alcohol dehydrogenase (ADH) enzymes convert the aldehydes into their respective higher alcohols, also known as fusel alcohols.

Besides the reduction step, there is a parallel oxidation of the aldehyde to form fusel acids, which play a minor role in yeast. The balance of these two reactions happening in the yeast cell depends on the fermentation conditions; normally, in \( S. cerevisiae \), the production of fusel alcohols is 90% higher than that of fusel acids, although it mainly depends on the redox status of the cell. At the end of the formation of the fusel alcohols and fusel acids, the cells release these compounds to the culture medium through simple passive diffusion for the higher alcohols or with the action of an exporter protein for the fusel acids.

Esters are another group of volatile compounds that significantly contribute to the aroma in alcoholic beverages. In wine and beer, esters define the final aroma depending on the concentrations produced by the yeast strain (Holt et al., 2019).
A low level of these compounds is enough to generate pleasant odours; on the contrary, when the compounds are overproduced, there is a negative effect on the final aroma of beer and wine (Pires et al., 2014; Querol and Fleet, 2006). Acetate esters are one of the major groups of esters produced by yeast. Their formation results from the transfer of an acetate group from acetyl-CoA to ethanol or a higher alcohol. The enzymes involved in this reaction are alcohol acetyltransferases (AATases), which are encoded by the genes \textit{ATF1} and \textit{ATF2}. The availability of acetyl-CoA in the cell is important for the production of acetate esters in anaerobic conditions.

**Yeast in the Food and Beverage Industry**

Currently, yeast is used for several purposes in the food and beverage industry. The most common uses of yeast are alcoholic beverage fermentation and baking. However, non-alcoholic beverages are also produced by yeast; in this case, the fermentation is stopped before the drink becomes alcoholic, but the modification of the beverage features is achieved. Thus, even non-alcoholic beer requires a yeast fermentation! Kefir and kombucha (see chapter by Lavelle and Boulé in this book) are other examples of non-alcoholic yeast products, and in these cases, bacteria also play a fermentation role. Moreover, yeast is used as a food additive; it is added to a large range of food products with different purposes such as food flavouring, replacing ingredients for vegan alternatives, and adding texture to the food. In other cases, yeast is used as a nutrient supplement for dietary purposes, thanks to yeast being a source of vitamins, minerals and high-quality protein. On top of that, some yeast species are commercialized as probiotics, especially \textit{Saccharomyces boulardii}, which is reported to confer health benefits.

In conclusion, yeast plays a significant role in the food and beverage industry, especially in the fermentation sector. Over the years, increasing research and knowledge generation on different strains of yeast has improved the quality of food products. It is fascinating to understand how the desired flavour and aroma profile can be accomplished by employing the right yeast strain.

**REFERENCES**


