Functional yeasts and molds in fermented foods and beverages

Fermented Foods and Beverages of the World

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Functional Yeasts and Molds in Fermented Foods and Beverages

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CONTENTS

4.1 Introduction

Fermented foods and beverages are produced as a result of the activities of microorganisms, principally yeasts, molds, and bacteria. Fungi (yeasts and molds) play a major role in traditional fermented foods that have long histories. Today, some of these fermented products have achieved industrial development and are produced on a large scale as a result of the application of modern technology, automation in production engineering, and biotechnology in the genetic manipulation of functional yeasts and molds. Yeasts play vital roles in the production of many traditional

TABLE 4.1

Main Functional Properties of Fungi in Fermented Foods

Genera	Species	Functional Properties
Fungi		
Zygomycetes		
Actinomucor	*A. elegans*, *A. taiwanesis*	*Production of enzymes*: Carbohydrases—α-amylase, amyloglucosidase, maltase, invertase, pectinase, β-galactosidase, cellulase, hemi-cellulase, and pentosan-degrading enzymes; acid and alkaline proteases; lipases; anti-nutritional properties, e.g., degradation of phytic acid, thus improving bioavailability of minerals
Amylomyces	*A. rouxii*	
Mucor	*M. circinelloides*, *M. rouxii*, *M. indicus*	
Rhizopus	*R. microsporus* var. *chinensis*, *R. oligosporus*, *R. oryzae*, *R. stolonifer*	
Ascomycetes		
Monascus	*M. purpureus*, *M. ruber*, *M. anka*	
Neurospora	*N. sitophila*, *N. intermedia*	
Aspergillus	*A. oryzae*, *A. sojae*, *A. glaucus*, *A. melleus*, *A. repens*, *A. candidus*, *A. tamarii*, *A. usamii*, *A. niger*	
Eurotiomycetes		
Penicillium	*P. glaucus*, *P. roqueforti*	
Basidiomycetes		
Ustilago	*U. maydis*	
Yeasts		
Brettanomyces	*B. anomalus*	Production of amylolytic enzymes, ethanol, aldehydes, isobutanol, isoamyl alcohol, esters, fusel oils, flavors compounds, 4-hydroxy-2 (or 5)-ethyl-5 (or 2)-methyl-3(2H)-furanone (HEMF), phenolic compounds—4-ethylguaiacol and 4-ethylphenol contribute to aroma
Candida	*C. javanica*, *C. famata*	
Geotrichum	*G. candidum*	
Hansenula	*H. anomala*	
Pichia	*P. burtonii*	
Rhodotorula	*Rh. glutinis*	
Saccharomycopsis	*Sm. fibuliger*	
Saccharomyces	*S. cerevisiae*, *S. dairensis*, *S. globosus*, *S. kluyveri*, *S. saké*	
Torulopsis	*Tor. versatilis*	
Trichosporon	*Tr. Pullulans*	
Zygosaccharomyces	*Zygos. rouxii*, *Zygos. sojae*.	

Source: Adapted from Nout, M.J.R. and Aidoo, K.E., Asian fungal fermented food, in *The Mycota*, ed. Osiewacz, H.D., Springer-Verlag, New York, 2002, pp. 23–47.

fermented foods and beverages across the world (Aidoo et al. 2006) that signify the food culture of the regions and the community (Tamang and Fleet 2009). About 21 major genera with several species of functional yeasts have been reported from fermented foods and beverages that include *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, Romano et al. 2006, Tamang and Fleet 2009). Molds in fermented foods and beverages are relatively limited, and include the genera *Actinomucor*, *Mucor*, *Rhizopus*, *Amylomyces*, *Monascus*, *Neurospora*, *Aspergillus*, and *Penicillium* (Hesseltine 1983, 1991, Samson 1993, Nout and Aidoo 2002).

This chapter deals with the main functional yeasts and molds in some of the major fermented foods of the world. Some of the benefits and problems associated with fungal fermented food are also discussed. The major functional properties of yeasts and molds in fermented foods are summarized in Table 4.1. Fermented foods and beverages of the world and their respective functional yeasts and molds are presented in Table 4.2.

4.2 Functional Roles of Yeasts and Molds in Fermented Foods of the World

4.2.1 Fermented Foods of Asian Origin

4.2.1.1 Furu

Furu, also known as *sufu*, is a flavor-rich Chinese fermented soybean product (Figure 4.1) (Han et al. 2001). According to the region and local preferences, there are different shapes, colors, and flavors of *furu*. The principle of preparation is as follows: first, soymilk is made by soaking, grinding, and extracting soybeans; next, the soy protein is coagulated by the addition of salt, and the resulting tofu is collected and pressed to a sheet of firm consistency. *Tofu* is cut into dices that are spray-inoculated with fungal spores (*Actinomucor elegans* and/or *A. taiwanensis*) and incubated at about 20°C–25°C to allow profuse mycelial growth (*pehtze*); the *pehtze* is matured during several months in brine that also contains coloring, spices, etc. The final product has a soft, spreadable consistency. It contains high levels of free amino acids, particularly glutamic acid (Han et al. 2004), and free fatty acids, and it is a very popular item at the breakfast table to go with rice, vegetables, etc. The key function of *Actinomucor* is its production of proteolytic and lipolytic enzymes. Studies on the modifications of soy protein before and during maturation have shown that at an early stage, the large protein molecules are decomposed to oligopeptides, followed by the gradual release of peptides, free amino acids, and nitrogenous degradation products such as NH_3. The optimum temperature for the production of extracellular enzymes by *Actinomucor* is 25°C; thus the production of *furu* during hot summers is problematic. It was found that "tropical" molds such as *Rhizopus* spp. can be used in the production of an acceptable *furu* as an alternative (Han et al. 2003).

TABLE 4.2

Some Functional Yeasts and Molds in Fermented Foods of the World

Yeasts (Y) and Molds (M)	Food	Main Ingredient(s)	Impact of Fermentation	References
Fermented foods of Asian origin				
A. elegans (M)	*Furu*	Soybean curd	Proteolytic activity modifies soy proteins into peptides and amino acids, and softens texture	Han et al. (2001)
S. dairensis (Y), *R. oligosporus* (M)	*Tempe*	Cooked soybeans	Edible mycelial biomass, flavor, isoflavone modifications, enzymatic degradation improves digestibility, anti-diarrhoeal activity	Nout and Kiers (2005)
M. purpureus (M)	Red *koji* rice (*angkak*)	Rice	Produces bio-colorants (pigments), flavor, and health beneficial strains (monacolin K)	Nout and Aidoo (2002)
C. javanica, *Tr. pullulans*, *Tri. versatilis* (Y)	*Idli*	Rice and black gram *dal*	Flavor, gas (leavening, improved digestibility)	Nout et al. (2007)
Sm. fibuligera, *H. burtonii* (Y), *A. rouxii* (M)	*Ragi*, *marcha* (fermentation starter)	Rice	Starch-degrading amyloglucosidase provides glucose for ensuing alcoholic fermentation	Dung et al. (2007)
H. anomala, *Tri. versatilis*, *S. saké*, *Zygos. rouxii*, *Zygos. sojae* (Y), *A. oryzae*, *A. sojae*, *A. niger* (M)	*Koji* (starter)	Rice, wheat, soybeans	*Koji* is used as an enzyme-rich starter for fermentations of, e.g., rice wines, and soy sauce	Fukushima (1985)
P. anomala, *S. cerevisiae*, *C. glabrata*, *Sm. fibuligera* (Y), *M. circinelloides*, *R. chinensis* (M)	*Kodo ko jaanr*	Finger millets	*Marcha* is used as mixed starter for fermentation; *Sm. fibuligera* and *Rhizopus* spp. play a major role in saccharification and the liquefaction process in the fermentation of jaanr; breaking starch of finger millets into glucose for ethanol production. *Mucor* spp., *P. anomala*, and *C. glabrata* may supplement the saccharification	Thapa and Tamang (2004, 2006)

Sm. fibuligera, *Sm. capsularis*, *P. anomala*, *P. burtonii*, *S. cerevisiae*, *S. bayanus* and *C. glabrata* (Y) *M. circinelloides*, *M. hiemalis*, *R. chinensis*, *R. stolonifer* (M)	*Bhaati jaanr*	Rice	*Sm. fibuligera* contributes in saccharification and liquefaction of glutinous rice, breaking starch of substrates into glucose for alcohol production, and also in aroma formation in bhaati jaanr preparation	Tamang and Thapa (2006)
Fermented foods of Australasian origin				
Sm. fibuligera, *Rh. glutinis*, *D. hansenii*, *C. parapsilosis*, *Tr. fennicum* (Y)	*Tapuy*	Rice	Starch hydrolysis, alcoholic fermentation, flavor compounds	Kozaki and Uchimura (1990)
S. cerevisiae (Y)	*Maori's kaanja-kopuwai*	Maize	Improved digestibility	Aidoo (1992)
S. cerevisiae (Y)	*Balao balao* (*Burong hipon*)	Rice, shrimps	Cheese-like flavor	
S. cerevisiae (Y)	*Burong dalag*	Cereal, fish	Cheese-like flavor	
S. cerevisiae (Y)	*Puto*	Leavened steamed rice cake	Texture, flavor, nutritional value	Steinkraus (1992)
A. oryzae, *Rhizopus* spp., (M), *Sm. fibuligera*, *A. rouxii*, *H. anomala* (Y)	Bubod starter	Glutinous rice, ginger, wild root	Alcoholic fermentation, sweet/sour alcoholic foods	Hesseltine et al. (1988)
Fermented foods of African origin				
I. orientalis (*Candida krusei*), *C. kefyr*, *C. glabrata*, *K. marxianus*, *S. cerevisiae* (Y)	*Mawè*	Maize	Flavor formation, nutrition, stimulation of lactic acid bacteria (LAB)	Hounhouigan et al. (1994, 1999)
Hanseniaspora uvarum, *Kluyveromyces* spp., *S. cerevisiae*, *Schizosaccharomyces pombe* (Y)	*Pito*, *tchoukoutou*	Sorghum	Alcoholic fermentation, flavor formation, and nutritional benefit	Kayode et al. (2007b)

(*continued*)

TABLE 4.2 (continued)

Some Functional Yeasts and Molds in Fermented Foods of the World

Yeasts (Y) and Molds (M)	Food	Main Ingredient(s)	Impact of Fermentation	References
P. anomala, *Zygosaccharomces* spp. (Y)	*Fufu*	Cassava	Flavor, nutrition, stimulation of LAB	Sobowale and Oyewole (2008)
Clavispora lusitaniae (Y)	*Amasi*	Cow milk	Flavor, stimulation of LAB	Gran et al. (2003)
S. cerevisiae and *I. orientalis* (Y)	Kachasu	Masau (*Z. mauritiana*)	Alcoholic fermentation, flavor formation	Nyanga et al. (2007, 2008)
Fermented foods of European origin				
Torulopsis holmii, *S. cerevisiae*, *Pichia saitoi*, *C. krusei* (M)	Sourdough	Wheat, rye	Organic acids, flavor, macro- and micronutrients	Sugihara et al. (1971)
P. roqueforti, *P. camemberti* (M) *G. candidum*, *D. hansenii*, *C. versatilis*, *K. marxianus*, *S. cerevisiae*, *Tor. delbrueckii*, *Tri. cutaneum* (Y)	Cheese—Roquefort, blue-veined, Camembert	Milk curd	Proteolytic activity, lipolytic activity contributes to maturation, breakdown of bitter peptides, flavor compounds, utilization of lactic or citric acid	Seiler and Bussie (1990), Auberger et al. (1997), Bintsis et al. (2000), Nout (2000), Fröhlich-Wyder (2003)
P. nalgiovense, *P. chrysogenum*, *P. camemberti* (M), *D. hansenii*, *C. famata* (Y)	Fermented ham	Pork	Proteolytic and lipolytic activities, flavor compounds	Andrade et al. (2008)
P. nalgiovense, *P. chrysogenum*, *P. camemberti* (M), *D. hansenii*. (Y)	Surface mold ripened sausages	Meat	Proteolytic and lipolytic activities, flavor compounds	Nout (1994)

Fermented foods of Middle East origin				
S. cerevisiae (Y)	*Tarhana*	Wheat flour, yoghurt	Minerals, vitamins, and improved digestibility	Ekinci (2005), Ozdemir et al. (2007)
P. nalgiovense, *P. chrysogenum*, *P. camemberti* (M), *D. hansenii*, *C. famata* (Y)	*Sucuk*	Beef, Lamb, seasoning	Proteolytic and lipolytic activities, flavor compounds	Genccelep et al. (2007)
K. marxianus, *S. cerevisiae* (Y)	*Kefyr*	Milk, *kefyr* grain	Sharp acid, yeasty flavor and aroma, yeasts also promote symbiosis between microorganisms, utilization of lactic or citric acid.	La Rivière (1969), Koroleva (1991), Fröhlich-Wyder (2003)
S. globosus, *B. anomalus* (Y)	*Kumiss*	Milk	Source of nutrition for those who are lactose intolerant	Steinkraus (1996), Nout and Aidoo (2002)
S. cerevisiae, *S. kluyveri* (Y)	*Naan*	Wheat flour	Starch hydrolysis, texture, flavor	Alam et al. (2007)
Fermented foods of Latin American origin				
Candida spp., *Tri. cutaneum* (Y)	*Pozol*	Nixtamalized maize	Sour beverage	Nuraida et al. (1995)
S. cerevisiae, *Z. mobilis* (Y)	*Pulque*	Juice of *Agave* spp.	Alcoholic fermentation, flavor formation	Nout (2003)
Dekkera anomala, *P. guilliermondii*, *P. membranifaciens*, *Cryptococcus albidus*, *Rhodotorula mucilaginosa*, *S. cerevisiae*) (Y)	*Tibi* grains (starter)	Microbiogleae consisting of dextran-embedding LAB and yeasts	Mixed fermentation to produce CO_2 gas, lactic acid, traces of alcohol and acetic acid in sugarcane juice	Armijo et al. (1991)
U. maydis (M)	Huitlacoche	Preharvest cobs of maize	Edible fungal mycelium (*Aztec caviar*)	Valverde et al. (1995)

FIGURE 4.1 *Furu*. (From Nout, M.J.R. and Aidoo, K.E., *The Mycota*, eds. Osiewacz, H.D., Springer-Verlag, New York, pp. 23–47, 2002. With permission.)

4.2.1.2 Idli

Idli is a small spongy cake obtained by steaming a naturally fermented mixed batter of parboiled rice (*Oryza sativa*) and black gram (*Phaseolus mungo*) (Figure 4.2). *Idli* is a popular food in South India and Sri Lanka, well appreciated for its pleasant acidic flavor and its easy digestibility. Although the batter undergoes a natural fermentation, usually a small portion of previously fermented batter (back-slop) is added to achieve better results. The fermentation takes place at ambient temperatures (25°C–35°C) for 16–24 h. Obligate heterofermentative lactic acid bacteria (LAB) (*Leuconostoc mesenteroides*) and yeasts (*Saccharomyces cerevisiae*, *Debaryomyces hansenii*, *Pichia anomala* and *Trichosporon pullulans*) co-ferment the product, later followed by *Trichosporon cutaneum*, and finally dominated by *S. cerevisiae* (Aidoo et al. 2006).

FIGURE 4.2 *Idli*. (Picture by Sarkar, P.K., 1998.)

As a result of the fermentation, the pH decreases, amylases and proteases are formed that modify carbohydrates and degrade proteins, and considerable quantities of gas are produced. The latter cause a leavening of the batter and the final open and flexible structure of the steamed cake. Another important functional aspect of the yeasts is their contribution to the nutritional value, by enhanced vitamin B levels, and the decomposition of anti-nutrients such as phytic acid. The latter have a detrimental effect on mineral availability, and the uptake of iron is greatly enhanced in dephytinized foods (Towo et al. 2006).

4.2.1.3 Ragi *and* Men

Ragi (Indonesia) and *men* (Vietnam) are examples of amylolytic starters for rice wine preparation (Figure 4.3). Such starters principally consist of uncooked rice flour to which a local mix of herbs and spices is added. The flour and herbs are moistened with some water to form a dough, which are made into small balls or flattened discs, spread on an incubation tray, and sprinkled with previously powdered *ragi* or *men* (Dung et al. 2007). The microflora of *ragi* or *men* can now proliferate in the fresh dough during an overnight period at high relative humidity and temperatures around 30°C. The next stage consists of a careful dehydration to stabilize and preserve the microbial starter; this is done by incubation in an artificially heated room of about 60°C for a few days. For commercial purposes, the tablets are packaged and labeled. Several researchers investigated the microflora of these and similar amylolytic starters. From their combined efforts, it can be generally concluded that there are three components of the microbiota. The first component comprises filamentous fungi such as *Amylomyces rouxii* and *Rhizopus* spp. that are able to degrade the native starch in the raw rice flour, by forming amylases especially amyloglucosidase, which degrades starch directly into glucose (Dung et al. 2006). The second component consists of fermentative yeasts such as *Saccharomycopsis fibuligera*, *Hyphopichia burtonii*, and *S. cerevisiae*; whereas some of these yeasts can degrade starch as well, their main function is in the alcoholic fermentation and the production of flavor components such as esters. The third component comprises the LAB, which do not seem to have a positive contribution to the quality of the fermented wine. However, they are present as a natural accompaniment of yeasts in the manufacture of *ragi* and *men*.

The similar mixed starters common in the Himalayas are *marcha*, *phab*, and *hamei* (Tamang et al. 1996, Thapa and Tamang 2004, Tamang et al. 2007), which are

FIGURE 4.3 *Ragi* or *men*. (Picture by Nout, M.J.R., 1986.)

used by the ethnic people to ferment alcoholic beverages and drinks. Molds, mostly species of *Mucor* and *Rhizopus*, along with the amylolytic yeast *Sm. fibuligera* and alcohol-producing yeasts *S. cerevisiae* and *P. anomala* are the dominant organisms in these mixed starters along with LAB, *Pediococcus*, and *Lactobacillus* (Tsuyoshi et al. 2005).

4.2.2 Fermented Foods of Australasian Origin

4.2.2.1 Bubod

Bubod is a dried, powdered starter used in the Philippines to prepare *basi* or sugarcane wine and other fermented products. *Bubod* starter is prepared by mixing powdered rice and ginger and then rolled and shaped into discs. These are then coated with 1–3-month old *bubod*, placed in bamboo baskets, and incubated at room temperature for up to 48 h. They are sun dried to about 14% moisture. *Bubod* is then used to prepare an activated starter, *binubudan*, for the production of sugarcane wine, *basi*, which contains up to 14% alcohol. Bubod contains molds, yeasts, and LAB. Functional molds and yeasts in *bubod* include *Aspergillus oryzae*, *Mucor*, *Rhizopus* spp., *Sm. fibuligera*, *A. rouxii*, *H. anomala*, and *S. cerevisiae*. The molds and amylase-producing yeasts produce enzymes to release assimilable carbon compounds from the carbohydrates of the rice flour. The alcohol fermentation is dominated by *S. cerevisiae*.

4.2.2.2 Puto

Puto is a leavened steamed rice cake usually consumed for breakfast or as a snack food in the Philippines. The production of *puto* is an important cottage industry. It is closely related to the Indian *idli* except it contains no legumes and is generally served with grated coconut (Steinkraus 1996). It is made from year-old rice stock grains that are soaked and ground with water to produce slurry (*galapong*), which is allowed to undergo a natural fermentation. A portion of the slurry is set aside as starter culture (*lebadura*) that is added to the *galapong*. The organisms responsible for the unique characteristics of *puto* are heterofermentative LAB like *Leuc. mesenteroides* and yeasts like *S. cerevisiae*. The yeasts are usually low at the first stage of the fermentation but increase steadily during the process resulting in the production of small amounts of alcohol. *S. cerevisiae* also plays an important role in the leavening of the batter.

4.2.2.3 Tapuy

Tapuy is a traditional alcoholic drink popular in the mountains of northern Philippines. *Tapuy* is produced by inoculating cooked rice with rice yeast, *bubod*, and allowed to ferment in a jar in a cool place for 2 weeks. The fermented mass is then pasteurized for 30 min and stored for a minimum of 6 weeks; after that the clear liquor is decanted. The liquor undergoes a final pasteurization before bottling. The alcohol content reaches about 5.6% after 48 h and increases with fermentation. One kilogram of glutinous rice may produce about a liter of rice wine. Yeasts, molds, and LAB bring about fermentation of *tapuy* with yeasts as the predominant organisms. Yeasts isolated from *tapuy* include *Sm. fibuligera*, *Rhodotorula glutinis*, *D. hansenii*, *Pichia burtonii*, *Candida parapsilosis*, and *Tr. fennicum* (Sakai and Caldo 1985, Kozaki and Uchimura 1990). During the fermentation, yeasts like *Sm. fibuligera* and *P. burtonii*, which are also amylase producers, dominate the early stages. *Mucor* and *Rhizopus* spp. also produce amylolytic enzymes that hydrolyze the rice starch to sugars for conversion to alcohol. LAB, *Lactobacillus viridescens* and *Lb. brevis*, also proliferate during fermentation and contribute to the production of organic acids. In the Philippines, drinking *tapuy* is a part of traditional ritual.

4.2.3 Fermented Foods of African Origin

4.2.3.1 Mawè

Mawè is an acidic fermented maize dough popular in Bénin, West Africa; it is an intermediate product that is used to prepare a variety of beverages, porridges, and dishes (Figure 4.4). It is made by cleaning, washing, and decorticating maize kernels. The maize endosperm is ground finely by a wet-milling procedure. The wet flour is adjusted to dough consistency by the addition of water, and it is left at ambient temperatures (25°C–30°C) for 2–3 days, under a cover of polythene sheeting. During the period, a natural microbiological succession will take place of a mixed biota of LAB and yeasts. Gradually, the microflora will be dominated by a few heterofermentative LAB (e.g., *Lb. fermentum*) and several yeasts including *Issatchenkia orientalis*, *C. kefyr*, *C. glabrata*, *Kluyveromyces marxianus*, and *S. cerevisiae* (Hounhouigan et al. 1994). Experiments with pure culture starters showed that both LAB and yeasts derive benefit from the presence of the other microorganisms, resulting in better growth and acid production (Hounhouigan et al. 1999). The additional functionality of yeasts in cereal foods is the degradation of phytic acid, resulting in a better availability of mineral micronutrients for the consumer.

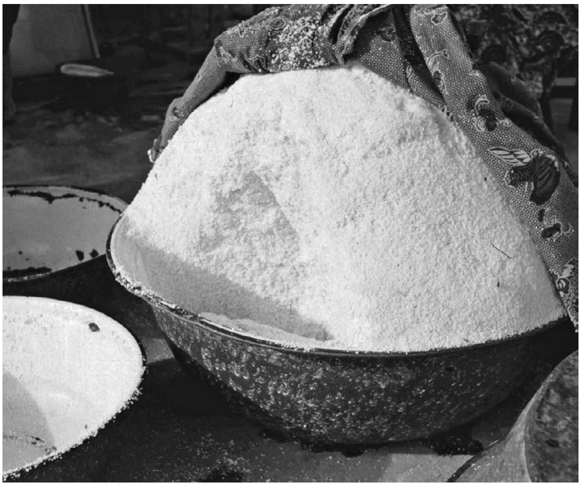

FIGURE 4.4 *Mawè* for sale at a market in Cotonou, Bénin. (Picture by Nout, M.J.R., 2002.)

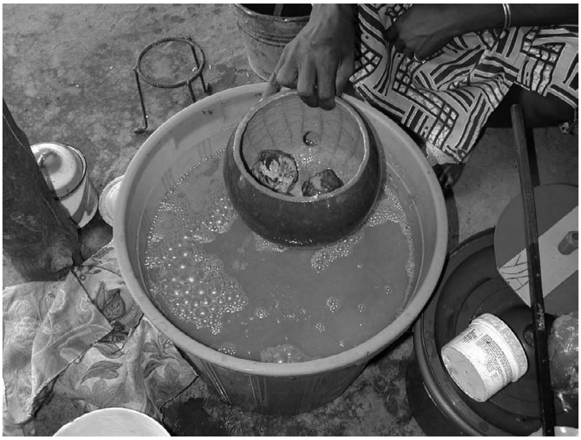

FIGURE 4.5 *Tchoukoutou* for sale, and calabash containing yeast starter. (Picture by Nout, M.J.R., 2006.)

4.2.3.2 Tchoukoutou

Tchoukoutou is one of the many different traditional African beers (Figure 4.5). It is made from red sorghum. First sorghum grains are cleaned, soaked, allowed to germinate (sprout) followed by a careful sun-drying in order to stabilize the obtained malt. After polishing and grinding the malt, it is mashed with water at a gradually increasing temperature until the final boiling, within a total period of 4–5 h (Kayode et al. 2007b). The wort obtained is decanted, cooled, and transferred to fermentation vessels that contain active yeast sediment. Extensive research on African beer yeasts has shown that most can be identified as *S. cerevisiae*. Although different genotypic clusters can be distinguished (beer, porridge, palm wine), these are distinctly different from the *S. cerevisiae* of industrial (European) origin (Jespersen et al. 2005). The alcoholic fermentation starts immediately. After 1 day, the beer is slightly alcoholic, effervescent, and sweet. Gradually, the sweet taste is replaced by alcohol and acidity. There is always a contaminant flora of acidifying bacteria (acetic and LAB) that limit the shelf-life to only 3 days. *Tchoukoutou* has a relatively low (3%–4% v/v) alcohol content and is locally regarded as a refreshing healthy beverage, which plays an important social role during market days and other festivities. In addition to the attractive sensory attributes, it was shown that the fermentation results in very good availability of iron (Kayode et al. 2007a).

4.2.3.3 Kachasu

Kachasu is an alcoholic spirit popular in Southern Africa, particularly Zimbabwe (Figure 4.6). Wild edible fruits locally called *masau* (*Ziziphus mauritiana*) are gathered from jungles as a part of the family diet in the rural areas. The fruits are also sold at the market to generate some income. Excess fruit is allowed to undergo natural fermentation, followed by distillation into *kachasu* (Figure 4.6). After fermentation, the pulp contains 2.1%–3.7% (v/v) alcohol, whereas the alcohol content of the distillate ranges from 23.8% to 45.6% (v/v) (Nyanga et al. 2008). A study was made of

FIGURE 4.6 *Kachasu*: distilling in a countryside in Zimbabwe. (From Nyanga, L.K. et al., *Ecol. Food Nutr.*, 47, 95, 2008. With permission.)

the yeasts on the fruits and during the fermentation of the fruit pulp. It was observed that the yeasts on the ripe fruit surface are dominated by *Aureobasidium pullulans*. This yeast was not detected anymore in the fermenting pulp which was populated by *S. cerevisiae* and *I. orientalis* (Nyanga et al. 2007). LAB are also encountered during this fermentation; these may stimulate yeast growth, and generate volatile flavor components.

4.2.4 Fermented Foods of European Origin

In this section, the roles of fungi, particularly yeasts, in the production of traditional bread, wine, beer, and other alcoholic beverages are not considered.

4.2.4.1 Sourdough

Although sourdough has a long history in Europe and North America, its origin goes back to the ancient Egyptian times. Sourdough (Figure 4.7) is a carbohydrate-based product with LAB and/or yeasts at low pH (<3.6). In bread dough, microorganisms, mainly LAB, predominate but yeasts also play a role in the development of organic acids, flavor, and nutrients. The yeasts tend to be acid tolerant and are unable to ferment maltose. It is thought that LAB benefit from yeast metabolites and the bacteria produce antimicrobial compounds for which yeasts are immune. In sourdough fermentation, the fermenting mass may be seeded with starter from previous batches and maintained at 20°C–30°C. Where such fermentation is propagated continuously, the microorganisms are usually heterofermentative, with the yeast *C. milleri* and *Lactobacillus sanfranciscensis* as predominant organisms. During fermentation, the lactobacilli produce maltose phosphorylase that hydrolyses maltose to glucose as a substrate for yeast. Lactobacilli also was reported to produce the antibiotic cycloheximide in the fermenting mass to which the yeast *C. milleri* is resistant (Sugihara et al. 1971). Wagner (2005) published on the production of sourdough bread to illustrate the role of industrial microorganisms in the food industry.

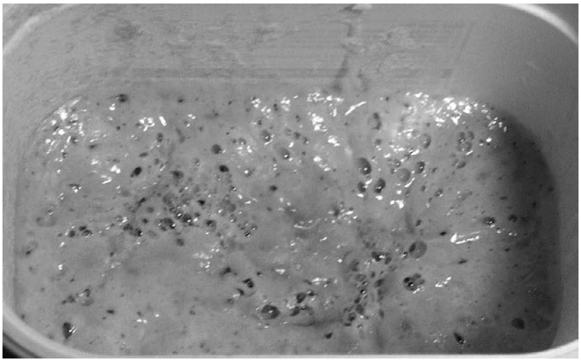

FIGURE 4.7 Sourdough. (From http://en.wikipedia.org/wiki/File:masa_madre.jpg, accessed March 20, 2009.)

4.2.4.2 Cheese

Mold-ripened cheeses are usually dominated by *Penicillium* spp. (Samson 1993). Camembert cheese is an example of a surface-ripened cheese with the mold *P. camemberti*. The cheese curd is sprayed with an aerosol of *P. camemberti* conidia and after brining and conditioning, the mold starts to develop at the surface during the incubation period. The starter strains have colors ranging from white to grayish blue and during ripening, lipolytic and proteolytic enzymes are produced by the mold that then diffuse into the cheese resulting in softening and the development of flavor. The product is ready for consumption in 3–5 weeks (Kosikowski 1997). Like molds, yeasts also play an important role in surface-ripened cheeses during maturation and develop a smear of growth on the surface that results in the development of flavor. The smear is a biomass of yeasts and bacteria, and the main yeasts species are *Trichosporon* spp., *Y. lipolytica*, *K. lactis*, and *Candida* spp. (Bockelmann and Hoppe-Seyler 2001, Petersen et al. 2002, Hansen and Jakobsen 2004).

4.2.4.3 Fermented Meats

In Europe, fermented meat products date back to Roman times and production spread to other European countries and the rest of the world; Europe is still a major producer and consumer of fermented dry sausages. LAB, molds, and yeasts all contribute to the development of the characteristic flavor and aroma of fermented meat and sausages. Molds, in particular *Penicillium* spp., are traditionally used as starter cultures. *Aspergillus* and *Eurotium* spp. also contribute to the fermentation and ripening of meat (Samson 1993, Josephsen and Jespersen 2004). Molds produce extracellular enzymes (amylases, proteases, lipases) and give a characteristic flavor and aroma. When carbohydrates becomes limiting in the protein-rich substrate, amino acids may be used as a carbon source. The most common molds used in mold-ripened fermented meat include *Penicillium nalgiovense*, *P. chrysogenum*, *P. camembertii*, and other molds include *P. commune*, *P. aurantiogriseum*, and *P. olsonii*. *P. nalgiovense* and *P. chrysogenum* are the main organisms in the production of salami. Yeasts produce proteolytic and lipolytic enzymes and help develop flavor in the fermentation of meat and meat products. *Debaryomyces polymorphus*, *C. zeylanoides*, *P. membranifaciens*, *P. guilliermondii*, and *Cryptococcus* spp. are known to be responsible for the ripening of cured ham.

4.2.5 Fermented Foods of Middle East Origin

4.2.5.1 Tarhana

Tarhana, a traditional Turkish fermented cereal food with sour acidic taste and yeast flavor, is produced principally by mixing wheat flour, yoghurt, yeast, vegetables (tomatoes, onions, green pepper, and paprika), salt, and spices (mint, thyme, dill, *tarhana* herb, etc.). *S. cerevisiae* and LAB (*Streptococcus thermophilus*, *Lb. lactis*, *Lc. diacetylactis*, *Lb. bulgaricus*, *Lb. acidophilus*, *Leuc. cremoris*, and *Lb. casei*) are the most important fermentative microorganisms. Studies on *tarhana* fermentation showed a significant increase in riboflavin, niacin, pantothenic acid, ascorbic acid, and folic acid contents of the product (Ekinci 2005). Ozdemir et al. (2007) reported that since *tarhana* is a good source of B vitamins, minerals, organic acids, and free amino acids with improved digestive properties, and since it is a product of yeast and LAB fermentation, it may be considered a functional and probiotic food. *Tarhana*-like products are known as *trahana* in Greece, *kishk* in Egypt, *kushuk* in Iraq, and *tahanya*/*talkuna* in Hungary and Finland.

4.2.5.2 Kefyr

Kefyr is an acidic, mild alcoholic fermented milk originating from central Asia but has become very popular in the Middle East. A review on the history and the symbiotic microflora of *kefyr* was reported by Fröhlich-Wyder (2003). Traditionally, *kefyr* was prepared from bags of goat hides of cow, sheep, or goat milk inoculated with the *kefyr* grain. The microflora of *kefyr* is known to be dependent on the fermentation process employed. However, Von Wiese (1986) and Koroleva (1991) reported that LAB and yeasts were the main symbiotic microflora. The production of *kefyr* is a two-stage process, namely fermentation occurring at 18°C–22°C for 18–20 h followed by a ripening process at 8°C–10°C for 1–3 days (Fröhlich-Wyder 2003). Yeasts like *K. marxianus*, *C. kefyr*, *S. cerevisiae*, and *S. delbreuckii* (*Torulaspora delbrueckii*) were isolated from *kefyr* (Wouters et al. 2002). On an industrial scale, addition of *kefyr* grains is rare; instead commercial mixed cultures isolated from the grains are used (Hansen and Jakobsen 2004).

4.2.5.3 Koumiss

Koumiss originates from Kazakhstan; it is a milk wine originally made from mare's milk; however, variants are now made from cow's milk (Figure 4.8). Koumiss usually contains about 2% alcohol and has a pH of about 4. The predominant microflora of *koumiss* are LAB and yeasts, particularly *S. unisporus* and *K. marxianus* (Hansen and Jakobsen 2004). Ni et al. (2007) isolated 87 yeast strains from traditional *koumiss* made from mare's milk in China. They also reported the two main yeasts as *S. unisporus* (48.3%) and *K. marxianus* (27.6%) with *P. membranifaciens* and *S. cerevisiae* accounting for 15.0% and 9.1% of the total yeast isolates, respectively.

FIGURE 4.8 *Koumiss*. (From http://en.wikipedia.org/wiki/File:kumis.jpg, accessed March 19, 2009.)

The starter culture used in traditional *koumiss* was thought to contain *Candida* spp. and LAB (Gordon 1997). Traditional *koumiss* has a uniform consistency; protein from mare's milk, unlike other types of milk, does not form visible curds when renneted. Although rennet is not used to make traditional *koumiss*, the acid produced during fermentation results in the formation of a fine precipitate that remains in suspension.

4.2.6 Fermented Foods of Latin American Origin

4.2.6.1 Pozol

Pozol is a Mexican fermented maize product. Traditionally, maize grains are boiled in lime water in order to enhance the swelling and ease of decortications. This method is referred to as "nixtamalization." The cooked grains are then dehulled, washed, and ground into wet flour that can be shaped into semicylindrical balls. These balls are wrapped in polythene or in banana leaves and undergo a natural fermentation for 2–5 days. During this period a complex microbiota develops, consisting of fungi and bacteria. As a result, the pH decreases to 3.5–4.0, and an acidic mixed flavor is formed. *Pozol* balls are used to make beverages by suspending them in water with added flavors such as salt, sugar, etc. (Figure 4.9). The microflora of *pozol* was investigated and was dominated by LAB (*Leuc. mesenteroides*, *Lb. plantarum*, *Lb. confusus*, *Lc. lactis*, and *Lc. raffinolactis*), yeasts, and the filamentous fungus *Geotrichum candidum* (*Galactomyces geotrichum*) (Nuraida et al. 1995). Although the latter could not hydrolyze starch, almost all of LAB and half of the yeasts studied degraded starch. This indicates that like in *ragi* and *men*, the substrate has a big impact on the natural selection and enrichment of the niche microbiota.

4.2.6.2 Pulque

Pulque is a fermented alcoholic beverage (Figure 4.10). Mexican *pulque* is made from agave juice (*Agave atrovirens* or *A. americana*). Essential microorganisms in the fermentation are *Lb. plantarum*, a heterofermentative *Leuconostoc*, *Zymomonas mobilis*, and *S. cerevisiae*. Other yeasts include *C. parapsilosis*, *C. rugosa*, *C. rugopelliculosa*,

FIGURE 4.9 A *pozol* ball and beverage made by diluting in water. (Picture by Wacher, C.)

FIGURE 4.10 *Pulque*. (From www.ianchadwick.com/tequila/pulque.htm, accessed March 6, 2009.)

Debaryomyces carsonii, *P. guilliermondii*, *P. membranifaciens*, and *Tor. delbrueckii*. Although *S. cerevisiae* appears to be the major producer of ethanol, it is *Z. mobilis* that transforms 45% of the glucose to ethanol (4%–6% v/v in final product) and carbon dioxide (Nout 2003).

4.2.6.3 Huitlacoche

Ustilago maydis is a basidiomycete that grows as a parasite on cobs of preharvest maize (Figure 4.11). The large fruiting body is edible, and is locally known as *caviar azteca*, *huitlacoche*, or "maize mushroom" (Valverde et al. 1995). In Mexico and other Latin American countries, *huitlacoche* is highly regarded as an interesting dish or condiment, containing diverse nutrients such as carbohydrates, proteins, fats, vitamins, and minerals. In addition, essential amino acids (especially lysine) and fatty acids (linoleate) are present in *huitlacoche*.

FIGURE 4.11 *Huitlacoche* (*U. maydis*) as grown on preharvest maize.

4.3 Benefits of, and Some Problems Associated with, Fungal-Fermented Foods

Fungal-fermented foods offer several benefits in relation to the nutrition and well-being of humankind. During the fermentation process, bio-enrichment of food materials occurs through a diversity of macro- and micronutrients, textures, enzymes, vitamins, trace elements, flavors, aromas, alcohols, and their derivatives. Food may also be preserved or attain an extended shelf-life through the production of alcohols, acids, esters, and other preservative compounds. The benefits of fungal-fermented foods also include improved digestibility and the production of essential nutrients, improved sensory properties, the production of edible fungal biomass (e.g., *Quorn* and *Huitlacoche*), natural food colors, carotenoids; furthermore, the processes involved in the fermentation system are usually simple.

Problems that may be associated with fungal-fermented foods include the formation of potential toxic substances, for example, ethyl carbamate produced as a result of yeast metabolism, mycotoxins (mold secondary metabolites) known to be carcinogens, yeasty off-flavors, film-forming yeasts, and undesirable discoloration. Many of the processes in fungal-fermented foods are based on solid substrate fermentation with various limitations such as mass and heat transfers, monitoring of fermentation parameters, and the relatively large inoculum size. However, the advantages of fungal-fermented foods outweigh the problems (Nout and Aidoo 2002, Nout 2003, Aidoo et al. 2006).

4.4 Conclusion

Yeasts and molds play a major role in the fermentation of foods during which there is bio-enrichment leading to the production of proteins, vitamins, minerals, aroma, alcohols, acids, esters, and also improvements in digestibility, preservation, and organoleptic properties. The authors have presented some examples of fermented foods that have received much attention and are produced with a high degree of technological advancement and automation, as well as some lesser-known and less-developed products. Some of the problems associated with fungal-fermented foods have also been highlighted. The majority of these fermented food products is of plant origin and could fulfill the ever-increasing worldwide demand for healthy foods, naturally fermented products, protein-rich meat substitutes, and exotic foods of plant origin. Although fungal-fermented foods are now receiving more attention, further developments are necessary to scale up and/or improve some of the lesser-known products to maximize substrate utilization, process control, yields, and hygiene.

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