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Yeast: World's Finest Chef

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1. Introduction

Yeast is the simplest eukaryotic organism of our days. They are unicellular microorganisms classified in the kingdom Fungi. Nevertheless, yeasts were probably the first microorganism to be domesticated and since early in human history have been used on a daily basis in bread making and in alcoholic beverages. Nowadays, yeast has become a key microorganism for many types of industrial and food processing manufactures, including the production of beer, wine, cheese and bread. In particular, its use in baking industry is quite relevant due to the central role of bread as a dietary product all over the world. Moreover, yeasts are regarded with reasonable interest as nutrients and health provider sources both for humans as well as for animals. We dare to appoint yeast as the one of the world finest chefs.

Yeasts are found in diverse natural environments; colonizing from terrestrial, to aerial and aquatic environments. They can be found on decomposing fruit, on soils, as opportunistic pathogens in human beings, in the gut of the fish and free living in the sea. In general they contribute to the decay of organic material, but their successful colonization is intimately related to their capacity of physiologically adapt at diverse milieus. Hitherto, it has been described approximately 1500 species [1].

This chapter aims at contribute to a comprehensible analysis of the role of yeasts on the actual feed lifestyle, mainly in what regards the yeast *Saccharomyces cerevisiae*. This yeast is known with by many appellations: "Baker's yeast" in baking and confectionery fields, "Brewer's Yeast" by all beer industrial and artisanal producers, and perhaps less familiar "Wine's Yeast" by wine-like alcoholic beverages producers. We will first go over several physiologic aspects of this yeast metabolism, specifically associated with glucose catabolism, under anaerobic environments (fermentation) as well as aerobic conditions. Most of our attention is given to glycolysis pathway and to alcoholic fermentation in order to prepare the reader for the issues discussed later. Considerable notice will be paid to the intervention of

yeast in alcoholic beverages, in particular beer and wine, important economical industries of our times. The particular role of *S. cerevisiae* in baking industry, interactions with lactic acid bacteria (LAB) in sourdoughs, and also the scientific approaches/advances for sustainability were exhaustively reviewed, recently by us in [2] but also by [3], therefore we will focus preferentially on the production of commercial yeast for baking. In the end, we will briefly review the other applications of *S. cerevisiae* in less familiar products, including animals and fish feeding rations and biotic supplements.

1.1. Yeast metabolism

Yeasts, resembling other heterotrophic organisms, have the energy and carbon metabolism operating in concert, *i.e.*, anabolism is coupled with catabolism. Their chemical energy, in the form of ATP, results from the oxidation of organic molecules and is used as energy resource by the cell. On the other hand, those organic molecules can also operate as carbon sources for biosynthesis. Yeast environmental diversity leads to a vast metabolic complexity, due to the multiplicity of carbon and the energy sources available in nature. This includes polyols, alcohols, organic acids and amino acids yet, yeasts preferentially metabolize sugars.

1.1.1. Greedy yeasts – Sugar metabolism

The yeast metabolize diverse sugars, hexoses such as glucose, fructose, galactose or mannose, some can use pentoses like xylose or arabinose, disaccharides as maltose or sucrose; yet, glucose and fructose are the preferred substrates. The metabolic routes for the dissimilation of hexoses and disaccharides share the same pathways, with the great majority of the metabolic elements arising from intermediaries of glycolysis, the tricarboxylic acid cycle (TCA) and the pentose phosphate pathway, and differ only in the initial basic steps of metabolism.

The sugar dissimilation may occur in anaerobic or in aerobic environment. In the first case is called **fermentation** and in the presence of oxygen is named **respiration**. The most common process is the glucose dissimilation, generally known as **alcoholic fermentation**, which occurs anaerobically and yields as final products: ethanol and CO₂.

For the sugar utilization, yeast has primarily to sense the presence of glucose in the environment and then to transport it across the plasma membrane [4, 5]. The presence and levels of glucose sensed by the yeast can influence the enzyme levels through several processes, alteration of mRNA translation rates; mRNA stability or protein degradation, but also the concentration of intracellular metabolites (for a review see [6]). Yet, the major outcome is the extensive transcriptional regulation of a large number of genes leading to the adaptation to fermentative metabolism (alcoholic fermentation). These encompasses the induction of genes required for the utilization of glucose, such as genes encoding glycolytic pathway enzymes (discussed below), whereas genes required for the metabolism of alternative substrates, and those encoding proteins in the gluconeogenic and respiratory pathways are repressed by glucose (for reviews see [6] and [7]).

The gene family of hexose transporters in *S. cerevisiae* consists of more than 20 members: i) 18 genes encoding transporters (*HXT1-HXT17*, *GAL2*), being the most relevant Hxt1p and Hxt3p, with a low affinity for glucose and high transport capacity, and Hxt2p, Hxt4p and Hxt7p, with a high affinity and low transport capacity and; ii) at least two genes encoding sensors (*SNF3*, *RGT2*), although several points of evidence suggest that *GPR1* and *HXK2* also sense and signal glucose levels [6, 8]. All these, sensors and transporters are therefore the primary interveners on sugar metabolism. After glucose uptake, it enters in the glycolytic pathway (Figure 1 – Steps from glucose to pyruvate) in order to be metabolized to pyruvate, whereby production of energy in form of ATP is coupled to the generation of intermediates and reducing power in form of NADH for biosynthetic pathways (for reviews see [5, 6, 9]) [10].

The first step of the glycolytic pathway consists on the phosphorylation of glucose to glucose 6-phosphate by the action of the hexokinases (Hxkp) and the glucokinase (Glkp); which are linked to high-affinity glucose uptake. Then glucose-6-phosphate is isomerized by the phosphoglucose isomerase, encoded by *PGI* gene, to fructose-6-phosphate. The next step, done by the phosphofructokinase (Pfkp) requires energy, in the form of ATP, to convert fructose-6-phosphate into fructose 1,6-biphosphate.

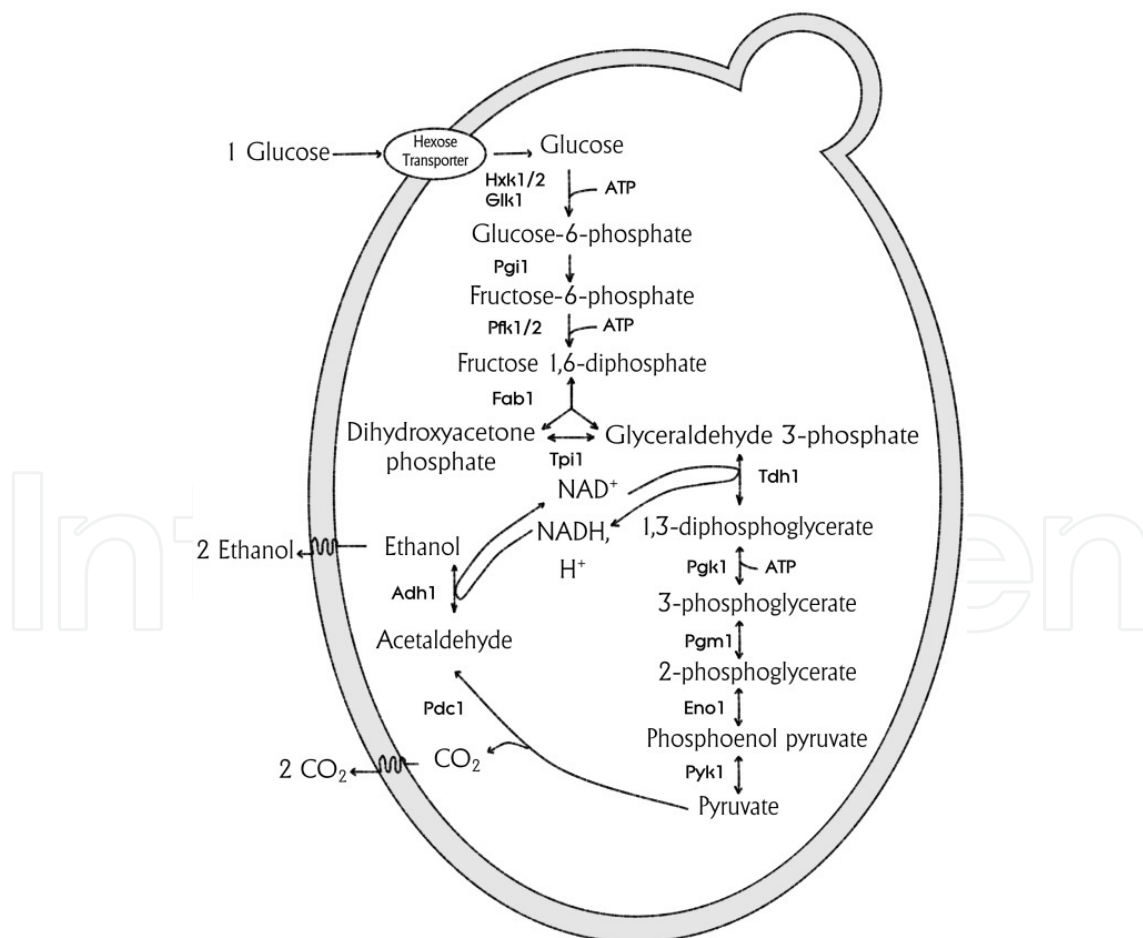


Figure 1. Alcoholic fermentation - enzymatic steps on *S. cerevisiae* (adapted from [11]).

Yeast phosphofructokinase, Pfkp, is a heterooctameric enzyme subject to a complex allosteric regulation. Aldolase (fructose 1,6-bisphosphate aldolase- Fbap) in turn, catalyses the reversible cleavage of fructose 1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. These two compounds can be converted one into another, again in a reversible way, by the triosephosphate isomerase (Tpip). Subsequently, glyceraldehyde 3-phosphate yields the pyruvate by the action of a series of acting enzymes, whereas some of the dihydroxyacetone phosphate follows gluconeogenesis. Glyceraldehyde 3-phosphate is firstly oxidised by NAD^+ (with the production of a reducing equivalent, which will take part in the latter steps of glycolysis when acetaldehyde gives ethanol (Figure 1)) and then phosphorylated, under the catalysis of the 3-phosphate dehydrogenase (Tdhp). The resulting 1,3-diphosphoglycerate, by the action of phosphoglycerate kinase (Pgkp), donates a phosphate group to an ADP molecule originating the 3 phosphoglycerate and releasing 1 molecule of energy (ATP). The next step is just a relocation of the phosphate group on position 2, done by the phosphoglycerate mutase (Pgmp); preparing this way the following reaction, the dehydration by the enolase (Enop) and from which results the phosphoenol pyruvate, a high energetic molecule. This is then phosphorylated by the pyruvate kinase (Pykp) giving the pyruvate and also releasing another molecule of ATP.

At this point, pyruvate can follow distinguished metabolic routes (Figure 2) depending on the environmental conditions, which in turn regulate the enzymes involved as well as their kinetics properties, but also of the yeast species [12]. Conversely, the carbon flux gets to a branching point in which may be divided among the respiratory and the fermentative pathways.

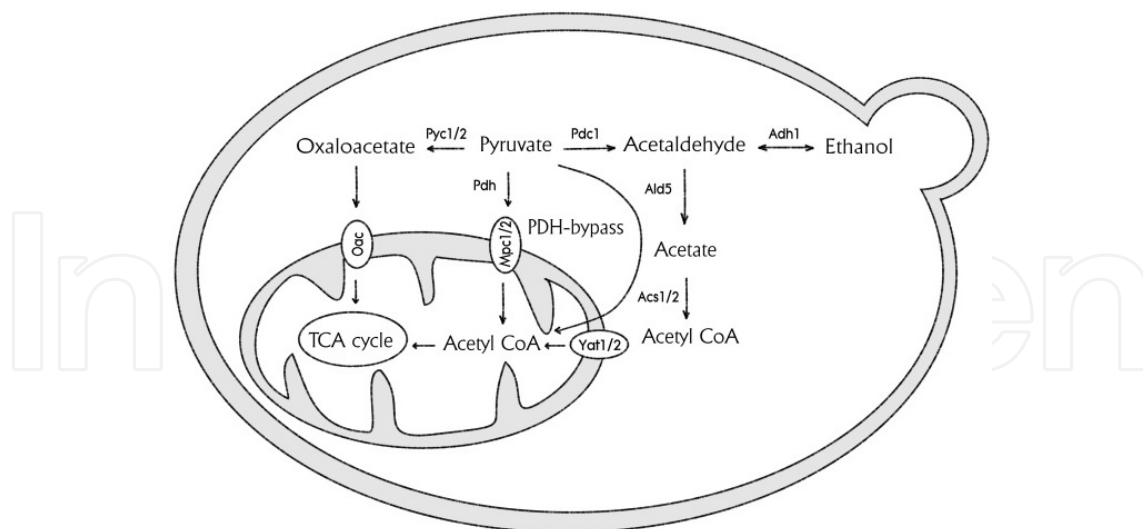


Figure 2. Pyruvate formed in glycolysis alternative metabolic routes. Pyruvate can be converted into 2 intermediates of TCA cycle: acetyl-CoA by the pyruvate dehydrogenase complex (Pdhp) and transported to the mitochondria by mitochondrial oxaloacetate carrier (Oacp); and/or oxaloacetate by pyruvate carboxylase (Pyc1p/2p) whose mitochondrial carrier is (Mpc1p/2p). Pyruvate can also be decarboxylated to give acetaldehyde by the pyruvate decarboxylase (Pdc1p). Adh1p - alcohol dehydrogenase; Ald5p - acetaldehyde dehydrogenase; Acs1p/2p - acetyl-CoA synthase; Yat1p/2p - carnitine acetyltransferase (adapted from [9]).

In alcoholic fermentation, pyruvate is decarboxylated to give acetaldehyde and CO₂, by the pyruvate decarboxylase (Pdc1p). In the final reaction, catalysed by the alcohol dehydrogenase (Adhp), acetaldehyde is reduced yielding the ethanol and promoting the re-oxidation of NADH to NAD⁺. At the same time, and in addition to the 2 molecules of CO₂ and of ethanol, formed per molecule of glucose, the sugar is incorporated into other by-products such as yeast biomass, acids (pyruvic, acetaldehyde, ketoglutaric, lactic) and also importantly glycerol. This is generated from dihydroxyacetone-phosphate and is, to a certain extent, very desired by the wine producers in order to get fuller bodied wines (discussed below). Furthermore, alcoholic fermentation is a redox-neutral process; given that the NADH produced during the oxidation of glyceraldehyde 3-phosphate is afterwards reoxidized in the reduction of acetaldehyde to ethanol [13]. Yet, one must keep in mind that with fermentation is associated culture growth and, biomass composition is more oxidized than glucose, consequently an excess of reducing equivalents may be attained. The way yeast circumvent this problem, under anaerobic conditions, consists on the production of glycerol by reduction of the glycolytic intermediate dihydroxyacetone phosphate to glycerol 3-phosphate catalysed by NAD⁺-dependent glycerol 3-phosphate dehydrogenase (encoded by the two isogenes *GPD1* and *GPD2*), and its subsequent dephosphorylation due to the action of glycerol 3-phosphatase (encoded by *GPP1* and *GPP2*) [14-16].

Although fermentation usually happens in the absence of oxygen, this is not a strict rule. Even in the presence of high levels of oxygen, if the sugars are fully accessible to be metabolized, yeasts choose to ferment instead of respire. This phenomenon is called the Crabtree effect [17], defined as the inhibition of aerobic metabolism when glucose is available, which occurs both in the presence or absence of oxygen. For instance, *S. cerevisiae* is known as Crabtree negative yeast, since is able to produce ethanol aerobically in the presence of high external glucose concentrations. These high concentrations promote the acceleration of glycolysis, producing appreciable amounts of ATP through substrate-level phosphorylation. Simultaneously, it reduces the need of oxidative phosphorylation done by the TCA cycle via the electron transport chain, inhibits respiration and ATP synthesis, and therefore decreases oxygen consumption. Conversely, Crabtree negative yeasts produce biomass via TCA cycle, but these should not be mystified with obligate aerobes: Crabtree-negative yeasts are able to ferment, yet usually only ferment under anaerobic conditions, since there is no inhibition of aerobic respiration in the presence of glucose and this is a more efficient form of energy metabolism. Obligate aerobe yeasts, on the other hand, cannot ferment and only respire aerobically, providing another category of metabolic diversity. Moreover, Crabtree effect is not specific to yeasts: many mammalian tumour cells display a Crabtree effect as well [18-20].

In **aerobic respiration** (Figure 3), the pyruvate is converted to Acetyl-CoA due to an oxidative decarboxylation, catalysed by the pyruvate dehydrogenase multi enzyme complex. In this way starts TCA cycle, which major issue is to supply the respiratory chain with reducing equivalents (in form of NADH and FADH₂) obtained from the oxidative decarboxylation of Acetyl-CoA, which is then used to generate energy through the highly conserved electron transport chain. Moreover TCA cycle also has anabolic functions, almost all intermediates

are utilized in other metabolic reactions; exception is made to isocitrate, including the synthesis of amino acids and nucleotides (for reviews see [9, 21]).

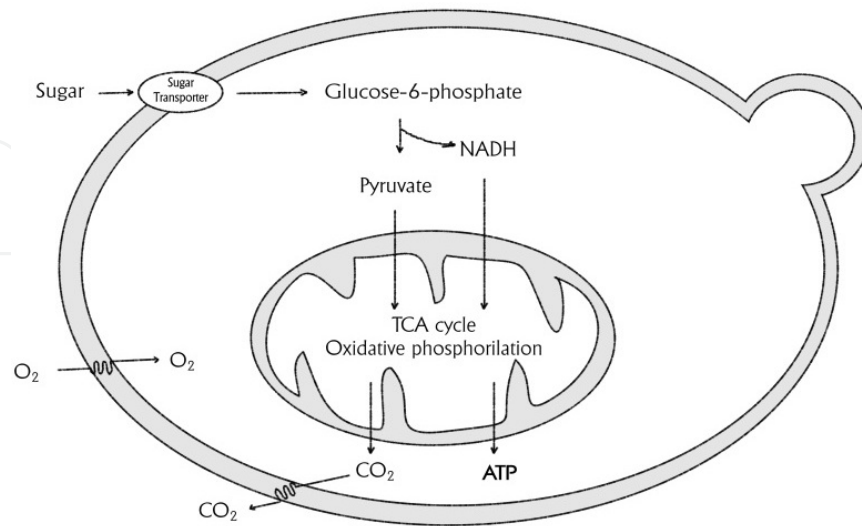


Figure 3. Aerobic respiration in *S. cerevisiae* (adapted from [22]).

2. *S. cerevisiae*, the party starter

Beverages with an alcoholic content are largely consumed by mankind since ancient times. Such beverages made from fermentation of sugar-rich goods, namely cereals and fruits, are present in oldest records [23]. Beer, made from germinated barley, and wine, produced from grapes, are among the most popular and their worldwide consumption is second only to non-alcoholic drinks as water, tea and coffee [24].

Wine and beer history is hand to hand with human civilization history, as most likely only the agriculture advent and the establishment of permanent settlements provided the conditions for its production. Nevertheless, wine and beer are most probably result of an “accident”, as some harvested grapes were not consumed rapidly enough or some cereal wet pulp was left aside, and *S. cerevisiae* took advantage of the sugary free meal. The result should have been pleasant enough, especially the mild psychotropic effect, therefore the early farmers must have tried to repeat such “accident”. The high ethyl alcohol content of this beverage and its analgesic, disinfectant and conservative properties contributed to a widespread utilization as a drug. Hence, our successful partnership with this yeast began.

In fact, our relationship with *S. cerevisiae* can be traced back as far as 7,000 years ago in China, with the first fermented beverages similar to beer, and Mesopotamia, with the first wines as well as domesticated vines [11, 25]. Conversely, the oldest known written account on the production of beer was found in Sumeria, modern day Iraq, in a stone tablet dating from 2,000 BC. This tablet, called “Hymn to Ninkasi”, describes the production of beer and its es-

pecially relevant role in religious ceremonies to Ninkasi, the Sumerian Goddess of beer [26]. Babylonians succeeded the Sumerians and kept producing the fermented beverage. They became so skilful at this art that at least 20 different brews were produced and exported, to as far as Egypt, at the zenith of Babylonian empire. Egyptians were so adept of this "imported beer" that they started producing their own from unbaked dough and even created a special hieroglyph for this new craft. Furthermore, records show that pyramid workers were paid in beer, a readily storable merchandise, and Pharaohs were entombed with model breweries to ensure an afterlife beer supply [26, 27]. Beer popularity grew and this beverage spread for the entire Europe, especially in the Mediterranean region [28].

Wine was also very popular in the ancient cultures, with references to this beverage in religious ceremonies of Egypt and Phoenicia. Pharaohs tombs were frequently adorned with vintage scenes and jars filled with wine accompanied the Kings afterlife [11, 23]. Wine consumption spread with the rise of the Greek and Roman Empires. Under the Greek and Roman influence, wine earned the status of "Civilized" drink, becoming very popular with the Empire upper classes, and beer was labelled as a "Barbarian" drink. Wine production and vine cultivation spread across Europe and replaced beer as the main drink in many countries. Some of these are still nowadays associated with wine production like Portugal, Spain and France. The production and consumption of beer continued mainly in northern borders of the Roman Empire, where Germanic tribes ruled and Roman influence was weaker [26, 28].

In Middle Ages, wine and beer production gained a new impetus with the shift from the familiar production to a more centralized production in monasteries [28]. Such happened because, at the time, water was frequently polluted, so alcoholic beverages were safer than water for the monks' consumption. Additionally, during the long fasting periods that the monks subjected themselves, the drinking of these highly nutritious beverages became common to satisfy hunger. This happened because wine and beer were considered similar to water and didn't constitute a breach of fast. In fact, in some monasteries monks were allowed to drink up to 5 litres of beer per day [28]. Southern monasteries produced mainly wine, as the weather was warmer and suitable for vines, but in the north the colder weather was more fitting barley and wheat growth and therefore Northern monasteries were more devoted to beer production. Each monastery developed its own methodologies for wine and beer making, leading to new wines and new brews and to a great technical improvement. Later these products become a source of income for the monasteries.

From the sixteenth century, with the discovery of the New World by Portuguese and the Spanish explorers, wine and beer spread to new territories. Vines were introduced in Brazil by the Portuguese around 1500 [29], and in Africa by the Dutch around 1650. In the Australian continent and North America this happened later, around 1800.

In the nineteenth century, wine and beer making suffered probably the major scientific advances. Around 1860 Louis Pasteur, a name forever associated with wine and beer production, developed studies on the conservation of wine through a heating-cooling process later known as "pasteurization", showing that wine could be stored for longer periods after such treatment. Moreover, in 1870 Pasteur made known to the world the role of *S. cerevisiae* in the fermentation process. Later, in 1876 Pasteur conducted similar studies in beer in his work

“Études sur la bière”. Such works were based in the first observations of yeast and bacteria by Antonie Van Leeuwenhoek in late seventeenth century [11, 26, 27]. Only almost a decade after it was isolated a pure yeast culture resulting from a single cell by Emil Christian Hansen at the Carlsberg brewery, Denmark and to that followed the name of several yeast species [30]. A few years later, based on Hansen’s work, Hermann Müller-Thurgau introduced the notion of inoculating wine fermentations with pure yeast starter cultures [11].

The intensive study of this amazing microorganism and its role in fermentation showed the specificities of each yeast species and strains. The necessity of consistent properties and quality in different fermentations, both in brewing and winemaking, paved the way for the selection of the right yeast for the job. The quest for stable and improved yeast began.

2.1. The “Right” yeast for the job

S. cerevisiae, known as “Wine’s Yeast” or “Brewer’s Yeast” (and also as “Baker Yeast”, see below), is the main responsible for some of the world’s most important fermented beverages. However, brewing and winemaking have inherent differences: i) the culture medium, ii) the bioreactor and iii) the yeast starter culture.

The final product, either wine or beer, is greatly influenced by the sugar-rich fermentable broth, grape juice or malted cereals, with different composition in fermentable sugars and nitrogen sources. The progression of the fermentation is another very important aspect of winemaking and brewing, *e.g.*, the oxygen available, the temperature and pH variations during substrate consumption and ethanol and CO₂ production. But the most significant difference is the yeast starter culture, its physiological state, whether it is dried yeast or a fresh inoculum, how well it ferments the available sugars and resists to fermentation by-products and its ability to flocculate at the right moment.

All *S. cerevisiae* strains described so far are capable of fermenting sugars to ethanol, but centuries of partnership with mankind directed the yeast evolution. Such evolutionary pressure resulted in a selection of distinct yeast strains for different applications, to produce wine and beer you need “Wine’s Yeast” and “Brewer’s Yeast”, respectively.

2.1.1. Yeast physiology

Beer is the denomination commonly attributed to a carbonated alcoholic beverage produced by fermentation of malted barley, while wine is made of the fermented juice of any of several types of grapes. However, there are as many different wines and beers as there are different producers, all with their unique character and flavour influenced by the selected ingredients, kind of fermentation and yeast selected.

As said, the choice of the ingredients greatly impacts the fermentation final product; usually beer is the product of malt, hops, water and yeast. Malt is the result of germinating and drying (kilning) barley, yet other cereals besides barley can be used to produce beer, as wheat and rye. Malt extract will provide the entirety of the carbohydrates and nitrogen to the fermentation process and as such, it will influence the final ethanol concentration as well as

colour and flavour development. Conversely, another important aspect is the intervention of hop, the female flower cluster of *Humulus lupulus*, which acts as bacteriostatic agent against Gram-positive bacteria, helping to control unwanted microorganism during brewing. It also functions as bittering agent, disguising beer natural sweet taste [31, 32]. In winemaking, the maceration of grapes is the starting point to wine production. The variety of grapevines, as well as the weather and cultivation/soil conditions, greatly influences the wine final properties. In fact, the environment has such influence that some type of wines can only be produced in certain regions, like the Porto wine in Douro region, Portugal, and Champagne wine in Champagne region, France.

Brewer's yeast can be distinguished in top fermenting and bottom fermenting yeasts, based in the position at which the fermentation occurs. This division accounts with the yeast flocculation behaviour, and it is such an important element of brewing that defines the two main classes: **ale beers** (top fermenting) and **lager beers** (bottom fermenting). Such categories were devised as soon as the first pure yeast culture was isolated. Hansen was able to purify two different species, a top fermenting appropriate for ale brewing, *S. cerevisiae*, and a bottom fermenting, *S. carlsbergensis*, suitable for lager beer [26]. Such taxonomic classification was reviewed several times [1], and the top fermenting yeasts are now included in the *S. cerevisiae* and *S. bayanus* species and the bottom fermenting yeasts fit to the *S. pastorianus* species, all belonging to the *Saccharomyces sensu stricto* genus [27, 33].

These brewer's yeasts present several differences in their genomes. **Lager yeast** strains present complex polyploidy genomes, with evidences of contribution from distinct *Saccharomyces* species [33]. Usually these complex genomes are tetraploid, which may result from the fusion of diploid parental strains or from duplication of the genetic information after the original cell fusion. Analysis on lager yeast genomes revealed other changes such as chromosome loss and/or duplications, likely due to human selection of relevant phenotypes [33, 34]. In reference [34] lager yeast genomes were analysed and classified in two groups. In group I, cells present one *S. cerevisiae* genome equivalent and, in group II, cells present two *S. cerevisiae* genome equivalents. Both groups exhibit one *S. bayanus* genome equivalent and the remaining genome was mostly hybrid chromosomes from both species. These different yeast must be related with the conditions they are exposed, meaning for instance to lager beer fermentation, yeast has to react to conditions inherent to beer production, as cropping and pitching, and to bottom fermentation specificities, e.g., temperature of reaction [35].

As for **ale yeasts**, studies revealed that these strains are closely to *S. cerevisiae* [34, 36]. In fact, a genotype analysis of 651 *S. cerevisiae* strains revealed that ale strains were more closely related to wine and bread strains (above referred as Baker's yeast), than to lager brewer's yeast strains [36]. Reports of hybrids in ale yeasts showed that strains traditionally classified as *S. cerevisiae* may indeed be the result of hybridization events [37]. Ale beer has less representation in worldwide markets, and as a consequence less studies and information are available on the corresponding yeasts. As such, the considerations on beer yeast physiology will be focused on lager strains.

In winemaking, most wine yeasts belong to *S. cerevisiae* species, but *S. bayanus* has also been detected. Yet, wine fermentations also present yeast, derived from vineyard environment,

belonging to the genera *Candida*, *Debaryomyces* and *Brettanomyces*. But, yeast is mainly selected for its resistance to ethanol, favouring *S. cerevisiae*. There is also selection for capacity to float or to flocculate, important for some specific wines. While in most wines the ability to flocculate is important to improve the filtration, in some wines such as sherry wine, the formation of a floating film is vital. This *vellum* is formed at the surface of the wine and promotes oxidative metabolism. Sherry wine is characterized by high ethanol content and low aldehyde. Its featured nutty flavour can be ascribed to partial oxidation of ethanol to acetaldehyde [11].

The utilization of dried yeast as a starter culture is very common in the wine industry. Cells are dehydrated through a cycle of filtrations and centrifugation to remove external water and then submitted to streams of dehumidified hot air. Such procedure can reduce yeast cells' content in water to as low as 6%. However, even though yeast cells can survive such treatment, it causes cellular damage. Damages to cell wall and plasma membranes caused by the changes on cell size and shape, as well as damages to proteins produced by free radicals were reported [35].

One of beer brewing specificities is the utilization of a freshly grown starter culture. In one hand, it ensures a healthy population fully adapted to growth medium. Cells are usually collected at the late exponential phase, preventing a large percentage of aged cells, and at the same time ensuring metabolic fitness. On the other hand, it meets the requirement for flavour consistency of the final product, even though it is more expensive than the alternatives. The pattern of metabolic products of yeast is highly dependent on its growth conditions, and cells fully adapted to *wort* produce a more consistent flavour. The first batches inoculated with dried yeast are often of inferior quality, with by-products of fermentation conferring off-flavours, which compromises the regularity of a brand product [30]. An alternative that meets the requirements of fresh grown cells and flavour consistency, but at the same time reduces the process duration and costs, is the fed-batch technology. Cells are kept in late exponential phase by leaving a certain amount of yeast in the reactor and adding fresh *wort*, shortening the fermentation time and maintaining the beers properties [38].

During fermentation, yeast is constantly facing new pressures. The high osmotic stress due to the sugar high content of *wort* and *must* is just the beginning. *Wort* is a rich and complex medium, composed of carbohydrates (90%), nitrogen sources (5%) and small amounts of inorganic ions, lipids and polyphenols. *Wort* composition, being highly dependable of the quality of the cereal and the process used to malt it, is usually enriched in fermentable sucrose (5%), monosaccharides (10%), maltotriose (15%) and maltose (50%). About 20-30% of total carbohydrates are non-fermentable dextrans, polysaccharides result from starch degradation [35]. On the other hand, grape juice is rich in fructose and glucose, presenting small amounts of sucrose. Grape variety influence the ratio glucose/fructose, Chardonnay is a high fructose variety, whereas Zinfandel is regarded as high glucose variety. Such high-gravity *worts*, 12-18 g of extract per 100 mL, subject cells to high osmotic pressure. The production of compatible solutes, as glycerol and trehalose, and a "robust" plasma membrane composition seem to be the main adaptations to withstand such stress. The cells fully adapted to *wort* used to inoculate (pitch) fermentations are important to avoid extended lag phases where

cells are adapting its physiology. In wine the use of active dried yeast (ADY) is common and no effect on fermentation time was reported [39].

Nitrogen assimilation is especially important in flavour development. The main sources of nitrogen are free amino acids and ammonium ions, which are used by the cell for protein formation [35, 40]. Such amino acids are also relevant for the production of alcohols and esters, important in these beverages flavour. During fermentation, amino acids are always used following a certain order, independent from the fermentation conditions. Group A, including arginine, asparagine, aspartate, glutamate, glutamine, lysine, serine and threonine, are used first. Group B amino acids are utilized slowly and include histidine, isoleucine, leucine, methionine and valine. Group C is composed by alanine, glycine, phenylalanine, tyrosine, tryptophan, and are only absorbed after the complete exhaustion of group A. Group D is composed of proline, which require an aerobic metabolism for its uptake and it is poorly used during fermentations [40].

In winemaking, fermentations are usually developed under anaerobic conditions, but it is common in brewing to oxygenate the *wort*. So another important stressor is the dissolved oxygen, which may lead to the formation of reactive oxygen species (ROS). ROS, as hydrogen peroxide or superoxide radical, can promote damages in cell main constituents, DNA, proteins and lipids. However, oxygen is very important for the synthesis of sterols and unsaturated fatty acids ensuring the physiological fitness for cell replication. The control of dissolved oxygen is vital to ensure a healthy population. An important problem is the excessive growth of yeast cells when exposed to high amounts of dissolved oxygen, at the expenses of ethanol production [41].

The inorganic ions are necessary, but at nanomolar concentrations. These trace elements, as calcium, zinc or copper, are mainly required as cofactors of enzymes or in the flocculation process. For instance, the response to oxidative stress is dependent on enzymes such as the different superoxide dismutase isoforms that require manganese, zinc or copper [32]. On the other hand, calcium is vital for the flocculation advance [42]. Conversely, insufficient amounts of such elements can lead to cellular damage and stress, and consequent stuck fermentations.

The use of antimicrobials in vineyards is common to control fungi that spoil grapes. But, when grapes are macerated these compounds are incorporated into the juice. Even though they may help to prevent the wine oxidation and microbial spoilage, a concentration to high may lead to off-flavours and in worst case, yeast death. So antimicrobials, especially sulphur dioxide, are an important stress to yeast during fermentation. Commercially available yeast also has to deal with toxins produced by wild yeasts derived from the vineyards. These toxins are produced to give those wild yeast advantages over others species in accessing to the nutrients. Isolation of strains resistant to both antimicrobials and natural toxins is an important research field [11, 43].

Certain compounds are extremely important for brewing and wine making not as substrates but as by-products. Such metabolites greatly influence the final product's colour and flavour, as well as its stability. In fact, the importance of these compounds is such that lager

beers are usually stored from several days to weeks, lagering, solely to remove diacetyl, an off-flavour causing metabolite. This time consuming maturation phase consists in a second fermentation at low temperature to eliminate the butter-like flavour caused by this vicinal diketone. Studies are being conducted in order to minimize this metabolite formation and reduce the maturation time [30]. Sulphur containing compounds are other family of by-products receiving great attention. Such group comprises sulphite, sulphide and dimethyl sulphide, and while sulphite is a beneficial and flavour stabilizing metabolite, the remaining compounds are responsible for off-flavours. The equilibrium of such compounds formation could lead to better wine and beer and shorter fermentations [44].

Ethanol is one of the most important by-products of beer fermentation. Nevertheless, it represents an important stressor for yeast cells due to its high toxicity. Ethanol concentration can reach 10% in higher gravity fermentations, and acts especially upon biological membranes [35]. Reports showed ethanol effects in growth inhibition [45], lipid modification and loss of proton motive force across the membrane and increased membrane permeability/fluidity [46]. Yet, cells exposed to oxygen, with high levels of sterols in membranes, and adequate levels of nutrients, amino acids and trace elements in the fermentation broth are able to respond efficiently to such effects [35].

Nutritional stress occurs at the end of fermentation and cells enter stationary phase. This occurs because fermentable carbon sources tend to be depleted, and cells have to change their metabolism from fermentative to respiratory (explained in section 1), entering in a quiescent state [30]. Such phenomenon induces flocculation, a cell-cell interaction process dependent of lectins and calcium that promotes sedimentation. Flocculation in turn is influenced by several other factors besides nutrient depletion. Reports showed the influence of ethanol content, calcium concentration, pH changes, oxygen concentration and temperature [47]. The onset of flocculation is an important area of interest in brewing. If flocculation occurs too soon, stuck fermentation may occur, which results in a high sugar and low ethanol content. If, on the other hand, happens in a later stage, it has a high impact in beer filtration as most cells tend to be kept in suspension.

Fermentation is the most yeast-dependent phase of these alcoholic beverages production, but yeast also interferes with others proceedings. The metabolic fitness of the starter culture, the storage and maintenance of both dried and fresh yeasts, and the storage of the products submit yeast to different conditions to which they have to respond/adapt. To obtain detailed information on such processes please see reviews [35] and [43].

2.2. Old beverages, new solutions

Wine and beer industrial production led to a demand for better and more efficient yeast. Yeasts with improved utilization of substrates, carbohydrates and nitrogen, and consistent flocculent behaviour, as well as high fermentative capacity and high ethanol production are the industry goal. Enhancing beer and wine flavour through modification of by-products formation is another field of intensive research [30, 39]. As it is the improvement of the fermentation process, through encapsulation/immobilization of yeast [48].

Large collections of yeast were assembled, as the Centraalbureau voor Schimmelcultures (CBS) collection, in The Netherlands, and the Carlsberg collection, in Denmark. Manipulation of these strains to improve wine and beer properties has been performed in several ways, from spores manoeuvring and natural mutants' survey to genetic engineering (GE). A rather recent and extensive review in strategies for the improvement of *S. cerevisiae* industrial strains can be found in [2]. As referred above, an efficient utilization of substrates by yeast during fermentation is extremely important for wine and beer industries. Such efficiency will yield higher amounts of by-products, as ethanol, and reduce the fermentation time. Furthermore, glucose repression on other sugars and consumption of unusual nitrogen sources are vital research areas [30].

The presence of glucose, even in small amounts, represses the simultaneous uptake and consumption of several sugars, namely maltose and galactose. Maltose (50%), maltotriose (15%) and sucrose (5%) are the main sugars in *wort* under glucose repression. Such repression leads to late fermentation of these sugars and slower fermentations, as their utilization is dependent on glucose depletion. This process is controlled at the transcriptional level, through the action of the proteins Mig1p, Ssn6p and Tup1p. Mig1p, a zinc finger protein, binds to specific sequences in the promoter region of the glucose-repressed genes and recruits the SSN6-TUP1 complex, the responsible for the actual repression [49]. Mig1p binding sites were found in genes associated with the utilization of sucrose (*SUC2*), maltose (*MALR*, *MALS* and *MALT*) and galactose (*GAL1-5*) [50]. Therefore, *MIG1* presents itself as a potential target to improve yeast sugar consumption.

Conversely, in sucrose metabolism, glucose repression addresses the sucrose conversion in fructose and glucose under the action of Suc2p. Studies showed that the disruption of *MIG1* lessen the glucose repression on the transcription of this excreted invertase in both lab and industrial strains. Therefore, the lag in sucrose utilization was greatly diminished. Besides Mig1p, another zinc finger protein, Mig2p, was associated with glucose repression of *SUC2* [51]. The interruption of both *MIG1* and *MIG2*, in *S. cerevisiae* strains led a high sucrose metabolism in the presence of high glucose concentrations [52].

Maltose metabolism is more complex than sucrose, as it responds to both glucose repression and maltose induction. Maltose induction is under the influence of the locus *MAL*, a closely integrated group of genes. This sugar presence induces *MALR*, a transcription factor, which in turn will induce *MALT*, coding for a maltose permease, and *MALS*, coding for a maltase [30]. Up to 5 different *loci* have been detected in *S. cerevisiae* industrial strains; still haploid lab strains present a single *locus*. In both situations these *loci* are under repression of Mig1p [53]. However, disruption of *MIG1* in industrial strains only alleviated the sucrose metabolism [50], but didn't cause any effect regarding maltose metabolism. Even though, in haploid lab strains *MIG1* disruption lifted the glucose repression; the same has not happen in the polyploid strains, presenting multiple *loci*. Complex regulation between the different genes must be under way and most probably not solely controlled by Mig1p [53].

Finally, maltotriose, a glucose tri-saccharide, is the second most abundant sugar in *wort* (15%). This is under similar regulation by the presence of glucose and maltose, therefore most studies have focused in an efficient uptake of this carbohydrate [54]. Those works

showed that overexpression of maltotriose transporters lead to positive effects on its metabolism [54, 55].

In winemaking and brewing, where flavour has such importance, amino acids metabolism has a notorious place. As said, amino acids are involved in formation of higher alcohols and esters that significantly contribute to beer and wine flavour. Since yeast cannot hydrolyse *must* and *wort* proteins, it depends on the available ammonium and amino acids in solution [11, 30]. However, the predominant amino acid in both *must* and *wort*, proline, is the less assimilated [40]. As such, improvements in yeast ability to uptake this amino acid has been attempted. Efficient proline uptake was reported in lager beer yeast expressing a mutagenized proline permease, Put4p. The site-directed mutagenesis stabilized the permease and enhanced amino acid utilization without affecting the beer quality [56]. The study of the same problematic in winemaking led to the disruption of *URE1*, a repressor of permease encoding *PUT1* and pyrroline-5-carboxylate dehydrogenase *PUT2*, with significant improvements in fermentation rate and vigour described [57].

Flocculation is a phenotype of industrial interest. It facilitates the filtration process in the end of fermentation, saving both time and money. In the case of brewing, it also serves the cropping (recover of part of the yeast population of the fermentation to pitch the next). Flocculation is a reversible aggregation of cells, where lectins recognize sugar residues in neighbour cells. Two industrially relevant flocculation phenotypes are well-known, Flo1 and NewFlo. Both are under the control of *FLO* genes, Flo1 phenotype is repressed by mannose and NewFlo by mannose, glucose and sucrose. Almost every industrial strain is NewFlo, associated with *FLO10* [30]. The main approach to improve these phenotypes is to put *FLO* genes under a promoter active only in stationary phase. Promoters of *HSP26* and *HSP30* were proven as the most suitable for induction at this late growth phase in lab strains [30].

The control of by-products production in order to improve wine and beer organoleptic properties is an expanding research area. The production of glycerol, to improve wine and beer's fullness, as well as sulphite, to improve stability, and the reduction in diacetyl and sulphide content are the main targets. Glycerol, as the second fermentation metabolite, is rather important to wine and beer; the increase of its concentration to improve these beverages sensory character is an active field. Overexpression of *GPD1*, encoding glycerol-3-phosphate dehydrogenase, is the main approach. However, this change resulted in a redox imbalance with increased production of unwanted metabolites [39]. This point has been fully discussed in [2].

The presence of sulphite, an antioxidant and flavour stabilizer, and reduction of off-flavour producing sulphide is another important problem addressed by the industry. Both these goals can be achieved at the same time with the directed mutagenesis of NADPH-dependent sulphite reductase, an important enzyme in sulphur-containing amino acids synthesis. This strategy lowered this enzyme activity and increased the amount of sulphite in wine while reducing the sulphide presence in wine [58].

The reduction of diacetyl has special importance, as the maturation time (lagering) is directly dependent on this compound concentration. The expression of the bacterial enzyme ace-

tolactate decarboxylase (ALDC) in yeast is the main approach to reduce the amounts of this compound. ALDC catalyses the reaction of α -acetolactate to acetoin, preventing the formation of diacetyl. However, after heterologous expression of ALDC, the yeast became auxotrophic for some amino acids and the growth rate was very low in *wort*. An alternative approach was the interruption of *ILV2*, encoding acetolactate synthase. Such strategy also resulted in an auxotrophic strain with slow growth. The search for natural mutants in *ILV2* with an appropriated growth rate is now the major strategy [30].

Improvement of yeast to render fermentations faster and cheaper is an industry goal, but the enhancing of the fermentation process itself is another alternative. The fed-batch technology has already proved its benefits [38], and improvements of such process with yeast immobilization/encapsulation are now under the spotlight. This results in much faster fermentation rates as compared to the existing free cell fermentations. However, it has some disadvantages, such as: i) complexity of production process including the choice of the suitable carrier materials, ii) bioreactors design, iii) fine-tuning of the flavour formation during fermentation processes, and iv) cost constraints [59].

3. Baker's yeast – Magic on bread making

The process of bread making relies on the fermentation carried out by a mixture of yeast and bacteria. Even when all this was unknown and the flour leavening seen as "magic", bread was already produced and extensively consumed. On those ancient times, the leavening resulted presumably due to the action (fermentation) of the natural microbial contaminants of flour or dough ingredients. This was obviously not a controlled process, yet with the practice of maintaining a fresh inoculum from one preparation to the next, promoted the selection of yeast and bacteria biodiversity. Nowadays, some types of bread are still prepared in this fashion, sourdoughs are one example (for a review see [2]), but the baking industry moved for the use of commercially baker's yeast, typically the strain *S. cerevisiae*, for the bread production. And, while the flour types, geographical origin and mixtures introduce organoleptic differences in bread, the globalization of commercial baker's yeast market decreased worldwide bread diversity (for a review see [2]).

3.1. Commercial baker's yeast production – The break of spell

Commercial baker's yeast is produced in several forms in order to meet specific requirements of climate, technology, methodology, transportation, storage and final product. As with all biotechnology processes, this is in constant development/undergoing research not only to optimize the process technology and its components, but as to produce faster growing strains with the characteristics to deliver better quality end products.

Molasses (beet and cane molasses), the common carbon and energy source used in the production of baker's yeast, is a by-product of sugar refining industries, therefore cheaper than the formerly used cereals grain. Furthermore the sugars present on those molasses (around 50%), consisting on a mixture of sucrose, fructose and glucose, are ready to be

fermented by the yeast. In order to obtain the proper broth for the optimum yeast biomass yield; the mixture of molasses has to be supplemented with nitrogen sources, minerals, salts and vitamins [60, 61].

After the preparation and sterilization of the broth, the production of baker yeast can take place. It begins by the inoculation of a small closed test flask containing the prepared sterilized broth with a pure yeast culture. The growth is allowed and carefully screened, and when the culture reaches an elevated density, it is transferred to larger vessels and supplied with more broth, fed-batch reactors. This scale-up process continues until a desirable biomass quantity is attained, the so-called commercial starter, able to inoculate industrial fermenters/reactors, which production ranges from 40,000 to 200,000L [62].

The entire fermentation process of baker's yeast has to be directed towards maximum biomass production; by-products such as ethanol are not desired. As we saw in section 1.1.1, in anaerobic dissimilation of sugars (alcoholic fermentation) the ATP yield is quite low comparing with respiratory dissimilation, affecting drastically the biomass yield. The way to avoid anaerobic ethanol production is the use of the mentioned fed-batch reactors, in which is possible to control the specific growth rate and sugar concentration by controlling the fed of reactors with fresh broth [63].

Nowadays, during the industrial large reactors the addition of nutrients and regulation of pH, temperature and airflow are carefully monitored and controlled by computer systems during the entire production process. In this way, the tones of baker's yeast obtained in the end of the fermentation have the same quality/characteristics/properties as the original pure yeast culture that started the process. These tones of yeast are suspended in a large amount of water, resulting in a creamy suspension of active yeast, being necessary the so-called **downstream processes** to obtain the concentrated yeast [64, 65]. At the end of the fermentation, the yeast culture is concentrated using a series of combined centrifugation and washing steps, into a yeast cream with concentration of approximately 20%. The yeast is then cooled to approximately 4°C, and stored. It can be sold in this form – **Cream Yeast**- however is quite expensive due to the manipulations required because of high content in water. Cream yeast can be further processed, compressed or dried originating the **Granular Yeast** or **Instant Dried Yeast**, if then converted in small granules, or **Cake Yeast** or **Active Dry Yeast**, if as an alternative the dried yeast is extruded or cut into blocks/cakes. All these yeast types are then packaged, typically vacuum packed to reduce the risk of contamination, and distributed to wholesalers or traders. The shelf life of Active Dry/Cake Yeast and Instant Dry/Granular Yeast at ambient temperature is 1 to 2 years.

3.2. Idol baker's yeast

Yeast has a significant role on bread making, greatly influencing the final product properties. The most important contribution is in the leavening phase; after the dough has been kneaded and the gluten network start to develop, yeast starts to consume the available fermentable sugars and to produce ethanol and CO₂, as mentioned in section 1. As fermentation occurs, the dough is gradually depleted of O₂ present in the air bubbles trapped in the dough, leaving small bubble *nuclei* full of N₂. As the metabolism is more and more stimulat-

ed, with incubation at optimal temperatures, the CO₂ starts to saturate the liquid phase of the dough and starts to accumulate in the bubble *nuclei* [66]. This leads to dough rising provided that a mature gluten network, capable of ensure the dough foam-like structure, is formed. The amount of time such process occurs will influence dough gumminess and rheology, as well as crust colour, crumb texture, and firmness of the bread (reviewed by [2, 3]). The amount of sugars present in the dough that are actually fermented, as well as the efficient secretion of enzymes as invertase, responsible for the conversion of sucrose to glucose and fructose, has a great impact in the flavour characteristics of the bread. The action of several enzymes, namely proteases, lecithinases, lipases α -glucosidase and β -fructosidase, leads to different utilization of the dough substrates. In bread making, the flavour is also greatly influenced by the metabolic by-products of the yeast fermentation, and while the most important by-product of yeast metabolism is certainly the CO₂, the production of metabolites such as alcohols, esters, and carbonyl compounds have also a deep impact. More than 300 volatile compounds associated with bread's flavour and aroma, are produced by yeast. Although, some are dependent more on the substrates, the vast majority of such compounds are yeast dependent, introducing an important variability to bread [67].

As mentioned, the common procedure of bread making today, at least in developed countries, consists of using this commercial baker's yeast. Its quality/individuality depends on storage stability, osmotolerance and freeze-thaw resistance. Considerable efforts have been made to obtain the Idol Yeast, including evolutionary engineering, genetic engineering (mainly to provide yeast with high capacity to tolerate freeze-thaw treatments). Yet, there is still considerable space for improvement. Those several strategies to achieve the Idol Yeast has been thoroughly revised and discussed in a previous work from the beginning of this year [2] as well as on [3, 68].

4. Yeast à la Carte

Fresh *S. cerevisiae* consists of approximately 30–33% of dry materials, 6.5–9.3% of nitrogen, 40.6–58.0% of proteins, 35.0–45.0% of carbohydrates, 4.0–6.0% of lipids, 5.0–7.5% of minerals and various amounts of vitamins, depending on its growth conditions [69]. So, today yeasts are acquiring increasingly more attention for other uses, besides the production of alcoholic beverages and the baking industry. The products of modern yeast biotechnology form the backbone of many commercially important sectors, including functional foods (for animals, fish and humans), health food supplements, including additives, conditioners and flavouring agents, as pharmaceutical products, for the production of microbiology media and extracts, as well as livestock feed or even as agents of detoxifying effluents containing heavy metals [70, 71].

4.1. Yeast treats for animals

The pioneering research conducted almost a century ago by Max Delbrück and his colleagues was the first to highlight the value of surplus brewer's yeast as a feeding supplement

for animals [72]. Yeasts have been fed to animals for more than a hundred years, either in the form of yeast fermented mash produced on the farm, yeast by-products from breweries or distilleries, or commercial yeast products specifically produced for animal feeding. In animals, including pets, this practice is used to compensate for the amino acid and vitamin deficiencies of cereals [73, 74], and in fish as a substitute for other ingredients [71].

Brewer's yeast biomass, as described above, which results from the cultivation of *S. cerevisiae* on malted barley, separated after the *wort* fermentation, debittered and then dried, is the second major by-product from brewing industry, just after the brewer spent grain [73]. This biomass is an excellent source of proteins, peptides and amino acids, vitamins (especially of B-group: B1, B2, PP, B5, B6, B8, B12), minerals and trace elements (calcium, phosphorus, potassium, magnesium, copper, iron, zinc, manganese, chromium, selenium), carbohydrates (glucans and mannans), as well as phospholipids [75]. The winemaking industry also generates a huge amount of microbial biomass - **leeds**. Yet, this incorporates the yeasts (that die due to nutrient depletion) but also other microorganisms, suspended solids, colloids, and organic matter, and have been shown to display quite low nutritional value to be considered for use as a supplement in animal feed [76].

Those yeast used for monogastrics food or feeding rations is generally inactivated because feeding of live yeast might cause avitaminosis due to the depletion of B-vitamins in the intestine [77]. They can also cause adverse fermentation in the digestive tract of swine leading to diarrhoea and bloating [75]. Yeasts can be killed through application of heat or using chemicals. High temperature destroys the yeast membrane, but does not necessarily inactivate all yeast enzymes, unless quite elevated temperatures are applied. Alternatively, there are the chemical treatments with propionic acid or formic acid, which also act as a preservatives for yeast, and contribute to the feed value of the yeast [73].

Yeasts have been used in diets of numerous species with varying levels of success. Yeast for swines is sold for feed applications as wet slurry, as dried brewer's yeast, or in mixtures with other brewery by-products [73]. It is ideal for their feed as a good protein source, it contains most of the essential amino acids in adequate quantities, and numerous vitamins, selenium, copper, and phosphorus. Selenium concentrations are much higher in yeast than in soybean meal, and deficiency of this compound in the swine's alimentation has been the cause of higher swine mortality [78, 79]. Additionally, dried brewer's yeast contains mannan oligosaccharides, which have been reported to increase the growth performance and intestinal health of pigs [80]. Benefits have been described as well for nursing and weanling pigs [81].

Either live or inactivated brewer's yeast have been used as well in ruminants diets, consequently it was observed an increase in productivity of animal meat or milk [73, 82], but also live yeast cultures have been used. These are prepared by inoculating wet cereal grains or grain by-products with live yeast, partially fermenting the mash, and then drying the entire medium without killing yeast or destroying vitamins and enzymes [73]. Live yeast is reported to stimulate fermentation in the rumen through its ability to stimulate the development of anaerobic, cellulolytic and acid lactic bacteria fermentations. In addition, the ingestion of yeast offers continuous supply of vitamins, dicarboxylic acids, removal of oxygen, buffering effect, and reduction in the number of protozoa. As a result, there is an improvement of the

digestion of the fibrous and cellulolytic portion of the diet, which leads to a greater intake of food and better performance [83, 84].

Dried yeast was used traditionally in poultry diets in the past as a source of amino acids and micronutrients, and though the broiler growth was improving this practice was largely discontinued for economic reasons [77]. On the other hand, brewer's yeast appears to be especially beneficial for breeder turkeys and laying hens [85]. Reproductive improvements are attributed to the high level of dietary biotin and selenium in yeast, which is more beneficial than inorganic selenium added to poultry diets [86] and it contributes for the prevention of biotin deficiency in poultry diets, which may result in reduced feed conversion, low egg production, and poor hatchability [87]. Brewer's yeast is also very rich in folic acid, an important vitamin for turkeys [73].

Brewer's yeast has been recognized to have potential as well as a substitute for live food in the production of certain fish or as a potential replacement for fishmeal [88-90]. In addition, it has low content in phosphorous, meaning less water and environmental contamination than common fish meal and other plant-based alternate protein sources [91]. Multiple studies have demonstrated the immunostimulant properties of yeasts, such as their ability to enhance non-specific immune activity [92]. That reaction can be related to β -glucans, nucleic acids as well as mannan oligosaccharides [93]. Brewer's yeast may serve as an excellent health promoter for fish culture as even when administered for relatively long periods is able to enhance immune responses as well as growth of various fish species, without causing immunosuppression [94, 95]. Furthermore, the relative high levels of nucleic acid nitrogen present (mostly in the form of RNA) that in humans and most monogastric animals can become toxic if taken in excess, as the capacity of excretion of the uric acid formed is limited [96], in fish does not happen due to their very active liver uricase [97].

4.2. Human little treats, big benefits

In a world of rapidly increasing population and low agricultural production, yeasts are relatively cheap and easily produced on an industrial scale representing a sustainable alternate protein source to cover the population nutritional demands. The first time that yeast was cultivated in large scale for human nutritional use was in Germany during both World Wars [72]. The yeasts *S. cerevisiae* as baker yeast, *Candida utilis* as torula yeast, and *Kluyveromyces fragilis* as whey yeast, when produced on suitable, food grade substrates (e.g., sugars, ethanol, and lactose), are permitted in many foods around the world [70]. Besides the alcoholic beverages and baking products referred above, yeast are used in the health food industry; as additives, conditioners, and flavouring agents; as sources of high nutritional value proteins, enzymes, nucleic acids, nucleotides, and cell wall polysaccharides [98] as well as for the production of food-grade yeast extracts and autolysates [69, 99]. Yeast extract from dried brewer's yeast cells can be used by enzymatic treatment in a wide variety of foods (e.g., meat products, sauces and gravies, soups, chips and crackers) as flavours enhancers or potentiators [73, 100]. β -Glucan obtained from brewer's yeast can be used in food products as a thickening, water-holding, or oil-binding agent and emulsifying stabilizer [101]. The probiotic activity is an additional role of some yeast that is attracting increasing interest [102].

S. cerevisiae has been studied extensively for its medicinal properties and several beneficial/probiotic effects on human health and well-being have been reported, including prevention and treatment of intestinal diseases and immunomodulatory actions, are the most well-known. Probiotics are viable microorganisms that are beneficial to the host when consumed in appropriate quantities [103]. The probiotic properties of yeasts reported refer the ability to survive through the gastrointestinal tract and interact antagonistically with gastrointestinal pathogens. As described above, *S. cerevisiae* have been used as supplements to animal and fish feeds with reported improvements on the growth and health of the hosts [104]. Regarding humans, *S. cerevisiae* var. *boulardii* has been successfully used as an oral biotherapeutic agent to treat patients with severe cases of diarrhoea (*e.g.*, antibiotic-associated diarrhoea and traveller's diarrhoea) and other gastrointestinal disorders (*e.g.*, irritable bowel syndrome and Crohn's disease) [105]. Several studies have shown that *S. cerevisiae* var. *boulardii* confer beneficial effects against various enteric pathogens, involving different mechanisms as: i) prevention of bacterial adherence and translocation in the intestinal epithelial cells, ii) production of factors that neutralize bacterial toxins and iii) modulation of the host cell signalling pathway associated with pro-inflammatory response during bacterial infection [106]. As reviewed in detail in [106] prevention of bacterial adherence and translocation in the intestinal epithelial cells is due to the fact that the cell wall of *S. cerevisiae* var. *boulardii* has the ability to bind enteropathogens, which results in a decrease of their adherence to host epithelial cells. This yeast also produces proteins that are responsible for degradation, neutralisation or dephosphorylation of bacterial toxins. Moreover, the mechanism by which *S. cerevisiae* var. *boulardii* modifies host cell signalling pathways associated with pro-inflammatory response is based on blocking activation of nuclear factor-kappa B (NF- κ B) and mitogen activated protein kinase (MAPK) which decreases the expression of inflammation-associated cytokines such as interleukin 8 (IL-8), tumor necrosis factor alpha (TNF- α) and interferon gamma (IFN- γ). Conversely, *S. cerevisiae* var. *boulardii* also stimulates the peroxisome proliferator-activated receptor gamma (PPAR- γ) expression in human colonocytes and reduces the response of human colon cells to pro-inflammatory cytokines. There are several studies indicating the stimulation of the host cell immunity, both innate and adaptive immunity, by yeast in response to pathogen infections. Furthermore, it has been shown that *S. cerevisiae* var. *boulardii* also has a role in the maintenance of epithelial barrier integrity; during bacterial infection the tight junctions are disrupted and this yeast enhances the ability of intestinal epithelial cells to restore the tight-junction structure and the barrier permeability [106].

The benefits from ingesting yeasts do not stop here, many other have been reported as: selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon [107], *e.g.*, fructooligosaccharides [108]; decreasing the serum cholesterol levels [109], in the treatment of diabetes (regulation of insulin levels) and chronic acne, reducing the appetite, for healthy hair and nails [70, 110, 111] and also for promoting the bioavailability of minerals through the hydrolysis of phytate, folate biofortification and detoxification of mycotoxins due to surface binding to the yeast cell wall [106]. Yet, still are some concerns about public health safety as cause of crescent reports associating the intake of yeast with cases of fungaemia [112, 113].

4.3. What the future holds

Since *S. cerevisiae* var. *boulardii* is recognised as a member of the species *S. cerevisiae*, it is most likely that also other strains within *S. cerevisiae* might show probiotics properties. So far, great efforts have been placed on utilising the probiotic effects of especially LAB, whereas rather limited emphasis has been placed on the beneficial effects offered by yeast. However, yeasts offer several advantages compared to LAB. They have a more diverse enzymatic profile and appear to have a more versatile effect on the immune system. They also provide protection against pathogenic bacteria and toxic compounds by surface binding and appear to be better suited for nutritional enrichment and delivery of bio-active molecules. Besides, yeast is much more robust than LAB and therefore easier to produce and to distribute, especially in less developed areas [106]. Furthermore and though there is still much room for improvement, also the encapsulation technology applied to probiotics has shown benefits, e.g., *S. cerevisiae* var. *boulardii* in microspheres protect the yeast from destruction in the gastrointestinal tract and therefore increase intestinal delivery of the viable probiotic [114].

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