FOOD BIOLOGY SERIES

Fermented Foods

Part I: Biochemistry and Biotechnology

Didier Montet and Ramesh C. Ray (eds)




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Fermented Foods

Part I: Biochemistry and Biotechnology

Food Biology Series

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Preface to the Series

Food is the essential source of nutrients (such as carbohydrates, proteins, fats, vitamins, and minerals) for all living organisms to sustain life. A large part of daily human efforts is concentrated on food production, processing, packaging and marketing, product development, preservation, storage, and ensuring food safety and quality. It is obvious therefore, our food supply chain can contain microorganisms that interact with the food, thereby interfering in the ecology of food substrates. The microbe-food interaction can be mostly beneficial (as in the case of many fermented foods such as cheese, butter, sausage, etc.) or in some cases, it is detrimental (spoilage of food, mycotoxin, etc.). The *Food Biology* series aims at bringing all these aspects of microbe-food interactions in form of topical volumes, covering food microbiology, food mycology, biochemistry, microbial ecology, food biotechnology and bio-processing, new food product developments with microbial interventions, food nutrification with nutraceuticals, food authenticity, food origin traceability, and food science and technology. Special emphasis is laid on new molecular techniques relevant to food biology research or to monitoring and assessing food safety and quality, multiple hurdle food preservation techniques, as well as new interventions in biotechnological applications in food processing and development.

The series is broadly broken up into food fermentation, food safety and hygiene, food authenticity and traceability, microbial interventions in food bio-processing and food additive development, sensory science, molecular diagnostic methods in detecting food borne pathogens and food policy, etc. Leading international authorities with background in academia, research, industry and government have been drawn into the series either as authors or as editors. The series will be a useful reference resource base in food microbiology, biochemistry, biotechnology, food science and technology for researchers, teachers, students and food science and technology practitioners.

Ramesh C. Ray
Series Editor

Preface

Traditional fermented foods are not only staple foods for most of the developing countries but also significant health food for developed countries. Fermented foods are food substrates that are processed by edible microorganisms (bacteria, yeasts and molds) whose enzymes (e.g., amylases, proteases and lipases) hydrolyze the polysaccharides, proteins and lipids to non-toxic products with flavors, aromas and textures desirable to the humans. As the health functions of these foods are being re-discovered, higher throughput biotechnologies are being used to promote the fermented food industries. As a result, microorganisms, process biochemistry, manufacturing and down-stream processing, as well as bioactive metabolites released by the fermenting organisms and above all, the health functions of these foods are being extensively researched. Furthermore, the applications and progress of biochemistry and biotechnology applied to traditional fermented food systems are different from each other, as the microorganisms and the food matrices vary widely.

Part I of the book, "Fermented Foods: Biochemistry and Biotechnology" covers general aspects on microbiology, biochemistry, and biotechnological applications involving yeasts, filamentous fungi, acetic acid and lactic acid bacteria in promoting and accelerating the development of multiple functional factors in fermented foods, the release of bioactive compounds during fermentation, development of starter cultures, and metagenomics of fermented foods. The impact of yeasts on food and beverage production beyond the original and popular application in bread, beer and wine has been described. Likewise, the importance of acetic acid bacteria, which are desirable and essential for the production of vinegar and cocoa, while they are sometimes involved in foods and beverages in detrimental way, such as in wine, beer, soft drinks and fruits, has been discussed. The classification, metabolism, and applications of lactic acid bacterial group, including their antimicrobial activities (bacteriocins-based) and effects on human health have been elucidated. Two chapters are devoted specifically to fermentation of cereals and vegetables by these bacteria and the resulting health benefits. A chapter is devoted to lactic acid fermentation of seaweeds (macro-algae), which can constitute a new raw material for the production of fermented foods and feeds. Microencapsulation of probiotics provides a good promising approach to overcome their loss during detrimental environmental conditions. The various techniques used for microencapsulation of probiotics and their applications are also outlined. Despite the focus on health benefits of probiotic yeasts and lactic acid bacteria, the negative aspect is the production of biogenic amines by some strains of lactic acid bacteria that has detrimental effects on health. These amines occur in a wide variety of fermented foods such as sausage, fish products, cheese and wine. Therefore, a chapter is devoted on biogenic amine in fermented foods, as well as a chapter on antimicrobial resistance of fermentative bacteria in the context of Whole Food Chain Approach. Other aspects such as microbial fructo-oligosaccharides and their relevance to human health, and technology adapted for bio-valorization of food wastes are covered in two separate chapters. Molecular methods used to engineer fermentative microorganisms are presented in a chapter dedicated to wine biotechnology. Other applications of molecular methods, and more specifically metagenomics, are also discussed in the light of monitoring ecosystems during fermented food elaboration. The 20 chapters in this book have been authored by highly reputed international contributors having an in-depth understanding of fermented food science and technology. We believe that this book will be a useful reference book for researchers, teachers, students, nutritional and functional food experts and all those working in the field of food science and technology.

The detailed technological interventions involved in different categories of fermented foods such as fermented cereals (bread and sourdough), fermented milk products (yogurt, cheese, kefir and koumiss), fermented sausages, fermented vegetables (kimchi, sauerkraut), fermented legumes (tempeh, natto, miso), coffee and cocoa fermentations, cassava and sweet potato fermentations, are discussed in Part II of this book (Fermented Foods: Technological Interventions).

Didier Montet
Ramesh C. Ray

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Yeasts in Fermented Foods and Beverages

Tek Chand Bhalla[1,*] and *Savitri*[1]

1. Introduction

For more than 6000 years, fermentation has been used as a means of improving the shelf life and adding quality to food. Earlier, the transformation of basic food materials into fermented foods was considered as a mystery. The first fermentation was probably discovered by accident when salt was added in food materials, causing the selection of some harmless microorganisms that could ferment the raw material to give nutritious and acceptable food. Since then, it has been practiced to improve both, the preservation and organoleptic characteristics of food.

Food fermentation technology utilizes the ability of growth of microorganisms on various substrates for the production of a variety of fermented foods, beverages and pickles, e.g., bread, beer, cheese, *idli, tempeh, miso*, sauerkraut, fermented sausages, etc. These fermentations involve the combined action of bacteria, yeast and fungi which may act in parallel, while others may act in a sequential manner with a changing dominant flora during the course of fermentation (Haard 1999). Among the various microorganisms, yeasts have an enormous impact on food and beverage production. They play a central role in the fermentation of foods and beverages, especially those with high carbohydrate content, as yeasts can survive and grow under stress conditions. In traditional fermentation processes, natural micro-organisms, including yeasts, are employed in the preparation and preservation of different types of foods. These processes add to the nutritive value of foods as well as enhance flavor and other desirable characteristics associated with digestibility and edibility (Kolawole et al. 2007).

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Today, the impact of yeasts on food and beverage production extends beyond the original and popular notions of bread, beer and wine fermentations by *Saccharomyces cerevisiae* (Fleet 2006). A variety of important food ingredients and processing aids are now obtained from yeasts. Some yeasts are being explored for their strong antifungal action and are used as novel agents in the biocontrol of food spoilage. The probiotic activity of yeast is another remarkable aspect that is attracting the attention of academia and industry. Unfortunately, yeasts are also involved in the spoilage of many commodities, with major economic losses to many food and beverage industries, while the impact of yeasts present in foods and beverages on public health is a matter of emerging concern.

2. History

Although, both yeast and bacteria were first reported to have been observed microscopically by Antonie van Leeuwenhoek in 1680, it was not until the year 1837 that Cagniard-Latour, suggested that the fermentation was intimately associated with the yeast cells. The history of yeast association with human society is synonymous with the evolution of bread, beer and wine as global food and beverage commodities, originating about 5,000 years ago (Fleet 2006, Chapter 1 in this book). Archaeologists have found evidence for the production of a fermented beverage in China about 7000 BCE (McGovern et al. 2004), and of wine in Iran and Egypt in 6000 BCE and 3000 BCE, respectively (Cavalieri et al. 2003). It is believed that the cultivation of grapevines and the production of wine spread all over the Mediterranean Sea, moving towards Greece in 2000 BCE, Italy in 1000 BCE, Northern Europe in 100 CE and America in 1500 CE (Pretorius 2000). Yeast is probably one of the earliest domesticated organisms among various microorganisms. It was used to carry out fermentation from the early beginnings of brewing. The microorganisms, or even yeasts, were not specifically known in the Middle Ages, but it was well-known that the best beers were produced next to bakeries (Kruif 1935).

3. Yeast

The term 'yeast' was derived from the Dutch word '*gist*', which refers to the foam formed during the fermentation of beer (Foligné et al. 2010). Yeasts are heterotrophic organisms occurring naturally on the surfaces of plant tissues, including flowers and fruits, and are fairly simple in their nutritional requirements. Yeasts are unicellular, although some species may become multicellular through the formation of strings of connected budding cells known as pseudohyphae, or false hyphae. Yeasts exhibit both asexual and sexual stages in their life cycle. The asexual stage of given yeast is called the anamorph, while the sexual stage is the teleomorph. Most yeasts reproduce asexually by mitosis, and many do so by an asymmetric division called budding. The budding yeasts or true yeasts are classified in the order Saccharomycetales (Kurtzman and Fell 2005).

Vegetative growth in yeasts is either entirely unicellular or a combination of hyphal and unicellular reproduction. The most common mode of vegetative growth of yeasts is by budding, which may be blastic or thallic. Anamorphic and teleomorphic genera may grow either as a 'yeast-like' unicellular organism or as a 'mold-like' filamentous organism, a phenomenon called as dimorphism. Moreover, some species are able to form a true mycelium, while genera such as *Candida* produce a well-developed pseudomycelium, or both pseudo and true mycelium as in the case of *Candida tropicalis* (Goldman 2008). Brewing yeast does have a sex life, but reproduces primarily by budding in production conditions. A single cell may bud up to 20 times, each time leaving a scar, the counting of which indicates how senile the cell has become.

Among the yeasts belonging to the phylum *Ascomycota*, the genus *Saccharomyces* is the most studied and widely used in industrial processes. *Saccharomyces cerevisiae* (Fig. 1) is spherical or ellipsoidal in structure. Laboratory strains of *S. cerevisiae* are haploid (have one copy of each of the 16 linear chromosomes), whereas industrial strains are polyploid (i.e., have multiple copies of each chromosome) or aneuploid (varying numbers of each chromosome). Some 6000 genes have been identified in yeast and the entire genome has now been sequenced (http://www.yeastgenome.org/).

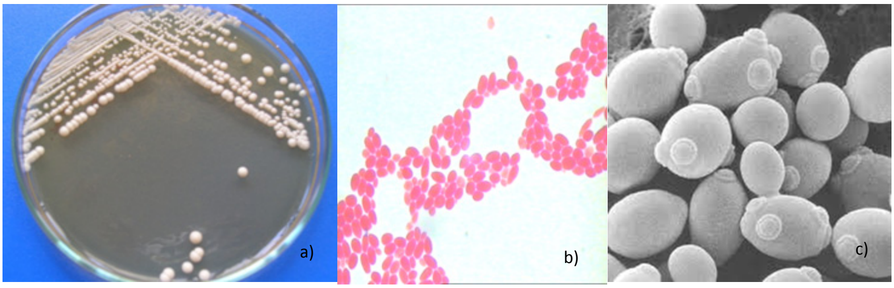

Figure 1. *Saccharomyces cerevisiae* (a) colonies on YPD agar medium, (b) microscopic view and (c) scanning electron micrograph.

3.1 Classification

Yeasts are eukaryotic microorganisms classified in the kingdom *fungi* with 1,500 species currently described, and estimated to be 1% of all fungal species (Kurtzman and Piškur 2006). They are widespread in natural environments including the normal microbial flora of humans, plants, airborne particles, water, food products, and many other ecological niches. Yeasts do not form a single taxonomic or phylogenetic group. The term 'yeast' is often taken as a synonym for *S. cerevisiae*, but the phylogenetic diversity of yeasts is shown by their placement in two separate phyla: the Ascomycota and the Basidiomycota whose vegetative growth results predominantly from budding or fission, and which do not form their sexual states within or upon a fruiting body (Kurtzman and Fell 1998). Phylogenetic analysis of the phylum Ascomycota has significantly changed yeast classification in recent years (Hibbett et al. 2007, Kurtzman et al. 2011).

3.2 Yeast Cell Structure

Yeasts consist of single cells which are smaller than animal and plant cells but larger than bacteria. There is a cell wall on the outside of the cell and the inside of the cell contains cytoplasm having a nucleus, mitochondria and ribosomes (Fig. 2). The cell wall in yeast is a two layered structure, the inner layer is made up of β-1–3-glucane linked covalently to chitin and is responsible for the stability of yeast cells. In contrast, the outer layer consists of densely packed glycosylated mannoproteins which decrease the permeability. In addition, hydrophilic attributes of the cell wall are achieved by phosphorylation of the mannoproteins (Lipke and Ovalle 1998). Due to the presence of phosphate groups attached to the mannan polysaccharides, the cell wall of yeast is negatively charged with underlying plasma membrane comprised primarily of sterols, unsaturated fatty acids, proteins and permeases.

Although, the function of the cell wall is to provide stabilization to the cell, the cell membrane, which consists of phospholipids and proteins along with sterols (mostly ergosterol and zymosterol), forms the main barrier for substrate exchange in and out of the cell. The membrane-building phospholipids vary from the outer to the inner layer (Zinser et al. 1991). Sphingolipids are mainly found on the outside and their function is to give the membrane a structure; they also have an important role in cell growth, cell regulation and have an influence on the stress reactions of the cell (Hannun and Obeid 1997). Proteins are also present in cell membrane and are spread asymmetrically over the bilayer. Intrinsic proteins are stretched through the whole

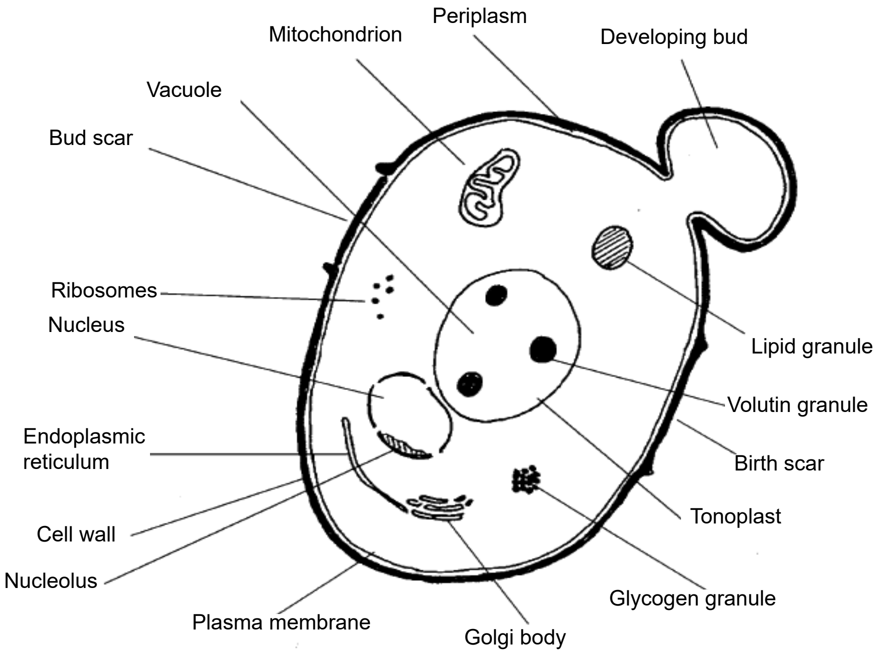

Figure 2. Diagrammatic representation of cross section of yeast cell (Source: Boulton and Quain 2001).

membrane, whereas extrinsic membrane proteins can only be found on the inner or the outer layers. Most of them are specialized to perform different tasks (Van der Rest et al. 1995). The cytoplasm of yeasts is an aqueous, slightly acidic fluid with several organelles, *viz*., vacuoles, ribosomes, endoplasmic reticulum, golgi apparatus and the nucleus. In addition, proteins, lipid particles and glycogen can also be found. The lipid particles play a role in sterol biosynthesis and serve as lipid storage for membrane synthesis. Glycogen granules are built up at the end of fermentation; these serve as an energy reserve during storage and as starting energy for new cell cycles (Tenge 2009).

3.3 Metabolism in Yeast

Yeasts are capable of utilizing a broad spectrum of carbohydrates and sugars. They require a reduced carbon source, various minerals and a supply of nitrogen and vitamins. The key vitamin requirements are biotin, pantothenic acid and thiamine. As oxygen is required for the desaturation reactions involved in the synthesis of the lipids, relatively small quantities of oxygen must be supplied to the yeast, even when it is growing anaerobically during fermentation. Metabolism in the yeast cell (i.e., by aerobic respiration or by fermentation) is based on the concentration of sugar to which yeast is exposed. For example, when sugar concentration is high, the cell is switched into the fermentative mode, and the pyruvate is metabolized *via* acetaldehyde to ethanol, yielding 2 molecules of ATP per sugar molecule. At low sugar concentrations, the pyruvate shunts into acetyl-CoA and the respiratory chain, and this yields 32 molecules of ATP per sugar molecules. This phenomenon is known as Crabtree effect. At high sugar concentrations, the cell does not need to generate as many ATP molecules per sugar molecule, whereas if the sugar supply is limited, the yeast must utilize the sugar efficiently. The significance of this in commercial fermentation processes is clear. In brewing, where the primary requirement is a high yield of alcohol, the sugar content in the feed stock is high, whereas in the production of baker's yeast, where the requirement is a high cell yield, the sugar concentration is always kept low, but the sugar is continuously passed into the fermenter (Bamforth 2005).

3.4 Diversity of Yeasts in Nature

Various yeasts have been isolated from natural and artificial environments (Lachance et al. 2001, Lee et al. 2001). In nature, *S. cerevisiae* is present on grape surfaces at extremely low density, but becomes the predominant species during alcoholic fermentation. The most extensive work has been focused on population change and the roles of yeasts during the fermentation process of food and beverages (Strauss et al. 2001). Studies on yeast biodiversity in the natural environment have been neglected because yeasts have been regarded as playing a major role in food fermentation and a rather minor role in the biosphere as compared to other microorganisms that may act as primary producers, predators, pathogens, or important agents of nutrient cycling in natural environments (Lachance and Starmer 1998). *S. cerevisiae*, also called winemaker's bug, is so closely associated with humans that it is rarely found in environments devoid of human inhabitation. In fact, its evolutionary success can probably be explained by its relationship with humans, particularly in the production of alcoholic beverages (McGovern et al. 2004).

4. Yeasts in Fermented Foods and Beverages

Yeasts form an inevitable part of the microflora of various fermented foods and beverages and are found in a wide range of foods from plant and/or animal origin (Fig. 3), where they have a significant role in food safety and imparting organoleptic properties (Foligné et al. 2010). They play important roles in the production of many traditional fermented foods and beverages across the world (Aidoo et al. 2006), foods that signify the food culture of the regions and the community (Tamang and Fleet 2009). More than 21 major genera of functional yeasts have been reported from fermented foods and beverages; the main genera being *Brettanomyces* (*Dekkera*), *Candida*, *Cryptococcus*, *Debaryomyces*, *Galactomyces*, *Geotrichum*, *Hansenula*, *Hanseniaspora* (*Kloeckera*), *Hyphopichia*, *Kluyveromyces*, *Metschnikowia*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Saccharomycodes*, *Saccharomycopsis*, *Schizosaccharomyces*, *Torulopsis*, *Trichosporon*, *Yarrowia*, and *Zygosaccharomyces* (Kurtzman and Fell 1998, Pretorius 2000, Romano et al. 2006, Tamang and Fleet 2009). Yeasts, alone or in stable mixed populations with mycelial fungi or with bacteria (usually lactic acid), have a significant impact on food quality parameters such as taste, texture, odour and nutritive value (Table 1). Molds are relatively limited in fermented foods and beverages, and include the genera *Actinomucor*, *Mucor*, *Rhizopus*, *Amylomyces*, *Monascus*, *Neurospora*, *Aspergillus*, and *Penicillium* (Nout and Aidoo 2002).

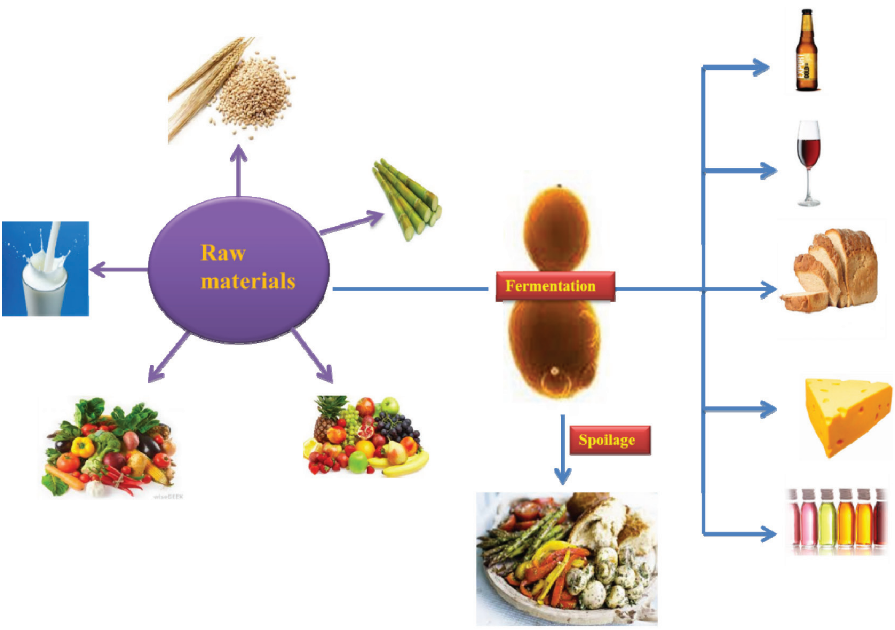

Figure 3. Role of yeast in food and beverages.

Table 1. Role of yeast in production of fermented food and beverages (modified from Romano et al. 2006).

Fermented Food/ Beverage	**Yeast**	**Function**
Beer, wine, sourdough, bread, cheese, indigenous fermented foods and beverages	*Saccharomyces* sp.	Sugar fermentation and formation of alcohol Improvement of flavor and texture Production of secondary metabolites Inhibitory effect on undesirable microorganisms
Sourdough, cocoa beans, soy sauce, indigenous fermented foods and beverages	*Candida* sp.	Production of different enzymes (protease, galactosidase and pectinase) Inhibition of undesirable organisms Secondary metabolite production
Cheese	*Clavispora lusitaniae*	Production of flavor compounds in cheese
Fresh fruits and fermented meat products	*Cryptococcus* sp.	Biocontrol agent against fungal pathogens (some species) Spoilage activity
Wine, sourdough, indigenous fermented foods and beverages	*Hansenula/Pichia* sp.	Production of volatile/aroma compounds in wine Inhibition of various moulds
Cheese	*Torulopsis* sp.	Flavour enhancement
Cheese, salami, rye sourdough	*Debaryomyces* sp.	Production of flavor compounds in cheese (nutty or malty flavor) Increase in pH Help in ripening of cheese
Cheese, salami	*Yarrowia lipolytica*	Lipolytic and proteolytic activities Reduction in fat rancidity
Fermented meat products, wine	*Rhodotorula* sp.	Lipolytic activities Production of carotenoids Spoilage of dairy foods
Cheese, cocoa beans	*Kluyveromyces* sp.	Fermentation of lactose Production of pectinase, lipase and rennet
Soy sauce	*Zygosaccharomyces* sp.	Osmotolerance

Apart from fermented foods and beverages, yeasts are also used for the production of enzymes, fine chemicals, single-cell proteins, and flavoring compounds (Gatto and Torriani 2004, Wang 2008). The non pathogenic *Saccharomyces unisporus* has shown a significant role in the ripening of cheese and production of fermented milk products such as *kefir* and *koumiss*. Two yeast species, *viz*., *S. unisporus* and *S. florentinus* are accepted by the International Dairy Federation and the European Food and Feed Cultures Association for food and feed applications.

4.1 Significance of Yeasts in Food and Beverage Production

Out of various microorganisms used for fermentation, yeasts are the most important group being exploited for commercial purposes. They are used in food fermentation processes to modify the cheaper raw materials organoleptically, physically and nutritionally into valuable products, and have been used for millennia in bread-making and the production of alcoholic beverages. In fermented foods and beverages, yeasts play a very important role in the bio-enrichment of foods, leading to the production of proteins, vitamins, minerals, aroma, alcohols, acids and esters. These compounds play a further role in improvements in the digestibility and preservation of these foods.

The significance of yeasts in food and beverage production includes (Fleet 2006):

- Fermentation and production of fermented foods and beverages
- Production of safe and suitable ingredients (flavor compounds) and additives for food processing
- Prevention of growth of spoilage microorganisms
- As animal and human probiotic
- Recombinant yeasts as biotherapeutic agents
- Spoilage of foods and beverages
- Source of food allergens

4.2 Yeasts in Dairy Products

Although dairy products are dominated by lactic acid bacteria, there is now substantial literature describing the important role of yeasts in flavor and texture development during the maturation stage of cheese production and in the production of fermented milks such as *kefir* and *koumiss* (Fleet 1990, Frohlich-Wyder 2003). Among the dairy products, yeasts are mainly used as single starter cultures for cheese production. In most cases, yeasts are used as secondary starter cultures which facilitate the production of aroma compounds and growth of other microorganisms. *Debaryomyces hansenii*, *Yarrowia lipolytica*, *Kluyveromyces marxianus* and *S. cerevisiae* are the predominant and most important species in dairy products but *Galactomyces geotrichum*, *Candida zeylanoides* and *Pichia* species are also significant. Dairy products which derive some of their character from the activity of fermenting yeasts are *kefir* and *koumiss*. The populations and yeast flora of *kefir* have been described by Marshall et al. (1984). In some cheese varieties, especially in semi-soft cheeses with surface films, yeasts are involved in the maturation process, contributing to ripening by utilization of lactic acid, thereby increasing the pH which encourages bacterial growth and initiates the second stage of maturation. During the production of surface-ripened cheeses such as Brick, Limburger, Port Salut, Taleggio, Tilsitter, Trappist, and the Danish Danbo cheese, *D. hansenii* is the key yeast involved. The surface smear of the above varieties of cheese consists of both yeasts and bacteria. In these cheeses, yeasts initiate the ripening by hydrolysis of lactic acid, thereby increasing the pH on the cheese surface and allowing the growth of a more acid-sensitive bacterial population (Leclercq-Perlat et al. 1999, Petersen et al. 2001). Further, *D. hansenii* might produce growth factors of importance for the bacteria as well as aroma components and lipolytic and proteolytic enzymes that contribute to the ripening process (Jakobsen and Narvhus 1996). In Gorgonzola cheese, *S. cerevisiae* is the main starter culture used in the production, but it apparently also occurs spontaneously, together with other yeasts, as an integral part of the microbial population of both blue veined cheeses and some types of soft cheese (Beresford et al. 2001, Hansen and Jakobsen 2001).

Under the genus *Saccharomyces*, 10 species are accepted in dairy products, of which *S. unisporus*, also called as *Kazachstania unispora* (Lu et al. 2004), is one. *S. unisporus* is found to be the dominant yeast in traditional dairy products (Rahman et al. 2009, Yildiz 2010). A unique synchronization of *Kluyveromyces marxianus* and *S. unisporus* (*Kazachstania*) species has been found in milk-fermented products, such as *dahi, suusac, gariss, kefir, shubat*, and *koumiss* (Narvhus and Gadaga 2003, Abdelgadir et al. 2008, Rizk et al. 2008). *S. unisporus* is the slowest producer of ethanol and performs a clean fermentation in milk and whey (Montanari et al. 1996).

4.3 Yeasts in Fermentation of Meats

In fresh meat, yeasts are usually present in very low numbers, but their counts may increase during storage at low temperature and they may eventually dominate the microflora in later stages (Cook 1995). Fermentation is a crucial phase of the curing stage of meat based products (e.g., sausages) since, at this stage, the principal physical, biochemical and microbiological transformations take place. The strains of *D. hansenii*, *D. kloeckeri*, *Y. lipolytica*, *Citeromyces matritensis*, *Trichosporon ovoides* and several other species of *Candida* (*C. intermedia* or *C. curvata*, *C. parapilosis*, *C. zeylanoides*), *Pichia*, *Cryptococcus*, and *Rhodotorula* are the yeasts which contribute significantly to the flavor of fermented meat products and meat-flavored products (Bolumar et al. 2003, Encinas et al. 2000). Various enzymes, *viz*., lipases and proteases, are secreted by these yeasts which contribute to the flavor of the products by offsetting the acidic pH caused by the activities of mixed bacterial starter culture through the degradation of lipids to free fatty acids and glycerol and the breakdown of nitrogenous compounds to amino acids with release of ammonia. Several recently published reports describe the isolation and characterization of several such enzymes from *D. hansenii* (Bolumar et al. 2003, Durá et al. 2002). Yeasts (*D. hansenii*, *Debaryomyces* species, *Y. lipolytica* and *Candida* species) play an important role in the fermentation of meat sausages and the maturation of hams where they contribute to flavor and color development in the products (Samelis and Sofos 2003). Although yeasts are not the part of the starter cultures used for salami processing, a high number of yeasts were observed during the later stages of maturation, suggesting that they play an important role in the ripening of salami (Abunyewa et al. 2000).

In addition, meaty flavor can be achieved in non-meat-derived products by the processing of aqueous yeast or yeast extracts by treatments that utilize heat in the presence of sugars (preferably xylose or lactose) with or without other amino acids such as methionine, cysteine, or cystine (Abbas 2006).

4.4 Role of Yeast in Cereal Fermentation

Cereals, in their dry state, are not subject to fermentation due to their low water content. Dried cereals contain less than 14% water which limits the growth of microorganisms and chemical changes during storage. However, on mixing grains or cereal flour with water or other water-based fluids, enzymatic changes occur that may be attributed to the enzymes inherently present in the grain and/or contributed by the microorganisms. These microorganisms can either be those present as the natural contaminating flora of the cereal, or they can be added as a starter culture (Narvhus and Sørhaug 2006).

Yeasts other than *S. cerevisiae* are found in the fermentation of various cereal products, including sourdough breads, where they affect product flavor and rheology. Prominent yeasts in sourdough fermentations include *S. exiguus*, *C. humicola/ C. milleri* and other species of *Candida* (e.g., *C. krusei/Issatchenkia orientalis*), *Torulaspora delbrueckii* and various *Pichia* species (Jenson 1998, Meroth et al. 2003, Hammes et al. 2005). *Zygosaccharomyces rouxii*, *C. versatilis*, and *C. etchellsii* are important osmotolerant species that play a key role in soy sauce fermentation (Hanya and Nakadai 2003). In sourdough, yeasts are responsible for the leavening process, while the bacteria determine the souring of the dough. The combined metabolic activities of these microorganisms lead to final products with particular sensory properties and longer shelf life. In addition, a vast range of traditional, fermented products are produced in Africa, Asia and Latin America where, along with bacteria, a diversity of yeast species make important contributions (Nout 2003). The yeasts *Geotrichum candidum*, *Torulopsis holmii*, *Torulopsis candida* and *Trichosporon pullulans* have also been identified in *idli* fermentation (Chavan and Kadam 1989).

Yeasts have well known association with bread, beer, wine and other alcoholic beverages. Many species, other than those of *Saccharomyces*, also make positive contributions to the production of fermented foods and beverages. Various species of *Hanseniaspora*, *Candida*, *Pichia*, *Metschnikowia*, *Kluyveromyces*, *Schizosaccharomyces* and *Issatchenkia* can contribute positively to the fermentation of wine from grapes and cider from apples (Fleet 1998, 2003, Pretorius 2000). *Dekkera* (*Brettanomyces*) species, in addition to *S. pastorianus* and *S. cerevisiae,* are significant in the production of some types of beer (Dufour et al. 2003), while *Schizosaccharomyces pombe* can be important in rum fermentations (Fahrasmane and Ganou-Parfait 1998).

4.5 Baker's Yeast

The first records of the use of yeast in bread making came from Ancient Egypt (Legras et al. 2007). *S. cerevisiae* is the most commonly used yeast as a leavening agent in baking, 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* (*S. minor*), 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 (Paramithiotis et al. 2000), by producing CO_2 via the alcoholic fermentation of the sugars thus increasing the dough volume and giving bread its 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 flavor (Damiani et al. 1996, Martinez-Anaya 1996).

In dough fermentation, formation of end products of yeast metabolism varies considerably with pH. In bread, the pH is usually below 6.0; above this pH, end products such as succinate, acetic acid and glycerol, along with ethanol and CO_2, are formed. *S. cerevisiae* is also able to degrade proteins and lipids, producing several flavor compounds (Narvhus and Sørhaug 2006).

Three different types of yeasts, *viz.*, wet compressed yeast (WCY), active dry yeast (ADY) and instant yeast (ITY) are produced commercially for different purposes. Wet compressed yeast is sold as fresh yeast compressed into a square cake and this form perishes quickly, so it must be used soon after production. In contrast to this, active dry yeast has solid levels of 92–97% and has shelf life of 4–12 months. Instant yeast, also called highly active dried yeast, has high solid levels and a shelf life of up to 2 years (Joshi and Pandey 1999, Wood 1998).

4.6 Yeast in Sourdough

Sourdough is defined as an ingredient containing cereal components, liquids and active microorganisms including lactic acid bacteria and yeast (Brummer and Huber 1987). Traditional sourdough bread technology is based on a spontaneous fermentation process from lactic acid bacteria and yeast occurring naturally in flour. Dense dough promotes the growth of lactic acid bacteria, whereas soft dough promotes the development of yeast (Gobbetti et al. 1995). Numerous yeasts have been isolated from sourdoughs but only a part of them can be considered to play a substantial role in fermentation processes. Table 2 shows the different yeast species isolated from sourdough. The most often reported yeast species in sourdough, regardless of the flour type and fermentation conditions, are *S. cerevisiae* (Gobbetti et al. 1994), *S. exiguus* (Gobbetti et al. 1994, Ottogalli et al. 1996), and *C. milleri* (Meroth et al. 2003, Vernocchi et al. 2004). Less often, *C. krusei*, *Torulaspora delbrueckii*, and *Pichia saitoi* are reported (Spicher and Stephan 1993). In the course of sourdough fermentation, yeast plays several important roles; for example, it produces carbon dioxide which expands the dough, resulting

Table 2. Yeast species identified in sourdoughs (Maloney and Foy 2003).

Yeast species	Sources
Saccharomyces cerevisiae	Rye, corn and wheat sourdough
Saccharomyces chevalieri	Rye and wheat sourdough
Saccharomyces curvatus	Rye and wheat sourdough
Saccharomyces exiguus	Rye and wheat sourdough
Saccharomyces fructuum	Wheat sourdough
Saccharomyces inusitalus	San Francisco wheat sourdough
Saccharomyces panis fermentati	Rye and wheat sourdough
Candida boidinii	Rye and wheat sourdough
Candida crusei	Wheat sourdough
Candida guillermondii	Wheat sourdough
Candida milleri	Wheat sourdough
Candida norvegensis	Wheat sourdough
Hansenula anomala	Wheat sourdough
Hansenula subpelliculosa	Wheat sourdough
Pichia salvi	Rye sourdough
Torulopsis delbrueckii	Corn and rye sourdough

in the proper porosity of the crumb and the proper volume of bread. Besides, yeast produces a variety of metabolites such as aldehydes, alcohols, acids, keto acids and esters, which alone or in combination with other compounds can create specific and unique flavors of bread (Makoto et al. 1990).

4.7 Yeasts in Alcoholic Fermentation

In general, yeasts (mainly the species of *Saccharomyces*) predominate during alcoholic fermentation, where the low pH and nutritional content of the substrate/juice lends itself to yeast growth. The use of yeast for the production of alcohol is found in the earliest human records. Beer, which is produced from hopped malt extract, is usually fermented by *Saccharomyces carlsbergensis* which differs from *S. cerevisiae* in that it is able to ferment the sugar melibiose. The two main types of beer, lager and ale, are fermented with strains of *S. carlsbergensis* and *S. cerevisiae*, respectively. The yeast used in beer production is called brewer's yeast (Gibson 2010).

4.8 Brewer's Yeast

Brewing yeasts may be classified as top-fermenting and bottom-fermenting. Top fermenting yeasts tend to be less flocculent and loose clumps of cells are carried to the fermenting wort surface, adsorbed to carbon dioxide bubbles. An example of a top fermenting yeast is *S. cerevisiae*, sometimes called an 'ale yeast'. Bottom fermenting yeasts are typically used to produce lager-type beers and these flocculate and collect at the bottom towards the end of fermentation, though they can also produce ale-type beers. These yeasts ferment well at low temperatures. An example of bottom fermenting yeast is *Saccharomyces pastorianus*, formerly known as *S. carlsbergensis* (Gibson 2010).

4.9 Yeast in Wine Preparation

Yeasts are the prominent organisms used in winemaking, where they convert the sugars present in grape juice (must) into ethanol. Yeasts determine several characteristics of the wine, including the flavor, through a range of mechanisms and activities (Fleet 2003). Yeasts are usually present as normal microflora on grape skin. Studies of spontaneous fermentations have identified a genetically diverse range of yeast populations, *S. cerevisiae* being the one which changes during fermentation (Schutz and Gafner 1993).

During wine preparation, fermentation can be done with endogenous 'wild yeast', but this gives an unpredictable product, which depends upon the exact types of yeast species present. So, a pure yeast culture is usually added to the must which ensures a reliable and predictable fermentation (González et al. 2001). Till date, more than 100 strains of wine yeasts are available in the market in the form of dry powder or tablet called 'dry wine yeast'. Based on the yeast usage of 0.1–0.2 g/l wine, approximately 5000 tonnes of dry wine yeast is required to produce the total estimated quantity (3.25×10^{10} L) of wine produced in different countries of world. But the actual market for the dry wine yeast does not exceed 600 tonnes per annum because many wineries either use the indigenous yeasts or propagate the wine yeast in-house. Most added wine yeasts are strains of *S. cerevisiae*, but not all strains of this yeast are suitable (González et al. 2001). Different *S. cerevisiae* yeast strains have different physiological and fermentative characteristics; therefore the actual strain of yeast selected for fermentation can have a direct impact on the finished wine (Dunn et al. 2005). Some of the desirable characteristics that wine yeast should possess are listed in Table 3.

In wine, brandy and other fruit based alcoholic beverages, yeast is responsible for the production of a variety of esters, *viz*., ethyl acetate, hexyl acetate, ethyl caproate, isoamyl acetate, ethyl caprylate and 2-phenylethyl acetate (Swiegers et al. 2005), which give the characteristic fruity odor to these beverages. Yeast cells synthesize these esters by alcohol acetyltransferases (AATases), using higher alcohols and acetyl-CoA as substrates. The *ATF1*- and *ATF2*-encoded alcohol acetyltransferases of *S. cerevisiae* are responsible for the synthesis of ethyl acetate and isoamyl acetate esters, while the *EHT1*-encoded ethanol hexanoyl transferase is responsible for synthesizing ethyl caproate (Lilly et al. 2006).

In addition, wine yeast strains have limited but varying capacities to produce aroma-enhancing thiols such as 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA), from their non-volatile counterparts in grape juice (Swiegers et al. 2007).

The non-*Saccharomyces* yeasts present in grape juice are *Hanseniaspora* (*Kloeckera*), *Candida*, *Pichia*, *Kluyveromyces* and *Metschnikowia,* which could proliferate to final populations of about 10^6–10^7 cfu/ml; and as the ethanol level exceeds

Table 3. Desirable characteristics of wine yeast (Bhalla et al. 2011).

A. Fermentation properties	**C. Technological properties**
• Rapid initiation of fermentation • Fermentation of variety of carbohydrates • High osmotolerance • High ethanol tolerance (up to 15% v/v or more) • Ability to ferment at low temperature and under pressure • Moderate biomass production	• High genetic stability • High sulfite tolerance • Low foam formation • Flocculation properties • Compact sediment • Resistance to desiccation • Resistance to copper • Zymocidal (killer) properties • Genetic marking • Low nitrogen demand • Secretion of pectinases, glucanases and proteases
B. Flavor characteristics	**D. Metabolic properties with health implications**
• Production of desirable aldehydes and ketones • Reduced sulphide/dimethyl sulphide/thiol formation • Low production of higher alcohol and volatile acidity • High glycerol production • Potential to produce b-glucosidase to enhance wine flavour • Enhanced production of desirable volatile esters	• Reduced ethyl carbamate (urea production) • Low biogenic amine formation • Least oxidation of polyphenols • Low sulfite formation

5–7%, their numbers start to decrease (Heard and Fleet 1988, Gao and Fleet 1988). Ethanol production by *S. cerevisiae* is the major factor affecting the growth of non-*Saccharomyces* yeasts. Significant research has been undertaken for the development of novel wine yeast strains that produce typical flavor profiles or increased complexity in wines (McBryde et al. 2006).

In addition, the growth of some yeasts such as *Zygosaccharomyces* and *Brettanomyces* in wine leads to its spoilage (Loureiro and Malfeito-Ferreira 2003).

5. Flavor Active Wine Yeasts

Besides the main production of ethanol and CO_2, yeast also produces a variety of flavor compounds which greatly influence the aroma and taste of wine. The aroma and flavor properties of wine can be enhanced by glycosidases, which catalyze the hydrolysis of non-volatile glycosidic precursors of the grapes. It must be underlined that yeasts involved in winemaking can be important producers of numerous enzymes. Thus, the various yeast species/strains that develop during the fermentative process metabolize grape juice components to a wide range of volatile and non-volatile end-products that contribute to the aroma and flavor characteristics of the wine. The flavor of fermented beverages such as beer, cider, sake and wine owe much to the primary fermentation yeast (*S. cerevisiae*) used in their production. The flavor compounds such as ethyl esters, acetate esters, fusel alcohols, carbonyls, and volatile fatty acids formed during yeast fermentation are also called ‘yeast bouquet’. Wine strains of *Saccharomyces bayanus* produce relatively high concentrations of 2-phenylethanol and 2-phenylethyl acetate compared to other higher alcohols and acetate esters, which may enhance 'rose' and 'floral' flavors (Masneuf-Pomarede et al. 2006). Flavor active wine yeast can also be arranged based on the production of sulfur containing compounds, which are associated with 'tropical' or 'sulfidic' flavors in wine.

These are the reasons why the alcoholic beverage industry is very much interested in isolation and identification of yeast strains that give a unique flavor to the fermented product, especially to the wine. So, intensive research is going on to understand the yeast flavor characteristics and on the selection of yeast strains yielding desired flavors (Fleet 2008).

6. Yeasts in Traditional Fermentations

The preparation of many indigenous/traditional fermented foods and beverages have remained a household art in homes, villages and small-scale industries. On the contrary, the preparations of others, such as soy sauce are now carried out on a large commercial scale (Bol and de Vos 1997). Most of the traditional fermented foods are prepared by the process of solid-substrate fermentation, in which the substrate is allowed to ferment either naturally or by adding starter culture (Tamang 1998). The association of yeasts and lactic acid bacteria is often encountered or used in the production of fermented foods and beverages (Gobbetti 1998). The most well-known example of stable coexistence of yeast and lactic acid bacteria is the presence of *C. milleri* and *Lactobacillus sanfransiscensis* in San Francisco sourdough (Kline and Sugihara 1971), in Dutch wheat sourdoughs (Nout and Creemers-Molenaar 1987) and in German commercial sourdoughs (Böcker et al. 1990). Table 4 shows various yeast species isolated from traditional fermented foods.

Demuyakor and Ohta (1991) reported *S. cerevisiae* as being the predominant species in Ghanaian *pito*, alongwith *Candida* sp. and *Kluyveromyces* sp. A similar prevalence of *S. cerevisiae* (38%) in Ghanaian *pito* was observed by Sefa-Deheh et al. (1999), who also isolated *Candida tropicalis* (19%), *Torulaspora delbrueckii* (14%) and members of four other species. Sanni and Lönner (1993) examined Nigerian sorghum beer and reported *Candida* spp., *Geotrichum candidum*, *S. cerevisiae*, *Kloeckera apiculata* and *T. delbrueckii* to be the predominant yeast involved in fermentation. From *dolo* originating from Togo, *S. cerevisiae* has been found to dominate with a prevalence of 55–90% of the yeast population, whereas *Candida krusei* (70%) was dominant in one sample (Konlani et al. 1996). Similarly, *S. cerevisiae*, *Cryptococcus laurentii* and *Torulospora delbrueckii* are the yeasts isolated from *seera*, a traditional fermented food of Himachal Pradesh in India (Savitri et al. 2012). Sake is a traditional alcoholic drink in Japan, and is made from steamed rice by fermentation with *Aspergillus oryzae*, which saccharifies rice starch, and sake yeast, which converts the resultant glucose to ethanol. Although, *S. cerevisiae* var. *sake* along with other yeast strains is used in sake fermentation (Jay 1991, Azumi and Goto-Yamamoto 2001), it differs from other yeast strains as it has many characteristics which makes it suitable for sake brewing. These characteristics include a good aroma and the production of a high concentration of ethanol at low temperature (Hosaka et al. 1998).

Table 4. Some important yeast species isolated from various fermented foods.

Yeast	Fermented food	Reference
Candida boidini	*Dosa*	Sandhu and Waraich (1984)
Candida cacaoi	*Idli*	Sandhu and Waraich (1984)
Candida curvata	*Warri, bhallae*	Sandhu and Waraich (1984)
Candida etchellsii	*Miso,* soy sauce	Sandhu and Waraich (1984)
Candida famata	*Bhallae, warri*	Sandhu and Waraich (1984)
Candida fragicola	*Idli*	Sandhu and Waraich (1984)
Candida glabrata	*Idli, dosa, kodo ko jaanr*	Venkatasubbaiah et al. (1985) Thapa and Tamang (2006)
Candida guillermondii	*Injera, torani*	Padmaja and George (1999)
Candida kefyr	*Idli*	Sandhu and Waraich (1984)
Candida krusei	Punjabi *warri*, cocoa beans	Sandhu and Soni (1989)
Candida lactose	*Tape ketan*	Cronk et al. (1977)
Candida melinii	*Tape ketan*	Cronk et al. (1977)
Candida menbranafaciens	*Bhallae*	Sandhu and Waraich (1984)
Candida milleri	Sourdough	Stolz (2003)
Candida parapsilosis	*Tape ketan*, Punjabi *warri*	Cronk et al. (1977) Sandhu and Waraich (1984)
Candida pseudotropicalis	*Idli, dosa*	Sandhu and Waraich (1984)
Candida sake	*Idli*	Venkatasubbaiah et al. (1985)
Candida tropicalis	*Torani, idli, pito*	Venkatasubbaiah et al. (1985), Sefa-Deheh et al. (1999)
Candida vartiovaarai	*Warri, bhallae*	Sandhu et al. (1986)
Candida vini	*Poi*	Padmaja and George (1999)
Cryptococcus sp.	*Seera*	Savitri et al. (2012)
Debaryomyces hansenii	*Bhallae, warri*	Sandhu et al. (1986)
Debaryomyces tamari	*Idli, warri*	Sandhu and Waraich (1984)
Endomycopsis fibuliges	*Thumba, tape*	Padmaja and George (1999)
Geotrichum candidum	Nigerian sorghum beer	Sanni and Lönner (1993)
Hansenula anomala	*Bhallae, idli*, Punjabi *warri, kanji, kecap, torani*	Soni and Sandhu (1999)
Hansenula malanga or H. subpelliculosa	*Tape ketan*	Cronk et al. (1977)
Hansenula polymorpha	*Bhallae, dosa*	Sandhu and Waraich (1984)
Issatchenkia torricola (*Pichia terricola, Saccharomyces terricolus*)	*Dosa, idli*	Sandhu and Waraich (1984)
Kluyveromyces marxianus	*Bhallae, warri*	Soni and Sandhu (1990)
Pichia anomala	*Kodo ko jaanr*	Thapa and Tamang (2006)

Table 4. contd....

Table 4. contd.

Yeast	Fermented food	Reference
Pichia membranefaciens	*Warri, bhallae*	Sandhu et al. (1986)
Pichia saitoi	*Sourdough*	Stolz (2003)
Rhodotorula flava	*Doenjang*	Chang et al. (1977)
Saccharomyces bayanus	*Jalebies*	Padmaja and George (1999)
Saccharomyces bisporus	Teekwass	Hesseltine (1983)
Saccharomyces carbajali	*Pulque*	Steinkraus (1998)
Saccharomyces carlbergensis	Beer and ale	Jay (1991)
Saccharomyces cerevisiae	*Bhallae*, beer, *burukutu*, cider, *fufu, ogi, puto, dosa, idli, papdam, lao chao*, scotch whiskey, *pito, bhatooru*	Padmaja and George (1999), Batra and Milner (1974), Soni and Sandhu (1990), Demuyakor and Ohta (1991), Savitri and Bhalla (2012)
Saccharomyces ellipsoideus	Wines	Jay (1991)
Saccharomyces exiguus	San Francisco sourdough	Jay (1991)
Saccharomyces intermedium	*Tibi*	Jay (1991)
Saccharomyces rouxii	*Miso*, Punjabi *warri*, Soy sauce	Batra (1981), Wang and Hesseltine (1982), Yokotsuka (1960)
Saccharomyces sake	*Sake* (rice beer)	Jay (1991)
Saccharomyces soyae	*Miso*	Winarno et al. (1977)
Saccharomycopsis fibuligera	*Tape ketan, lao chao, kodo ko jaanr*	Hesseltine (1983), Thapa and Tamang (2006)
Saccharomycopsis malanga	*Tape ketan, lao chao*	Hesseltine (1983)
Schizosaccharomyces pombe	*Teekwass, traditional African beverages*	Hesseltine (1983)
Torula sp.	*Kefir, kumiss*	Jay (1991)
Torulopsis candida	*Idli*	Batra and Milner (1974)
Torulopsis dattila	*Doenjang*	Chang et al. (1977)
Torulopsis etchellsii	*Miso*	Padmaja and George (1999)
Torulopsis holmii	*Idli*	Venkatasubbaiah et al. (1985)
Torulospora delbrueckii	*Seera*	Savitri et al. (2012)
Zygosaccharomyces soyae	*Tauco*	Winarno et al. (1977)

7. Yeasts as Source of Ingredients and Additives for Food Processing

With the increase in consumer demand for more natural foods, there is increasing interest in using microorganisms, including yeasts, as unique sources of food ingredients and additives, such as flavors, colors, antioxidants and vitamins. Since yeasts have a positive image with consumers, they are considered as a safe source of ingredients and additives for food processing (Demain et al. 1998). Preparations of baker's and brewer's yeasts have been available for many years as dietary and nutrient supplements because of their high contents of B vitamins, proteins, peptides, amino acids and trace minerals. Yeasts are also often considered as alternative source of protein for human consumption (Harrison 1993).

Yeasts serve as a source of antioxidants, aromas, flavors, colors, vitamins, glutamic acid, and nucleotides. Flavor ingredients based on yeast extracts, yeast autolysates and dried yeast preparations represent the most commercially significant products extracted from yeasts, and are used extensively in the food industry as a source of savory, roasted, nutty, cheesy, meaty and chicken flavors. In addition, some extracts are specifically enriched in their contents of glutamic acid and nucleotides that function as strong flavor enhancers (Stam et al. 1998). Although, baker's and brewer's yeasts have been the traditional sources of these products, some other yeasts such as *C. utilis* (*Pichia jadinii*) and *K. marxianus* are also used for this purpose (Lukondeh et al. 2003).

In addition to the above mentioned compounds, yeasts are frequently mentioned as potential sources of high value aroma and flavor substances (Vandamme and Soetaert 2002) such as vanillin (*S. cerevisiae*, *Rhodotorula glutinis*), citronellol, linalool and geraniol (*K. marxianus*), and γ- and δ-decalactones (*Sporidiobolus sulmonicolor*, *Y. lipolytica*). The yeast cell wall is composed principally of b-(1→3) and b-(1→6)-glucans that have gelling, thickening and fat-sparing functional properties and offer a range of applications in food processing (Seeley 1977).

Food colorants such as astaxanthin and other carotenoid pigments (Lyons et al. 1993, Johnson and Schroeder 1995) and several vitamins (Sauer et al. 2004) can also be derived from yeasts. Carotenoids belong to the most important components in foods. They are natural colorants and, as yellow to red colors, have great influence on the acceptability of many foods. Yeasts are reliable microbial source of carotenoids, e.g., species of the genus *Rhodotorula, viz.*, *R. glutinis*, *R. minuta, R. mucilaginosa, R. acheniorum* and *R. graminis* have been recognized as carotenoid producers. Other yeasts such as *Sporobolomyces roseus*, *Sporidiobolus salmonicolor* and *S. patagonicus* also produce carotenoids. Most of these yeasts are known to produce β-carotene but these also produce other carotenoids such as torulene, torularodine, and γ-carotene (Tinoi et al. 2005, Moliné et al. 2010). Moreover, some carotenoids are precursors of vitamin A; in terms of human health, they are among the bioactive compounds credited to reduce the risks of degenerative diseases such as cancer, cardiovascular diseases, macular degeneration and cataract. Yeast, *Xanthophyllomyces dendrorhous* has been reported to produce astaxanthin which is widely used a food colorant (Mata-Gómez et al. 2014).

8. Probiotic Yeasts

Probiotics are live microorganisms, mainly lactic acid bacteria, that are beneficial to the host when consumed in appropriate quantities. Although, lactic acid bacteria are widely accepted as the main probiotic species, interest in probiotic yeasts has increased (Klaenhammer 2001), especially in relation to animal feed applications. Preparations of live *S. cerevisiae* have been used as feed supplements to animal and poultry for many years, and have been reported to improve the growth and health of the animals (Lyons et al. 1993). Milk production increased by 7% in Tunisian Holstein Friesian cows whose feed was supplemented with probiotic yeast (Maamouri et al. 2014). There is increasing interest in the use of yeasts as probiotics in the aquaculture industry (Gatesoupe 1995). In humans, *S. cerevisiae* var. *boulardii*, has been successfully used over the last 20 years as an oral, biotherapeutic agent to treat patients with severe cases of diarrhea and other gastrointestinal disorders (Czerucka and Rampal 2002). Both baker's and brewer's yeasts (*S. cerevisiae*) are used as dietary supplements because of their high contents of vitamin B, proteins, peptides, amino-acids and trace minerals. Regardless of their non-human origin, such non-pathogenic yeasts fulfill the major criteria for probiotic definition (http://www.who.int/foodsafety/fs_management_probiotic_guidelines.Pdf).

The mechanisms behind their probiotic activities are based on the secretion of proteases and other inhibitory proteins, stimulation of immunoglobulins and the elimination of toxins secreted by other microorganisms (Fooks and Gibsen 2002). Recently, yeast strains obtained from local fermented foods of Ethiopia were reported to have antimicrobial activities against *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus* (Mariam et al. 2014).

Over the past few decades, *Saccharomyces boulardii* has been extensively studied for its potential probiotic use (Buts 2009). Most studied aspects of yeasts as probiotic organisms in clinical trials are in treatment of (1) antibiotic associated diarrhea, (2) infectious diarrhea including recurrent *Clostridium difficile* (*C. difficile*) related diseases, (3) irritable bowel syndrome, and (4) inflammatory bowel diseases (Rajkowska and Kunicka-Styczinska 2010). Saurabh et al. (2012) screened around 23 indigenous yeast isolates obtained from the traditional fermented foods of the Western Himalayas for various probiotic attributes.

However, there are serious concerns about its public health safety because of the reports on the association of yeasts with cases of fungaemia (Cassone et al. 2003). Apart from health benefit and safety issues, probiotic yeasts will also require certain technological properties for use in foods, e.g. (i) to remain viable in the food, (ii) not to grow in foods, (iii) not spoil the food and (iv) not adversely affect sensory acceptability of the food (Heenan et al. 2004).

9. Yeast Starters

In fermentation, the raw materials are converted by microorganisms or their enzymes to products that have acceptable food qualities. Fermentation initiated without the use of a starter inoculum, also called spontaneous fermentation, has been applied to food preservation for millennia. However, spontaneous fermentations are neither predictable nor controllable as the natural microflora of the raw material is inefficient, uncontrollable, and unpredictable, or is destroyed altogether by the heat treatments given to the food. Modern fermentations are initiated by starter cultures consisting of different microorganisms (bacteria, yeast and molds) that are inoculated into food materials to bring about desired changes such as novel functionality, enhanced preservation, reduced food safety risks, improved nutritional or health value, enhanced sensory qualities, and increased economic value of the finished product (Kolawole et al.

2007). Since the beginning of the 1980s, the use of *S. cerevisiae* yeast starters has been extensively applied in the industrial and homemade beverage production processes. Depending upon the adaptation of microorganisms to a substrate or raw material, starter cultures are selected either as single strain or multiple strains. Currently, most of the wine production processes depend on *S. cerevisiae* strains that allow rapid and reliable fermentations, reduce the risk of sluggish or stuck fermentations and prevent microbial contaminations (Romano et al. 2003). A wine starter culture is normally able to dominate intrinsic yeasts in the grape must during fermentation (Pretorius 2000). Yeast starter cultures that are specifically selected for the winemaking process on the basis of scientifically verified characteristics typically complement and optimize the raw material quality and individual characteristics of the wine, creating a more desirable and acceptable product (Swiegers et al. 2005). It has been reported that the wines produced with selected yeast starters have a higher quality than wines produced by spontaneous fermentation (Srivastava et al. 1997).

Numerous studies have proposed the use of mycocin producing yeasts as starter cultures to prevent the growth of spoilage yeast strains and secondary fermentation of wines (Boone et al. 1990, Comitini et al. 2004). Calmette (1892) was the first to report the presence of several wild yeast species accompanied by molds *Amylomyces, Mucor, Aspergillus,* and about 30 different bacteria in starters used in India and China to produce alcohol.

Traditional fermentation starters are referred to as *chu* in Chinese, *nuruk* in Korean, *koji* in Japanese, *ragi* in Southeast Asian countries, *bakhar ranu* or *marchaar/murcha* in India (Batra and Millner 1974) and *phab* (Fig. 4) in the North Western Himalayan region of India (Thakur et al. 2004). *Saccharomyces bayanus*, *Candida glabrata*,

Figure 4. '*Phab*' traditional yeast based fermentation starter of the North Western Himalayas.

Pichia anomala, *Saccharomycopsis fibuligera*, *Saccharomycopsis capsularis* and *Pichia burtonii,* have been isolated from the *marcha. Hamei,* is used in the Eastern Himalayas for the preparation of alcoholic beverages, e.g., *bhaati Jaanr*, *aitanga* and *kodo ko jaanr* (Tamang et al. 2007). *Phabs* is an indigenous inoculum of the Trans-Himalayan Ladakh region of India and used in fermentation of two traditional barley based alcoholic beverages, *chhang* and *arrak* (Angmo and Bhalla 2014).

In addition to their numerous roles in food and beverage production, some of the yeasts play a role in the spoilage of foods and beverages, mainly those with high acidity and reduced water activity (aw). In these foods, yeasts are able to change the sensory characteristics in undesirable ways and are thus regarded as ‘food spoilage yeasts’.

10. Food Spoilage Yeasts

Yeast spoilage is a constant threat in the food and beverage industries. Typically foods and beverages with high-acid, low-pH, high sugar, i.e., more than 10% w/v or high salt, i.e., more than 5% NaCl content, and products preserved with weak organic acids (e.g., sorbic, benzoic, acetic) are susceptible to spoilage by yeasts. Fruits, fruit juices, fruit drinks, fruit pulp, fruit juice concentrates, sugar and flavor syrups, confectionery products, alcoholic beverages, carbonated beverages, vegetable salads with acid dressings, salt based and acid based sauces, fermented dairy products and fermented or cured meat products are the prime candidates for yeast spoilage (Tudor and Board 1993). A list of the most common yeasts as contaminants in different food products and spoilage yeasts is given in Table 5.

The most noticeable indication of yeast spoilage is the production of excess gas, leading to the swelling of containers or cans and in extreme cases, explosions of the containers occur (Grinbaum et al. 1994). The second most apparent sign of yeast spoilage is the visible appearance of yeast colonies on the food surface, causing discoloration of the surface and formation of mucous slimes due to the production of extracellular polysaccharides. In beverages, the growth of spoilage yeast may cause the development of hazes, clouds, particulates, surface films or colonies and sediments. In some cases, spoilage by yeast is characterized by distinct off-flavors like the mouse flavor caused by *Brettanomyces intermedius* (Beech and Carr 1977), yeasty aldehyde flavor due to the formation of high levels of acetic acid, acetaldehyde (Lafon-Lafourcade 1986) and esters, including ethyl acetate (Lanciotti et al. 1998) by *Pichia* species. Off-flavor is also caused by high levels of acetoin and acetaldehyde (Romano et al. 1999) as reported in *Saccharomycodes ludwigii*, petroleum-like off-odor due to the degradation of sorbic acid to 1,3-pentadiene by *Zygosaccharomyces rouxii* and *Debaryomyces hansenii* (Casas et al. 1999), while *K. apiculata* causes off-flavors in cider comprised of high levels of esters and volatile acids (Beech and Carr 1977). In addition, the growth of lipolytic yeasts such as *Yarrowia lipolytica* on fat-rich substrates such as cheese or meat, may result in free fatty acid rancidity. Sometimes, the addition of preservatives to foods may make them more susceptible to spoilage by off-flavors.

Table 5. Different yeast species involved in food contamination and spoilage.

Most frequent contaminants	**Foods**	**Spoilage species**	**Foods**
Candida albidus	Fruit, vegetables and cereal grains	*Brettanomyces intermedius*	Soft drinks and fruit juices
Candida guilliermondii	Fish	*Candida dattila*	Canned fruit products
Candida parapsilosis	Fruit juices	*Candida globosa*	Fruits in sugar syrup and condensed milk
Candida tropicalis	Wine	*Candida holmii*	Soft drinks and fruit juices
Candida zeylanoides	Raw and processed poultry meat	*Candida humicola*	Refrigerated fishery products
Debaryomyces hansenii	Gherkins, fruit juices, marzipan and canned figs	*Candida krusei*	Citrus fruits, soft drinks and wine
Hanseniaspora uvarum	Wine	*Candida lactis-condensi*	Soft drinks
Issatchenkia orientalis	Meat products and processed poultry products	*Candida lipolytica*	Ready-to-drink beverages
Kluyveromyces marxianus	Fruit juices and concentrates, marzipan, salted and dry-cured meats, olives and cheeses	*Candida sake*	Unpasteurized orange juice
Pichia anomala	Grape juice concentrates	*Candida versatilis*	Wine
Pichia fermentans	Orange juice	*Cryptococcus* sp.	Cheese and frozen food products
Pichia membranifaciens	Wines and tomato sauce	*Hansenula anomala*	Italian cream-filled cakes
Rhodotorula glutinis	Strawberries	*Hansenula subpelliculosa*	Mango pickle
Rhodotorula mucilaginosa	Wines	*Kloeckera apiculata*	Figs, tomatoes, canned black cherries, strawberry toppings and yogurt
Saccharomyces cerevisiae	Grape juice concentrates, olive oil	*Pichia burtonii*	Bread and bakery products
Saccharomyces exiguus	Sugar-rich foods, maple sap, syrup, concentrated juices and condiments	*Schizosaccharomyces pombe*	Wines and high sugar syrups
Torulaspora delbrueckii	Dairy products, juices, wines and ready-to-eat (RTE) salad products	*Sporobolomyces roseus*	Proteinaceous foods and fruit products
Trichosporon pullulans	Idli, meat and different meat products	*Trichosporon cutaneum*	Fruits and vegetables, cereal grains, etc.
Zygosaccharomyces bailii	Fruit juices and concentrates, marzipan, salted, dry-cured meats, olives, cheeses, mayonnaise and salad dressings	*Zygosaccharomyces bisporus*	Mayonnaise based salad dressings
Zygosaccharomyces rouxii	Concentrated grape juice		

(Compiled from: Battey et al. 2002, Comitini et al. 2004, Fleet 2006, Lanciotti et al. 1998, Loureiro 2000, Loureiro and Malfeito-Ferreira 2003, McNamee et al. 2010, Saez et al. 2011, Saranraj and Geetha 2012, Schuller et al. 2000).

11. Health Significance of Yeasts in Food and Beverages

The presence of yeasts in foods and beverages is a subject of emerging interest in public health where in one context, the yeasts could serve as probiotic microorganisms, and in other circumstances, could lead to infections and other adverse effects to the consumer. Unlike many bacteria and viruses, yeasts are not known as aggressive infectious agents. However, some species of yeast, especially *C. albicans* and *Cryptococcus neoformans*, fall into the category of opportunistic pathogens. These cause a range of mucocutaneous, cutaneous, respiratory, central nervous, systemic and organ infections in humans (Hazen and Howell 2003). Generally, individuals with weakened health and immune function are at greater risk, including persons with AIDS and cancer, hospitalized patients and those undergoing treatments with immunosuppressive drugs, broad-spectrum bacterial antibiotics and radio chemotherapies.

12. Genetically Modified Yeasts for Food and Beverages

Initially, yeast for various purposes was obtained from breweries and distilleries, but with the growth of industrial population, there was an increase in the demand of yeast with higher yield of product and better technical performance. Considerable progress has been made in breeding new strains of yeast, and recombinant DNA techniques have been extensively used for the construction of new recombinant yeast strains, having the following desirable characteristics (Verstrepen et al. 2006):

- Improved performance and product quality
- Ability to ferment a wider range of carbohydrates
- Altered flocculation properties
- Produce products with modified flavors
- Better oligosaccharide (dextrin) utilization
- Fermentation of branched oligosaccharides and polysaccharides
- Antimicrobial properties
- Improved stress tolerance
- Improved sensory qualities of fermentation products
- Reduction in diacetyl levels in alcoholic beverages
- Improved flavor profiles of alcoholic beverages

In addition to the above properties, yeast strains are engineered with altered levels of ethanol and glycerol production, control acid levels in wine, produce decreased levels of hydrogen sulfide and ethyl carbamate and to make alcoholic beverages with increased storage/antioxidative potential. Attempts have also been made to change fermentation rates using genetically modified yeast strains engineered to produce higher levels of glycolytic enzymes (Verstrepen et al. 2006).

13. Food Yeast Collections

Research in the field of fermentation and fermented foods has led to isolation, identification and characterization of large numbers of yeast strains around the world. These yeast strains are deposited and preserved in different culture collections (Table 6). The primary aim of these culture collections is to preserve the microbial diversity, as well as to provide pure cultures for scientific research and academic purposes.

Table 6. List of some important yeast depositories and culture collections of the world.

Name of culture collection	**Address and website address**
American Type Culture Collection (ATCC)	12301 Parklawn Drive, Rockville, MD 20852, USA www.atcc.org.general/html
Bioresource Collection and Research Center (BCRC)	Academia Sinica, Biodiversity Research Center, 128Sec. 2, Academic Road, Nangang District Taipei, Chinese Taipei China http://www.gbif.org
Central Bureau Schimmelculture (CBS)	P.O. Box 85167, 3508 AD Utrecht, The Netherlands http://www.cbs.knaw.nl
China General Microbiological Culture Collection (CGMCC)	China General Microbiological Culture Collection, Institute of Microbiology, Chinese Academy of Sciences, No. 1, West Beichen Road, Chaoyang District Beijing, 100101, China http://www.cgmcc.net
Colecao de Culturas Tropical (CCT)	Colecao de Culturas Tropical, Fundacao Tropical de Pesquisas e Tecnologia 'André Tosello', Campinas - SP, Brazil
Spanish Type Culture Collection (CECT)	Spanish Type Culture Collection (CECT) University of Valencia, Parc Científic Universitat de València Catedrático Agustín Escardino, 9, 46980 Paterna (Valencia) Spain http://www.uv.es/uvweb/spanish-type-culture-collection/en/location-contact/contact/contact-details-1285872233467.html
Culture Collection of Yeast (CCY)	Institute of Chemistry Slovak Academy of Sciences Dúbravská cesta 9 Bratislava 845 38 Slovakia http://www.ccy.sk/
Industrial Yeast Collection (DBVPG)	Industrial Yeasts Collection DBVPG Department of Agricultural, Food and Environmental Science, University of Perugia, Borgo XX Giugno, 74 I-06121 Perugia, Italy dbvpg@unipg.it
Institute for Fermentation Osaka (IFO)	Institute for Fermentation, Osaka, Yodogawa-ku, Osaka, Japan http://trove.nla.gov.au/version/9088375

Table 6. contd....

Table 6. contd.

Name of culture collection	**Address and website address**
Japan Collection of Microorganisms (JCM)	Microbe Division/Japan Collection of Microorganisms RIKEN Bioresource Center, 3-1-1 Koyadai, Tsukuba, Ibaraki, 305-0074, Japan http://jcm.brc.riken.jp
Microbial Type Culture Collection (MTCC)	Institute of Microbial Technology, Sector-39 A, Chandigarh, India http://mtcc.imtech.res.in
Belgiun Coordinated Collection of Microorganisms (MUCL /BCCM)	croixduSud 2, box L7.05.06, B-1348 Louvain-La-Neuve http://bccm.belspo.be
National Bureau of Agriculturally Important Microorganisms (NBAIM)	National Bureau of Agriculturally Important Microorganisms, Kushmaur, Post Box No. 6, Mau Nath Bhanjan, Pin: 275103, Uttar Pradesh India www.nbaim.org.in
National Collection of Agricultural and Industrial Microorganisms (NCAIM)	National Collection of Agricultural and Industrial Microorganisms, Department of Microbiology and Biotechnology, University of Horticulture and Food Industry, Budapest, Hungary
National Collection of Industrial Microorganisms (NCIM)	Council of Industrial Research, National Chemical Laboratory (NCL), Dr. Homi Bhabha Road, Pune, India www.ncl-india.org/files/NCIM/
National Collection of Yeast Culture (NCYC)	National Collection of Yeast Cultures, Institute of Food Research, Norwich Research Park, Norwich, NR4 7UA, UK http://www.ncyc.co.uk
Northern Regional Research Center (NRRL)	Northern Regional Research Center, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, US Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604, USA
Portuguese Yeast Culture Collection (PYCC)	Departamento de Ciências da Vida (DCV), Faculdade de Ciências e Tecnologia Universidade Nova de Lisboa Campus da Caparica 2829-516 Caparica Portugal http://pycc.bio-aware.com/
RCDM (Catch Rotary Culture Rev1)	MDC: Molecular Diagnostics Center, Biomolecular Technologies S.L. and Universidad Miguel Hernández, Orihuela E-03300, Alicante, Spain
All Russian Collection of Microorganisms (VKM)	Russia, 142290, Moscow Region, Pushchino, pr. Nauki, 5, IBPM Russia http://www.vkm.ru
Culture Collection of Industrial Organisms (CCIM)	Culture Collection of Industrial Microorganisms, Institute of Microbiology, 7b, A. Kadiry street, Tashkent 700128, Uzbekistan

14. Safety of Yeasts

The impact of yeasts on the quality and safety of foods and beverages is closely related to their biological activities. These activities are determined by the physical and chemical properties of the ecosystem, and by how yeasts respond according to their physiology, biochemistry and genetics. The close relationship with *S. cerevisiae* in food and beverage production over millennia supports the fact that it is safe to work with, and thus designated 'Generally Recognized as Safe' (GRAS) by the United States Food and Drug Administration (Verstrepen et al. 2006).

15. Future Prospects

A wide variety of yeasts are involved in the production of different fermented foods and beverages all over the world. The diversity of products prepared by using yeasts ranges from bread and bread like products to alcoholic beverages such as beer and wines and traditional fermented foods, flavor compounds, food ingredients, etc. Additionally, yeasts are also involved in spoilage of a variety of food products. Recently, interest in probiotic yeast for application in animal feed has also increased. Although considerable progress has been made in the isolation, identification, characterization and breeding of new strains of yeast, there is a need for further research in the areas of flavor formation in yeast, the factors affecting yeast growth, yeast flocculation and probiotic aspects. These require more precise characterization by using recent techniques of genomics, proteomics and metabolomics. Continued research in these fields will further our understanding and eventually lead to the development of yeast strains that can produce predictable products with specific metabolic profiles, thus allowing the producers to 'shape' their fermented products to suit certain consumer preferences and add value to existing fermentation. This will further enable the development of new products based on the exploitation of new and improved strains of yeasts.

Keywords: Yeast, fermentation, fermented foods, beverages, *Saccharomyces cerevisiae,* wine

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