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Review

# Baker's Yeast: Historical Development, Genetic Characteristics, Biochemistry, Fermentation and Downstream Processing

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## Abstract

Baker's yeast is the most widespread food microbial starter and its main function is to produce gas, more specifically to raise dough made from flour and to give bakery products with an aerated structure. Since, the beginnings of the baker's yeast industry, much effort has been devoted to optimize growth conditions to get high biomass yield in fermentation tanks and gassing power in dough. However, there is a limit to the effect of growth conditions to improve the performance of commercial yeast. The latter is a concentrate of yeast cells obtained from a single strain of *Saccharomyces cerevisiae*, a strain being a particular line of descendants sharing the same properties. This is why baker's yeast manufacturers have given much interest to the selection of the strains they grow. In this present review, we discussed about Baker's yeast *Saccharomyces cerevisiae*, Historical development of Baker's yeast, Genetic characteristics, Biochemistry, Biochemical and molecular identification, Fermentation, Downstream processing of Baker's yeast and Future perspectives of Baker's yeast.

**Keywords:** Baker's yeast, *Saccharomyces cerevisiae*, molecular identification, fermentation, downstream process.

## Introduction

Fermentation is the process of using microorganisms to produce valuable products such as antibiotics, industrial enzymes, food, and chemicals. Microorganisms which multiply predominantly by budding are collectively called "yeasts". Phaff (1990) gave the definition for yeasts as unicellular eukaryotes which, at some stage in their life cycle, divide by budding. The best known of these organisms is the strain of *Saccharomyces cerevisiae* used in the brewing and baking industries. If the term "yeast" is not further specified, *Saccharomyces cerevisiae* is the organism commonly referred. This strain of yeast has been extensively studied and applied widely both in the laboratory and in industry. Baker's yeast (*Saccharomyces cerevisiae*) is one of the oldest products of industrial fermentation. It is still one of the most important fermentation products based on volume of sales and its use for bread-making, a stable food for large section of world population. The Baker's yeast *Saccharomyces cerevisiae* has been associated with human beings for more than 6000 years due to its use in food production, baking, wine and beer making. Potable and industrial ethanol production constitutes the majority of use of *Saccharomyces cerevisiae* in biotechnological applications (Sivasakthivelan *et al.*, 2014). However, baker's yeast also plays an important role as a model organism in the field of biochemistry, genetics and molecular biology.

*Saccharomyces cerevisiae* was the first eukaryotic organism to be sequenced in 1996 (Goffeau *et al.*, 1996), and is clearly the most ideal eukaryotic microorganism for biological studies. The impact of Baker's yeasts on the production, quality and safety of foods and beverages is intimately linked to their ecology and biological activities. Recent advances in understanding the taxonomy, ecology, physiology, biochemistry and molecular biology of Baker's yeasts have stimulated increased interest in their presence and significance in foods and beverages. This has led to a deeper understanding of their roles in the fermentation of established products, such as bread, beer and wine, and greater awareness of their roles in the fermentation processes associated with many other products. As the food industry develops new products and processes, yeasts present new challenges for their control and exploitation. Food safety and the linkage between diet and health are issues of major concern to the modern consumer and Baker's yeasts have emerging consequences in this context. On the positive side, there is increasing interest in using Baker's yeasts as novel probiotic and biocontrol agents, and for the nutrient fortification of foods (Gelinas, 2006; Prem Kumar *et al.*, 2015a). Baker's yeast, *Saccharomyces cerevisiae*, is still one of the most important biotechnological products because it has several industrial applications.

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Baker's yeast as a commercial product has several formulations that can be grouped into two main types: compressed yeast, called fresh yeast, and dried yeast (Beudeker *et al.,* 1990). Compressed yeast is the traditional formulation of baker's yeast and is ready for immediate use. Dried yeast is available in two forms: active dry yeast (ADY) and instant dry yeast (IDY). Active dry yeast (ADY) is normally sold in airtight packages, vacuum seal or filled with an inert gas such as nitrogen. It is not a problem to maintain quality, but it should be rehydrated before use. Unlike ADY, instant dry yeast (IDY) does not have the cell damage during rehydration. IDY is the most expensive among the three type of baker's yeast. Baker's yeast is marketed in two ways, either as compressed cakes or as a dry powder, however there is also a saleable intermediate of the process known as 'Cream yeast'.

## Baker's yeast–*Saccharomyces cerevisiae*

Yeast are a unicellular fungi or plant-like microorganism that exists in or on all living matter i.e. water, soil, plants, air, etc. They are microbial eukaryote, associated with ascomycetes and are rich in protein and vitamin B (Dunn *et al*., 2015). As a living organism yeast primarily requires sugars, water and warmth to stay alive. In addition, albumen or nitrogenous material is also necessary for yeast to thrive. There are hundreds of different species of yeast identified in nature, but the genus and species most commonly used for baking is *Saccharomyces cerevisiae*. The scientific name *Saccharomyces cerevisiae*, means 'a mold which ferments the sugar in cereal (saccharo-mucus cerevisiae) to produce alcohol and carbon dioxide'. Yeasts are usually spherical, oval or cylindrical in shape and a single cell of *S. cerevisiae* is around 8 µm in diameter. Each cell has a double-layered wall, which is permeable to certain substances and in this way food material is taken into the cell and metabolites. Cell division or cell reproduction generally takes place by budding. In the budding process, a new cell forms as a small outgrowth of the old cell, the bud gradually enlarges and then separates. Although, most yeast reproduce only as single cells, under some conditions some yeasts can form filaments (Madigan *et al.,* 2003; Sivasakthivelan *et al*., 2014). Yeasts flourish in habitats where sugars are present, such as fruits, flowers and bark of trees. However, commercial yeasts of today are quite different from wild strains due to genetic manipulation, allowing them to grow in previously unsuitable conditions (Madigan *et al.,* 2003; Prem Kumar *et al*., 2015b). Yeasts are of great economic importance. Yeasts, especially different strains of *Saccharomyces cerevisiae* have long been used for the production of alcoholic beverages, solvents and other chemicals. In the modern bakery, yeasts are used for manufacturing of different kinds of bread and confectionaries. It is responsible for leavening the dough and imparting a delicious flavour to the product.

Yeast converts the fermentable sugars present in the dough into carbon dioxide. This causes the dough to expand or rise as the carbon dioxide forms pockets or bubbles. When the dough is baked it 'sets' and the pockets remain, giving the baked product a soft and spongy texture. The aroma of bread is created during baking by thermal reactions within some of its individual components formed by yeast fermentation and between these compounds and other dough constituents such as some amino acids. When there is an excess of amino acids, they are degraded to aromatic carbonyl compounds6. In addition, yeast is a valuable source of protein and vitamins, especially certain amino acids like lysine, methionine and threonine and B-group vitamins. Hence, many bakery products are currently being prepared with the addition of inactive dried yeasts (Jahan *et al.,* 2007; Gil *et al.,* 2009; Kanchana *et al*., 2015). Most of the baking industries of Bangladesh use baking powder (mixture of $NaHCO_3$, potassium hydrogen tartarate). In recent years, the use of baker's yeast has increased in bread making in our bakery industries. Its use is expensive than chemical because baker's yeasts are not produced in our country. It is being imported in huge amount every year for baking purpose. Since in the recent years, growing concern in making quality bread, the import size has gradually been increased. So, a considerable amount of foreign currency has to be spent for this purpose (Jahan *et al.,* 2007; Sivasakthivelan *et al*., 2014; Dunn *et al*., 2015; Kanchana *et al*., 2015; Prem Kumar *et al*., 2015a).

## Historical development of Baker's yeast

Fermented cereal-based foods are obtained with a wide variety of microbial starters, either from pure or mixed yeasts and bacteria (Gelinas and McKinnon, 2000). For bread making, yeast strains are the result of a long-term domestication of species bred in captivity and modified in ways to make it more useful to humans (Legras *et al.,* 2007). In the beginnings of the baker's yeast industry, brewing by-products were readily available and brewer's yeast was often used for bakery applications. Contrary to bottom yeast which tends to sediment in fermentors, ale yeast was easier to harvest from the top of fermentation tanks. Although, the exact nature of strains was not clear at this time, specific yeast types were later requested by distillers who found profitable to jointly manufacture alcohol and yeast for miscellaneous applications including baking (Hansen, 1896). Only the most promising yeast strains from brewing or alcohol production were probably retained based on yield and sales potential for specific applications. In this context, this is likely that typical strains of yeast well adapted to hydrated flour were also screened because pieces of dough were often used as starters for bread dough. Efforts were made to purify such starters, to eliminate acid-forming microorganisms later recognized as bacteria.

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Whatever their source, only the best yeast strains growing rapidly in saccharified worts and showing high gassing power were kept and grown commercially (Sivasakthivelan *et al*., 2014; Dunn *et al*., 2015; Kanchana *et al*., 2015; Prem Kumar *et al*., 2015a). With the advent of modern industrial baker's yeast manufacturing around 1920, yeast strains for bakery applications further differentiated themselves from the other food yeasts. To improve yeast yields, the baker's yeast industry slowly departed from the growth conditions prevailing in breweries and distilleries. For example, the use of pure cultures became widespread and optimized growth conditions of baker's yeast were slowly discovered. Introduced around 1890, pure yeast seed cultures became essential to get high biomass yields and led to the development of banks or libraries of yeast strains (Hansen, 1896). Other key growth conditions included: strong aeration, strict low pH control, and the use of diluted molasses added at increased concentrations during cell growth instead of concentrated grain-based mash used in breweries and distilleries (Gelinas, 2006; Saranraj and Geetha, 2012). Nowadays baker's yeast strains are very different from wine or brewer's yeast strains. Dawson (1994) showed that modern baker's yeast strains differ from other industrial yeasts because of their high maximum growth temperature, moderate tolerance to low pH and high growth rate on glucose. Compared to baker's yeast strains, wine yeast strains would be better adapted to highly acidic (pH lower than 3.5) and osmotic environments (up to 30% sugar in grape musts), as well as low fermentation temperatures (0-35°C, preferably lower than 20°C) (Jenson, 1998). In addition, wine yeasts strains are expected to develop specific aroma after several days while dough fermentation typically lasts only a few hours (Sivasakthivelan *et al*., 2014; Dunn *et al*., 2015; Kanchana *et al*., 2015; Prem Kumar *et al*., 2015b).

Baker's yeast is a widely distributed commercial product. This means that strains are widely available and they may be grown in different yeast factories worldwide. This situation is very different from brewer's yeast strains which are generally proprietary strains: brewers propagate and breed these strains in-house and, at the completion of the fermentation process, yeast is harvested and is not distributed commercially. Marking production strains, for example with gene inserting has been considered (Legras *et al.,* 2007) because yeast manufacturers find it very difficult to protect or retain their own strains which are freely available to competitors. According to this author, this has led to an uncontrolled exchange of strains and this inhibits any one of manufacturer committing substantial effort to strain improvement. Such situation probably stimulated interest for legal protection for Baker's yeast strains (Donalies *et al.,* 2008; Sivasakthivelan *et al*., 2014; Dunn *et al*., 2015).

Contrary to common belief and well before the advent of genetic engineering, this has been practiced at least since 1904 for pure strains of baker's yeast with strong aroma properties called *Brettanomyces* (Donalies *et al.,* 2008). This was probably the first patent to claim protection for a specific microorganism: The employment in the manufacture of English beers such as ale, stout and porter, of cultures of the new species of microorganisms hereinbefore called *Brettanomyces* (which do not form endospores and thus differ from the saccharomycetes) in order to produce the flavour and condition peculiar to such beers. The first baker's yeast strain to get patent protection appears to have been claimed by Balls in 1927. Earlier patents were granted in the 19$^{th}$ century for methods for obtaining brewer's or baker's yeast biomass but, before 1904, no specific line of yeast cells (strain) appears to have been patent protected (Tanghe *et al.,* 2003; Sivasakthivelan *et al*., 2014; Prem Kumar *et al*., 2015a). The first records of the use of yeast in bread making came from Ancient Egypt (Kanchana *et al*., 2015). *Saccharomyces cerevisiae* is the most commonly used yeast in baking as a leavening agent, where it converts the fermentable sugars present in dough into carbon dioxide. This causes the dough to expand or rise as gas forms pockets or bubbles. When the dough is baked, the air pockets "set", giving the baked product a soft and spongy texture. Most yeast strains used in baking are of the same species common in alcoholic fermentation. In addition, *Saccharomyces exiguus*, wild yeast found on plants, fruits, and grains, is occasionally used for baking. In bread making, the yeast initially respires aerobically, producing carbon dioxide and water. When the oxygen is depleted, fermentation begins, producing ethanol as a waste product, however, this evaporates during baking. In bread making, the most important function of baker's yeasts is leavening (Dunn *et al*., 2015), by producing $CO_2$ *via* the alcoholic fermentation of the sugars which increases the dough volume and giving bread characteristic light and spongy texture. For improved performance during bread making, the yeast strains should possess different characteristics like, (i) high $CO_2$ production, (ii) the ability to quickly start utilizing maltose when the level of glucose in the flour is depleted, (iii) the ability to store high concentrations of trehalose, which will give tolerance to freezing and to high sugar and salt concentrations (iv) tolerance to bread preservatives and chemicals, and (v) viability and retained activity during various storage conditions. Baker's yeast also influences the development of the gluten structure in dough, brought about by expansion of the dough owing to $CO_2$ production. Furthermore, yeasts produce primary and secondary metabolites, such as alcohols, esters and carbonyl compounds which contribute to the development of the characteristic bread flavour (Kanchana *et al*., 2015; Dunn *et al*., 2015).

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## Genetic characteristics of Baker's yeast

Commercial yeasts are mainly *Saccharomyces cerevisiae* strains domesticated under artificial selection conditions. These domestication events were dependent on the desired function of the yeast: baking, brewing, wine making, or bioethanol production (Fay and Benavides, 2005; Legras *et al.,* 2007; Gill *et al.,* 2013). As a result of this selective scheme, we currently have a large number of *S. cerevisiae* strains with highly specialized phenotypes that suit specific applications. In fact, the comparison of available genome sequences of natural and commercial yeasts shows clear genetic signatures for each defined industrial class of yeast (Liti *et al.,* 2009; Borneman *et al.,* 2011; Warringer *et al.,* 2011; Gill *et al.,* 2013). For example, ale strains share the *RTM1* cluster, which has been involved in resistance to an inhibitory substance found in molasses (Ness and Aigle, 1995; Gill *et al.,* 2013), whereas wine strains exhibit a five-gene cluster that contains two potential transcription factors, which were horizontally acquired from *Zygosaccharomyces* spp. (Novo *et al.,* 2009). Unfortunately, the origin and expansion of yeast-raised bakery products is poorly documented, and it is unclear whether bread strains were yeasts present in cereals and maintained and selected in unbaked dough or the surface of actively fermenting beer was used to leaven bread dough. Nevertheless, in the nineteenth century, frequent exchanges between brewers and bakers were reported (Diamond, 2002; Gill *et al.,* 2013).

In general, *Saccharomyces cerevisiae* is an diplontic yeast with clonal reproduction; in fact, it only completes one meiotic cycle for every 1,000 mitotic divisions and 99% of these sexual cycles correspond to self-fertilization in homothallic strains (Ruderfer *et al.,* 2006). This capacity to switch their sexual mating type could explain the high rate of homozygote strains found in some geographically isolated lineages. Industrial strains are diploid, triploid, tetraploid, and polyploid, and some of them are aneuploids, which is the state characterized by having an abnormal number of certain chromosomes. This could be a way for cells to develop phenotypic innovation and adaptation to the various environments by modifying the abundance of key proteins (Pavelka *et al.,* 2010). In addition, aneuploidy may induce genomic instability, as has been recently demonstrated (Zhu *et al.,* 2012), and thus could facilitate the development of genetic variants. For example, aneuploid strains display increased mitotic recombination as well as defective DNA damage repair (Sheltzer *et al.,* 2011). High ploidy and aneuploidy may also confer variable sporulation ability, from zero to high frequency (>50%) sporulation rates, and variable spore viability, which is characteristic of industrial strains (Magwene *et al.,* 2011). With respect to baking strains, a comparative study of industrial yeasts clarified that these strains are heterothallic (mating type switches rarely if at all) and are tetraploids, which concretely display four complete chromosome sets arising from the same species (Albertin *et al.,* 2009). It has been proposed that actual bread yeast strains could originate from a tetraploidization event between beer and a wine strain. This fact could explain the presence in bread strains of a combination of alleles intermediate between beer and wine strains (Legras *et al.,* 2007). Moreover, these tetraploid strains display four alleles at several microsatellite loci, suggesting again that they have only *S. cerevisiae* ancestors. In agreement with this, karyotype analysis and restriction fragment length polymorphism (RFLP) markers identified *S. cerevisiae* strains and excluded interspecific hybrids. The analysis of marker segregation revealed tetrasomic inheritance at meiosis, giving additional evidence for autotetraploidy (Albertin *et al.,* 2009). Although, currently only three whole genomes from bread strains are available, their analysis demonstrates the mosaic nature of these genomes. Thus, it is possible to find some regions very similar to those present in wine strains, with others being more related to sake, oak, or palm wine yeast populations and some that are unique. In the near future, the massive sequencing technology will give new insight into the effects of human and natural selection in commercial yeast strains, and this will provide new opportunities to obtain strains with better or novel properties according to the needs of producers and bakers.

## Biochemistry of Baker's yeast growth

The Baker's yeast (*Saccharomyces cerevisiae*) primarily uses molasses as its growth substrate, the main constituent of which is the disaccharide sucrose. Sucrose is composed of the two monosaccharides glucose and fructose. Glucose degradation can proceed via two distinct pathways, depending on the availability of oxygen within the system. Fructose is degraded in much the same way, differing in the initials reactions for its utilization due to its different conformation. In the presence of oxygen, the oxidative pathway is followed, producing biomass at the expense of glucose breakdown. As oxygen is consumed to support the degradation of glucose, a critical point arises where the reduced availability of oxygen forces the cell to commence anaerobic metabolism as its primary means of growth. This second pathway is known as the reductive pathway and results in the production of biomass along with ethanol ($C_2H_6O$). Furthermore, in the presence of oxygen, ethanol can be utilized as a substrate in the oxido-reductive pathway to produce further quantities of biomass. However, baker's yeast production simply requires the production of biomass, therefore the additional production of ethanol is undesired and so the system is designed as to purposely direct cells into the oxidative pathway, producing only biomass. For this reason, methods have been studied to optimize the growth of *S. cerevisiae* on glucose by regulating the oxygen uptake and glucose consumption by

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the yeast cell, consequently regulating which growth pathway the cell will enter (Sonnleitner and Kappeli, 1986). The findings of such research concluded that in baker's yeast fermentation, the use of fed-batch fermentation allows the constant supply of sugar, such that sugar substrate will not accumulate but instead accommodate the constant growth of *S. cerevisiae* i.e. the sugar was utilized as it was added making the process more efficient. Also constant oxygen sparging within the reactor allows the emergence of the oxidative pathway as the sole route of yeast growth.

## Biochemical and molecular identification of Baker's yeast

Traditional methods of identification are based on diversified morphological and physiological properties of yeast. In such methods, even tens of various tests need to be performed, which in turn is time and labour consuming. Rapid physiological tests in the form of a strip with mounted wells filled with already prepared media are somewhat convenient. Another simplification is the automation of read outs and analysis of results. Still, the above mentioned methods may yield unreliable results and may not be suitable for the identification or discrimination of strains (Arias *et al.,* 2002, Lipinska *et al.,* 2009). Malt extract yeast extract glucose peptone (MYGP) medium, Urease test broth, Bromocresol purple broth, Nitrate broth, Yeast extract agar media and Actidione agar media were used to isolate and identify indigenous yeast isolates. Indigenous yeasts were isolated from different decomposed fruits such as mango, banana peel, apple, grape, lichi, black berry, date fruit, jack fruit, orange, pine apples as well as fermented sugar cane juice and fermented rice (Nasrin Jahan *et al.,* 2007). The isolation and identification of yeasts were carried out as described by Perkins (1976).

The broadening of knowledge in the field of molecular biology enabled the elaboration of new methods for the identification and differentiation of yeast (Lipinska *et al.,* 2009). Predominant among such techniques are the techniques based on the PCR reaction that makes it possible to investigate differences and similarities at the level of nucleic acids. The PCR has a number of variations. One of them is a RAPD technique based on the application of only one primer containing 5 – 15 nucleotides. Moreover, the starter is arbitrary, *i.e.* with any optional sequence, thus its construction does not require the knowledge of, for instance, a matrix fragment of DNA. In the course of reaction, the primer attaches to the matrix at many complementary sites and, once the distance and orientation between two subsequent starters are appropriate, products of reactions are formed. Differences in DNA occurring between organisms result in variations in the number and size of the products formed for each species or strain. Comparison of electrophoretic profiles of RAPD reaction products is a basis for identifying either similarity or difference between the analyzed organisms (Williams *et al.,* 1990). The above method has been successively used by Foschino *et al.* (2004). They examined a yeast starter mix from eight Italian bakeries for diversifying their yeast flora and on that basis identified *S. cerevisiae*. In turn, in a study by Vernocchi *et al.* (2004), the analysis of RAPD profiles of cells collected at a few sites of a production line of a traditional Italian bakery product called Cordoba enabled the identification of *S. cerevisiae*. Fadda *et al.* (2004) investigated yeast flora during the ripening of Fiore Sardo cheese produced using traditional methods in a cheese-manufacturing unit in Sardinia. They noted the occurrence of 18 yeast species whose presence and percentage in cheese changed with time. DNA of isolates of five prevailing species and of standard strains was used in the RAPD reaction, which was proved to be effective in the identification process. The authors suggest that RAPD may be an alternative to conventional identification methods (Lipinska *et al.,* 2009; Sivasakthivelan *et al*., 2014; Dunn *et al*., 2015; Kanchana *et al*., 2015; Prem Kumar *et al*., 2015a).

The literature provides a number of examples that indicate the high popularity of the above technique. Successful experiments using RAPD are improved in order to undertake other trials, yet caution should be exercised due to some restrictions linked with the above-mentioned method. The first obstacle is changeable repeatability of reaction results. Different conditions of PCR as well as various composition of a reaction mixture result in the formation of different products. Similar results may be evoked by: application of enzymes from various sources and application of different protocols or thermocyclers. To facilitate the comparison of the results between laboratories, it would be advisable to combine the applied procedures, reagents, and equipment (Penner *et al.,* 1993). However, as reported by Tyler *et al.* (1997), even after unification and optimization of reaction conditions, results are not always repeatable, both between different research centers and within them (Lipinska *et al.,* 2009). According to Josepa *et al.* (2000) in the analysis of yeast differentiation using the RAPD method, the selection of starter is very significant. Appropriate oligonucleotides make it possible to obtain profiles which, in turn, enable easy identification of similarities and differences between the examined organisms. The number of products should not be high as it would render the analysis difficult. Researchers postulate checking some number of primers before the selection of a proper one for a given organism. It is also advisable to carry out several reactions with different starters and then to analyze their joint results or to compile RAPD with other methods, which has been the subject of the reported study.

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In the production of baker's yeast, use is made of strains belonging to the *S. cerevisiae* species selected for their technological usability. Yeast factories possessing a number of strains (originating from various sources) not always observe distinct diversification of their technological characteristics in the production process. It is likely that strains of different origin are the same strains (Lipinska *et al.,* 2009).

## Fermentation of Baker's yeast

Baker's yeast is a fermentation product used primarily in the preparation of bread dough. It is manufactured by large scale aerobic fermentation of selected strain, *S. cerevisiae.* Aerobic growth of *S. cerevisiae* on fermentable sugars has been studied mainly in batch culture experiments. The growth characteristics of *S. cerevisiae* are variable depending on the condition to which yeast cells are subjected. Many researchers have studied the factors affecting the growth patterns under aerobic conditions. Slonimski (2013) reported the influence of aeration on the yeast growth. Bruver *et al.* (2005) defined the different growth patterns for different carbon sources. Scragg (2011) investigated the adaptation of yeast to variation in sugar concentration. Subsequently, studies in applications of genetic engineering techniques become very popular due to the increasing demands of the industry to improve the strains of yeasts. Molasses is the substrate used in the production of baker's yeast. Molasses is a by-product of sugar refining and contains about 50% sugar. It serves as the source of carbon and energy for the process. It is supplemented with a number of nitrogenous compounds and vitamins such as biotin (Ringbom *et al.,* 2006), which are required for the proper and efficient growth of the yeast cells. Commercial yeast production starts in the laboratory where a small quantity of a yeast culture is injected into a closed flask containing a sterile solution of molasses, ammonium salts to provide a source of nitrogen, and phosphate, necessary for yeast development and reproduction. The yeast culture is made up of a particular yeast strain, which is normally kept on an agar slant. An enormous number of strains of *Saccharomyces cerevisiae* exist, many of which have already been selected for baking (Gil *et al.,* 2009). The closed flask that the culture is injected into is kept at a constant temperature and the yeast grows vigorously for 12 hours. It is then transferred to a larger flask containing a further solution of molasses and nutrient material and more growth takes place. The transfer process is repeated again until a large enough culture of yeast is obtained to start the main yeast production process in the factory's large fermentation vessels. Fermentation vessels for yeast production range from 40,000 to 200,000 L. The progressive increase of fermentor size used is known as scale-up.

Until this stage the yeast cultures have been grown in the absence of air; this is known as anaerobic fermentation. Anaerobic fermentation is, however, inefficient in terms of yeast growth, and subsequent stages of yeast production take place with sterile air being blown through the growing yeast cultures; this is known as aerobic fermentation. The reason why the early stages of yeast production take place in the absence of air is to favour the growth of yeast cells instead of other organisms, such as bacteria, which may gain access to the culture, since these would also grow rapidly and could decrease the efficiency of the process and affect the final yeast quality. A small amount of alcohol is produced during the early stages, which inhibits the growth of foreign organisms (Ringbom *et al.,* 2006).

The fermentation process continues with air being blown through the yeast cultures and molasses solution and nutrients being added continuously, at a constantly increasing rate that is directly proportional to the yeast cell population. By maintaining this supply at a level just sufficient for the amount of yeast present, together with an adequate supply of air, maximum yeast cell reproduction takes place with the minimum production of alcohol as indicated previously. At the end of the first stages of yeast growth about 12 tonnes of yeast is produced and this is known as seed or mother yeast. The seed yeast is divided into portions and these are used to start other fermentations. These fermentations are carried on as before with increasing addition of air, molasses solution and nutrients, and each 3 tonnes of seed yeast produces about 11 tonnes of the final baker's yeast (Sivasakthivelan *et al.*, 2014; Dunn *et al.*, 2015; Kanchana *et al.*, 2015; Prem Kumar *et al.*, 2015a).

Control strategies in industrial aerobic fermentation have been developed to maximize the growth of yeast and minimize the detrimental factors affecting the yeast's growth patterns. Around 1920, the technique of substrate-feeding, referred to as fed-batch culture, was developed to maintain high biomass yields. Fedbatch operation is the process whereby nutrients necessary for cell growth are fed intermittently during the production phase (Reed and Nagodawithana, 2011). This technique has been found to be particularly effective for processes in which effects such as substrate inhibition, catabolite repression, product inhibition and glucose effects are important (Modak *et al.,* 2006). In fermentation of Baker's yeast, fedbatch processes were devised to prevent a detrimental effect to yeast growth because of a high glucose concentration. Fedbatch fermentation takes advantage of the fact that yeast grows most efficiently when glucose is present in small amounts. Currently, the fed-batch technique has been recognized as the best method of commercial yeast fermentation.

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Throughout the whole fermentation process stringent checks are carried out to ensure that yeast growth and quality are maintained, so that the final 40-50 tonnes of baker's yeast are of the same quality and have the same characteristics and properties as the original few milligrams of pure yeast culture that started the process. At the end of the fermentation stage the yeast is present as a suspension of cells in a dark brown liquid containing the residues of the molasses. The yeast is removed from the fermentation liquid by a process of washing and separating in centrifugal separators, signaling the end of the fermentation and beginning of the downstream processing stage (Ringbom *et al.,* 2006).

## Downstream processing of Baker's yeast

Downstream Processing can be defined as the stages of processing that take place after the fermentation or bioconversion stage. The yeast broth which is produced by fermentation, containing approximately 5% solids, can be manipulated into two main types of baker's yeast product and an additional intermediate saleable product. These are cake yeast, granular yeast and cream yeast, each of which requires a downstream process to arrive at the desired product. These downstream processes are investigated in the subsections below.

***Cream yeast:*** Cream yeast is not typically termed a 'baker's yeast product' but is relevant as it represents a major step in the process and is a marketable product itself. At the end of the fermentation, the fermentor/yeast broth is concentrated using a series of combined centrifugation and washing steps, into a yeast cream with a solids concentration of approximately 20% (Gil *et al.,* 2009). The yeast is then cooled to approximately 4°C, an ideal temperature to restrict the growth of any contaminating mesophilic microorganisms. The cooled yeast cream is stored in a stainless steel cream tank, which is insulated and equipped with agitators and cooling pipes (Ringbom *et al.,* 2006), effectively preventing heat exchange with the surrounding atmosphere, keeping the cream at 4°C. Following storage either of two pathways can be followed. The first involves the preparation for sale of the cream yeast itself. Cream Yeast is basically the liquid product and can therefore be transferred into sterile tanks/containers and distributed to bakeries, where it is used to produce yeast based products. The advantage of this is that it excludes any human handling and therefore reduces the risk of contamination by handling, however due to its high (water) volume, transport costs can be expensive. For this reason, distribution is generally confined to a particular area (Randez Gil *et al.,* 2009).

***Granular yeast:*** Granular Yeast, also known as Instant Dried Yeast, is a form of compressed yeast. Stored cream/liquid yeast is passed through a filter, usually a filter press or rotary vacuum filter, which removes water increasing its solids content to approximately 30%. Salt may also be added to the cream yeast prior to filtration to aid the removal of water. The filtered yeast is then dried using fluid-bed dryers. As the yeast is dry it generally does not require refrigeration as the low water content reduces the risk of microbial contamination. Emulsifiers and oils can be added at this point to texturize the yeast and aid the cutting process. As the name implies, granular yeast is crumbled into granules, the granulation process being carried out by a granulator. Granular broths are typically used to make restoring drinks to serve in a cup; the practicality of granular products coming both from their instantly soluble nature and the fact that they are easily measured (Gil *et al.,* 2009).

***Cake yeast:*** The filtered and dried yeast can alternatively be used to make cake yeast. Cake yeast is another form of compressed yeast and can be categorized as active dry yeast. It differs from granular yeast in that rather than granulation, the dried yeast is extruded or cut into blocks/cakes. Similar to granular yeast cake yeast also contains about 30% solids (70% water). The composition of solids may vary depending on the growth rate of the yeast as lower growth rates give lower protein, lower activity, higher carbohydrate, and higher stability (Gil *et al.,* 2009). Both types of compressed yeast are then packaged, typically vacuum packed to reduce the risk of contamination by aerobic bacteria, 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.

## Conclusion

Nowadays, the man-made baker's yeast strains, as well as their associated technological process particularities are fast disappearing due to globalization of yeast and dough industry. Nevertheless, sustainability demands ask for solutions empowering local populations with tools that allow their own survival. Yeasts, as always, have a role in this desired change of paradigm. The baker's yeast industry is a major market, grossing several billion dollars per year. The low value, high volume product is produced under stringent environmental conditions to obtain the maximum biomass yield which is dependent on process design, cost and the strain of *Saccharomyces cerevisiae* used. The fermentation and scale-up stage is the critical to the process as it dictates the volume and quality of the product. The industry is expanding after a recent slump and with the potential for large profits it can be expected to continue. As with all biotechnology processes, research and development in this area is continuously ongoing to create more beneficial strains of the *S. cerevisiae* fungi and optimize the fermentation and processing steps which make up the baker's yeast process.

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