Traditional fermented products from Africa, Latin America and Asia

Yeasts in Food - Beneficial and Detrimental Aspects

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17.1 Introduction

Fermentation is regarded as one of the oldest-known methods of food processing and preservation. Fermented foods and beverages are obtained by the action of micro-organisms (bacteria, yeasts and mycelial fungi) and their enzymes. Traditional fermented foods, also referred to as indigenous fermented foods, are those popular products that are known since early history and that can be prepared in the household or cottage industry using relatively simple techniques and equipment. The world-wide diversity of traditional fermented foods, their preparation methods and safety aspects are the subject of several textbooks and encyclopedia [1, 7, 61]. This chapter will deal with some of the documented foods of Africa, Latin America and Asia. As other chapters deal specifically with shoyu (Chapter 15), cocoa (Chapter 16), coffee (Chapter 16), wine (Chapter 14) and kefyr (Chapter 8), this chapter will not include those products.

Yeasts occur in a wide range of fermented foods, made from ingredients of plant as well as animal origin. When yeasts are present as an abundant group of organisms, they usually have a significant impact on food quality parameters such as taste, texture, odour, as well as nutritive value. There are many products however, in which yeasts take part in the fermentation in small numbers next to the presence of bacteria or mycelial fungi. Such mixed food fermentations sometimes are characterized by synergistic and very stable communities of e. g., yeasts and lactic acid bacteria (LAB), or yeasts and amylolytic mycelial fungi that depend on each other for nutrient supply [38]. On the other hand, the presence of some yeasts in fermented foods can result in undesirable reactions and can thus be considered as spoilage. The present chapter will deal with the occurrence of yeasts (biodiversity), their beneficial effects in fermented foods, as well as some cases of negative effects. Future prospects for development and industrialization will be discussed.

17.2 Yeast biodiversity related to specific fermented products

A large variety of yeasts can be found as functional flora in traditional fermented foods world-wide. Table 17.2-1 summarizes some major catogories of fermented foods and beverages. Some representative examples will be discussed in more detail.

In all continents, yeasts play a predominant role in the preparation of alcoholic beverages made from cereals or sugary juices. Likewise, fermented doughs and batters are encoun-

Table 17.2-1 Major categories of traditional fermented foods involving yeasts

Continent	Alcoholic beverages	Starters for alcoholic fermentation	Doughs and Batters	Miscellaneous
Africa	**made from maize, sorghum or millets, using malt for brewing:** busaa [36, 37], pito [56]), ting [34]. **palmwine** [65]	**natural fermentation (no starters added), or yeast grown on inoculation belt** [56]	**made from cereals (maize, sorghum, millets):** kenkey [50], kisra [42], mawè [23], **or root-crops (cassava)**: agbelima [2], fufu [48], lafun [42], **mostly fermented before cooking.**	**fermented milk products:** amasi [14], m'bannick [35], **fermented vegetables:** kawal [18], **non-or low-alcoholic cereal beverages:** togwa [42]
Latin America	**made from sugary juices:** aguardente [33], pulque [8, 60], toddy [30] found world-wide	natural fermentation	**made from maize, cooked before fermentation:** pozol [45]	**non-or low-alcoholic beverages:** sugary kefyr [38], tibicos [3, 8].
Asia	**made from rice, using amylolytic starters for brewing: beers or wines:** brem bali [39], chongju [29], ou [39], sake [29, 37], samsu [30], sato [39], shaohing [39], takju [29], tapuy [39], yakju [39], **or pasty snacks:** khaomak [29], peuyeum (= tape ketella) [29, 37], tapai pulut [29], tape ketan [9].	**yeasts present in mixed fungal amylolytic starters** [42]: bubod [52, 53], koji [39], murcha [63], nuruk [29], ragi-tape [37].	**made mostly from rice and leguminous seed flour (dal), fermented before steaming (some other cereals such as millets may be used):** dhokla [29], dosa [5], idli [43, 58, 66], jalebies [5], nan [39], phool waries [5], punjabi waries [5].	**fermented milk products: kumiss [32], fermented mass of leguminous seeds (mainly soybeans):** hamanatto [37], kinema [55], miso [37], tempe [41, 44], **non-or low-alcoholic beverages:** kombucha [37, 49, 64].

tered in all continents. These doughs and batters may be leavened (i. e., have a spongy texture due to gas produced) or not, but in most cases they are cooked or steamed prior to consumption as a staple food providing starch, energy and some protein. Unlike alcoholic beverages, doughs and batters are fermented by a majority population of bacteria usually dominated by lactic acid bacteria. Nevertheless, yeasts are present in significant numbers and contribute to flavour, texture and nutritive value of the products.

17.2.1 Alcoholic beverages

A distinction will be made on the basis of principles of generating fermentable sugars. Plant or fruit juices containing sufficient levels of glucose or sucrose are the easiest to ferment. They simply need to be inoculated, naturally from the environment or using an enrichment starter or pure culture. Pulque is an example of such a fermentation.

On the other hand, when cereals are used as ingredients, endosperm starch needs to be converted into fermentable sugars (maltose and glucose). This can be achieved using added amylolytic enzymes, sources of which are germinated (sprouted) cereal grains or mixed fungal amylolytic starters. In African beermaking, germinated sorghum and millets are commonly used in brewing; the preparation of pito beer from sorghum is a representative example. In most Asian countries, amylolytic starters are used in the form of starchy tablets containing mixed cultures of starch degrading moulds and yeasts. Such starters are used for the manufacture of beers, wines and pasty snacks from various kinds of rice, sorghum, and cassava. The preparation of takju in Korea, using a starter called nuruk is a representative example.

Mexican pulque (Figure 17.2-1) is made from Agave juice (*Agave atrovirens* or *A. americana*). Essential micro-organisms in the fermentation are *Lactobacillus plantarum*, a heterofermentative *Leuconostoc*, *Zymomonas mobilis* and *Saccharomyces cerevisiae*. Other yeasts include, *Candida parapsilosis*, *C. rugosa, C. rugopelliculosa, Debaryomyces carsonii, Pichia guilliermondii*, *P. membranifaciens* and *Torulaspora delbrueckii* [28]. 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 [60].

Pito beer (Figure 17.2-2) from Ghana is obtained by mixed activities of lactic acid bacteria and yeasts. It is a yellow to brown coloured sorghum beer that is obtained from previously germinated sorghum which is extracted, boiled and inoculated. Depending on the type of pito, the inoculation is achieved by immersing a woven "inoculation belt" (Figure 17.2-3) which allows entrapment of microbial cells. Other inoculation methods include back-slop-

cut *Agave* inflorescence
↓
collect juice
↓
inoculate with previous juice
↓
ferment 8-30 days (15-30°C)
↓
pulque
(white viscous, sour-alcoholic beverage)

Fig. 17.2-1 Manufacture of pulque (Mexico)

sorghum or maize grains
↓
soak and germinate
↓
dry and grind resulting malt
↓
prepare and boil malt extract
↓
inoculate (e.g. with belt)
↓
ferment
↓
serve **pito** beer

Fig. 17.2-2 Manufacture of pito (Ghana)

ping (addition of previous beer), or adding dried scum (foam) of previous beer [56]. After fermentation it typically contains 1.5–3.5 % v/v ethanol and 0.7–1.0 % w/w lactic acid and a corresponding pH of about 3.5.

Takju (Figure 17.2-4) is a Korean rice beer, which can also be prepared from other cereals. The nuruk starter is made by solid-state fermentation of wheat flour with *Aspergillus usamii*. After approximately 2 months fermentation, nuruk also contains *Rhizopus*, *Aspergillus niger* and yeasts such as *Debaryomyces hansenii*, *D. occidentalis, Pichia anomala, P. fabianii* and *Saccharomycopsis fibuligera*. Nuruk contains fungal amylolytic enzymes to saccharify starch (brewing), as well as the yeasts needed for alcoholic fermentation. Takju is a turbid beer with suspended insoluble solids and living yeasts, containing 7–10 % ethanol, approx. 1 % titratable acidity and has pH 4 after 3 days fermentation [29, 39].

17.2.2 Fermented doughs and batters

In Africa, fermented doughs form the basis for a variety of staple foods. These doughs are first fermented, then cooked.

Mawè, an uncooked fermented maize dough from Bénin, is not consumed as such but it is used as an ingredient for the preparation of a wide variety of beverages, cooked and fried meals and snacks. Its fermentation (Figure 17.2-5) is dominated by heterofermentative lactic acid bacteria (> 9 Log CFU/g) but a minority of yeasts (7–8 Log CFU/g) are important for the correct taste [23].

Fig. 17.2-3 Inoculation belt for pito fermentation

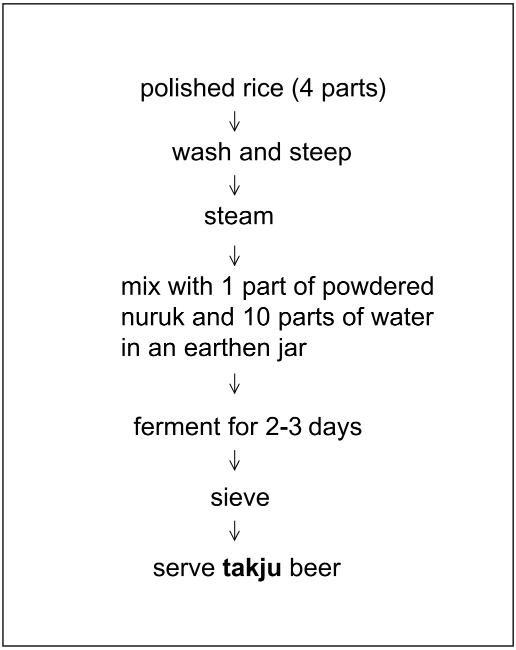

Fig. 17.2-4 Manufacture of takju (Korea)

Ghanaian Kenkey, a fermented and cooked stiff maize dough is fermented by mixed lactic acid bacteria and yeasts. Although yeasts (*Issatchenkia orientalis* and *S. cerevisiae*) are a minority with about 6–7 Log CFU/g compared to the LAB, they contribute to the taste and odour of kenkey [17, 42].

Nigerian Fufu is a product obtained by submerged cassava fermentation [48]. Yeasts are present in relatively high numbers (8 Log CFU/g) and comprise predominantly *Issatchenkia orientalis*, *Candida tropicalis* and *Zygosaccharomyces bailii*. These yeasts co-exist with lactic acid bacteria such as *Lactobacillus plantarum*. The growth of the latter was reported to be enhanced in the presence of *Issatchenkia orientalis*.

In Mexico, a typical alkaline maize dough (nixtamal) is fermented after having been cooked. Mexican pozol (Figure 17.2-6) is a refreshing beverage prepared from fermented nixtamal, which is a dough made from maize cooked in alkali. In this fermentation, which takes about 12–60 h, yeasts are a minority (2–7 Log CFU/g), and the fermented dough contains a majority of lactic acid bacteria resulting in pH 4.7–5.7 and 0.35–0.75 % titratable acidity. It was reported that 50 % of the yeasts isolated from this product can hydrolyze starch [8].

In Asia, leavened batters of rice and leguminous flour are obtained by fermentation. Subsequently they are steamed. Idli is popular throughout India and Sri Lanka because of its typ-

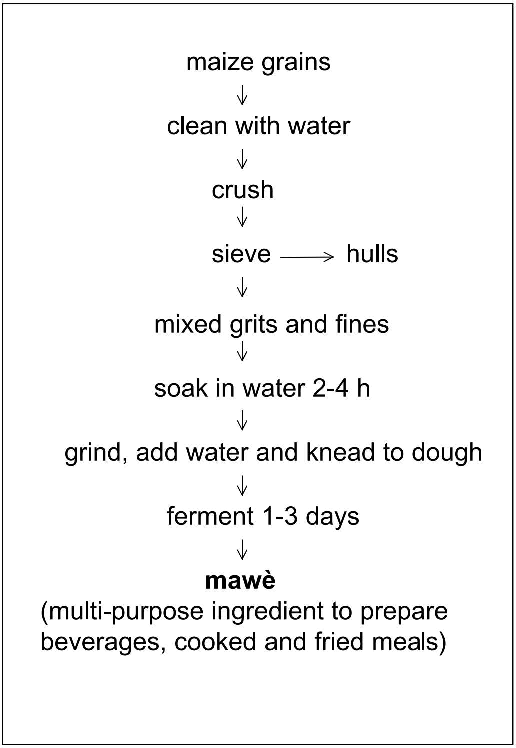

Fig. 17.2-5 Home-scale preparation of mawè (Bénin)

ical sour flavour and spongy texture, nutritional quality and improved digestibility. It is fed to infants as a weaning food, and as a main dish in diets in hospitals [43].

The main ingredients used in the traditional preparation of idli (Figure 17.2-7) are white polished rice (*Oryza sativa* L.) and black gram (*Phaseolus mungo* L.) dal, which are washed, soaked separately in water at room temperature for 5–10 hours before grinding in a stone mortar or other grinders. While rice is coarsely ground, the dal is ground to a fine smooth paste. The rice and dal slurries are mixed and stirred to form a thick batter. Salt is added to taste. The batter, put in a closed container, is kept at a warm place to ferment overnight or longer. The fermentation period must allow a definite leavening of the batter and development of a pleasant acid flavour. The fermented batter is poured in small cups or in a special idli pan having cups (8–10 cm diam), and steamed until the starch is gelatinized and the idli cakes are soft and spongy. The fermented batter is consumed the same day and there is no effort to preserve it.

Idli is a natural fermented food; no inoculum is added generally for fermentation. This is because the essential microorganisms have been found to be naturally present in the ingre-

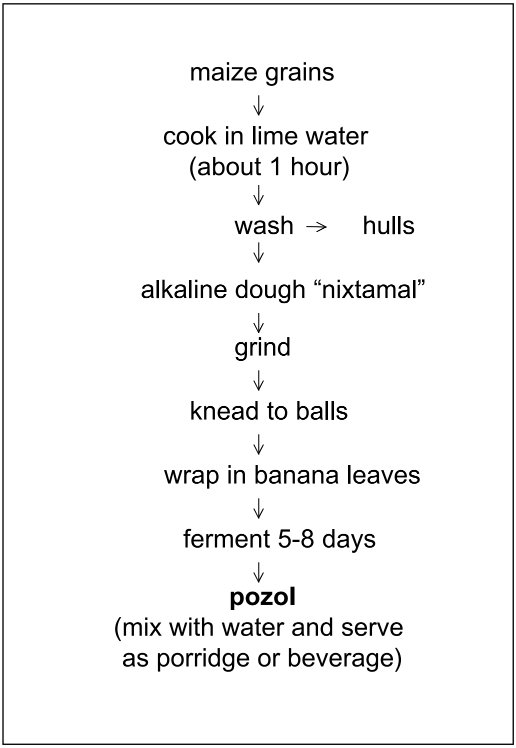

Fig. 17.2-6 Preparatio and use of pozol (Mexico)

dients. When the product is made daily, it is often the practice of adding a bit of freshly fermented batter (“backslop”) to the newly ground one. In addition to lactic acid bacteria (*Leuconostoc mesenteroides, Enterococcus faecalis*, *Lactobacillus fermentum* and *Pediococcus cerevisiae)* the slightly acid environment favours the growth and activity of yeasts, mainly *Saccharomyces cerevisiae*, *Debaromyoces hansenii* var. *hansenii*, *Pichia anomala*, *Candida saitoana* and *Trichosporon cutaneum* var. *cutaneum.*

The major functions of the fermentation include the leavening of the batter and the improvement of taste and nutritional value of idli. *Leuconostoc mesenteroides* is the main species responsible for the production of CO_2 which results in about 2–3 times increase in the original volume of batter [58].

17.2.3 Some other products

In western Sudan, kawal, sigda and furundu are fermented products made by solid-state fermentation from plant leaves and seeds [18]. The fermentation of kawal is dominated by *Ba-*

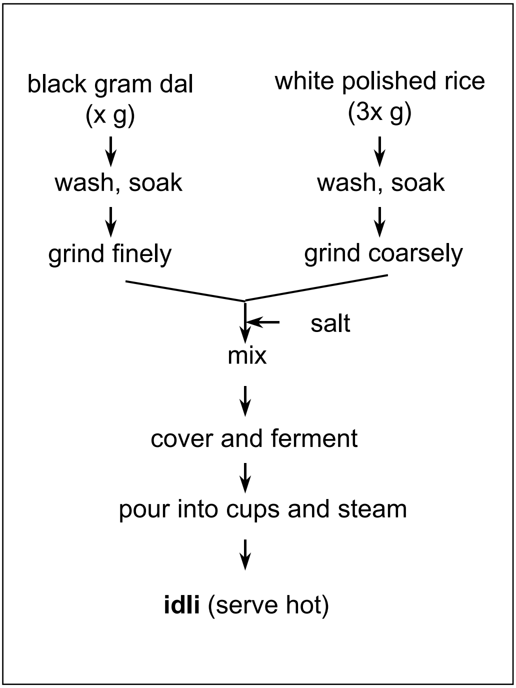

Fig. 17.2-7 Preparation of idli (India, Sri Lanka)

cillus subtilis, *Lactobacillus plantarum*, *Propionibacterium* sp. and *Staphylococcus sciuri*, and two yeasts *Issatchenkia orientalis* and *Saccharomyces* sp. During later stages of the fermentation, *Debaryomyces* and *Candida* spp. are detected in low numbers. The role of the yeasts is to degrade starch into fermentable sugars in the early stages of fermentation, and in later stages they consume lactic acid. It is not clear whether amylolytic yeasts are to be considered desirable or not for this type of fermentation.

Mexican tibicos, also called tibi grains, and probably similar to sugary kefyr, are microbiogleae consisting of dextran with embedded bacteria and yeasts (*Dekkera anomala, Pichia guilliermondii, P. membranifaciens* var. *membranifaciens, Cryptococcus albidus, Rhodotorula mucilaginosa* var. *mucilaginosa* and *Saccharomyces cerevisiae*) that live in symbiosis. Tibicos are used to prepare a kind of soft drink from sugar cane juice, containing approximately 3 % sugar, 0.6 % lactic acid and traces of alcohol and acetic acid, after 5 days of fermentation [51].

Kinema from Nepal and north-east India [55] is one of several "alkaline fermented foods" made from protein-rich seeds (e. g., soy beans) with a predominant *Bacillus subtilis* fermentation. During the fermentation a sticky mass is formed of pH 8.5 that is consumed as a flavouring condiment. Yeasts, particularly *Candida parapsilosis* are a minority (<5.2 Log CFU/g) in the microflora and their contribution to the product quality is still uncertain.

Tempe from Indonesia is a fungal solid-state fermented cake-like product, usually made from soy beans [41]. It's principal functional micro-organisms are *Rhizopus* and *Mucor* spp. but various bacteria and yeasts may be present, in relatively high (4–8 Log CFU/g) numbers. The predominant yeasts include *Debaryomyces, Rhodotorula, Candida, Pichia* and *Cryptococcus* spp. (VAN LAARHOVEN, unpubl. observ.).

Kombucha from Central and East Asia is a beverage obtained by fermentation of sweetened boiled tea with a mixed culture of yeasts and acetic acid bacteria [7].

17.3 Beneficial aspects of yeasts in fermentations

Yeasts can have several beneficial effects:

Functional effects, by the production of alcohol, gas, flavour and taste, as well as a contribution to food preservation by scavenging of sugars and other compounds that could otherwise serve as assimilable carbon sources for spoilage-causing micro-organisms. The production of alcohol improves the aroma of the product. In addition, alcohol at a certain concentration makes the substrate unsuitable for spoilage-causing microorganisms. This effect is increased in the presence of organic acids produced by bacteria. However, this preservative effect is not always a guarantee for long shelf-life as was observed in African beers such as Burukutu and Pito made from maize and sorghum [54]. These underwent spoilage within 72 h after the end of fermentation. The spoilage was associated with a decline of the yeast flora, and a concomitant increase and dominance of acetic acid bacteria including *Acetobacter aceti*, *A. pasteurianus* and *A. hansenii*.

The typical attractive volatiles produced by yeasts in fermentations of cereals such as maize include ethanol, propanol, 2-methyl-1-propanol, 3-methyl-1-butanol (isoamylalcohol), 2,3 butanediol, acetaldehyde, acetoin, diacetyl (2,3 butanedione), acetic acid, and ethyl acetate [10, 17, 40]. In agbelima, a fermented cassava dough [2], the volatile aroma was also attributed to the yeasts present, which produce various alcohols as well as ethylacetate and acetoin. In particular, the ability to assimilate or ferment the available carbohydrates, determines the evolution of attractive odours. These are produced almost exclusively by fermentation [40]. In Table 17.3-1, the ability to ferment glucose is mentioned for all relevant yeast species.

A study of palm wine volatile aroma [65] pointed out that *Saccharomyces* spp. were the major responsible organisms for the attractive odour. About 17 esters, 4 alcohols, 4 terpenes and 2 hydrocarbons were detected in the headspace volatiles.

Nutritional improvement due to increasing the digestibility of foods by the degradation of anti-nutritional factors such as phytate, and by the synthesis of nutrients such as vitamins. For example, *S. cerevisiae* and *Issatchenkia orientalis* occurring in African sorghum

Tab. 17.3-1 Yeast genera and species predominating in fermented foods

Genus	Species	Synonyms reported in literature sources	Fermentation of glucose	Traditional Fermented Foods
Candida	*fennica*	*Trichosporon fennicum*	+	tapuy
Candida	*glabrata*		+	idli, mawè
Candida	*inconspicua*	*Torulopsis inconspicua*	–	yakju
Candida	*intermedia* var. *intermedia*	*Candida intermedia*	+	tempe
Candida	*maltosa*		+	tempe
Candida	*parapsilosis* var. *parapsilosis*	*Candida parapsilosis*	+	bubod, kinema, pulque, tapuy
Candida	*rugopelliculosa*		+	pulque
Candida	*rugosa* var. *rugosa*	*Candida rugosa*	–	pulque
Candida	*saitoana*	*Torulopsis candida*	w	bubod, idli, dosa, dhokla
Candida	*sake*		v	aguardente, idli, tempe
Candida	*sake*	*Torulopsis sake*	v	yakju
Candida	spp.			brem bali, fufu, kombucha (= teekvass), lafun, ou, pito, pozol, sato, toddy
Candida	*stellata*		w	kombucha
Candida	*tropicalis* var. *tropicalis*		+	agbelima, bubod, fufu, idli, pito
Candida	*versatilis*	*Torulopsis versatilis*	w	hamanatto, idli, kombucha, miso
Clavispora	*lusitaniae*	*Candida lusitaniae*	+	amasi, tempe
Cryptococcus	*albidus* var. *albidus*	*Cryptococcus albidus*	–	tibicos
Cryptococcus	*humicola*		–	tempe
Debaryomyces	*carsonii*	*Pichia carsonii*	–	pulque
Debaryomyces	*hansenii* var. *hansenii*	*Candida famata*	v	nuruk, tibicos

Tab. 17.3-1 Continued

Genus	Species	Synonyms reported in literature sources	Fermentation of glucose	Traditional Fermented Foods
Debaryomyces	*hansenii* var. *hansenii*		v	idli, tapuy, tempe
Debaryomyces	*occidentalis* var. *occidentalis*		+	tempe
Debaryomyces	*occidentalis* var. *occidentalis*	*Schwanniomyces occidentalis*	+	nuruk
Debaryomyces	*polymorphus* var. *polymorphus*	*Pichia polymorpha*	v	yakju
Dekkera	*bruxellensis*	*Brettanomyces bruxellensis*	w	kombucha
Dekkera	*anomala*	*Brettanomyces claussenii*	+	tibicos
Hanseniaspora	*uvarum*	*Kloeckera apiculata*	+	pito, sugary kefir
Issatchenkia	*orientalis*	*Candida krusei*	+	busaa, fufu, idli, kawal, kenkey, kisra, mawè, phool waries, punjabi waries, togwa
Kluyveromyces	*africanus*		+	pito
Kluyveromyces	*marxianus* var. *marxianus*	*Candida kefyr*	+	kumiss, mawè
Kluyveromyces	*marxianus* var. *marxianus*	*Candida pseudotropicalis*	+	m'bannick
Kluyveromyces	*marxianus* var. *marxianus*	*Kluyveromyces fragilis*	+	kumiss
Kluyveromyces	*marxianus* var. *marxianus*		+	aguardente, kumiss, pulque
Lodderomyces	*elongisporus*		+	tempe
Pichia	*anomala*		+	bubod, fufu, murcha
Pichia	*anomala*	*Candida javanica*	+	amylolytic starters, idli, kombucha, murcha

Tab. 17.3-1 Continued

Genus	Species	Synonyms reported in literature sources	Fermen-tation of glucose	Traditional Fermented Foods
Pichia	*anomala*	*Hansenula anomala*	+	bubod, idli, jale-bies, koji, murcha, nuruk, pito, tape ketan, saké, yakju
Pichia	*fabianii*	*Candida fabianii*	+	nuruk
Pichia	*fabiani*	*Hansenula fabiani*	+	tempe
Pichia	*fermentans* var. *fermentans*	*Candida lambica*	+	sugary kefir
Pichia	*fluxuum*	*Candida mycoderma*	w	ting
Pichia	*guilliermondii*	*Candida guilliermondii*	w	bubod, pulque, tibi-cos
Pichia	*membranifaciens* var. *membrani-faciens*	*Candida valida*	w	sugary kefir, tibicos
Pichia	*membranifaciens* var. *membranifa-ciens*	*Pichia membranaefaciens*	w	pulque, ting
Pichia	*pini*		v	tempe
Pichia	spp		+	khaomak, tapai pu-lut
Pichia	*subpelliculosa*	*Hansenula subpelliculosa*	+	yakju
Rhodotorula	*glutinis* var. *glutinis*		–	tapuy
Rhodotorula	*minuta* var. *minuta*		–	tempe
Rhodotorula	*mucilaginosa* var. *mucilaginosa*		–	tempe
Rhodotorula	*mucilaginosa* var. *mucilaginosa*	*Rhodotorula rubra*	–	tibicos, ting

Tab. 17.3-1 Continued

Genus	Species	Synonyms reported in literature sources	Fermentation of glucose	Traditional Fermented Foods
Saccharomyces	*cerevisiae* var. *cerevisiae*		+	aguardente, amasi, amylolytic starters, bubod, busaa, chongju, fufu, idli, jalebies, kenkey, mawè, nan, palm wine, pito, pulque, punjabi waries, saké, shaohing, sugary kefir, takju, tibicos, ting, yakju
Saccharomyces	*cerevisiae* var. *cerevisiae*	*Saccharomyces chevalieri*	+	palm wine
Saccharomyces	*dairenensis*		+	amasi, tempe
Saccharomyces	*bayanus*	*Saccharomyces globosus*	+	kumiss
Saccharomyces	*exiguus*	*Torulopsis holmii*	+	idli
Saccharomyces	*kluyveri*		+	nan
Saccharomyces	spp		+	brem bali, kawal, khaomak, kombucha (= teekvass), m'bannick, ou, pito, sato, toddy
Saccharomyces	*unisporus*		+	bubod, kumiss
Saccharomycodes	*ludwigii* var. *ludwigii*		+	kombucha
Saccharomycopsis	*fibuligera*	*Candida lactosa*	w	samsu
Saccharomycopsis	*fibuligera*	*Endomycopsis fibuliger*	w	bubod, murcha, nuruk, peuyeum (= tape ketella), ragi-tape, tapai pulut, tapuy
Saturnispora	*saitoi*	*Pichia saitoi*	+	fufu
Schizosaccharomyces	*pombe* var. *pombe*		+	kombucha, pito
Schizosaccharomyces	spp		+	toddy

Tab. 17.3-1 Continued

Genus	Species	Synonyms reported in literature sources	Fermentation of glucose	Traditional Fermented Foods
Torulaspora	*delbrueckii*		+	kombucha, pito
Torulaspora	*delbrueckii*	*Candida colliculosa*	+	amasi, pulque
Torulaspora	*pretoriensis*		+	sugary kefir
Trichosporon	*cutaneum* var. *cutaneum*	*Trichosporon beigelii*	–	idli, tempe
Trichosporon	*cutaneum* var. *cutaneum*		–	pozol
Trichosporon	*pullulans*		–	idli
Yarrowia	*lipolytica*		–	tempe
Zygosaccharomyces	*bailii* var. *bailii*	*Zygosaccharomyces bailii*	+	fufu, kombucha
Zygosaccharomyces	*florentinus*		+	sugary kefir
Zygosaccharomyces	*rouxii*		w	hamanatto, miso
Zygosaccharomyces	*rouxii*	*Saccharomyces rouxii*	w	punjabi waries, ting
Zygosaccharomyces	spp.			agbelima

beer can contribute to human nutrition by the prodution of valuable proteins and amino acids [27]. Phytic acid and polyphenols occur as anti-nutritional factors in cereals such as pearl millet (*Pennisetum typhoideum*). Fermentations of cooked pearl millet [26] with single cultures of *S. cerevisiae* (reported as *S. diastaticus*) result in slight reductions of these compounds. Added impact can be obtained when mixed or sequential yeast-lactic acid bacteria fermentations are carried out, resulting in an improved protein efficiency ratio and higher digestibility values measured in in-vitro rat feeding studies [25].

Another health-related effect is found in the kombucha or "tea-fungus". The mixed yeast-bacterial culture growing on sugary tea extract accumulates lactic (0.1 %), acetic (traces) and gluconic (0.01–0.3 %) acids, and some ethanol (0.3 %). The pH decreases steadily to about 2.5 [49]. The resulting beverage is considered healthy, and it may be expected that in addition to the acids, some vitamins and minerals will be accumulated.

The antimutagenic activity of milk products fermented by various lactic acid bacteria was enhanced by co-culturing it with *Saccharomyces cerevisiae* [62]. Since the mechanisms of these phenomena are not known, it will be of interest to carry out more research on these functional aspects.

Safety improvement by contributing towards the degradation of potentially toxic naturally-occurring substances in food ingredients. For instance, cyanogenic glycosides such as amygdalase can be degraded efficiently by β-glycosidase [EC 3.2.1.21] activities (amygdalase and linamarase) produced by *Saccharomycopsis fibuligera* [6].

17.4 Detrimental aspects of yeasts in (fermented) foods

In particular, oxidative (non-fermenting) yeasts are associated with spoilage of (fermented) foods. Such yeast-associated spoilage can manifest itself as:

Degradation of organic acids, which can be achieved by assimilation or by direct oxidation, causing an increase of pH and concomitant loss of microbial stability [13].

Formation of yeasty off-odours. In fish paste fermentations, it was found that exclusion of oxygen enabled a better control of undesirable levels of yeast growth and resulting off-odours [4]. In tempe, *Cryptococcus humicola*, *Pichia* spp. and *Rhodotorula minuta* were associated with off-odours during the storage of tempe (van Laarhoven, unpubl. observ.). In miso, film-forming yeasts such as *Pichia* sp. and *Zygosaccharomyces rouxii* (reported as *Z. halomembranis*) are detrimental because of their strong unpleasant odour [12].

Formation of discolorations, turbidity and/or gas.

Formation of potentially toxic substances. Ethyl carbamate can accumulate in fermented products undergoing alcoholic fermentation followed by some form of heat treatment. Ethyl carbamate (urethane) is formed by the reaction of carbamic acid with ethanol [11]. Carbamic acid is a yeast metabolite of citrulline. Ethyl carbamate is carcinogenic; the highest levels (100–300 ppb, exceptionally 1 ppm) are found in distilled alcoholic beverages (brandy, bourbon, sake). In view of the relatively limited consumption of these beverages the risks of ethyl carbamate are considered small.

17.5 Physiological key properties

Physiological aspects of importance in the ecology of natural fermentations are the formation of functional enzymes, the assimilation of carbon and nitrogen sources, microbial interactions, tolerance to ethanol and lysis of cells.

Formation of functional enzymes to release assimilable carbon sources. Obviously these enzymes are valuable in brewing and flavour development. For example, glucoamylase (glucan 1,4-alpha-glucosidase) [EC 3.2.1.3] is a key enzyme in rice wine fermentation, converting starch directly into glucose. Glucoamylase from *Pseudozyma* (= Candida) *tsukubaensis* was reported to be constitutive and inducible by glucose, starch, maltose and glycerol [59]. Also, β-glucosidases formed by e. g. *Zygosaccharomyces bailii* [15] occur as extracellular and intracellular enzymes and can degrade a variety of polysaccharides. This ability would enable the yeast to mobilize assimilable carbon sources. On the other hand, glucosidase activity can also contribute to flavour development as a number of flavour precursors in fruits are glycosides.

Assimilation of wide or narrow ranges of carbon and nitrogen sources that correspond with the nutrients available in natural substrates. Using molecular typing techniques for *Saccharomyces cerevisiae*, it was observed that in naturally fermenting African maize dough, *Saccharomyces cerevisiae* strains are involved throughout the fermentation period [20]. The ability to assimilate galactose, saccharose, lactate, raffinose, maltose and glucose was common in these isolates and corresponds very well with the naturally occurring assimilable carbohydrates in uncooked maize. The same group investigated the occurrence of *Issatchenkia orientalis* [21], which is also a fermentative yeast that contributes to the attractive flavour of maize dough.

Microbial interactions that improve chances for survival and growth. Examples are the proto-cooperative interaction of yeasts and lactic acid bacteria in sourdoughs [38] and the formation of killer-toxins by yeasts that reduce competitive yeasts e. g., in wine fermentations [38].

Tolerance to ethanol, especially related to cytoplasmic membrane composition and of practical importance in alcoholic fermentations that must yield high (> 15 % v/v) levels of alcohol content.

Lysis of yeast cells is associated with many of the nutritional benefits but also to enzymatic spoilage phenomena. The lysis is determined by proteases that influence the hydrolysis and solubility of complexes, not involving the cell wall as such [68].

17.6 Future prospects and conclusions

The traditional foods mentioned in this chapter are well accepted, affordable and use local resources. It is important to ensure that their quality and safety meet the requirements of present-day and future consumers. Upgrading traditional home-scale processes is needed so that they can compete successfully with imported products. Whereas small-scale manufacture has advantages of short distribution lines, income generation for families etc., urbanization and the resulting growing demand for ready-to-consume foods requires larger-scale industrial production. Examples of industrialized traditional fermented foods are: alcoholic pastes and rice wines in Asia, such as tapai which is now produced at a small cottage scale in Malaysia using commercially available pure culture starters of the starch degrading mould *Amylomyces rouxii* and the yeast *Saccharomycopsis fibuligera* [31]; palm wine and sorghum beer [16] in S. Africa are prepared at industrial scale in processes involving souring of sorghum mash with *Lactobacillus delbrueckii* at 48–50 °C. After boiling and straining the obtained wort, alcoholic fermentation is performed at 20–35 °C using pure strains of *Saccharomyces cerevisiae*; African doughs such as mawè in Bénin, in which the performance of added starter cultures was tested. Although mawè can be prepared using only lactic acid bacteria such as *Lactobacillus brevis*, the addition of *Issatchenkia orientalis* (commonly found in maize dough) enhances the growth of the lactic acid bacteria and the performance of the fermentation [24]. Although it was observed that traditionally fermented dough had a better flavour, added starter cultures can be very useful in semi-industrial settings to achieve predictability of short fermentation times. Mageu, a non-alcoholic sour maize porridge, is produced at an industrial scale in South Africa. A similar product is known in Kenya as uji. Although these porridges are fermented using lactic acid bacteria only, yeasts mainly *Pichia* spp., as well as *Acetobacter liquefaciens* are involved in the process as spoilage microorganisms [22]. Spoilage yeasts are kept under control using high fermentation temperatures and chemical preservatives such as benzoate, sorbate and propionate. Ogi, a sour fermented starch cake processed from maize, sorghum or millet grains has been industrialized in Nigeria [47]. The fermentation is not inoculated and depends on the natural fermenting flora in which *Lactobacillus plantarum* is considered essential. However, yeasts such as *Saccharomyces cerevisiae* and *Pichia fluxuum* contribute to the acceptability of the flavour. A cost analysis showed that inoculation with pure culture starters would be unacceptably expensive, considering the infrastructure needed to propagate and maintain appropriate quality and safety of such cultures.

Yeast products such as enzymes, vitamins of the B-group, trace elements (Selenium, Chromium), glycans, flavour components and carotenoid pigments [19] occur in traditional foods, but could be exploited more effectively as purified substances and food ingredients. New processing methods including the use of immobilized yeast cells, are promising for obtaining a higher efficiency of starch degradation and ethanol production in rice wines [57]. The development of starters for commercial processes continues. Miso, a fermented salted paste of soybeans, rice and barley, is produced at a large industrial scale. The fermentation takes place in two stages, a mould solid state fermentation initiated by the inoculation with a koji starter, and a brine fermentation during which halophilic lactic acid bacteria (*Tetragenococcus halophila*) and yeasts (*Zygosaccharomyces rouxii* and *Candida versatilis*) are essential for acidity and flavour development. These are grown as defined mixed cultures and are available commercially for processing [12]. Modern molecular biotechnology for starter culture development resulted in the insertion of the α-amylase gene of *Saccharomycopsis fibuligera* into *Saccharomyces cerevisiae* [67] with the advantage of more rapid growth and fermentation. Another example is the insertion of a synthetic gene for lysine into *Saccharomyces* sp. [46]. It was shown that lysine was overproduced and excreted. When used as a fermentation starter culture, the yeast could be used to enrich protein-poor products such as fufu. However, in view of the cost aspects of industrial production, it is doubtful whether such expensive GMO techniques could be applied in practice.

In conclusion, a wide variety of yeasts is involved in traditional fermented foods. Those that contribute to desirable product properties require characterization in view of more efficient exploitation, whereas the undesirable yeasts need further study in order to develop consumer-friendly strategies to avoid their metabolic activity.

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