Chapter 9
Yeasts Diversity in Fermented Foods and Beverages

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Contents

Abstract People across the world have learnt to culture and use the essential microorganisms for production of fermented foods and alcoholic beverages. A fermented food is produced either spontaneously or by adding mixed/pure starter culture(s). Yeasts are among the essential functional microorganisms encountered in many fermented foods, and are commercially used in production of baker's yeast, breads, wine, beer, cheese, etc. In Asia, moulds are predominant followed by amylolytic and alcohol-producing yeasts in the fermentation processes, whereas in Africa, Europe, Australia and America, fermented products are prepared exclusively using bacteria or bacteria-yeasts mixed cultures. This chapter would focus on the varieties of fermented foods and alcoholic beverages produced by yeasts, their microbiology and role in food fermentation, widely used commercial starters (pilot production, molecular aspects), production technology of some common commercial fermented foods and alcoholic beverages, toxicity and food safety using yeasts cultures and socio-economy.

Keywords Amylolytic yeasts, fermented foods, alcoholic beverages, food safety, yeasts, food fermentation

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9.1 Introduction

A fermented food or beverage is defined as an edible product prepared from the raw or cooked materials of plant or animal origins by microorganisms either naturally or by adding mixed or pure culture(s) (Campbell-Platt, 1994; Holzapfel, 2002). The essential objective of food fermentation is to carry over supplies from the time of plenty to those of deficit (Tamang, 2000). Traditionally people knew how to culture the beneficial microorganisms, mostly lactic acid bacteria, yeasts and filamentous moulds, for production of foods for consumption. What was the scientific explanation and identity of these microorganisms were unknown to them.

Microorganisms are present in or on the ingredients, utensils, environment, and are selected through adaptation to the substrate for fermentation (Hesseltine, 1983; Tamang, 1998). 'Rice-soybean-fish-alcoholic beverage' diet is the characteristic food culture of the East and South East Asia, whereas 'wheat-milk and milk products-meat-wine' is the basic diet in the Western part of the world. In East Asia rice is a staple food whereas in West Asia wheat or barley is a staple food. Milk products similar to West and alcoholic beverages similar to East are encountered in food habits of the people of the Himalayas (Tamang, 2005). In Asia, moulds are predominant microorganisms in the fermentation processes, whereas in Africa, Europe and America, fermented foods are prepared exclusively using bacteria or bacteria-yeasts mixed cultures; moulds seem to be little or never used (Tamang, 1998). However, in the Indian sub-continent, mostly due to wide variation in agro-climatic conditions and diverse form of dietary culture, all major groups of micro-organism (bacteria-yeasts-moulds) are associated with fermented foods showing the transition of food culture (Tamang and Holzapfel, 1999).

Yeasts play vital roles in production of many traditional fermented foods and beverages across the world (Aidoo et al., 2006) signifying the food culture of the regions and the community. Functional yeasts genera associated with fermented foods and beverages are mostly *Brettanomyces* (its perfect stage, *Dekkera*)*, Candida, Cryptococcus, Debaryomyces, Galactomyces, Geotrichum, Hansenula, Hanseniaspora* (its asexual counterpart *Kloeckera*), *Hyphopichia, Kluyveromyces, Metschnikowia, Pichia, Rhodotorula, Saccharomyces, Saccharomycodes, Saccharomycopsis, Schizosaccharomyces, Torulopsis, Trichosporon, Yarrowia* and *Zygosaccharomyces* (Kurtzman and Fell, 1998; Pretorius, 2000; Nout and Aidoo, 2002; Tsuyoshi et al., 2005). Table 9.1 shows the common fermented foods and beverages, mostly prepared by yeasts, or in combination with bacteria and moulds. This chapter deals with the role of yeasts in production of traditional fermented foods and beverages mostly concerning their diversity and identity.

9.2 Fermented Meat Products

Fermented meat products are divided into two broad groups: those made from whole meat pieces or slices, such as dried meat, cured hams and jerky, and those made by chopping or comminuting the meat, usually called sausages and salami-type

Table 9.1 Foods and beverages fermented by yeasts

Food	Substrate	Nature and use	Microorganisms	Country
Fermented legume products				
Bhallae	Black gram	Deep-fried patties; snack	LAB, yeasts	India
Dhokla	Bengal gram	Spongy cake; snack	LAB, yeasts	India
Maseura	Black lentil	Dry, ball-like, brittle; condiment	LAB, Yeasts	India, Nepal
Papad	Black gram	Circular wafers; spicy condiment, snack	LAB, yeasts	India
Vadai	Black gram	Deep fried patties; snack	LAB, yeasts	India
Wari	Black gram	Dry, ball-like hollow, brittle; condiment	LAB, yeasts	India
Fermented cereal products				
Dosa	Rice (finely grounded), black gram	Spongy, pan cake; staple food	LAB, yeasts	India
Enjera	Tef flour, wheat	Sour, leavened, pancake-like bread; staple food	LAB, yeasts	Ethiopia
Idli	Rice (coarsely grounded), black gram	Steamed, spongy pancake; breakfast	LAB, yeasts	India
Jalebi	Wheat flour	Crispy, deep fried pretzels; confection snack	Yeasts, LAB	India, Nepal, Bangladesh
Kenkey	Maize	Acidic, maize dumpling, steamed; staple food	LAB, yeasts	Ghana
Kisra	Sorghum	Thin pancake bread; staple food	LAB, yeasts	Sudan
Masa	Rice/millet or sorghum	Shallow fried millet or sorghum flour cake; snack food	LAB, yeasts	Nigeria
Nan	Wheat flour	Leavened flat baked bread; staple food	Yeasts, LAB	India, Pakistan, Afghanistan
Puto	Rice	Rice cake; breakfast and snack food	LAB, yeasts	Philippine, China
Rabadi	Barley, maize, wheat, pearl millet	Cereal flour with buttermilk; staple food	LAB, yeasts	India
Selroti	Rice-wheat flour-milk	Pretzel-like, deep fried; bread	Yeasts, LAB	India, Nepal, Bhutan
Sourdough	Wheat flour	Leavened baked bread; staple food	Yeasts, LAB	America

(continued)

Table 9.1 (continued)

Food	Substrate	Nature and use	Microorganisms	Country
Fermented milk products				
Cheese	Animal milk	Fermented dairy product; side-dish	LAB, yeasts	World-wide
Chhu or *sheden*	Cow/Yak milk	Soft, strong flavoured; curry	LAB, yeasts	India, Nepal, Bhutan, China (Tibet)
Chhurpi	Cow/Yak milk	Soft, cheese-like; curry, pickle	LAB, yeasts	Nepal, India, Bhutan
Dahi	Cow milk	Curd; savory	LAB, yeasts	India
Mohi	Cow milk	Butter-milk	LAB, yeasts	India
Kefir	Goat, sheep, or cow milk	Acidic, mildly alcoholic, effervescent milk	LAB, yeasts	East Europe, middle east countries, north America
Kishk	Sheep milk, wheat	Milk-wheat mixture; dried balls	LAB, yeasts	Greece, Turkey, Egypt, Libya, Iran
Koumiss	Horse, donkey or camel milk	Acid/alcoholic milk	LAB, yeasts	North European conutries, Russia, Mongolia, China
Fermented meat products				
Cured hams	Beef/pork	Whole meat product, side-dish	LAB, micrococci, Yeasts	World-wide
Kargyong	Beef/yak/pork	Sausages; curry	LAB, micrococci, Yeasts	India, Bhutan, China (Tibet)
Kheuri	Beef/yak/pork	Sausages; curry	LAB, micrococci, Yeasts	India, Bhutan, China (Tibet)
Salami	Beef/pork	Meat product, side-dish	LAB, micrococci, Yeasts	World-wide
Sausages	Beef/pork	Sausages, side-dish	LAB, micrococci, Yeasts	World-wide
Fermented/dry fish products				
Gnuchi	River fish	Smoked, sun-dried; curry	LAB, micrococci, *Bacillus*, yeasts	India
Hentak	Fish	Dried fish mixed aroid plants, fermented; curry	LAB, yeasts	India
Ngari	Fish	Fermented fish product; curry	LAB, yeasts	India
Sidra	Fish	Dried fish; curry	LAB, micrococci, yeasts	India, Nepal
Sukako maacha	River fish	Smoked, sun-dried; curry	LAB, micrococci, *Bacillus*, yeasts	India, Nepal

Sukuti	Fish	Dried fish; curry	LAB, micrococci, yeasts	India, Nepal
Tungtap	Fish	Fermented; pickle	LAB, yeasts	India
Mixed-amylolytic starter cultures				
Appon dobai	Rice, wild herbs	Starter culture	Filamentous moulds, Yeasts, LAB	India
Bubod	Rice, wild herbs	Dry, ball-like starter culture	Filamentous moulds, Yeasts, LAB	The Philippines
Chiu-yueh	Rice, wild herbs	Starter culture to ferment alcoholic beverages	Filamentous moulds, Yeasts, LAB	China, Singapore
Hamei	Rice, wild herbs	Starter culture to ferment alcoholic beverages	Filamentous moulds, Yeasts, LAB	India
Longpang	Rice, wild herbs	Starter culture to ferment alcoholic beverages	Filamentous moulds, Yeasts, LAB	Thailand
Marcha	Rice, wild herbs, spices	Dry, ball-like starter culture	Filamentous moulds, Yeasts, LAB	Nepal, India
Men	Rice, wild herbs, spices	Dry, ball-like starter culture	Filamentous moulds, Yeasts	Vietnam
Nuruk	Rice, wild herbs	Starter culture to ferment alcoholic beverages	Filamentous moulds, Yeasts, LAB	Korea
Phab	Wheat, wild herbs	Starter culture to ferment alcoholic beverages	Filamentous moulds, Yeasts, LAB	China (Tibet), Bhutan
Ragi	Rice, wild herbs	Dry, ball-like starter culture	Filamentous moulds, Yeasts, LAB	Indonesia
Alcoholic beverages				
Aara	Cereals	Clear distilled liquor; alcoholic drink	Yeasts, LAB	India
Appo	Finger millet	Mild-alcoholic, beverage	Filamentous moulds, Yeasts, LAB	India
Atingba	Rice	Alcoholic, sweet-sour paste beverage	Yeasts, LAB	India
Bhaati jaanr	Rice	Mild-alcoholic, sweet-sour, food beverage; paste	Filamentous moulds, Yeasts, LAB	India, Nepal, Bhutan

(continued)

Table 9.1 (continued)

Food	Substrate	Nature and use	Microorganisms	Country
Beer	Barley, hubs	Mild alcoholic beverage	Yeasts	World-wide
Chyang/ Chee	Finger millet	Mild-alcoholic, slightly sweet-acidic; beverage	Filamentous moulds, Yeasts, LAB	Bhutan, China (Tibet)
Cider	Apple	Clear alcoholic drink	Yeasts	France, Spain, Ireland, Slovenia
Gahoon ko jaanr	Wheat	Mild-alcoholic, slightly acidic, beverage	Filamentous moulds, Yeasts, LAB	India, Nepal, Bhutan
Jou	Rice	Alcoholic beverage	Yeasts, LAB	India
Kodo ko jaanr	Finger millet	Mild-alcoholic, slightly sweet-acidic; beverage	Filamentous moulds, Yeasts, LAB	India, Nepal
Makai ko jaanr	Maize	Alcoholic, sweet-sour, food beverage; paste	Filamentous moulds, Yeasts, LAB	India, Nepal, Bhutan
Ruou nep	Rice	Alcoholic drink	Moulds, Yeasts	Vietnam
Oh	Rice-millet	Soft, beverage	Yeasts, LAB	India
Pona	Rice	Alcoholic, sweet-sour, beverage	Yeasts, LAB	India
Raksi/Arak	Cereals	Clear distilled liquor; alcoholic drink	Filamentous moulds, Yeasts, LAB	India, Nepal, Bhutan
Simal tarul ko jaanr	Cassava tuber	Mild-alcoholic, sweet-sour, food beverage; paste	Filamentous moulds, Yeasts, LAB	India, Nepal
Wine	Grapes	Clear alcoholic drink	Yeasts	World-wide
Zutho	Rice	Alcoholic beverage	Yeasts, LAB	India
Non-alcoholic beverages				
Kombucha or tea fungus	Tea liquor	Non-alcoholic drink	LAB, Yeasts	China, Japan, Indonesia, Russia

Note: LAB, lactic acid bacteria

products (Campbell-Platt and Cook, 1995). Meat processing is the combination of chemical curing, microbial fermentation and drying which together give stable, safe, ready-to-eat products (Bacus, 1984). Microorganisms involved in the production of fermented meats are lactic acid bacteria, staphylococci, micrococci, moulds and yeasts (Cook, 1995).

Although bacteria are considered to have the dominant role in meat fermentation, the contribution of yeasts nevertheless is significant (Romano et al., 2006). There are two stages during processing where yeasts growth and activity are considered relevant. They exhibit limited growth along with bacterium during the initial stage of fermentation (e.g. as in the production of salami-type sausages), and their populations remain high and active during subsequent storage and maturation of the product (e.g. as for salami and dry-cured hams).

The ingredients for sausage production include chopped or dried meat (beef, pork or sheep), sodium chloride, sodium nitrite/nitrate, sucrose (obtained) and a range of species according to the product (Bacus, 1986). Initial populations of yeasts are generally low (less than 10^3–10^4 cfu/g), and originate from the ingredients and processing equipment. Yeasts are naturally present on the hides of animals and readily contaminate fresh meat during slaughtering (Dillon and Board, 1991). Studies in several countries have now shown that yeast grow during the initial stages of fermentation, along with bacteria, growing final populations of 10^6–10^7 cfu/g. The total populations usually remain at these levels or decrease slightly during the subsequent stages of maturation and storage, which may last for several months (Samelis et al., 1993; Abunyewa et al., 2000; Coppola et al., 2000; Encinas et al., 2000; Gardini et al., 2001; Ferreira et al., 2006). Similarly total yeast populations of 10^6–10^8 cfu/g are frequently found during the storage or ripening of dry-cured hams (Nú ez et al., 1996; Saldanha-da Gama et al., 1997). Numerous factors can alter the growth of yeast during the production of fermented sausages and cured hams and include smoking, addition of spices, salt concentration, relative humidity and temperature. Encinas et al. (2000) found difference in yeast counts between smoked and non-smoked sausages. Inhibitory effects of garlic powder on yeast growth during sausage production have been reported (Ghamnnoum, 1990; Olesen and Stahnke, 2000).

A diversity of yeast species has been isolated from fermented sausages and cured hams produced in different countries with little exception (Metaxopoulos et al., 1996; Martin et al., 2006). *Debaryomyces hansenii* and its anomorph *Candida famata* are the most frequently isolated and quantitatively the most significant yeasts in the production of fermented sausages and cured hams. Various other species in the genera *Candida, Cryptococcus, Pichia, Rhodotorula, Trichosporon, Metschnikowia* and *Yarrowia* have been associated with these products, with *Y. lipolytica, R. mucilaginosa, C. zeylanoides* and *M. pulcherrima* deserving special mention. Recently, both culture and culture-independent-DNA analyses have confirmed the predominance of *D. hansenii* in sausage fermentations (Rantsiou et al., 2005). *D. hansenii* is reported from a traditional South Italian processed sausages along with bacteria (Baruzzi et al., 2006). Moreover, molecular analyses have demonstrated substantive strain heterogenecity in the strains of *D. hansenii* and *Y. lipoltica* isolated from these products (Gardini et al., 2001).

It is generally considered that yeasts make a positive contribution to colour and flavour developments in fermented sausages and cured-hams (Mauriello et al., 2004), but the precise mechanisms are not understood. Significant metabolic properties in this context include their oxygen scavenging ability for production of extracellular proteases and lipases, utilization of organic acids such as lactic acid, and production of flavour volatiles such as alcohols, acids, esters and various carboxyls (Romano et al., 2006). *Debaryomyces hansenii* is known for its ability to hydrolyze meat proteins (Martin et al., 2003; Flores et al., 2004), while *Y. lipolytica* has strong lipase and proteolytic activities (Gardini et al., 2001). However, there is significant strain variation among these properties. Both *D. hansenii* and *Y. lipolytica* have been proposed as candidates for development of novel starter cultures for production of sausage and ham (Gardini et al., 2001; Martin et al., 2006) where they could enhance, flavour, texture and colour applied and continue to decrease processing times.

There are few reports regarding the contribution of yeasts to biogenic amines production in fermented foods. In *Debaryomyces* and *Candida* isolated from fermented meats, a histidine decarboxylase activity was found, which was higher than that observed for lactic acid bacteria and staphylococci (Suzzi and Gardini, 2003).

Tamang and Rai (unpublished data) found the populations of yeasts in kargyong and kheuri, ethnic fermented sausages of Sikkim, Bhutan and Tibet at the level of 10^5 cfu/g along with predominant lactic acid bacteria.

9.3 Fermented Milk Products

Fermented milk products are generally classified as (i) low acid products (e.g. cultured buttermilk and cultured cream); (ii) medium acid products (e.g. yogurt and cheese); (iii) high acid products (e.g. Bulgarian sour milk); and (iv) acid alcohol products (e.g. kefir, koumiss) (Kosikowski, 1977; Oberman and Libudzisz, 1998). In terms of microbiology, the production of these commodities is usually associated with the growth and metabolic activities of lactic acid bacteria during the initial fermentation of milk. The contribution of yeasts to the manufacture of these products is a relatively recent observation that is reviewed by Fleet (1990), Jakobsen and Narvhus (1996) and Frohlich-Wyder (2003). It is now widely demonstrated that yeasts make a significant and positive contribution to the maturation process of many types of cheese and may grow in association with lactic acid bacteria in the fermentation of products such as kefir and koumis (Stanley, 1998).

Low populations of yeasts (less than 10^3 cfu/ml) occur in raw or pasteurized milks (Fleet and Mian, 1987; Deak and Beuchat, 1995). Milk is an excellent substrate for their growth and in the absence of competing bacteria; they quickly grow to populations as high as 10^7–10^8 cfu/ml (Roostita and Fleet, 1996a). However, they exhibit little growth in milk inoculated with high levels of lactic acid bacteria. Consequently, they are not significant in the milk fermentation stages of cheese or

yogurt production (Viljoen, 2006). Surveys of cheeses in the retail markets, globally, consistently show the presence of high populations of yeasts (10^6–10^9 cfu/g), especially in soft-brined cheese and mould ripened soft to semi-soft varieties such as Brie, Camembert and blue veined cheeses (Roostita and Fleet, 1996b). Detailed investigations of the microbial ecology of cheese production have revealed that yeasts are major component of the microflora of cheese maturation. The coagulated curd from milk fermentation contains little yeast. However, soon after the curd is brined, yeasts quickly grow to 10^6–10^9 cfu/g, and generally remain at these levels throughout subsequent maturation. They are distributed throughout the entire curd, but populations on the outer curd are usually 10–100-fold higher than those of the inner curd (Devoyod, 1990; Addis et al., 2001). These yeasts originate as natural contaminants of the processing environment, principally coming from the brine (salt), surface of equipment, the air and workers.

A significant diversity of yeast species has been isolated from cheeses and includes species of *Candida, Debaryomyces*, *Kluyveromyces*, *Saccharomyces*, *Pichia*, *Torulospora*, *Yarrowia*, *Rhodotorula*, *Cryptococcus, Galactomyces* and *Trichosporon* (Frohlich-Wyder, 2003; Vasdinyei and Deak, 2003; Romano et al., 2006). However, the most prevalent species are *D. hansenii*, *Y. lipolitica, S. cerevisiae, K. marxianus* and *G. geotrichum* (Addis et al., 2001; Romano et al., 2006). *D. hansenii* is notable for its tolerance to salt and occurrence in cheese brines, and exhibits dominant growth in cheese throughout the maturation process. DNA-based molecular typing and phenotypic analyses have demonstrated significant strain heterogenecity within cheese isolates of the species (Suzzi et al., 2000; Hansen and Jakobsen, 2001; Petersen et al., 2002).

The function of yeasts during cheese maturation and their contribution to cheese quality requires further investigation. However, they are considered to impart on cheese flavour and texture through proteolytic and lipolytic activities, (as *D. hansenii* and *Y. lipolytica*), fermentation of residual lactose (e.g. *K. marxianus*), and utilization of organic acids such as lactic and citric acids (Fleet, 1990). Moreover, they may stimulate or inhibit the growth of bacteria and filamentous fungi that contribute to maturation of some cheeses (Viljoen, 2006). Generally, their activities during maturation are considered positive, creating commercial interest in using selected strains of *D. hansenii*, *S. cerevisiae* and *Y. lipolytica* as novel starter cultures in cheese manufacture (Frohlich-Wyder, 2003; Romano et al., 2006).

Kefir is an acidic mildly alcoholic beverage prepared by fermenting the milk of goats, sheep or cows with kefir grains obtained from the previous batch of kefir. It is popular in Eastern and Coastal European as well as in Scandinavia and is considered as a health promoting, probiotic beverage (Oberman and Libudzisz, 1998; Frohlich-Wyder, 2003). Milk fermentation is conducted at 18–22°C for about 20–24 h, after which newly formed kefir grains are removed and the liquid cooked for consumption. Microbiologically, the fermentation is a symbiotic interaction between lactic acid bacteria, yeasts and acetic acid bacteria. During fermentation and subsequent refrigeration of the product, the yeasts grows to about 10^5–10^8 cfu/ml. Species of *K. marxianus* and *S. cerevisiae* generally dominate but less frequently isolated species include *Torulaspora delbrueckii*, *S. unisporus* and *S. exiguus* (Beshkova

et al., 2002; Frohlich-Wyder, 2003). The yeasts contribute characteristic gassiness (CO_2), ethanol and other flavours to the product (García Fontán et al., 2006).

Koumiss is an effervescent acid or alcoholic fermented milky white-greyish liquid made primarily from mare milk (Kosikowski, 1977). The primary fermenting microorganisms in koumiss are *Lb. bulgaricus*, yeasts *Candida kefyr,* and *Torulopsis* spp. (Kosikowski, 1977; Tamime, 1981).

Laban rayeb (laban) is a traditional Lebanese fermented milk product, which has slightly acid taste with aroma resembling that of buttermilk (Morcos et al., 1973; Oberman, 1985). The predominating organisms are *Lactococcus lactis* subsp. *lactis, Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* (Chammas et al., 2006) and lactose fermenting yeasts (Vedamuthu, 1982).

Misti dahi (sweetened dahi, mishti doi, lal dahi or payodhi) is a sweetened fermented milk product from the eastern part of India (Ray and Srinivasan, 1972). Gupta et al. (2000) optimized the production of misti dahi from buffalo milk using starter combinations comprising (i) *Streptococcus salivarius* subsp. *thermophilus*, *Lb. acidophilus* and *Lb. delbrueckii* subsp. *bulgaricus*; and (ii) *Lb. acidophilus*, *Lactococcus lactis* subsp. *lactis* and *S. cerevisiae*.

The people of the Eastern Himalayan regions of India, Nepal, Bhutan and Tibet in China, prepare a variety of ethnic fermented milk products for long centuries (Tamang, 2005). Some of these milk products are dahi, mohi, gheu, chhurpi, chhu**,** somar and philu (Dewan and Tamang, 2007). Species of lactic acid bacteria are the dominant microorganisms in these ethnic fermented milk products with high population levels up to 10^8 cfu/g, followed by yeasts with a load of 10^7 cfu/g (Tamang et al., 2000; Dewan and Tamang, 2007). Prevalence of yeasts in fermented cow-milk and yak-milk products of the Sikkim Himalayas was 60% and 45.5%, respectively (Dewan, 2002). *Saccharomycopsis crataegensis* and *Candida castellii* are reported from chhu, an indigenous fermented cheese-like product of Sikkim (Dewan and Tamang, 2006). Presence of high number of yeasts (10^6 cfu/g) indicates some role during spontaneous fermentation of chhu. Yeasts bring about desirable fermentation changes in fermented milk products (Westall and Filtenborg, 1998). Lactic acid bacteria and *Candida kefyr* constitute a part of microflora in amasi, Zimbabwean fermented cow-milk product (Gadaga et al., 2001).

9.4 Fermented Cereal Products

The production of bread is an ancient biotechnological process that is based on the fermentation of a wheat flour-dough, but flours from other cereals such as rye, are also used. It has evolved into a modern industrialized process, the details of which are well-reviewed (Jenson, 1998; Hammes and Ganzle, 1998; Decock and Cappelle, 2005). Sourdough bread is acidic-tasting aerated bread, made from rye, wheat or mixed flours of Europe and South America (Campbell-Platt, 1987). Sourdough fermentation is a mixture of flour, water and salt and if kept at room temperature, it will undergo a natural fermentation that involves the growth of

indigenous yeasts and LAB (Brandt, 2007). Essentially, bread dough is a mixture of flour, water and salt and if kept at room temperature, it will undergo a natural fermentation that involves the growth of indigenous yeasts and lactic acid bacteria. The microbial ecology of these fermentations has been well studied, leading to the recognition that *S. cerevisiae* is the principal yeast of most bread fermentations (Jenson, 1998; Hammes et al., 2005). Today, selected strains of *S. cerevisiae* (baker's yeast) are extensively produced at commercial products for bread production. Typically, the bread manufacturer buys baker's yeast as a dried, compressed or liquid product and adds it to the dough mixture to give populations of 10^8–10^9 cfu/g. The dough mixture is then incubated at 30–35°C for varying times (depending on the process) for fermentation. The substrates for fermentation are mainly maltose and glucose that are released by the breakdown of wheat starch by endogenous amylases of the flour. Yeast fermentation serves several functions. Gas (CO_2) production causes expansion and leavening of the dough, ultimately affecting bread texture, density and volume. Some of the CO_2 dissolved to form carbonic acid. Ethanol produced by the yeast, yeast enzymes that affect cereal proteins and carbonic acid, influence the rheological properties of the dough again impacting on final bread texture and structure. A vat assay of secondary metabolites (alcohols, acids, esters, aldehydes, ketones, etc.) produced by the yeast and any bacterium associated with the fermentation, constitute distinctive flavour to the bread. Most of these metabolites are volatile and are lost during baking, but they also undergo complex reactions with other dough components during baking to affect flavours (Jenson, 1998; Hammes and Ganzle, 1998; Decock and Cappelle, 2005). There has been extensive selection development and genetic improvement of specific strains of *S. cerevisiae* for the bread processing industries (Rande-Gil et al., 1999; Dequin, 2001; Bonjean and Guillaume, 2003). The principle requirements of the strains are rapid fermentation and CO_2 production from maltose and glucose, and generation of good bread flavours. However, strains used for fermenting sweet doughs that certain added sucrose need to be more osmotolerant and strains used for the production of frozen dough must be freeze-thaw tolerant. In addition, the various strains of baker's yeasts need to have properties that enable them to be produced efficiently and economically on a large commercial scale and to have good storage and stability properties as dried yeasts, either compressed yeasts or yeast slurries. Strains to be processed as dried yeasts need to be more tolerant of heat stresses than compressed yeasts. Thus, there is significant physiological, biochemical and genetic diversity in strains of *S. cerevisiae* used for bread production (Jenson, 1998; Rondez-Gil, 1999). In some cases, species other than *S. cerevisiae* could offer better functionality in some criteria. For example, *Torulaspora delbrueckii* and *Kluyveromyces thermotolerans* are more freeze tolerant than *S. cerevisiae* and could be used to prepare frozen dough breads (Jenson, 1998; Alves-Araújo et al., 2004).

Many types of bread, especially in European countries, are still produced by traditional processes where no commercial strains of baker's yeast (*S. cerevisiae*) are added. Indigenous yeasts and lactic acid bacteria conduct dough fermentation, and the resultant products are generally called sourdough breads because they have

higher contents of lactic acid and acetic acid due to the bacterial growth (Hammes and Ganzle, 1998; Hammes et al., 2005; De Vuyst and Neysens, 2005; Rehman et al., 2006). San Francisco sourdough bread falls into this category. Various studies have been conducted in recent years to understand the microbial ecology of this fermentation. While indigenous *S. cerevisiae* is still prominent in many of these fermentations, the presence and growth of other yeast species are significant and these include *Saccharomyces exiguus, Candida milleri, Candida humilis, Candida krusei* (*Issatchenkia orientalis*), *Pichia anomola, Pichia membranifaciens* and *Yarrowia lipolytica* (Gobbetti, 1998; Corsetti et al., 2001; Paramithiotis et al., 2000; Gullo et al., 2002; Foschino et al., 2004; Veinocchi et al., 2004). These yeasts have evolved to grow in temperature with the lactic acid bacteria of these dough, including *Lactobacillus sanfranciscensis* (unique to these ecosystems), *Lactobacillus plantarum*, and various other species *of Lactobacillus, Pediococcus* and *Leuconostoc* (Hammes et al., 2005; de Vuyst and Neysens, 2005). Commercial starter cultures of these yeast-bacterial combinations are now available (Decock and Cappelle, 2005). Finished bread products are not immune to spoilage by yeasts if not properly stored. If slightly moist, they can develop a fermentative odour and flavour due to growth of *S. cerevisiae*. Growth of *Pichia burtonii* produces visible, white or chalky discoloration (Legan and Voysey, 1991).

Consumption of rice as a staple food in Asia has resulted in a traditional cereal fermentation with moulds and yeasts (Haard et al., 1999). Varieties of traditional non-alcoholic cereal-based fermented foods are mostly prepared and consumed in Africa as staple foods (Nout, 2001; Blandino et al., 2003). Fermented cereal-based gruels are generally used as naturally fortified weaning foods for young children in Africa (Efiuvwevwere and Akona, 1995; Tou et al., 2007).

Enjera (Injera) is thin soft bread, with numerous eyes, or gas holes, baked in Ethiopia from the cereal tef (*Eragrotis tef*) and eaten at nearly every meal with meat, vegetable or legume stew, with each person eating two or three per day. Stewart and Getachew (1962) isolated fungi including yeasts *Pullaria, Aspergillus, Penicillium, Rhodotorula, Hermodendrum, Candida guilliermondii* and a number of unidentified bacteria from samples of *enjera* batter. Yeasts appeared in all stages of *enjera* fermentation but disappeared later due to decrease in pH of the product (Gashe, 1985).

Idli is an acid-leavened and steamed cake made by bacterial fermentation of a thick batter made from coarsely ground rice and dehulled black gram. Idli cakes are soft, moist and spongy, have desirable sour flavour, and is eaten as breakfast in South India. Dosa batter is very similar to idli batter, except that both the rice and black gram are finely grounded. The batter is thinner than that of idli and is fried as than, crisp pancake and eaten directly in South India. Though, lactic acid bacteria are predominant microflora in idli and dosa fermentation, yeasts have also been reported from the product. Soni and Sandhu (1989, 1990, 1991) reported the principal yeasts in idli and dosa as *S. cerevisiae, D. hansenii, H. anomala, T. candida* and *Trichosporon beigelii*. Addition of yeasts in idli and dosa fermentation contributes to leavening and flavour development and results in enhanced contents of thiamine and riboflavin. However, the presence of yeasts can interfere with acidification of

the batter since the yeasts utilize a portion of the fermentable sugars that otherwise would be used for production of lactic acid supplementation of the batter ingredients (Venkatasubbaiah et al., 1985; Steinkraus, 1996).

Jalebi is a traditional Indian crispy sweet, deep fried pretzel made from wheat flour and eaten as confection snack food (Chitale, 2000). The batter is prepared by mixing wheat flour with curd and then fried in oil and the fried jalebi are taken out from the pan and soak in sugar syrup immediately for 4–5 h (Batra, 1986). LAB along with yeasts *Saccharomyces bayanus, S. cerevisiae* and *Hansenula anomala* have been reported from jalebi (Batra and Millner, 1974; Soni and Sandhu, 1990).

Rabadi is a fermented cereal-based food prepared by mixing flour of wheat, barley, pearl millet or maize with buttermilk of North-West India (Gupta et al., 1992a). Single as well as mixed culture fermentation of pearl millet with yeast (*S. cerevisiae* or *S. diastaticus*) and LAB (*Lb. brevis* or *Lb. fermentum*) was developed for utilization of pearl millet by fermentation (Khetarpaul and Chauhan, 1990a). Fermentation of pearl millet with pure cultures of yeast and lactobacilli has been found effective method for improving its nutritive value: increased bioavailability of minerals (Khetarpaul and Chauhan, 1989); improved starch and protein digestibility (Khetarpaul and Chauhan, 1990a); increased total soluble sugar, reducing and non-reducing sugar content with decrease in starch (Khetarpaul and Chauhan, 1990b); elimination of anti-nutrients (Khetarpaul and Chauhan, 1991a); and brought an improvement in biological utilization (Khetarpaul and Chauhan, 1991b; Gupta et al., 1992b).

Kenkey is a popular fermented maize product of Ghana that is acidic, dumpling like, wrapped in leaves or maize cob sheaths, and usually steamed, eaten as a staple food with soup (Amoa and Muller, 1976). *Leuconostoc mesenteroides, Pediococcus acidilactici* and *Lactobacillus fermentum*, and yeast *Geotrichum candidum* were reported from kenkey (Christian, 1970; Halm et al., 1993).

Kisra is a thin pancake like fermented bread made from whole sorghum flour and it is the staple food of Sudan served regularly for at least in one of the three meals of the day (Elkhalifa, 2000). Mohammed et al. (1991) reported species of LAB and yeasts *Candida intermedia*, *Debaryomyces hansenii*, and few moulds in samples of kisra.

Masa is a popular shallow fried fermented product which is obtained through fermentation of rice, millet or sorghum and is widely produced in Northern Nigeria (Efiuvwevwere, and Ezeama, 1996). A wide range of microorganisms was isolated during the early stage of masa fermentation between 0–6 h with *Bacillus* spp., *Lactobacillus* spp., *Saccharomyces* spp., *Enterobacter* spp., *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* spp., however, after 8 h, all disappear except *Saccharomyces* spp. with few *Bacillus* spp. which become dominant in the fermentation (Efiuvwevwere and Ezeama, 1996).

Puto is a fermented rice cake consumed as a breakfast and snack food in the Philippines and is generally served with grated coconut; it is closely related to Indian *idli* except that it contains no legume (Sanchez, 1996). During puto fermentation, the yeasts and microaerophilic bacteria increased in number with time where the predominant organism was always *Leuc. mesenteroides*, followed by *S. faecalis* and then *S. cerevisiae* (Cooke et al., 1987; Rosario, 1987). It was found out that the

yeast along with *Leuc. mesenteroides* played an important role in leavening the batter for puto (Tongananta and Orillo, 1996).

Selroti is a popular fermented rice-based ring shaped, spongy, pretzel-like, deep-fried food item commonly consumed by the Nepalis of Sikkim and the Darjeeling hills in India, Nepal and Bhutan (Tamang, 2005). Selroti is mostly prepared at home (75.6%) comparable to market purchase (Tamang et al., 2007a). The microbial population of selroti batters revealed that LAB present in viable numbers above 10^8 cfu/g, followed by yeasts around 10^5 cfu/g, respectively, which included *Saccharomyces cerevisiae*, *Saccharomyces kluyveri*, *Debaryomyces hansenii, Pichia burtonii* and *Zygosaccharomyces rouxii* (Hannah, 2007). It was found that selroti batters produced using a mixture of pure culture strains of *Leuc. mesenteroides* BS1:B1 and *S. cerevisiae* BA1:Y2, selected on the superior technological property, at 28°C for 4h had organoleptically scored the highest acceptability among the consumers (Hannah, 2007).

9.5 Alcoholic Beverages

Yeasts are intimately involved with the production all alcoholic beverages. This association depends on the ability of some yeast species to rapidly and efficiently ferment sugar into ethanol and also their ability to tolerate ethanol concentration of 15–20% v/v. Alcoholic beverages represent a vast diversity of products ranging from table wines, fortified wines, sparkling wines, beer, sake, cider and other fruit wines, distilled alcoholic products to many traditional fermented beverages (Fleet,1998; Lea and Piggott, 2003).

Wines generally refer to those products originating from the alcoholic fermentation of grape juice. The international wine industry is extensive and produces many different types and styles of wines depending on the cultivar of the grape, the geographical origin of the grape and wine making process. Until 50–75 years ago, most wines were produced by so called spontaneous or natural alcoholic fermentation of the grape juice by indigenous yeast flora. These yeasts originated from the surface of the grape berry, the surface of winery equipment that come in contact with the juice during crushing, pressing, pumping and fermentation, and the air (Raspor et al., 2006). Many years of research have identified various strains of *S. cerevisiae* and *S. bayanus* as the principal yeasts of wine fermentation. Today many wine makers purchase commercially prepared dried preparation of these yeasts for inoculation into grape juice and initiation of the alcoholic fermentation (Fleet,1998; Pretorius, 2000) However, it needs to be understood that indigenous yeasts are always present in the juice and usually grow in cooperation and competition with any inoculated strain (Fleet, 2003).

Very few yeasts 10^4–10^3 cfu/g are found on immature grape berries but they increase to 10^4–10^6 cfu/g of the berries mature to harvest. Unripe grapes harbour a predominance of *Rhodoturula, Sporobolomyces, Cryptococcus* and *Candida* species, along with the yeasts-like fungus *Aureobasidium pullulans*. Most of these

species also occur on mature grapes but at this stage, species of *Hanseniaspora* (anamorph *Kloeckera*) and *Metschnikowia* predominate. Damaged grapes with increased availability of fermentable carbohydrate have increased population of *Hanseniaspora* and *Metschnikowia* species as well as the other yeasts, including *Saccharomyces* and *Zygosaccharomyces*. *Saccharomyces* yeasts are rarely isolated from healthy mature grapes berries by plate culture methods but they occur at low populations detectable by enrichment culture procedures.

Freshly extracted grape juice harbours a yeast population of 10^3–10^5 cfu/ml, comprised mostly of *Hanseniaspora/Kloeckera* species, but species of *Candida, Metschnikowia, Pichia, Kluyveromyces* and *Rhodotorula* also occur. The juice will also contain low populations of indigenous species of *Saccharomyces*, depending on the extent of their occurrence on grapes and equipment used to process the juice. Fermentation is initiated by the growth of various species of the non-Saccharomyces yeasts (e.g. *Hanseniaspora uvarum, Kloeckera apiculata, Candida stellata, Candida colliculosa, Metschnikowia pulcherrima, Kluyveromyces thermotolerans*) as well as *Saccharomyces* yeast is generally limited to the first 2–4 days of fermentation, after which they die off (Moreira et al., 2005). They achieve maximum populations of 10^6–10^7 cfu/ml before death, thereby imparting on the metabolic behaviour of the fermentation and products released into the wine are, consequently, wine flavour and quality. Their death is attributed to an inability to tolerate the increasing concentrations of ethanol which is largely produced by the *Saccharomyces* species. After 4 days or so, the fermentation is continued and completed by the *Saccharomyces* species especially strains of *S. cerevisiae*, *S. bayanus* and in some cases *S. paradoxus*. Molecular fingerprinting of isolates from the wine fermentation has isolated further ecological complexity and diversity, in the most species are represented by more than one strain. For example, many genetically distinct strains of *S. cerevisiae* have been isolated from the one fermentation. Thus, wine fermentations reflect not only an ecological succession of different yeast species, but also successional growth of strains within a species (Fleet, 2003). Apart from ecological diversity, the species and strains of wine fermentations also reflect significant metabolic or biochemical diversity. Different species and even different strains within a species can produce substantially different profiles of metabolic end products such as organic acids, higher alcohols, esters and sulphur volatiles (Romano et al., 2003). Apart from contributing to the alcoholic fermentation, yeasts can also spoil the wine, especially during the stages of bulk storage and maturation in the cellar and after packaging. Ethanol-tolerant fermentative species such as *Zygosaccharomyces bailii, Saccharomycodes ludwigii* and *Dekkera* (*Brettanomyces*) *bruxellensis* are particularly notable and lead to a variety of spoilage off-flavour. Wine exposed to air quickly develops a surface flora of weakly fermentative or oxidative yeasts in the genera *Candida, Pichia* and *Hansenula,* with *Pichia membranifaciens* being significant (Sponholz, 1993; du Toit and Pretorius, 2000; Loureiro and Malfeito-Ferriera, 2003).

Beer is the fermented extract of malted cereal grains principally barley. It has an ethanol content of 2–8%, and a distinctive flavour which originates from constituents of the malt extracts of hops, and products of yeast metabolism. Barley is germinated

and kilned in a process called malting. The malted barley is extracted with water under carefully controlled conditions (mashing) to give the extract, termed wort, which contains fermentable carbohydrates and other nutrients for yeast metabolism. The wort is boiled with hops, clarified and then fermented. After fermentation, the beer is clarified, conditioned or matured if necessary and then packaged (Hammond, 1993; Fleet, 1998; Dufour et al., 2003). The boiled wort is essentially sterile and brewers conduct the fermentation by inoculating (pitching) it with pure cultures of yeasts. Generally brewers maintain and propagate their own stocks of yeasts principally, species and strains of *Saccharomyces*. Current taxonomy has these strains in the species, *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*, although former literature describes brewing yeasts within the species *Saccharomyces carlsbergensis* and *Saccharomyces uvarum*. Strains within these species have been merged into either *S. cerevisiae* or *S. pastorianus* (Kurtzman, 2003; Kurtzman and Robnett, 2003).

There is great diversity in the styles of beer produced internationally and indeed within the one brewery (e.g. ale, lager, stout, pilsner, etc.) and this depends on the raw materials, mashing, fermentation and conditioning processes and the strain of yeast. Ecologically, the fermentation should be relatively homogeneous being conducted by the strain of yeast that is inoculated (10^6 cfu/ml) into the boiled wort. However, there is significant metabolic, physiological and genetic diversity in the strains used, and these determine the final flavour and quality of the beer and the efficiency of the process (Dufour et al., 2003). Some key technological properties used by brewers in selecting strains for particular beers include: profile of aroma volatiles produced (as higher alcohols, esters, carbonyl compounds, sulphur volatiles); rate and extent of sugar (glucose, maltose, maltotriose fermentation; fermentation at lower (7–15°C) or higher (25–30°C) temperatures; flocculation and sedimentation characteristics; oxygen requirement; ethanol and osmotolerance-especially in relation to performance in high gravity brewing. Most brewers recycle their yeasts in order to improve efficiency and decrease wastes. After fermentation, the yeast cells are harvested, acid washed to decrease bacterial contamination, stored at 4–5°C and then used to inoculate the next batch of wort. Generally, brewers yeasts can be recycled about 7–8 times, before their performance decreases. This process can lead to increase in the proportion of mutant strains (as respiratory deficient petite mutants) and killer strains within the total yeast population and comprise beer quality (Russel and Stewart, 1995; Dufour et al., 2003).

Growth of any yeast other than the desired strains during fermentation and maturation or any yeast after packaging will lead to off-flavours and turbidity, essentially the beer is spoiled. Many fermented species within the genera *Saccharomyces, Kluyveromyces, Torulaspora, Zygosaccharomyces* and *Dekkera* (*Brettanomyces*) are capable of causing such spoilage. Exposure of beer to air will encourage the growth of oxidative species in the genera of *Pichia*, *Debaryomyces, Candida* and *Hansenula*. Species of Dekkera produce high levels of acetic acid and esters, *Pichia* and *Hansenula* species give excessive ester production while others can give phenolic off-flavours and ferment residual dextrans in the beer (Fleet, 1998; Dufour et al., 2003; Romano et al., 2006). Good manufacturing and hygiene practices are needed to prevent these outcomes.

Some beers, such as those produced in African countries and the limbic beers of Belgium are still produced by traditional processes where mixtures of wild yeasts and bacteria conduct the fermentations. In these cases, the yeasts represent various mixtures of *Saccharomyces* and *Dekkera* spp. (Jespersen, 2003; Viljoen, 2006).

Sparkling wines and fortified wines are all produced from a base wine that is fermented from grape juice with yeasts in a similar way to the table wines just described (Fleet, 1998, 2001). With sparkling wines, the base wine is subjected to a secondary fermentation which traditionally is conducted in the bottle to retain the dissolved carbon dioxide (fizz or sparkle) produced during this fermentation. About 2% of fermentable sugar is added to the base wine, along with a selected strain of *S. cerevisiae*. The mix is bottled and sealed, and fermented for 6 months to 2 years. The yeast grows, ferments the sugar, sediments out and autolyses to contribute unique flavour and other physical properties to the wine. Finally, the yeast is removed from the wine in a specialized process called disgorgement (Howe, 2003). Special strains of *S. cerevisiae* are required for the secondary fermentation. Criteria for these strains include: fermentation under conditions in the bottle- high ethanol concentration (8–12%), low pH (as low as 3.0), low nutrient availability, and increasing pressure of carbon dioxide (upto 600 kpa); ability to flocculate and sediment to facilitate yeast cell removal from the wine; undergo autolysis and give good flavours (Fleet, 2001).

Fortified wines include products such as sherry and port that have ethanol concentrations of 15–22%. This higher ethanol concentration is achieved by addition of ethanol (usually derived from the distillation of wine products) at certain stages during the process (Reader and Dominiquez, 2003). Generally, the higher ethanol concentration suppresses further yeast growth so that they no longer have a role in the process. However, in the case of 'finos', sherries, the wine is fortified in wooden casks by a staged, maturation process called the 'solera' system. This process encourages the formation of a surface film or velum of yeast growth (flor) at the air-wine interface. Essentially, the velum is a thick, wrinkled layer of yeast biomass that largely consists of a unique mixture of hydrophobic strains of *S. cerevisiae*, but strains of *Torulaspora delbureckii, Dakkera bruxellensis, Candida* spp. and *Zygosaccharomyces* spp. may be found. The oxidative metabolism of the velum yeasts decreases the concentrations of wine acid, glycerol and alcohol and substantially increases the concentration of acetaldehyde, thereby contributing unique flavours to these sherry products (Esteve-Zarzoso et al., 2001, 2004).

Using the principles of wine and beer production, almost any fruit juice or cereal extract can be fermented by yeasts to yield an alcoholic product. The raw material must give a supply of fermentable carbohydrates (glucose, fructose, sucrose) and the initial pH should be sufficiently low (as pH 3.0–4.0) to restrict the growth of bacteria. If the pH is not sufficiently low, then the extract will need to be heat processed to reduce the bacterial population, and starter cultures of yeast then inoculated (e.g. as for beer production).

Among the fruit wines, cider produced from apple juice has received most study (Beech, 1993). Like grape wines, it is produced by traditional indigenous fermentation or by inoculation with selected strains of *S. cerevisiae* or *S. bayanus*. In either

case, there is a successional development of yeast species and strains throughout the process. Initially, of *Hanseniaspora uvarum/Kloeckera apiculata* contribute to the fermentation along with species of *Metschnikowia, Candida* and *Pichia*. These give way to a predominance of *S. cerevisiae* and *S. bayanus* that complete the fermentation and demonstrate significant strain diversity (Naumov et al., 2001; Morrissey et al., 2004). Later during maturation *Dekkera, Zygosaccharomyces*, *Saccharomycodes* and *Hanseniaspora* species may develop, either enriching the product flavour or contributing to spoilage (Valles et al., 2007).

Distilled liquor or 'wines' produced from the fermentation of rice extracts are popular in East and South East Asia. Saké production in Japan is well known (Fleet, 1998). Initially, the rice is fermented in a solid substrate process with amylolytic and proteolytic filamentous fungi (*Aspergillus*, *Rhizopus* spp.) to produce a mix (Koji) that is rich in fermentable sugar and starch degrading enzymes. The koji is then mixed with steamed rice, water and a traditional or selected yeast starter culture for alcoholic fermentation. After fermentation, the liquid material is separated from the solids to give the wine. Unique strains of *S. cerevisiae* have evolved to conduct those fermentations generating products with high ethanol content (12–20%), attractive flavour and aroma and odour (Kodama,1993; Dung et al., 2005, 2006).

Distilled products (spirits) form a large part of the market for alcoholic beverages. Although a diverse range of spirit product is available, a general scheme for their production can be presented as (i) selection of the raw material (ii) processing of the raw material to give a fermentable extract (iii) alcoholic fermentation by yeast, principally by strains of *S. cerevisiae* (iv) distillation of the fermented material to give the distillate product and (v) post-distillation processing (Watson, 1993; Bluhm, 1995). Whisky is a distillate from fermented cereals such as malted barley, rum is made by distilling fermented sugar cane or molasses, brandies are prepared from distillates of fermented fruit juices, such as grapes. In addition, there is a large range of flavoured spirits, such as gin, vodka, etc. They are generally produced by alcoholic fermentation of extracts from cereals or their agricultural commodities and then distilled. Specific flavours are added to the distillate to give particular products (Bluhm, 1995).

Specific distillers strains of *S. cerevisiae* are generally purchased from yeast-producing companies and inoculated into the raw material extract of 10^6–10^7 cfu/ml to initiate and conduct the fermentation. However, they must compete with the growth of any other yeasts and bacteria that may be naturally present in the extract. The key criteria for strain selection include efficient production of high concentrations of ethanol, and production of a desired profile of flavours volatile that carry over into the distillate (Watson, 1993). In some cases, traditional processes are still conducted, where indigenous yeasts make a significant contribution to the fermentation and product flavour. In tequila production from fermenting agave juice, species of *Kloeckera africana*, *Candida magnolia* and *Candida krusei* contribute to the fermentation in addition to *S. cerevisiae* (Lachance, 1995) Rum production from molasses fermentation may involve contributions from *Schizosaccharomyces pombe*, as well as *S. cerevisiae* (Fahrasame and Ganow Parfact, 1998). Brazilian rum, cachucha, is distilled from fermented cane juice where the successional

growth of several yeast species occur. A diversity of different strains of *S. cerevisiae* eventually dominates the fermentation after initial growth of other species within the genera *Pichia*, *Candida* and *Kluyveromyces*. In some case, *Schizosaccharomyces pombe* was dominant yeast (Pataro et al., 2000; Schwan et al., 2001).

9.5.1 Mixed Starter Cultures

In Asia, three types of inocula as starters are commercially produced to convert starchy materials to sugars and subsequently to alcohol and organic acids (Hesseltine et al., 1988; Fleet, 1998; Thapa, 2001):

i. In koji, pure cultures of *Aspergillus oryzae* and *Aspergillus sojae* are used in combination. At the same time, they produce amylases that convert starch to fermentable sugars, which are then used for the second stage yeast fermentation to make miso and shoyu, while proteases are formed to break down the soybean protein.
ii. In second-type, whole-wheat flour with its associated flora is moistened and made into large compact cakes, which are incubated to select certain desirable organisms. The cakes, after a period of incubation, are used to inoculate large masses of starchy material, which is then fermented to produce alcohol. Cakes contain yeasts and filamentous moulds. This inoculum is used in the so-called kao-liang process for making alcohol.
iii. The third type of starter is a mixed culture of yeast, filamentous moulds and bacteria. This starter is in the form of flattened or round balls of various sizes, compact in texture, and dry. The starter is inoculated with some previous starter. This mixed flora is allowed to develop for a short time, then dried, and used to make either alcohol or fermented foods from starchy materials. The starters have a variety of names such as marcha in India, Nepal and Bhutan, ragi in Indonesia, bubod in the Philippines, chiu-chu in China and Taiwan, loogpang in Thailand, nuruk in Korea, men in Vietnam (Tamang et al., 1996; Thapa, 2001; Dung et al., 2007), which are used as starters for a number of fermentations based on rice and cassava or other cereals in Asia.

Marcha or murcha is the mixed dough inocula prepared as a dry, round to flattened; creamy white to dusty white, solid ball like starter which is used to produce sweet-sour alcoholic drinks, commonly called jaanr in the Himalayan regions of India, Nepal, Bhutan and Tibet in China (Shrestha et al., 2002; Tamang, 2005). During production of marcha, mainly soaked glutinous rice are crushed, mixed with wild herbs and spices, made into paste by adding water, starter (marcha) from previous batch is mixed, shaped into balls, wrapped in fern leaves, covered with jute sags, left to ferment for 1–3 days and sun-dried. Marcha is a Nepali word. Bhutia calls it phab, and the Lepcha calls it buth (Tamang et al., 1996). Microorganisms of marcha included filamentous moulds *Mucor circinelloides*, *Mucor hiemalis, Rhizopus chinensis* and *Rhizopus stolonifer* variety *lyococcus*; yeasts *Saccharomycopsis fibuligera*,

Saccharomycopsis capsularis, Pichia anomala, Pichia burtonii, Saccharomyces cerevisiae, *Saccharomyces bayanus* and *Candida glabrata*, and lactic acid bacteria *Pediococcus pentosaceus, Lb. bifermentans* (Tamang and Sarkar, 1995; Thapa, 2001; Tsuyoshi et al., 2005).

Based on phylogenetic, morphological and physiological characterization, yeast strains isolated from marcha were first classified into four groups (Group I, II, III, IV), and were identified as *Saccharomyces bayanus* (Group I), *Candida glabrata* (Group II), *Pichia anomala* (Group III), *Saccharomycopsis fibuligera*, *Saccharomycopsis capsularis,* and *Pichia burtonii* (Group IV) (Tsuyoshi et al., 2005). Among them, the Group I, II, and III strains produced ethanol. The isolates of Group IV had high amylolytic activity. Because all marcha samples tested contained both starch degraders and ethanol producers, it was hypothesized that all groups of yeasts (Group I, II, III, and IV) contribute to starch-based alcohol fermentation. *Rhizopus* spp. and amylolytic yeasts (mostly *Saccharomycopsis fibuligera*) degrade starch and produce glucose, and alcohol-producing yeasts (species of *Saccharomyces* and *Pichia*) rapidly grow on the resultant glucose to produce ethanol (Thapa, 2001). Marcha making technology reflects the traditional method of sub-culturing desirable inocula from previous batch to new culture using rice as base substrates. This technique preserves the microbial diversity essential for beverages production. Marcha retains its potency *in situ* for over a year or more. In Manipur, a similar mixed starter is called hamei (Tamang *et a*l., 2007b).

Ragi is a starter like marcha, used in Indonesia where rice is used as a substrate (Saono et al., 1974). Went and Prinsen-Geerligs (1896) found *Monilia javanicus* (*Pichia anomala*) and *Saccharomyces cerevisiae* as principal yeasts in ragi. The dominant yeasts species present in ragi are *Candida parapsilosis, C. melinii, C. lactosa, Hansenula subpelliculosa, H. anomala* and *H. malanga* (Dwidjoseputro and Wolf, 1970; Ardhana and Fleet, 1989). Studies of Saono and Basuki (1978) revealed that ragi and its fermented products such as tape keté la, tapé ketan hitam, oncom hitam and oncom mérah from various places in West Java contained *Candida* spp as dominant yeasts, and *Mucor* spp and *Rhizopus* spp as dominant among moulds.

Bubod is used as a starter in the Philippines (Tanimura et al., 1978). Kozaki and Uchimura (1990) reported the presence of *Mucor circinelloides*, *M. grisecyanus*, *Rhizopus cohnii*, *Saccharomyces cerevisiae* and *Saccharomycopsis fibuligera* in bubod. Hesseltine and Kurtzman (1990) reported that *Saccharomycopsis fibuligera* was dominant in bubod.

Nuruk is the starter for preparing Korean alcoholic drink yakju and takju, prepared from rice or wheat (Steinkraus, 1996). Generally, nuruk is prepared by natural inoculation of molds, bacteria, and yeasts; however, it can be prepared by inoculation with *Aspergillus usamii*. Kim (1968) isolated *Aspergillus oryzae* (10^7 cfu/g), *A. niger* (10^7 cfu/g), *Rhizopus* sp (10^6 cfu/g), anaerobic bacteria (10^7 cfu/g), aerobic bacteria (10^6 cfu/g) and yeasts (10^5 cfu/g) from nuruk.

Chiu-yueh or peh-yueh is the Chinese starter for lao-chao, fermented rice product of China. It is a gray-white ball containing yeasts and fungi grown on rice flour, which is closely related to ragi. Wei and Jong (1983) isolated yeasts and moulds

from chiu-yüeh and tested the ability of these microorganisms to convert steamed glutinous rice into a good quality lao-chao.

Loogpang is the starter commonly used in Thailand to prepare alcoholic drink and vinegar (Vachanavinich et al., 1994). Dhamcharee (1982) showed that the yeasts present were *Saccharomycopsis*, *Hansenula*, and *Saccharomyces* along with molds in loogpang. Sukhumavasi et al. (1975) isolated a strain of *Endomycopsis* (*Saccharomycopsis*) *fibuligera* from loogpang with high glucoamylase activity. Uchimura et al. (1991) reported the presence of *Saccharomycopsis fibuligera* and *Pediococcus* sp. in loogpang.

9.5.2 Asian Alcoholic Beverages

Alcoholic foods and beverages, in which amylolytic moulds and yeasts accomplish starch hydrolysis and fermentation, range from very primitive (Steinkraus, 1996). The main yeasts, which ferment saccharified rice starch to alcohol, are *Endomycopsis* (*Saccharomycopsis*) *burtonii*, *Saccharomycopsis fibuligera*, *Saccharomyces cerevisiae* and *Candida lactosa*; *Saccharomycopsis fibuligera* also produces amylolytic enzymes (Reiser and Gasperik, 1995; Yip et al., 1997; Brimer et al., 1998; Dung et al., 2005). Other yeasts genera *Hansenula, Pichia* and *Torulopsis* have also been isolated from rice wine. Esters, fusel oils, acids and other compounds which contribute to flavour are also produced (Nout and Aidoo, 2002). Some of common indigenous alcoholic beverages prepared by a mixed starters are kodo ko jaanr, bhaati jaanr, tapé, etc. *Saccharomycopsis fibuligera* which is one of the common yeasts in Asian starter cultures have amylolytic as well as some ethanol producing capacity (Limtong et al., 2002).

Kodo ko jaanr is the most common fermented mild-alcoholic beverage prepared from dry seeds of finger millet (*Eleusine coracana*), by using marcha in Sikkim, the Darjeeling hills and North East hills in India, Nepal, Bhutan and Tibet in China (Tamang, 2005). Kodo ko jaanr contributes to the mineral intake in daily diet of the local people (Thapa, 2001). Because of high calorie, ailing persons and post-natal women consume the extract of kodo ko jaanr to regain the strength. Population of yeasts and lactic acid bacteria was detected at the level of 10^7 cfu/g and 10^5 cfu/g, respectively. Yeasts consisted of *Pichia anomala*, *Saccharomyces cerevisiae*, *Candida glabrata*, *Saccharomycopsis fibuligera*, and lactic acid bacteria consisted of *Pediococcus pentosaceus* and *Lactobacillus bifermentans* in kodo ko jaanr samples. Microorganisms necessary for fermentation of finger millets into kodo ko jaanr are supplemented by marcha (Thapa and Tamang, 2004).

Thapa and Tamang (2006) studied the microbiological and physico-chemical changes during fermentation of kodo ko jaanr and found that population of yeasts increased significantly ($P < 0.05$) from 10^5 cfu/g to 10^7 cfu/g within second day. Maximum activities of saccharification and liquefaction of millets were observed on second day of fermentation. It was revealed that *Saccharomycopsis fibuligera* and *Rhizopus* spp. play the dominant role in saccharification process of finger millet in kodo ko jaanr fermentation.

On the basis of amylolytic activity 4 strains of *Rhizopus* spp., 2 strains of *Mucor* spp., 5 strains of *Saccharomycopsis fibuligera*, 4 strains of *Pichia anomala*, 4 strains of *Saccharomyces cerevisae* and 3 strains of *Candida glabrata* were selected for liquefying and saccharifying activities (Thapa and Tamang, 2006). None of the lactic acid bacteria showed amylolytic activity. Saccharifying activities were mostly shown by *Rhizopus* spp. and *Saccharomycopsis fibuligera* whereas liquefying activities were shown by *Saccharomycopsis fibuligera* and *Saccharomyces cerevisiae*. *Saccharomycopsis fibuligera* played the main roles in amylase production whereas *Rhizopus* seemed to supplement the saccharification (Wei and Jong, 1983; Uchimura et al., 1990). The result indicated that *Saccharomycopsis fibuligera* and *Rhizopus* spp. play the important role in saccharification process of kodo ko jaanr fermentation breaking starch of substrates into glucose for ethanol production (Thapa and Tamang, 2006). *Mucor* spp., *Pichia anomala* and *Candida glabrata, Saccharomyces cerevisiae* may supplement the saccharification.

Bhaati jaanr is an inexpensive high calorie mild-alcoholic beverage prepared from the steamed glutinous rice, consumed as a staple food beverage in the Eastern Himalayan regions of Nepal, India and Bhutan. It was revealed that *Saccharomycopsis fibuligera* and *Rhizopus* spp. play the important roles in saccharification process of rice in bhaati jaanr fermentation (Tamang and Thapa, 2006).

Tapé is a sweet-sour paste with an alcoholic flavour, prepared from glutinous rice or cassava or other cereals by using starter ragi in Indonesia (Ko, 1972). A combination of *Aspergillus rouxii* and *Endomycopsis* (*Saccharomycopsis*) *burtonii* reduced total solids by 50% in 192 h at 30°C, which raised the crude protein in tapé ketan by 16.5% on a dry basis (Cronk et al., 1979). Suprianto et al. (1989) reported that *Saccharomycopsis fibuligera* produced mainly α-amylase and *Rhizopus* sp. produced glucoamylase in tapé fermentation. They also found that liquefaction was not caused by amylases of *Saccharomycopsis* even though it produced high activity of α-amylase.

Tapai is the Malaysian fermented food-beverage produced by adding pulverized ragi or jui-piang. It is consumed as a desert but in East Malaysia it is the rice wine with lighter colour and less sweetness (Merican and Yeoh, 1989). *Candida* spp, *Saccharomycopsis fibuligera*, *Amylomyces rouxii*, *Mucor circinelloides*, *M. javanicus*, *Hansenula* spp, *Rhizopus oryzae*, and *R. chinensis* have been found in tapai (Wang and Hesseltine, 1970; Ko, 1972). Merican and Norrijah (1985) showed that the organisms necessary to produce a good tapai pulut consist of a mixture of *Amylomyces rouxii, Saccharomycopsis fibuligera* and *Hansenula anomala*, and for a good quality tapai ubi, the essential microorganisms are *Amylomyces rouxii,* and *Saccharomycopsis fibuligera*.

Lao-chao is a popular Chinese fermented food with sweet taste and fruity aroma, made from rice by using chiu-yueh or peh-yueh as starters (Wang and Hesseltine, 1970). It is served as a dessert and is a traditional diet for new mothers who believe that it helps them regain their strength. Wei and Jong (1983) reported the presence of *Rhizopus*, *Amylomyces*, *Torulopsis*, and *Hansenula* in lao-chao. Pure culture fermentation method of lao-chao was developed by Wang and Hesseltine (1970) and showed that good fermented rice was made when *Rhizopus chinensis* NRRL 3671, and *Saccharomycopsis* sp. NRRL Y7067, used as inocula instead of a commercial starter.

Yakju is the Korean alcoholic beverages, made from rice by using nuruk. The lower or diluted concentration of yakju is known as takju (Steinkraus, 1996). Microbial studies of yakju revealed the presence of yeasts, *Bacillus* spp. and *Lactobacillus* sp. and *Leuconostoc* spp. (Shin and Cho, 1970; Kim, 1970; Lee and Rhee, 1970). Kim and Lee (1970) reported that *Saccharomyces cerevisae* is the most important organism in alcohol production while *Hansenula* spp. play an important role in flavour development.

Basi is a traditional alcoholic beverage of the Philippines made by fermenting boiled, freshly extracted sugarcane juice with a mixture of yeast, bacteria and moulds or with organisms found in 'samac' (*Macharanga tanarius*) leaves, bark, or fruit (Tanimura et al., 1978). Kozaki (1976) reported that the dominant organisms in basi are *Saccharomyces*, *Saccharomycopsis* and lactic acid bacteria.

Ruou nep is the Vietnamese fermented rice wine prepared from rice using a traditional mixed starters called men (Aidoo et al., 2006). Defined granulated starters containing *A. rouxii* and *S. arvensis* in men make high-quality Vietnamese rice wine (Dung et al., 2005).

Zutho is an ethnic rice beer prepared and consumed by the Nagas in Nagaland in India. Teramoto et al. (2002) reported *S. cerevisiae* as main yeast in zutho fermentation.

9.6 Other Commodities

Yeasts have a key role in the production of a diverse range of other products that are commercially significant globally, or have significance as local, traditional products.

Cocoa beans are the raw material of chocolate manufacture, and require fermentation as one of the first stages in the chocolate production chain (Schwan and Wheals, 2004). The beans are contained in pods of the tree, *Theobroma cocao*, mostly cultivated in the tropical equatorial regions of the world. After harvesting, the beans are removed from the pods and placed as large masses in wooden boxes, on trays or as heaps covered with plantain leaves. They undergo a spontaneous, indigenous fermentation from the growth of naturally associated yeasts, bacteria and fungi. Numerous studies in different countries have described the microbial ecology of this fermentation (Schwan and Wheals, 2003). Yeasts have a prominent role in the fermentation, exhibiting a successional development through *Hanseniaspora uvarum* (*Kloeckera apiculata*), *Hanseniaspora quilliermundii* (*Kloeckera apis*), *Saccharomyces cerevisiae*, *Pichia membranifaciens* and other *Pichia* species, *Issatchenkia orientalis* (*Candida krusei*), various Candida species and *Kluyveromyces* species (Ardhana and Fleet, 2003; Jespersen et al., 2005).

During fermentation, the yeasts and other organisms degrade the previous pulp surrounding the seeds, ferment pulp sugars and create ethanolic acidic conditions that kiln the beans. Thereafter, the beans undergo endogenous biochemical reactions that develop the chemical precursors to chocolate flavour. Products generated

by yeast metabolism also diffuse into the beans to impart on their flavour (Schwan and Wheals, 2003, 2004).

Coffee beans are harvested from trees of the green coffee. Subsequently, they are processed by either wet or dry methods to remove pulp and mucilaginous materials that surround the seeds (Schwan and Wheals, 2003). Various species of yeasts and bacteria grow throughout these processes, producing an array of pectinolytic, hemicellulolytic and other enzymes that facilitate pulp and mucilage degradation. Other metabolic reactions may contribute either positively or negatively to coffee flavours and character, but precise linkages of these reactions to specific organisms are not clear. A diversity of yeast species has been isolated from coffee bean fermentations, including various species of *Candida*, *Saccharomyces*, *Kluyveromyces, Saccharomycopsis, Hanseniaspora, Pichia* and *Arxula*, but further studies are required to describe definitive associations and functionality with respect to the process (Silva et al., 2000; Masoud et al., 2004)

Soy sauce is another global product of major economic significance. The raw materials, soybeans and wheat, are initially fermented with the filamentous fungus *Aspergillus oryzae* or *Aslergillus sojoe,* in a solid substrate mode. During this stage, starch, proteins and other macromolecules in the raw materials are partially degraded to fermented sugars, amino acids and other nutrients necessary for subsequent fermentation by yeasts and lactic acid bacteria (Hanya and Nakadai, 2003). This material is then mixed with brine (16–20% NaCl) for the next stage of fermentation which generally takes several months. Lactic acid bacteria (*Tetragenococcus halophilus*) initiate the fermentation, which is then dominated by the growth of osmotolerant yeasts, *Zygosaccharomyces rouxii* followed by *Candida versatilis* or *Candida etchellsii*. These yeasts evolve naturally or may be added as starter cultures. *Zygosaccharomyces rouxii* conducts an alcoholic fermentation while the *Candida* spp. contribute characteristic flavours from the production of phenolic compounds. The yeasts also accumulate and produce significant amounts of glycerol and other polyols in response to the high salt environment. At the completion of fermentation, the product is clarified and packaged.

9.7 Conclusions

Yeasts food fermentation is practiced in nearly all the countries, along with bacterial and fungal fermentation, or in combination with them. It is concluded that in fermentation of any substrate, *Saccharomyces* ferments sugar, produces secondary metabolites; inhibits growth of mycotoxin-producing moulds and has several enzymatic activities such as lipolytic, proteolytic, pectinolytic, glycosidasic and urease activities. *Debaryomyces* contributes in sugar fermentation, increases pH of the substrates, and produces growth factors for bacteria. *Hanseniaspora* and *Candida* also contribute in sugar fermentation, production of secondary metabolites, and enzymatic activities. *Yarrowia lipolytica* also plays role in sugar fermentation, lipolytic, proteolytic and urease activities and reduction of fat rancidity in the product.

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