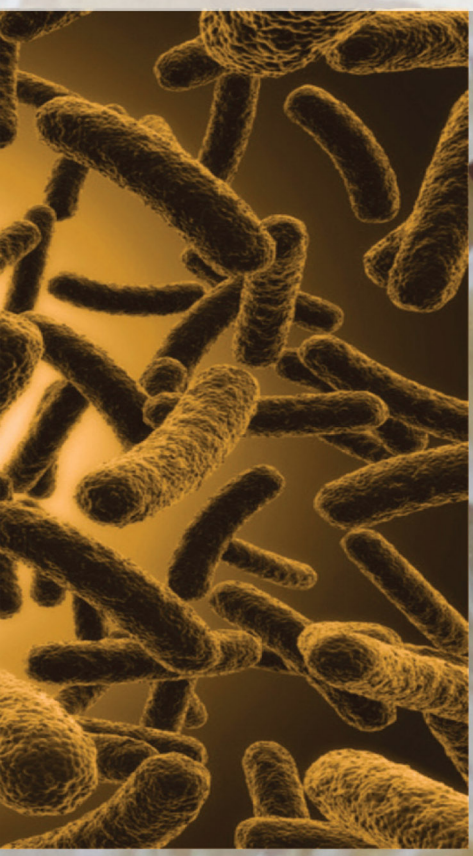
Food Biology Series

Microorganisms and Fermentation of Traditional Foods



Editors
Ramesh C. Ray
Didier Montet

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About the Series

Food is the essential source of nutrients such as carbohydrates, proteins, fats, vitamins, and minerals, that provides energy for all living organisms to sustain life. A large part of the daily human efforts is concentrated on food production, processing, packaging and marketing, product development, preservation, storage, and ensuring food safety and quality. It is obvious therefore, our food supply chain can contain microorganisms that interact with the food, thereby interfering in the ecology of food substrates. The microbe- food interaction is mostly beneficial (as in the case of many fermented foods such as cheese, butter, sausage, etc.), but in some cases it is detrimental (spoilage of food, mycotoxin, etc.). The series *Food Biology* aims at bringing all these aspects of microbe-food interactions in form of topical volumes, covering food microbiology, biochemistry, microbial ecology, food biotechnology, new food product developments with microbial interventions, mycology, food authenticity, food origin traceability, and food science and technology. Special emphasis is placed on including new molecular techniques in food biology research or on monitoring and assessing food safety and quality, as well as new interventions in biotechnological applications in food processing and development. The key topics include food fermentation, food safety and hygiene, microbial interventions in food processing and food additive development, molecular diagnostic methods in detecting food borne pathogens and food policy, etc. Leading international authorities with background in academia, research, industry and government will contribute or have contributed to the series either as authors or as editors. The series will be a useful reference resource base in food microbiology, biochemistry, biotechnology, food science and technology for researchers, teachers, students and food science and technology practitioners.

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Preface

Fermentation is one of the oldest technologies for processing of food and beverages to improve qualities such as extended shelf-life and organoleptic properties. Fermented foods usually have an improved microbial stability and safety, alongwith acceptable taste, and some products can be stored even at ambient temperatures. The common microorganisms involved in food fermentations are bacteria, yeasts and moulds. The lactic acid bacteria, notably lactobacilli and streptococci are the most commonly found microorganisms in fermented foods, having the ability to produce lactic acid from carbohydrates. Other important bacteria in fermented foods are the acetic acid producing *Acetobacter* and the *Bacillus* species. The most important beneficial yeasts in terms of desirable food fermentations belong to the *Saccharomyces* family, especially *S. cerevisiae*. These yeasts play a crucial role in the food industry as they produce enzymes that bring about various desirable biochemical reactions involved in the production of alcoholic beverages. Also, few fungi are usually used to produce a number of popular cheeses.

The role of fermented foods in human health and well being is a matter of recent interest, particularly the involvement of probiotic bacteria that include several members of lactobacilli and bifidobacteria. Probiotics are live microorganisms that confer beneficial effects on host such as anti-microbial activity, improvement in lactose metabolism, reduction in serum cholesterol, immune-modulation properties etc, when administered in appropriate quantities. All the traditional fermented foods are rich sources of beneficial microorganisms and some of them show probiotics characteristics. Fermented foods especially dairy products, play a predominant role as carriers of probiotics.

The book aims at providing comprehensive information about the involvement of microorganisms in fermented foods. It contains 11 chapters covering the history, current scenario and future prospects of fermented foods; microbial diversity in fermented foods and health benefits; microbiology, biochemistry and biotechnology of fermented products processed from plant-(cereals, vegetables and fruits) and animal-based raw materials (milk, fish and meat). It also covers the traditional fermented foods

and beverages of Oriental, African and Latin-American countries, as well as their culinary practices, microorganisms involved and health benefits either claimed or scientifically proven. The last chapter discusses the food safety issues associated with traditional fermented foods and also the intervention procedures to improve the safety of fermented foods.

The book brings out updated scientific information and new research findings in microbiology, biochemistry, food science and technological aspects of fermented foods. The 11 chapters have been authored by 19 outstanding international contributors in the forefront of fermented food science and technology. We believe the book will be a useful reference book and valuable resource for researchers, teachers, students, nutritional and functional food experts and all those working in the field of fermented foods.

Ramesh C. Ray
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Contents

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1

Fermented Foods: Past, Present and Future

Ramesh C. Ray[1,*] and *V.K. Joshi*[2]

1 Introduction

Fermentation is one of the oldest biotechnologies for the production of food products with desirable properties such as extended shelf-life and good organoleptic properties (Smid and Hugenholtz 2010). Finished fermented foods usually have an improved microbial stability and safety and some can be stored even at ambient temperatures. Furthermore, there are several examples of fermentation processes which lead to an increase in nutritional value or digestibility (Jägerstad et al. 2005) of food raw materials. Finally, food fermentation processes also deliver products with increased palatability for consumers. All these arguments have boosted the interest to explore natural food fermentation processes and more precisely to link the diversity of the community of fermenting microbes and their properties to the energetics of the process and to product quality.

From a biochemical point of view, fermentation is a metabolic process of deriving energy from organic compounds without the involvement of an exogenous oxidizing agent. Fermentation plays different roles in food processing. Major roles attributed to fermentation are: (1) Preservation of food through formation of inhibitory metabolites such as organic acid (lactic

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acid, acetic acid, formic acid, and propionic acid), ethanol, carbon dioxide, diacetyl, reutrin, bacteriocins, etc., often in combination with decrease of water activity (by drying or use of salt) (Gaggia et al. 2011); (2) improving food safety through inhibition of pathogens (Adams and Nicolaides 2008) or removal of toxic compounds (Ray and Panda 2007); (3) improving the nutritional value (Poutanen et al. 2009, van Boekel et al. 2010); and (4) organoleptic quality of the food (Lacroix et al. 2010, Sicard and Legras 2011).

The common groups of microorganisms involved in food fermentations are bacteria, yeasts and moulds. The most important bacteria in the fermentation of foods are the *Lactobacillaceae*, which have the ability to produce lactic acid from carbohydrates. Other important bacteria are the acetic acid producing *Acetobacter* (mainly from fermentation of fruits and vegetables) and *Bacillus* (from fermentation of legumes) species. The beneficial yeasts in terms of desirable food fermentation are from the *Saccharomyces* family, especially *S. cerevisiae*. Yeasts play an important role in the food industry as they produce enzymes that result in desirable biochemical reactions such as the production of wine, beer and ethanol, and leavening of bread (Sicard and Legras 2011). The lactic acid bacteria (LAB) are, however, the most commonly found microorganisms in fermented foods (Sengun and Karabiyikli 2011). Their crucial importance is associated with their physiological features such as substrate utilization, metabolic capabilities and probiotic properties. Their common occurrence in foods coupled with their long historical use contributes to their acceptance as GRAS (Generally Recognized As Safe) for human consumption (Silva et al. 2002). The various LAB have been isolated from different fermented foods. Their functions during or after food fermentation have gradually been elucidated. This chapter focuses briefly on the types of microorganisms involved in food fermentations, especially on the roles of LAB in fermented foods. In addition, the current research activities in the field of fermented foods are also discussed. The roles of other microorganisms such as yeasts and moulds in food fermentations have been reviewed briefly.

2 Microorganisms Involved in Food Fermentations

The most common groups of microorganisms involved in food fermentation are:

- Bacteria
- Yeasts
- Moulds

2.1 Bacteria

Several bacteria are present in foods, the majority of which are concerned with food spoilage, while some like *Clostridium* are the causative agent for production of toxin like botulin, causing botulism in man (Joshi et al 2006). As a result, the important role of bacteria in the food fermentations is often overlooked. Lactic acid bacteria like *Lactobacillus, Pediococcus, Streptococcus, Oenococcus,* etc. are the most important bacteria in fermented foods, followed by *Acetobacter* species, which oxidize alcohol to acetic acid. The acetic acid fermentation has been used extensively to produce fruit vinegars including cider vinegar (Joshi and Thakur 2000, Joshi and Sharma 2010). A third group of bacteria of significance in fermentation are the *Bacillus* species (*Bacillus subtilis*, *B. licheniformis* and *B. pumilus*), which bring about alkaline fermentation. *Bacillus subtilis* is the dominant species causing the hydrolysis of protein to amino acids and peptides and releasing ammonia, which increases the alkalinity and makes the substrate unsuitable for the growth of spoilage organisms. Alkaline fermentations are more common with protein-rich foods such as soybeans and other legumes, although there are few examples utilizing plant seeds. For example, water melon seeds (ogiri in Nigeria) and sesame seeds (ogiri-saro in Sierra Leone) are the substrates for alkaline fermentation (Battcock and Azam Ali 2001).

2.2 Yeasts

Yeasts and yeast like fungi are widely distributed in nature. They are present in orchards and vineyards, in air and soil, and in the intestinal tract of animals. Like bacteria and moulds, yeasts can have beneficial and non-beneficial effects in food fermentations. Some of the yeasts like *Pichia* are viewed as spoilage of food products while those like *Candida* are utilized for the single cell protein production. The most beneficial yeast in terms of desirable food fermentations are from the *Saccharomyces* family, especially *S. cerevisiae* involved in bread making and alcohol in wine fermentations. *Saccharomyces cerevisiae* var. *ellipsoideus* is employed extensively in wine making (Joshi et al. 2011). *Schizosaccharomyces pombe* and *S. boulderi* are the dominant yeasts in the production of traditional fermented beverages, especially those derived from maize and millet (Battcock and Azam Ali 2001). *Saccharomyces cerevisiae* var. *carlbergenisis* is the yeast involved in beer production. *Schizosaccharomyces pombe* has been found to have capacity to degrade malic acid into ethanol and carbon dioxide, and has been used successfully to lower the acidity in the grape and plum musts (Vyas and

Joshi 1988, Joshi et al. 1991). A number of yeasts like *Rhodotorula*, *Crptococcus* have capacity to produce pigment to be used as biocolur (Joshi et al. 2003).

2.3 Moulds

Moulds are also important organisms in food processing both as spoilers and preservers of foods. Many moulds have capacity to produce enzymes of commercial importance such as pectinase by *Aspergillus niger* (Joshi et al. 2006). Species of *Aspergillus* are involved in the production of citric acid from waste like apple pomace (Joshi et al. 2009, Joshi and Attri 2006). The *Aspergillus* species are often responsible for undesirable changes in foods causing spoilage. On the other hand, *Penicillium* species are associated with the ripening and flavour development in cheeses. While the species of *Ceratocystis* are involved in fruit flavour production, at the same time, *Penicillium* is the causal agent for production of toxin like patulin (Joshi et al. 2013).

3 History of Fermented Foods

Fermentation as a food processing technique can be traced back to thousands of years (Table 1). The history of fermented foods is lost in antiquity. It seems that the art of fermentation originated in the Indian Sub-continent, in the settlements that predate the great Indus Valley civilization (Prajapati and Nair 2003). The art of cheese making was developed as far back as 8000 yr ago in the fertile Crescent between Tigris and Euphrates rivers in Iraq, at a time when plants and animals were just being domesticated (Fox 1993). Later, alcoholic fermentations involved in wine making and brewing are thought to have been developed during the period 4000–2000 BCE by the Egyptians and Sumerians. The Egyptians also developed dough fermentations used in the production of leavened breads way back 4000–3500 BCE (Prajapati and Nair 2003). However, the scientific rationale behind fermentation started with the identification of microorganisms in 1665 by van Leeuwenhoek and Hooks (Gest 2004). Louis Pasteur revoked the "spontaneous generation theory" around 1859 AD by elegantly designed experimentation (Farley and Geison 1974). The role of a sole bacterium "bacterium" lactis (*Lactococuus lactis*), in fermented milk was shown around 1877 by Sir John Lister (Santer 2010). Fermentation, from the Latin word Fevere' was defined by Louis Pasteur as "la vie sans l'air" (life without air). Coincidentally, this was the time of the industrial revolution in Europe which resulted in large scale migration of populations from villages to larger cities. There was therefore a dramatic shift from the food production for local communities to large scale food production, necessary to meet the requirements of expanding

Table 1. Milestones in the history of fermented foods.

Milestone	Development/Location
6000–4000 BCE	Dahi—Coagulated sour milk eaten as a food item in India
7000 BC	Cheese production in Iraq, following the domestication of animals
6000 BC	Wine making in the Near East
5000 BC	Nutritional and health value of fermented milk and beverages described
4000 BC	Egyptians discovered how to use yeasts to make leavened bread and wine
2000 BCE-1200 CE	Different types of fermented milks from different regions
1750 BCE	Sumerians fermented barley to beer
1500 BCE	Preparation of meat sausages by ancient Babylonians
500 BCE	Mouldy soyabean curd as antibiotic in China
300 BCE	Preservation of vegetables by fermentation by the Chinese
500–1000 CE	Development of cereal-legume based fermented foods
1276 CE	First whisky distillery established in Ireland
1500 CE	Fermentation of sauerkraut and yoghurt
1851 CE	Louis Pasteur developed pasteurization
1877 CE	*Bacterum lactis* (*Lactococcus lactis*) was shown in fermented milk by John Lister
1881 CE	Published literature on koji and sake brewing
1907 CE	Publication of book *Prolongation of Life* by Eli Metchnikoff describing therapeutic benefits of fermented milks
1900–1930 CE	Application of microbiology to fermentation, use of defined cultures
1928 CE	Discovery of nisin—antagonism of some lactococci to other LAB shown by Rogers and Whittier
1970 CE–present	Development of products containing probiotic cultures or friendly intestinal bacteria
1953 CE	Nisin marketed in UK and since approved for use in over 50 countries
1990 CE–Present	Deciphering of genetic code of various LAB isolated from fermented foods
2002 CE	First authoritative list of microorganisms to be used in dairy culture was released by IDF and EFFCA
2012 CE	The list of Microbial Food Cultures regarded as GRAS to be used in all types of food fermentations has been released by IDF and EFFCA

Source: Ross et al. (2002), Prajapati and Nair (2003), updated

and more distant markets. This in turn led to the development of large scale fermentation processes for commercial production of fermented foods and alcoholic beverages, with the most widely used microorganisms including yeast for the production of beer, wine and spirits, and LAB for a variety of

dairy, vegetable and meat fermentations (Ross et al. 2002). Modern large scale production of fermented foods and beverages is dependant almost entirely on the use of defined strain starters, which have replaced the undefined strain mixture traditionally used for the manufacture of these products. This switch over to defined strains has meant that both culture performance and product quality and consistency have been dramatically improved, while it has also meant that a smaller number of strains are intensively used and relied upon by the food and beverage industries. This intensive use of specific starters has, however, some drawbacks and can lead to production problems resulting in unsatisfactory strain performance. In the case of lactococcal fermentations, bacteriophage proliferation can affect cheese starter performance (Klaenhammer and Fitzgerald 1994).

In 1928 CE, Rogers and Whittier discovered nisin produced by some LAB and demonstrated its antagonistic activity against other food-borne bacterial pathogens. In 2002, a complete list of microorganisms that can be used as safe microbial food culture in dairy industry has been released by the International Dairy Federation (IDF) (Mogensen et al. 2002a, 2002b). The "2002 IDF inventory" has become a *de facto* reference for food cultures in practical use. In 2002, an updated inventory of microorganisms (bacteria, fungi, filamentous fungi and yeasts) used in food fermentations covering a wide range of food matrices was prepared by the members of IDF Task force (Bourdichon et al. 2012).

4 Advantages of Food Fermentation

Food fermentations have been practiced for millennia resulting the existence of a tremendous variety of fermented foods ranging from those derived from cereals, fish and meat to those derived from milk and dairy products (Table 2). In each case, the fermentation process involves the oxidation of carbohydrates to generate a range of products which are principally organic acids, alcohol and CO_2 (Ray and Panda 2007). Such products have a preservative effect through limiting the growth of spoilage and/or pathogenic flora in the food product (Dalié et al. 2010). In addition, a number of desirable products, which affect the quality of the food may be produced, including the flavour compounds diacetyl and acetaldehyde (Ross et al. 2002, Jacqes and Casergola 2008), as well as compounds which may have positive health implications such as vitamins, antioxidants and bioactive peptides (Hugenschmidt et al. 2010). When considering food fermentations (as distinct from alcoholic fermentations involving yeast), the LAB is primarily responsible for many of the microbial transformations found in the more common fermented food products (Table 2). This group is composed of a number of genera including *Lactoctoccus, Lactobacillus*, *Enterococcus, Streptococcus, Leuconostoc* and *Pediococcus,* and generally

Table 2. Representative examples of fermented foods.

Product	Country	Microorganism(s)	Substrate
Bread	International	*Saccharomyces cerevisiae*, other yeasts, lactic acid bacteria (LAB)	Wheat, rye, other grains
Cheese	International	LAB (*Lactobacillus lactis, Streptococccus thermophilus, Lb. shermanii, Lb. bulgaricus*), *Propionibacterium shermanii*, sometimes moulds (*Penicillium* spp.)	Milk
Fufu	West Africa	LAB, *Citrobacter freundii*, *Geotrichum* sp., *Candida* sp. and *Saccharomyces* sp.	Cassava root
Gari	West Africa	*Corynebacterium manihot*, yeasts, LAB (*Lb. plantarum, Streptococcus* spp.)	Cassava root
Idli	Southern India	LAB (*Leuconostoc mesenteroides, Enterococcus faecalis*), *Torulopsis, Candida, Trichosporon pullulans*	Rice and black gram
Injera	Ethiopia	Yeasts, some fungi including *Pullaria* sp., *Aspergillus* sp., *Penicillium* sp., *Rhodotorula* sp., *Hormodendrum* sp., *Candida* sp. and number of unidentified bacteria	Cereals such as teff and sorghum
Kefir	North Africa	LAB	Milk
Kenkey	Ghana	LAB (*Pediococcus cerevisiae*, *Leuconostoc mesenteroides*, and *Lc. fermentum*	Maize
Kimchi	Korea	LAB	Cabbage, vegetables, sometimes seafood, nuts
Nan	India	*Saccharomyces cerevisiae*, LAB	White wheat flour
Ogi	Nigeria, West Africa	Lactic bacteria *Cephalosporium, Fusarium, Aspergillus, Penicillium* spp., *Saccharomyces cerevisiae, Candida mycoderma, C. valida*, or *C. vini*	Maize
Olives	Mediterranean	*Lc. mesenteroides, Lb. plantarum*	Green olives
Pickles	International	*Pediococcus cerevisiae, Lb. plantarum*	Cucumber
Plara	Thailand	*Bacillus* sp., *Bacillus cerus, B. circulans, B. licheniformis, B. megaterium, B. pumilus* and *B. subtitils*	Fresh water and marine fish

Table 2. contd....

Table 2. contd.

Product	Country	Microorganism(s)	Substrate
Nam pla and Budu	Thailand	*Lentibacillus salicampi, Lentibacillus juripiscarius Lantibacillus halophilus, Halococcus thailandensis*	Marine fish
Pulque	Mexico	LAB (*Pediococcus parvulus, Lb. brevis, Lb. composti, Lb. parabuchneri*, and *Lb. plantarum*)	Juice of *Agave* species
Sausages	Southern and Central Europe USA	LAB (lactobacilli, pediococci), Catalase positive cocci (*Streptococcus carnosus*), sometimes yeasts and/or moulds	Mammalian meat, generally pork and/or beef, less often poultry
Sauerkraut	International	LAB (*Lc. mesenteroides, Lb. brevis, Lb. plantarum, Lb. curvatus, Lb. sake*	Cabbage
Tempeh	Indonesia, Surinam	*Rhizopus oligosporus*	Soybeans
Wara	North Africa	*Lactobacillus* sp., *Leuconostoc* sp., *Pediococcus* sp., *Lactococcus* sp., yeasts	Sodom apple plant or pawpaw leaves
Yoghurt	International	*S. thermophilus, Lb. bulgaricus*	Milk, milk solids

produces lactic acid as their major end product. The lactic acid produced may be L (+) or, less frequently (–) or a mixture of both. It should be noted that D (–) lactic acid is not metabolized by humans and is not recommended for infants and young children. The LAB are strictly fermentative and lack functional heme-linked electron transport chains and a functional Krebs cycle, they obtain energy *via* substrate level phosphorylation (Montet et al. 2006). The most common members of the group which are exploited for food uses include lactococci for cheese manufacture, *Streptococcus salivarius* subsp. *thermophilus* for cheese and yoghurt manufacture and various members of the *Lactobacillus* genus for a variety of cereals, dairy, meat and vegetable fermentations (Liu et al. 2011) (Table 2).

Members of the LAB can be sub-divided into two distinct groups (Table 3) based on their carbohydrate metabolism. The homo-fermentative group composing *Lactococococcus, Pediococcus, Enterococcus*, *Streptococcus* and some lactobacilli use the Embden-Meyerhof-Parnas pathway to convert 1 mol of glucose into 2 mol of lactate. In contrast, hetero-fermentative bacteria produce equi-molar amounts of lactate, CO_2 and ethanol from glucose using the hexose-monophosphate or pentose pathway (Caplice and Fitzgerald 1999, Montet et al. 2006, Di Cagno et al. 2013) (Fig. 1), and in so doing generate only half the energy of the homo-fermentative group. Members of this group include *Leuconostoc, Weissella* and some lactobacilli.

Table 3. Major lactic acid bacteria in fermented foods.

Homofermenter	***Facultative homofermenter***	***Obligate heterofermenter***
Enterococcus faecium	*Lactobacillus bavaricus*	*Lactobacillus brevis*
Lactobacillus acidophilus	*Lactobacillus casei (syn. Lb. rhamnosus)*	*Lactobacillus buchneri*
Lactobacillus delbrueckii	*Lactobacillus coryniformis*	*Lactobacillus cellobiosus*
Lactobacillus lactis	*Lactobacillus curvatus*	*Lactobacillus confusus*
Lactobacillus leichmannii	*Lactobacillus plantarum*	*Lactobacillus coprophilus*
Lactobacillus salivarius	*Lactobacillus sakei*	*Lactobacillus fermentum*
Pediococcus acidilactici	*Lactobacillus manihotivorans*	*Lactobacillus sanfrancisco*
Pediococcus cerevisiae		*Leuconostoc dextranicum*
Pediococcus damnosus		*Leuconostoc lactis*
Pediococcus pentosaceus		*Leuconostoc mesenteroides*
Streptococcus agalactiae		*Leuconostoc paramesenteroides*
Streptococcus bovis		
Streptococcus faecalis		
Streptococcus mutans		
Streptococcus salivarius		
Streptococcus thermophilus		

Source: Ray and Panda (2007)

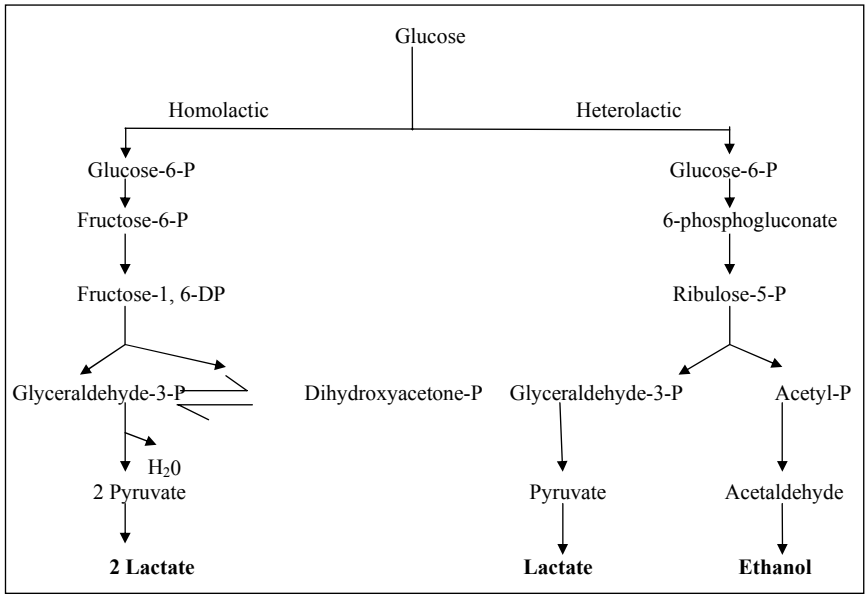

Fig. 1. Generalized scheme for the fermentation of glucose to lactic acid bacteria (Caplice and Fitzgerald 1999).

The metabolism of the disaccharide lactose is of primary importance in those LAB that are used in dairy fermentations (Shah 2007). Lactose may enter the cell using either a lactose carrier, lactose permease, followed by cleavage to glucose and galactose or *via* a phosphor-enolpyruvate-dependent phosphor-transferase (PTS) followed by cleavage to glucose and galactose-6-phosphate. Glucose is metabolized *via* glycolytic pathway, galactose *via* the Leloir pathway and galactose-6-phosphate *via* the tagatose 6-phosphate pathway. Most *Lactobacillus lactis* strains used as starters for dairy fermentations use the lactose PTS, the genes for which are plasmid located. Among some thermophilic LAB, only the glucose moiety of the sugar is metabolized and galactose is excreted into the medium, although mutants of *Streptococccus thermophilus* have been described, which metabolize galactose *via* the Leloir pathway (Caplice and Fitzerald 1999, Hansen 2002).

Citrate metabolism is important among *Lb. lactis* subsp. *lactis* (bv. *diacetylactis*) and *Leuconostoc mesenteroides* subsp. *cremoris* strains used in the dairy industry, as it results in excess pyruvate in the cell. The pyruvate may be converted *via* α-acetolactate to diacetyl, an important flavour and aroma component of butter and some other fermented milk products (Hansen 2002) (Fig. 2).

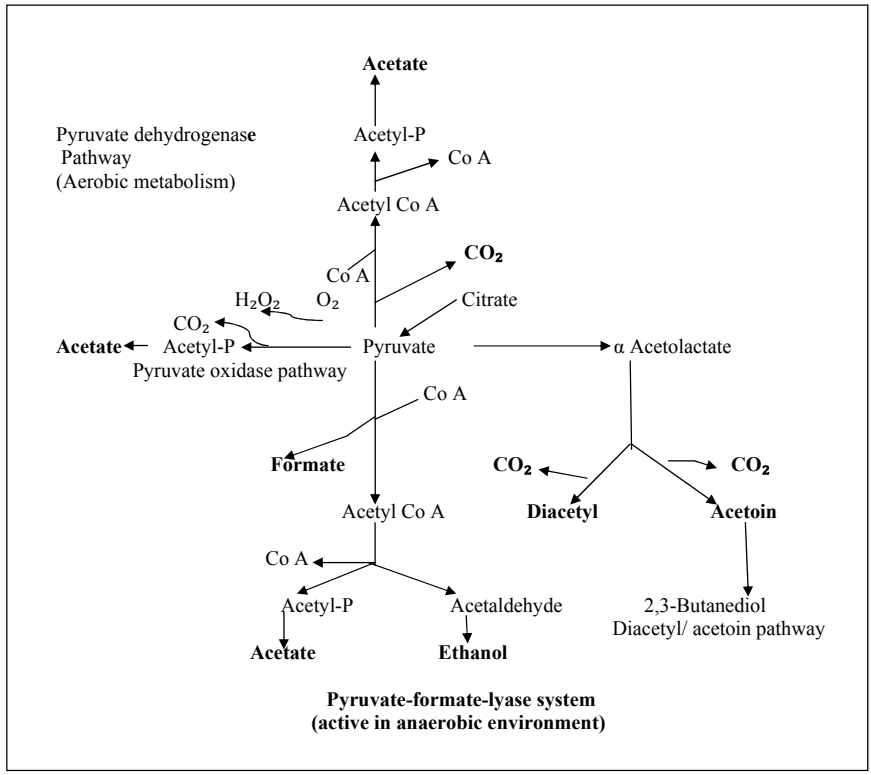

Fig. 2. Generalized scheme for the formation of important metabolic products from pyruvate in lactic acid bacteria (Caplice and Fitzgerald 1999, Hansen 2002).

4.1 Production of Anti-Microbial Compounds

The LAB produce several antimicrobial compounds such as organic acids, hydrogen peroxide, carbon dioxide, diacetyl, broad-spectrum antimicrobials such as reuterin and the production of bacteriocins (De Vuyst and Vandamme 1994a, 1994b, Adam and Nicolaides 2008, Jacqes and Caseregola 2008).

4.1.1 Organic acids, acetaldehyde and ethanol

The antimicrobial effects of organic acids (lactic, acetic and propionic) is believed to result from the action of the acids on the bacterial cytoplasmic membrane which interferes with the maintenance of membrane potential and inhibits active transport. Acetic acid is more inhibitory than lactic acid and can inhibit yeasts, moulds and bacteria (Panda et al. 2007, 2009, Settanni

and Corsetti 2008). Propionic acid inhibits fungi and some gram positive bacteria (Ross et al. 2002). The contribution of acetaldehyde and ethanol to biopreservation is minor since the flavour threshold is much lower than the levels that are considered necessary to achieve inhibition of microorganisms (Ross et al. 2002, Dalié et al. 2010).

4.1.2 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) generated during lactic acid fermentation can be inhibitory to some microorganisms (Hansen 2002). Inhibition is mediated through the strong oxidizing effect on membrane lipids and cell proteins. H_2O_2 may also activate the lactoperoxidase system of fresh milk with the formation of hypothiocyanate and other antimicrobials (De Vuyst and Vandamme 1994a, Ross et al. 2002).

4.1.3 Carbon dioxide

Carbon dioxide, formed from hetero-lactic fermentation, can directly create an anaerobic environment and is toxic to some aerobic food microorganisms through it action on cell membranes and its ability to reduce internal and external pH (De Vuyst and Vandamme 1994a). At low concentration, it may be stimulatory to the growth of some bacteria (Caplice and Fitzgerald 1999, Ray and Panda 2007).

4.1.4 Diacetyl

Diacetyl is a product of citrate metabolism (Fig. 2) and is responsible for the aroma and flavour of butter and some other fermented milk products (Caplice and Fitzgerald 1999, Ross et al. 2002). Many LAB including strains of *Leuconostoc*, *Lactococcus*, *Pediococcus* and *Lactobacillus* may produce diacetyl although production is repressed by the fermentation of hexoses. Gram-negative bacteria, yeasts and moulds are more sensitive to diacetyl than gram-positive bacteria and its mode of action is believed to be due to interference with the utilization of arginine (De Vuyst and Vandamme 1994a). Diacetyl is rarely present in food fermentations at sufficient levels to make a major contribution to antibacterial activity (de Bok et al. 2011).

4.1.5 Reuterin

Reuterin is produced during stationary phase by the anaerobic growth of *Lactobacillus reuteri* on a mixture of glucose and glycerol or glyceraldehyde. It has a general antimicrobial spectrum affecting viruses, fungi and protozoa

as well as bacteria. Its activity is thought to be due to inhibition of ribonucleotide reductase (Caplice and Fitzgerald 1999).

4.1.6 Bacteriocins

It has been known for some time that many members of the LAB produce proteinaceous inhibitors that are collectively referred to as bacteriocins. These inhibitors generally act through depolarization of the target cell membrane or through inhibition of cell wall synthesis (Settanni and Corsetti 2008), and range in specificity from a narrow spectrum of activity (lactococcins which only inhibit lactococci) to those which have a broad range of activity such as the lantibiotic nisin (De Vuyst and Vandamme 1994b, Settanni and Corsetti 2008). Based on the groupings proposed by Klaenhammer (1993), bacteriocins can be divided into the following three main groups.

4.1.6.1 Class I: the lantibiotic family

These are generally small bacteriocins composed of one or two peptides of approximately 3 kDa, collectively called lantibiotics. An unusual feature of this group is that they are post-translationally modified to contain lanthionine, hmethyl lanthionine and dehydrated amino acids, while at least two members of the group also contain Dalanine (Ray and Panda 2007). Nisin and lacticin 3147 both belong to the lantibiotic family and inhibit a broad range of Gram-positive bacteria (De Vuyst and Vandamme 1994b, Settanni and Corsetti 2008). The lantibiotics were originally subdivided into two groups, A and B. Type A includes the elongated flexible molecules that have a positive charge and act *via* membrane depolarization, such as nisin (Settanni and Corsetti 2008). Type B lantibiotics are globular in structure and interfere with cellular enzymatic reactions and examples include mersacidin and ctagardine (Settanni and Corsetti 2008).

Nisin is approved for use, to varying degrees, as a component of the preservation procedure for processed and fresh cheese, canned foods, processed vegetables and baby foods, in up to 50 countries (De Vuyst and Vandamme 1994b, Di Cagno et al. 2013). Typical levels that are used in foods range between 2.5 and 100 ppm. It is most stable in high-acid foods (Settanni and Corsetti 2008). The addition of a nisin-producing strain of *Lactobacillus lactis* to the starter culture used in the manufacture of nitrate-free Gouda cheese has been demonstrated to result in the prevention of the outgrowth of *Clostidium tyrobutyricum* spores (Giraud et al. 2010) and it has also been shown to inhibit the growth of *Lysteia monocytogenes* in cottage and Camembert cheese (Fernadez-Bodega et al. 2009).

Lacticin 3147, produced by a lactococcal isolate from Irish Kefir grains used in the manufacture of buttermilk, is effective against a wide spectrum of gram-positive bacteria (McAuliffe et al. 1999). Unlike nisin, lacticin 3147 is effective at neutral pH.

4.1.6.2 Class II: small non-modified peptides

These are generally small unmodified peptides of < 5 kDa, which are subdivided into two groupings. The first (Class IIa) group is composed of the pediocin-like bacteriocins with anti-listerial activity. Pediocins are produced by *Pediococcus* spp. and while they are not very effective against spores they are more effective than nisin in some food systems such as meat (Leroy et al. 2013). Pediococci are the main starter culture used in the manufacture of American-style fermented meats (Leroy et al. 2013) and they are also important in the fermentation of many vegetables such as Korean kimchi and sauerkraut (Ray and Panda 2007). Many studies report the inhibition of *L. monocytogenes* by pediocins or pediocin-producing cultures in fermented Spanish pork sausages (GarcíaFontán et al. 2007) and in sourdough fermentation (Leroy et al. 2007).

4.1.6.3 Class III: large heat-labile proteins

The class III bacteriocins are the least characterized group and consist of heat-labile proteins which are generally > 30 kDa. The group includes Helvetin J produced by *Lactobacillus helveticus* (and enterolysin produced by *Enterococcus faecium* (Ross et al. 2002).

5 Global Fermented Foods

Fermented foods are now regarded as part of our staple diet. Today the fermentation technology has moved from artisanal practices and empirical science to industrialized and life science driven technology. The main substrates used in the commercial production of the most familiar fermented products are cereals, milk, meat, cucumber and cabbage. Fermented foods are the products of acidic, alkaline or alcoholic fermentation, and are mediated either by bacteria, yeasts, moulds, or mixed (bacteria and yeasts) microbial cultures.

5.1 Cereal-based Fermentation

Fermented cereals play a significant role in human nutrition in all parts of the world where cereals grow. Among all food fermentations (e.g.,

milk, meat, fish, vegetables, soya or fruits), cereal fermentations reach the highest volume (Brandt 2014). The major cereal based foods are derived mainly from maize, sorghum, millet, rice or wheat. In terms of texture, the fermented cereal foods are either liquid (porridge) or stiff gels (solid). Some examples of cereal porridges (gruels) include ogi, mahewu and mawe, the cereal gels are for example kenkey, kisra and injera (Osungbaro 2009). LAB play a critical role in cereal fermentations (Blandino et al. 2003, Kohajdová, Chapter 3 in this book).

5.2 Dairy-based Fermentation

Fermented dairy products represent about 20% of the total economic value of fermented foods produced world-wide. The market share of such products continues to grow. Dairy industry is a prime user of various LAB strains such as *Lactobacillus, Lactococcus*, and *Leuconostoc.* Cow, sheep, goat, and mare milk has been adopted as a raw material for dairy-based fermentation. The LAB, which are naturally present in air, raw dairy material, and containers are responsible for the fermentation. The LAB for dairy-based fermentation are desirable for their ability to create homogenous textures and particular flavour providing different traits attributed by different microbes (Wouters et al. 2002). Yoghurt is the most popular fermented milk in the world. It is mostly prepared from cow milk which is fermented by two species of LAB: *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Manufacture of acidophilus milk (North America), laban (Middle East), leben (Arab World) and dahi (India) is very close to that of yoghurt. Kefir and koumiss (Central Asia) are fermented milks made with kefir grains composed by little clumps of yeast, lactic acid bacteria and milk proteins (Liu et al. 2011). Cheeses, another popular fermented diary product, are still made from non-pasteurized milk and may even depend on natural lactic flora for fermentation; most are produced on a commercial scale using the appropriate starter culture. These can contain mesophilic *Lb. lactis* subsp. *lactis* and *Lb. lactis* subsp. *cremoris* or thermophilic *Streptococcus thermophilus*, *Lb. helveticus*, and *Lb. delbrueckii* subsp. *bulgaricus*, depending on the specific application (Liu et al. 2011). Detailed descriptions of fermented dairy products are given in Chapter 5 in this book by Catherine and Sandra.

5.3 Meat-based Fermentation

Fermentation is a traditional processing and preservation method that provides relatively stable meat products with acceptable sensory characteristics. Fermented meat is produced with the addition of microbes when different condiments are mixed together with meat (Leroy et al.

2013). The microbiota involved in the fermenting process is diverse and complex, and closely related to the ripening technique. The LAB are usually present in high hygienic quality raw meat at low amounts and dominate the fermentation later (GarcíaFontán et al. 2007, Tu et al. 2010). Their presence effectively prevents harmful bacterial growth and controls the fermentation processes. During the fermentation, acids and alcohols are produced, leading to a decrease of pH. Meanwhile, proteins are broken down into peptides and amino acids (Leroy et al. 2013). The characteristics of fermented meat include special flavours, a longer shelf-life, and convenience for consumption. A variety of fermented meat products are available such as fermented sausage, bacon, and ham (Liu et al. 2011, Leroy et al. 2013, El Sheikha and Baker, Chapter 7 in this book).

5.4 Fish-based Fermentation

There are two kinds of fermented fish products available worldwide; i.e., fish sauce and fish paste. In Southeast and east Asian countries, fish sauces (i.e., nuoc-mam, nam-pla, patis, budu, bakasang, etc.) are very popular. Some fish sauces are made from raw fish, others from dried fish, some from only a single species, others from a variety of fishes. The most common microorganisms isolated from fish sauce are *Bacillus, Lactobacillus, Pseudomonas, Pediococcus* (all bacteria), *Debaryomyces* and *Hansenula* (yeasts) (Sanni et al. 2002). The common fish pastes available are hentak and ngari in India, bagoong from the Philippines, terasi from Indonesia, belacan from Malaysia, ngapi from Myanmar (Panda et al. 2011). The bacteria involved in fish paste fermentation are mainly halophilic bacteria such as *Lentibacillus jeotgali* (Korean fermented seafood), *Gracilibacillus thailandensis* (from Plara), *Paenibacillus tyraminigenes* (from Myeolchi-jeotgal, a traditional Korean salted and fermented anchovy), *Piscibacillus salipiscarius* (from Plara), etc. (Panda et al. 2011).

5.5 Vegetable-based Fermentation

Plant-based foods contribute to the core daily dietary intake in Asia. Traditionally, people fermented mixed vegetables such as cabbage, radishes, cucumbers, turnips and beets (Ray and Panda 2007). The LAB can bind to the surface of vegetables without decomposing cellulose or proteins (Li 2001), contributing to the characteristics of the final product in addition to preservation. The traditional method for fermentation is to place vegetables into clean containers and add ingredients for natural fermentation. Addition of salt is indispensible even if the vegetable species or manufacturing

processes differ from region to region (Di Cagno et al. 2013). This facilitates the production of flavour, controls against undesirable microorganisms, extracts water and nutrients, and constitutes soft tissue (Panda et al. 2007, Montet et al., Chapter 4 in this book). Finally, anaerobic environment, salt addition and acid production result in unique features of the products and a high degree of hygienic safety.

5.6 Soybean-based Fermentation

Soybean is one of the most widely cultivated plants in the world and is a good source of protein and essential amino acids, particularly, lysine. China is the place of origin of the soybean, which may date back to more than two millennia (Li 2003), and it has a long tradition of soybean production and processing, especially in the preparation of the main product of soybean, tofu. Fermented soybean products with high nutrition and health benefits have gained much attention. During the fermentation process, useful active substances are released through metabolic processes of microorganisms, providing additional health benefits. Resources and expertise in producing and developing soybean-based fermented foods are abundant. Typical products are sufu, stinky tofu, and lobster sauce. The characteristic aroma and flavour of soybean-based fermented foods are partially generated by LAB (Liu et al. 2011).

5.7 Fruit-based Fermentation

Traditionally, fruits have been fermented to produce low alcoholic beverages like wines which are produced and consumed all over the world (Joshi 2009, 2011, Jackson 2011). Wines are produced mainly from grapes but other fruits like plum, peach, pear, apple, citrus, strawberry, etc. are also used in it production (Joshi and Attri 2005). The fermentation is carried out by *Sacchromyces cerevisiae* var. *ellipsoideus* (Joshi et al. 1999). Several types of wines are made like table, sweet and dry wines, fortified wines, sparkling wines (Joshi et al. 2011). The wines are distilled to make brandy also. Wine yeast, *Sacchromyces cerevisiae* var. *ellipsoideus* and several of its strains like UCD 595, UCD 502 and UCD 522 are employed to conduct alcoholic fermentation of foods to make wine. The process consists of preparation of must, culture preparation, fermentation, siphoning, clarification and maturation (Amerine et al. 1980). For more details see comprehensive treatise by Jackson (2011) and Joshi (2011).

6 Starter Cultures

A starter culture can be defined as a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating and steering its fermentation process. The LAB occupy a central role in these processes, and have a long and safe history of application and consumption in the production of fermented foods and beverages (Leroy and De Vuyst 2004). They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. Also, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes is of importance. In this way, they enhance shelf-life and microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product (van Boekel et al. 2010).

6.1 Backslopping

The earliest production of fermented foods was based on spontaneous fermentation due to the development of the microflora naturally present in the raw material. The quality of the end product was dependent on the microbial load and spectrum of the raw material. Spontaneous fermentation was optimized through backslopping, i.e., inoculation of the raw material with a small quantity of a previously performed successful fermentation. Hence, backslopping results in dominance of the best adapted strains.

Backslopping is still in use, for instance in the production of sauerkraut and sourdough, and particularly for products for which the microbial ecology and the precise role of successions in microbial population are not well known (Bartkiene et al. 2011). Today, the production of fermented foods and beverages through spontaneous fermentation and backslopping represents a cheap and reliable preservation method in less developed countries (Holzapfel 2002), whereas in Western countries the use of starter culture in large-scale production of fermented foods has become an important routine procedure of the food industry (Moroni et al. 2009).

6.2 Functional Starter Cultures

The use of functional starter cultures in the food fermentation industry is being explored (De Vuyst 2000). Functional starter cultures are starters that possess at least one inherent functional property. The latter can contribute to food safety and/or offer one or more organoleptic, technological, nutritional, or health advantages. The implementation of carefully selected strains as starter cultures or co-cultures in fermentation processes can help to achieve *in situ* expression of the desired property, maintaining a perfect natural and

healthy product. Examples are LAB that are able to produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, useful enzymes, or nutraceuticals, or LAB with health-promoting properties, so called probiotic strains (Gareau et al. 2010). The functional starters have several applications in food fermentations (Table 4). The important characteristics of LAB functional starter cultures are discussed.

6.2.1 Food preservation and safety

Chemical food additives such as nitrite, sulfite, propionic acid, sorbic acid, and benzoic acid are commonly applied in food preservation technology. As an alternative, the antimicrobial activity (as described earlier) displayed by LAB strains may help to combat microbial contamination (Lücke 2000). Acetic acid, for instance, contributes to the aroma and prevents mould spoilage in sourdough (Messens and De Vuyst 2002).

6.2.2 Improvement of texture

To give a desired texture and mouth feel to yoghurt, skim-milk powder or whey is frequently added to the milk. In some countries, however, gelatine or plant (e.g., starch, pectin, guar gum, and alginate) (Mohapatra et al. 2007) and microbial polysaccharides (e.g., xanthan and pullulan) (Ray and Moorthy 2007) are added. Polysaccharides increase the viscosity and firmness, improve the texture, and contribute to the mouth feel of low-fat products. Functional, exo-polysaccharide-producing starters of *Lb. delbrueckii* subsp. *bulgaricus* or *S. thermophilus* can be added directly in the food matrix to impart the same result (De Vuyst et al. 2001, De Vuyst and Marshall 2001). Another application of using exo-polysaccharide producing strains can be found in the bakery industry for a beneficial effect on bread volume and staling (Tieking et al. 2003).

The texture improvement of foods through functional starter cultures can also be attained by the use of amylase producing LAB. The LAB producing thermostable amylases have potential in cereal, root and tuber (starch) fermentations (Panda et al. 2008), in particular in sourdough technology for the natural inhibition of staling in bread (Tieking et al. 2003).

6.2.3 Production of aroma and flavour

The LAB contribute to the aroma and flavour of fermented products (Ayad et al. 2001). They acidify the food, resulting in a tangy lactic acid taste, frequently exert proteolytic and lipolytic activities, and produce aromatic

Table 4. Typical examples of functional starter cultures or co-cultures and their advantages for the food industry.

Advantage	**Functionality**	**Lactic acid bacteria**[a]
Food preservation	Bacteriocin production	
	-Dairy products	*Lactococcus lactis* subsp. *lactis* *Enterococcus* spp.
	-Fermented meats	*Lb. curvatus* *Lb. sakei* *Pediococcus acidilactici* *Enterobacter faecium*
	-Fermented olives	*Lb. plantarum*
	-Fermented vegetables	*L. lactis, Lb. plantarum*
Organoleptic	Production of exo-polysaccharides	Several lactobacilli and streptococci
	Production of amylase	Several lactobacilli
	Aroma generation	Several strains of lactobacilli
	Enhanced sweetness	
	-Homoalanine-fermenting starters	*L. lactis*[b]
	-Galactose-positive/ glucose-negative starters	*Lb. delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*
	Malolactic fermentation	*O. oeni*
Technological	Bacteriophage resistance	Several strains
	Prevention of over-acidification in yoghurt	lactose-negative *Lb. delbrueckii* subsp. *bulgaricus*
	Autolyzing starters	
	-Phage-mediated	*L. lactis* subsp. *lactis*
	-Bacteriocin-induced	*L. lactis*
Nutritional	Production of nutraceuticals	
	-Low-calorie sugars	*Lb. plantarum, L. lactis*
	(e.g., sorbitol and mannitol)	
	-Production of oligosaccharides	*L. lactis*
	-Production of B-group vitamins (e.g., folic acid)	*Lb. delbrueckii* subsp. *bulgaricus, L. lactis, S. thermophilus*
	-Release of bioactive peptides	Several strains of lactobacilli
	Reduction of toxic and anti-nutritional compounds	
	-Production of l(+)-lactic acid isomer	L(+)-lactic acid-producing lactobacilli
	-Removal of lactose and galactose	*S. thermophilus*
	-Removal of raffinose in soy	Several strains of lactobacilli
	-Reduction of phytic acid content, amylase inhibitors, and polyphenolic compounds	*Lb. plantarum* *Lb. acidophilus*
	-Decreased production of biogenic amines	*E. faecalis*

[a]*E.=Enterococcus, L.=Lactococcus, Lb.=Lactobacillus, O.=Oenococcus, P.=Pediococcus, S.=Streptococcus*
[b]Recombinant strain
Source: Leroy and De Vuyst 2004, modified

compounds from, for instance, amino acids upon further bioconversion (van Kranenburg et al. 2002). Control over the activities of peptidases from LAB is a key target of cheese ripening technology (Law 2001, Grattepanche et al. 2008, 2010). As an example, over-expression of certain peptidases of *Lb. lactis* subsp. *cremoris* improved the sensory quality of cheese (Powell et al. 2011).

6.2.4 Phage-resistant starters for the dairy industry

Bacteriophages pose a serious problem to the dairy industry. In addition to strict sanitary conditions, the use of appropriate media, the rotation of starter cultures and the use of phage-resistant starter cultures offers a solution. Phage resistance may be caused by natural resistance mechanisms (restriction and modification enzymes), prevention of intracellular phage development through phage adsorption and abortive phage infection, or by intracellular defense strategies (Smid and Hugenholtz 2010). Strains that have acquired natural mechanisms of phage resistance, e.g., through *in vivo* recombination (conjugation) or *in vitro* self-cloning, are currently applied on a large scale in the dairy industry (Brenner et al. 2008).

6.2.5 Production of nutraceuticals

Nutraceuticals are food components that, through specific physiological action, contribute to the health of the consumer. Several nutraceuticals from bacterial origin have been added to food products (Hugenholtz et al. 2002). Through strain selection and process optimization, the activity of LAB can be modified to increase the content of nutraceuticals in fermented foods such as fermented dairy products (Guldfeldt et al. 2001, Santos et al. 2008). As an example, fermented milks can be produced with LAB starter strains that produce high amounts of low-calorie polyols so as to reduce the sugar content (Wisselink et al. 2002).

6.2.6 Reduction of toxic or anti-nutritive factors

The fermentative action of specific LAB stains may lead to the removal of toxic or anti-nutritive factors, such as lactose and galactose from fermented milks to prevent lactose intolerance and accumulation of galactose (Wouters et al. 2002). Other examples are the removal of raffinose, stachyose, and verbascose from soy to prevent flatulence and intestinal cramps (Holzapfel 2002, Hou et al. 2000), proteinase inhibitors from legumes and cereals to prevent maldigestion (Holzapfel 2002), phytic acid and tannins from cereals and legumes to increase mineral bioavailability (Holzapfel 2002), and natural toxins such as cyanogenic glucosides from cassava (Ray and

Ward 2006) as well as biogenic amines from traditional fermented foods (Holzapfel 2002, Ray and Panda 2007).

6.2.7 Probiotics

Probiotics are live microorganisms that confer a beneficial effect on the host when administered in proper amounts (Shah 2007). Multiple reports have described their health benefits on gastrointestinal infections, antimicrobial activity, improvement in lactose metabolism, reduction in serum cholesterol, immune system stimulation, anti-mutagenic properties, anti-carcinogenic properties, anti-diarrheal properties, improvement in inflammatory bowel disease and suppression of *Helicobacter pylori* infection by addition of selected strains to food products (Imasse et al. 2007, Shah 2007).

Microorganisms that have probiotic qualities are receiving increasing attention. These microorganisms are mostly of human or animal origin; however, some studies show that strains recognized as probiotics are also found in non-dairy fermented substrates (Schrezenmeir and de Vrese 2001). As fermentation process involves mixed cultures such as yeast, LAB and fungi (Blandino et al. 2003), traditional fermented foods are a plentiful source of microorganism and some of them show probiotic characteristics (Lei and Jacobsen 2004, Psani and Kotzekidou 2006, Todorov et al. 2008), although the research of these matrices as raw material for probiotic microorganisms is still scarce compared with their dairy counterpart.

Various studies have shown that probiotic organisms survive poorly in fermented foods (Lücke 2000) and there is little information about the microorganisms' challenges for survival, the criteria for fermentation, their use as starters, and their relationship with other microorganisms (Kedia et al. 2007). The information provided by traditional fermented foods and scientific research could help develop new probiotic products for the food industry, which could help when lactose intolerance and cholesterol content are drawbacks, when people refuse to ingest dairy product for particular reasons or when the milk products are inaccessible.

Nonetheless, probiotic organisms may be ''encapsulated'' before addition to food matrices (Lacroix and Yildirim 2007). Probiotic bacteria are sold mainly in fermented foods, and dairy products play a predominant role as carriers of probiotics (Heller 2001). In order to deliver these microorganisms efficiently, the survival of probiotic bacteria in non-fermented food matrices and non-dairy products is being studied (Sheehan et al. 2007). Fermented foods are well suited to promote the positive health image of probiotics because consumers are familiar with the fact that they contain living bacteria (Saxelin 2000, Heller 2001). Most probiotics belong to the genera *Bifidobacterium* and *Lactobacillus*. However, species belonging to the genera *Lactococcus, Enterococcus, Saccharomyces* and *Propionibacterium*

are also considered due to their health-promoting effects (Blandino et al. 2003, Vinderola and Reinheimer 2003, Ray et al., Chapter 9 in this book). Bifidobacteria are normal inhabitants of the human and animal gastrointestinal tract and it is not surprising to find them in the mouth and feces. The intestinal tracts of newborns are colonized with *Bifidobacterium* within days after birth and the population is influenced by age, diet, antibiotics, and stress. The effectiveness of this organism is related to its ability to colonize the intestinal tract and control undesirable intestinal bacteria, but the adhesive factor and survivability are not presented in all isolated Bifidobacteria. Numerous studies have screened, characterized and selected those strains that meet the criteria for colonization of intestine and colon (Del Re et al. 1998). The optimum pH for the growth of Bifidobacteria is 6.0–7.0 and there is virtually no growth below 4.5 or above 8.5. The optimum temperatures of growth are 37–41ºC, the minimum are 25–28ºC, and the maximum are 43–45ºC. Some *Bifidobacterium* cultures used as probiotic are *B. adolescentis, B. longum, B. infantis*, and *B. Breve* (Rivera-Espinoza and Gallardo-Navarro 2010).

7 Microbe-Microbe Interactions in Mixed Starter Food Fermentation

Most food fermentation processes depend on mixtures of microbes which act in concert to produce the desired product characteristics. In general, it can be stated that complex microbial consortia perform more complex activities (versatility) and tolerate more variation in the environment (robustness) as compared to pure cultures. The versatility and robustness can be explained by two features (Brenner et al. 2008). First, members of the consortium communicate with one another by trading metabolites or by exchanging molecular signals. The process of quorum sensing mediates bacterial cell-cell communication by secretion and detection of inducer molecules and serves the culture in monitoring population density (Smid and Lacroix 2012). Detection of inducer molecules leads to synchronized gene expression of (sub)-populations in bacterial cultures. Quorum sensing plays a role in many microbiological processes such as pathogenicity, biofilm formation, competence, sporulation and the production of antimicrobial compounds. As a result, each individual cell in the mixture responds to the presence of others in the consortium (Keller and Surette 2006). The second key feature is the division of labour between the members of the consortium leading to an overall output that can only be explained by combining tasks performed by constituent individuals or sub-populations. This is of particular importance for the design of fermented foods containing probiotic strains as both their

ability to survive gastric stress and the production of probiotic effect or molecules may be highly dependent on growth conditions.

All types of interactions (competition, mutualism, commensalism, amensalism and parasitism), potentially play a role in consortia of microbes found in fermenting foods. The best well-known example of mutualism is the proto-cooperation between *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in yoghurt fermentation with clear links to product functionality (van Hylckama Vlieg et al. 2011). Harvesting on the availability of multiple genome sequences, Liu et al. (2009) demonstrated that during the long history of co-cultivation in milk multiple events of horizontal gene transfer have occurred, as for instance the transfer of a gene cluster for the production of sulfur-containing amino acids from *Lb. bulgaricus* to *S. thermophilus* that probably optimize their combined growth. In a study by Herve´-Jiminez et al. (2009), transcriptomic and proteomic analysis revealed regulatory responses in purine biosynthesis related to co-cultivation. In addition, the production of H_2O_2 by *Lb. bulgaricus* modifies iron metabolism in *S. thermophilus* likely protecting it from the generation of reactive oxygen species.

In another example, Bover-Cid et al. (2000) reported control of biogenic amine production in dry fermented sausages by using mixed starter cultures of *Lactobacillus sakei*, *Lb. plantarum*, and *Streptococcus xylosus*.

Another recent example of proto-cooperation is the application of LAB—propionic acid bacteria (PAB) co-cultures is vitamin production. Following a broad screening of a natural biodiversity of LAB and PAB for folate and vitamin B12 production, a highly efficient fermentation process was developed using a co-culture of *Lactobacillus plantarum* SM39 and *Propionibacterium freudenreichii* DF13 (Hugenschmidt et al. 2010, 2011). Using a two-step process consisting of an initial anaerobic incubation period followed by an aerobic phase and whey permeate medium supplemented with vitamin precursors, a very high yield of total folate was reached, up to 8400 ng/ml, which is comparable to genetically engineered strains (Santos et al. 2008).

8 Molecular Techniques for Microbial Community Profiling of Fermented Foods

Over the last decade, the application of molecular technologies with emphasis on the application of novel sequencing technologies to generate phylobiomes, metagenomes and metatranscriptomes for microbial community profiling that complement culturing studies has greatly facilitated monitoring of fermentation ecosystems and characterization of the microbial species (Van Hijum et al. 2013).

8.1 Microbial Community Profiling

Molecular community profiling for fermented foods commenced around a decade ago when PCR was used to amplify variable 16S rRNA gene regions of total DNA and denaturing gradient gel electrophoresis (DGGE) to visualize profiles from fermented foods such as pozol (maize dough) and artisanal cheeses (Van Hijum et al. 2013).

More recently, sequencing of PCR-amplified rDNA gene regions is being used to characterize and profile microbial fermentation ecosystems. Although often referred to as the metagenome or to the sequencing method currently used, that is, pyro-sequencing, the term 'phylobiome' provides a better description in terms of referring to the phylogeny or taxa information generated. An increasing number of fermented foods, with a recent focus on Asian types were analyzed with this technique including pulque (alcoholic beverage) (Escalante et al. 2004), pozol (fermented maize dough) (Ampe et al. 1999) kimchi (cabbage and radish) (Nam et al. 2009, Park et al. 2012), doenjang (soybean paste), or kochujang (red pepper, rice, soybean mix) (Nam et al. 2012b) and rice bran (Van Hijum et al. 2013). Table 5 summarizes the major findings of these 16S rRNA-sequencing based studies.

8.2 Genomics of Microbes from Traditional Fermentations

Metatranscriptome sequencing could provide a more comprehensive view on metabolic processes and trophic chains during indigenous fermentations. A metatranscriptomics analysis of kimchi using a microarray approach has also been described that indicated that later in the kimchi fermentation next to *Leuconostoc mesenteriodes* and *Lc. sakei,* less abundant microorganisms namely *Lactobacillus graminis*, *Lactobacillus curvatus*, *Weisella viridescens*, and *Weisella* minor, had relatively high transcriptional activity and presumably still contributed to the fermentation process (Nam et al. 2009, 2012a, 2012b). Microarray-based metatranscriptomics has also been performed for sourdough showing strongest transcriptome contribution of *Lb. plantarum* and *Lb. fermentum* in the stabilized ecosystem (Weckx et al. 2010).

9 Microbial Food Cultures and Legislation

The concept of "history of safe use" of microbial species used in food fermentations has appeared recently in regulations and in safety assessment guidance. One definition of "history of safe use" proposes "significant human consumption of food over several generations and in a large, genetically diverse population for which there exist adequate toxicological and allergenicity data to provide reasonable certainty that no harm will result from consumption of the food" (Health Canada 2003). In order

Table 5. Major findings from sequencing-based community profiling of indigenous fermentations.

Fermented food type	Molecular analysis technique	Major findings
Pulque (alcoholic beverage)	16S rRNA gene amplicon sequencing, ARDRA	Large biodiversity discovered, which is referred to as "regionality". Major species identified by sequencing of 16S clone libraries were *Lactobacillus acidophilus, Leuconostoc mesenteroides, Gluconobacter oxydans* and *Hafnia alvei*
Kimchi (cabbage, raddish)	16S rRNA gene amplicon sequencing	Community dynamics studied to find differences between starter-inoculated and non-inoculated kimchi at the early stages of fermentation, but overall there were no significant differences in the late phases. Kimchi is dominated by *Leuconostoc, Lactobacillus*, and *Weissella*
Narezushi (fermented fish)	16S rRNA gene amplicon sequencing	Time dependent changes detected, *Lactobacillaceae* increase in early fermentation phase, high variation between products analyzed. *Lactobacillus plantarum* and *Lactobacillus brevis* dominate the process as identified by culturing
Nam pla and budu (fermented fish)	16S rRNA gene amplicon sequencing	LAB diversity discovered: *Lentibacillus salicampi, Lentibacillus juripiscarius Lantibacillus halophilus, Halococcus thailandensis. Filobacillus* sp. RP 2–5, *Piscibacillus salipiscarius, Tetragenococcus halophilus, T. muriaticus, Halobacterium salinarum*
Doenjang (soy bean paste)	16S rRNA gene amplicon sequencing	High diversity of bacterial species discovered in artisanal samples, which is referred to as "regionality". Commercial brands contained simple microbial communities dominated by *Tetragenococcus* and *Staphylococcus*
Kochujang (red pepper, ice, soybean mix)	16S rRNA gene amplicon sequencing	*Bacillaceae* showed the highest abundance in most samples except one, in which *Leuconostocaceae* were dominant. The second-most dominant bacterial families widely differed between (regional) samples and included *Paenibacillaceae, Lactobacillaceae, Enterococcaceae* or *Staphylococcaceae*
Seafood	16S rRNA gene amplicon sequencing DGGE	Predominant types included halophilic archaea related to the family *Halobacteriaceae*; various uncultured mesophilic Crenarchaeota discovered for the first time in fermented food

Nukadoko (rice bran)	16S rRNA gene amplicon sequencing	Structure and dynamics of the bacterial community studied, revealing stabilization of high biodiversity of *Lactobacillaceae* to the predominating role of (inoculated) *Lactobacillus namurensis* and *Lactobacillus acetotolerans* during the refreshment and fermentation cycles
Nuruk/Makgeolli (starchy starter tablets/ fermented drinks made thereof)	Fungal spacer region 2 and 16S rRNA gene amplicon sequencing	Community shift towards *Saccharomyces* and lactic acid bacteria during fermentation with *Saccharomycetaceae* significantly increasing, and the major bacterial phylum of the samples shifting from γ-Proteobacteria to Firmicutes
Cocoa bean	Taxon-indicative (e.g., 16S rRNA) sequences from metagenomic data	The metagenomic sequencing analysis identified *Hanseniaspora uvarum, Hanseniaspora opuntiae, Saccharomyces cerevisiae, Lactobacillus fermentum*, and *Acetobacter pasteurianus* as the dominant species

Source: Namwong et al. 2005, 2007, Tanasupawat et al. 2007, 2009, Van Hijum et al. 2013

to evaluate the history of safe use of a microorganism, it is necessary to document not just the occurrence of a microorganism in a fermented food product, but also to provide evidence whether the presence of the microorganism is beneficial, fortuitous, or undesired (Stevens and O'Brien Nabors 2009, Bourdichon et al. 2012).

9.1 Definition of Microbial Food Cultures

Microbial food cultures (MFC) have not been defined legally. To alleviate this, European Food and Feed Cultures Association (EFFCA) has proposed the following definition: "Microbial food cultures are live bacteria, yeasts or moulds used in food production". MFC preparations are formulations, consisting of one or more microbial species and/or strains, including media components carried over from the fermentation and components which are necessary for their survival, storage, standardization, and to facilitate their application in the food production process (Bourdichon et al. 2012).

9.2 Regulatory Frameworks

Microbial food cultures have directly or indirectly come under various regulatory frameworks in the course of the last decades. Several of those regulatory frameworks put emphasis on "the history of use", "traditional food", or "general recognition of safety" for use of microorganisms in food fermentations. Bourdichon et al. (2012) briefly reviewed the US regulatory and European regulatory frameworks. Therefore, an authoritative list of microorganisms with a documented use in food has come into high demand. One such list was first published in 2002 as a result of a joint project between the International Dairy Federation (IDF) and EFFCA (Mogensen et al. 2002a, 2002b). The "2002 IDF inventory" has become a *de facto* reference for food cultures in practical use. The focus mainly was on commercially available dairy cultures, however, recently, an updated inventory of microorganisms (bacteria, fungi, filamentous fungi and yeasts) used in food fermentations covering a wide range of food matrices (dairy, meat, fish, vegetables, legumes, cereals, beverages, and vinegar) has been prepared by the members of IDF Task force (Bourdichon et al. 2012). The detailed list of microorganisms (bacteria, fungi, filamentous fungi and yeasts) that include 62 genera and 264 species with demonstration in food usage can be found in this publication.

10 Future Prospects

We are now entering the post-genomic age of microbiology at a time when many microorganisms used for food fermentation or microorganisms isolated from food fermentations, have already been sequenced. This offers a new knowledge-based approach to the exploitation of microorganisms for food fermentation, from metabolic engineering of microorganisms to produce antimicrobials or nutritionals, to the molecular mining of activities as yet unknown but which could benefit food production. In addition, the availability of the genomes of many food pathogenic and spoilage bacteria may open up new possibilities for the design of novel antimicrobials which target essential functions of these problematic bacteria. The real challenge of the genomics and proteomic era, as it applies to food systems, is the harnessing of this wealth of information for improved culture performance and activities, thereby improving the safety and quality and composition of our food supply.

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Keywords: Fermentation, Lactic acid bacteria, Mould, Yeasts, Backslopping, Microbial food cultures, PCR-DGGE, phylobiomes, Metagenomes, Metatranscriptomes

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2

Microbial Diversity in Fermented Foods with Emphasis on Bacterial Fermentation and Health Benefits

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1 Introduction

Fermentation is "a form of energy-yielding microbial metabolism where an organic substrate, usually a carbohydrate, is incompletely oxidized" (Adams 1990). This bioprocess resulting in biomass and biosynthesis of metabolites is commonly induced by microorganisms, or by enzymes of plant or animal origin through utilization of amounts of carbon, nitrogen, and oxygen, etc. Food fermentation technologies have evolved through years of experience and contributed one-third of the total food consumption all over the world particularly in rural households and village communities. As one of the oldest means used for food preservation, food fermentation maintains the

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desirable biochemical changes and unique properties of raw materials and provides popular, wholesome and nutritious food for daily diet. Products including alcoholic foods or beverages, vinegar, sauerkraut, sausages, cheeses, yogurts, and breads are consumed in a considerable portion in the food market. Increasing consumption of these foods either as main food, side dish or cooking condiment reflects the economic importance of food fermentation. Principally there are five attributes arising from fermentation: (1) Enrichment of the diet through developing a variety of food flavors, aromas, and textures in the substrates, (2) Low-cost and energy-efficient method in food preservation to prevent food spoilage and extend the shelf life, (3) Biological enrichment of food substrates with proteins, essential amino acids, essential fatty acids, and vitamins, etc., (4) Detoxification of the substrates (e.g., vegetable-originated materials) during fermentation processing, and (5) Saving cooking time and fuel requirements (Steinkraus 1996).

2 Food Fermentation Products

Fermented food products can be categorized into the following groups.

2.1 Fermented Dairy Products

Fermented dairy products take account of 20 percent of the total economic value of fermented foods throughout the world. Compared with the perishable milk, fermented dairy products have advantages of long shelf-life, high biological value of protein as well as unique organoleptic attributes. Microorganisms naturally present in air, raw dairy material, and containers are responsible for the fermentation. Dairy cultures ferment lactose to lactic acid, providing a low pH environment for prevention of spoilage microorganisms, coagulation or calcium solubilization, and generating desirable flavor and texture (Hutkins 2011). The microorganisms involved in some important fermented dairy products are given in Table 1 and three important fermented milk products are discussed in brief.

2.1.1 Yogurt

Yogurt is perhaps the oldest and most popular dairy product consumed in many parts of the world. This word came from Turkish language and is characterized by a smooth and viscous gel with acidic taste and potential health benefits. It is also called *Dahi* when the culture is undefined and used for fermenting milk from cows, buffalos or goats (Vijayendra and Gupta 2012, Younus et al. 2002). Microbiologically yogurt is a product of controlled

Table 1. Several important fermented dairy products (Panesar 2011, modified).

Name (Country)	Type of milk	Microorganisms
Curd (South Asian countries)	Buffalo's or cow's milk	*Lactococcus lactis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum*, *Streptococcus lactis*, *Streptococcus thermophilus*, *Streptococcus cremoris*
Yogurt	Cow's milk	*Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*
Cultured butter milk	Buffalo's or cow's milk	*Streptococcus lactis* subsp. *diacetylactis*, *Streptococcus cremoris*
Lassi (India)	Buffalo's or cow's milk	*Lactobacillus delbrueckii* subsp. *bulgaricus*
Acidophilus milk	Cow's milk	*Lactobacillus acidophilus*
Bulgarian butter milk	Cow's milk	*Lactobacillus delbrueckii* subsp. *bulgaricus*
Shrikhand (India)	Buffalo's or cow's milk	*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*
Koumiss (Mongolia, Kazakhstan, Kirgizstan and part of Russia)	Mare's, camel's or ass's milk	*Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Saccharomyces*, *Micrococci*
Kefir (north Caucasus Mountains)	Sheep's, cow's, goat's or mixed milk	*Streptococcus lactis*, *Leuconostoc* subsp., *Saccharomyces*, *Kefir*, *Torula kefir*, *Micrococci*
Leben (Middle East and North Africa)	Goat's or sheep's milk	*Streptococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus delbueckii* subsp. Lactose fermenting yeast
Cheese	Cow's, buffalo's, goat's or sheep milk	*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *diacetylactis*, *Streptococcus thermophilus*, *Lactobacillus delbueckii* subsp. *bulgaricus*, *Priopionibacterium shermanii*, *Penicillium roqueforti*, etc.

milk fermentation with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* for milk acidification and synthesis of aromatic compounds respectively (Murevanhema and Jideani 2013). Milk is then incubated at 42ºC–45ºC after inoculation until the pH falls to 4.4 and the titratable acidity reaches 0.9–1.2 percent (Surono and Hosono 2003). A cooling process is followed at 10ºC for 5–6 hr before storing in refrigerator. The types of yogurt can be stirred, set, frozen or in a liquid form. Nutrients in milk are maintained in a more digestible form and the storage period is extended up to four weeks.

2.1.2 Koumiss

Koumiss, also called "*airag*" or "*chigee*", is a slightly alcoholic fermented milk beverage and also has medical remedies. It is prevalent among people of Mongolia, Kazakhstan, Kirgizstan and part of Russia (Ishii and Konagaya 2002, Wang et al. 2008). Traditionally, koumiss is made from fresh mare or camel milk by mixing with prepared cultures of lactobacilli and lactose fermenting yeasts as the primary bacterial flora (Wu et al. 2009). The product can be divided into three categories named "mild", "medium", and "strong" koumiss depending on the level of fermentation. The strong type has the highest acidity and alcohol content among them. Fermentation results in a special sour flavor and homogeneous milky liquid.

2.1.3 Kefir

Kefir, also known as *Kefyr*, *Kephir*, *Kefer*, *Kiaphur*, *Knapon*, *Kepi* and *Kippi*, is a self-carbonated refreshing cultured milk beverage that originated in the Caucasian mountains (Assadi et al. 2000). Kefir means "feel good" in Turkish and is produced by the action of bacteria and yeasts that exist in symbiotic association in kefir grains, composed of microorganisms immobilized on a polysaccharide and protein matrix (Leite et al. 2013). Due to its nutritional and therapeutic effects, kefir is consumed in many parts of the world. There are a large number of microorganisms present in kefir. Bacteria identified from kefir were *Lactobacilli*, *Leuconostoc*, *Lactococcus*, *Enterococcus* and *Streptococcus*. Predominant yeasts were *Zygosaccharomyces*, *Candida*, *Saccharomyces* and *Klyveromyces* (Sarkar 2007).

2.2 Fermented Meat Products

Meat fermentation emerged as a preservation strategy and makes use of salting and drying to prevent spoilage of nutritious fresh meat (Zeuthen 2007). This technology leads to lower water activity value, low pH value, and thus inhibits the growth of spoilage and pathogenic microorganisms. Usually, salted materials are stuffed together in casings. In the anaerobic environment, lactic acid fermentation is the predominant process followed by a drying phase to further stabilize and mature the product (Leroy et al. 2013). Different sorts of sausage and smoke-cured meat products are produced in this way. During fermentation, lactic acid, pyruvic acid, alcohols, aldehydes, ketones, and carboxylic acids are yielded, contributing to the quality and storage stability of the final products (Kołożyn-Krajewska and Dolatowski 2009).

2.2.1 Fermented sausage

As one of the most successful meat products, fermented sausage with its special flavors, extended shelf-life, and convenience for consumption, represents the characteristics of fermented meat. Lactic acid bacteria (LAB), *Staphylococccaceae*, *Debaryomyces* and *Penicillia* are related strains added when different condiments are mixed together with meat (Franco et al. 2010). Fermented dry sausages are non-heated meat products developed for preservation of raw meat. The pH and water activity are decreased during fermentation and drying processes, contributing to food safety (Leroy et al. 2006). Most common LAB in spontaneously fermented dry sausages are *Lactobacillus sakei*, *Lactobacillus curvatus* and *Lactobacillus plantarum* (Ravyts et al. 2012). Chinese-style sausage is a kind of popular spontaneously fermented meat product in China. It is prepared by mixing ground pork with curing ingredients, which are incubated for several days before stuffing in pork casing and drying (Rai et al. 2010). Western-style sausage is widely produced by pure cultures fermentation after the starter was introduced. Rapid sausage fermentation is adopted in the US whereas most European manufacturers use lower temperatures for longer time periods (Candogan et al. 2008). The adoption of starter cultures with specific functions ensures that the final products have stable quality and high safety. Nowadays, with the development of modern preservation technology, in particular the introduction of the cold chain, the popularity of fermented meat products is explained as their attractive organoleptic characteristics, ease in consumption, and also traditional culture heritage.

2.3 Fermented Vegetable Products

Vegetables including cabbage, radishes, cucumbers, turnips, and beets are great sources of fermented foods. In the traditional way, vegetables are put into clean containers. Salt and other ingredients are added. Fermentation takes place in an anaerobic environment by putting weights on the top of the containers. This economic way for food storage is popular in areas where vegetables are limited in winter. Nowadays, most products are still produced by spontaneous fermentation revealing the flexibility and simplicity for household handling. The naturally occurring microbial population in the raw material, environmental pH, temperature, and salt concentration are some of the key factors determining the outcome of spontaneous vegetable fermentation (Paramithiotis et al. 2010).

2.3.1 Kimchi

Kimchi, a traditional fermented food in Korea, is made mainly from Chinese cabbage, radish and cucumber, etc. Kimchi fermentation is the method for preservation of the fresh and crispy texture of vegetables during winter (Rhee et al. 2011). The preparation processes include pretreatment of oriental cabbage (or radish), brining, blending with various spices and other ingredients, and fermentation (Cheigh et al. 1994). Fresh vegetables contain diverse types of microorganisms such as aerobic bacteria, LAB and yeasts. Then the LAB (*Lactobacillus plantarum*, *Lactobacillus brevis*, *Enterococcus faecalis*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceous*, and *Weissella koreanis*) become prevalent as the fermentation conditions (temperature, salt levels, pH values, etc.) favor the growth of these microorganisms (Chun 2011).

2.4 Fermented Grain Products

Grain-based fermented indigenous foods such as bakery products, beer and vinegar account for an important part of people's diet. As a staple food, bread feeds much of the world population due to its simple and cheap production technology. Bread-making can be generally classified into three steps: mixing, fermentation, and baking. Raw materials are composed of flours, drinking water, yeast, sugar, and baking soda, etc. In order to gain leavened bread, gas formation mainly by yeast and LAB is necessary. The dough must contain a great deal of gas and gas retention in reserve is also needed before baking (Angioloni et al. 2006). Fermentation is decisive to the formation of aroma, flavor, and texture of the final products. In modern bakeries, baker's yeasts are widely used in bread preparation.

Various other products in different types, shapes and sizes are consumed either as main foods or snack. In beer production, key procedures are malting, mashing, boiling, fermentation, finishing, and packing (Shellhammer 2011). Strains of *Saccharomyces cerevisiae* and *Saccharomyces uvarum* are used for metabolization of sugar to produce ethanol, carbon dioxide, organic acids, and esters, etc. Raw materials including cereals and rice are also used to produce vinegar, a seasoning agent with sourness as its most obvious feature. Traditionally, acetic acid bacteria are cultured on the surface of the liquid. The modern way is using submerged culture system where the acetic acid bacteria are suspended in the liquid supplied with oxygen or air (Tesfaye and Troncosco 2011). The final compounds are formed during the acid fermentation or aging process depending on different types of vinegars. Other indigenous foods include red mold rice,

produced through the solid-state fermentation of the *Monascus* species on long-grain rice in Asian countries (Wang and Lin 2007); soy sauce, invented afterwards by fermentation with LAB, fungi and other microorganisms; miso, a fermented soybean food product consumed as breakfast in Japan, China and East Asia; and sufu, a fungal solid-state fermented soybean curd (tofu) consumed as a side dish in China (Han et al. 2004a).

2.5 Fermented Fish Products

Fermented fish products are traditional food especially in eastern and southeastern Asian countries. Fermented seafood can be consumed as a side dish or with other foods for improving taste. These kinds of foods provide calories, proteins, and vitamins at a relatively low cost (Steinkraus 1993). LAB were reported as the dominant microorganisms in fermented fish (Ostergaard et al. 1998, Paludan-Müller et al. 1999).

Jeotgal is a Korean fermented seafood product made from fish or shellfish such as shrimp, oysters, fish roes, and fish tripes with lots of salt. During microbial fermentation, fish proteins are degraded and contribute to the unique feature (Roh et al. 2010). Bekasam is an Indonesian fermented fish product obtained by spontaneous fermentation of freshwater fish, supplemented with salt and rice of fermented cassava (Choesri et al. 2013). In Thailand, a fermented fish sausage named som-fug is popular among indigenous people. The ripening process leads to slightly sour and salty flavor as well as relatively firm and springy texture (Riebroy et al. 2005).

3 Microorganisms and Food Fermentation

As one of the oldest ways of food processing and preservation, food fermentation plays a significant role in the diet of many indigenous communities. The key element for successful fermentation and desirable features of the products is predominantly decided by microbial biodiversity. Three important groups of microorganisms are directly involved in food fermentation, i.e., bacteria, yeast and molds.

3.1 Bacterial Fermentation

Broadly, LAB and *Bacillus* species play pivotal roles in fermentations of many indigenously fermented foods, although other bacteria such as *Corynebacterim*, *Micrococcaceae* and *Acetobacter* are also often implicated in food fermentation.

3.1.1 LAB Fermentation

Although the microbial and enzymatic processes involved in food fermentation have not been fully unraveled until now, the significant contribution of LAB to the properties of many fermented foods has been well recognized. LAB are a group of Gram-positive bacteria, non-respiring, non-spore forming, cocci or rods, which produce lactic acid as the major metabolic end product of the fermentation of carbohydrates (Calo-Mata et al. 2008). LAB consist of 13 genera and the main species cover *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Oenococcus* and *Streptococcus*. The long-lived use endows their natural acceptance as GRAS (Generally Recognized as Safe) for human consumption by the US Food and Drug Administration (Silva et al. 2002). They are crucial bacteria in desirable food fermentations, being responsible for the fermentation of bread, beer, dairy product, sausages, seafood, fruit, and vegetables. One of the most important applications, for instance, is their use as starter strains in the production of fermented dairy products. LAB are in charge of lactose fermentation, protein hydrolysis, formation of organoleptic characteristics of the final cheese (Menéndez et al. 2000). Besides, LAB fermentation serves also as a low-cost method for food preservation since LAB exert antagonistic effect to inhibit pathogenic bacteria and undesirable spoilage microflora such as *Listeria*, *Clostridium*, *Staphylococcus*, and *Bacillus* species. The antagonistic activities mainly come from decreasing of the pH in the food, competition for nutrients and production of inhibitory metabolites (Stiles 1996). Great effort has been made by scientists to clarify the function of different LAB species in the processes of fermentation.

Lactococci are dominant strains in spontaneous dairy fermentation as starter cultures. They play part in the rapid acidification, proteolytic pathways for desirable texture, and flavor-forming. *Lactococci* involvement represents nearly 20 percent of the total economic value of fermented foods produced throughout the world with *Lactococcus lactis* as the prominent bacterium in cheese production. The rapid fall of pH in the medium requires large cell population obtained by metabolism of lactose and milk proteins. Caseins, the main protein in milk are decomposed by lactococcal proteolytic system producing aroma compounds (Smit et al. 2005).

Lactobacillus plantarum, essential for the production of fermented milk, vegetable, fruit, and meat products generates high amounts of lactate during homo-fermentation, and also ethanol, acetate and carbon dioxide during hetero-lactic fermentation. For vegetable and fruit fermentation, low population of *Lactobacillus plantarum* exists naturally on the surface of the raw materials. It grows rapidly and produces lactate at low level of oxygen and pH conditions despite of the presence of high salt concentrations or plant phenolic compounds (Marco 2011). *Lactobacillus plantarum* functions

as a non-starter LAB (NSLAB) in cheese production and as main microbiota in sausages and salami (Ammor and Mayo 2007). Strains belonging to *Lactobacillus plantarum* have been applied in production of mozzarella cheese, fata cheese, kefir and many dry fermented sausages. Other *Lactobacillus* such as *Lactobacillus curvatus* and *Lactobacillus sakei* are also widely isolated microorganisms from fermented sausages.

Pediococcus species are homo-fermentative, spherical and arranged in tetrads. Among them, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, and *Pediococcus halophilus* are often related to food fermentation either as indigenous microflora or as starters (Papagianni and Anastasiadou 2009). Normally *Pediococci* are not capable of utilizing lactose and function as non-starters in cheese production (Caldwell et al. 1996). *Pediococcus acidilactici* and *Pediococcus pentosaceus* are widely identified in fermented vegetables and sausages, contributing to the unique features as well as improvement of food safety by secreting antimicrobial peptides. *Pediococcus acidilactici* is the first industrial starter culture adopted in sausage manufacturing because of fast acidification. *Pediococcus halophilus* takes part in soy-based fermentation such as miso and soy sauce as a salt-tolerant and homo-fermentative LAB (Wood 1997).

Leuconostoc species are hetero-fermentative strains used in dairy and vegetable fermentation. They can also be used as adjunct cultures in combination with fast-acid-producing *Lactococci*, and producer of aroma and flavor compounds (Alegría et al. 2013).

3.1.2 Bacillus fermentation

The genus *Bacillus* is comprised of a group of Gram-positive, aerobic and facultative anaerobic endospore-forming rods found in a wide range of habitats. *Bacillus* becomes an attractive cell factory for efficient conversion of inexpensive raw materials to industrially important enzymes, biochemicals and fuels. For example, *Bacillus* species ferment pentoses and hexoses to L-lactic acid, showing a potential biocatalyst for efficient L-lactic acid production (Wang et al. 2013). Normally the *Bacillus* group are essential microorganisms in alkaline fermentation, which turn proteins to digestible peptides and amino acids. With a recognized history of safe use in foods, *Bacillus* can also be good producer of the antimicrobials against many pathogenic microorganisms such as *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus* (Compaoré et al. 2013). A variety of *Bacillus* species have been identified (Table 2) from different foods including cereal, legume and vegetable-based fermented products (Ogbadu et al. 1990, Wang et al. 2010, Gadaga et al. 1999, Roy et al. 2007, Valero et al. 2007).

Table 2. *Bacillus* strains identified in foods.

Microorganisms	Identification	References
Bacillus subtilis	Korean fermented soy food (cheonggukjang), fermented congee from Inner Mongolia of China, fermented African locust bean, fermented soybean dawadawa, fermented rice-noodle, Chinese fermented soybean food (sufu), traditional sausage from southern Italy	Kindoli et al. 2012, Wang et al. 2010, Ouoba et al. 2004, Amoa-Awua et al. 2006, Ikeda et al. 2005, Han et al. 2004b, Caputo et al. 2011
Bacillus licheniformis	fermented congee from Inner Mongolia of China, fermented soybean (Chunkook-Jang, Meju), fermented Indian Shad	Wang et al. 2010, Kim et al. 2004, Ham et al. 2004, Majumdar et al. 2008
Bacillus amyloliquefaciens	fermented congee from Inner Mongolia of China, fermented soybean products (Meju, Doenjang)	Wang et al. 2010, Lee et al. 2010, Hong et al. 2012
Bacillus pumilus	fermented African locust bean, kimchi	Ouoba et al. 2004, Yamanaka et al. 2007
Bacillus cereus	food pathogen	Drobniewski 1993

Bacillus-fermented soybean foods are known as good sources of protein, which have a long historical standing in Japan, Chinese, Korea, and Thailand. As the major biochemical activity in the fermentation, proteolytic activity of *Bacillus* leads to release of amino acids and other volatile compounds. For instance, *Bacillus subtilis* is the principal microflora in the soybean-based natto fermentation. The production of γ–D-polyglutamic acid (-PGA) and a levan-type fructan provide the stringy characteristic of the fermented mixture (Schallmey et al. 2004). The starch and proteins of the raw materials are converted to amino acids, vitamins, and enzymes during fermentation. Besides, *Bacillus subtilis* has also been found in the production of Chungkookjang, a Korean fermented unsalted soybean (Kwon et al. 2006).

Apart from soybean-based food, *Bacillus subtilis* is responsible for the production of sufu, a Chinese fermented soybean food (Han et al. 2004b), and plays important role in the fermentation of okpehe, a traditional soup condiment produced from *Prosopis africana* seeds and consumed in Nigeria (Oguntoyinbo et al. 2010). *Bacillus subtilis* TR50, isolated from a traditional sausage from southern Italy, contributes to the development of texture and organoleptic features in the sausage and also exerts antimicrobial activity (Caputo et al. 2011). In addition, *Bacillus* has been recognized as one of the few important microorganisms in fish fermentation. For instance, Majumdar et al. (2008) have identified *Bacillus licheniformis* from salt fermented Indian Shad.

3.2 Yeast Fermentation

Yeasts are among the essential functional strains which are crucial to the production of many traditional fermented foods. They ferment sugars, generate secondary metabolites, and prevent the growth of harmful molds. To most biologists, "yeast" is baker's or brewer's yeast. *Saccharomyces cerevisiae*, is the most popular yeast strain for bread application and is undoubtedly an important yeast species used in fermentation as starter culture. There are four commercial forms: liquid yeast, compressed yeast, active dry yeast, and instant active dry yeast (Bonjean and Guillaume 2003). Species other than *Saccharomyces cerevisiae* for example, *Torulaspora delbrueckii* and *Kluyveromyces thermotolerans* may also be used to produce frozen dough breads due to their good tolerance to freezing temperature (Alves-Araújo et al. 2004). For successful fermentation, the selected strains meet the criteria of high and constant gas production in dough, reasonable fermentation rate in a range of temperature, degradation of different sugars, surviving in unfavorable growth conditions, and good storage properties, etc. Gas formation is the most typical function for yeast fermentation. The expansion and leavening of the dough contributes mainly to the bread texture. Various metabolites produced by the yeast positively influence the formation of flavors as well as structure.

Wine fermentation is a complex process associated with the sequential growth of various yeasts strains which play prominent roles in determining the fermentation speed and representative attributes (Lv et al. 2013). Yeasts, originated from grapes and cellar, are tightly related to "spontaneous" alcoholic fermentation. They come in contact with the juice during crushing, pressing, pumping and fermentation, and the air (Raspor et al. 2006). It has been documented that the freshly extracted grape juice maintains a yeast population of 1,000–100,000 cfu (colony forming units) per milliliter consisting of mainly *Hanseniaspora* (*Kloeckera*) (Tamang and Fleet 2009). Besides, *Candida*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, and *Torulaspora* are also common yeast strains in alcoholic fermentation (Pretorius 2000). Usually the non-*Saccharomyces* initiate the fermentation by growing to the maximum population. During this early process, wine aroma and flavor are improved. Then *Saccharomyces* yeasts such as *Saccharomyces cerevisiae* and *Saccharomyces bayanus* are involved in wine fermentation. They increase the ethanol concentration during mid-to-final process (Calabretti et al. 2012).

For other food matrix, yeast fermentation is also an indispensible part leading to the unique characteristics of final products. In the dairy industry, yeasts may contribute either positively to the formation of aroma and flavor or negatively to the product spoilage (Fadda et al. 2004). The common species identified from cheeses are *Debaryomyces hansenii*, *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus* and *Galactomyces geotrichum*

(Addis et al. 2001, Romano et al. 2006). Their functions are considered as improving the cheese flavor and texture by producing proteases and lipases. In the fermentation of kefir grain, lactose is converted to ethanol and carbon dioxide in the presence of *Kluyveromyces marxianus*, *Candida inconspicua*, *Candida maris*, *Torulopsis kefir*, and *Saccharomyces cerevisiae* (Kabak and Dobson 2011). Further more, yeasts are naturally present in raw material of meats and their populations remain high during maturation (Tamang and Fleet 2009). The contributions of yeasts in meat fermentation lie in their positive impact on color, flavor and texture formation. To be specific, yeasts secrete proteases and lipases; utilize organic acids to produce flavor volatiles and carboxyls (Kabak and Dobson 2011).

3.3 Molds (fungi) Fermentation

Fungal fermentation is closely related to food production, such as for beer, wine, bread, and cheese. Fungi are used widely to provide supplements for instance, methionine, glutamic acid, riboflavin and vitamin B_{12} for normal and healthy growth. The advantages for using food-grade fungi are summarized as low cost of raw material, fast growth of cells, non-allergenic properties and lack of pathogenicity. Nowadays, diverse food substrates have been adopted for fungal fermentation to make desirable foods. Molds, in particular are predominant in the processes of many Asian fermented foods, followed by amylolytic and alcohol-producing yeasts. Molds are used as starter cultures for some fermented meat products including dry fermented sausages and dry cured hams (Hierro et al. 2005). Molds have been the most important microorganisms in sausage ripening apart from LAB. They may improve the external appearance, aroma and flavor of the products by degradation of lipid and protein, and become the major part of the microflora at the end of the drying process (Selgas et al. 2003).

Tempeh is made from soybean fermentation after soaking and cooking. It has been agreed that *Rhizophus oligosporus* is the dominant fungus together with *Rhizophus oryzae* and *Mucor* species responsible for the texture, flavor and nutritional value of the final products (Wiesel et al. 1997). *Rhizopus* starter culture is mixed to the beans to initiate fermentation. In successful tempeh fermentation, the individual beans will be bound into a solid cake by the mold mycelium firstly. Then the soybean will be partially digested by the mold enzymes (Babu et al. 2009). The antimicrobial compounds secreted by *Rhizophus oligosporus* during fermentation may inhibit the growth and toxin accumulation of some undesirable microorganisms (McCue et al. 2005).

Another case is koji which is often used for the saccharification of starch from grain or soybeans in preparation of traditional fermented foods such as miso, sake, and some pickles in Japan. Koji mold (*Aspergillus oryzae*) is cultivated on steamed rice or other grains for growth and enzymes production. Noticeably, *Aspergillus oryzae* has great power of producing enzyme and transforming starches, proteins and lipids into bioactive compounds at high level (Murooka and Yamshita 2008).

4 Fermented Foods and Health Benefits

Food fermentation covers a wide range of microbial and enzymatic processing to attain desirable features of the final products, such as enhancing food safety, improving organoleptic attributes, enriching nutrients, and promotion of health. Nowadays, the awareness of consumers towards health-promoting foods with "friendly bacteria" has resulted in increasing demand for fermented food products. Since the well established concept of clear relationship between diet and health, the market for these "functional foods" has been growing remarkably (Batdorj et al. 2007). Functional foods designed as "food for specific health use" was first established in 1991 by Japanese government (Rebucci et al. 2007). A food which affects beneficially one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease can be regarded as functional (Diplock et al. 1999). Functional foods bring good balance of ingredients with claimed health-specific advantages as well as basic nutrition in foods. Expectation of longevity, increasing cost of medical care, and desire of the elderly to improve the quality of their lives, have motivated development in the area of functional foods (Stanton et al. 2005).

4.1 Probiotic as Functional Food

Diet greatly influences the consumer's own state of health and is responsible for obesity, cardiovascular disease, cancer and other diet-related diseases. One of the reasons for the popularity of fermented foods is that people believe these foods may promote health in many positive ways that are primarily associated with the GI (gastrointestinal) tract. As people's awareness of healthier diet in modern society is rising, functional foods associated with safe and "natural" therapy represent a bright future in food market. A growing body of scientific evidence has been continually demonstrated by microbiologists.

The word "probiotics" means *for life*. This concept started being used in the early years of the 20th century and was coined by Lilly and Stillwell in 1965 to denote viable microorganisms which provide health benefit to the host when administered in appropriate amounts (Lilly and Stillwell 1965). Since then the definition has been broadened to a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert health effects in this host primarily by promoting the proliferation of beneficial GI indigenous microflora (Schrezenmeir and de Vrese 2001, Lye et al. 2009). Fermented foods are the basic sources of probiotics and dietary supplements in our society. Usually, probiotics are more effective when ingested in a food medium than in the form of tablets or capsules. Thus, the use of probiotics as starters combining with the positive impact of fermentation is becoming a tendency in the modern food industry.

A probiotic must fulfill the following criteria (Gupta and Abu-Ghannam 2012): (1) It has scientifically validated health properties and demonstrated safety. (2) It has good technological properties for large scale production and incorporation into food products without loosing viability and function. (3) It does not create unpleasant flavor or texture in food products. (4) It exhibits high survival rates in downstream processes and in food products during storage (e.g., tolerance to acid and bile, adhesion to the intestinal epithelium of the hosts, and antagonistic activity against pathogenic bacteria). Besides, probiotics contain no undesirable properties like virulence factors, detrimental biochemical characteristics, and antibiotic resistance (Ryu and Chang 2013, Lin et al. 2007).

Probiotic foods are those foods which carry live single or mixed culture of microorganisms and when consumed beneficially affect the host. The world probiotic market is estimated at $ 15 billion and is growing at a pace of 5 to 30 percent depending on the country and product type (Bhadoria and Mahapatra 2011). Yogurt is a successful example of good delivery of probiotics and a total of 78 percent of current probiotic sales in the world are delivered through yogurt (Granato et al. 2010).

Nowadays, probiotics can be used both for human and animals. Intake of certain amount of viable microbial cells leads to health benefits not only in the GI tract, but also in the respiratory and urogenital tracts. Commercial probiotic brands including Actimel, ProViva, Actifit, Yakult, Gefilus, and LCI have been gradually developed and recognized by consumers (Baker 2005). Their preparations may consist of one single strain (e.g., Yalkult, Japan-*Lctobacillus casei* Shirota) or mixed cultures (e.g., Bacilac, Belgium-*Lactobacillus acidophilus* and *Lactobacillus rhamnosus*; food supplement VSL-3, Italy-8 different LAB strains) (Nagpal et al. 2012). Some commercial examples of probiotic products are listed in Table 3.

Table 3. Some commercial examples of probiotic products (Gupta and Abu-Ghannam 2012).

Brand/Trade name	Description	Producer
Actimel	Probiotic drinking yogurt with *Lactobacillus casei Imunitass*® cultures	Danone, France
Activia	Creamy yogurt containing *Bifidus ActiRegularis*®	Danone, France
Gefilus	A wide range of *Lactobacillus rhamnosus* GG products	Valio, Finland
Hellus	Dairy products containing *Lactobacillus fermentum* ME-3	Tallinna Piimatööstuse AS, Estonia
Jovita Probiotisch	Blend of cereals, fruit and probiotic yogurt	H&J Bruggen, Germany
Pohadka	Yogurt milk with probiotic cultures	Valašské Meziříčí Dairy, Czech Republic
ProViva	Refreshing natural fruit drink and yogurt in many different flavors containing *Lactobacillus plantarum*	Skåne mejerier, Sweden
Rela	Yogurts, cultured milks and juices with *Lactobacillus reuteri*	Ingman Foods, Finland
Revital Active	Yogurt and drink yogurt with probiotics	Olma, Czech Republic
Snack Fibra	Snacks and bars with natural fibers and extra minerals and vitamins	Celigüeta, Spain
SOYosa	Range of products based on soy and oats and includes a refreshing drink and a probiotic yogurt-like soy-oat product	Bioferme, Finland
Soytreat	Kefir type product with six probiotics	Lifeway, USA
Wholegrain liquid	Organic ingredients including the grains, beans, and seeds	Grainfields, Australia
Yakult	Milk drink containing *Lactobacillus casei* Shirota	Yakult, Japan
Yosa	Yogurt-like oat product flavored with natural fruits and berries containing probiotic bacteria (*Lactobacillus acidophilus*, *Bifidobacterium lactis*)	Bioferme, Finland
Vitality	Yogurt with pre- and probiotics and omega-3	Müller, Germany
Vifit	Drink yogurts with *Lactobacillus rhamnosus* GG, vitamins and minerals	Campina, the Netherlands
Vita Biosa	Mixture of aromatic herbs and other plants, which are fermented by a combination of lactic acid and yeast cultures	Denmark

4.2 Origin of Potential Fermented Probiotic Strains

Our intestine is one of the most diversely colonized parts in the body with microbial populations comprising approximately 10^{11}–10^{12} cfu per gram of contents (Slavin 2013). As a favorable condition for growth, GI tract gains benefits from microbiota which build up a barrier to prevent detrimental

factors and keep the tract in a good state. The composition of the microflora may change depending on the physiological conditions of the host (e.g., age stress, and health status), ingredients of diet, and environment such as antibiotic therapy, and hygiene, etc. (De Fillippo et al. 2010). Various species of microorganisms delivered in fermented foods are in the spotlight for their probiotic properties. Table 4 lists some common strains used as probiotics.

Table 4. Strains used as probiotics (Hoover 2011).

Genus	**Species**
Lactobacillus	*acidophilus*, *brevis*, *delbrueckii* (subsp. *bulgaricus*), *fermentum*, *gasseri*, *johnsonii*, *paracasei*, *plantarum*, *reuteri*, *rhamnosus*, *salivarius*
Bifidobacterium	*adolescentis*, *animalis* (subsp. *lactis*), *bifidum*, *breve*, *infantis*, *longum*
Pediococcus	*acidilactici*
Streptococcus	*thermophilus*, *salivarius*
Enterococcus	*faecium*
Bacillus	*coagulans*, *clausii*
Escherichia	*coli*
Yeast	*Saccharomyces cerevisiae*

4.2.1 Lactobacillus and Bifidobacteria

Probiotic products are a fast growing area of functional food with increasing number of strains used in the food market. Undoubtedly a majority of accepted probiotics belong to the LAB species. LAB are naturally acid tolerant and capable of surviving in GI tract of human and animals as normal microflora. Especially *Lactobacillus* and *Bifidobacteria* are the most commonly used probiotic strains in animal feeds and human foods as well as in therapeutic, prophylactic and growth supplements (Coeuret et al. 2004, Kailasapathy and Chin 2000, Kesarcodi-Watson et al. 2008). These two kinds of probiotic cultures are often used in combination in commercial food products. Their growths require sufficient nutrients such as carbohydrates, amino acids, vitamins, salts and nucleic acid derivatives. They have a long recorded history of safe use in fermented and probiotic foods, showing positive effects in producing antagonistic compounds to pathogens, yielding bioactive health-beneficial peptides from raw materials, strengthening intestinal barrier, and modulating immune response (Savijoki et al. 2006, Marco et al. 2006). These promotions for GI health have shown specificity among strains and the exact molecular mechanisms remain unclear.

The genus *Lactobacillus* belongs to Gram-positive, rod-shaped, catalase-negative, non-motile, non-sporulating, and strictly fermentative facultative anaerobes (Singh et al. 2009). As the largest genus within the LAB, *Lactobacillus* takes part in the development of a unique organoleptic profile

and food safety, being widely used in the food and feed industries. More than 140 described species are included, displaying a variety of phenotylic, biochemical and physiological traits (Nezhad et al. 2013). They are common indigenous microorganisms and are often located in the ileum of the small intestine. *Lactobacillus* strains have been isolated from kefir grains, Maasai fermented milk, European cheeses and other traditional fermented products, showing the functional and metabolic properties of probiotics (Baruzzi et al. 2011). Typical probiotics are members of the *Lactobacillus acidophilus* group (such as *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus gasseri*, *Lactobacillus reuteri*, and *Lactobacillus plantarum*) (Tham et al. 2012). Species including *brevis*, *delbrueckii* (subsp. *bulgaricus*), *fermentum*, *johnsonii*, *pentosus* and *salivarius* are also considered. The potential mechanisms include producing antimicrobial substances, competition for nutrients, competition for binding sites to the intestinal cells and modulation of the immune system (Parvez et al. 2006).

Lactobacillus plantarum has been isolated from different ecological niches such as meat, vegetables, milk, fish and grain products. As a GRAS status, *Lactobacillus plantarum* is widely used as starter culture in different fermentation processes contributing to the organoleptic properties, flavors, and texture as well as food safety. *Lactobacillus plantarum* is natural inhabitant of the human gut and is a source of several commercial probiotics such as *Lactobacillus plantarum* 299v and *Lactobacillus plantarum* NCIMB8826 (de Vries et al. 2006). Research data showed that *Lactobacillus plantarum* has the ability to protect against EIEC (Enteroinvasive *Escherichia coli*) by inducing damage of the epithelial monolayer barrier function (Qin et al. 2009).

Lactobacillus casei is the predominant species of the *Lactobacillus* genus, being isolated from a variety of conditions such as fermented dairy, meat and vegetable foods as well as the human GI tract. Several strains of *Lactobacillus casei* have been studied for their potential probiotic properties with satisfying utilization in health-promoting foods (Guerin-Danan et al. 1998, Spanhaak et al. 1998). *Lactobacillus* (*casei* subsp.) *rhamnosus* GG is a probiotic strain of human origin, which excellently survives in the stomach and small intestine and transiently colonizes in the GI tract (Saxelin 1997). The efficacy of *Lactobacillus rhamnosus* GG fermented milk product in health has been demonstrated earlier.

On the other hand, probiotic bifidobacteria are Gram-positive, rod-shaped, non-spore forming, non-motile, catalase-negative, and obligate anaerobes including *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Bifidobacterium infantis* (Cheikhyoussef et al. 2008). Bifidobacteria primarily reside in the large intestine, especially in the area of the caesum and have the ability of proliferating and colonizing neonatal intestines after birth (Liu et al. 2007). It is well documented that bifidobacteria take effect in inhibition of pathogenic bacteria (e.g., *Salmonella*, *Shigella*, *Campylobacter*

jejuni, *Staphylococcus aureus*) both *in vitro* and *in vivo*. Species of *adolescentis*, *animalis* (subsp. *lactis*), *bifidum*, *breve*, *infantis*, and *longum* have been studied.

4.2.2 Pediococcus

Pediococci are usually isolated from fermented vegetable foods (Papagianni and Anastasiadou 2009), and less frequently from fermented sausage, cheese and alcoholic beverage (Holzapfel et al. 2006). Ryu and Chang (2013) demonstrated that *Pediococcus pentosaceus* MP1, isolated from kimchi showed high tolerance to acid and bile environment, antimicrobial activity against four pathogens, and adherence to Caco-2 and HT-29 cells, suggesting potential candidature for functional purpose.

4.2.3 Enterococcus

The genus of *Enterococcus* has a predominant habitat in the GI tract of human and animals (Ambadoyiannis et al. 2005). They can also be found in some dairy products and positively influence the development of organoleptic characteristics of cheeses. Some enterococci strains have been acclaimed for their health benefits of preventing the growth of food-borne pathogens and alleviating diarrhea, etc. (Ambadoyiannis et al. 2005, Underdahl 1983).

4.2.4 Bacillus

Bacillus species are promising candidates of probiotics as they bear spores, which provide potential benefit of a live passage through the stomach to the intestine (Patterson and Burkholder 2003). The typical strain *Bacillus subtilis* has been isolated from the human GI tract and regarded as gut commensals (Hong et al. 2009). It has been proved that *Bacillus* species exert a synergistic effect in pathogen inhibition (Mante et al. 2003). Research also indicated that natto, a *Bacillus* fermented food has the potential of scavenging dietary mutagenic heterocyclic amines (Rajendran and Ohta 2001). *Bacillus* has been reported to possess a number of beneficial health effects, including preventing pathogenic bacteria, reducing blood pressure, and exhibiting anticancer activity although the specific modes of action have not been fully clarified. Some beneficial effects of *Bacillus* in probiotic are related to the production of useful metabolites to improve intestinal microbial balance, as well as to reduce viable pathogen cells and their toxin production (Dunne and Shannahan 2002).

4.2.5 Yeast

Saccharomyces boulardii is a common probiotic yeast which converts glucoside isoflavones into aglycones and in hence increase the antioxidant activity of fermented soymilk (Pyo et al. 2005). It may also promote the growth of LAB during fermentation and the survival of LAB during shelf-life (Rekha and Vijayalakshmi 2008). Another potential probiotic yeast from kefir is *Kluyveromyces marxianus* CIDCA 8154, which may inhibit the innate response of the intestinal epithelium triggered by different proinflammatory pathways (Romanin et al. 2010).

4.2.6 Other strains and species

Other strains and species belonging to *Streptococcus*, *Enterococcus*, and *Escherichia* are also used as probiotics cultures (Hoover 2011). Exceptional properties contain catalase activity, immune enhancing ability, easier commercial preparations due to spore formation, etc. (Patel et al. 2010).

4.3 Mode of Action of Probiotics

Currently, the interaction among probiotics, diet and host remains partially understood due to the niche's complexity, although plenty of clinical evidence has emphasized the importance of probiotics (Kleerebezem and Vaughan 2009, Lebeer et al. 2008). The mechanisms of action by which probiotics promote health appear to be a combination of direct interaction with the host and indirect function in microbiota modulation (Ceapa et al. 2013). Possible mechanisms are summarized as: (1) enhancing the natural barrier function of the normal intestinal mucosa, (2) modulation of the immune system, (3) inhibition of pathogens and (4) production of enzymatic activities and/or beneficial metabolites for the host (McFarland 2009).

There are several reasons for probiotic strains to inhibit pathogens. Firstly, probiotics produce inhibitory compounds (e.g., bacteriocins, hydrogen peroxide, and reuterin) directly into the environment. These compounds may inhibit the growth of deleterious bacteria. Bacteriocins are ribosomally synthesized peptides which have potential application in enhancement of food safety. For instance, a bacteriocinogenic strain of *Lactobacillus plantarum*, isolated from the traditional pearl millet-based African fermented food effectively inhibited *Bacillus cereus*, *Escherichia coli* O157:H7 and *Salmonella enterica* cells within 48 hr (Valenzuela et al. 2008). Reuterin is another antimicrobial agent with a broad inhibitory spectrum and hypothesized to prevent microbial growth by inhibiting the ribonucleotide

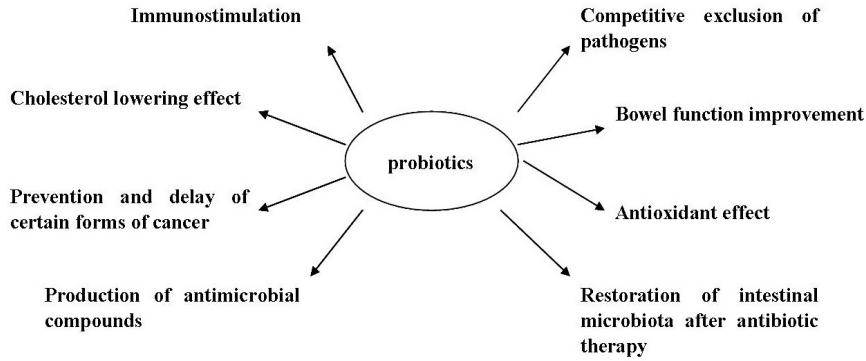

Fig. 1. Mode of action of probiotics (Divya et al. 2012).

reductase, related proteins and other small molecules (Divya et al. 2012). Secondly, probiotics usually produce some short chain fatty acid (e.g., lactic acid) to reduce the luminal pH. Thirdly, probiotics compete for nutrients and space for growth. Furthermore, stimulation of the immune system and regulation of colonocyte gene expression, maintenance of mucosal integrity also contribute to the antimicrobial effect (Steer et al. 2000, Magnusson et al. 2003). It has been demonstrated that LAB and particularly *Lactobacillus plantarum* are capable of inhibiting *Aspergillus*, *Fusarium*, *Penicillium* and other molds in bakery products (Todorov and Franco 2010).

4.4 Health Effects of Fermented Foods

Generally, health benefits of probiotic foods are decided by the strains presented in the foods and their remaining viabilities after passing through the host's intestine. According to the joint Food and Agriculture Organization (FAO)/WHO Working group, the principal outcome of efficacy studies for probiotics needs to demonstrate proven benefits in human trials (Blandino et al. 2008). To colonize and take effect in the intestine, a probiotic strain is required to tolerate with low pH, presence of bile salts and some enzymes. Since lactic acid is the main metabolic acid produced by LAB, members of these strains can adapt to acidic conditions.

Regular consumption of probiotic products with daily intake of approximately 10^9 viable cells has been recommended (Sohrabvandi et al. 2010). Specific reasons for probiotic administration have been summarized as follows: (1) constipation; (2) treatment of irritable bowel syndrome, diarrheal and inflammatory bowel diseases; (3) allergy development; (4) protection of liver function; (5) bile salts deconjugation; (6) relieving aging process; (7) prevention of vaginal infections; (8) improving blood lipid profiles and reducing blood pressure; (9) treating *Helicobacter pylori* infection;

(10) body weight control; (11) improving infant health and nutrition; (12) degradation of nitrosamines; (13) enhancing calcium absorption; (14) antitumorigenic activity; and (15) recolonization after clinical treatment (Hoover 2011).

Many commercial probiotic strains were originally isolated from the GI tract of human beings and the composition of the gut microbiota maintains consistency in adults. Taken into consideration that there are hundreds of microflora normally existing in the human intestine, maintenance of microbial balance is crucial to prevent colonization by potentially pathogenic microorganisms. Other important functions include relieving lactose intolerance, improvement of digestibility of dairy products, enhancing immunomodulatory activity, preventing infection, decreasing risk of cancer, decreasing serum cholesterol levels, and reducing the development of allergy, etc. (Table 5). As a result, the combination of probiotic bacteria with food products to increase therapeutic value is becoming more and more popular (Williams 2010).

4.4.1 Antioxidant activity

Dietary components are dominant in protecting the human body from oxidative damage. Research revealed that soybean fermented foods may lead to the increase of anti-oxidative capacity (Berghofer et al. 1998). It has been proved that soy sauces have high antioxidant activity which can protect against lipid peroxidation and probably come from proteins (LeBlanc 2011). Chen et al. (2006) have also reported that kefir contained a series of bioactive compounds and presented a good antioxidant activity. Vinegar is rich of phenolic compounds which are studied for the antioxidant activities. The health benefits of vinegar in addition to its use as a seasoning agent are supported by sufficient evidence (Seki et al. 2008).

4.4.2 Maintenance and restoration of normal intestinal balance

The complex composition of the intestinal microbiota is relatively stable in healthy people (Awaisheh et al. 2005). Any disturbance in the intestinal microbiota composition may lead to the growth of undesirable microorganisms and consequently causes infectious diseases (Oliveira et al. 2001). Pathogens can be established when the integrity of the microbiota is impaired by stress, illness, antibiotic treatment, changes in diet or physiological alterations in the gut (Macfarlane and Cummings 1999).

Diarrhea is often caused by the imbalance of normal intestine. Consumption of probiotic foods are reported to reduce the risk of acute or rotavirus-induced diarrhea in humans (Saavedra et al. 1994). It has been well

Table 5. Probiotic bacteria and their reported health benefits.

Reported effects	Probiotic species	References
Modulation of immune system	*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus delbrueckii, Lactobacillus rhamnosus, Bifidobacterium bifidum*	Steer et al. 2000, Zanini et al. 2007, Khailova et al. 2010, Matsuguchi et al. 2003
Balancing of gut microbiota and prevention of pathogens	*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus paracasei, Lactobacillus reuteri, Bifidobacterium longum, Bifidobacterium bifidum*	Steer et al. 2000, Sameshima et al. 1998, Muthukumarasamy and Holley 2006
Reducing faecal enzyme activities (enzymes involved in activation of carcinogens)	*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus gasseri, Lactobacillus delbrueckii*	Steer et al. 2000
Antitumorigenic activity	*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus gasseri, Lactobacillus delbrueckii, Lactobacillus plantarum, Bifidobacterium infantis, Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifidobacterium longum, Lactobacillus rhamnosus* GG	Steer et al. 2000, Fuchs et al. 2008, Goldin et al. 1996
Prevention of traveller's diarrhea	*Saccharomyces* spp., *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus rhamnosus* GG, *Bifidobacterium bifidum, Streptococcus thermophilus*	Steer et al. 2000, Hilton et al. 1997, Briand et al. 2006
Prevention of rotavirus diarrhea	*Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus, Bifidobacterium bifidum*	Steer et al. 2000, Guandalini 2000, Simakachorn et al. 2000
Antibiotic associated diarrhea; *Clostridium difficile*	*Lactobacillus rhamnosus* GG, *Lactobacillus* casei, *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus, Streptococcus boulardii, Streptococcus faecum, Bifidobacterium longum, Bifidobacterium lactis*	Steer et al. 2000, Hickson et al. 2007, McFarland 2010, Shiby and Mishra 2013
Prevention of other diarrhea	*Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum*	Steer et al. 2000
Prevention of infant diarrhea	*Lactobacillus rhamnosus, Bifidobacterium lactis, Streptococcus thermophilus*	Bhadoria and Mahapatra 2011, Saavedra et al. 1994
Reduction of serum cholesterol levels	*Lactobacillus plantarum, Lactobacillus helveticus, Pediococcus acidilactici*	Mandal et al. 2009, Bilige et al. 2009, Wang et al. 2009
Prevention of allergy	*Lactobacillus* spp., *Bifidobacterium* spp.	Isolauri et al. 1999, 2000

documented that *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* shortened the duration of rotavirus diarrhea (Guandalini 2000, Simakachorn et al. 2000). Formula milk supplemented with *Bifidobacterium bifidum* and *Streptococcus thermophilus* reduced rotavirus shedding and episodes of diarrhea in children in the hospital (Saavedra et al. 1994). The efficacy of *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* in traveler's diarrhea treatment has also been found (Hilton et al. 1997, Briand et al. 2006). Supplementation of infant formulas with *Bifidobacterium lactis* and *Streptococcus thermophilus* protected the infants from nosocomical diarrhea (Saavedra et al. 1994).

Diarrhea associated with antibiotic use and caused by *Clostridium difficile* is a complication occurring in about 5–25 percent of patients (Bergone-Berezin 2000). At least 10 percent of all antibiotic treatments are related to diarrhea, vomiting, and abdominal pain (Marteau and Rambaud 1993). Disturbance of intestinal microbiota may induce the overgrowth of *Clostridium difficile* causing severe diarrhea (Thompson-Chagoyán et al. 2007). Antibiotics exert toxic effects on intestinal mucosa, gut motility, and carbohydrate metabolism, etc. (Pirker et al. 2013). Lactobacilli, bifidobacteria, and *Streptococcus* species have been studied for the treatment of antibiotic-associated diarrhea (AAD). *Streptococcus boulardii* convincingly showed evidence of reducing incidence of AAD and/or *Clostridium difficile*-associated diarrhea (McFarland 2010). Probiotics including *Bifidobacterium longum*, *Bifidobacterium lactis*, *Lactobacillus rhamnosus* GG, *Lactococcus* La-5, *Streptococcus faecum*, and *Lactobacillus casei* DN-114-1 with the yogurt cultures *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* have also been reported to reduce antibiotic induced diarrhea (Shiby and Mishra 2013, Hickson et al. 2007). The yeast *Saccharomyces boulardii* has also been studied for prevention and treatment of diarrhea caused by *Clostridium difficile* infection (Surawicz et al. 1989). Usually probiotics have little influence on the microbial composition and will not lead to microbial disturbances. Therefore, they may also play an important role in the treatment of inflammatory bowel disease (IBD) caused by the alterations of intestinal microbiota.

4.4.3 Relieving lactose intolerance and improvement of digestibility of dairy products

The action of microorganisms during the preparation of food fermentation or in the GI tract improves the quantity, availability and digestibility of some dietary nutrients. LAB were shown to release different enzymes into the intestinal lumen and exert synergistic effects on digestion (Kopp-Hoolihan 2001). Lactose is the main carbohydrate disaccharide naturally present in all mammalian milks. When people are not capable of producing

enough lactase, the undigested lactose is utilized by the bacteria in the intestine. Maldigestion of lactose leads to loose stools, abdominal bloating, pain, flatulence, nausea and borborygmi (Tuohy et al. 2003). This common symptom is called "lactose intolerance" and a great portion of world population is suffering from this ailment. It is estimated, the lactose intolerance varies from about 2 percent in Scandinavia to about 70 percent in Sicily within Europe (Vesa et al. 2000). It has been accepted that the lactose-intolerance can be reduced with the consumption of probiotic dairy products, making the use of fermented dairy products as the primary solution to this problem. Lactose concentration can be lower in fermented products either by bacteria degradation of lactose or introduction of bacteria enzymes with high lactase activity. Strains of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Bifidobacterium longum* used in fermented milk products deliver enough bacterial lactase to the intestine and stomach (Kopp-Hoolihan 2001). Therefore, consumption of fermented dairy foods containing these strains reduces the symptoms of lactose intolerance.

4.4.4 Antitumorigenic activities and decreasing risk of cancer

Increasingly, research data indicate that probiotic is capable of preventing certain cancers in several ways. Early studies focused on the consumption of fermented dairy products in reducing the risk of breast tumors (van't Veer et al. 1989). Evidence showed that yogurt helped reduce incidence of colon cancer in some population groups (Ganjam et al. 1997). Extracts of fermented kefir inhibited 29 percent of the proliferation of human mammary cancer cells (Chen et al. 2007). *Lactobacillus acidophilus* resulted in a decrease of the mycotoxins ochratoxin A and supplementation with *Lactobacillus acidophilus* in diet significantly suppressed the total number of colon cancer cells in rats (Fuchs et al. 2008, Rao et al. 1999). Mutagenic activity was eliminated by fermented soymilk (Hsieh and Chou 2006). Mechanisms for antitumorigenic activities have not been fully explained. However, it is accepted the bioactive compounds have the ability of preventing cancer initiation by hindering related enzymes and activating the immune system (Ahmed et al. 2013). Chalova's group have demonstrated the selected strains from genera *Lactobacillus* and *Bifidobacterium* biosynthesized and released the extracellular bioactive compounds which have antimutagenic properties against benzopyrene and sodium azide as a function of growth phase (Chalova et al. 2008). The anti-carcinogenic actions include reduction of colonic pH, immuno-stimulation, anti-mutagenicity, and reduction in the activity of enzymes responsible for the conversion of procarcinogens to carcinogens (Nagpal et al. 2012). It has been documented that lactobacilli degraded carcinogens such as N-introsamines and *Bifidobacterium infantis* induced activation of phagocytes for destroying tumor cells (Rowland

and Grasso 1975, Sekine et al. 1994). Feeding of *Lactobacillus rhamnosus* GG prior to the carcinogen challenge decreased the incidence of colonic tumors (Goldin et al. 1996).

4.4.5 Immunomodulating activities

The immune system is a dynamic section in regulation of our body states and there is growing interests in research of immunomodulating strains for the stimulation of the GI immune system. Although the mechanisms by which the probiotics enhance the immune system have not been fully clarified, extensive documents have indicated that fermented products have a potential to improve immune systems both in animals and human models. Since each probiotic influences the immune system in a particular fashion, knowledge of immunomodulating properties is strain-specific. It has been demonstrated that probiotics may induce immune responses and intestinal barrier integrity. The cell wall components of probiotic strains such as peptidoglycans, polysaccharides, and teichoic acid may take immunostimulatory effects (Divya et al. 2012). Studies have been focused on the ability of probiotics to modulate cytokine production ranging from stimulation to inhibition of pro-inflammatory and anti-inflammatory cytokines and chemokines (Osmanagaoglu et al. 2013).

LAB or fermented milks have been reported to enhance NK cell activity, produce antibodies, and proliferate T and B cells (Pujol et al. 2000). A previous study showed bifidobacteria induced formation of large amounts of IgA *in vitro* (Yasui and Ohwaki 1991). For *in vivo* study, *Bifidobacterium breve* fed in fermented milk induced macrophage-like cells in mice (Yasui et al. 1992). Administration of LAB and *Bifidobacterium bifidum* strains increased the NK activity and reduced apoptosis in the intestinal epithelial cell line (IEC-6) in the testing models respectively (Zanini et al. 2007, Khailova et al. 2010). *Lactobacillus casei* Shirota and *Lactobacillus rhamnsus* Lr23 have been shown to trigger the formation of regulatory dendritic cells and stimulate macrophages to produce TNF-α *in vivo* (Matsuguchi et al. 2003). Studies revealed that the fermented milks with *Lactobacillus casei* contributed to modulating the response of cells of the innate immune system during intensive exercise (Pujol et al. 2000).

4.4.6 Reduction of serum cholesterol levels

The mechanisms of cholesterol removal of probiotics remain unclear until now. Research suggested some probiotic strains have the ability of producing enzymes for cholesterol utilization, and reduce total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein

(LDL) cholesterol (Dilmi-Bouras 2006, Xiao et al. 2003). Therefore, the risk of cardiovascular diseases caused by hypercholestremia is reduced. Research in hypercholesterolemic mice showed that administration of low levels of *Lactobacillus reuteri* for seven days decreased total cholesterol and triglyceride levels by 38 percent and 40 percent, respectively, and increased the HDL:LDL ratio by 20 percent (Taranto et al. 1998). Studies on *Pediococcus acidilactici* LAB5 isolated from vacuum packed fermented meat product, *Lactobacillus plantarum* MA2 from kefir and *Lactobacillus helveticus* MG2-1 from home-made koumiss in Mongolia have also confirmed their probiotic properties in cholesterol reduction (Mandal et al. 2009, Bilige et al. 2009, Wang et al. 2009).

4.4.7 Enrichment of nutrients and bioactive compounds

People will get benefits from the improved nutritional value of fermented foods. The bioavailability of protein and fat are enhanced by bacterial enzymatic hydrolysis. For instance, short chain fatty acids (SCFA) such as lactic acid, propionic acid and butyric acid are produced by microorganisms, contributing to the available energy source (Rombeau et al. 1990).

Kefir, a typical fermented dairy product contains nearly all of the important nutrients. The fermentation process leads to a higher nutritional value with balanced composition. Researchers found that vitamins such as pyridoxine, vitamin B_{12}, folic acid, and biotin are increased whereas thiamine and riboflavin are reduced during kefir fermentation (Kneifel and Mayer 1991, Liut Kevicius and Sarkinas 2004). Protein contents are increased in ammonia, serine, lysine, alanine, and threonine (Guzel-Seydim et al. 2003). Minerals such as calcium, potassium, phosphorus, magnesium, and other micro-elements are also enhanced (Liut Kevicius and Sarkinas 2004).

Soybean is rich in genistein, an isoflavone which has been studied for protection against cancers. In miso and tempeh, free isoflavones are liberated from genistein and daidzein by β-glucosidase during fermentation (Chen et al. 1999). The protein digestibility, protein efficiency ratio and net protein utilization are increased after tempe fermentation (Tchango 1995). Besides, the antinutritional factors such as trypsin inhibitors and phytic acid contained in raw soybeans decreased while the contents of calcium, zinc and iron increased significantly during tempe fermentation (Nout and Kiers 2005).

Meat fermentation is also related to the nutrient enrichment. Stadnik and Dolatowski (2013) found that inoculation of dry-cured pork loins with a probiotic strain *Lactobacillus casei* ŁOCK 0900 significantly increased the content of peptides and free amino acids.

Bioactive compounds are produced during the fermentation process when microorganisms degrade substrates to various small molecules. In

milk fermentation, for instance, α-, β-, κ-caseins and whey proteins are activated to digestible peptides, providing health-promoting benefits (Korhonen and Pihlanto 2006). Meat-based bioactive compounds include conjugated linoleic acid (CLA), carnosine, anserine, L-carnitine, glutathione, taurine, and creatine (Ravyts et al. 2012). It is reported that LAB may increase the content of the vitamins of the B group (riboflavin, niacin, vitamin B_6, and vitamin B_{12}) and active peptides such as opiates, angiotensin converting enzyme inhibitors, platelet aggregating inhibitors and some casein-related peptides in fermented foods (Deeth and Tamime 1981, Matar et al. 2001, Nout and Kiers 2005). The immunoenhancing properties might also come from these bioactive compounds which are released during fermentation. *Bifidobacterium animalis* hydrolyzed isoflavone glucosides into bioactive and bioavailable aglycones in fermented soybean milk. The enrichment of isoflavone aglycone together with balance of intestinal microbiota contributed to the improved bioavailability of isoflavones (Tsangalis et al. 2005). *Monascus*-fermented rice in Asian countries contains different pigments and dimerumic acid whose functions including antibiotic activities and immunosuppressive activities against mouse T splenocytes have been studied (Martinkova et al. 1999). It is also documented that *Monascus*-fermented products may effectively inhibit inflammation, suppress atherosclerosis, promote eNOS, improve dyslipidemia and blood pressure regulation resulting in hypertension prevention (Lee and Pan 2012).

4.4.8 Other effects

Allergic diseases developed by the differences in intestinal microbiota composition are prevalent (Björkstén et al. 2001, Kalliomäki et al. 2001). The main approach to the treatment of food allergy is removal of food materials containing allergens. Probiotics including lactobacilli and bifidobacteria promote normalization of the increased intestinal permeability caused by the exposure to food allergens (Isolauri et al. 1999). Addition of probiotics such as *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12 in formula showed effect on eczema alleviation in infants with atopic eczema probably due to the anti-inflammatory properties of the probiotic strains (Isolauri et al. 2000, Martín et al. 2013). Colonization of the GI tract with bifidobacteria induces oral tolerance and alleviates allergic disorders (Isolauri et al. 2012).

Some probiotic microorganisms show inhibitory activities to food-borne and spoilage pathogens. For example, *Lactobacillus rhamnosus* FERM P-15120 and *Lactobacillus paracasei* subsp. *paracasei* FERM P-15121 prevented the growth and enterotoxin production of *Staphylococcus aureus* (Sameshima et al. 1998). *Lactobacillus reuteri* ATCC 55730 and *Bifidobacterium longum* ATCC 15708 inactivated *Escherichia coli* O157: H7 during sausage manufacturing (Muthukumarasamy and Holley 2006).

It is also suggested that probiotic administration provides benefits for protection of liver function, prevention of vaginal infections, reduction of blood pressure and management of diabetes, etc. (Hoover 2011, Yadav et al. 2007). LAB are promising candidates for development as oral delivery vehicles which deliver biologics to targeted locations and tissues for digestive enzymes and vaccine antigens (Klaenhammer et al. 2002). It has also been studied that consumption of a certain amount of probiotic ham regularly provided positive effect to cardiovascular disorders (Kołożyn-Krajewska and Dolatowski 2012).

4.5 Safety Evaluation

Although LAB have been widely accepted as GRAS status, trails and tests are necessary to ensure that proper amount of high viable probiotic culture can reach to the intestinal tract and take effect. Factors associated with food safety should also gain more attention and be evaluated carefully.

An important concern related to probiotics is the potential high level of biogenic amines (BA), namely histamine, tyramine, 2-phenylethylamine, agmatine, putrescine, and cadaverine which might represent a food poisoning hazard (Alberto et al. 2002). BA are low molecular weight organic molecules and are normally formed by microbial decarboxylation of their precursor amino acids. These molecules are the neurotransmitters or precursors for the synthesis of hormones, alkaloids or other metabolites. In a proper fermentation process, BA concentration is low and cannot cause food poisoning. Detrimental psychoactive and vasoactive effects by getting into the blood circulation system may be induced when accumulation of BA occurs (potentially in fermented protein-rich food products) (Buňková et al. 2013). *Lactobacillus* is active in accumulation of histamine, tyramine and putrescine, and enterococci are recognized as tyramine formers (Bover-Cid and Holzapfel 1999). Since LAB fermented foods may contain traces of histamine, tyramine, putrescine, and cadaverine, BA amounts should be monitored in related fermented foods such as dairy and soybean products. BA have been reported to occur in cheese, tempeh, miso, natto, douchi, stinky tofu, sufu, dry fermented sausages, and fish, etc. (Buňková et al. 2013, Guan et al. 2013, Bover-Cid et al. 2001, Dapkevicius et al. 2000).

Mycotoxins such as patulin and ochratoxin A, produced by several fungal species are also risk factors in some fermented foods (Pattono et al. 2013). Mycotoxinogenic species of filamentous fungi such as *Aspergillus ochraceus*, *Penicilium nordicum*, and *Penicillium verrucosum* may grow on the surface of fermented sausages and become potential detrimental factors to the consumers (Iacumin et al. 2009).

5 Conclusion and Future Prospects

During the past decade, significant scientific evidences and technological innovations associated with food fermentation have been achieved. The upgrading of indigenous fermented foods is undertaken with the innovation of food technologies. With the development of industrialization and urbanization, there is increasing demand for large-scale production of fermented foods with stable high quality. For one thing, effective process control is necessary to ensure smooth and desirable fermentation, and for another, identification and characterization of microbial compositions and interactions are required for better properties and useful treatment. Intense research efforts are under way in unraveling the microflora in different traditional fermented foods as well as their specific functions contributing to the unique features of these foods. Desire for well-being will continue having great impact on consumers' purchasing behavior. There will be a growing trend of consuming fermented foods with health benefits globally. More fundamental research is needed to elucidate the health benefits in different fermented products. Meanwhile, standard protocol for probiotic usage as well as convenient and viable forms of probiotic cultures is to be considered.

Keywords: Food fermentation, LAB, Bacillus, Yeast, Mold, Probiotic, Health benefit

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3

Fermented Cereal Products

Zlatica Kohajdová

1 Introduction

Fermentation is one of the oldest technologies used for food preservation (Kalui et al. 2010). As a technology, food fermentation dates back at least 6000 yr (Holzapfel 2002, Kohajdová 2010), and probably originated from microbial interactions of an acceptable nature. Fermentation has enabled our ancestors in temperate and cooler regions to survive winter season and those in the tropics to survive drought periods, by improving the shelf life and safety of foods. Through the ages, fermentation has had a major impact on nutritional habits and traditions, on culture and on the commercial distribution and storage of food. Traditional fermentation process still serves as a substitute where refrigeration or other means are not available for the safekeeping of food (Holzapfel 2002).

Fermentation plays at least five roles in food processing (Steinkraus 2002, Kohajdová and Karovičová 2007): enrichment of the human dietary through development of a wide diversity of flavours, aromas and textures in food, preservation of substantial amounts of food through lactic acid, alcoholic, acetic acid and alkaline fermentations, enrichment of food substrates biologically with protein, essential amino acids, essential fatty acids and vitamins, detoxification during food fermentation, a decrease in cooking times and fuel requirements. Its importance in modern-day life is underlined by the wide spectrum of fermented foods marketed both in developing and industrialized countries, not only for the benefit

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of preservation and safety, but also for their highly appreciated sensory attributes (Holzapfel 2002, Kohajdová and Karovičová 2007, Kohajdová 2010). Fermentation is an inexpensive and the most important economical form of production and preservation of food for human consumption (Nyanzi and Jooste 2012, Adebayo et al. 2013).

Fermented foods and beverages are defined as those products that have been subjected to the effect of microorganisms or enzymes to cause desirable biochemical changes (Blandino et al. 2003, Kohajdová and Karovičová 2007), and are produced world-wide using various manufacturing techniques, raw materials and microorganisms (Blandino et al. 2003). Each fermented food is associated with a unique group of micro-biota (Kumar et al. 2012). At the present, a variety of fermented foods are produced all over the world in household as well as industrial level, in both small scale and large commercial enterprises (Kalui et al. 2010). It is estimated that 25 percent of the European diet and 60 percent of the diet in many developing countries consist of fermented foods (Adebayo et al. 2013).

Steinkraus (2002) classified fermentations into the following four groups: alcoholic, lactic acid, acetic acid and alkali fermentation. Alcohol fermentation results in the production of ethanol, and yeasts are the predominant organisms (e.g., wines and beers). Lactic acid fermentation (e.g., fermented milks and cereals) is mainly carried out by lactic acid bacteria. A second group of bacteria of importance in food fermentations are the acetic acid producers from the *Acetobacter* species. *Acetobacter* converts alcohol to acetic acid in the presence of excess oxygen. Alkali fermentation often takes place during the fermentation of fish and seeds that are popularly used as condiment (Blandino et al. 2003).

Fermented cereals play a significant role in human nutrition in all parts of the world where cereals grow. Among all food fermentations (e.g., milk, meat, fish, soy or wine) cereal fermentations reach the highest volume (Brandt 2013). Fermented cereal based foods can be classified on the basis of either the raw cereal ingredients used in their preparation or the texture of the fermented products. The major cereal based foods are derived mainly from maize, sorghum, millet, rice or wheat. In terms of texture, the fermented cereal foods are either liquid (porridge) or stiff gels (solid). The cereal porridges (gruels) include for example ogi, mahewu and mawe, the cereal gels are for example kenkey, kisra, injera (Osungbaro 2009).

2 Nutritional Aspects of Cereal-based Fermented Foods

Cereal grains have been one of man's earliest sources of food (Osungbaro 2009). Their cultivation dates back to 7000 BC for wheat and barley, 4500 BC for rice and maize, 4000 BC for millet and sorghum, 400 BC for rye, and 100 BC for oats (Hammes et al. 2005, Kohajdová 2010, Coda et al. 2013).

The total global production of food crops amounts to roughly 3.6 billion tonnes, and 60 percent thereof are cereals (Hammes et al. 2005). Several cereals are cultivated, but on the worldwide basis, wheat and rice are the most important crops accounting for over 50 percent of the world cereal production (Coda et al. 2013). In developed countries up to 70 percent of the cereal harvest is used as animal feed. The remaining part plus nearly all cereals in developing countries are used for human nutrition (Hammes et al. 2005).

Cereal grains are considered to be one of the most important sources of proteins, carbohydrates, minerals and dietary fibre for people all over the world (Rivera-Espinoza and Gallardo-Navarro 2010, Das et al. 2013). Cereals and cereal constituents could be used as fermentable substrate for growth of different microorganisms (Das et al. 2013), including probiotic microorganisms (microorganisms that when ingested act on the intestinal microflora of the host providing beneficial effects beyond the nutritional ones) (Asmanah and Muna 2009a, Coda et al. 2013, Das et al. 2013), especially lactobacilli and bifidobacteria because they contain native prebiotic substances (such as β-glucan, arabinoxylan, galacto- and fructooligosaccharides) that protect probiotic microorganisms from the adverse conditions of the gastrointestinal tract. Furthermore, cereals can also be used to encapsulate probiotics and maintain the viability and functionality of these microorganisms (Charalampopoulos et al. 2002, Martins et al. 2013, Sharma and Mishra 2013). The nutritional value of a particular food depends on its digestibility and its content of essential nutrients. Both digestibility and its nutrient content may be improved by fermentation (Karovičová and Kohajdová 2003, 2005). During fermentation the enzymatic activity of microbial culture may predigest the macronutrients (Karovičová and Kohajdová 2003). The different ways by which the fermentation process can affect the nutritional quality of foods include improving the nutrient density and increasing the amount and bioavailability of nutrients (Karovičová and Kohajdová 2003, 2005). The latter may be achieved by degradation of anti-nutritional factors, pre-digestion of certain food components, synthesis of promoters for absorption and by influencing the uptake of nutrient by the mucosa (Karovičová and Kohajdová 2003).

Fermentation of cereals leads to a decrease in the level of carbohydrates as well as some non-digestible poly- and oligo-saccharides (Blandino et al. 2003, Kalui et al. 2010, Martins et al. 2013) and the availability of proteins and B-group of vitamins such as thiamine, fiboflavin and niacin may be improved (Karovičová and Kohajdová 2003, Blandino et al. 2003, Kohajdová 2010, Martins et al. 2013).

Fermentation of cereals by lactic acid bacteria has been reported to increase essential amino acids (such as lysine, methionine and tryptophan)

and their derivatives by proteolysis and/or by metabolic synthesis (Mugula et al. 2003b, Kohajdová and Karovičová 2007, Kohajdová 2010). The free amino acids also enhance the growth of yeasts and contribute directly or as precursors of flavour development during cereal-based fermentation (Mugula et al. 2003b, Kohajdová 2010).

Improvement in starch digestibility during fermentation can be related to enzymatic properties of fermenting microflora that bring about the breakdown of starch oligosaccharides. The enzymes bring about cleavage of amylose and amylopectin to maltose and glucose. Reduction in amylase inhibition activity may also be responsible for the starch digestibility (Sindhu and Khetarpaul 2001, Kohajdová and Karovičová 2007, Kohajdová 2010). Similarly an improvement in protein digestibility of fermented products is mainly associated with an enhanced proteolytic activity of the fermenting microflora (Sindhu and Khetarpaul 2001, Kohajdová and Karovičová 2007).

Fermentation, by certain lactic acid bacteria and yeasts, removes or reduces the levels of anti-nutritional factors such as phytic acid, tannins and polyphenols present in some cereals (Kohajdová and Karovičová 2007, Nyanzi and Jooste 2012). Fermentation provides optimum pH conditions for enzymatic degradation of phytate (due to the phytase activity) and releases minerals such as manganese (which is important growth factor of lactic acid bacteria), iron, zinc and calcium (Blandino et al. 2003, Kalui et al. 2010, Rivera-Espinoza and Gallardo-Navarro 2010). Tanin levels may be reduced as a result of lactic acid fermentation, leading to increased absorption of iron, except in some high tanin cereals, where little or no improvement in iron availability has been observed (Kohajdová and Karovičová 2007, Kohajdová 2010). Diminishing effect of fermentation on polyphenols may be due to the activity of polyphenol oxidase present in the food grain or microflora (Sindhu and Khetarpaul 2001).

Fermented foods may reduce the serum cholesterol concentration by reducing the intestinal absorption of dietary and endogenous cholesterol or inhibiting cholesterol synthesis in liver. Fermentation also imparts attributes of robust stability and safety to the product, and thereby pre-empts disease infections such as diarrhoea and salmonellosis (Karovičová and Kohajdová 2003, 2005).

Fermentation leads to a general improvement in texture, taste and aroma of the final products (Blandino et al. 2003). The traditional foods made from cereal grains usually lack flavour and aroma (Charalampopoulous et al. 2002, Kohajdová 2010). During cereal fermentations several volatile compounds are formed, which contribute to a complex blend of flavours in products. The presence of aromas represented by diacetyl, acetic acid and butyric acid makes fermented cereal-based products more appetizing (Table 1) (Blandino et al. 2003, Kohajdová 2010).

Table 1. Compounds formed during cereal fermentation.

Organic acids	**Alcohols**	**Aldehydes and ketones**	**Carbonyl compounds**
Butyric Heptanoic	Ethanol	Acetaldehyde	Furfural
Succinic Isovaleric	*n*-propanol	Formaldehyde	Methional
Formic Propionic	Isobutanol	Isovaleraldehyde	Glyoxal
Valeric *n*-Butyric	Amyl alcohol	*n*-Valderaldehyde	3-Methyl butanal
Caproic Isoburyric	Isoamyl alcohol	2-Methyl butanol	2-Methyl butanal
Lactic Caprylic	2,3-Butandieol	*n*-Hexaldehyde	Hydroxymethyl furfural
Acetic Isocaprilic	β-Phenylethyl alcohol	Acetone	
Capric Pleagronic		Propionaldehyde	
Pyruvic Mevulinic		Isobutyraldehyde	
Plamitic Myristic		Methyl ethyl ketone	
Crotonic Hydrocinnamic		Butanone	
Itaconic Benzylic		Diacetyl	
Lauric		Acetoin	

3 Microorganisms for Cereal Fermentation

A large proportion of the world cereals production is processed by fermentation prior to consumption (Nout 2009, Das et al. 2013). Traditional fermented foods prepared from most common types of cereals (such as rice, wheat, corn or sorghum) are well known in many parts of the world (Blandino et al. 2003). Cereal fermentation processes are affected by characteristics variables, the control of which is the basis of all technological measures that are used to obtain the various products at a defined quality. These variables include the following (Hammes et al. 2005): type of cereal determining the fermentable substrates, nutrients, growth factors, minerals, buffering capacity and efficacy of growth inhibiting principles, water content, degree and moment of comminution of grains, i.e., before or after soaking, duration and temperature of fermentation, components added to the fermenting substrate, such as salt, sugar, hops and oxygen, and source of amylolytic activities that are required to gain fermentable sugars from starch or even other polysaccharides. Among these variables, the type of cereal plays a key role. It affects the amount and quality of carbohydrates as primary fermentation substrates, nitrogen sources, and growth factors such as vitamins, minerals, buffering capacity and the efficacy of growth inhibitors (Hammes et al. 2005).

The microbiology of many cereal-based fermented products is quite complex and not known (Blandino et al. 2003). Changes occur during fermentation that are as a result of the activity of microorganisms: bacteria,

yeasts and moulds (Kalui et al. 2010). These microorganisms either singularly or in combination contribute to the creation of great variety of products (Hammes et al. 2005). Lactic acid bacteria are the predominant organisms involved in the fermentation of cereal based foods and beverages (Nyanzi and Jooste 2012), yeasts are also frequently reported, but at much lower orders of magnitude (Guyot 2012). The common fermenting bacteria are species of *Leuconostoc, Lactobacillus* and *Pediococcus* and the yeasts most frequently found are of the genera *Saccharomyces* (Blandino et al. 2003, Kohajdová 2010).

The term lactic acid bacteria is used to describe a broad group of Gram-positive, catalase-negative, non-sporulating rods and cocci, usually non-motile, that utilize fermented carbohydrates and form lactic acid as the major end product (Blandino et al. 2003, Kalui et al. 2010). In accordance to the pathways by which hexoses are metabolized, they are divided into two groups: homo-fermentative and hetero-fermentative. Homo-fermentative such as *Pediococcus*, *Streptococcus*, *Lactococcus* and some lactobacilli produce lactic acid as the major or sole end product of glucose fermentation. Hetero-fermenters such as *Weisella* and *Leuconostoc* and some lactobacilli produce equimolar amounts of lactate, CO_2 and ethanol from glucose (Blandino et al. 2003). Lactic acid bacteria have strong inhibitory effects on the growth and toxin production of the other bacteria. This antagonistic activity can be the result of (Karovičová and Kohajdová 2003): competition for available nutrients, decrease in redox potential, production of lactic acid and acetic acid and the resulting decrease in pH, production of other inhibitory metabolites such as hydrogen peroxide, carbon dioxide or diacetyl, production of special antimicrobial compounds such as bacteriocins and antibiotics. Each of these properties and especially the combination of some of them can be used to extend the shelf-life and safety of fermented products (Karovičová and Kohajdová 2003, 2005). Lactic acid bacteria also claimed to have health benefits. These include antimicrobial effects against pathogenic bacteria such as *Shigella, Salmonella* and *Escherichia coli* (Nyanzi and Jooste 2012), anti-tumor effects *via* binding, inhibition or activation of mutagens *in vitro*, reductions in carcinogen-generating faecal enzymes *in vivo*, stimulation of immune system, suppression of tumour formation (Karovičová and Kohajdová 2003) and protection against diarrhoea associated with antibiotics or food allergy (Kalui et al. 2010).

In case when the cereal grains are used as natural medium for lactic acid fermentation, amylase needs to be added before or during fermentation or amylolytic bacteria need to be used because these microorganisms contain enough amylase which is necessary for saccharification of the grain starch (Kohajdová and Karovičová 2007). Amylolysis is not common among lactic acid bacteria but amylolytic lactic acid bacteria have frequently been isolated from starchy fermented foods and can represent around 10

percent of the lactic acid bacteria population (Guyot 2010). Their role has yet to be elucidated since mono- and di-saccharides such as glucose and sucrose, which occur naturally in cereals, are readily available for lactic acid fermentation (Reddy et al. 2008, Kohajdová 2010). This capacity has been reported in different strains of *Lactobacillus plantarum* and *Lactobacillus fermentum*, which are commonly isolated from this type of food, but amylolytic strains of *Lactococcus lactis*, *Streptococcus* spp. and of *Leuconostoc mesenteroides* have also been reported (Guyot 2012). The use of amylolytic lactic acid bacteria offers another alternative by combining both amylase production and acidification in one microorganism (Kohajdová and Karovičová 2007).

Yeasts also occur in symbiotic relationships with lactic acid bacteria in fermented cereals (Kohajdová 2010). It has been suggested that the proliferation of yeasts in foods is favoured by the acidic environment created by lactic acid bacteria while the growth of bacteria is stimulated by the presence of yeasts, which may provide growth factors, such as vitamins and soluble nitrogen compounds. Association of lactic acid bacteria and yeasts during fermentation may also contribute metabolites, which could impart taste and flavour to fermented food (Mugula et al. 2003b, Kohajdová and Karovičová 2007, Asmanah and Muna 2009a).

4 Cereal-Based Fermented Foods and Beverages

Lactic acid fermentation of cereals is a long-established processing method and is being used in Asia and Africa for the production of foods in various forms such as beverages, gruels and porridges (Hansen 2002, Charalampopoulos et al. 2002, Kohajdová and Karovičová 2007). Examples of some fermented cereal-based foods and beverages are presented in the Table 2 (Blandino et al. 2003, Helland et al. 2004, Osungbaro 2009, Champagne 2009, Guyot 2012). Pre-fermentation treatments of cereals are largely dependent on the type of cereal and on the desired end product. Generally, treatments such as drying, washing, steeping, milling and sieving are some of the processing steps applied in the preparation of these fermented cereal foods (Osungbaro 2009).

Although differences exist between regions, the preparation procedure could be generalized. Cereal grains, mainly maize, sorghum, or millet grains are soaked in clean water for 0.5–2 d. Soaking softens the grains and makes them easier to crush or wet-mill into slurry from which hulls, bran particles, and germs can be removed by sieving procedures. Fermentation takes place during the slurry making or dough making stage, which lasts for 1–3 d (Charalampopoulos et al. 2002, Kohajdová and Karovičová 2007, Kohajdová 2010). Traditional fermentation is usually done spontaneously without the addition of a commercial starter culture. The fermenting microorganisms

Table 2. Examples of fermented cereal-based foods.

Product name	Countries of production	Substrate	Fermenting microorganism	Textural characteristics
Ogi	Nigeria, Benin	Maize, sorghum or millet	*Lactobacillus* spp., *Aerobacter, Corynebacterium*, yeast, moulds	Liquid (porridge)
Mahewu	East African Countries	Maize, sorghum and millet	*Lactobacillus delbruckii, Lactobacillus bulgaricus, Streptococcus lactis*	Liquid (porridge)/beverage
Mawe	South Africa, Benin	Maize	*Lactobacillus fermentum, Candida krusei, Saccharomyces cerevisiae*	Liquid (porridge)
Kenkey	Ghana, Botswana	Sorghum, maize, millet	*Lactobacillus* sp., yeasts	Dough (solid)
Injera	Ethiopia, Sudan	Sorghum, tef, corn, finger millet	Yeasts, *Lactobacillus* sp.	Dough (solid)
Kisra	Sudan, Ethiopia	Sorghum	*Lactobacillus bulgaricus*, yeasts	Dough (solid)
Dosa	India	Rice, black gram	*Lactobacillus fermentum, Leuconostoc* spp., *Sacharomyces* spp.	Dough (solid)
Dhokla	India, Srí Lanka	Rice, black gram	Lactic acid bacteria, yeasts	Dough (solid)
Idli	India	Rice, black gram	*Leuconostoc mesenteroides, Lactobacillus delbruckei, Lactobacillus fermentum, Lactobacillus lactis, Sacharomyces cerevisiae*	Dough (solid)
Tarhana	Grece, Turkey	Wheat, milk (yoghurt)	*Lactobacillus* sp. *Sachcaromyces cerevisiae*	Dough (solid)
Uji	Kenya, Uganda, Tanzania	Sorghum, maize, cassava	*Leuconostoc mesenteroides, Lactobacillus plantarum*	Beverages
Boza	Bulgaria, Romania, Turkey, Albania	Wheat, rye, millet, Maize, etc.	*Lactobacullus* spp., *Leuconostoc, Sachcaromyces cerevisiae*	Beverage
Bushera	Uganda	Sorghum, millet		Beverage
Pozol	Mexico	Maize	*Lactobacillus pantarum, Lactococcus lactis, Lactobacillus casei, Lactobacillus delbruckii, Lactobacillus fermentum*	Beverage
Togwa	East Africa	Maize	*Lactobacillus pantarum, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus cellobiosus, Sacharomyces cerevisiae, Candida tropicalis*	Beverage

come from the raw materials or from a previous batch of the fermented products (back slopping) (Gadaga et al. 2013). During fermentation, the pH decreases with a simultaneous increase in acidity, as lactic and other organic acids accumulate due to microbial activity (Charalampopoulos et al. 2002, Kohajdová and Karovičová 2007, Kohajdová 2010).

4.1 Wheat-based Fermented Foods

One of the ways to increase the nutritional value of cereal-based products is by combining cereals with milk (Helland et al. 2004). Traditional dried fermented milk-cereal foods are widely used in the diet of people in the Middle East, Asia, Africa and some part of Europe (Georgala 2013). Tarhana and kishk are fermented milk-wheat mixtures containing lactic acid bacteria that have some probiotic properties and are considered as important foods in the diet of many populations (Blandino et al. 2003, Rivera-Espinosa and Gallardo-Navarro 2010). Even tarhana has been considered one of the oldest probiotic foods (Rivera-Espinosa and Gallardo-Navarro 2010, Kohajdová 2010).

4.1.1 Tarhana

Tarhana is a traditional Turkish cereal-based lactic acid fermented food product mainly produced at home or at home-scale level. It is also made commercially on small and large scales (Settanni et al. 2011). It is produced by mixing cereal flour (mainly wheat flour), yoghurt (stirred or set yoghurt), bakers yeast and a variety of cooked vegetables (tomatoes, onions, green peppers and red peppers), salt and spices (mint, thyme, dill, tarhana herb, etc.) (Blandino et al. 2003, Kohajdová 2010, Settanni et al. 2011, Sengun and Karapinar 2012, Georgala 2013). Tarhana can also be enriched with soybean (Kose and Cagindi 2002, Koca et al. 2002), lentil or chickpea (Yilmaz et al. 2010, Georgala 2013), germ and bran (Biglici et al. 2007), corn (Tarakci et al. 2004), barley (Erkan et al. 2006), whey concentrate (Taracki et al. 2004) and buckwheat (Biglici 2009).

Tarhana dough is prepared by mixing and kneading the ingredients, following by lactic and alcoholic fermentation for 1–7 d (Blandino et al. 2003, Kohajdová 2010, Yilmaz et al. 2010, Georgala 2013). The temperature of fermentation is in the range of 30–40°C depending on the procedure applied (Kohajdová 2010). Owing to its low pH (3.8–4.2) and moisture content (6.0–9.0 percent), tarhana is a medium that is not conducive to pathogenic microorganisms and therefore provides a naturally safe product with a long shelf-life (Yilmaz et al. 2010, Herken and Con 2012). The fermented slurry is air-dried and used in soup making, adding high nutritional

content of proteins and vitamins to the product. Regional diversity of the amount and type of ingredients, as well as the processing techniques, affect chemical compositions, nutritional content and sensory attributes of tarhana (Georgala 2013). This product has an acidic and sour taste with a strong yeasty flavour (Herken and Con 2012).

The fermentation of tarhana is a result of the action of a mixed population of microbes (Georgala 2013). Although many microorganisms may be present, principal organisms are lactic acid bacteria from yoghurt (*Lactobacillus bulgaricus, Streptococcus thermophilus, Lactococcus lactis, Lactococcus diacetylactis, Lactobacillus acidophilus, Leuconostoc cremoris* and *Lactobacillus casei*) (Kohajdová 2010, Settanni et al. 2011, Lar et al. 2013). Other bacteria such as *Lactobacillus plantarum* and *Lactobacillus brevis* were also identified in tarhana (Herken and Con 2012). Settani et al. (2011) isolated 224 lactic acid bacteria from tarhana samples fermented at 30 and 40°C and defined them as *Pediococcus acidilactici* (50.4 percent), *Lactobacillus plantarum* (31.1 percent) and *Lactobacillus brevis* (18.5 percent). *Pediococcus acidilactici* was found to have higher population rates at 40°C, while at 30°C, the rates were higher for *Lactobacillus plantarum* and *Lactobacillus brevis*. The presence of yeasts mainly belonging to the species *Saccharomyces cerevisiae*, is generally attributed to the addition of baker's yeasts during ingredient mixing (Settanni et al. 2011, Georgala 2013).

There are similar products as tarhana with different names such as kishk in Egypt, Syria, Lebanon and Jordan, kushuk in Iraq, trahanas in Greece and Cyprus, tarhonya/talkuna in Hungary and Finland and stole in Scotland (Settanni et al. 2011, Herken and Con 2012, Georgala 2013). Methods for preparation of such products vary from place to place, but cereals and fermented milks are always the two major components (Herken and Con 2012, Georgala 2013).

4.1.2 Kishk

Kishk is one of the traditional food products in Upper Egypt (Bahnasawy and Shenana 2004, El-Nawawy et al. 2012), Lebanon, Syria (Kohajdová 2010) and Jordan (Mahasneh and Abbas 2010). It is typically prepared by adding strained yoghurt to bulgur (cracked and bran-free parboiled wheat) and allows the mix to ferment at an ambient temperature for different periods of time. The wheat grains are boiled until soft, dried, milled and sieved in order to remove the bran. Milk is separately soured in a container, concentrated and mixed with the moistened wheat flour. The milk undergoes a lactic fermentation and the resulting paste is dried to a moisture content of 10–13 percent and then ground into a powder. The product is stored in the form of dried balls, brownish in colour with a rough surface and hard texture (Blandino et al. 2003, Kohajdová 2010). The microorganisms responsible

for the fermentation include *Lactobacillus plantarum, Lactobacillus casei* and *Lactobacillus brevis, Bacillus subtilis* and yeasts (Blandino et al. 2003, Kohajdová 2010). Probiotic strains *Lactobacillus rhamnosus* and *Lactobacillus sakei* are also usefully applied for kishk preparation (El-Nawawy et al. 2012).

Some modifications, such as the substitution of cow milk with soy milk yoghurt (El-Nawawy et al. 2012), and whole wheat-meal for bulgur have been proposed in the formulation of kishk (Blandino et al. 2003). It was shown that kishk prepared from soy yoghurt had higher protein content than milk kishk (El-Nawawy et al. 2012) and that substitution of whole wheat-meal for bulgur enhances the availability of Ca, Fe, Mg and Zn and provide a better means for the utilization of wheat nutrients (Blandino et al. 2003).

4.2 Corn-based Fermented Foods

Fermented maize meals or dough are intermediate products used widely in many part of the West African sub-region for the preparation of various staple dishes (Annan et al. 2003).

4.2.1 Ogi

Ogi is an example of traditional fermented gruel consumed in Nigeria (Evans at al. 2013) obtained by fermentation of a suspension of maize in water (Greppi et al. 2013), although sorghum, millet and soybean flours are also used (Kohajdová and Karovičová 2007, Kohajdová 2010, Nyanzi and Jooste 2012). In Nigeria the name of ogi depends on the locality and the type of cereal. Ogi is the generic name in the West states of Nigeria where it is usually processed from white maize. Ogi from sorghum is known as ogi-baba while ogi-gero is prepared from millet. In Northern Nigeria, ogi is known as akamu or eko gbona, while in the Republics of Togo, Benin and Ghana, ogi from maize is known as koko (Nyanzi and Jooste 2012).

Ogi is traditionally produced by washing the grains, steeping for 12–72 hr, wet milling and wet-sieving. The sieved material is allowed to sediment and ferment for 1–3 d, and is marketed as wet cake wrapped in leaves (Nyanzi and Jooste 2012, Evans et al. 2013). Wet ogi usually has a smooth texture, a sour flavour resembling that of yoghurt and a characteristic aroma that differentiates it from starch and flour. The colour of ogi depends on the type of cereal used: cream-white for maize, light browns for sorghum and greenish to grey for millet (Omemu 2011).

Traditional fermentation processes of ogi production are usually spontaneous and uncontrolled (Kohajdová 2010). Lactic acid bacteria, yeasts and moulds are responsible for the fermentation although *Lactobacillus*

plantarum is the predominant microorganism (Blandino et al. 2003, Kohajdová and Karovičová 2007, Osungbaro 2009, Ojokoh 2009, Kohajdová 2010, Evans et al. 2013). *Lactobacillus brevis* and *Lactobacillus fermentum* were also isolated from Nigerian ogi samples (Ojokoh 2009, Omemu 2011). Other bacteria such as *Corynebacterium* hydrolyze corn starch, and then yeasts of the *Saccharomyces cerevisiae, Candida krusei* and *Candida tropicalis* also contribute to flavour development (Blandino et al. 2003, Kohajdová and Karovičová 2007, Osungbaro 2009, Asmahan et al. 2009a, Omemu 2011). Optimum pH for ogi is 3.6–3.7. The concentration of lactic acid may reach 0.65 percent and that of acetic acid 0.11 percent during fermentation (Kohajdová and Karovičová 2007, Kohajdová 2010).

4.2.2 Kenkey

Kenkey is a stiff gruel or dumpling (Annan et al. 2003) from maize consumed in Ghana (Nout 2009). There are two types, namely *Fanti-* and *Ga*-kenkey which differ in salt content and packing material (Nout 2009, Nyanzi and Jooste 2012). This product is made from the raw fermented dough and a partially cooked portion of the dough (1:3). The mixture, which is referred to as aflata, is made into balls which are wrapped in leaves, and boiled to cook for up to 3 hr. The final product is a ready-to-eat staple food eaten with soups or stews (Annan et al. 2003).

Kenkey fermentation is spontaneous and is dominated by lactic acid bacteria, particularly *Lactobacillus fermentum* and *Lactobacillus reuteri*, and *Candida krusei* is the exisiting dominant yeast species, while *Saccharomyces cerevisiae* also contributes to the flavour (Blandino et al. 2003, Annan et al. 2003, Nout 2009, Champagne 2009, Kohajdová 2010, Nyanzi and Jooste 2012). During the kenkey production process, the level of available lysine increased from 1.3 in maize to 3.3 g/16 g nitrogen in ready-to-eat kenkey. In addition, flavour components (2, 3-butanediol, butanoic acid, lactic acid, 3-methylbutanoic acid, octanoic acid, 2-phenylethanol and propionic acid) are also formed (Nout 2009, Nyanzi and Jooste 2012).

4.2.3 Mawe

This is fermented product consumed in Benin and Togo (Hounhouigan et al. 1993, Nout 2009, Nyanzi and Jooste 2012, Greppi et al. 2013). Mawe is an uncooked fermented maize dough, which is made by washing, wet extraction of endosperm, kneading to a dough, following by fermentation for about 3 d at ambient temperature (approx. 30°C) (Hounhouigan et al. 1993, Nout 2009). The pH of fermented mawe is about 3.5–4.0 (Nout 2009). Mawe serves as an important ingredient for the preparation of cooked

beverages (koko), stiff gels (akassa, agidi, eko) and steamed cooked bread (albo) in Benin (Nout 2009, Nyanzi and Jooste 2012, Greppi et al. 2013).

Hetero-fermentative lactic acid bacteria (*Lactobacillus fermentum, Lactobacillus cellobiosus, Lactobacillus brevis, Lactobacillus curvatus, Lactobacillus buchneri, Weissella confuse*) (Agati et al. 1998, Amoa-Awua et al. 2007, Nout 2009), pediococci and yeasts (*Candida krusei, Candida kefyr, Candida glabrata, Saccharomyces cerevisiae*) predominate in the fermented mawe dough (Amoa-Awua et al. 2007, Nout 2009, Champagne 2009, Nyanzi and Jooste 2012, Greppi et al. 2013).

4.3 Rice and Legume-based Fermented Foods

A global interest in rice and its fermented product is increasing due to their calorie, unique quality characteristics and high acceptability. In most of the countries, rice is fermented either by using mixed culture(s) into alcoholic beverages, or by natural fermentation into leavened batter formed dough breads which are usually baked or steamed (Das et al. 2013).

4.3.1 Idli

Idli, a fermented steamed product with a soft and spongy texture is a highly popular and widely consumed snack food (Das et al. 2013) in the Indian sub-continent made mainly from rice (75–80 percent) and black gram (20–25 percent) (Balasubramanian and Viswanathan 2007, Nout 2009, Manickavasagan et al. 2013). Soybean, green gram and chickpea can be substituted for black gram (Balasubramanian and Viswanathan 2007, Sekar and Mariappan 2007, Maheswari and Shetty 2013). Common finger and foxtail millet are substituted for a proportion of rice. Normally idli fermentation is a natural fermentation. Sometimes, sour buttermilk or yeasts are added to enhance fermentation (Sekar and Mariappan 2007).

Preparation of idli involves soaking (for 3–4 hr) and grinding rice and black gram separately, mixing batters of rice and black gram together and fermenting the batter overnight at room temperature (Aachary et al. 2011, Durgadevi and Shetty 2012, Manickavasagan et al. 2013). The fermented batter should be steamed in greased idli pans for 10–15 min. The prepared idli should hold soft and fluffy texture, white colour with good flavour and taste (Aachary et al. 2011).

Idli fermentation is a mixed auto-fermentation as the organisms present in the ingredients as well as the environment determine the nature of microflora involved (Aachary et al. 2011). *Leuconostoc mesenteroides, Lactobacillus delbrueckii, Lactobacillus fermenti, Lactobacillus lactis,* and *Leuconostoc mesenteroides* and yeasts especially *Saccharomyces cerevisiae*

are responsible for idli fermentation process (Blandino et al. 2003, Balasubramanian and Viswanathan 2007, Sekar and Mariappan 2007, Nout 2009, Kohajdová 2010, Das et al. 2013). The function of these organisms is diverse. They contribute to the leavening of the batter and flavour formation. Lactic acid bacteria reduce pH from 6.0 to about 4.2 which favours yeasts growth. Yeasts contribute to starch degradation and gas formation, as well as to the accumulation of vitamin B and free amino acids (Nout 2009).

4.3.2 Dosa

Dosa batter is similar to idli batter but the batter is thinner. After fermentation, the leavened dosa batter is baked on hot pan as a thin, crisp pancake and eaten with chutney and sambar [a curry prepared with mixed vegetables and flavoured with chilli powder, *Asa foetida* (hing) powder and other spices] (Sekar and Mariappan 2007, Das et al. 2013).

A dosa suspension is prepared by grinding wet rice and black gram separately with water. The two suspensions are then mixed and allowed to undergo natural fermentation, usually for 8–20 hr. To make a dosa, the fermented suspension is spread in a thin layer (of 1–5 mm thickness) on a flat heated plate, which is smeared with a little oil or fat. A sol to gel transformation occurs during the heating and within a few minutes, a circular, semi-soft to crisp product resembling a pancake, ready for consumption is obtained (Blandino et al. 2003, Kohajdová 2010, Das et al. 2013).

Traditional dosa batter fermentation has already revealed the occurrence and role of several bacteria alone or in combination with yeasts in bringing about various biochemical changes. *Leuconostoc mesenteroides*, *Streptococcus faecalis* and *Lactobacillus fermentum* are predominant among bacteria while *Saccharomyces cerevisiae*, *Debareomyces hansenii* and *Trichosporon beigelii* are the common yeasts involved in fermentation (Sandhu and Soni 1988, Kohajdová 2010).

4.3.3 Dhokla

Dhokla is also similar to idli except that Bengal gram dhal is used instead of black gram dhal in its preparation (Blandino et al. 2003, Sekar and Mariappan 2007, Kohajdová 2010, Das et al. 2013). A mixture of rice and chickpea flour is also used as the substrate for the fermentation. As in idli preparation, the fermented batter is poured into a greased pie tin and steamed in an open steamer (Blandino et al. 2003, Kohajdová 2010, Das et al. 2013).

4.3.4 Puto

Puto is a leavened steamed rice cake, which is consumed daily in many parts of the Philippines as a breakfast, dessert, or snack food. The product has a short shelf-life, and is made from rice that is washed and soaked overnight, ground and mixed with sugar and coconut milk. The resulting batter is then fermented for several hours, during which time acidification and leavening occur. The fermented batter is steamed for approximately 30 min before serving (Kelly et al. 1995, Kohajdová 2010). *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Leuconostoc pseudomesenteroides*, *Leuconostoc citreus* and *Leuconostoc fallax* were found to be the predominant bacteria isolated from puto (Kelly et al. 1995).

4.4 Sorghum-based Fermented Foods

Sorghum is widely grown in the semiarid topics of Africa and Asia, and constitutes a major source of carbohydrates and proteins for people living in this region (Nyanzi and Jooste 2012, Das et al. 2013). It is estimated that over 60 milion people use sorghum as part of their staple food in Africa both in the fermented or unfermented form (Nyanzi and Jooste 2012).

4.4.1 Injera

Injera is a leavened, flat round Ethiopian traditional bread made from cereals such as tef and sorghum (Yetneberk et al. 2004, Anyango et al. 2011) or from different cereal mixtures: teff and white sorghum, wheat and red sorghum and barley and wheat (Baye et al. 2013a,b). The preparation of injera from sorghum has considerable economic benefits over tef, as sorghum commands a much lower price (Yetneberk et al. 2004). Injera is made from flour, water and starter "ersho" (fluid saved from previously fermented dough) (Abiyu et al. 2013). The dough is fermented for 2 or 3 d (Blandino et al. 2003, Kohajdová 2010). After fermentation the dough is thinned down to a thick batter and poured onto a lightly oiled pan, which is then covered with a tightly fitting lid to retain the steam. Within about 2–3 min it is ready to be removed from the pan and then is placed in a basket. The storage period does not usually exceed 3 d at room temperature (Blandino et al. 2003, Kohajdová 2010).

The microorganisms involved in fermentation of injera are mainly yeasts, some fungi including *Pullaria* sp., *Aspergillus* sp., *Penicillium* sp., *Rhodotorula* sp., *Hormodendrum* sp., *Candida* sp. and number of unidentified bacteria (Blandino et al. 2003, Champagne 2009, Kohajdová 2010). A normal and typical injera is round, soft, spongy and resilient, about 6 mm thick, 60 cm in diameter with uniformly spaced honeycomb-like ''eyes'' on the

top. The major quality attribute of a good injera is its slightly sour flavour. Injera has a very high nutritional value, as it is rich in calcium and iron (Blandino et al. 2003, Kohajdová 2010).

4.4.2 Kisra

Kisra is an indigenous staple food to the majority of Sudanese people. It is a pancake-like bread made from sorghum or millet flour (Asmahan and Muna 2009a). Kisra fermentation is a traditional process whereby sorghum or millet flour is mixed with water in a ratio of about 1:2 (w/v) (Asmahan and Muna 2009a,b, Rahman et al. 2010). Usually a starter is added by a back-slopping using mother dough from previous fermentation as a starter at a level of about 10 percent. Fermentation is completed in about 12–19 hr by which time the pH drops from about 6.0 to less than 4.0 (Asmahan and Muna 2009a,b). The fermented dough is baked into thin sheets and it is eaten with certain types of stew prepared from vegetables and meat (Blandino et al. 2003, Kohajdová 2010).

Lactobacillus fermentum and *Lactobacillus amylovorus* have been suggested to be the predominant microorganisms during kisra fermentation (Halm et al. 1983, Asmahan and Muna 2009a,b). Other microorganisms such as *Lactobacillus brevis*, *Pediococcus pentosaceas*, *Acetobacters* sp. and *Saccharomyces serevisiae* were also identified from kisra (Blandino et al. 2003, Asmahan and Muna 2009a,b, Champagne 2009, Rahman et al. 2010).

4.5 Cereal-based Fermented Beverages

Nowadays, cereals alone or mixed with other ingredients are used for the production of traditional fermented beverages as well as for the development of new food with enhanced healthy properties (Coda et al. 2012). They can be classified based on the raw materials used or the type of fermentation involved in the manufacturing process. Alcohol fermented beverages can be classified into wines and beers, while the great majority of non-alcoholic fermentations are souring, mainly lactic acid fermentations (Blandino et al. 2003).

4.5.1 Bushera

Bushera is a traditional fermented beverage widely consumed in Uganda (Muyanja et al. 2003a). The sorghum or millet flour from the germinated sorghum and millet grains is mixed with boiling water and left to cool to ambient temperature (Muyianja et al. 2003a, Prado et al. 2008). Germinated millet or sorghum flour is then added and the mixture is left to ferment at

ambient temperature for 1–6 d (Muyianja et al. 2003a,b, Kohajdová 2010, Vashuda and Mishra 2013).

The lactic acid bacteria from household bushera included *Lactobacillus plantarum, Lactobacillus paracasei*, subsp. *paracasei, Lactobacillus fermentum, Lactobacillus brevis*, *Lactobacillus delbruckii* subsp. *delbruckii*, and *Streptococcus thermophillus*. The isolates from laboratory fermented bushera belong to the genera *Lactococcus, Leuconostoc, Lactobacillus, Weissella* and *Enterococcus* (Muyianja et al. 2003a, Nyanzi and Jooste 2012).

4.5.2 Mahewu

Mahewu (amahewu) is a sour maize-based fermented beverage (Chelule et al. 2010, Nyanzi and Jooste 2012) consumed in Africa and some Arabian Gulf countries (Blandino et al. 2003, Kohajdová 2010). Mahewu is prepared from the maize porridge (Prado et al. 2008), which is mixed with the water (Vashuda and Mishra 2013). This porridge, if thin, is mixed with water or if thick (*sadza*) is mashed into small pieces and mixed with water (Gadaga et al. 1999, Kohajdová 2010). The sorghum, millet malt, or wheat flour is then added and left to ferment (Blandino et al. 2003, Vashuda and Mishra 2013). The spontaneous fermentation process is carried out by the natural flora of the malt at the ambient temperature. The predominant microorganism found in African mahewu is *Lactococcus lactis* subsp. *lactis* (Gadaga et al. 1999, Blandino et al. 2003, Champagne 2009). The commercial production of mahewu is successfully realised in South Africa. The commercial mahewu is prepared using *Lactobacillus delbruckii* and the product is pasteurized after fermentation (Nyanzi and Jooste 2012).

4.5.3 Boza

Boza is a traditional fermented beverage (Kohajdová 2010) consumed in countries of the Balkan region including Bulgaria, Romania, Albania and Turkey (Cosansu 2009, Kivanc et al. 2011, Nyanzi and Jooste 2012). It is a viscous liquid product with a pale yellow colour and sweet or sour taste (Altay et al. 2013). It is produced from millet, maize, wheat, rye, or rice and other cereals (Zorba et al. 2003, LeBlanc and Todorov 2010, Nyanzi and Jooste 2012, Kancabas and Karakaya 2013). In the preparation of boza, the milled cereals are mixed in water and then cooked in an open or steam-jacketed boiler. The gruel is cooled and strained to remove the bran and hull. Sugar/saccharose powder is added and then fermented at 30°C for 24 hr (Todorov 2010, Altay et al. 2013) by back-slopping or use of sourdough and/or by adding yoghurt starter cultures (Nyanzi and Jooste 2012). Fermented boza is then cooled to refrigeration temperatures and distributed into plastic

bottles (Todorov 2010, Nyanzi and Jooste 2012). The shelf life of boza is fairly short; up to 15 d. Boza is not appropriate for storage below 10°C. Boza is acceptable for consumption at every stage of the fermentation until pH drops to about 3.5 (Gotcheva et al. 2001, Altay et al. 2013).

Spontaneous fermentation of boza involves lactic acid bacteria and yeasts (Cosansu 2009). A number of lactic acid bacteria (*Leuconostoc paramesenteroides*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Leuconostoc mesenteroides* subsp. *dextranicum*, *Leuconostoc oenus*, *Leuconostoc raffinolactis Lactobacillus coryniformis*, *Lactobacillus sanfrancisco*, *Lactobacillus fermentum, Lactobacillus plantarum*, *Lactobacillus acidophilus, Lactobacillus coprophilus, Lactobacillus brevis, Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus pentosus*, and *Lactobacillus rhamnosus*) isolated from boza have been reported (Zorba et al. 2003, Prado et al. 2008, Kivanc et al. 2011, LeBlanc and Todorov 2011, Nyanzi and Jooste 2012, Östürk et al. 2013).

Hancioglu and Karapinar (1997) isolated 77 lactic acid bacteria and 70 yeast strains from the Turkish boza. Among the lactic acid bacteria, *Leuconostoc paramesenteroides* (25.6 percent) was predominant, followed by *Lactobacillus sanfrancisco* (21.9 percent) and *Leuconostoc mesenteroides* subsp. *mesenteroides* (18.6 percent). On the other hand, Gotcheva et al. (2000) reported that *Lactobacillus plantarum* (24 percent of lactic acid bacteria), *Lactobacillus acidophilus* (23 percent of lactic acid bacteria) and *Lactobacillus fermentum* (19 percent of lactic acid bacteria) were predominantly isolated from Bulgarian boza. Yeasts belonging to the genus *Saccharomyces* and *Candida* (Guyot 2010) such as *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis, Saccharomyces uvarum, Candida tropicalis*, *Candida glabrata*, *Geotrichum penicillatum* and *Geotrichum candidum* were found in boza from Bulgaria or Turkey (Guyot 2010, LeBlanc and Todorov 2011, Nyanzi and Jooste 2012, Vashuda and Mishra 2013).

Diversity in fermentation flora and variations in numbers of lactic acid bacteria and yeast from the different samples could be due to different raw materials, production processes and storage conditions (Gotcheva et al. 2001, Altay et al. 2013). Many of the lactic acid bacteria such as *Lactobacillus plantarum* (Todorov and Dicks 2006, Todorov 2010), *Lactobacillus pentosus, Lactobacillus rhamnosus, Lactobacillus paracaesi* (Todorov and Dicks 2006), *Lactococcus lactis* (Todorov 2010) and *Leuconostoc lactis* (Todorov 2010, Altay et al. 2013) isolated from boza produce antimicrobial compounds, including bacteriocins, increasing the shelf life of the product and possibly demonstrate health benefits (Kabadjová et al. 2000, Todorov and Dicks 2006, LeBlanc and Todorov 2011, Kivanc et al. 2011).

Boza also inherently includes a number of probiotic species such as *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, and *Lactobacillus pentosus* (Todorov et al. 2008, Altay et al. 2013, Kancabas and Karakaya 2013, Östürk et al. 2013). The selection of boza strains with

probiotic properties is currently under way with the aim of developing novel healthy cereal-based foods (Gotcheva et al. 2000, Altay et al. 2013).

4.5.4 Togwa

Togwa a traditional lactic acid fermented product (Mugula et al. 2001, Kohajdová 2010), is prepared either from cereals (maize, sorghum, millet) (Mugula et al. 2003a, Kohajdová and Karovičová 2007, Hellström et al. 2012), root tuber of cassava (Hellström et al. 2012) or their combination (Mugula et al. 2001, Kohajdová and Karovičová 2007, Kohajdová 2010). Togwa is widely consumed in Africa (Vashudha and Mishra 2013) for use directly as a weaning food for younger children or diluted for use as a refreshment drink for adults (Kohajdová 2010, Hellström et al. 2012).

The cereal or cassava flour is cooked in the water. After cooling at 35°C, starter culture (old togwa) and/or cereal flour from the germinated grains are added (Molin 2001, Vashudha and Mishra 2013). The mixture is allowed to ferment for 12–24 hr before being consumed (Hjortmo et al. 2008). The fermentation process finishes at pH 4.0–3.2 (Vasudha and Mishra 2013). Microbial communities of togwa are diverse and consist both of lactic acid bacteria belonging to genera *Lactobacillus* and *Pediococcus* (*Lactobacillus brevis*, *Lactobacillus cellobiosus*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *Pediococcus pentosaceus*) (Mugula et al. 2001, 2003b, Hjortmo et al. 2008, Champagne 2009, Kohajdová 2010) and of yeasts belonging to *Candida*, *Saccharomyces* and *Issatchenkia* (*Candida pelliculosa*, *Candida tropicalis*, *Saccharomyces cerevisiae*, and *Issatchenkia orientalis*) (Mugula et al. 2001, 2003b, Hjortmo et al. 2008, Asmahan and Muna 2009a,b, Champagne 2009, Kohajdová 2010) at concentration 10^9 cfu (colony forming units)/cm^3 and 10^7 cfu/cm^3 (Mugula et al. 2003b).

4.5.5 Kvass

Kvass is a non-alcoholic cereal beverage which is traditionally produced from rye and barley malt, rye flour, and stale rye bread in eastern European countries (Dlusskaza et al. 2008, Nyanzi and Jooste 2012). It is a beverage that is similar to boza, a Turkish, non-alcoholic fermented beverage, with respect to the composition of the final product as well as the microflora (Hancioglu and Karapinar 1997, Dlusskaza et al. 2008, Kohajdová 2010).

Kvass is manufactured using two techniques. One technique involves the use of stale dough bread in which the sugars for the yeast fermentation are obtained from the bread-making process, while the second technique involves the use of malt enzymes to hydrolyse the gelatinized starch (Dlusskaza et al. 2008, Nyanzi and Jooste 2012). Prior to fermentation, the

kvass batter is diluted in boiling water and clarified by sedimentation. Sucrose is added to the kvass wort and fermentation is initiated by addition of baker's yeast or a previous batch of kvass (Dlusskaza et al. 2008, Kohajdová 2010). The fermentation process is terminated by cooling the kvass to 4°C and the product contains proteins, amino acids, vitamins and organic acids either from the raw materials or from the activity of the fermenting microorganisms (Dlusskaza et al. 2008, Nyanzi and Jooste 2012). Kvass has a golden brown colour, the pleasant flavour of rye bread, low sweetness, is not obviously alcoholic and has sufficient carbonate to give kvass a sparkle on the palate.

The microflora of kvass fermentation is consistently composed of lactic acid bacteria and yeasts (Dlusskaza et al. 2008, Kohajdová 2010). The predominant microorganisms in kvass fermentation were found to be *Lactobacillus. casei*, *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*. Kvass is not heat-treated after fermentation and as a result high counts of viable cells can be found in the beverage. The isolation of *Lactobacillus casei* from kvass (in which it was highly viable), is indicative of the potential that cereal-based beverages such as this can be used as alternatives to milk products in the delivery of probiotics and other functional ingredients to the consumer in the developing world (Dlusskaza et al. 2008, Nyanzi and Jooste 2012).

4.5.6 Pozol

Pozol is a maize acid beverage consumed in the South-eastern Mexico (Diaz-Ruíz et al. 2003). During pozol preparation (Nyanzi and Jooste 2012), maize grains (white or yellow) (Mendéz-Albores et al. 2004) are cooked in an approximately 1 percent (w/v) lime solution in water (Sharma and Mishra 2013) during approximately 90 min (Díaz-Ruíz et al. 2003) to obtain nixtamal (nixtamalization is a process in which maize, or other grains are treated by soaking and cooking in limewater) (Nyanzi and Jooste 2012). The nixtamalized products are then cleaned by washing in water to separate the husks (Díaz-Ruíz et al. 2003, Nyanzi and Jooste 2012). The grains are ground to make dough, shaped into balls, wraped in banana leaves and left to ferment at ambient temperature for 0.5–4 d (Prado et al. 2008, Vashuda and Mishra 2013). The pH of pozol is usually in range of 3.7–4.7 after 48 hr of fermentation. Pozol balls at different stages of fermentation can be mixed with water to make gruel of desired viscosity and then consumed as a beverage by adults, children and infants (Nyanzi and Jooste 2012). Some fibrous components are not completely solubilized by nixtamalization and sediment is present in the beverage when the dough is suspended in the water (Prado et al. 2008, Kohajdová 2010, Sharma and Mishra 2013).

The microbiology of pozol has been the subject of different studies, which show the importance and diversity of lactic acid bacteria (Guyot et al. 2003, Díaz-Ruíz et al. 2003, Blandino et al. 2003, Méndez-Albores et al. 2004). It is believed that nixtamalization plays an important role in affecting the microbial community by decreasing the initial concentration of readily available mono- and disaccharides. Recent studies showed that amylolytic streptococci and enterococci could have a great influence on structuring the pozol microbial community (Guyot et al. 2003, Díaz-Ruíz et al. 2003). *Lactococcus lactis, Streptococcus suis, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus alimentarium* and *Lactobacillus delbruekii* have also been identified in pozol (Blandino et al. 2003, Champagne 2009, Kohajdová 2010).

4.5.7 Uji

Uji is a non-alcoholic cooked beverage which can be made from maize, sorghum or finger millet. Uji is very popular in East Africa where it is used as a convenient breakfast, and as a food for children (Nout 2009). There are two types of uji, fermented and unfermented. The unfermented uji is prepared by boiling water and adding the flour while stirring to obtain the desired drinkable viscosity (Nout 2009, Nyanzi and Jooste 2012). The fermented uji can be prepared by spontaneous or back slop fermentation in a submerged culture state (Kohajdová 2010). The preparation of spontaneously fermented uji involves soaking of the grains until soft, wet grinding by stone machine, slurrying and removal of coarse particles and bran by filtration. The filtrate is allowed to ferment while the fine flour particles sediment. The supernatant (or some of it) is brought to the boil, and the wet sediment is gradually stirred into the boiling liquid to obtain a thin cooked beverage, to which often some sugar or salt is added for taste (Onyango et al. 2004, Nout 2009). The back slop technique involves adding a small amount of previously fermented uji into the fresh slurry before the latter is incubated. The slurry (30 percent, w/v solids) is allowed to ferment near a fire or at ambient temperature for 1±3 d after which it is diluted with water (to 8±10 percent w/v solids), boiled and sweetened and consumed while still hot (Kohajdová 2010).

During fermentation of uji *Lactobacillus plantarum* has been found to be the dominant *Lactobacillus* species, while *Lactobacillus fermentum, Lactobacillus cellobiosus* and *Lactobacillus buchneri, Pediococcus acidilactici* and *Pediococcus pentosaceus* are also reported to be part of the fermenting microorganisms in uji (Onyango et al. 2004, Nout 2009, Kohajdová 2010, Nyanzi and Jooste 2012).

4.5.8 New cereal-based probiotic beverages

Along with the antimicrobial effects of the lactic acid bacteria from cereal-based fermented foods, the use of these microorganisms and their fermented products for the production of new probiotic foods is also a new trend. The term probiotic refers to a products containing mono- or mixed cultures of live microorganisms, which when ingested will improve the health status and/or affect beneficially the host by improving its microbial balance (Blandino et al. 2003).

Proviva

Proviva is known to be the first commercial oats-based probiotic beverage (Prado et al. 2008). Proviva is produced by Skane Dairy and it has been a commercial product in Sweden since 1994 (Sharma and Mishra 2013). Proviva has malted barley added as a liquefying agent and the active probiotic component is *Lactobacillus plantarum* 299v (Champagne 2009, Nyanzi and Jooste 2012). The final product which is a mixture of fruit juice and 5 percent oat meal has a probiotic bacterial population count in the region 5 x 10^{10} cfu (colony forming units)/cm^3 (Nyanzi and Jooste 2012).

Yosa

Yosa is a new probiotic beverage made from oat bran (Rivera-Espinosa and Gallardo-Navarro 2010, Zubaidah et al. 2012). It is mainly consumed in Finland and other Scandinavian countries (Blandino et al. 2003, Kohajdová and Karovičová 2007, Kohajdová 2010, Rivera-Espinoza and Gallardo-Navarro 2010). This product has a flavour and texture comparable to that of dairy yoghurt and is made by cooking the oat bran pudding in water and fermenting it with (Kohajdová and Karovičová 2007, Kohajdová 2010, Nyanzi and Jooste 2012) probiotic bacteria *Lactobacillus acidophilus* LA5 and *Bifidobacterium lactis* Bb12 (Nyanzi and Jooste 2012, Zubaidah et al. 2012). Apart from probiotic bacteria, yosa also contains oat fibre, a source of β-glucan that has (Blandino et al. 2003, Nyanzi and Jooste 2012) a potency as a symbiotic beverage (combines prebiotic effect of β-glucan and probiotic effect of lactic acid bacteria) (Zubaidah et al. 2012).

Grainfields Wholegrain Liquid®

Grainfields Wholegrain Liquid® is a refreshing, effervescent liquid that delivers active, friendly lactic acid bacteria and yeasts as well as vitamins, amino acids, and enzymes. It is made from organic ingredients including

the grains, beans, and seeds such as the malted organic oats, maize, rice, alfalfa seed, pearl barley, linseed, mung beans, rye grain, and wheat, millet. The liquid is fermented to achieve high levels of active probiotic bacteria sustained in a liquid medium that is immediately available for use within the digestive system. Grainfields Wholegrain Liquid® is fermented with lactobacili and yeasts cultures: *Lactobacilus acidophilus*, *L. delbreukii*, *Saccharomyces boulardii* and *S. cerevisiae*. The liquid is dairy-free, contains no genetically modified ingredients and has no added sugar (Prado et al. 2008, Kohajdová 2010, Vashuda and Mishra 2013).

Other experimental cereal-based fermented beverages

Several workers have endeavoured to develop non-dairy cereal-based probiotic food products (Angelov et al. 2006, Gupta et al. 2010, Coda et al. 2012, Hassan et al. 2012, Nyanzi and Jooste 2012).

A symbiotic functional drink from the oats by combining a probiotic starter culture *Lb. plantarum* A28 was developed. The levels of starter culture concentration, oat flour and sucrose content were established for completing a controlled fermentation for 8 hr. The addition of aspartame, sodium cyclamate, saccharine and Huxol (12 percent cyclamate and 1.2 percent saccharine) had no effect on the dynamics of the fermentation process and on the viability of the starter culture during the product storage. The viable cells counts reached at the end of the process were about 7.5×10^{10} cfu/cm^3. The shelf-life of the oat drink was estimated to 21 d under refrigerated storage (Angelov et al. 2006).

In another study related to the oat, Box-Behnken optimization design was used to optimize three different levels of oat, sucrose and starter culture concentration on the final viable cells population of *Lb. plantarum* ATCC8014 for the production of a fermented drink. Oat and sugar concentration were found to be the factors with the greatest influence on *Lb. plantarum* growth. The oat based drink was successfully fermented with the optimized parameters (5.5 percent oats, 1.25 percent sugar and 5 percent inoculums) to obtain a growth of 10.4 log cfu/cm^3 and was found to be stable for 21 d with a reduction of less than 1 log cfu/cm^3 (Gupta et al. 2010).

The potential of two *Lb. plantarum* strains isolated from emmer flour and blackberries to ferment gelatinised cereal (rice, barley, emmer and oat) substrates combined with soy flour and concentrated red grape must was investigated. It was found that during fermentation lactic acid bacteria consumed glucose, fructose and malic acid, which was supplied with grape must. Prepared beverages had values of pH lower than 4.0 and both starters remained viable at ca 8.4 log cfu/cm^3 through storage at 4°C for 30 d (Coda et al. 2012).

Rice and millet grain were fermented with commercial probiotic starter culture ABT-2 (*Streptococcus thermophillus, Lactobacillus acidophilus, Bifidobacterium* BB-12) to obtain probiotic beverages, fortified with pumpkin and sesame seed milk. During product preparation, the rice and millet milks were heated (90°C, 20 min), cooled to 37°C, subsequently 5 percent of honey and 5 percent of starter culture were added. Then the material was fermented for 16 hr at 37°C and the plain beverage was enriched with 10 percent of sesame or pumpkin seed milk. At the end of the fermentation, viable cell counts reached 4.3 x 10 cfu/cm^3. The shelf life of these beverages was estimated to be 15 d under refrigerated storage; pH and acidity of beverages remained above 4.0 and lower than 1 percent, respectively, while starter culture remained above 8.0 log cfu/cm. It was also concluded that fermentation with ABT-2 starter culture improved colour, flavour, texture and overall acceptance of beverages (Hassan et al. 2012).

5 Conclusions and Perspectives

Cereals are considered one of the most important sources of dietary nutrient for people all over the world (Blandino et al. 2003, Coda et al. 2013). Nevertheless the nutritional quality of some cereals and the sensory properties of their products are sometimes inferior or poor compared to other staple-foods (Kohajdová 2010, Coda et al. 2013).

A variety of technologies (e.g., cooking, sprouting and milling) are used for cereal processing but fermentation still remains the best choice for improving the nutritional, sensory and shelf-life properties (Blandino et al. 2003, Kohajdová and Karovičová 2007, Coda et al. 2012). This is the main reason why a large proportion of cereals are processed into food and beverages by fermentation prior to consumption (Nout 2009, Coda et al. 2013).

Fermentation comprises the chemical changes in foods accelerated by enzymes of microorganisms resulting in a variety of cereal-based fermented foods (Nyanzi and Jooste 2012, Adebayo et al. 2013). In most of these products the fermentation is natural and involves mixed cultures of bacteria (mainly lactic acid bacteria), yeasts and fungi. Some of the microorganisms may participate in parallel, while others act in a sequential manner with a changing dominant flora during the course of the fermentation (Blandino et al. 2003). The understanding of the microbial ecology of cereal fermentations needs the knowledge of the fermentation substrates, i.e., the grains of the various cereal plants, as well as the products obtained thereof (Hammes 2005).

The food and beverage industry has re-discovered fermentation as a crucial step in product innovation (Hugenholtz 2013). In today's world the development and utilization of different cereal-based fermented probiotic

foods is a challenging task (Das et al. 2013). There is a need to isolate more microorganisms, mainly lactic acid bacteria, and to perform extensive studies on their probiotic properties (Kumar et al. 2012). Invention of newer technologies for processing of cereals to improve their nutritional value *vis-á-vis* their acceptability by the end users will be the focus area in the near future (Das et al. 2013).

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4

Lactic Acid Fermentation of Vegetables and Fruits

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1 Introduction

Lactic acid (LA) fermentation is considered a simple and useful form of biotechnology to keep and/or enhance the safety, nutritional, sensory and shelf life properties of vegetables and fruits (Demir et al. 2006). As shown in the data from literature of the last decade, the combination of this ancient method of bio-preservation with the current biotechnology tools should allow controlled fermentation processes and the selection of starter cultures to increase the consumption of fresh-like vegetables and fruits (McFeeters 2004, Di Cagno et al. 2013). Lactic acid bacteria (LAB) convert the carbohydrate contents of the vegetables and fruits into LA, which decreases the pH of the fermented products to around 4.0 ensuring stability. Lower pH value restricts the growth of spoilage flora and pathogenic bacteria. These bacteria improve the human intestinal microbial balance and enhance health by inhibiting the growth of pathogens such as *Escherichia coli*, *Salmonella* and *Staphylococcus* (Ohmomo et al. 2000, Ross et al. 2002). They are often considered as probiotic, beneficial for human health and active in lowering the serum cholesterol level (Kaur et al. 2002). They also stimulate immune responses and prevent tumour formation by inhibiting

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carcinogenic compounds in the gastro-intestinal tract through reducing fecal bacteria enzyme activity (Nakphaichit et al. 2011) or breaking down certain enterotoxins (Bernardeau et al. 2006).

Fruits are commonly processed for alcoholic fermentation of wine and beer as they are rich in sugars, vitamins, minerals. As juices are slightly acidic, they are therefore a suitable medium for the growth of yeasts, and fruit sugars are rapidly converted into ethanol. Vegetables on the other hand, have low sugar content but are rich in minerals, vitamins, have neutral pH and thus provide a natural medium for fermentation by LAB. Fermentation of fruits and vegetables can occur 'spontaneously' by the natural lactic acid bacterial surface microflora, i.e., *Lactobacillus*, *Leuconostoc*, *Pediococcus*, etc.; however, the use of starter culture such as *Lactobacter plantarum*, *Lb. rhamnosus*, *Lb. gasseri* and *Lb. acidophilus* (all probiotic strains) provides consistency and reliability of performance (Di Cagno et al. 2013). Pasteurizing or adding preservatives after fermentation, which are commonly done during the industrial production of lactic acid fermented vegetables (e.g., sauerkraut), destroy most of the LAB present, thus cancelling any possible probiotic effects (Montet et al. 2006).

This chapter describes the exploitation of vegetables and fruits through LA fermentation. The LAB microbiota, the occurring spontaneous fermentation, the main features of commercial/allochthonous and autochthonous starters, and the emerging and traditional fermented vegetable and fruit products are reviewed. The botanical names of vegetables and fruits mentioned in this chapter are given in Table 1.

2 Lactic Acid Bacteria

Lactic acid bacteria (LAB) are a group of organisms that ferment sugar (i.e., glucose) predominantly to LA. They are gram positive, non-sporulating rods and cocci having low guanine-cytosine content. Most of the LAB grow an-aerobically but they are also aero-tolerant. This group of bacteria is divided into two sub-groups.

(i) Homo-fermentative

This sub-group of bacteria produces a single fermentation product, i.e., LA *via* the glycolytic (Embden- Meyerhof) pathway (Steinkraus 2002). Members of the genera are *Pediococcus*, *Streptococcus* and *Lactococcus*. The fermentation of one mole of glucose yields two moles of LA.

$$C_6H_{12}O_6 \longrightarrow 2CH_3CHOHCOOH$$

Glucose Lactic acid

Table 1. Common and botanical names of the fruit and vegetables mentioned in the text.

Common Name	**Botanical Name**
"Almagro" eggplants	*Solanum melongena* L. var. *esculentum depressum*
Artichoke	*Cynara cardunculus* var. *scolymus*
Apple	*Malus sylvestris* L.
Bamboo	*Bambusa glaucescens* L.
Beet root	*Beta vulgaris* L.
Black berry	*Rubus fruticosus*
Black pepper	*Piper nigrum* L.
Brinjal (syn. Aubergine/Eggplant)	*Solanum melongena* L.
Cabbage	*Brassica oleracea* var. *capitata* L.
Capsicum (Yellow or green pepper)	*Capsicum annum* L.
Carrot	*Daucus carota* L.
Cassava	*Manihot esculenta* Crantz
Casper berry	*Capparis spinosa* L.
Cauliflower	*Brassica oleracea* var. *botrytis* L.
Celeriac	*Apium graveolens* var. *rapaceum* L.
Chinese cabbage	*Brasicca chinensis* L.
Cucumber	*Cucumis sativus* L.
False banana	*Ensete ventricosum*
Fennels	*Phaseolus vulgaris* L.
French beans	*Foeniculum vulgare* L.
Garlic	*Allium sativum* L.
Ginger	*Zingiber officinale* L.
Green pea	*Pisum sativum* L.
Horseradish	*Lactuca sativa* L.
Immature palm	*Phoenix dactylifera* L.
Lemon	*Citrus lemon* L.
Lime	*Citrus aurantifolia* L.
Kiwi fruits	*Actinidia deliciosa* L.
Mango	*Mangifera indica* L.
Mustard	*Brassica juncea* L., *B. campestris* L.
Okra	*Abelmoschus esculentus* L.
Olives	*Olea europea* L.
Onion	*Allium cepa* L.
Pak-sian	*Gynadropsis pentaphylla*
Papaya	*Carica papaya* L.
Parsley	*Petroselinum crispum* L.
Pear	*Pyrus communis* L.
Peas	*Pisum sativum* L.
Pepper	*Piper nigrum* L.
Pineapple	*Ananas comosus* L.
Pomegranate	*Punica granatum* L.
Plums	*Prunus domestica* L.

Table 1. contd....

Table 1. contd.

Common Name	Botanical Name
Radish	*Raphanus sativus* L.
Red chilli	*Capsicum frutescens* L.
Sesame	*Sesamum indicum* L.
Spinach	*Spinacea oleracea*
Sweet cherry	*Prunus avium* L.
Sweet orange	*Citrus sinensis* L.
Sweet potato	*Ipomoea batatas* L.
Taro	*Colocasia esculenta* var. *antiquorum* L.
Tomato	*Lycopersicum esculentum* L.
Turnip	*Brassica rapa* L.

(ii) Hetero-fermentative

This sub-group of bacteria produces LA plus appreciable amount of ethanol, acetate and CO_2 *via* the 6-phosphogluconate/phosphoketose pathway (Steinkraus 2002). Bacteria involved in this group belong to genera *Leuconostoc* and *Lactobacillus*. The biochemical pathway is as follows.

$$C_6H_{12}O_6 \longrightarrow 2CH_3CHOHCOOH + C_2H_5OH + CO_2$$

Glucose Lactic acid Ethanol Carbon dioxide

2.1 Information on D (–) Lactic Acid

Lactic acid is a three carbon carboxylic acid with the chemical formula $C_3H_6O_3$. In water solution, LA can lose a proton from the acidic group, producing the lactate ion CH_3CH (OH) COO; its pKa is 3.86. The higher acidity is the consequence of the intra-molecular hydrogen bridge between the α-hydroxyl and the carboxylate group, making the latter less capable of strongly attracting its proton. Two different isomers of LA may be produced during fermentation. They are classified depending on whether the polarized light rotates to the right L (+) or to the left D (–). The L (+) LA isomer is absorbed by the intestinal mucus and is used as energy substrate during the metabolic activity. On the other hand, the D (–) form is not assimilated and is eliminated by the kidneys in salt forms, leading to a loss of calcium and magnesium. Both of the LA isomers are usually present in the homemade or small-scale fermented vegetable preparations. However, the D (–) LA isomer concentrations in LA fermented vegetables are reported to be generally low.

2.2 Bacteriocin Production

LAB produce bacteriocins, which are peptides or small proteins that are frequently inhibitory towards many undesirable bacteria, including food-borne pathogens (e.g., *Listeria monocytogenes*, *Salmonella*, *Staphylococcus*, *Escherichia coli* and *Clostridium botulinum*) (Leroy et al. 2002). They can be subdivided into four groups:

- Class I of bacteriocins consists of lanthibiotics. These are small and heat stable peptides that contain thio-ether amino acids such as lanthionine (Hernandez et al. 2005)
- Class II is divided into three sub-groups of which Class IIa is the most common. This group is composed of pediocins like bacteriocins with anti-listerial activity. Pediocins are produced by *Pediococcus* spp. and while they are not very effective against spores, they are more effective than nisin in some food systems (O'Sullivan et al. 2002)
- Class III comprises large heat labile proteins (Eijsink et al. 2002), and
- Class IV is a complex of bacteriocins with glyco-and/or lipid moieties (Rodriguez et al. 2003)

An advantage of bacteriocins over classical antibiotics is that digestive enzymes destroy them. Bacteriocin-producing strains can be used as part of or adjuncts to starter cultures for fermented foods in order to improve safety and quality.

3 Principles of Lactic Acid Vegetable Fermentation

Lactic acid fermentations are carried out under three basic types of conditions: dry-salted, brined and non-salted. Salting provides a suitable environment for the growth of LAB, which imparts acidic flavour.

3.1 Dry-Salted Fermented Vegetables

In this process, dry salt is voluntary added to vegetables. For 100 kg of vegetables, approximately 3 kg of salt is needed. Salt extracts the juice from the vegetables and creates the brine. The vegetable is sliced, washed in potable water and drained. Then they are placed in a layer of about 2.5 cm depth in the fermenting container (a barrel or keg). Salt is sprinkled over the vegetables. Another layer of vegetables is added and more salt is added. This is repeated until the container is three quarters full. Usually, weight (stones) is placed to compress the vegetables and assists the formation of brine, which takes about 24 hr. As soon as brine is formed, fermentation starts and bubbles of CO_2 begin to appear. Fermentation takes place between

1 to 4 wk depending on the ambient temperature. Fermentation is complete when no more bubbles appear, then the pickle can be packaged in a variety of mixtures, i.e., vinegar and spices or oil and spices (Liu et al. 2011).

3.2 Brine-Salted Fermented Vegetables

In this process, a brine solution is prepared by dissolving salt in water (15 to 20% salt solution). Brine is used for vegetables that inherently contain lower water content. Best fermentation takes place in brine of about 12.5 to 20° Salometer (Liu et al. 2011). The strong brine solution draws sugar and water out of the vegetables, which decreases the inner salt concentration. It is crucial that the salt concentration does not fall below 10%; otherwise, conditions will not allow fermentation (Panda et al. 2009). To achieve this level, extra salt is added periodically to the brine mixture.

Once the vegetables have been brined and the container sealed, a rapid development of microorganisms is observed in the brine. The natural parameters that affect the microbial populations of the fermenting vegetables include the concentration of salt and temperature of the brine, the availability of fermentable materials and the numbers and types of microorganisms present at the start of fermentation. The rapidity of the fermentation is correlated with the concentration of salt in the brine and its temperature (Ray and Panda 2007).

Most vegetables can be fermented at 12.5 to 20° Salometer. If so, the sequence of LAB generally follows the classical sauerkraut fermentation. At higher salt levels of about 40° Salometer, the sequence is skewed towards the development of a homo-fermentation, dominated by *Lactobacillus plantarum*. At highest salt concentrations as 60° Salometer, lactic fermentation stops and if any acid is detected during brine storage, it is acetic acid, presumably produced by acid-forming yeasts which are still active at this salt concentration (Montet et al. 2006).

3.3 Non-Salted Lactic Acid Fermented Vegetables

Some vegetables can be fermented by LAB, without prior addition of salt or brine. Examples of non-salted products include gundruk (consumed in Nepal), sinki and other wilted fermented leaves (Dahal et al. 2005, Tamang et al. 2005). The detoxification of cassava through fermentation includes an acid fermentation, during which the cyanogenic glycosides are hydrolyzed to liberate the toxic cyanide gas (Onabolu et al. 2002a,b). The fermentation process relies on the rapid colonization of the food by LA-producing bacteria, which lower the pH and make the environment unsuitable for the growth of spoilage organisms. Oxygen is also excluded

as the lactobacilli favour an anaerobic atmosphere. Restriction of oxygen ensures that yeasts do not grow.

4 Different Processes of Lactic Acid Fermentation

Lactic acid fermentations are of the following types:

- Spontaneous Fermentation
- Controlled Fermentation

4.1 Spontaneous Fermentation

Spontaneous fermentation leads to variations in the sensory properties of the products which differ according to the quality of raw material, temperature and harvesting conditions (Paramithiotis et al. 2010, Wouters et al. 2013). Fresh vegetables and fruits may present high microbial loads (around 10^5 to 10^7 microorganisms/g) after harvesting, most of which are Gram (–) ve bacteria and Gram (+) ve bacilli, yeasts and moulds. Lactic acid bacteria are least prevalent, accounting for less than 0.1% of the autochthonous microbial population as these bacteria require high nutrients such as amino acids, fatty acids, vitamins and certain minerals for their growth and metabolism. Therefore, the plant environment is not suitable for their development. But the plant medium can be enriched by salting or by the addition of certain protein ingredients (whey, bran, etc.) for their growth (Rao et al. 2004). During the fermentation process, lactic and acetic acids are formed and pH decreases thus inhibiting the Gram (–) ve and sporulating bacteria. In these conditions, only LAB are able to grow.

Some of the LAB isolated from naturally fermented vegetables are *Lactobacillus plantarum*, *Lb. brevis*, *Lb. lactis, Lb. paraplantarum, Lb. hilgardii, Pediococcus cerevisiae*, *Leuconostoc mesenteroides,* and *Lactococcus lactis,* etc. (Table 2) (Dahal et al. 2005, Tamang et al. 2005, Montet et al. 2006, Ponce et al. 2008, Paramithiotis et al. 2010, Di Cagno et al. 2013). Lactobacilli do not only produce LA but also H_2O_2 and bacteriocins, which inhibit the growth of pathogenic bacteria (Ray and Joshi, chapter 1 in this book). Along with organic acids, the hetero-fermentative lactobacilli produce CO_2 which also has a preservative effect on foods.

Recently, metagenomic and metabolomic approaches were used to characterize the microbial community during spontaneous fermentation (Jung et al. 2011). The microbiota responsible for the spontaneous fermentation of kimchi was one of the largely investigated (Lee et al. 2002, 2005, Kim and Chun 2005, Park et al. 2010, Jung et al. 2011). In particular, *Lc. mesenteroides* and *Pediococcus pentosaceus* started the first stage of fermentation, and the combination of *Lb. plantarum* and *Lb. brevis*

Table 2. Microorganisms isolated from indigenously lactic acid fermented vegetables and fruits.

Product name	Country	Main Ingredients	Microorganisms
Burong mustala	Philippines	Mustard	*Lactobacillus brevis* *Pediococcus cerevisiae*
Cucumbers	Asia, USA	Cucumbers, vinegar, salt	*Lactobacillus plantarum, Pediococcus pentosaceus*
Dakguadong	Thailand	Mustard leaf, salt	*Lactobacillus plantarum*
Dhamuoi	Vietnam	Cabbage, various vegetables	*Leuconostoc mesenteroides* *Lactobacillus plantarum*
Gundruk	Nepal	Cabbage, radish, leafy vegetables	*Lb. plantarum, Lb. casei* subsp. *casei, Lc. pseudoplantarum, Lb. fermentum, P. pentosaceus*
Hardaliye	Turkey	Vegetables	*Lb. paracasei* subsp. *paracasei, Lb. casei Lb. pontis, Lb. brevis, Lb. acetotolerans, Lb. sanfranciscoensis*
Jiang-gua	Rep. of China	Cucumbers, salt, sugar, vinegar, soy sauce	*Enterococcus casseliflavus, Leuconostoc lactis, Lc. mesenteroides, Lb. pentosus, Lb. plantarum, Lb. paraplantarum, Lactococcus lactis* subsp. *lactis, Weissella hellenica, Weissella cibaria*
Kanji	India and Pakistan	Carrots	*Lactobacillus plantarum* *Lactobacillus brevis*
Khalpi	Nepal	Cucumber	*Lactobacillus plantarum, Lb. brevis, Leuconostoc fallax, Pediococcus pentosaceus*
Kimchi	Korea	Cabbage, radish, various vegetables and spices (ginger, pepper, garlic, onion)	*Leuconostoc mesenteroides, Leuconostoc kimchii, Leuconostoc citreum, Leuconostoc gasicomitatum, Lc. pseudomesenteroides, Lactobacillus plantarum, L. brevis, Lactobacillus curvatus, Lactobacillus sakei, Lactobacillus maltaromicus, Lactobacillus bavaricus, P. pentosaceus, Weissella confusa, Weissella kimchii, Weissella koreensis*

Table 2. contd....

Table 2. contd.

Product name	Country	Main Ingredients	Microorganisms
Olive	Spain, Italy	Olive	*Lactobacillus plantarum*, *Lb. paracasei*, *Lb. pentosus*, *Lb. casei*, *Lb. vaccinostercus*, *Lb. suebicus*, *Lb. paracollinoides* *Lactobacillus brevis* *Pediococcus cerevisiae* *Leuconostoc mesenteroides*, *Lc. lactis*
Pak-sian-dong	Thailand	Leaves of Pak-sian	*Lactobacillus brevis* *Pediococcus cerevisiae* *Lactobacillus plantarum*
Salgam	Turkey	Black/Violet carrots, turnip, bulgur flour, sourdough, salt and water	*Lb. plantarum*, *Lb . paracasei* subsp. *paracasei*, *Lb. fermentum*, *Lb. brevis*
Sauerkraut	International	Cabbage	*Leuconostoc mesenteroides* *Lactobacillus plantarum* *Lactobacillus brevis*
Sinki	India, Nepal and Bhutan	Radish	*Lactobacillus plantarum* *Lactobacillus brevis* *Lactobacillus fermentum* *Leuconostoc fallax* *Pediococcus pentosaceus*
Sunki	Japan	Leaves of otaki- turnip	*Lactobacillus plantarum* *Lactobacillus brevis* *Pediococcus pentosaceus* *Bacillus coagulans*
Suan-tsai	Taiwan	Mustard leaves	*Pediococcus pentosaceus* *Tetragenococcus halophilus*
Tursu	*Turkey*	Cucumbers, cabbage, green tomatoes, green peppers and other vegetables	*Lb. plantarum*, *Lc. mesenteroides*, *Lb. brevis*, *P. pentosaceus*, *Enterococcus fecalis*

Source: Montet et al. 2006, Ray and Panda 2007, Hurtado et al. 2012, Di Cagno et al. 2013
Lb. = *Lactobacillus*, Lc. = *Leuconosto*c, P = *Pediococccus*

or *Lb. maltaromicus* and *Lb. bavaricus* further dominated depending on the temperature of incubation (20–30ºC or 5–7ºC, respectively). Distinct kinetics of growth characterized the three genera, *Leuconostoc, Lactobacillus* and *Weissella*, which dominated the fermentation. Similarly, phylogenetic analysis based on partial 16S-rRNA gene sequences exhibited that spontaneous cauliflower fermentation was characterized by an initial hetero-fermentative stage driven by strains belonging to *Lc. mesenteroides*-group that was followed by a homo-fermentative one with strains of *Lb. plantarum*-group dominating. Strains belonging to *Enterococcus faecium*-group and *Enterococcus faecalis*-group were also isolated but only at the early stages of fermentation (Paramithiotis et al. 2010).

4.2 Controlled Fermentation

Quality control is essential for the industrialization of fermentation process (Ray and Sivakumar 2009). For controlled LA fermentation, conditions must be created which favour the growth of commensal and/or inoculated LAB while excluding other microorganisms (Gardner et al. 2001, Di Cagno et al. 2008a,b, 2011a). Authorized lists of microorganisms with certified use in food fermentations, which cover a wide range of food matrices, including vegetables and fruits, were recently published (Bourdichon et al. 2012). These lists may represent a *de facto* reference of food cultures, which should be consulted to select starters for fermentation of raw vegetables and fruits.

Two main options may be pursued for the controlled LA fermentation of vegetables and fruits: the use of autochthonous or allochthonous starters (Di Cagno et al. 2008a,b, 2009, 2010, 2011b). Autochthonous starters mean isolated from and re-used on the same raw matrix, apart from the geographical origin. Allochthonous starters means isolated from certain raw matrices but used to ferment various products. Obviously, commercial starters, which are used to ferment a variety of vegetables and fruits, mostly coincide with the above definition of allochthonous strains (Di Cagno et al. 2013).

4.2.1 Commercial/allochthonous starters

Majority of the reports show the use of commercial/allochthonous starters in LA fermentation of vegetables and fruits (Gardner et al. 2001, Plengvidhya et al. 2004, Demir et al. 2006, Johanningsmeier et al. 2007). Few examples are cited here. Peeled and blanched garlic was fermented with commercial *Lb. plantarum* (de Castro et al. 1998). The allochthonous starter grew well in blanched garlic after two days of fermentation and LA was the main fermentation end-product. Allochthonous starters (e.g., *Lb. plantarum* RSKK

1062) were also used for making vegetable juices, aiming at favouring the activity of pectolytic enzymes, which increases the juice yield (Wong 1995), and at rapidly decreasing the value of pH, when the matrix was poorly acid (carrots) (Demir et al. 2006). Usually, commercial starters are not previously selected to ferment a specific vegetable or fruit matrix. Only one report described the selection of *Lb. plantarum* NK-312, *Pediococcus pentosaceus* AFERM 772 and *Lc. mesenteroides* BLAC to ferment a mixture of cabbages, carrots, beets and onions (Gardner et al. 2001). However, Di Cagno et al. (2013) have outlined some limitations in using allochthonous culture such as: (i) the selection time (days) did not consider other features except rapid acidification; (ii) the adaptation to the main sensory and functional properties of the matrix is poor; (iii) the metabolic flexibility is low; and (iv) the diversity did not reflect the ecosystem where they have to be used.

4.2.2 Autochthonous starters

Selection of starter cultures within the autochthonous microbiota of vegetables and fruits should be recommended since autochthonous cultures may ensure prolonged shelf life and targeted nutritional, rheology and sensory properties (Di Cagno et al. 2013). Autochthonous *Lb. plantarum* starters were compared to allochthonous strains (isolated from green olives) during fermentation of tomato juice (Di Cagno et al. 2008b). Compared to selected autochthonous strains, these allochthonous strains showed longer latency phases of growth and acidification. Tomato juices fermented with autochthonous strains maintained the highest values of ascorbic acid, glutathione and total antioxidant activity during storage. In another study, when fermented with selected autochthonous starters (*Lb. plantarum* M1, *Lc. mesenteroides* C1 and *P. pentosaceus* F4), carrots, French beans and marrows showed a rapid decrease of pH, marked consumption of fermentable carbohydrates, and inhibition of *Enterobacteriaceae* and yeasts (Di Cagno et al. 2013). Allochthonous starters, belonging to the same species, did not show the same performance. The differences between autochthonous and allochthonous strains were also pronounced regarding the concentration of vitamin C, colour indexes, firmness and sensory properties. The use of autochthonous strains (*Lb. plantarum* PE21, *Lb. curvatus* PE4 and *Weissella confusa* PE36) was preferable to the spontaneous fermentation during processing of red and yellow peppers (Di Cagno et al. 2009). Sweet cherry (*Prunus avium* L.) puree added with stem infusion was fermented with selected autochthonous *P. pentosaceus* SWE5 and *Lb. plantarum* FP3 (Di Cagno et al. 2011b). Although the environment was hostile (pH 3.9 and high presence of phenolic compounds), the above strains grew well, showed

metabolic adaptation to environment and remained viable during 60 days of storage at cell numbers, which exceeded those of potential probiotic beverages (Yoon et al. 2004). Autochthonous *Lactobacillus pentosus* and *Lb. plantarum*, and *Candida diddensiae* were used as starters for the traditional fermentation of Arbequina naturally green olives (Hurtado et al. 2012, Aponte et al. 2012). Compared to the spontaneous fermentation, the survival of *Enterobacteriaceae* was inhibited. *Lb. pentosus* showed a very short latency phase of acidification and rapidly decreased the pH of the brine. Fourteen days more were needed by spontaneous fermentation to reach the same value of pH.

5 Factors Affecting Lactic Acid Fermentation

There are seven factors that influence the growth and activity of LAB in fermenting fruits and vegetables. Those are pH, moisture and water activity, O_2 concentration, temperature, nutrients, selected starter culture and inoculum concentration (Lee and Salminen 1995, Ballesteros et al. 1999).

5.1 pH

The pH is a critical factor in preservation and developing aroma and flavour of many fermented fruits and vegetables like cabbage, olives, etc. (Muyanja et al. 2003, Rao et al. 2004). Most LAB favour conditions with a near neutral pH (Battcock and Azam-Ali 2001). Certain bacteria are acid tolerant (i.e., *Lactobacillus* and *Streptococcus*) and can survive at reduced pH levels (3.0–4.0) (Ray and Panda 2007).

5.2 O_2 Availability

The O_2 requirements vary from species to species. Unlike many anaerobes, however most LAB are not sensitive to O_2 and can grow in its presence as well as in absence. They are aero-tolerant anaerobes (Molenaar et al. 2005).

5.3 Temperature

Temperature is a critical factor for vegetable fermentation. Most LAB have a temperature optimum between 20ºC to 30ºC, there are some (thermophiles) which prefer high temperatures (50–55ºC) and those with colder temperatures optima (15–20ºC) (Ross et al. 2002). Most LAB work best at temperatures of 18–22ºC (Ray and Panda 2007).

5.4 Salt Concentration

Salting is an important step in vegetable fermentation. Sodium chloride concentration can range from 20 to 80 g/l during fermentation. LAB can tolerate high salt concentrations. This salt tolerance gives them an advantage over less tolerant species and allows LA fermentation that inhibits growth of non-desirable organisms (Rao et al. 2004). Salt induces plasmolysis in plant cells which releases mineral salts and nutrients from the vacuole and creates anaerobic conditions for proper growth of LAB around the submerged product (Gardner et al. 2001, Rakin et al. 2004, Wouters et al. 2013).

5.5 Water Activity

In general, LAB require a fairly high water activity (0.9 or higher) to survive. There are a few species, which can tolerate water activities lower than this, but usually, the yeasts and fungi will predominate on foods with a lower activity (Ray and Panda 2007).

5.6 Nutrients

All bacteria require a source of nutrients for metabolism. The fermentative bacteria require carbohydrates, either simple sugars such as glucose and fructose or complex carbohydrates such as starch or cellulose (Ray and Panda 2007, Wouters et al. 2013).

5.7 Selected Starter Cultures

The selection of starter cultures (either allochthonous or autochthonous) is based principally on the competitiveness between the starter and the natural flora, as well as on the sensory properties of the resulting products (McFeeters 2004). They are selected on the following criteria for fermentation of fruits and vegetables:

- Lack of production of toxic chemicals
- Ability to produce only (L+) lactic acid
- Low or nil production of biogenic amines
- Genetic stability of the species
- Rapid brine acidification
- Production reproducibility between different batch cultures
- Total depletion of fermentable sugars
- Resistance to bacteriocins and bacteriophages from natural strains
- Potential of strain preservation by drying, freezing or freeze-drying

6 Lactic Acid Fermentation of Vegetables and Fruits

Vegetables are rich in nutrients, vitamins and minerals and some of them contain coloured pigments such as flavonoids, lycopene, anthocyanin, β-carotene and glucosinolates, which act as antioxidant in the body by scavenging harmful free radicals implicated in degenerative diseases like cancer, arthritis and ageing (Kaur and Kapoor 2001).

Lactic acid fermentation of vegetables and fruits can be divided into four main categories:

A. Vegetable fruits such as cucumbers, tomatoes, peppers, okra and green-peas (Dahal et al. 2005).
B. Fruits such as olives, apples, pears, immature mangoes, immature palms, lemons and fruit pulps (Zhang et al. 2000).
C. Root and tubers such as carrots, turnips, beetroot, radishes, celeriac, cassava and sweet potato (Ray and Sivakumar 2009).
D. Innovative juices and smoothies from fruits and vegetables (Yoon et al. 2006, Di Cagno et al. 2013).

6.1 Lactic Acid-Fermented Vegetables

There are several traditional and non-traditional fermented vegetables available around the globe. Few important ones are discussed.

6.1.1 Sauerkraut

This is an example of dry salted fermented vegetables. It results from the natural LA fermentation of salted and shredded cabbage. It is a common way of preserving fresh vegetable in the Western world, in China and Korea (Liu et al. 2011). The high nutritive value of sauerkraut is mainly due to the increased digestibility in comparison to raw cabbage and relatively low vitamin C losses. For at least 150 yr, sauerkraut has been made in the home for saving fresh cabbage before spoilage (Steinkraus 2002, Viander et al. 2003). Other vegetables which are fermented by sauerkraut process are carrots, onions, garlic and beets (Gardner et al. 2001). Presently, the production of sauerkraut has become an important food industry in Korea and Vietnam.

Sauerkraut processing at industrial scale consists of trimming the mature and sound heads of cabbage to remove the outer green, broken or dirty leaves (Karovicova and Kohajdova 2002). The head of cabbage is then sliced by rotary knives into long and fine shreds. Salt is sprinkled on the shreds which are conveyed to the fermentation tanks. Spices could also be added. A salt concentration in the range of 2.25–2.5% is used for facilitating

LA fermentation. Forks are used to uniformly distribute the shreds which are dumped and squeezed into the vat. Once the tank has been filled to the proper level, it is closed. The shredded cabbage should be completely immersed to favour anaerobic conditions and prevent undesirable darkening and flavour changes. Within a few hours the brine is formed and the fermentation is started, the latter is initiated by *Lc. mesenteroides*, which produces lactic and acetic acids and CO_2. The pH is quickly lowered, thus limiting the activity of undesirable microorganisms and enzymes that might soften the cabbage shreds. In the next phase, homo-fermentative bacteria such as *Lactobacillus* and *Pediococcus* continue the fermentation to a final pH of 3.5 to 3.8 (Yoon et al. 2006, Xiong et al. 2012). The CO_2 replaces air and creates an anaerobic atmosphere which prevents the oxidation of ascorbic acid and the darkening of the natural cabbage colour. The optimal temperature for sauerkraut fermentation is 18ºC (Viander et al. 2003). At this temperature fermentation is completely performed in three weeks.

6.1.2 Kimchi

Kimchi is the name given to a group of traditional fermented vegetables in Korea (Cheigh and Park 1994, Cho et al. 2009). It is a popular side dish that is served at every meal with rice. Kimchi production in Korea is estimated at over 1.5 million tonnes, mainly at household level and daily consumption is estimated at 150 to 250 g (Cho et al. 2009). The main ingredient of kimchi is either Chinese cabbage to which radish and cucumber may be eventually added. Cabbages are cut and brined in salt (5–10%) solution for 12 hr or in 15% brine for 3–7 hr. Salting is followed by rinsing and draining the water. Minor ingredients such as garlic, onions, black pepper, ginger, mustard, parsley, sesame grains and fermented anchovies or shrimps are then added at 10% w/w of the main ingredient (Jung et al. 2011). The mixture is finally left to ferment in jars. Due to its nutritional properties, kimchi was recently included in the list of the top five World's Healthiest Foods (http://eating.health.com/2012/02/01/worlds-healthiest-foods-kimchi-korea/). These beneficial effects are attributed either to functional components (vitamins, minerals, fibre and phytochemicals) or to fermentation by LAB (Lee et al. 2011). The main kimchi are tongbaechu-kimchi, tongkimchi and bossam-kimchi (Di Cagno et al. 2013).

The LAB profile during kimchi fermentation varies with pH and acidity. In a multiplex PCR assay, *Lc. mesenteroides* was observed during early fermentation (pH, 5.64–4.27 and acidity, 0.48–0.89%), and *Lb. sakei* become dominant later in fermentation (pH, 4.15 and acidity, 0.98%) (Cho et al. 20). The other organisms found in kimchi are *Lb. plantarum*, *Lb. brevis*,

Lc. mesenteroides, *Streptococcus faecalis* and *Pediococcus cerevisiae*, and aerobic bacteria such as *Achromobacter*, *Flavobacterium* and *Pseudomonas* spp. (Lee et al. 2005, Kim and Chun 2005). The main microorganism responsible for kimchi fermentation is *Lc. mesenteroides* and the main acidifying microorganism is *Lb. plantarum* (Lee et al. 2011).

6.1.3 Kocho

Kocho is a fermented product from false banana (*Ensete ventricosum*) pseudostem. It is produced in Ethiopia (Steinkraus 2002). False bananas are peeled before placing in the pit and left to ferment for three to six weeks, after which it becomes soft, has a strong odour and a paste-like consistency. During fermentation, CO_2 builds up in the pit creating an anaerobic atmosphere. As a result of bacterial activity, the temperature rises much higher than the ambient temperature. The pH of the fruit within the pit decreases from 6.7 to 3.7 within about four weeks. Inoculation of the fruit in the pit with LAB greatly speeds up the process. The pit therefore provides a good, reliable, cheap means of storage (Ray and Panda 2007).

6.1.4 Cucumbers

Pickled cucumbers are made in Africa, Asia and Latin America. Cucumbers undergo lactic acid fermentation and change from a pale product to a darker green and more transparent product. Khalpi is a popular cucumber pickle in Nepal during summer months (McGee 2004, Dahal et al. 2005). The gherkin (Fig. 1), popularly known as pickling small cucumber, has emerged as a potential export-oriented delicacy, fetching foreign exchange of US$ 33.3 million from 50,000 tonnes every year from India (Kapur and Singh 2003).

Fully ripe undamaged cucumbers are washed in potable cold water and drained. One kg of salt is added to the cucumbers. As soon as the brine is formed, fermentation starts and bubbles of CO_2 appear. Fermentation takes in between one to four weeks depending on the ambient temperature. Proper salt concentration exerts a selective effect on natural flora, resulting in the growth of lactic acid bacteria. When the pH is about 4.7, the brine is inoculated with either *Lb. plantarum* or *P. pentosaceus* or a combination of these organisms (Steinkraus 2002). At the end of fermentation, salt (16% w/w of brine) is traditionally added to the LA fermented cucumbers in order to stop any undesirable bacterial growth during storage (Kapur and Singh 2003). Cucumber pickle is usually stored in clean capped jars. They keep well if stored in a cool place. Due to high acid level (3.1–3.5) of the final product, the risk of food poisoning is low (Tamang et al. 2005).

Fig. 1. Fermented gherkin (Ray and Panda 2007).

Color image of this figure appears in the color plate section at the end of the book.

6.1.5 Gundruk

Gundruk is popular pickle consumed in Nepal (Dahal et al. 2005, Tamang et al. 2005). It is obtained by LA fermentation of leafy vegetables such as cabbage and radish. It is an important source of minerals, particularly when the diet consists of mostly starchy roots, tubers and maize which are low in minerals. This is mainly served as side dish with the main meal and also used as an appetizer.

Shredded leaves are tightly packed in an earthen pot and warm water is added to cover all the leaves. After 5 to 7 days, a mild acidic taste indicates the end of fermentation. The ambient temperature at the time of fermentation is 18ºC (Steinkraus 2002). *Pediococcus* and *Lactobacillus* species are the predominant microorganisms during fermentation (Tamang et al. 2005).

6.1.6 Ethnic Pickled Vegetables

'Almagro' eggplants (*Solanum melongena* L. var. *esculentum depressum*) is a pickle, virtually exclusive to Spain and more specifically to the town of Almagro and surrounding areas in the province of Ciudad Real (Sanchez et al. 2000b). The effect of a commercial *Lactobacillus* starter and sodium chloride concentration on the fermentation of "Almagro" eggplants was studied. The results of fermentation using added starter and varying salt concentrations (4, 6, and 10% w/v) in brine were compared with the results of spontaneous fermentation taking place in brine with a salt concentration of 4%. Fresh fruits, medium in size (34–44 g), were used in all cases; all fruits were blanched under identical conditions. Temperature in the fermenters was 32ºC. The results indicated that addition of a suitable starter shortened

the fermentation process, provided the salt concentration in the brine did not exceed 6%. In the conditions tested, the eggplants obtained after fermentation were found to be of good quality though somewhat bitter which may explained by the starter employed (Ballesteros et al. 1999). Other ethnic pickled vegetables are:

- Brovoda: Pickled turnips (*Brassica rapa*) (Maifreni et al. 2004)
- Naw-mai-dong: pickled bamboo shoots (*Bambusa glaucescens*) from Thailand (Tanasupawat and Komagata 1994)
- Hom-dong: pickled red onions from Thailand (Tanasupawat and Komagata 1994)
- Jeruk: pickled vegetables including ginger and papaya from Malaysia
- Pickled carrots and turnips are produced in Asia and Africa. They are known as hua-chai po in Thailand and tai tan tsoi in China (Liu et al. 2011)
- Nukamiso-zuke: vegetables fermented in rice bran, salt and water in Japan
- Dak-dua-dong: fermented mustard leaves from Thailand (Tanasupawat and Komagata 1994)
- Dhamuoi, fermented cabbage and other vegetables in Vietnam
- Torshi felfel: Fermented sweet peppers (capsicum) are produced in West Asia and Africa
- Cauliflower stalks are fermented to produce achar tandal in India
- Torshi bentigen: Aubergines (brinjal) are pickled in West Asia
- Pak-sian-dong: Pak Sian (*Gynadropsis pentaphylla*) vegetables fermented in Thailand
- Sayur asin: fermented wilted mustard and cabbage (*Brassica juncea*) from Indonesia
- Kaktugi: fermented radish produced in Korea
- Tursu: fermented vegetables and fruits such as cucumbers, cabbages, green tomatoes, green peppers, carrots, red beets, eggplants or melon, in Turkey (Kabak and Dobson 2011)
- Suan-tsai and fu-tsai: Fermented mustard products of Taiwan (Chao et al. 2009)
- Poi: Fermented taro tubers in Papua New Guinea and other Pacific islands (Ray and Ward 2006)

6.2 Lactic Acid-Fermented Fruits

Most of the research works have concentrated on olives. Nevertheless, there are some other fruits which have been LA fermented.

6.2.1 Olives

Green olives are lactic acid fermented following sodium hydroxide (lye) treatment to remove bitterness (Sanchez et al. 2000a). The bacteria involved are *Lb. plantarum, Lb. brevis, P. cerevisiae* and *Lc. mesenteroides* (Borcakli et al. 1993, Duran-Quintana et al. 1999). The pH varied between 4.0–4.5. The optimum fermentation temperature is 24ºC. The fermentation period usually takes between 2–3 mon. Once the fermentation is complete, the olives are packed in air-tight jars and sterilized, which produces a good quality product with a long storage life. Salting is normally done at 1–10% brine solution (Tsapatsaris and Kotzekidou 2004). With the aim of formulating a probiotic food, the green olive was used as a vehicle for incorporating probiotic bacterial species such as *Lactobacillus rhamnosus*, *Lb. paracasei*, *Bifidobacterium bifidum* and *B. longum.* All these strains showed a good survival rate with a recovery of about 10^6 cfu (colony forming units)/g in olives after 30 d of storage (Lavermicocca et al. 2005).

Naturally black fermented olives are of equal importance in the international market as green olives (Nychas et al. 2002). After harvesting, black olives are transported to the factory, sorted to separate damaged fruits, washed to remove superficial dirt and finally brined in a 6–10% salt solution (Ozay and Borcakli 1996). In a recent study, the effect of controlled fermentation processes on the microbial association and biochemical profile of cv. *Conservolea* naturally black olives processed by the traditional anaerobic method was studied. The different treatments included (a) inoculation with a commercial starter culture of *Lactobacillus pentosus*, (b) inoculation with a strain of *Lb. plantarum* isolated from a fermented cassava product and (c) un-inoculated spontaneous process. Microbial growth, pH, titratable acidity, organic acids and volatile compounds were monitored throughout the fermentation. The initial microbiota consisted of Gram (–) ve bacteria, LAB and yeasts. Inhibition of Gram (–) ve bacteria was evident in all processes. Both starter cultures were effective in establishing an accelerated fermentation process and reduced the survival period of Gram-negative bacteria by 5 days compared with the spontaneous process, minimizing thus the likelihood of spoilage. Higher acidification of the brines was observed in inoculated processes without any significant difference between the two selected starter cultures (113.5 and 117.6 mM for *Lb. plantarum* and *Lb. pentosus*, respectively). *Lb. pentosus* was also determined as the major species present during the whole process of spontaneous olive fermentation. It is characteristic that LA fermentation was also initiated rapidly in the spontaneous process, as the conditions of fermentation, mainly the low salt level (6%, w/v), favoured the dominance of LAB over yeasts. Lactic, acetic and propionic were the organic acids detected by HPLC in considerable amounts, whereas citric and malic acids were also present

at low levels and degraded completely during the processes. Ethanol, methanol, acetaldehyde, ethyl acetate were the major volatile compounds identified by gas chromatography (Panagou et al. 2008).

6.2.2 Sweet Cherry

Sweet cherry fruits have a very short life since they are subject to rapid microbial spoilage. Fermentation by LAB is a simple technological option for maintaining and/or improving the safety, nutritional, sensory and shelf-life properties of these fruits. Di Cagno et al. (2011b) isolated several strains of LAB such as *Pediococcus acidilactici, P. pentosaceus, Lb. plantarum,* and *Lc. mesenteroides* subsp. *esenteroides* from spontaneous fermentation of eight cultivars of these fruits by partial 16S rRNA gene sequence and subjected to typing by Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analysis. Lactic fermentation has increased the anti-oxidant activity, anthocyanin content and sensory quality of these fermented fruits.

6.2.3 Caper berries

Caper berries are the fruits of *Capparis* species (mainly *Capparis spinosa* L.), a Mediterranean shrub cultivated for its buds and fruits. Fermented capers are typical of Mediterranean countries (e.g., Greece and Italy). Fruits are harvested during June or July, immersed in tap water, and subjected to spontaneous LA fermentation for 5–7 days at ambient temperature, which may vary markedly from 23–43ºC. Subsequently, fermented capers are placed into brine and distributed for consumption. *Lb. plantarum* is the main species, which was isolated from the brine of capers (Pulido et al. 2012).

6.3 Lactic Acid-Fermented Roots and Tubers

There are several traditional products such as sunki, kanji, etc. obtained by lactic acid fermentation of roots and tubers.

6.3.1 Sunki

Sunki is a non-salted fermented vegetable prepared from the leaves of the otaki-turnip in the Kiso District of Japan. Sunki is eaten with rice or in miso soup. The otaki turnip is boiled, mixed with zumi (a wild small apple) and dried sunki from the previous yr and allowed to ferment for one to two months. Sunki is produced under low temperature (in winter season).

Microorganisms involved include *Lb. plantarum*, *Lb. brevis*, *Bacillus coagulans* and *Pediococcus pentosaceus* (Battcock and Azam-Ali 2001).

6.3.2 Sinki

Sinki is a sour pickle prepared from radish tap roots. It is consumed traditionally in India, Nepal and parts of Bhutan (Dahal et al. 2005). Fresh radish roots are harvested, washed and wilted by sun-drying for one to two days. They are then shredded, re-washed and packed tightly in glass jars, which are sealed and left to ferment. The optimum fermentation time is 12 days at 3ºC. Sinki fermentation is initiated by *Lactobacillus fermentum* and *Lb. brevis*, followed by *Lb. plantarum*. The pH drops from 6.7 to 3.3. After fermentation, the radish substrate is sun-dried to a moisture level of about 21%.

6.3.3 Kanji

In Northern India and Pakistan, carrots, especially a variety that is deep purple in colour, are fermented to make a traditional ready-to-serve drink known as kanji. Kanji is very popular and considered to have cooling and soothing properties and to be of high nutritional value. After thorough washing, the carrots are finely grated. Each kg of grated carrot is mixed with 7 L of water, 200 g of salt, 40 g of crushed mustard seed and 8 g of hot chilli powder. The mixture is then placed in a glazed earthenware vessel, which is almost entirely sealed, leaving only a tiny hole for gases released during fermentation to escape. The mixture is then allowed to ferment naturally for seven to ten days. The final product is slightly acidic in taste and has an attractive purple-red colour. After fermentation, the drink is strained through fine muslin and has to be consumed within three to four days after which it goes bad. Each kg of grated carrot yields just over 7 L of kanji (Ray and Panda 2007).

6.3.4 Onion and garlic

Lactic acid fermentation was conducted on sweet, white, and yellow storage onions to produce sour onion. The onions were sliced to 0.3 cm thick, salt was added at 1.5, 2.0, and 2.5 g/100 g without or with sugar at 1.0 and 2.0 g/100 g, and the fermentation temperature was 18ºC. Since onions did not have the necessary LAB for anaerobic fermentation, they were inoculated using either brine from sauerkraut or slices of cabbage. The

fermentation produced sour onion with pH between 3.25–3.35 and 1.2–1.5 g LA/100 ml, which is in the range as that of sauerkraut. Sensory evaluation showed that the yellow storage sour onion was a favourable product with respect to colour, texture and flavour. The sour onion had a tartaric acidic taste, characteristic of sauerkraut, with the onion flavour but without the pungency of raw onions (Roberts and Kidd 2005).

The controlled fermentation of peeled, blanched garlic, using a starter culture of *Lb. plantarum*, was studied and compared with that of un-blanched garlic. Blanching was carried out in hot water (90ºC) for 15 min. The starter grew abundantly in the case of blanched garlic, producing mainly LA and reaching a pH of 3.8 after 7days, but its growth was inhibited in un-blanched garlic. Ethanol and fructose, coming from the enzymatic activities of garlic, and a green pigment were formed during the fermentation of un-blanched garlic, but not of blanched garlic. The blanched garlic fermented by *Lb. plantarum*, even without a preservation treatment (such as pasteurization), was microbiologically stable during storage at 30ºC in an acidified brine (approximately 3% (w/w) NaCl and pH 3.5 at equilibrium), but the fructans were hydrolyzed (De Castro et al. 1998).

6.3.5 Sweet potato

Bio-fortified sweet potato roots, rich in colour pigments such as anthocyanin or β-carotene, were pickled by LA fermentation by brining both cut and blanched roots in 2–10% NaCl solution and subsequently inoculated with a probiotic strain of *Lb. plantarum* MTCC 1407 for 28 days (Fig. 2). Treatment with 8–10% brine solution was found to be organoleptically most acceptable (based on texture, taste, aroma, flavour and after tasting). The pickle prepared from anthocyanin-rich sweet potato had a pH (2.5–2.8), titratable acidity (1.5–1.7 g/kg), LA (1.0–1.3 g/kg), starch (56–58 g/kg) and anthocyanin content (390 mg/kg) on fresh weight basis (Panda et al. 2009). Similarly, β-carotene-rich pickle had a pH (2.9–3.0), titratable acidity (2.9–3.7 g/kg), lactic acid (2.6 3.2 g/kg), starch (58–68 g/kg) and β-carotene (163–165 mg/kg) (Panda et al. 2007) (Fig. 2).

Sweet potato roots (non-boiled/fully-boiled) rich in β-carotene pigments were fermented with *Lb. plantarum* MTCC 1407 at 28± 2ºC for 48 hr to make lacto-juice. There were no significant variations in biochemical constituents (pH, 2.2–3.3; LA, 1.19–1.27 g/kg root and titratable acidity, 1.23 1.46 g/kg root) of lacto-juices prepared from non-boiled and fully-boiled sweet potato roots except β-carotene concentration [130 ± 7.5 mg/kg (fully-boiled roots) and 165± 8.1 mg/kg (non-boiled roots)] (Panda and Ray 2007).

Fig. 2. Lacto-pickle from orange fleshed sweet potato (Panda et al. 2007).

Color image of this figure appears in the color plate section at the end of the book.

6.3.6 Cassava

Cassava is another tropical crop in which roots are consumed as food in Africa and Latin America. Several fermented foods (gari, fufu, lafun, dawa dawa, chickwanghe, agbelima, attieke, kivunde and peujeum in Africa, gaplek and putto in Indonesia and cheese bread and sour starch in Latin America) are prepared from cassava roots based on LAB and yeast fermentations (Ray and Ward 2006). Fermentation reduces the cyanogenic toxicity and enhances flavour, taste and aroma of the fermented products (Onabolu et al. 2002a,b).

6.4 Innovative Juices and Smoothies from Fruits and Vegetables

Innovation in food technology has an important role to develop new LA fermented products to improve the nutritional features; few of them are described below.

6.4.1 Lactic acid-fermented vegetable juices

Lactic acid bacteria have been added to a variety of dairy-based products such as fermented milks and yoghurts for their probiotic human health benefits. In recent yrs, consumers' demand for nondairy-based probiotic

products particularly lacto-juice prepared from vegetables has gained importance (Karovicova et al. 1999, 2002b). Lacto-juices have been prepared from vegetables such as carrot, turnip, tomato pulp, onion, sweet potato, beet and horseradish (Karovicova et al. 1999, 2002b, Klewicka et al. 2004). For fermentation of juices of highest quality, it is imperative to use commercially supplied probiotic starter cultures such as *Lactobacillus acidophilus, Lb. plantarum*, *Lb. bavaricus*, *Lb. xylosus*, *Lb. bifidus,* and *Lb. brevis* (McFeeters 2004).

Lu et al. (2001) carried out the fermentation of cucumber juices inoculated by *Lb. plantarum*. The juices were fortified with glucose, fructose or a mixture of glucose and fructose. When the cucumber juice was supplemented with fructose and/or glucose, the starter culture continued to ferment fructose, but not glucose, resulting in an increase in lactic acid production and decrease in terminal pH. Karovicova et al. (2002a) performed spontaneous fermentation of cabbage juices, one fermentation by *Lb. plantarum* 92H and another by a mixed culture of *Lb. plantarum* and *Saccharomyces cerevisiae* C11-3. It was found that the highest amount of lactic acid was produced in the juice inoculated by *Lb. plantarum* 92H and the highest decrease in pH was observed in juice inoculated by the mixed culture. The spontaneously fermented juice had the highest intensity of harmonic taste, acceptance of taste, odour and flavour. Yoon et al. (2004) studied the probiotic strain of tomato juice by *Lb. acidophilus* LA39, *Lb. plantarum* C3, *Lb. casei* A4 and *Lb. delbrueckii* D7. Tomato juice was inoculated with a 24 hr-old culture and incubated at 30ºC. The LA fermentation reduced the pH to 4.1 and increased the acidity to 0.65% and the viable cell count reached nearly 1.0–9.0 x 10^9 cfu/ml, after 72 hr fermentation. Similar studies were carried out by Yoon et al. (2006) for production of probiotic cabbage juice.

Red beets were evaluated as a potential substrate for the production of probiotic beet juice by four species of LAB (*Lb. acidophilus*, *Lb. casei*, *Lb. delbrueckii,* and *Lb. plantarum*). All the lactic cultures were found capable of rapidly utilizing beet juice for cell synthesis and LA production. However, *Lb. acidophilus* and *Lb. plantarum* produced a greater amount of LA than other cultures and reduced the pH of fermented beet juice from an initial value of 6.3 to below 4.5 after 48 hr of fermentation at 30ºC (Yoon et al. 2005). In another study, Bergqvist et al. (2005) reported that LA fermentation by *Lb. pentosus* FSC1 and *Lc. mesenteroides* FSC2 strongly improved iron solubility in carrot juice. Tomato, carrot, cabbage, artichokes and reed beet juices were proven to be particularly suitable for probiotic fermentation, allowing a rapid growth of the strains and viable cell population above ca. 10^8 c/ml (Rivera-Espinoza and Gallardo-Navarro 2010).

6.4.2 Lactic acid-fermented fruit juices

Lactobacillus and *Bifidobacterium* strains survived for a longer time in orange and pineapple juices than in cranberry juice. *Lactobacillus casei*, *Lb. rhamnosus*, and *Lb. paracasei* remained viable in orange juice at a cell number higher than 10^7 during 12 weeks of storage (Sheehan et al. 2007). *Lactobacillus plantarum* and *Lb. delbrueckii* were able to survive at 10^8 cfu (colony forming units)/ml for 2 weeks in pomegranate juice, while *Lb. paracasei* and *Lb. acidophilus* showed a marked decrease of the viability (Mousavi et al. 2010). In a more recent study, Filannino et al. (2013) reported enhanced concentration of polyphenolics (ellagic acid), anti-oxidant activity and anti-microbial activity of LA fermented probiotic pomegranate juices that were fermented with *Lb. plantarum* (POM 1 and POM 2) strains isolated previously from tomatoes and carrots.

6.4.3 Smoothies

The manufacture of smoothies is based on the use of a mixture of fruits and vegetables which are processed into pulp or puree, after removing seeds and peel (Qian 2006). In most cases, the selection of the mixtures is based on the colour, flavour, drinkable texture and, especially, to ensure high concentration of nutrients with low energy content (Watzl 2008). Recently, a novel protocol for the manufacture of fermented smoothies was set up (Di Cagno et al. 2011a). White grape juice and *Aloe vera* extract were mixed with red (cherries, blackberries prunes and tomatoes) or green (fennels, spinach, papaya and kiwi) fruits and vegetables, and subjected to fermentation with mixed autochthonous starters, consisting of *Lb. plantarum, Lb. pentosus* and *Weissella cibaria* strains. Lactic acid fermentation by selected starters positively affected the content of antioxidant compounds and enhanced the sensory attributes.

7 Health and Nutritional Benefits of the Fermented Vegetables and Fruits

Lactic acid fermented vegetables and fruits have several health and nutritional attributes beside the scope of food preservation. These are discussed below in brief.

7.1 Food Preservation

The main motive of LA fermentation is to preserve food, increase its shelf-life, and improve food quality and palatability (Ray and Sivakumar 2009).

There are several options for preserving fresh fruits and vegetables including drying, freezing, canning and pickling (Karovicova et al. 1999). But, many of these techniques are not suitable for small-scale or household use in developing countries (Steinkraus 2002). For instance, the small-scale canning of vegetables has food safety problems and contamination by foodborne pathogens such as *Listeria monocytogenes* and *Escherichia coli* might occur (Reina et al. 2005). Lactic acid fermentation requires very little sophisticated equipment for carrying out the fermentation process (Steinkraus 2002). Further, the pathogenic microflora is inhibited by LAB (Reina et al. 2005).

7.2 Removal of Anti-Nutritional Factors

Many vegetables and fruits contain naturally-occurring toxins and anti-nutritional compounds (Drewnowski and Gomez-Carneros 2000). For example, cassava roots contain two cyanogenic glucosides, linamarin and lotaustralin (Ray and Ward 2006). When the roots are naturally fermented by a mixed population of yeasts (*Saccharomyces cereviseae* and *Candida* spp.) and LAB (*Lactobacillus*, *Leuconostoc* and *Pediococcus*), the cyanogen level is reduced drastically (Kostinek et al. 2005). Likewise, LAB reduce the toxic elements in African locust beans and in leaves of *Cassava obtusifolia* during preparation of kawal, a Sudanese food (Dirar 1993).

7.3 Mineral and Vitamin Preservation

The micronutrient availability is enhanced in LA fermented vegetables because of significant reduction in the phytase enzyme. Iron bioavailability was also reported to be higher in carrots (Rakin et al. 2004), beet (Yoon et al. 2005) and sweet potato (Panda et al. 2007) after LA fermentation.

7.4 Improvement of Food Digestibility

Lactic acid bacteria contain various intracellular and extracellular food digestive enzymes, i.e., α-amylase (Guyot et al. 2000, Rao et al. 2004), pectinase (Kunji et al. 1996) and proteinase (Shurkhno et al. 2005). These enzymes aid in improving the digestibility of fermented fruits and vegetables. Proteases digest vegetable proteins during fermentation and some indigestible compounds such as sulfur compounds (aliin, allicin, ajoene, allylpropl, trisulfide, sallylcysteine, vinyldithiine and S-allylmercaptocystein) in garlic or onion improving the food digestibility (Di Cagno et al. 2013). Single culture lactic fermentation (*Lb. casei*, *Lb. plantarum* and sequential culture fermentation (*Saccharomyces boulardii* + *Lb. casei*, *S. boulardii* + *Lb. plantarum*) drastically reduced the content of

phytic acid, polyphenols and trypsin inhibitors of a food mixture containing rice flour, green paste and tomato pulp while significantly improved the *in vitro* digestibility of starch and protein (Sindhu and Khetarpaul 2002).

7.5 Some Disadvantages of Lactic Acid Fermentation

Microbial de-carboxylation of amino acids results in the formation of biogenic amines that can be found in fermented foods. These amino-compounds can confer an unpleasant flavour to the product or can be toxic (Arena and Manca de Nadra 2001, Garcia- Ruiz et al. 2011). Ingestion of certain amines by human can cause headaches, fever and vomiting, symptoms similar to those of microbial food poisoning. Histidine, ornithine, tyrosine and lysine are the main amino acids that can be de-carboxylated into biogenic amines such as histamine, putrescine, tyramine, and cadaverine, respectively. A minimum histamine level of 1 g/kg in a foodstuff or the consumption of 0.07 to 1 g of histamine per meal is supposed to elicit histamine toxicity. The European regulation 2073/2005 stated that the average histamine content must be 100 mg/kg or less in food.

Some bacteria commonly involved in LA fermentation, such as *Lc. mesenteroides*, have been found to contain the amino acid decarboxylase, which induces the biogenic amine formation (Arena et al. 1999). Conversely, other bacteria such as *Lb. plantarum* and *Pediococcus* can limit biogenic amines through the production of amine oxidase enzymes (Garcia-Ruiz et al. 2011).

8 Perspectives and Conclusion

The daily intake of fruits and vegetables is estimated to be lower than the recommended dietary intake of 450 and 500 g of fruits and vegetables, respectively, as advised by the Food and Agricultural Organization (Rome). Fruits and vegetables are an essential part of the human nutrition. In particular, they are rich in water-soluble vitamins (vitamin C and group B vitamins), pro-vitamin A, phytosterols, and show a high variety of minerals and phytochemicals depending on the plant species. The major part of fruits and vegetables are consumed fresh or as industrially processed such as canned, dried, juice, paste, salad, sauce and soup preparations. Nevertheless, LAB perform an essential role in the preservation and production of wholesome foods ranging from fermented fresh vegetables such as cabbage, cucumber to fermented fruits (olive). Due to LA fermentation, foods become resistant to microbial spoilage and to development of toxins, and enriched with vitamins, minerals, dietary fibres and antioxidants. Regular consumption of LA fermented food products

enriched with natural pigments such as anthocyanin, lutein and β-carotene would be helpful in combating several chronical diseases such as night blindness, liver injury, aging, and related ailments. This is in addition to health benefits from probiotics.

Keywords: Food preservation, Lactic acid bacteria, Lactic fermented vegetables, Food fermentation

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5

Yogurt and Other Fermented Milks

*Catherine Béal** and *Sandra Helinck*

1 Introduction

Fermented milks are dairy products obtained mainly from lactic acid fermentation, thus leading to acidification and milk coagulation without any action of rennet. In addition, the fermentation process provides increased food safety as a result of low pH, without altering the nutrients of the milk, thus allowing consumption of milk constituents for a longer period of time than for milk itself. These fermented products are first mentioned 9000 yrs BCE in the Middle East (Mesopotamia and Egypt), India, and North East Africa and have historically expanded to help preserving milk. Among them, yogurt that originates from the Eastern Mediterranean countries and the Balkans is the most popular and the best known.

Fermented milks include various products that differ according to four main different characters that correspond to the kind of milk, the microbial species and strains, the possible addition of ingredients and the process technology.

- Most of the fermented milks are made from cow milk, but depending on the country, milks from goat, sheep, buffalo, zebu, camel or yak are also employed. For each kind of milk, differences may occur by considering the fat content, thus leading to full-fat, low-fat or not-fat products;

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- The fermentation is either carried out by specific combinations of lactic acid bacteria, or by an association of these bacteria with yeasts, thus conferring the products different sensory characteristics;
- Some ingredients are sometimes included during products manufacture in order to modify their sensory properties (flavor, color and texture). Addition of sweetening agents (sugar or other sweeteners for low-calorie products) allows reducing the perception of sourness. Addition of flavoring agents (mainly fruits and vanilla), fruits (in the form of jam), spices or salt (depending on the country) is done to better fit consumers demand. In some countries, stabilizers such as pectin, starch or gelatin or emulsifiers can be included in order to improve thickness and creaminess of the fermented milks;
- If the technology used for the manufacture always involves a fermentation step, differences are observed by considering the milk pretreatment (fat and solids-not-fat standardization, homogenization, heat treatment) or the fermented milk post-treatment (stirring, concentration, mixing with water, drying, freezing). These various post-treatments generate different textures such as set-type (or firm), stirred, drinking, frozen, concentrated or powder yogurts and fermented milks.

Many different kinds of fermented milk products are available all over the world, and are being produced either at industrial scale or at smaller scale, in artisanal production units.

Yogurt is the most popular fermented milk in the world. It is mostly prepared from cow milk, sometimes from goat or sheep milk. Two species of lactic acid bacteria are involved in yogurt manufacture: *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The products are fermented at 42°C, until pH 4.5–4.4 is achieved. They are put on the market as natural yogurts, sweetened, flavored or added with fruits or with cream (Béal and Sodini 2003, Leroy and De Vuyst 2004). They are also available as frozen yogurts (Soukoulis et al. 2007) or as long life yogurts (Guldas and Atamer 1995). Manufacture of acidophilus milk (North America), laban (Middle East), leben (Arab World) and dahi (India) is very close to that of yogurt. Differences mainly concern the microorganisms involved and corresponding growth temperature and duration.

Kefir and koumiss (Central Asia) are fermented milks made with kefir grains composed by little clumps of yeast, lactic acid bacteria and milk proteins. Originated from the Caucasus mountains, kefir is prepared by inoculating cow, goat, or sheep milk with kefir grains, whereas koumiss is made from mare's milk. After fermentation with stirring, the product has a slightly sour flavor due to lactic acid fermentation and a faint effervescence, as a result of alcoholic fermentation. Viili is a Finnish fermented milk that is

characterized by a slightly acid taste, a good diacetyl flavor and a stringy or ropy texture (Kahala et al. 2008). It is obtained by co-fermentation of lactic acid bacteria strains and molds.

Many kinds of fermented milks are available as beverages. Mixing yogurt with salt and water leads to popular drinks in many countries: dough in Iran, tan in Armenia, ayran in Syria and Turkey, laban arbil in Iraq, shenina in Iraq and Jordan, lassi in Punjab (India), Pakistan and Bangladesh. These products are fermented by unknown mixtures of bacteria and are sometimes added with herbs, mint, spices (cumin, red chillies), rose water or fruit juices. Some of them can be carbonated with carbonated water. Cultured buttermilk is another slightly sour liquid fermented milk, which is obtained from fermentation of whey by lactic acid bacteria (Leroy and De Vuyst 2004). It is generally salted and displays a tangy flavor. In Europe, USA and many countries, drinking yogurts are popular, generally in sweetened and flavored form. These products are not added with water, but strongly mixed after fermentation in order to break the curd before being packed. They can be added with stabilizers and/or thickeners to obtain the desired texture (Janhøj et al. 2008). Developed in 1935 by M. Shirota in Japan, Yakult is a liquid product obtained from milk fermentation by lactobacilli and bifidobacteria.

Strained fermented milks are more and more consumed in the world, as a consequence of the increasing consumption of Greek yogurt in the USA, due to its thick, creamy and tangy characteristics. Traditional products such as srikhand in India and labneh in Lebanon are conventionally manufactured by straining the fermented products in bags until the desired texture is achieved or, more recently, by ultra-filtration (Nsabimana et al. 2005). As a consequence of these operations, traditional Greek yogurt has a high fat content of 8–10%. Greek yogurt and industrial labneh are obtained by straining the product after cultures have been added to milk, by using mechanical separators that use centrifugal force or by ultra-filtration (Ur-Rehman et al. 2010). In addition to modified sensory properties, popular US-versions of Greek yogurt contains less carbohydrate but almost double the protein of regular yogurt, together with a low fat content, thus it is preferred by people who want to manage their weight.

In addition to these traditional products, probiotic fermented milks are obtained by involving probiotic bacteria during manufacture (Aureli et al. 2011, Quigley 2011, Shiby and Mishra 2013). According to the recommendations of Food and Agriculture Organization of the United Nations, World Health Organization, these bacteria are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO 2001). Among the benefits for human health, which are defined strain by strain, the following are the most

recognized (Quigley 2011): Role on host metabolism (lactose intolerance); Prevention of microbial infections (infectious disorders, diarrheal illness); Stimulation of the immune system (respiratory infections, oral vaccines); Regulation of immune responses (some inflammatory bowel diseases, irritable bowel syndrome, allergies). In Europe, these health benefits have to be demonstrated before using the probiotics in foods and supplements, pursuant to European regulation EC n° 1924/2006 (Official Journal of the European Union 2006).

Though many of these fermented milks are still produced on artisanal scale, the increasing world demand has led to the industrialization of yogurt manufacture since 1950. Large multinational companies are involved in yogurt and fermented milk production, the most important being Danone, Yoplait, Chobani, Fage and Stonyfields. Nowadays, the interest for these dairy products is increasing worldwide, as a result of their pleasant sensory properties (fresh character, acidity, distinctive flavor, smooth texture) and their ability to offer a wide range of products for the consumers. They also provide the consumers live and active cultures in significant numbers, which may offer specific health benefits beyond conventional nutrition.

By considering that yogurt is the most consumed fermented milk in the world, this chapter focuses mainly on this product, with additional information about the other fermented milks when required.

2 Microbiology of Fermented Milks

2.1 Microorganisms

Fermented milks are produced by using specific combinations of microorganisms, which are defined according to national or international rules (FAO/WHO 2011) or to industrial specifications. These microorganisms possess the 'Generally Recognized as Safe' (GRAS) status in USA and the 'Qualified Presumption of Safety' (QPS) status in the European Union, due to a long history of safe use in food production and to an absence of pathogenicity and potentially harmful metabolites (Bernardeau et al. 2008, Delorme 2008).

Streptococcus thermophilus and *Lb. delbrueckii* subsp. *bulgaricus* are used in combination in all types of yogurt, including frozen and long-life products. However, other species of lactic acid bacteria and bifidobacteria can be present in fermented milks (Shiby and Mishra 2013). The most used species in lactobacilli is *Lactobacillus acidophilus* (notably in acidophilus milk), *Lb. johnsonnii*, *Lb. reuteri*, *Lb. rhamnosus* and *Lb. paracasei*. Laban and leben implicate mainly *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (Chammas et al. 2006) and depending on the author, *Lb. acidophilus*, *Leuconostoc lactis*, *Lactococcus lactis* subsp. *lactis*, *Lc. lactis* subsp. *lactis biovar.*

diacetylactis, *Lc. lactis* subsp. *cremoris*, *Lb. plantarum* and two kinds of yeasts, *Kluyveromyces fragilis* and *Saccharomyces cerevisiae* (Baroudi and Collins 1976, Guizani et al. 2001). A mixture of yogurt bacteria, together with *Lc. lactis* subsp. *lactis*, *Lb. acidophilus*, *Lb. lactis* and *Lb. casei* characterizes dahi production (Mehmood et al. 2009). The liquid fermented milk Yakult is obtained from milk fermentation with *Lb. casei* Shirota, sometimes combined with *Bifidobacterium breve*, whereas cultured buttermilk is fermented by *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis biovar. diacetylactis* and *Ln. mesenteroides* subsp. *cremoris* (Leroy and De Vuyst 2004).

The use of *Bifidobacterium* in dairy industry is widespread: *B. bifidum*, *B. adolescentis*, *B. breve*, *B. infantis*, *B. longum* and *B. lactis* species are currently used in commercial probiotic fermented milks, and they are widely exploited as a tool for the development of novel functional products (Vasiljevic and Shah 2008). Some of the commercial cultures used in fermented dairy products and having health claims are listed in Table 1.

Table 1. Main species of lactic acid and probiotic bacteria involved in commercial fermented milk manufacture (adapted from Vasiljevic and Shah 2008).

Strains	**Source**
Lactobacillus reuteri SD2112	Biogaia
Lb. acidophilus LA1/LA5 *Lb. delbrueckii* subsp. *bulgaricus* LB12 *Lb. paracasei* CRL431 *Bifidobacterium animalis* subsp. *lactis* BB12	Chr. Hansen
Lb. acidophilus NCFM, La *Lb. paracasei* Lpc *B. lactis* HOWARU™/Bl	Danisco
Lb. casei immunitas *B. animalis* DN173010 (Bioactivia)	Danone
Lb. acidophilus LAF L10	DSM Food Specialties
Lb. rhamnosus LB21 *Lc. lactis* L1A	Essum AB
B. lactis HN019 (DR10) *Lb. rhamnosus* HN001(DR20)	Fonterra
Lb. rhamnosus R0011 *Lb. acidophilus* R0052	Institut Rosell Lallemand
Lb. acidophilus LB	Lacteol Laboratory
Lb. paracasei F19	Medipharm
B. longum BB536	Morinaga Milk Industry Co. Ltd.
Lb. plantarum 299V *Lb. rhamnosus* 271	Probi AB
Lb. acidophilus SBT-20621 *B. longum* SBT-29281	Snow Brand Milk Products Co. Ltd.
Lb. salivarius UCC118	University College Cork
Lb. rhamnosus GG1	Valio Dairy
Lb. casei Shirota *B. breve* Yakult	Yakult

Other fermented milks such as kefir, koumiss and viili are obtained by combining lactic acid bacteria and other microorganisms, such as yeasts and molds. Traditional kefir grains contain 83–90% bacteria including *Lactobacillus kefiranofaciens* subsp. *kefirgranum, Lb. parakefiri, Lb. kefiri, Lb. helveticus, Lb. casei* subsp. *pseudoplantarum, Lb. brevis* and 10–17% yeasts such as *Kluyveromyces marxianus* var. *lactis, Saccharomyces cerevisiae, Candida inconspicua, C. maris, K. marxianus, Kazachstania exigua* and *Rhodosporidium kratochvilovae* (Simova et al. 2002, Vardjan et al. 2013). Three types of koumiss exist, depending on the lactic acid content. 'Strong' koumiss is generated by *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. rhamnosus,* 'moderate' koumiss contains *Lb. acidophilus*, *Lb. plantarum*, *Lb. casei*, *Lb. fermentum* and 'light' koumiss is acidified by *S. thermophilus* (Danova et al. 2005). In koumiss, *K. marxianus* and *S. cerevisiae* are the dominant species of yeast but other genera such as *Kazachstania*, *Dekkera*, *Candida*, *Geotrichum* and *Pichia* sp. are also found (Mu et al. 2012). The Finnish fermented milk viili is obtained with a microflora that includes *Lc. lactis* subsp. *lactis, Lc. lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis biovar diacetylactis, Ln. mesenteroides* subsp. *cremoris* and *Geotrichum candidum* (Kahala et al. 2008).

2.2 General Characteristics of Major Bacteria Involved In Fermented Milks

S. thermophilus and *Lb. delbrueckii* subsp. *bulgaricus* are thermophilic lactic acid bacteria. They are Gram-positive, anaerobic, aerotolerant, catalase negative, non spore forming, and have less than 55% G+C content in their DNA.

S. thermophilus is part of the genus *Streptococcus* that comprises more than 40 species, including several pathogens such as *S. agalactiae*, *S. pneumoniae* and *S. pyogenes*. Among the *Streptococcus* genus, *S. thermophilus* is the only safe species that is used as a starter culture in foods. It is frequently isolated from dairy environments, but strains have also been isolated from plant sources (Michaylova et al. 2007). Technological and functional attributes of this species in yogurts and cheeses are gathered in a recent review (Iyer et al. 2010). The entire genome sequence of *S. thermophilus* has been deciphered (Bolotin et al. 2004). Comparative genomics suggest that this species recently emerged and evolved by combination of loss-of-function and horizontal gene transfer events. These gene transfer events have originated from other dairy species, such as *Lc. lactis* and *Lb. delbrueckii*, and contribute to its adaptation to the milk environment (Delorme 2008). In milk, it grows with linear chains of ovoid cells, at 42°C and up to 50°C, but not at 10°C (Stiles and Holzapfel 1997). Lactose is converted into glucose and galactose, which is not metabolized. Glucose is fermented predominantly to L (+) lactic acid, thus corresponding to homofermentative metabolism.

Lb. delbrueckii subsp. *bulgaricus* is part of the genus *Lactobacillus*, which is more heterogeneous (G+C content ranging from 33 to 55%) and includes 60 species. The classical partition of the lactobacilli is based on their fermentative characteristics (Stiles and Holzapfel 1997), which allow identifying obligatory homofermentative (*Lb. delbrueckii* subsp. *bulgaricus, Lb. acidophilus, Lb. johnsonnii*), facultative heterofermentative (*Lb. paracasei, Lb. rhamnosus*) and obligatory heterofermentative (*Lb. reuteri*) species. Homofermentative lactobacilli convert glucose to D (–), L (+) or DL lactic acid, whereas heterofermentative bacteria ferment glucose to equimolar amounts of lactic acid, CO_2 and ethanol (and/or acetic acid). Lactobacilli are ubiquitous in the nature and are mostly found in carbohydrate rich environments. *Lb. delbrueckii* subsp. *bulgaricus* is frequently isolated from dairy environments, but strains have also been isolated from plant sources (Michaylova et al. 2007). It is a Gram-positive rod that grows between 45°C and 50°C (Stiles and Holzapfel 1997). Complete genome sequence of *Lb. delbrueckii* subsp. *bulgaricus* has been described (Van de Guchte et al. 2006).

Lb. acidophilus, which was firstly isolated from the human gastro-intestinal tract, is involved in the manufacture of various fermented milks. It is a Gram-positive rod that grows optimally in the range 35 to 40°C (Gomes and Malcata 1999). It displays a homofermentative metabolism with production of 34% D(–) and 66% L(+) lactic acid. The complete genome sequence of *Lb. acidophilus* NCFM has been determined and analyzed (Altermann et al. 2005).

Bifidobacteria are Gram-positive, non-spore forming, non-motile, anaerobic and catalase-negative bacteria. They display various shapes including short, curved rods, club-shaped rods and bifurcated Y-shaped rods. Presently, 30 species are included in the genus *Bifidobacterium.* Among them, *B. animalis* subsp. *lactis, B. infantis, B. longum, B. bifidum* and *B. breve* are often encountered in fermented milks. Bifidobacteria represent an important group of human gut commensal bacteria, accounting for 5 to 25% of the microbiota in adults and being dominant in the microflora of children (Gomes and Malcata 1999). They have a high (G+C) content, which varies from 54 to 67%. Many strains have been sequenced (Chervaux et al. 2011, Garrigues et al. 2010). Their optimum growth temperature is comprised between 37 and 41°C, with maximum growth at 43–45°C and no growth at 25–28°C (Gomes and Malcata 1999). These bacteria convert lactose and glucose to lactic and acetic acids *via* a metabolic pathway that is characterized by the presence of the enzyme fructose-6-phosphate phosphoketolase. Some strains of *Bifidobacterium* possess probiotic properties such as stimulation of immune system, prevention of some microbial gastroenteritis and prevention and treatment of some bowel diseases such as irritable bowel syndrome or Crohn's disease (Vasiljevic and Shah 2008, Aureli et al. 2011).

2.3 Associative Growth in Milk

Different types of interactions are observed between the microorganisms involved in fermented milk manufacture. They are classified into positive (commensalism, mutualism, protocooperation), negative (competition, amensalism, antagonism) and neutral (neutralism) interactions. They are achieved either directly (with a physical contact between microorganisms) or indirectly (through compounds produced or consumed by the microorganisms).

In the milk, *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* exhibit a positive interaction that is mutually favorable. This interaction is called protocooperation and is well documented (Courtin and Rul 2004, Sieuwerts et al. 2008). By comparing co-cultures and pure cultures, it is reflected by more rapid growth and acidification (Béal and Corrieu 1994) and by higher production of aroma compounds and exopolysaccharides and proteolysis (Monnet et al. 2008, Sodini et al. 2000). Recently, from mixed-culture transcriptome analysis, the molecular response associated with mixed-culture growth in milk of yogurt bacteria has been related to purine, amino acids and long-chain fatty acid metabolism (Sieuwerts et al. 2010). *Lb. delbrueckii* subsp. *bulgaricus* is stimulated by formic acid, folic acid and CO_2 that are produced by *S. thermophilus* in milk (Crittenden et al. 2003). The positive effects of formic acid and folic acid on the growth of *Lb. delbrueckii* subsp. *bulgaricus* are related to the biosynthesis of purines. CO_2 comes from the decarboxylation of urea by urease excreted by *S. thermophilus* (Zotta et al. 2008). It is a precursor for the synthesis of aspartate, glutamate, arginine, and nucleotides. *S. thermophilus* is stimulated by free amino acids and small peptides liberated from milk proteins by the cell-wall protease PrtB of *Lb. delbrueckii* subsp. *bulgaricus* (Sieuwerts et al. 2008). The absence of this enzyme resulted in a higher final pH, as the amount of branched-chain amino acids present in milk and directly consumable, is not sufficient for an optimal growth of *S. thermophilus* (Courtin et al. 2002). However, proteolysis by *Lb. delbrueckii* subsp. *bulgaricus* is insufficient to meet the biosynthetic demands for sulfur and branched-chain amino acids, as demonstrated from the up-regulation of genes associated with these amino acids in mixed cultures (Sieuwerts et al. 2010). The main compounds involved in the relevant interactions between *S. thermophilus, Lb. delbrueckii* subsp. *bulgaricus* and their environment are summarized in Fig. 1.

In probiotic fermented milks, lactic acid starters (*S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*) are associated with probiotic bacteria (*Bifidobacterium* or other *Lactobacillus* strains). Numerous interactions exist, depending on association of strains: stimulation, delay, complete inhibition of growth or no effect (Vinderola et al. 2002b). Among them,

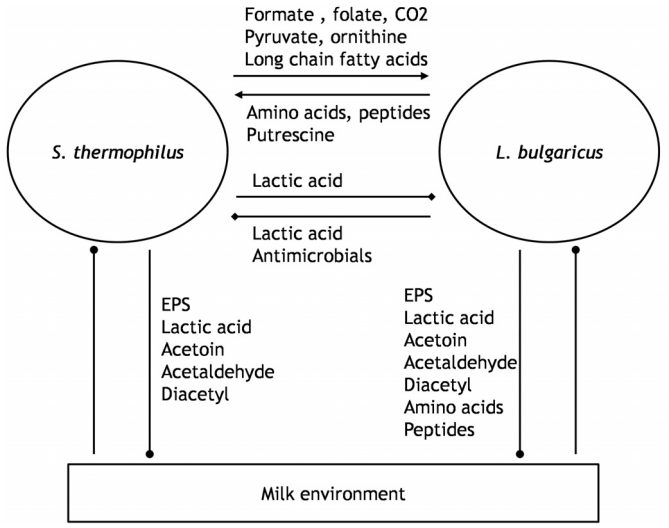

Fig. 1. Schematic representation of the validated and hypothesized interactions that occur between *S. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, their environment, and the compounds relevant for yogurt characteristics (adapted from Sieuwerts et al. 2008). Positive interactions (▼); negative interactions (♦); interactions that do not specifically promote or decrease the growth of the other species (●).

typical combinations of cultures including *B. animalis* subsp. *lactis* and *S. thermophilus* increase the milk concentration of folic acid, as a result of folate synthesis during growth of the bifidobacteria (Crittenden et al. 2003). The possible interactions among the strains selected to manufacture a probiotic fermented dairy product is a compulsory criterion so as to define the best combination in order to optimize their performance in the process.

In kefir, many interactions have been described. The co-culture of *Lb. kefiranofaciens* and *S. cerevisiae* reduces the lactic acid concentration in the product, since it is consumed by the yeast. It also stimulates cell growth and kefiran production by the lactobacilli, as a result of the physical contact between the two microorganisms (Cheirsilp et al. 2003). The co-aggregation between *Lb. kefir* and *S. lipolytica* was shown to be due to the lectin-like activity of a surface protein of the bacteria (Golowczyc et al. 2009). Co-cultivation of *Zygotorulaspora florentina* and *Lb. hordei* in water kefir medium significantly increases cell yield of all microorganisms. Acidification of the medium by the lactobacilli stimulates the yeasts, which, in return, makes available some essential nutrients (amino acids such as arginine and vitamin B_6) for the bacteria (Stadie et al. 2013).

3 Biochemistry and Physico-Chemistry of Lactic Acid Fermentation

3.1 Lactose Metabolism

Glycolysis is the main source of energy for lactic acid and probiotic bacteria and the ATP formed during glycolysis is used for anabolism and bacterial growth. Homofermentative metabolic pathway is operated by *Lactococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus* and group I lactobacilli (including *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* and *Lb. helveticus*), whereas heterofermentative metabolism is used by *Leuconostoc*, *Oenococcus*, *Weissella* and groups II and III lactobacilli (including *Lb. rhamnosus*, *Lb. casei* and *Lb. brevis*).

Homofermentative pathway takes place in three steps as shown in Fig. 2. Lactose is first internalized into the cell *via* the phospho-transferase system coupled to phospho-enolpyruvate (PTS-PEP) or *via* a lactose permease energized by the proton gradient. Secondly, intracellular lactose

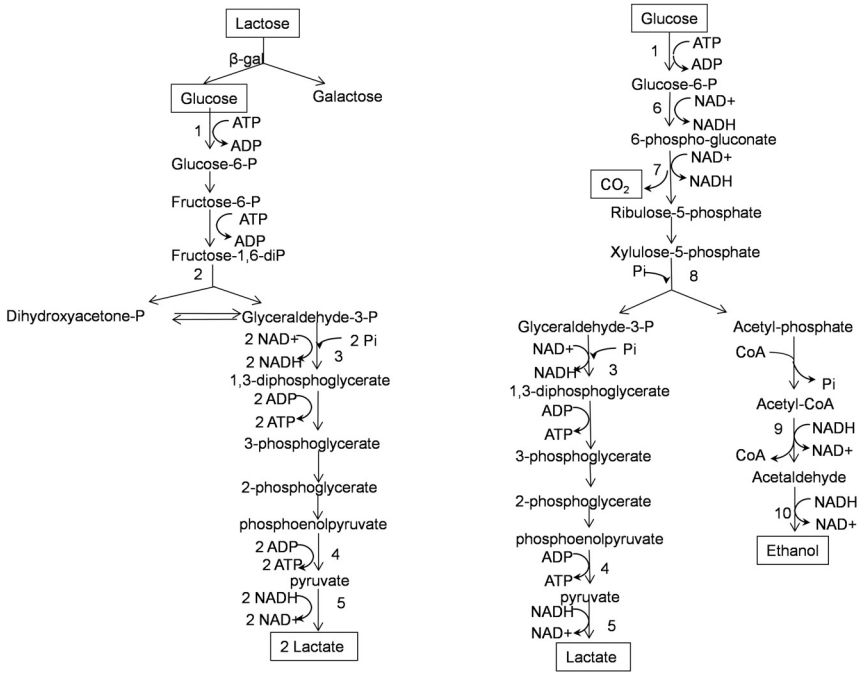

Fig. 2. Scheme of the homofermentative and heterofermentative metabolisms in lactic acid bacteria. 1: glucokinase; 2: fructose-1,6-diphosphate aldolase; 3: glyceraldehyde-3-phosphate dehydrogenase; 4: pyruvate kinase; 5: lactate dehydrogenase; 6: glucose-6-phosphate dehydrogenase; 7: 6-phosphogluconate dehydrogenase; 8: phosphoketolase; 9: acetaldehyde dehydrogenase; 10: alcohol dehydrogenase.

is hydrolyzed into glucose and galactose by the enzyme β-galactosidase. Glucose is catabolized to pyruvate *via* the glycolytic pathway (Embden-Meyerhof-Parnas). *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* do not metabolize galactose which is secreted out of the cell. However, some species of lactic acid bacteria such as mesophilic strains are able to metabolize galactose into pyruvate, through the tagatose-6-phosphate pathway (Hugenholtz et al. 2002). Finally, lactic acid is formed from pyruvate through a lactate dehydrogenase activity. This reaction allows the re-oxidation of NADH formed during the earlier glycolytic steps. The intracellular lactate is excreted out of the cell *via* a symport with H^+, either on L(+), D(–) or DL form, depending on the bacterial species. Homolactic fermentation of glucose results theoretically in two moles of lactic acid and two moles ATP per mole glucose consumed. Accumulation of lactate in the extracellular medium and subsequent pH decrease cause inhibition of bacterial growth.

Heterolactic fermentation of glucose, through the pentose-phosphate pathway, is characterized by an initial dehydrogenation step with the formation of 6-phosphogluconate, followed by a decarboxylation that leads to one molecule of CO_2 (Fig. 2). This pathway allows the formation of one mole of pyruvate from glyceraldehyde-3-phosphate and one mole of acetyl-phosphate, which generates either ethanol or acetic acid, depending on environmental conditions. Consequently, heterofermentative catabolism of one mole of glucose theoretically leads to one mole of lactic acid, one mole of ethanol or acetic acid and one mole of ATP, together with the reoxydation of the coenzyme NADH.

Bifidobacteria strains are able to catabolize galacto-oligosaccharides such as lactose, galactose and glucose (Gomes and Malcata 1999). From glucose, they synthesize acetic and (L+) lactic acid without generation of CO_2 as shown in Fig. 3. Hetero-fermentation is initiated by splitting fructose-6-phosphate into one C2 and one C4 moiety. As a consequence, the fermentation of two moles of hexose results theoretically in three moles of acetate, two moles of lactate and two moles of ATP. Despite ATP synthesis, acid production by bifidobacteria is slow, thus resulting in prolonged fermentation time and low lactic acid final level (Ostlie et al. 2003). Consequently, *Bifidobacterium* strains and lactic acid bacteria strains belonging to *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* species are often associated in dairy industry, for the production of fermented milks.

3.2 Proteolysis

During milk fermentation, the proteolytic system of lactic acid bacteria degrades caseins into peptides and free amino acids. These compounds are essential to the growth of the strains, they affect the physical structure

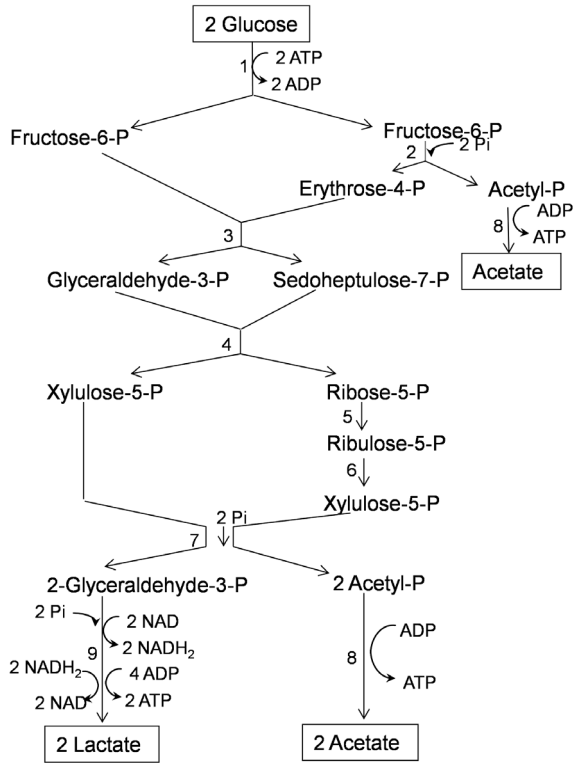

Fig. 3. Formation of acetate and lactate from glucose by *Bifidobacterium* (adapted from Gomes and Malcata 1999). 1: hexokinase and fructose-6-phosphate isomerase; 2: fructose-6-phosphate phosphoketolase; 3: transaldolase; 4: transketolase; 5: ribose-5-phosphate isomerase; 6: ribulose-5-phosphate-3-epimerase; 7: xylulose-5-phosphoketolase; 8: acetate kinase; 9: enzymes as in homofermentative pathway

of the curd and they can be converted into flavor compounds (such as acetaldehyde). Proteases of lactic acid bacteria are key enzymes of the proteolytic system, initiating the breakdown of caseins into oligopeptides.

The unique cell-wall proteinase PrtS of *S. thermophilus* is essential to the growth and acidification activity of this species in milk (Fernandez-Espla et al. 2000). However, it is present only in a minority of *S. thermophilus* strains, since among 30 strains, 12 were $PrtS^+$, three displayed a slight PrtS activity and 15 were $PrtS^-$, despite the presence of the corresponding gene (*prtS*) in eight of them (Galia et al. 2009). In mixed cultures, this enzyme is not essential since nitrogen compounds necessary for *S. thermophilus* growth are supplied by the proteinase of *Lb. delbrueckii* subsp. *bulgaricus* (Courtin et al. 2002).

The cell-wall proteinase of *Lb. delbrueckii* subsp. *bulgaricus* PrtB is responsible for the first step of caseinolysis (Siezen 1999) and is essential for the growth of this species in milk. Indeed, the biomass of a $PrtB^-$ strain of *Lb. delbrueckii* subsp. *bulgaricus* grown in milk is only 22% of that obtained by the corresponding $PrtB^+$ strain (Gilbert et al. 1997). Most *Lb. delbrueckii* subsp. *bulgaricus* strains are characterized by a high activity of this cell-surface proteinase, resulting in fast growth and rapid fermentation of milk. In addition, the global proteolytic activity in *Lb. delbrueckii* subsp. *bulgaricus* is higher than in *S. thermophilus* (Courtin et al. 2002).

The ability of some specific strains to produce natural bio-active peptides from proteolytic activity has been investigate in some countries (Finland, Japan), in order to develop fermented milks with health claims. These strains act on the cardiovascular system of humans through the release of bioactive peptides from caseins during lactic acid fermentation. These peptides display hypotensive properties that inhibit the angiotensin converting enzyme (ACE). ACE plays a major role in the control of blood pressure and ACE inhibition may result in an overall reduction of blood pressure by a decrease of the vasoconstrictory peptide and an increase of the vasodilatory peptide. ACE inhibitor peptides are produced by various strains of proteolytic lactic acid bacteria: *Lb. helveticus*, *Lb. acidophilus*, *Lb. rhamnosus*, *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. lactis* (Choi et al. 2012, Fitzgerald and Murray 2006). Some *B. bifidum* strains also display an ACE inhibitor activity in fermented milk (Gonzalez-Gonzalez et al. 2011). Among these species, *Lb. helveticus* is a good candidate because of its high cell-wall proteinase activity and high ACE inhibitor activity of its bio-peptides. It produces two peptides, Val-Pro-Pro and Ile-Pro-Pro that exhibit hypotensive activity (Fitzgerald and Murray 2006). Two milk drinking products fermented with *Lb. helveticus* and containing these two tri-peptides have been developed and are commercialized in Japan and in Finland.

3.3 Production of Flavor Compounds

Starter cultures are responsible for the production of most of the aroma compounds of fermented milks. More than hundred volatile compounds, including carbonyl compounds, alcohols, acids, esters, hydrocarbons, aromatic compounds, sulfur-containing compounds and heterocyclic compounds are found in yogurt, at low to trace concentrations (Cheng 2010). Among them, lactic acid, acetaldehyde, diacetyl and acetoin, as well as their relative proportions, are essential for the final aroma of yogurt (Routray and Mishra 2011). Their final level in the fermented products depends on the bacterial strains, the composition of milk base and the processing conditions.

Acetaldehyde is suggested as the major flavor compound of yogurt, where it reaches a final concentration of 5 to 40 mg/kg, depending on the strain used (Chaves et al. 2002, Routray and Mishra 2011). It confers a pleasant fresh and fruity aroma to the product (Cheng 2010). The formation of acetaldehyde in fermented milks takes place during lactic acid fermentation by *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, according to two metabolic pathways (Chaves et al. 2002). It is firstly synthesized directly from pyruvate, through the action of the pyruvate decarboxylase, or indirectly from acetyl coenzyme A, through the action of pyruvate dehydrogenase and aldehyde dehydrogenase. Furthermore, threonine is converted into acetaldehyde and glycine, by the action of serine hydroxyl-methyl transferase by *Lb. delbrueckii* subsp. *bulgaricus*. The balance between these pathways is strain dependent and more than one metabolic pathway may operate simultaneously.

Diacetyl (2,3-butanedione) and acetoin (3-hydroxy-2-butanone) are two carbonyl compounds present in fermented milks such as viili, the aroma of diacetyl being 100-fold more powerful than that of acetoin. Diacetyl has a typical butter aroma and its concentration in yogurt ranges from 0.2 to 3 mg/kg, depending on the strains (Cheng 2010, Routray and Mishra 2011). Acetoin concentration in yogurt varies between 1.2 and 28.2 mg/kg and confers a butter-like flavor that is similar to that of diacetyl (Cheng 2010). Diacetyl is mainly synthesized by *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc* from lactose and citrate. These species degrade citrate (1.7 g/L in milk) to form acetate and oxaloacetate that is decarboxylated into pyruvate. Pyruvate is metabolized by an additional pathway into α-acetolactate and subsequently to acetoin *via* α-acetolactate decarboxylase, or diacetyl *via* a spontaneous oxidative decarboxylation (Martin et al. 2011). However, other lactic acid bacteria that are not able to metabolize citrate may produce low levels of diacetyl from acetolactate. In *Lc. lactis* subsp. *cremoris*, acetoin and diacetyl are formed as a result of aspartic acid catabolism (Le Bars and Yvon 2008). The production of diacetyl and acetoin is highly dependent on the pH of the medium (optimal at pH 7–8). *S. thermophilus* also produces α-acetolactate that can be partially metabolized into diacetyl or acetoin (Monnet et al. 2003). Indeed, one of the physiological functions of the α-acetolactate decarboxylase in *S. thermophilus* is to regulate leucine and valine biosynthesis by diverting the flux of α-acetolactate towards acetoin, when the branched-chain amino acids are present at a high concentration.

In kefir, lactic acid, acetaldehyde, diacetyl, acetone and 2-butanone are produced by lactic acid bacteria (*Lb. delbrueckii* subsp. *bulgaricus*, *Lb. helveticus* and *S. thermophilus*). In addition, ethanol and CO_2 are produced by *S. cerevisiae* in amounts (3.98 g/L and 1.8 g/L) that confer specific flavor and aroma of kefir (Beshkova et al. 2003).

In laban, acetaldehyde, ethanol, diacetyl and acetoin are the four major volatile compounds produced by thermophilic and mesophilic lactic acid bacteria and yeasts (Chammas et al. 2006, Samet-Bali et al. 2010).

3.4 Formation of the Coagulum

Acidification of milk helps to destabilize the casein micelles by progressively converting the colloidal calcium-phosphate complex (CCP) to the soluble calcium phosphate fraction which diffuses into the aqueous phase of the milk. This phenomenon results in the depletion of calcium in the micelles, leading to the coagulation of caseins at their isoelectric point (pH 4.6–4.7) and the formation of the yogurt gel. These physico-chemical mechanisms are summarized in the review of Lee and Lucey (2010). In milk, the CCP becomes completely solubilized in casein micelles around pH 5. As caseins approach their isoelectric point (pH 4.6), the net negative charge on casein micelles is reduced, which decreases electrostatic repulsion between charged groups, including the phosphoserine residues that are exposed when the CCP is solubilized. Finally, electrostatic and casein-casein attractions increase due to enhanced hydrophobic interactions, thus resulting in the formation of a three-dimensional network consisting of clusters and chains of caseins.

3.5 Production of Exopolysaccharides

Some strains of lactic acid bacteria contribute to the physical properties of stirred fermented milks through biosynthesis of extracellular polysaccharides (EPS), which prevent syneresis and limit the use of stabilizers. Stirred yogurts fermented with EPS producing cultures show higher mouth thickness, higher ropiness, higher creaminess but lower gel firmness than yogurts fermented with non EPS-producing strains (Béal et al. 1999, Folkenberg et al. 2006). In addition to the important role of EPS in the rheological behavior of fermented milks, some of them exert some health benefits that are related to their immunomodulatory and antioxidant potential (Liu et al. 2011). As no correlation between viscosity of fermented milks and EPS concentration exists, their rheological properties are related to their intrinsic viscosity or molar weight of EPS, their chemical composition and structure, as well as their interactions with the milk proteins.

Depending on the strain, either homo- or heteropolysaccharides are synthesized. Homopolysaccharides are composed of one type of monosaccharide (D-glucopyranose or D-fructofuranose) whereas heteropolysaccharides are characterized by repeated units of D-glucose, D-galactose and L-rhamnose in various ratios (De Vuyst et al. 2001). In

S. thermophilus, N-polymers containing N-acetyl-D-galactosamine and N-acetyl-D-glucosamine have also been reported (Iyer et al. 2010). EPS from lactic acid bacteria possess apparent molecular masses that range from 4×10^4 to 6×10^6 Da. Homopolysaccharides are mainly produced by *Ln. mesenteroides* in the form of dextrans. Heteropolysaccharides are synthesized by a great variety of mesophilic and thermophilic lactic acid bacteria and by few strains of *Bifidobacterium* (De Vuyst et al. 2001, Prasanna et al. 2012).

The amount of EPS produced by lactic acid bacteria generally varies between 30 to 600 mg/L, depending on species, strains and culture conditions (De Vuyst et al. 2001). Higher amounts are obtained with some strains of *S. thermophilus* (1.5 g/L) and *Lb. rhamnosus* (2.7 g/L) in specific media (Badel et al. 2011, De Vuyst et al. 2001). Thermophilic lactic acid bacteria produce the highest amount of EPS during growth and under optimal environmental conditions, whereas mesophilic lactic acid bacteria synthesize maximum amounts of EPS under sub-optimal growth conditions (De Vuyst et al. 2001). Some strains of *Bifidobacterium* are able to produce 6 to 600 mg/L of EPS (Prasanna et al. 2012).

Exopolysaccharides are synthesized intracellularly by the polymerization of repeated unit precursors formed in the cytoplasm. These precursors are assembled by the sequential addition of activated carbohydrates (UDP-glucose, UDP-galactose and dTDP-rhamnose) by specific glycosyl-transferases to a growing repeated unit that is coupled to a lipid carrier. To date, ten repeated unit structures of EPS have been found in *S. thermophilus* and the genetic organization of the different types of *eps* gene clusters is already characterized (De Vuyst et al. 2011). The repeated units are then translocated across the membrane to the outside of the cell before polymerization, which allows obtaining a polymer composed by several hundred to thousand repeated units (Badel et al. 2011, De Vuyst et al. 2001).

3.6 Influence of Environmental and Biotic Factors

The quality of milk that results from milk composition and heat treatment, the fermentation conditions such as temperature and fermentation time, the strains used and the presence of additives or bacteriophages in milk affect the growth of the starter cultures and the rate of acid development.

Heat treatment of milk is used for fermented milks manufacture to permit the destruction of the majority of pathogens and spoilage microflora. Moreover, some antibacterial compounds such as agglutinins and lactoperoxidases, present in raw milk, are inactivated by heating conditions. These phenomena promote the growth of the lactic acid bacteria during fermentation step. In addition, the heating of milk results in the release

of compounds such as peptides, free amino acids and formic acid that stimulate the growth of *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (Courtin and Rul 2004).

Recently, the fatty acid content of milk was shown to affect the fermentation kinetics, which are more rapid with organic milk as compared to conventional milk. This difference results from the higher poly-unsaturated fatty acid content in organic milk (Florence et al. 2012).

Temperature and fermentation time are important parameters which impact on the technological and sensory properties of fermented milks. Indeed, the rate of fermentation is linked to the metabolic activity of lactic acid bacteria and bifidobacteria, which depends on the fermentation temperature (Béal et al. 1999). The amounts of lactic acid, acetaldehyde and acetoin vary among the bacteria and according to temperature and fermentation time (Ostlie et al. 2005), and are related to the final pH (Béal et al. 1999). Moreover, incubation temperature and fermentation time have major effects on the rheological properties of yogurt (Abbasi et al. 2009, Béal et al. 1999, Lee and Lucey 2004).

The strains and the strain combinations used also affect the fermentation, acidifying strains leading to shorter fermentation times than other strains (Béal et al. 1999, Florence et al. 2009).

Common additives involved in fermented milk manufacture are sugar, sweeteners, colorings and flavorings, which can have an impact on the growth of lactic acid and probiotic bacteria. The presence of sucrose in milk (15%) reduces the growth of lactic acid bacteria and bifidobacteria, but differently according to the strains (Vinderola et al. 2002a). In contrast, the addition of sweeteners such as aspartame at the concentration normally used in fermented dairy products (0.03%) has no impact on growth of lactic acid bacteria. Some flavoring-coloring commercial mixtures have an important inhibition potential on the growth of lactic acid starters and probiotic bacteria, at the concentrations recommended by suppliers (Vinderola et al. 2002a). The effects of added fruit juices or fruit powders on fermentation kinetics differ, depending on bacteria and type of fruits (Sun-Waterhouse et al. 2013, Vinderola et al. 2002a). Moreover, these compounds influence the texture of fermented milks (Do Espirito Santo et al. 2012).

Bacteriophages of dairy cultures are a very significant problem in the yogurt industry, leading to product variability and reduced productivity that results in important economic losses. They can come from various sources such as raw milk, starter cultures, air or surfaces in the dairy plant (Garneau and Moineau 2011). Phages of lactic acid bacteria, phage-host interactions and host response to infection have been extensively studied (Mahony et al. 2012). Presence of bacteriophages induces low production of lactic acid, reduced milk coagulation and a culture lysis. Consequently,

lactic acid and probiotic bacteria suppliers regularly assess their starters for the presence of phages. The traditional strategies used by dairy industries to avoid phage development include the use of phage resistant bacteria and rotation of cultures, as well as performing aseptic processing conditions. Effective cleaning procedures are also applied in industrial plants, peracetic acid and combinations of biocides showing a good capacity to eliminate phage particles. However, some phages show resistance to thermal treatments and biocides (Ebrecht et al. 2010), and nowadays, strain rotation remains the most efficient phage control system.

3.7 Nutritional Value

Yogurts and fermented milks possess nutritional advantages in comparison with milk. Yogurt contains higher levels of calcium and potassium (199 and 255 mg/100 g) than milk (123 and 166 mg/100g) (Caroli et al. 2011). In addition, calcium availability in dairy products is affected by the amount and the nature of the calcium complex. However, a recent study performed with rats and humans indicate that no or little difference exists among different dairy products such as milk, cheese and yogurt in terms of calcium bioavailability (Caroli et al. 2011).

It is well established that yogurt consumption reduces lactose intolerance in lactase-deficient humans that are characterized by a low lactase activity. For these intolerant people, 90% of residual lactose is fermented in the colon by bacteria, thus leading to the production of organic acids, hydrogen, methane and carbon dioxide (Marteau et al. 1990). In fermented milks, lower residual lactose content (30 g/L) is metabolized by the lactase of lactic acid bacteria in the human gut, thus limiting the formation of these compounds.

Milk and fermented milks contain folic acid which is an essential vitamin in the human diet, as a low folate intake (<400 μg per day for adults) is associated to a number of health disorders. Folate concentration is higher in fermented milks than in milk, as a result of biosynthesis by lactic acid bacteria involving three precursors: GTP, *p*-aminobenzoate and glutamate (Iyer et al. 2010). It is produced by *S. thermophilus* in the range of 20 to 150 µg/L, depending of the considered strain (Van Hylckama Vlieg and Hugenholtz 2007). However, *Lb. delbrueckii* subsp. *bulgaricus* consume folic acid, thus leading to a decreased level in yogurt (80 µg/L). Thereby, the folic acid concentration in the fermented products can be controlled by using convenient strains selection. As an example, a combination of *B. animalis* and *S. thermophilus* resulted in a six-fold increase in folate concentration, compared to that obtained with yogurt starters (Crittenden et al. 2003).

4 Industrial Fermented Milks Manufacture

As the main fermented milk consumed in the world, yogurt is manufactured mostly at very large scale. Production at a lower scale, such as in a farm, is also encountered but it concerns a negligible part of the world production.

From the Codex Alimentarius (FAO/WHO 2011), yogurt is a milk product obtained by fermentation of milk, with or without compositional modification, by the action of two specific lactic acid bacteria, *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*, resulting in reduction of pH and leading to protein coagulation. The starter microorganisms shall be viable, active and abundant in the product to the date of minimum durability. If the product is heat-treated after fermentation the requirement for viable microorganisms does not apply and according to countries regulation, it may be impossible to keep the name of "Yogurt".

4.1 General Diagrams

By considering industrial yogurt and fermented milk production, differences occur depending on the strains used (yogurt or fermented milks), the kind of fermented milk (set-type, stirred or drinking yogurt), the fat level (low or high fat yogurt) and the plain or flavored character. However, whatever the considered product, the industrial process involves three main steps, which may be completed by the eventual addition of ingredients such as fruits, sugar or other ingredients: (1) The milk pretreatment that includes standardization and physical and thermal treatments of the mix; (2) The inoculation with the starters and the lactic acid fermentation; (3) The harvesting that consists of cooling, filling, packaging and cold storage. Figure 4 summarized the main steps of manufacture of different types of yogurts, which are described in the next paragraphs.

4.2 Milk Pretreatment

As milk composition varies according to the breed, the feeding and the lactation step of the animals, as well as to the region and the season, it has to be standardized before starting the fermentation. The pretreatment consists of fat and protein standardization in order to meet the nutritional and sensory specifications of the products, and to deliver well normalized products, in accordance with FAO/WHO code and principles (FAO/WHO 2011). More specifically, protein standardization is required to get a stable and constant product quality. Pretreatment also includes a step of homogenization and a thermal treatment that aim to stabilize the product by considering microbiological and physical concerns. When required,

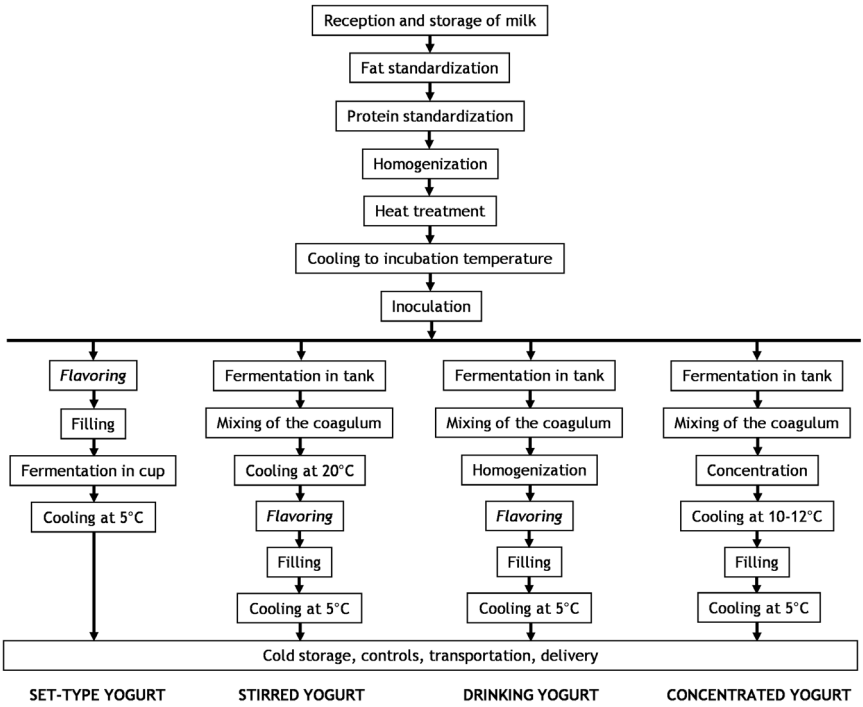

Fig. 4. General diagram of production of set-type, stirred, drinking and concentrated yogurts.

addition of sugar or other sweetening agents and stabilizers or emulsifiers is done before homogenization and thermal treatment of the mix, which will be maturated.

4.2.1 Reception and storage

After each milking session at the farm, milk is cooled to a temperature of 6°C or lower, and stored in refrigerated storage tanks made of sanitary stainless steel. Fresh milk is generally collected every two days and transported to the dairy factory in special bulk milk trucks. After passing quality control tests at the factory, milk is pumped and filtrated to eliminate any solid impurities such as soil particles, straw or leaves. It is stored at 4°C for maximum one day in large insulated tanks (25,000–200,000 liters) equipped with a propeller agitator to prevent fat separation. If longer storage duration is required, a short thermization (63–65°C for 15 seconds) is performed to inactivate psychrotrophic bacteria.

4.2.2 Fat standardization

The first step of milk pretreatment consists in separating fat from the milk. As fat content in milk varies between 3.8% and 4.2%, standardization of milk fat is compulsory at an industrial scale, to meet the fat specifications of the products: skim (0–0.1%), low (1%), light (2%) or whole (3.25–8%) fermented milks.

The separation process relies on centrifugal force, by using a cream separator working under controlled pressure. Most of all separators employed are three phases machine, if this operation is also used to eliminate sediments from milk during discharge phase. Fat removal is done at 55°C in order to improve the efficiency of fat recovery without creating lipolysis, thus allowing a maximum of 0.05% of fat in skimmed milk. After being removed, the cream is incorporated into the skimmed milk by direct in-line standardization, to reach the desired proportion. Batch standardization is also possible by mixing preset volumes of whole milk and skimmed milk. This in-line operation requires different sensors (flow-meters, densimeters, thermometers, and pressure sensors) in order to calculate on-line the fat content in the cream and to automatically adjust the cream rate into the milk. Industrial systems are designed to work between 7,000 and 45,000 L/hr, with an accuracy of fat rate higher than 0.01%.

4.2.3 Fortification of the solids-not-fat content in milk

The second step of milk standardization allows increasing the solids-not-fat content in milk (lactose, protein and minerals) up to 15–16%. This operation is needed as protein content in milk varies from 2.9 and 3.7%, whereas a final protein content ranging between 3 and 5% is generally wanted in a firm yogurt. It aims at improving the body of the product and reducing syneresis, and also modifies its nutritional value. However, in order to limit addition of proteins, syneresis is today mainly controlled by homogenization parameters implemented during mix pasteurization. Four main ways are employed: addition of milk proteins (skimmed milk or milk concentrate) or milk replacers (ultrafiltration retentate); water separation with membrane processes that is promising; partial evaporation of water that is the most energy consuming process; addition of stabilizers or thickeners, which is sometimes undertaken when these components are authorized by local regulation authorities. The most common used method is the addition of milk powder. In contrast, water evaporation is not often used because the global running cost is very high or it is only used by factories having on same production site, evaporation activity and yogurt production.

4.2.3.1 Addition of milk proteins

Increasing the protein content in milk can be achieved by incorporating milk powder, concentrated milk, or milk proteins. The quantity of proteins to be added depends on the desired texture. High protein skimmed milk powder is the most common ingredient, at a concentration generally comprised between 3 and 5%. Incorporation of milk powder or concentrated milk leads to similar physical properties of the fermented products. In addition, it has the advantage that only "milk" is indicated in the ingredients list of the product. Incorporation of concentrated milk in milk is easier than that of milk powder, from a practical point of view. Milk replacers such as whey, caseinates or buttermilk powders can be used, in concentrated form or not. They allow reducing the costs, but specific regulations are required to be taken into account before adding these high protein components into the mix. Whey proteins efficiently reduce the syneresis in the products, as a result of their high water retention capacity. Casein powders and sodium caseinates allow increasing the viscosity of the product. Whey and buttermilk are generally used to replace part of skimmed milk powder, in a range of 25 to 50%, as they may induce off-flavors at higher concentration. In this case, all processing parameters during thermal treatment and homogenization of the mix shall be carefully adjusted.

Mixing dry ingredients with milk requires a complete dispersion and hydration of the powders into the liquid phase, without any formation of lumps. The equipment must also prevent the incorporation of air during the mixing, in order to avoid foam generation as well as longer uncontrolled fermentation time. Depending on the system, discontinuous or continuous addition is achieved, generally at 40–50°C to facilitate the solubilization of the ingredients. The use of a mixing device is recommended for discontinuous addition. This equipment allows incorporating the milk powder in the aqueous milk by limiting air incorporation. The system is composed of a tank installed on a recirculation loop where milk is circulated at 10–25 m^3/h. All powders and ingredients are transferred from a silo or added manually into the mixing device. In advanced solutions, a vacuum is created in the tank to allow suction of powder and introduction under liquid phase level. Under these conditions, the mixing is usually effective within 20 min. Mixing tanks, equipped with impellers or paddles as agitation systems (low production capacity), or in line blenders with open hoppers are also employed to incorporate dry ingredients into the milk for specific process. In any case, influence of air included in the mix would be taken into consideration in further processing steps.

4.2.3.2 Addition of stabilizers or thickeners

When it is correctly produced, natural yogurt does not require addition of stabilizers, as a firm gel with a high viscosity will naturally occur. However, low-fat yogurts, fruit yogurts and pasteurized yogurts require stabilizers, in order to improve their consistency (Janhøj et al. 2008). Addition of these components is generally done at the same time and with the same technology as for milk powder or other milk proteins.

The list of authorized compounds, with permitted concentrations, is given by the Codex Alimentarius (FAO/WHO 2011). As addition of stabilizers is authorized only in some countries, national legislative regulations in the country of sale to the final consumer should be checked before any use.

Different gums derived from seaweeds (agar, alginates, carrageenan), microbial fermentation (xanthan), animals (gelatin) and cellulose derivatives (carboxymethylcellulose) are listed by the Codex Alimentarius. Pectin, gelatin and starch can also be added at a final concentration of 10 g/kg, whereas the permitted level of the other stabilizers is 5 g/kg.

4.2.3.3 Membrane concentration

Membrane separation is an alternative method for milk concentration (whole and/or skim). Depending on the cut-off threshold of the membrane, ultrafiltration or reverse osmosis are used for milk concentration. The differences between these two systems rely firstly, on the operational pressure that is higher in the case of reverse osmosis (1 to 5 Mpa) than in the case of ultrafiltration (0.1 to 1 Mpa). Secondly, ultrafiltration membranes are more permeable (pore size 0.1 to 0.01 µm) than those used in reverse osmosis (pore size 0.001 to 0.0001 µm).

Within the yogurt industry, ultrafiltration is mostly operated as it allows increasing protein concentration but not lactose in the milk base. However, it also leads to an increase in the calcium content that finally gives a metallic taste to the product. If filtration is used, one key additional step will be diafiltration to decrease calcium content in the final product. In addition, as degradation of the physical properties of the fat may occur in an ultrafiltration plant, the fat standardization takes place after fortification.

4.2.4 Sugar or sweetening agents addition

In order to tone down the acidity of the fermented milks, sweetening agents are sometimes added either before or after the fermentation step. However, as these compounds often inhibit the bacterial growth, as a consequence of

an adverse osmotic effect, they are preferably added after the fermentation in the case of stirred and drinking yogurts. In addition to saccharose, other carbohydrates (fructose, invert sugar), polyols (sorbitol) or aspartame are commonly used for yogurt sweetening. The interest of using invert sugar relies in its liquid form. These compounds are added by considering their relative sweetness as compared to sucrose and the desired taste of the final product. In order to avoid any denaturation or chemical reaction (such as Maillard reaction), these compounds are generally added after the heat-treatment, using the same technology than for other solid materials.

4.2.5 Deaeration

In order to reduce and better control the fermentation time, air contained in the milk is removed, especially when incorporation of dry components such as milk powder or other milk proteins is carried out with improper device. As an illustration, Fig. 5 shows the positive effect of reduced oxygen concentration on the development of acidity during yogurt manufacture (Horiuchi et al. 2009). In this example, fermentation time is significantly lower under low dissolved oxygen condition, as 180 min instead of 220 min are needed to reach 7 g/g of lactate in the fermented product. In addition to this effect on fermentation time, deaeration improves the texture and the stability of the fermented products (Martin et al. 2010), reduces the risk of fouling during further heat treatment and helps removing off-flavors.

Vacuum deaeration is generally used to expel air bubbles and to reduce dissolved oxygen content in milk. This operation is usually done on mix pasteurizer, just before homogenization. Preheated milk is provided to an expansion vessel under vacuum, which is installed upstream and fed at a temperature of around 72°C. The drop in pressure expels the dissolved air, which boils off and is removed by a vacuum pump, together with a temperature decrease of 4 to 6°C. In advanced deaerator, a condenser is included to avoid feed concentration.

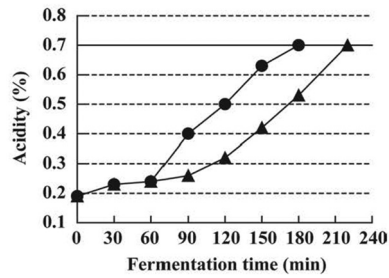

Fig. 5. Effect of dissolved oxygen (DO) concentration on the acid production by the yogurt culture LB81 during fermentation at 37°C under reduced DO (●) or during control fermentation (▲). From Horiuchi et al. (2009).

4.2.6 Homogenization

After fat and protein standardization, and possible sugar addition, the mix is homogenized to prevent creaming during the incubation period and to assure uniform distribution of the milk fat, in order to achieve a stable fat-in-water emulsion. Homogenization also allows increasing the viscosity of the product, reducing syneresis during storage of set-type yogurt, and obtaining a more white color of the product.

For hygienic reasons and to avoid any recontamination of milk, homogenization is conducted before thermal treatment, during temperature increase. However, some recent works demonstrate that homogenization improves the yogurt viscosity when it is done during cooling (Kulozik et al. 2003). The thermal treatment induces denaturation of serum proteins and favors their interaction with κ-casein. When it is carried out before homogenization, the resulting complex κ-casein–serum proteins bind to the fat globules during homogenization, thus leading to higher gel aggregation. As a consequence, one of the coming best practices is to carry out homogenization at pasteurization temperature, after the holding section to stabilize whey proteins that are present in the mix.

During yogurt manufacture, single or double stage high pressure homogenizers can be used, the second ones being recommended for high-fat products. These systems aim at forcing the mix through a very small gap (0.1 mm diameter), under pressure and at high velocity, in order to reduce the width of the fat globules. The cavitations effect that results from homogenization allows molecules rearrangement. The diameter of the fat globules is lowered and standardized, from 1–10 μm to 2 μm, and links are created between them and hydrophilic proteins. The velocity of the liquid is generally set between 100 and 400 m/s in the narrow annular gap and homogenization is complete in 10–15 μs. The pressure is comprised between 20–25 MPa and the temperature depends on equipment location. If the homogenizer is installed upstream, temperature is comprised between 60 and 70°C. If it installed downstream, temperature is set either between 60 to 70°C or between 95 to 98°C, depending on the required heat treatment. The flow rate is defined by that used for the heat treatment of the mix, as these two operations are generally coupled. It is generally comprised between 4,000 and 30,000 L/hr.

4.2.7 Heat treatment and cooling

For most commercial fermented dairy products, heat treatment is compulsory as pasteurized milk is used for their manufacture. As previously stated, heat treatment of raw milk leads to several effects: removal of pathogens and spoilage microorganisms, inactivation of lactoperoxidases, reduction of toxic

sulfur content, production of stimulatory factors (nitrogenous compounds, and formic acid), denaturation of whey proteins and modification of salt balance. These changes allow stabilizing the product and improving the properties of milk as a substrate for the lactic acid and probiotic bacteria. They also influence the physical properties and microstructure of the fermented product, as heating of milk (>70°C) induces denaturation of whey proteins (β-lactoglobulin). During denaturation, β-lactoglobulin interacts with the κ-casein on the casein micelle surface, through disulfide linkages and hydrophobic interactions, which results in increased gel firmness and viscosity of yogurt and reduced whey separation. Moreover, high heating temperature of milk allows increasing perceived mouth coating attributes and decreasing in the chalkiness attribute of stirred yogurts (Lee and Lucey 2004). Time-temperature combinations vary, depending on the desired product: From 65°C to 95°C, for 15 s to 30 min. For yogurt manufacture, temperature of 90–95°C for 3 to 7 min is generally employed.

Two main types of pasteurization equipments are used. Batch processes are recommended for small production plants. Heating of milk is achieved either by direct steam injection in milk or by circulation of water heated by steam injection into the water jacket of the tank. Time-temperature combinations are generally 85–90°C for 15–30 min. Continuous processes are used in large scale production units. Heat exchangers consist of plate, tubular or scraped surface systems, using steam of hot water. The two first systems are convenient for milk treatment, the third one for the treatment of fruit preparations. Time-temperature combinations are generally 90–95°C for 3–7 min, with flow rates comprised between 4,000 and 30,000 L/hr. The calculation of the surface area of the heat exchangers takes into account different factors: the design of the heat exchanger, the overall heat transfer coefficient, the temperatures of the hot and cold fluids, the physical properties of the fluid to be processed (specific heat and density) and the flow rate of the product.

Equipments for continuous processes generally include different sections: preheating (or regeneration) of cold milk by using already heated milk (e.g., from 5°C to 45°C); heating of prewarmed milk by hot water to the desired temperature (e.g., from 45°C to 90°C); holding the heated milk at the right temperature for the specified period of time (e.g., 90°C for 3–7 min); cooling the heated milk by regeneration (e.g., from 90°C to 50°C) and further cooling with cold water (e.g., from 50°C to fermentation temperature: 30°C or 42°C).

4.3 Milk Fermentation

By considering commercial products, fermentation of the white mass obtained after the pretreatment is achieved under controlled conditions,

by adding well defined starter cultures. Two main types of processes are carried out: For set-type products, fermentation takes place in the retail containers, whereas stirred products are fermented in tanks.

4.3.1 Inoculation

Bacterial cultures, known as starters, are used for milk fermentation. Depending on the fermented milk to be manufactured, different bacterial species will be used, as previously stated. Concentrated starters are available since about thirty years, for direct use in the production tank or for the bulk starter in large dairy companies. Small dairy plants propagate their own starters, by successive cultures, from a mother strain. However, the use of these precultures is time consuming, leads to insufficient reproducibility and may be subjected to unsatisfactory hygienic level.

By considering concentrated starters, strains combinations are defined according to the customer's specifications, by considering the type of product to be manufactured, the legal rules in each country, as well as biological (strains interactions) and technological issues (acidification rate, flavor, texture). Industrial starter manufacturers are now able to provide adequate mixings of strains with well defined specifications. Industrial starters are available in the form of frozen starters, at a concentration of about 1×10^{10} CFU/g (storage at <–40°C) or freeze-dried starters at a concentration of about 5×10^{10} CFU/g (storage at <4°C).

They are added in sufficient amount in the white mass (e.g., between 1×10^6 and 1×10^7 CFU/mL) and under strict hygienic rules, in tanks containing pretreated milk at the fermentation temperature (generally 30°C or 42°C). A good mixing is required to obtain homogenous suspension. Today, on-line incorporation of starters is also used, by culture injection during transfer from mix pasteurizer to incubation tanks (for stirred yogurt) or to packaging machines (for set type yogurt). This operation is done either automatically from buffer vessel under controlled atmosphere, or manually by using a laminar flow hood. In both cases the storage and injection device are cleanable and sterilizable in place.

4.3.2 Fermentation

During fermentation, bacterial concentrations increase as a result of consumption of lactose and nitrogenous compounds. As an illustration, Fig. 6 shows typical fermentation curves obtained during yogurt production. Growth of *S. thermophilus* starts rapidly, from $6{\cdot}10^6$ to $1.6{\cdot}10^9$ CFU/mL. Since it needs growth factors from the metabolic activity of the streptococci, growth of *Lb. delbrueckii* subsp. *bulgaricus* begins after 2 hrs, thus increasing the concentration from $6{\cdot}10^6$ to $6{\cdot}10^8$ CFU/mL. It continues for 4 hrs, whereas

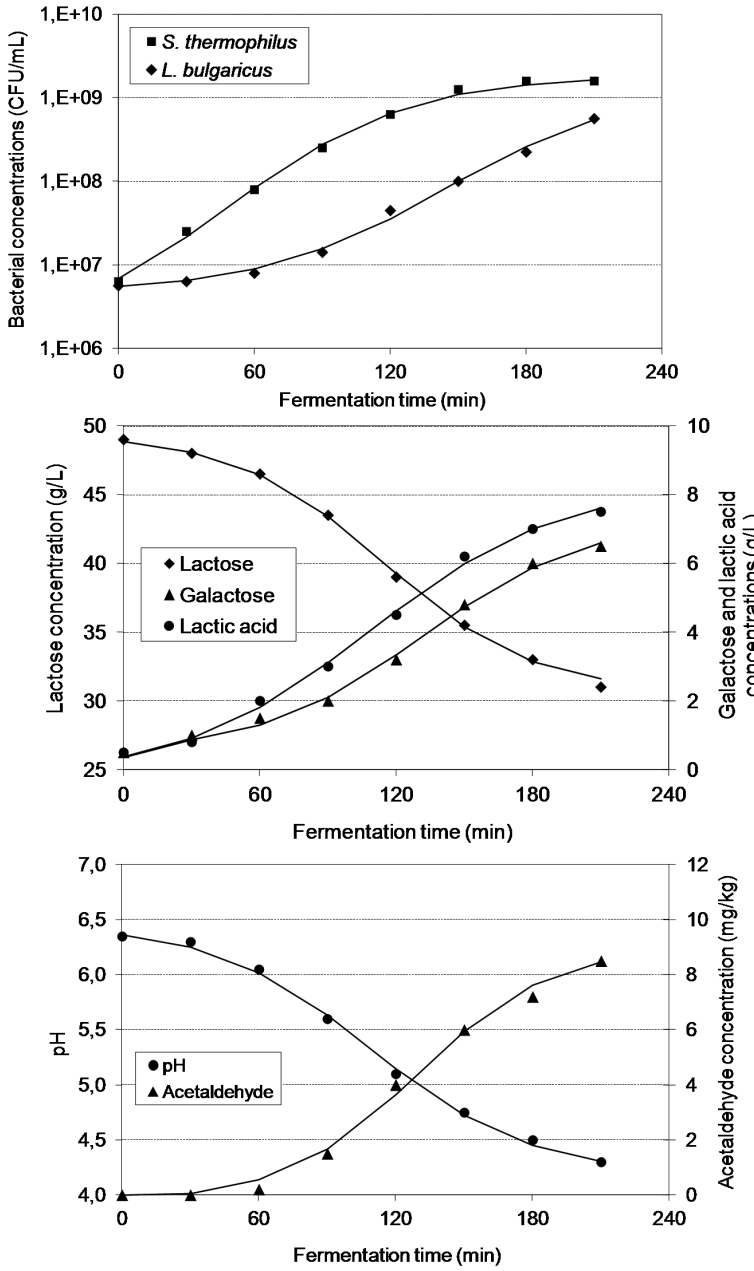

Fig. 6. Time course of growth of *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*, together with lactose consumption, galactose and lactic acid synthesis, pH decrease and acetaldehyde evolution during yogurt manufacture.

that of *S. thermophilus* stops after 3.5 hrs. Even if the initial ratio between the two species is commonly 1:1, the final balance moves in favor of the streptococci at the end of the fermentation (2.6:1 in this case). The lactose concentration decreases from 49 g/L (initial lactose concentration in milk) to about 30 g/L, thus leading to significant residual lactose content in the final product. In the same time, the galactose and lactic acid concentrations increase, to attain 7 g/L and 8 g/L, respectively. The molar lactic acid conversion yield (ratio between lactic acid produced and lactose consumed) is close to 90%, thus characterizing homofermentative metabolism. Lactic acid is excreted in the milk base, thus inducing pH decrease, from 6.4 to 4.2, and proteins coagulation. When adequate strains and convenient fermentation temperatures are employed, production of aroma compounds such as acetaldehyde may occur (Fig. 6).

Depending on the type of product, fermentation occurs before (stirred products) or after (set-type products) the packaging, according to a chosen process. The fermentation temperature depends on the microorganisms involved in the starter, as they possess different optimal characteristics (Table 2).

Table 2. Fermentation temperature and duration during manufacture of various fermented milks.

Fermented milks	Fermentation temperature	Fermentation duration
Yogurt	42°C	3 hr–4 hr
Laban, leben, labneh	37°C–42°C	3 hr–6 hr
Acidophilus milk	37°C	20 hr–24 hr
Dahi	37°C	6 hr–10 hr
Kefir, koumiss	18°C–20°C	<24 hr
Viili	20°C	18 hr
Buttermilk	20°C	12 hr
Yakult	37°C	7 days

When we consider set-type fermented milks, inoculated milk is delivered into the retail containers by using automatic filling machines. The filled and sealed containers are placed into crates that permit air circulation during warming and cooling. The crates are stacked on pallets, which are moved into the incubation room where the fermentation takes place. Flavoring and coloring compounds are added in the milk before packaging, whereas fruit preparations are delivered into the retail container, before the inoculated milk. Incubation generally takes place in insulated rooms, where forced air is circulated at the desired temperature. As an alternative for large scale units, tunnels are used for yogurt production. The length of the tunnel and the speed of the pallets are controlled in order to achieve the desired acidity in the product.

Considering stirred products, fermentation takes place in a tank equipped with insulation and agitation control. In the industry, two main types of tanks are employed: fermentation only, or fermentation and cooling. The volume of the stainless steel maturation vessels is designed according total production capacity required and requested fermentation time, it may reach thousands of liters. The tanks are insulated by a double jacket into which water circulates at the desired temperature. They comprise an agitation system to facilitate the homogenous dispersion of starters and their rehydration, the heat transfers and the breaking of the coagulum at the end of the fermentation. These tanks are available in atmospheric or ultra-clean pressurized blanketed version.

Fermentation is stopped when the required pH is achieved. As further cooling and storage leads to low but significant post-acidification, final fermentation pH is higher than that of the final product. For example, when the pH of the product is equal to 4.5, the final fermentation pH is set at pH 4.7. For stirred products, the coagulum needs to be broken at the end of the fermentation to make it pumpable, together with preserving it as much as possible to recover high consistency afterwards. This objective is achieved first by mixing the coagulum inside the tank. Removal of the gel is then carried out by using pumps and stretching devices that help getting final consistency after filling and storage in retail containers at cold temperature.

4.4 Downstream Treatments

4.4.1 Cooling

In the final stage of incubation, e.g., when the required pH is achieved (typically pH 4.7), the fermented milk is cooled to stop any enzymatic and metabolic reactions, such as acidification. Depending on the process, cooling is conducted either in one (set-type products) or two steps (stirred fermented milks). As this step is critical for the final consistency of the fermented milk, it is carried out very carefully to avoid destruction of the coagulum.

For set-type products, one-step cooling is achieved by decreasing the temperature from 42°C to 5°C, within one or two hours. The cooling time depends on the depth of the crate stack, the design of the crates as well as the size, design and material of the packages. Cooling is performed either in insulated rooms, in which forced air is circulated at low temperature, or in tunnels. Tunnels enclose cooling sections, where the pallets containing the products move with the aid of conveyors, thus permitting continuous cooling.

For stirred yogurt, a two-steps cooling is performed. The first target temperature is generally 18–20°C, before attaining the final temperature

of 5°C. The first phase of cooling is carried out either directly in the tank, and now more and more in line during transfer to storage tanks to ensure more rapid cooling. The coagulum is pumped from the tank and moves through a plate or tubular exchanger at a defined flow rate, to ensure gentle mechanical treatment of the product. The cooling rate is defined by the geometry and the surface of the heat exchanger, the flow rate and the temperature of cold water. In order to get a uniform product quality, the pump and the cooler are sized to empty the maturation tank within 30–40 min, at flow rates comprised between 1,000 and 30,000 L/hr. The precooled fermented milk is then stored in buffer tanks before being sent to the packaging machines. The stirred products are packed at 18–20°C, because their viscosity is lower at higher temperature, which helps maintaining the physical properties of the coagulum. Fruits, flavoring and coloring matters are added in the coagulum during their transfer from the buffer tank to the filling machine. This operation is done in-line, using a variable-speed metering pump that operates simultaneously with the yogurt feed pump. The final products are chilled to 5°C in the retail containers, either in tunnels or in the chilled store. During this phase, products recover the final desired consistency. The packages are then kept at 5°C during their storage, transportation and delivery.

4.4.2 Partial dehydration of the coagulum

For the manufacture of concentrated products, such as labneh or Greek yogurt, mechanical separation (centrifugation) or tangential filtration (ultrafiltration) are used to partially remove whey from the fermented product. They allow eliminating up to 75% of the whey from the original product, thus reducing by a half the residual carbohydrate content. This concentration step also leads to an increase in protein content in the fermented milk, which attains 7 to 11% instead of 3 to 5% in regular yogurt. When such a treatment occurs, denaturation of whey proteins is previously achieved during pasteurization, before maturation. This operation allows obtaining the required final consistency and mouth feel, with less fat and proteins. Consequently, the sensory properties of these products differ from those of not strained yogurt as they are thicker and heavier in the mouth (Mutz-Darwell et al. 2012).

4.4.3 Smoothing of the coagulum

After fermentation and cooling, a final smoothing step can be carried out. It aims at increasing the smoothness of the product and improving its shine. This operation is conducted by using smoothing disks, smoothing valves or

smoothing homogenizers or pumps, especially in the case of yogurts with high protein content (>10%). Now, this smoothing step is part of normal yogurt production as it also improves firmness of final product.

4.4.4 Addition of food additives

Different food additives can be added in the milk or the fermented milks, in order to modify their sensory properties. The complete list of authorized additives is available in Codex Alimentarius (FAO/WHO 2011). Apart from some specific recommendations given in Codex Alimentarius, maximum levels are defined from the good manufacturing practices used in the dairy plant. The main authorized additives are listed below:

- Acidity regulators (mainly, tartrates and adipates);
- Carbonating agents (carbon dioxide);
- Colors (curcumin, carmines, indigotine, chlorophyll, caramel, β-carotene, riboflavine, anthocyanins, etc.);
- Emulsifiers (for example, polyoxyethylene (20) sorbitan monolaurate, sucrose and polyglycerol esters of fatty acids, sorbitan monostearate, etc.);
- Stabilizers and thickeners (carrageenan, xanthan gum, methyl cellulose, salts of myristic, palmitic, stearic and oleic acids with ammonia, calcium, potassium and sodium, lactic and fatty acid esters of glycerol, dextrines, modified starch, etc.);
- Sweeteners (sugar, sorbitol, mannitol, saccharine, aspartame, etc.);
- Flavor enhancers (monosodium L-glutamate, disodium 5′-guanylate, inosinic acid, 5′, calcium 5′-ribonucleotides, maltol, etc.);
- Preservatives (sorbic acid and potassium sorbate that are present in fruits).

In addition to these additives, aroma compounds as well as fruits, fruit pulps and jams can be included in the products. The maximum level of non dairy ingredient is limited at 30% in weight.

By considering set-type products, the addition of the additives is performed either before the fermentation (sweeteners, colors, aroma compounds) or during packaging (fruits). Stirred and drinking fermented milks are generally supplemented with additives during their transfer from the buffer tank to the filling machine. On-line injection of fruits can also be done directly on the filling machine, with automatic change over.

4.5 Packaging

Packaging of fermented milks is essential to guarantee that nutritional and sensory properties of the product reach the consumers, to protect the

product from mechanical shock, light and oxygen, to ensure product hygiene during distribution and to deliver product information.

4.5.1 Packaging materials

Yogurts are generally packed in two main types of packaging containers: preformed cups or plastic thermoformed packages. Preformed packs correspond to glass cups and rigid plastic bottles and cups. They are decontaminated before filling with the product, by emission of H_2O_2 vapor or irradiation with UV. Thermoformed containers are composed of multilayer thermoplastic materials, such as polypropylene, polyethylene or polystyrene, the last one being the most used. Thermoforming is ensured at 150–200°C, depending on the material. This temperature is sufficient to complete sterilization of the packs. These materials can be cut easily, resist acidity, limit flavor loss and inhibit oxygen transfer to restrain potential growth of yeasts and moulds.

Sealing material is generally composed by an aluminum foil coated with a plastic layer to support the acidic character of the fermented products. It is supplied preprinted, either in a precut form or as a reel. It is thermosealed at the top of the cups and labeled for traceability and use-by date.

4.5.2 Packaging machines

Different types of packaging machines are found in the dairy industry for fermented milk packaging. Their design varies according to the objectives (forming, filling and/or sealing), the type of container used (size, preformed or thermoformed), the needs for hygiene standards (controlled atmosphere, aseptic conditions) and the level of automation. The total packing capacity ranges between 8,000 to 66,000 cups/hr.

When flavored yogurts are produced, the flavorings and processed fruits are introduced on-line into the milk, before packaging, either in batch (small scale) or continuously (large scale). Continuous fruits/yogurts mixers are composed by a metering unit that doses the desired volume of flavorings or fruits, a metering device that measures the required volume of yogurt and a mixing chamber that combines the two ingredients. The mixing occurs either statically, with a pipe equipped with helical blades, or dynamically, with a circulation loop inside the mixing chamber, driven by a rotating helical displacer. In some cases, fruits can be added directly into the cups before dosing the yogurt, thus leading to two layers in the final product.

Machines used for filling into preformed plastic containers are composed of an automatic cup loader and dispensing unit, a filling conveyor

sized for several cups and working under clean air, and a system of heat sealing to close the cups with an aluminum foil lid. These machines may work under sterile air overpressure, in a hermetically closed environment and they are linked to the cleaning-in-place system. After being closed, the packages can be grouped into multipacks of 2, 4, 8 or 16 cups, with an automatic tray packer.

When plastic material containers are used, machines are designed for form, fill and seal. The packaging material is delivered continuously in the form of large reels of preprinted plastic sheets. It is fed to the preheating section of the filing machine, thermoformed by forming plugs under sterile air overpressure, transferred to the filling head where the yogurt is dosed and then to the heat sealing system. The high temperature implicated during thermoforming helps for sanitization. However, UV irradiation, H_2O_2 decontamination, steal barriers and use of pressurized sterile air may be used to extend the shelf life of the products. As an illustration, Fig. 7 shows a photograph of an aseptic form, fill and seal packaging machinery for yogurt cups.

4.5.3 Labeling

After being sealed, packages are printed with a code for traceability and the use-by date. In addition, compulsory or facultative information is added, depending on the country. The main indications are listed below:

- Indication of animal species, except for the use of cow milk;
- Fat content;
- Presence of non dairy ingredients (sugar, sweeteners, flavorings, fruits, stabilizers or thickeners, etc.).

In some countries, strict rules govern the use of pictures and package designs.

4.5.4 Commercialization

After their manufacture, fermented milks have to be maintained at a maximum temperature of 5°C, during storage and transportation. The temperature shall not exceed 8°C during distribution to the consumers.

These temperature limits aim at maintaining the hygiene of the product and at limiting the post-acidification phenomenon (pH decrease up-to 0.5 pH unit) that may occur after manufacture.

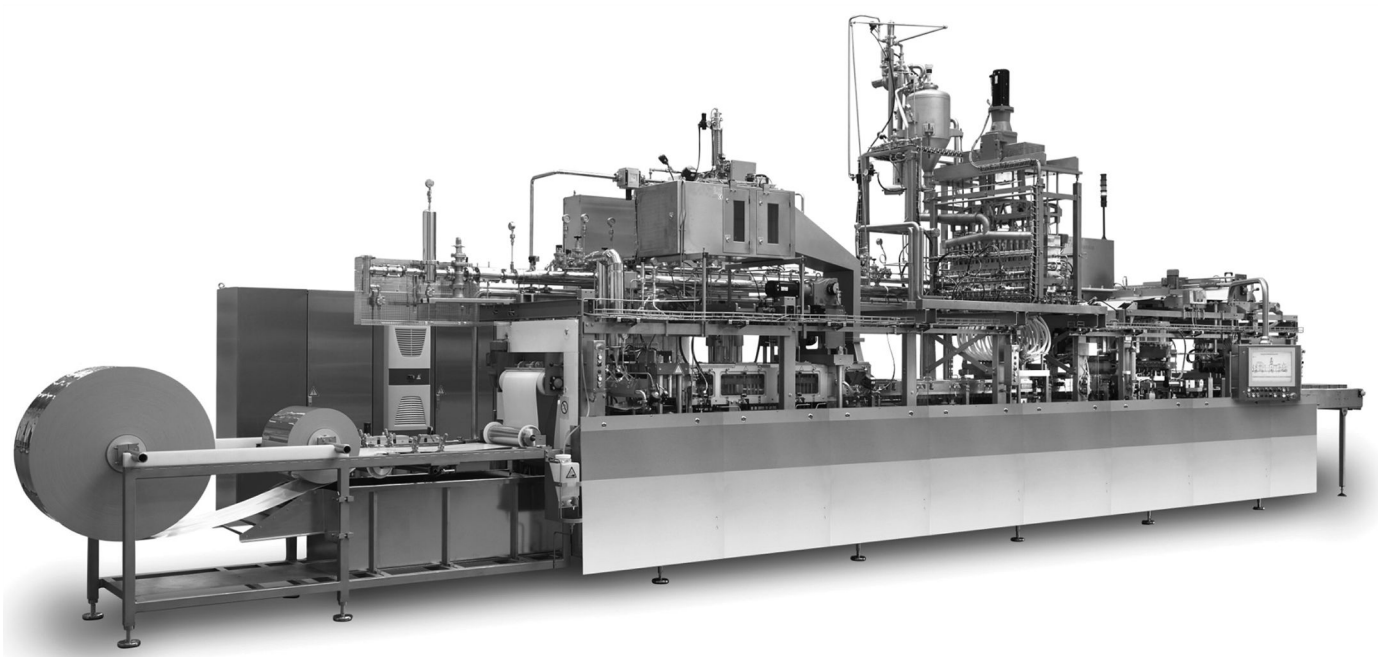

Fig. 7. Manufacturing aseptic form, fill and seal packaging machinery for cups (Reproduced with courtesy of Oystar Hassia Holding GmbH, Stutensee, Germany).

Color image of this figure appears in the color plate section at the end of the book.

4.6 Industrial Processing Plants

4.6.1 Integrated production lines

At large industrial scale, the different steps of manufacture are, in most cases, conducted continuously. Consequently, equipment suppliers may provide complete lines that combine fat standardization, on-line addition of solids-not-fat and other solid materials (stabilizers, sweeteners), deaeration, homogenization, heat-treatment and cooling, inoculation, incubation, cooling, addition of fruits, packaging and cold storage. Among these industrial unit operations, the mechanical handling of the coagulum should be conducted carefully in order to limit the breakdown of the coagulum or the impact on the viscosity in the case of stirred products. As an illustration, a scheme of a production line for stirred yogurt is shown on Fig. 8.

Each stage of manufacture of fermented milks, from raw materials to packaging, shall take place in accordance with Good Manufacturing Practices (GMP) to guarantee the quality and traceability of the product.

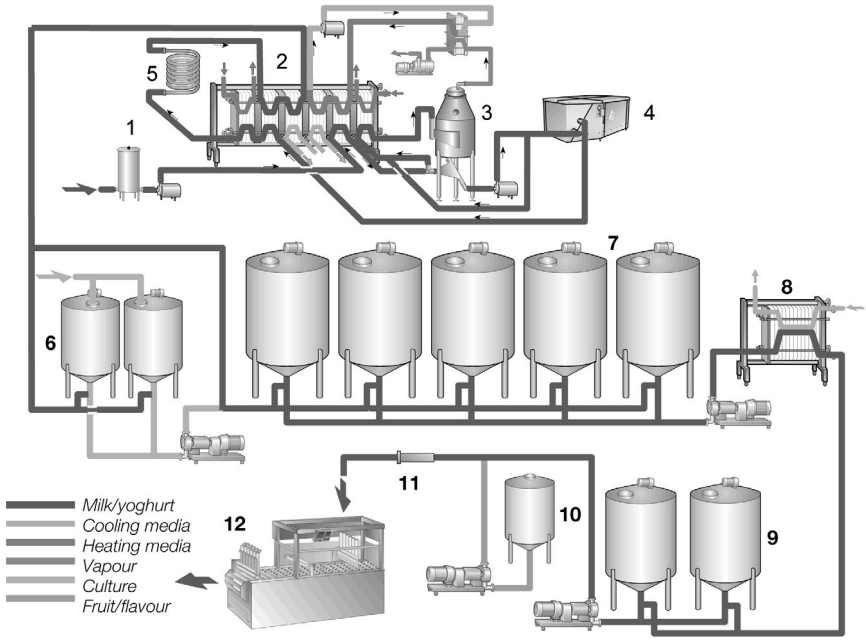

Fig. 8. Scheme of a stirred yogurt production line (Reproduced with courtesy of Tetra Pak, Processing Systems Division A/B, Lund, Sweden). 1: balance tank; 2: plate heat exchanger; 3: deaerator; 4: homogenizer; 5: holding tube; 6: bulk starter tank; 7: incubation tanks; 8: plate cooler; 9: buffer tanks; 10: fruit/flavors; 11: mixer; 12: packaging.

Color image of this figure appears in the color plate section at the end of the book.

4.6.2 Control and automation

In order to ensure manageability, security and reproducibility of the industrial processes, automation and control of all operations are necessary. Automation level varies from one process to another, from manual, to semi-automatic or to fully automatic systems. Automatic systems allow controlling the main functions of the industrial process, with the help of monitoring devices (probes and sensors) that are connected to a controller. It allows comparing the measured value (for example the temperature in the tank) to the set-point value (the desired temperature in the tank). In the case of a significant difference, it takes the relevant action to decrease the difference between measured and target values, e.g., by heating or by cooling. Latest automation developments now provide full traceability of processing operations, ensuring full quality control and food safety.

4.6.3 Cleaning in place

All contact surfaces with the product (processing equipments, pipes, containers, filling equipments) have to be effectively cleaned and sanitized before each use, in order to secure the process and ensure hygienic quality. The cleaning procedure consists of applying a combination of different detergents such as inorganic alkalis, inorganic acids, surface-active agents, chelating agents, sequestering agents and sterilizing agents, at well defined concentrations. The cleaning efficiency is affected by the contact time, the temperature and the flow rate of the solutions. As an example, a typical cleaning procedure is given in Fig. 9.

In large industrial plants, a cleaning in place (CIP) module is often set up, where all steps have to be controlled and registered. It consists of a CIP kitchen where detergents are prepared and driven to the different parts of the process where they are needed. Either single use or re-use systems, where detergents and acids are recycled, can be employed.

4.6.4 Wastewater treatment

Since dairy wastes of yogurt factories are mainly composed by organic matter (lactose and proteins), treatment of effluents is accomplished by biological degradation, either aerobically or anaerobically. Because of the high level of BOD (biological oxygen demand) of the wastes, effluent treatment plants can be integrated in the dairy factory.

Currently, dairy factories are more and more using white water recovery units to concentrate the edible part of the effluent and re-inject it in upstream processing to improve plant efficiency.

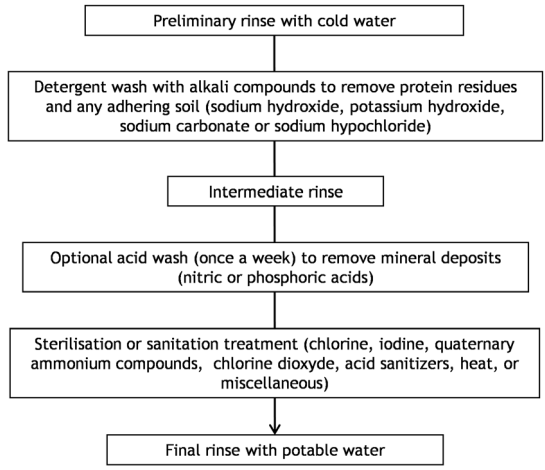

Fig. 9. Typical cleaning procedure used in a dairy plant.

5 Quality Control

5.1 Control of Raw Materials

The major ingredients of fermented milks are milk, milk powder, starters and fruit preparations. These incoming materials are tested for safety assessment to meet the standards set by the dairy companies. In addition, packaging materials are also controlled.

Various tests are performed on a representative sample collected from each refrigerated tank of raw milk. Temperature on arrival (<10°C), total colony count (<100 000 CFU/mL), somatic cell count (<4.10^5 cells/mL) and titratable acidity (<0.2% lactic acid) provide information on the sanitary quality of milk. Moreover, chemical composition (fat and protein levels) is determined to determine payment and freezing point is checked to verify adulteration with water.

Quality controls are performed on milk powder and fruit preparations to ensure that agreed specifications are respected. Milk powder is controlled by considering solubility, moisture, fat content, antibiotics and total colony counts. Yeasts and molds contaminations are checked in fruits and sweeteners' preparations. Assessment of the physico-chemical quality of fruits (pH, viscosity, Brix level) is also operated.

For starter cultures, the acidification activity is assessed. This determination is generally performed by using the Cinac system (Corrieu et al. 1988). It allows measuring the pH continuously during standardized lactic acid fermentation and leads to the determination of various quantitative descriptors. Among them, the time necessary to reach a given

pH (for example pH 5.5) is often used: The higher is the pH 5.5, the lower is the acidification activity (Chammas et al. 2006). As an example, Fig. 10 shows the acidification curves of two different starters: starter A that is characterized by a low tpH 5.5 value (126 min) is highly acidifying, whereas starter B is less active as it demonstrates a high tpH 5.5 value (168 min).

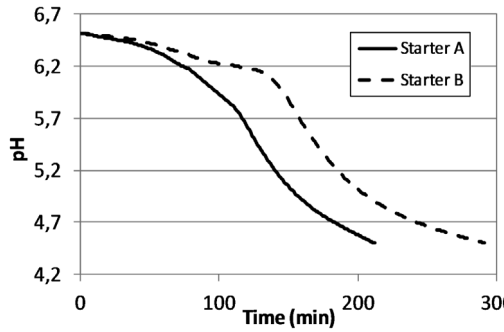

Fig. 10. Acidification activity of two different starters, as measured by the Cinac system.

5.2 Controls during Manufacture

During fermented milk manufacture, controls have to be performed in order to ensure the quality of the products and the repeatability of the production.

Food safety management systems (FSMS) such as ISO 22000, BRC (British Retail Consortium) or IFS (International Food Standards) are used in the dairy industry for controlling food borne safety hazards and ensuring that safe dairy products will reach the consumers (Papademas and Bintsis 2010). The Hazard Analysis and Critical Control Point system (HACCP), described by all FSMS, is implemented in dairy companies all around the world. It is a systematic approach to the identification, evaluation, and control of hazards in these steps that are critical to food safety. During yogurt manufacture, pasteurization of milk, packaging and refrigerated cold storage of fermented milks are identified as a Critical Control Point (CCP) to control microbiological hazards. Packaging is also a CCP with regards to physical hazards. In addition, control measurements are necessary to ensure an optimum level of food safety and quality of the end products.

In set-type products, pH control is achieved manually, by taking samples and measuring the pH directly in the containers. When the expected pH is obtained, the pallets are moved from the incubation room to the cooling room, in order to stop the fermentation.

During manufacture of stirred fermented milks, samples are removed from the tank in order to determine the pH evolution versus fermentation time. However, this procedure is more difficult when the coagulum is

formed. As an alternative procedure, indirect pH assessment from bulk temperature measurements has been proposed (Corrieu et al. 2006). By considering that pH decrease is accompanied by a slight temperature increase (from 0.7 to 1.3°C), as a consequence of exothermic behavior of lactic acid fermentation in adiabatic conditions, a polynomial relationship between these two variables was established. A careful calibration of the sensors is necessary, and the accuracy of the relationships is better if the composition of the milk base is taken into account. Such software sensor can be used to define, at each time, the pH from bulk temperature measurement, and to predict the pH in the fermented milk within a given range of time (accuracy of 0.1 pH unit) as well as the fermentation end time (at a chosen end pH).

5.3 Control of Final Products

Various microbiological, chemical and physical analyses are conducted on fermented milks in accordance with the standard official procedures of countries, to ensure quality and safety of final products. In addition, sensory evaluations allow guaranteeing that the product meets the required standards defined by the dairy company.

For microbiological analyses of fermented milks, counts of specific microflora are realized at the end of production. Target values of counts depend on countries regulations, health claims and specification given by the producer. As an example, for yogurt, total counts of *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* must be higher than 10^7 CFU/g at shelf life. The presence of spoilage microflora such as coliforms, yeasts or molds, and pathogenic bacteria including *Listeria monocytogenes* and *Salmonella* subsp. has to be checked. Yeast and molds have to be detected in yogurts samples, especially in fruits yogurts that have been incubated at 30°C or 25°C during 3 or 10 days. As an example, the target values that are used in France correspond to the absence of *L. monocytogenes* and *Salmonella* subsp. in 25 g of product, less than 10 CFU/g of *Enterobacteriaceae* and total coliforms and less than 100 CFU/g of yeasts and molds. The frequency of sampling is defined by each dairy plant, according to its own good hygiene practices.

By considering chemical analyses, the fat and total solid content are controlled in the fermented milks. Acidity of the products is measured, either from pH and/or titratable acidity.

Physical characteristics of yogurt such as the lack of visual whey separation and the viscosity are important parameters of the quality and of consumers' acceptance. They are generally determined one day after packaging. Set yogurts, stirred yogurts and drinking yogurts have different physical properties that must be checked. As yogurt can be either a viscoelastic fluid (stirred or drinking yogurt) or a viscoelastic solid (set

yogurt), different texture parameters such as firmness, consistency and viscosity are estimated, by using specific instruments such as penetrometer, viscometer or rheometer (Chammas et al. 2006).

Sensory evaluations are performed with panelists experienced in the evaluation of fermented milks in order to describe the sensory characteristics of the products. Appearance (color, syneresis, smooth), flavor (odor, aroma), taste and aftertaste (acid, fresh, persistency) and texture (palatability, firmness, consistency, fluidifying) of yogurt samples are evaluated in order to verify the consumer acceptability (Martin et al. 1999, Soukoulis et al. 2007). An example of sensory attributes used to describe the sensory characteristics of yogurts is showed in Table 3.

Table 3. Definitions of the descriptors (sensory attributes) used for the evaluation of the yogurts. Adapted from Soukoulis et al. (2007).

Attribute	**Description**
Color	Color of the product (white, whitish, yellowish)
Syneresis	Visual observation of the yogurt surface; Level of whey drainage after inserting the spoon into the curd
Odor	Intensity of acetaldehyde immediately after removing the lid
Aroma	Flavor defect (e.g., unclean, masked, unnatural, cooked, lacks freshness) by smelling and oral perception of samples
Palatability	Taste of samples considering several attributes associated with taste (e.g., unclean, unnatural, whey, refreshing perception) and aftertaste (e.g., sourness, astringency, sweetness, bitterness, saltiness)
Firmness	Hardness, brittleness, gumminess, gelatin-like texture of the coagulum
Consistency	Viscosity when stirring the samples with the spoon; rheological behavior of yogurt in the mouth
Overall acceptance	Overall score of samples considering the appearance, taste, texture and flavor profiles

6 Conclusion

Whatever the considered fermented milk, consumers are looking for products with elevated shelf life and stability, good consistency or viscosity, pleasant taste and mouth feel, together with high and reliable quality. In addition, other factors such as usefulness (drinkable or spoonable, on the go, different sizes), nutritional and functional benefits (probiotic microorganisms, bioactive peptides, low-fat products), ethnical constraints (Halal, Kosher), variety of the products (traditional, regional, fun) are often considered. More recently, investigations look for its availability to everybody (in the context of food security) and the consideration of environmental factors (energy and water sobriety, sustainable development).

These requirements, together with an increasing demand, allow developing and modifying the yogurt industry to reach high quality standards, by considering different criteria:

- Food quality: Consistency of product, sensory properties, critical quality assurance in production;
- Food safety: Microbiological safety, chemical contamination, traceability;
- Environment: GHG emissions, carbon footprint, packages waste, energy and water saving, life-cycle assessments;
- Supply capability: supply flexibility, delivery without defects and with high accuracy;
- Operational efficiency and costs: Overall equipment effectiveness and time utilization, total effective equipment performance, product losses and yields, media consumption, staff time utilization, operating and investment costs.

Even if these developments principally concern the yogurt industry, the industrial manufacture of other fermented milks in the world is also concerned with the aim to increase their level of quality.

Finally, some changes regularly occur concerning the market of the fermented milks. The most recent one concerns the very quick development of the Greek type yogurt chain, as a result of consumer's demand, that raises the global demand for fermented milks in the USA. In addition, yogurt consumption has strongly expanded in Asia, due to increasing purchasing power and spending patterns.

Keywords: Fermented milk, Industrial process, Lactic acid bacteria, Organoleptic quality, Probiotic bacteria, Yogurt

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6

Fermented Fish and Fish Products: Snapshots on Culture and Health

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1 Introduction

Culturally, fish forms the main dish in a meal for people residing near coasts, rivers, streams, lakes, and ponds where it is available in plenty. Fish is an extremely perishable proteinaceous food. Fermentation, salting, drying, and smoking are the principal methods of fish preservation innovated by people to enrich their diets (Tamang 2010). Historically, fermentation of fish was associated with salt production, irrigated rice cultivation, and the seasonal behavior of fish stock (Lee et al. 1993). The Mekong basin was most probably the place of origin of fermented fish in Asia, and Han Chinese (200 BCE–200 CE) learned of it when they explored south of the Yangtze River (Ishige 1993). Fish products prepared by lactic acid fermentation remain common in Laos, Cambodia, and in North and Northeast Thailand (Pringsulaka et al.

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2012). Southeast Asia has a wide variety of ethnic, fermented fish products that are deeply associated with the food culture of ethnic Asians (Tamang 2010, Panda et al. 2011).

Many fermented fish products are prepared in different parts of the world and the method of processing depends upon various factors, viz., availability of raw materials, consumers' preference and the climatic conditions of the region (Al-Jedah et al. 2000). Fish fermentation technology is a home-based traditional technique where varieties of fermented fish products, mostly fish sauce, are prepared and used as a staple food, side dishes, and condiments in Asia. Some of the ethnic fish products are patis from the Philippines, nam pla and pla ra from Thailand, shottsuru and shiokara from Japan, jeot kal from Korea, pindang from Indonesia, budu from Malaysia, nga pi from Myanmar, and sukuti, sidra, ngari, hentak and tungtak from the Himalayas in India. In Africa, salting and drying of fish for preservation is accompanied by fermentation, but the period is short and the product is not transformed into a paste or sauce. Although fermented fish products are a good source of protein, they can be consumed only in limited quantities because of the high salt content of these products. Nevertheless, they continue to play a vital role in hot countries to add protein and flavor. Fermentation of fish is especially used in situations where drying of fish is not possible because the climate is too wet and where cooling and sterilization of the product is too expensive.

In this chapter, three important traditionally fermented fish and fish products for human consumption, i.e., fish sauce, fish paste and dried/salted fish have been discussed with special emphasis on health hazards of fermented fish consumption.

2 Fermentation of Fish Products

Fermented fish can be described as any fishery product that has undergone degradative changes through enzymatic or microbiological activities either in the presence or absence of salt (sodium chloride) (Zakhia and Cuq 1993). The fish protein is mainly broken down by enzymes which come from the fish itself. These enzymes are mainly present in the gut (Paludan-Müller et al. 2002). In the traditional fermentation methods in which the intestines are removed from the fish, fermentation will often be slower as there are fewer enzymes present in the flesh.

The term "fermented fish" covers two categories of fish products: (1) fish–salt formulations, e.g., fish sauce products such as fish paste and sauce that tend to contain relatively high levels of salt, typically in the range of 15–25 percent and are used mainly as a condiment; and (2) fish–salt–carbohydrate mixtures, e.g., pla ra in Thailand and burong isda in the Philippines (Panda et al. 2011).

2.1 Spontaneous Fermentation

The fermentation methods described in this chapter are mostly traditional methods. That is to say that the fermentation is allowed to take place naturally (spontaneous fermentation) and is guided by experience. No control is exerted over the fermentation.

2.2 Fermentation Using Starter Culture

The use of starter culture in food fermentation has become a means to increase processing rates and product consistency. Starters are used to improve the sensory characteristics and microbiological quality and to shorten the fermentation time of fermented foods (Visessanguan et al. 2006). Recently, lactic acid bacteria starters such as *Lactobacillus plantarum*, *Lb. brevis*, *Lb. fermentum*, *Pediococcus acidilactici*, *P. pentosaceus*, etc. are used commercially for production of traditional fish products like som fug (Riebroy et al. 2008), plaa som (Saithong et al. 2010, Hwanhlem et al. 2011), suan yu (Zeng et al. 2013b) and other fish-based food products (Gelman et al. 2000).

3 Types of Fermented Fish Products

There are three kinds of fermented fish products:

- Fish sauce. The fish flesh is converted into a liquid fish sauce;
- Fish paste. The fish is converted into a paste;
- Dry/salted fish. The fish, whole or in pieces, retains as much as possible of its own structure.

Fermented fish products such as fish sauce and fish paste are eaten mainly in South-East Asia, whereas dried fish are consumed in many parts of Asia and Africa. Fermented fish products are an important protein supplement. They contain a number of essential amino acids which can form an important addition to the daily diet. For example, fish sauce contains a lot of the lysine (Khem 2009, Dincer et al. 2010). This amino acid is found only in small quantities in rice. The quality of the resulting product depends on the fat content of the fish, the enzyme activity in the fish flesh, contamination of the salt used and the temperature.

4 Fish Sauce

In Southeast and East Asian countries, fish sauce has been used extensively as condiment in cooking. This is due to the deep umami taste of fish

sauce. Fish sauce is known by different names according to the country, for example, bakasang in Indonesia, patis in Philippines, yu-lu in China, ngapi in Burma, nam-pla in Thailand, ishiru in Japan and nuoc-mam in Cambodia and Vitenam (Sim et al. 2009, Panda et al. 2011).

Although fermented fish sauce itself may not be directly used as a functional food because of its high concentration of salt, it may be useful as a source of biologically active substances such as amino acids and vitamins, traditional food supplements in the diet, as condiments, flavoring material, and sometimes as a substitute for soy-sauce (Ichimura et al. 2003, Watanabe et al. 2004). Some fish sauces are made from raw fish, others from dried fish, some from only a single species, others from whatever is dredged up in the net, including some shell fish; some from whole fish, others from only the blood or viscera. Some fish sauces contain only fish and salt, and others a variety of herbs and spices. A number of fish sauces that are manufactured by traditional methods are listed in Table 1, and the most important ones are described below.

4.1 Nuoc-mam

This is the most common fish sauce produced in the Indo-China Peninsula (Vietnam, Laos, and Cambodia) and is mostly consumed entirely within the country of manufacture. The sauce is a clear brown liquid with a distinctive mealy/sharp aroma. The sauce is prepared as follows. The fish, usually anchovies or mackerel, which are not usually cleaned, are kneaded and pressed by hand. They are then placed in layers with salt in an appropriate ration of 3:1 fish to salt (w/w) in earthenware jars that are almost buried in the ground. After filling, the jars are highly sealed and left for several (16–18) mon. After fermentation, the pots are carefully removed and after a few days of setting, the supernatant liquor are decanted off carefully (Panda et al. 2011). The liquid is known as nuoc-mam.

Often caramel, roasted rice or molasses are added to fish to get a dark color and a certain taste. This improves the keeping quality of the qualitatively inferior nuoc-mam. At a fermentation temperature higher than 45°C, the nuoc-mam loses its characteristic taste. It is therefore best to keep the vats somewhere cool.

4.2 Nam-pla

This is the equivalent of nuoc-mam and is reported to be very similar (Beddows 1985). The major species of fish used is *Stolephorus* and *Sardinella* but smaller *Scomber* and *Ristrelliger* may be used as well as certain *Clupeids*. The process is more commercialized than that of nuoc-mam. The

Table 1. Fish Sauces.

Country	Name	Fish species	Method (fish:salt) and time of fermentation
Egypt	-	*Affinis affinis* (gambusia)	4:1 salt; for 150 d then salted fish was manually drained using cheese cloth to separate fish sauce to keep out salted fish
Sudan	Terkin	*Hydrocynus* sp. (kass or tiger fish) *Alestes* sp. (kawara)	-; 6 mon
Japan	Shottsuru Uwo-shoytu Ikashoyu	*Astroscopus japanicus* (sandish) *Clupea pichardus* (sardine) *Omnastrephis sloani* (squid) *Omnastrephis pacificus* (squid)	5:1 salt + malted rice and koji (3:1) added; 6 mon
Korea	-	-(shrimp)	Salt 4:1; 6 mon
Khmer Republic	Nuoc-mam	*Stolephorus* sp., *Ristrelliger* sp., *Engraulis* sp., *Decapterus* sp., *Darasoma* sp., *Clupea* sp.	3:1–3:2 salt; 3–12 mon
	Nuoc-mam-gau-ca	*Clarius* sp., *Ophicephalus* sp.	Livers only 10:1 salt; for 6 d then boiled and filtered
Cambodia	Nuoc-mam	*Stolephorus* sp., *Ristrelliger* sp., *Engraulis* sp., *Decapterus* sp., *Darasoma* sp., *Clupea* sp.	3:1–3:2 salt; 3–12 mon
Thailand	Nam-pla	*Stolephorus* sp., *Ristrelliger* sp. *Cirrhinus* sp.	5:1–1:1 salt; 5–12 mon
Malaysia	Budu	*Stolephorus* sp.	5:1–3:1 salt + palm sugar and tamarind; 3–12 mon
Myamar	Ngapi	-	5:1 salt; 3–12 mon
Philippine	Patis	*Stolephorus* sp., *Clupea* sp., *Decapterus* sp., *Leionathus* sp.	3:1–4:1 salt; 3–12 mon
Indonesia	Ketjap-ikan	*Stolephorus* sp., *Clupea* sp., *Leiagnathus* sp., *Osteochilus* sp., *Puntius* sp., *Ctenaps* sp.	6:1 salt; 6 mon
	Bakasang	*Stolephorus* sp.	5; 1.5–3.5 fish; salt ratio; 3–6 wk
India and Pakistan	Colomba cure	*Ristrelliger* sp., *Cybium* sp., *Clupea* sp.	Gutted fish with gills removed and tamarind added 6:1 salt; up to 12 mon

Hong Kong	Yeesui	*Sardinella* sp., *Jelio* sp., *Carangidae* sp., *Engraulis pupapa*, *Teuthis* sp.	4:1 salts; 3–12 mon
Ghana	Momoni	*Caranx hippos*	10:3 fish:salt ratio; 1–5 d
Greece	Gaross	*Scomber colias*	Liver only 9:1 salt; 8 d
France	Pissala	*Aphya pellucid*, *Gobius* sp., *Engraulis* sp., *Atherina* sp., *Meletta* sp.	4:1 salt; 2–8 wk (depending on size)
	Anchovy	*Engraulis encrasicholus*	Beheaded and gutted fish 2:1 salt; 6–7 mon

Source: Sanni et al. 2002, Ibrahim 2010, Abu-Hassan and Sulieman 2011

fermentation takes 6–12 mon. The sauce is 'run off' and exposed to the sun for 1–3 mon (ripening) (Panda et al. 2011). The liquid is blended with meiki, a concentrated by-product obtained from the bacterial production of monosodium glutamate. In general, less salt is used in the production of nam-pla than with nuoc-mam (4:1 ratio, fish: salt). Padack, a more aromatic version of nam-pla, is also produced.

4.3 Ngan Byar Yay

Similar to nam pla and is indigenous to Myanmar.

4.4 Patis

A sauce called patis is produced in the Philippines which is comparable to nuoc-mam. The procedure for making patis is more or less the same as that for nuoc-mam. After the first patis yield, which has a characteristic taste, a saturated brine solution is used to obtain the second yield of patis with an inferior quality. Patis is usually made of small fish (*Stolephorus*, *Sardinella*, *Leignathus* or *Decapterus macrosoma*). Small shrimp or alamang, goby fry, herring fry and anchovies give the best results. Enough salt must be added to saturate the moisture which oozes from the fish. One kg of salt to 3.5–4 kg of fish gives a final product with 20–25 percent salt content (Panda et al. 2011).

4.5 Budu

This is a fish sauce similar to nam pla and nuoc mam and is produced in the North Eastern states of Malaysia. The species of fish used is mostly *Stolephorus* (*Ikanbilis*), *Sardinella* sp. or *Decaterus macrosoma* (Beddows 1985). Budu is prepared by fermentation of fish with the addition of salt (3:2 fish: salt ratio; w/w) in circular earthen pots covered with a plastic sheet. Weights are placed on the top which help the fish mass become immersed in the pickle produced by osmotic dehydration. The pickle is picked up at irregular intervals. Usually in budu manufacture, tamarind and caramelized palm sugar are added to the pickle that sweetens/sours the product and gives it a darker appearance (Rosma et al. 2009). The flow-chart for production of budu is given in Fig. 1.

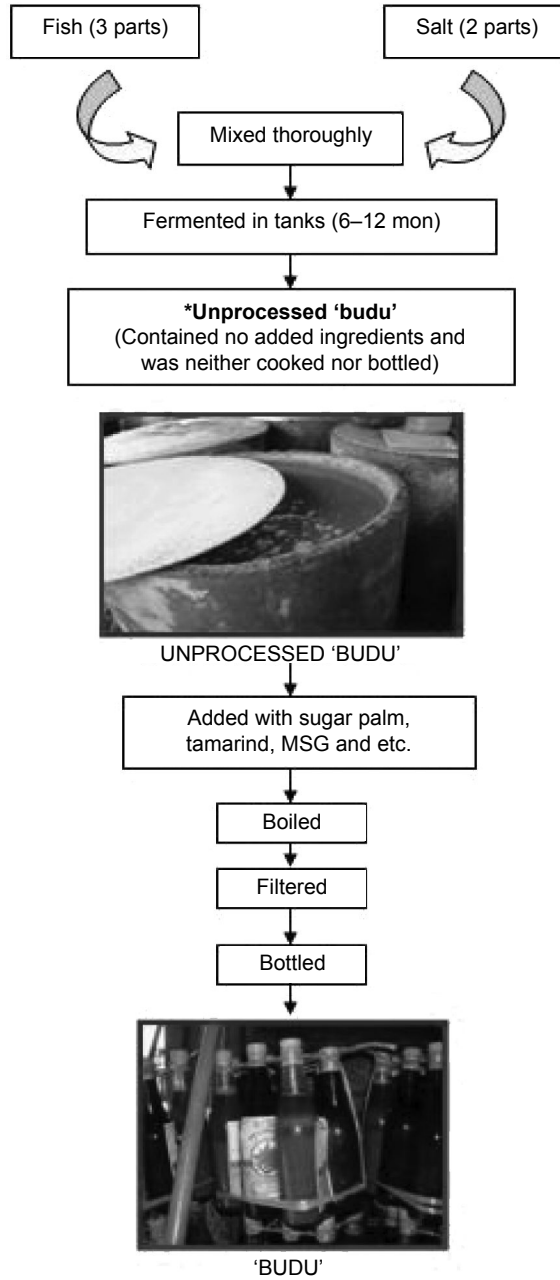

Fig. 1. Flow chart for production of budu (Source: Rosma et al. 2009).

Color image of this figure appears in the color plate section at the end of the book.

4.6 Bakasang

Bakasang is a traditional fish sauce produced in Indonesia by fermenting small whole sardines (*Sardinela* sp. or *Stelophorus* sp.). It is prepared by mixing fish and salt (5:1.5–3.5 fish: salt ratio; w/w), packed in small bottles and placed in the kitchen near the fire space. The temperature ranges from 30–60°C and the fermentation is allowed for about 3–6 wk. The product has become closely integrated with the eating habits of eastern Indonesian people, especially the Manadonese people (in the north Colebes Island). It is usually used as flavoring in many dishes or mixed with red chillies, tomato, red onion and garlic and then sautéed with coconut oil. The sautéed sauce is eaten with hot porridge mixtures of rice and vegetables called tinutuan (Ijong and Ohta 1995, 1996).

4.7 Momoni

In Ghana, a type of fermented fish product, momoni is popularly used as condiment for preparing sauces for the consumption of yam, cocoyam and apetum (boiled unripe plantain). Momoni is similar to feseekh of Egypt which is prepared from bouri (*Mugil cephalus*). For the preparation of momoni, different types of freshwater fish can be used; usually African jack mackerel (*Caranx hippos*) is employed. They can be scaled and gutted followed by washing in tap water, and salting (294–310 g/kg) is done with the gill and gut regions being heavily salted. The fish are arranged in baskets covered with aluminum trays or jute bags and fermentation is allowed for 1–5 d. Before retailing, the fermented fish are washed in brine water, rubbed with salt and cut into small pieces. The cut pieces are sun-dried on a wooden tray in the open air for a few hours. Momoni is a solid product that is added to boiling stew consisting of ground red pepper, tomato, onion and little quantity of palm oil. The finished product is usually of low quality with a high salt concentration and deteriorates rapidly during retailing and storage (Sanni et al. 2002).

4.8 Feseekh

In Egypt, feseekh is a traditional fish dish consisting of fermented salted and dried gray mullet, of the *Mugil* family, a saltwater fish that lives in both the Mediterranean and the Red Seas. The traditional process of preparing it is to dry the fish in the sun before preserving it in salt. Feseekh is traditionally eaten during Sham El Nessim "Smelling the Breeze", which is a spring celebration in Egypt from ancient times (Rabie et al. 2009). El-Tahan et al. (1998) considered the feseekh as one of the traditional dishes in Egypt which described the heritage of Egypt.

4.9 Other Traditional Sauces

Southeast Asians use fish sauce as a cooking sauce. In Thailand, the fish sauce is used in cooking and is also kept in a jar on the table for use as a condiment. This jar often contains a mixture of fish sauce and chopped hot chillies, called nam pla prik. In Korea, it is called ack jeot (usually from myul chi or kanari, meaning anchovies), and is used as a crucial ingredient of kimchi, both for taste and fermentation. Sae woo jeot (shrimp) is also popular as side sauce. Shottsuru is made in Japan from sandfish. Sardines, anchovies and mollusks can also be used as starting material. The fluid is filtered and boiled and can be kept for years. Soybean sediments or koji, which is fermented with wheat, can be added to shottsuru (Panda et al. 2011).

4.10 Novel Fish Sauces

A new method for producing fermented fish sauce was developed to improve the taste of the fish sauce. The method involved the production of a fermented sauce, seasoning liquid from salmon that was enzymatically hydrolyzed with the wheat gluten koji, lactic acid bacteria and yeast (*Saccharomyces cerevisiae*). The fish sauce produced using wheat gluten was very light-colored and had a higher content of free amino acids, especially glutamic acid (Indoh et al. 2006).

The gambusia (*Affinis affinis*) fish, a small freshwater species not normally used for human consumption has been fermented to produce fish sauce. The fish sauce obtained was composed of 65.97 percent moisture, 12.37 percent crude protein, 1.56 percent lipid, 19.33 percent ash and 9.08 percent salt. In addition, the pH value was 6.08 and the non-essential amino acids (3.864 mg/100 ml) were higher than the essential amino acids ones (2.172 mg/100 ml) (Ibrahim 2010).

A fish sauce was produced by pounding clupeids (*Pellonula afzeliusi*) fish with salt and fermenting it for 12 wk at 28°C (± 4.5°C). The biochemical characteristics of the sauce were similar to budu and aekjeot, the Malaysian and Korean fish sauce, respectively. The pH of the sauce was in the range of the standard sauce (6.8–7.6) produced from Asia (Olubunmi et al. 2010).

5 Fish Paste

Fermented fish pastes are common fermented products other than fish sauce that have a special place in ethnic culinary. Fish pastes are used mainly as a flavoring agent or in condiment preparations. Almost all South and Southeast Asian countries prepare this product. Hentak, ngari and tungtap in India, bagoong in the Philippines, terasi in Indonesia, belacan in Malaysia,

ngapi in Myanmar, kapi in Thailand and many other names specific to the surrounding countries, are some examples of fermented fish pastes. Fish pastes are made from various species of freshwater and marine fish as well as shrimps (Salampessy et al. 2010).

There are two kinds of fish and shrimp pastes in South-East Asia:

- Fish or shrimp-salt mixtures.
- Produces, which are fermented in the presence of cooked or roasted rice on which yeasts and moulds are present.

The general method of preparation of fish and shrimp pastes is the same as that described for fish sauces. Only the fermentation time is shorter, as not all of the fish flesh needs to be broken down. Fish paste must be mixed regularly to help the salt be evenly distributed.

5.1 Shiokara

Shiokara is consumed mostly as a side dish, and is important in the cuisines of Cambodia, Laos, North and Northeast Thailand, Lower Myanmar, the Philippines (Luzon and the Visayas), and Korea. In Japan, shiokara was formerly an important side dish, but is now just a specialized, savory product. Among the Han Chinese, shiokara is now a local and mostly forgotten food. The paste form can be dissolved in water and used as a soup stock or for dipping. The liquid in shiokara (the nam pla deak of North Thailand, for example) is always drained off during production. Shiokara products contain salt plus a range of other ingredients to enhance the taste. Sometimes, a small quantity of boiled rice is added, making it difficult to distinguish chemically between, for example, Thai pra la and narezushi, although they are defined differently in folk taxonomy (Ruddle and Ishige 2010).

5.2 Bagoong

Bagoong, a fish paste from the Philippines, is made by fermenting well-cleaned whole or minced fish, shrimp, fish or shrimp eggs in the presence of salt (3:1 fish: salt ratio; w/w) (Fig. 2). The fish used for bagoong include anchovies, sardines, herring, silverside, shrimp, oysters, clams, and other shellfish. The fish-salt mixture is put into earthenware pots and covered with cheesecloth for 5 d. The covered pots are then put in the sun for 7 d. After that, the product is fermented for a further 3 to 12 mon (Panda et al. 2011).

As a by-product, the fish sauce patis can be harvested by separating the liquid above from the paste. The paste is sometimes colored by adding 'angkak'—colored rice which has been treated with the red yeast-like organism *Monascus purpureus*. Bagoong can be stored for several years.

Fig. 2. Fish paste "bagoong".

Color image of this figure appears in the color plate section at the end of the book.

5.3 Hentak

Hentak is a fermented fish product made from a mixture of sun-dried fish (*Esomus danricus*) powder and petioles of aroid plants (*Alocasia macrorhiza*). Hentak preparation includes crushing sun-dried fish to a powder and mixing with an equal amount of petioles of aroid plants, forming a ball-like thick paste and fermenting in an earthen pot for 7–9 d. Hentak is consumed as a curry as well as a condiment with boiled rice and is a typical product from Manipur, India (Thapa et al. 2004).

5.4 Balao Balao

Balao balao, which has its origin in the Philippines, is a fermented rice-shrimp (*Penaeus indicus* or *Macrobrachium* sp.) product. It is prepared by mixing boiled rice, whole raw shrimps and salt (5:1; shrimp: salt ratio). The product is stored in jars and fermented for 7–10 d. The mixture becomes less sour the longer the fermentation takes place. The shells of the shrimp become red and soft, and the mixture including the rice, becomes liquid. In the general preparation it is fried with garlic and onion after fermentation. It is eaten as a sauce or as a complete meal in itself (Steinkraus 1997).

5.5 Belachan

Belachan is a Malay-Indonesian shrimp paste made of small shrimps to which a relatively small amount of salt (20:1 shrimp: salt ratio), chilli pepper, and belasan is added. The mixture is dried on mats on the ground in the

sun. After 4–8 hr of drying, during which 50 percent of the moisture is lost, any contaminants in the shrimp are removed. The shrimp are then chopped up and squeezed into wooden vats so that no more air is present. The paste which results is fermented for 7 d. After 7 d the substance is taken out of the barrel and is dried for 3–5 hr in the sun. The paste is again ground up after which it is put back in the wooden vats. The paste is fermented further for one more mon (Panda et al. 2011).

5.6 Ngapi Yay

A watery dip or condiment that is very popular in Myanmar, especially amongst the Burmese and Karen ethnic groups. The ngapi (either fish or shrimp, but mostly whole fish ngapi is used) is boiled with onions, tomato, garlic, pepper and other spices. The result is a greenish-grey broth-like sauce, which makes its way to every Burmese dining table. Fresh, raw or blanched vegetables and fruits (such as mint, cabbage, tomatoes, green mangoes, green apples, olives, chilli, onions and garlic) are dipped into the ngapi yay and eaten. Sometimes, in less affluent families, ngapi yay forms the main dish, and also the main source of protein (Panda et al. 2011).

5.7 Trassi (Terasi)

Trassi, an Indonesian variant of dried shrimp paste, is usually purchased in dark blocks, but is also sometimes sold ground. The color and aroma of terasi varies depending on which village produced it. The color ranges from soft purple-reddish hue to darkish brown (Panda et al. 2011).

5.8 Bagoong Alamang

Bagoong alamang is a Filipino shrimp paste, made from minute shrimp or krill (Alamang) and is commonly eaten as a topping on green mangoes or used as a major cooking ingredient. Bagoong paste varies in appearance, flavor, and spiciness depending on the type. The paste can be sautéed with various condiments, and its flavor can range from salty to spicy-sweet (Panda et al. 2011).

5.9 Hom Ha

This Chinese shrimp paste is popular in South-Eastern China. This shrimp paste is lighter in color than many Southeast Asian varieties and is often used to cook pork. The shrimp paste industry has historically been important in the Hong Kong region (Panda et al. 2011).

5.10 Hae Ko

Hae Ko means prawn paste in the Hokkien dialect. It is also called petis udang in Malay. This version of shrimp/prawn paste is used in Malaysia, Singapore and Indonesia. This thick black paste has a molasses-like consistency instead of the hard brick like appearance of belacan. It also tastes sweeter because of the added sugar. It is used to flavor common local street foods like popiah spring rolls, laksa curry, chee cheong fan rice rolls and rojak salad (Panda et al. 2011).

The list of fish and shrimp pastes from countries in Asia is given in Table 2.

Table 2. Some Fish Pastes of Asian Continent.

Country	Name	Ingredients
Cambodia	Pra-hoc	Fish (cyprinid, ophiocephalid)/salt
	Phaak, Paak or mam-chao	Fish/salt + glutinous rice
	Mam-ca-sat	Fish/salt + roasted rice
	Mam-ca-sak	Fish/salt roasted rice + pineapple or papaya
	Mam-ca-lok	Fish/salt +roasted rice+ sugar + ginger + pineapple + color
	Mam-ruot	Fish entrails/salt
	Mam-seing	Fish eggs+ salt + roasted rice
	Mam-ruoc	Freshwater shrimps/salt
Thailand	Kapi	Marine shrimps/salt
	Pla-mam	Freshwater fish/salt + roasted rice + pine apple
	Pla-chao	Freshwater fish/salt + glutinous rice
	Kung-chao	Marine or freshwater shrimp/salt + color (and sometimes roasted rice or sesame)
Malaysia	Blachan	Shrimp/salt
Philippines	Bagoong	Fish or shrimp/salt (sometimes + color)
Indonesia	Trassi	Fish/salt + sun drying, e.g., Trassi-udung from shrimp. Trassi-ikan from small fish
Myamar	Nga-ngapi	Fish/salt
Japan	Shiokara tyupe	Squid or skipjack/salt + malted rice, e.g., Unishiokara-ovary of sea urchin + salt, kakishiokara-oyster + salt
Pakistan and North Eastern India	Sidal	Small fish (*Bambus* sp.) –salting and drying, then crude fish oil is added

Source: Adapted from Beddows (1985), Panda et al. (2011)

6 Fermented Dried/Salted Fish

Apart from fish sauces and pasta, different processing techniques are employed in fish fermentation. This is greatly influenced by factors such as the availability of salt and food habits of the local people. Three main techniques have emerged as methods commonly practiced by people in Asian and African countries for fish fermentation. These are:

- Fermentation with salting and drying;
- Fermentation and drying without salting; and
- Fermentation with salting but without drying.

6.1 Asian Fish Products

6.1.1 India and Bangladesh and Sri Lanka

Tungtap

It is a popular fermented fish (*Puntis* sp. and/or *Danio* sp.) product, commonly prepared and consumed by the Khasi and Jaintia tribes of Meghalaya in North-Eastern state of India. This practice is similar to traditional processing of fish such as fermentation salting, drying and smoking which are the principal methods of fish preservation in South-East Asia (Rapsang and Joshi 2012).

Lona Ilish

It is a salt fermented product prepared from Indian shad (*Tenualosa ilisha*), a high-fat fish (fat content of adult hilsa ranges from 14–25 percent) (Majumdar et al. 2006). This fish is popularly known as hilsa due to its earlier generic name hilsa. Lona ilish is a very popular product and widely consumed in Northeast India and Bangladesh mainly due to its typical flavor, aroma and texture (Majumdar and Basu 2010). Lona ilish is traditionally prepared by dry salting the diagonally cut hilsa chunks followed by fermentation in saturated brine (previously boiled and cooled) in a metal container till the appearance of the characteristic flavor and texture. It is kept immersed in the fermenting medium (saturated brine) till consumption. The fermentation period is usually 4–6 mon. The traditional method for preparation lona ilish is shown in Fig. 3.

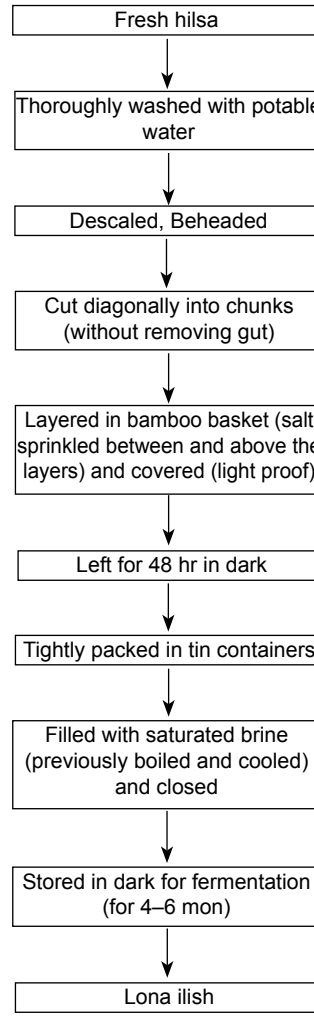

Fig. 3. Procedure of lona ilish production by the traditional method.

Colombo cure

It is a fermented fish product of India and Sri Lanka. The intestines and gills are removed from mackerel or non-fatty sardines after which the fish are washed in drinking water. The fish are mixed with salt (3:1; fish: salt ratio, w/w) and put into jars. Dried fruit pulp or tamarind (a tropical fruit)

is added to the salt and fish to lower the pH (8 kg of tamarind to 100 kg of fish). The fish are kept covered with brine with the help of weighted mats and are fermented for 2 to 4 mon. They are transferred to wooden barrels and care is taken to keep them covered with brine. The fermented fish can be kept for one year (Lopetcharat et al. 2001, Prajapati and Nair 2003).

6.1.2 Thailand

Plaa som

It is a low salt product, typically composed of freshwater fish, salt, boiled rice or steamed sticky rice and garlic (Kopermsub and Yunchalard 2010) and is mainly produced in the central and North-Eastern part of Thailand. However, in the Songkhla Province in Southern Thailand, a local variety of plaa som is produced, in which garlic and boiled rice are replaced by palm syrup and from time to time by roasted rice, thus resembling plaa uan, another type of Thai fermented fish (Phithakpol et al. 1995).

For plaa som, whole fish is mixed with salt (8:1; fish: salt ratio, w/w) and left overnight. Cooked rice and minced garlic are added (ratio 20 fish/salt: 4 rice: 1 garlic; w/w), then the mixture is packed in jars and fermented at ambient temperature for 5–7 d. The shelf-life is reportedly 3 wk (Phithakpol et al. 1995).

Som-fug

Som-fug is a Thai traditional fermented minced fish, which is composed of fish mince, salt (2.5 percent), ground steamed rice (2–12 percent) and minced garlic (4 percent) (Riebroy et al. 2008). The mixture is tightly packed in banana leaves or plastic bags and left to ferment for 2–5 d at 30°C. Som-fug can be served either as a main dish or as a snack with vegetables. Som-fug is highly nutritious and is an excellent source of protein. The fish species include giant snake-head fish (*Ophicephalus micropeltes*), rohu (*Labeo rohita*), spotted feather back (*Notopterus chitala*) and grey featherback (*Notopterus notopterus*) (Lopetcharat et al. 2001, Riebroy et al. 2008).

Use of starter culture of different lactic acid bacteria on the fermentation and quality of som-fug from big eye snapper was investigated. Som-fug inoculated with *Pediococcus acidilactici* at 10^4 colony forming units (cfu)/g had a greater acceptability than those inoculated with *Lactobacillus plantarum* and *Pediococcus pentosaceus* at either 10^4 or 10^6 cfu/g and the control (without inoculum). From the result, inoculation with *P. acidilactici* resulted in a reduction of fermentation time and improved the quality of som-fug. Therefore, *P. acidilactici* can be used as a potential starter for som-fug fermentation (Riebroy et al. 2008).

Som fak

For som fak (similar to som fug), fish fillets are minced. Cooked rice, minced garlic and salt (ratio 120 fish mince: 20 rice: 7 garlic: 7 salt, w/w) are added to the minced fish and the mixture is divided into small portions and packed in banana leaves or plastic sheets. The product ferments for 3–5 d at ambient temperature. The shelf-life is reported to be two wk; however, the product is best consumed within a few days of fermentation (Phithakpol et al. 1995).

Plara (Plaa-raa, Plaa-ra)

Plara is a popular product, especially in the North and Northeast of Thailand (Phithakpol et al. 1995). There are several kinds of freshwater fish normally used: *Channa striatus* or *Ophicepharus striatus* (striped snake-head fish; pla chon); *Puntius gonionotus* (silver barb, pla ta-pian); Trichogaster trichopterus (gouramy, pla kra-dee); *Cirrhina jullieni* (jullien's mud carp, pla soi); *Cyclocheilichthys* sp. (soldier river barb, pla ta-kok) and *Oreochromis niloticus* (*tilapia*, pla nin). Recently marine fish (*Rastrelliger neglectus* and *Rachycentron canadus*)—based plaara is also being prepared (Sangjindavong et al. 2008).

Generally, freshwater fish are scaled, headed, eviscerated and washed with tap water and then rapidly dried. The prepared fish is mixed with salt in a fish to salt ratio of 3-5:1 by weight and then left at ambient temperature for 12–24 hr before packing in an earthenware jar and letting it ferment for 1 mon. Then, salted fish is added with roasted rice or rice bran with a salted fish to roasted rice or rice bran ratio of 4-5:1 by weight. It is put in earthenware jars and held at least 6 mon. The shelf life of plara is approximately 6 mon to 3 yr, depending on the fermentation period and good handling, such as tight packaging and occasionally mixing to exchange the upper and lower portions in the jar.

Hoi Dorng

It is a high salt product from Thailand and is produced from sea mussel meat washed in brine (10 percent salt) and water. After drainage, sea salt is added (7:1; meat: salt ratio, w/w) and mixed well. The product matures for 4–5 d and is packed in sealed glass jars. It has a shelf-life of 3–6 mon (Phithakpol et al. 1995).

Pla-paeng-daeng (Red fermented fish)

It is prepared by fermenting fish and ang-kak rice (red rice) with the mould *Monacus purpureus* which gives it a red color and specific flavor (Phithakpol et al. 1995). Some of the fermented fish products are given in Fig. 4.

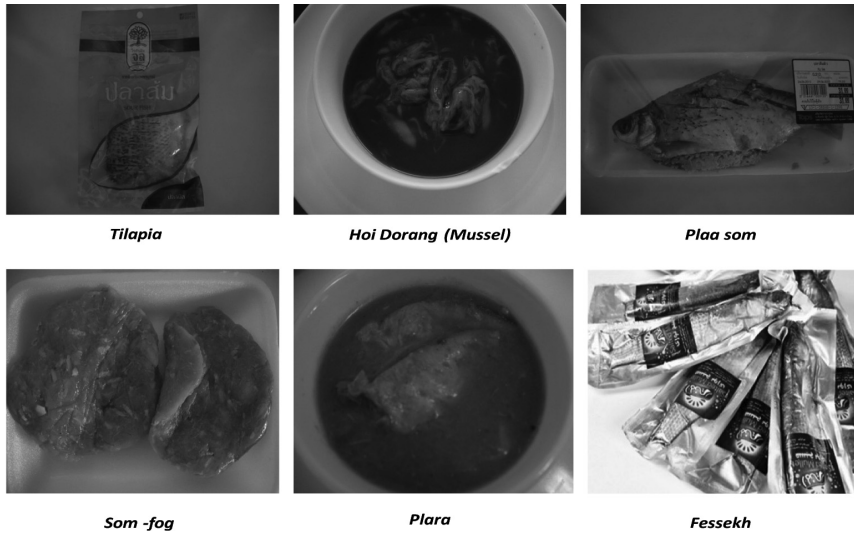

Fig. 4. Some fermented fish products.

Color image of this figure appears in the color plate section at the end of the book.

6.1.3 Korea

Jeotkals

Jeotkals are the Korean traditional salted and fermented fish products and are popular not only as side dishes but also as ingredients in preparing kimchi in Korea. To prepare jeotkals, salt should be added at the level of 5–20 percent to raw fish and then allowed to ferment for a long period of time to develop taste. The fermentation period varies depending on the salt concentration and fermentation temperature; 2 mon for most jeotkals with low salt level (6–18 percent). Thus, these fermented fish products contain relatively high amounts of amino acids, the degradation products of fish proteins (Mah et al. 2002, 2008, Cheorun et al. 2004).

6.1.4 The Philippines

Burong isda

This product is a typical food in Central Luzon in the Philippines prepared by fermenting cooked rice and freshwater fish (about 20 percent, w/w). Previously consumed as condiment, it is now a main dish because of economic conditions. The fish is scaled, eviscerated, and filleted before

mixing with cooled cooked rice. Fermentation is carried out for 7–10 d at room temperature (Olympia et al. 1995).

Burong isda is available in two forms depending on the consumers' preferences in a particular area. One is called white burong isda, which has a natural product color and the other is red burong isda, which is colored by addition of angkak, culture of *Monascus purpeveus* grown on rice. Also, there are several varieties of burong isda based on the type of fish used in preparation. One example is burong dalang, a fermented rice-fish mixture using mud fish, *Ophicephalus striatu* (Olympia et al. 1995).

6.1.5 Indonesia

Ale-ale

It is a fermented shellfish product popular in West Kalimantan, Indonesia. It is made from *Meritrix meritrix* meat. There are two types of fermented ale-ale based on its color: white and red (Fig. 5). White fermented ale-ale is prepared by mixing ale-ale *M. meritrix* meat with salt and rice porridge in certain ratio, and then pressed tightly in a plastic bottle. It is fermented for 7 d at room temperature before selling to the domestic market. Processing of red fermented ale-ale also uses white fermented ale-ale but rice porridge is replaced with angkak (Monascus-fermented rice). The ratio of raw materials differs for every product. The taste of the product is slightly sour and salty (Nofiani et al. 2010).

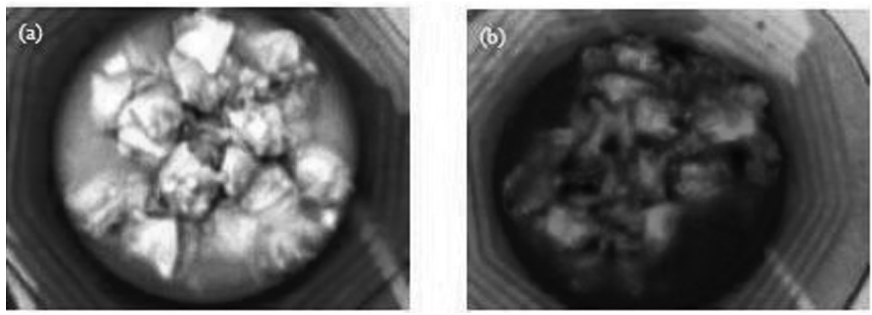

Fig. 5. Fermented ale-ale. (a) White fermented ale-ale. (b) Red fermented ale-ale.

Color image of this figure appears in the color plate section at the end of the book.

Wadi Betok

It is the other popular traditional fermented fish product in South Kalimantan, Indonesia (Petrus 2009). This freshwater fish, Climbing Perch

(*Anabas testudineus* Bloch) with protein content in the range of 10–15 percent is used as raw material in its production (Arianti 2004). Furthermore, Petrus (2009) reported that whole freshwater fish after added with 30–100 percent (w/ w) salt were layered in plastic jars and left for spontaneous fermentation for 7 d to 4 mon at room temperature.

Peda

It is a wet fermented mackerel (*Rastrilliger* sp.), where fresh mackerel is put in layers in basket and topped with 30 percent (w/w) salt. It is fermented at room temperature for 3 mon or more (Petrus et al. 2013).

6.1.6 China

Suan yu

It is a Chinese low-salt fermented whole fish snack with a characteristic flavor. It is stable during storage and free of fishy odor and taste and retains all the nutritional advantages of fish compared with other traditional fermented fish products. It is manufactured using traditional technologies without the addition of starter cultures in small-scale processing units.

Fish used are mainly freshwater fish mixed with cooked carbohydrate, salt, and spice. Long-term spontaneous fermentation is conducted to develop flavor in anaerobic conditions (Zeng et al. 2013a).

6.1.7 Myanmar

Pedah-siam

This Myanmar product is made of salted mackerel. During the preparation, the intestines are removed through the mouth. The fish are then salted, 3 kg of fish to 1 kg of salt, and stored for 24 hr. Ripening takes place under anaerobic conditions and the brine formed is removed regularly. A red color appears after ripening (Berkel et al. 2004).

6.1.8 Japan

Sushi

Sushi is a group of preserved fish products in Japan which are formed through the addition of boiled rice to fermented fish and salt. The general preparation is as follows. The intestines of the fish are removed and the fish is mixed with 20 to 30 percent salt. After being stored for 1 to 2 mon the fish

is de-salted and the liquid is removed. Boiled rice and koji (fermented wheat) are placed on the bottom of a basket and the de-salted fish is alternated in layers with boiled rice or koji. The amount of boiled rice added is equal to 40 or 50 percent of the weight of the fish, the amount of koji is half the amount of boiled rice (rice:fish:koji = 2:4:1). The fermentation continues for another 10 d (Berkel et al. 2004).

6.2 Latin American Fish Products

6.2.1 Anchoa

It is a product found in a few South American countries, including Brazil, Peru, Chile and Argentina. Whole anchovies, traditionally prepared from fish belonging to the *Engraulidae* family (*Engraulis* sp.) are mixed with 35 percent salt and placed in barrels. The fermentation, a result of enzyme activity, takes place for a period of 3 to 4 mon (Oetterer et al. 2003, Berkel et al. 2004).

6.3 African Fish Products

In Africa, there are many dried and salted fish products; however, the few popular fish products are described below.

6.3.1 Ghana

Momoni

It is a product from Ghana. In its general preparation, the intestines and gills of the fish are removed and the fish are washed in water. They are then rubbed with salt and packed in layers in barrels, alternating with layers of salt. The salt: fish ratio is 1:9. Fermentation takes place for 7 d. After that the fish are dried for 1 to 3 d on mats in the sun (Kerr et al. 2002, Berkel et al. 2004).

6.3.2 Benin

Lanhouin

This fermented fish is used for flavor and is mostly produced in the coastal regions of the Gulf of Benin. For traditional lanhouin production, the fresh fish is scaled, gutted, washed and left overnight before the seemingly deteriorate fish is treated with salt and allowed to ferment for 3 to 8 d (Anihouvi et al. 2007).

6.3.3 Burundi

Ndagala

The fish (*Limnothrissa miodon*, *Stolothrissa tanganicae*) is sun-dried soon after harvest. The drying period is 2–5 d on the ground or a rack. Slight fermentation for 2–5 d (normally during drying, no salting) takes place during sun-drying but this is pronounced when drying is delayed due to low ambient temperatures (15–20°C) or during the rainy season. The product is hard, dry and brittle with a silvery color. Fermentation is not the objective but it occurs during drying. The product can keep for about 3 mon (Essuman 1992).

6.3.4 Uganda

Dagaa

The fish (*Haplochromis* sp., *Rastineobola* sp.) is usually dried by passing a stick through the eyes of 10–12 individual fish or spread on the ground or a mat to dry. Ten such sticks of fish are joined to form a mat which is then hung in the open air for the fish to dry. The drying process takes 2–5 d on ground or rack. Fermentation occurs during drying for 3–6 hr without salting. The final products are grayish in color, with a very hard and dry texture. They have a very mild smell which becomes pronounced if drying is inadequate (Essuman 1992).

6.3.5 Mali

Djege/Djadan

Djege/Djadan are local names that refer to two Malian traditional fermented and dried fish products, with light brown color and a mild fermented odor (Essuman 1992). For processing, medium and large species of fish are washed, dressed, headed, salted and then put into water in an earthenware pot or oil drum and allowed to ferment for 12 hr (Fig. 6). The fermented fish is then immersed in a solution of Gardona or K-Othrine for few minutes to prevent it from being attacked by blowflies during drying. Concerning smaller species, the fish is usually dried immediately after washing and fermentation occurs during drying. Fish species such as *Tilapia*, *Clarias* sp., *Alestes* sp., *Schilbe* sp. and *Hydrocynus* sp. are commonly used to process djege and djadan (Anihouvi et al. 2012).

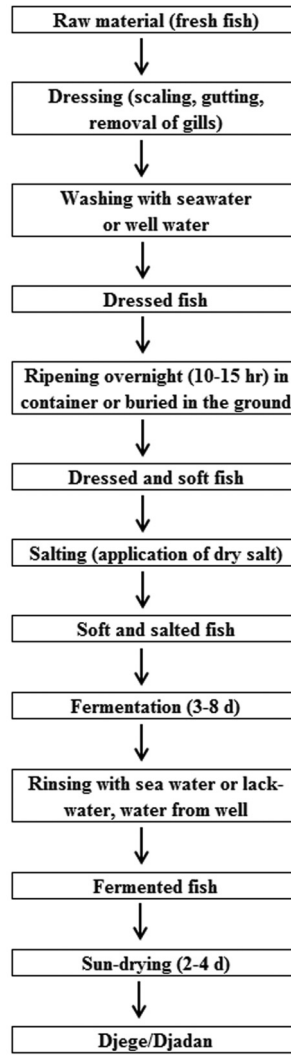

Fig. 6. Flow diagram of traditional processing of Djege/Djadan.

7 Microbiology and Biochemistry of Fermented Fish Products

7.1 Microbiology

Fish sauces contain high concentrations of salt (25–30 percent NaCl, w/v), thus microorganisms found during sauce production are generally classified as halo-tolerant or halophilic bacteria (Tanasupawat et al. 2009).

Earlier studies have identified the following microorganisms from samples of nam-pla; mostly *Bacillus* sp.; *Bacillus cerus*, *B. circulans*, *B. licheniformis*, *B. megaterium*, *B. pumilus* and *B. subtitils* (Crisan and Sands 1975). The important roles of bacteria in fish sauce/paste are protein degradation and flavor-aroma development. Bacteria involved in fish sauce and paste can be classified into two major groups (Lopetcharat et al. 2001).

- Bacteria that produce proteolytic enzymes. These include *Bacillus*, *Pseudomonas*, *Micrococcus*, *Staphylococcus*, *Halococcus* and *Halobacterium*. Highly concentrated NaCl (25 percent) does not have any effect on the proteolytic activity of enzymes from *Halobacter*.
- Bacteria that relate to flavor and aroma development. Ten out of 17 *Bacillus* type isolates produced a measurable amount of volatile acids in nam pla. *Staphylococcus* strain 109 also produced a significant amount of volatile acid in nam-pla.

The patis liquid contained strains of *Bacillus pumilus*, *Micrococcus colpogenes*, *M. varians* and *Candida clausenii* (Crisan and Sands 1975). These organisms are halo-tolerant rather than halophiles, as they could grow on 10 percent salt, but not beyond that level (Crisan and Sands 1975). When microbiological changes during bakasang processing were monitored, a variety of bacteria grew during the first 10 d of fermentation; however after 20 d, *Streptococcus*, *Pediococcus*, and *Micrococcus* were dominant (Ijong and Ohta 1996). The microflora found from Korean anchovy sauce in the final stage of fermentation included *Bacillus cereus*, *B. megaterium*, *B. pumilis*, *Pseudomonas halophilus* and *Serratia marcesens* (Sands et al. 1974).

In some recent studies, strains of aerobic, spore forming, gram positive, moderately halophilic bacteria were isolated from nam pla and budu. They grow optimally in the presence of 10 percent salt (NaCl) at 37°C and pH 7.0. Three strains were identified as *Lentibacillus salicampi* and the remaining strains were proposed as *Lentibacillus juripiscarius* based on 16S rRNA-based phytogenetic analysis (Namwong et al. 2005). Some other strains isolated from fish sauce in Thailand were: *Lantibacillus halophilus*, *Halococcus thailandensis*. *Filobacillus* sp. RP 2–5, *Piscibacillus salipiscarius*, *Tetragenococcus halophilus*, *T. muriaticus*, and *Halobacterium salinarum* (Namwong et al. 2007, Tanasupawat et al. 2007, 2009). The bacteria were identified based on phenotypic and chemotaxonomic characteristics including DNA-DNA relatedness and phylogenetic properties. Likewise, *Lentibacillus kapialis* sp. nov. and *Oceanobacillus kapialis* were isolated from fermented shrimp paste in Thailand (Pakdeeto et al. 2007, Namwong et al. 2009).

A total of 67 microbial strains were isolated from momoni (momoni fermented fish sauce) obtained from retail outlets. The strains belonged to 9 genera of microorganisms, i.e., *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Pediococcus*, *Staphylococcus*, *Klebsiella*, *Debaryomyces*, *Hansenula* and

Aspergillus, with *Bacillus* having a predominant occurrence of 37.7 percent (Sanni et al. 2002).

Other halophilic bacteria isolated from fermented fish products were: *Lentibacillus jeotgali* (Korean fermented seafood) (Jung et al. 2010), *Gracilibacillus thailandensis* and *Salinivibrio siamensis* (from Plara) (Chamroensaksri et al. 2009, 2010), *Paenibacillus tyraminigenes* (from Myeolchi-jeotgal, a traditional Korean salted and fermented anchovy (Mah et al. 2008), *Piscibacillus salipiscarius* (from Plara) (Tanasupawat et al. 2007). Anihouvi et al. (2007) studied the microbiological changes in naturally fermented mixture cassava-fish (*Pseudotolithus* sp.) for lanhouin fermentation. A total of 224 isolates belonging to the genera *Bacillus*, *Staphylococcus*, *Micrococcus*, *Streptococcus*, *Corynebacterium*, *Pseudomonas*, *Achromobacter* and *Alcaligenes* were isolated from the fermenting fish samples. *Paenibacillus tyraminigenes* sp. Nov. was isolated from Myeolchi-jeotgal, a traditional Korean salted and fermented anchovy (Mah et al. 2008).

Lactic acid bacteria are also found as the dominant microorganisms in many fermented fish products (Paludan-Müller et al. 2002). The primary role of lactic acid bacteria is to ferment the available carbohydrates and thereby cause a decrease in pH. The combination of low pH and organic acids (mainly lactic acid) is the main preservation factor in fermented fish products. In plaa-som, the major lactic acid bacteria isolated were *Pediococcus pentosaceus*, *Lactobacillus alimentarius*, *Lb. planatrum*, *Lb. garviae*, *Lb. reuteri* and *Weisella confusa*, based on studies of phenotypic tests, ITS-PCR, carbohydrate fermentations and 16S rRNA gene sequencing (Paludan-Müller et al. 2002, Saithong et al. 2010). Forty-two percent isolated strains were *P. pentosaceus*. In som-fak (a Thai low-salt fermented fish product), a succession of aciduric, homo-fermentative lactobacillus species, dominated by *Lactobacillus plantarum*, *Lb. pentosus*, *Lactococcus lactis*, and *Leuconostoc citreum* was found during fermentation (Paludan-Müller et al. 1999). Further, at the start of fermentation, *Leuconostoc* sp., *Leuconostoc lactis* subsp. *lactis* and *Lactobacillus brevis* were dominant, followed by more acid tolerant species of *Lb. planatrum*, the latter dominating the lactic acid bacteria microflora towards the end of fermentation. In a more recent study, a total of 762 lactic acid bacteria (LAB) were isolated during plaa-som fermentation by culture on $CaCO_3$-MRS (Man-Rogosa-Sharpe) agar plates. They were screened and grouped by amplified ribosomal DNA restriction analysis (ARDRA), giving six groups that were identified by ribosomal DNA sequencing as *Lactococcus garvieae*, *Streptococcus bovis*, *Weissella cibaria*, *Pediococcus pentosaceus*, *Lactobacillus plantarum*, and *Lactobacillus fermentum*. Freshly mixed ingredients contained low populations of LAB (less than 10 cfu/g) that subsequently grew during fermentation to final populations of approximately 10^7 cfu/g. Early stages of the process were dominated by the presence of *Lc. garvieae*, *S. bovis*, and *W. cibaria*. At 48

hr into fermentation, *W. cibaria*, *P. pentosaceus*, and *Lb. plantarum* were prevalent, and gave way to dominance of *Lb. plantarum* that completed the fermentation (Kopermsub and Yunchalard 2010). *Tetragenococcus halophilus* and *Tetragenococcus muriaticus*, moderately halophilic lactic acid bacteria, were found in fermentation period of various salted and fermented foods, such as soy sauce and fish sauce. In this study, a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) method was developed for the rapid identification of *T. halophilus* and *T. muriaticus* (Kuda et al. 2014).

Bacteriocin production by lactic acid bacteria such as *Weissella confusa* N31 and *W. cibaria* N 23, isolated from fermented Thai fish products was reported (Pringsulaka et al. 2012).

7.2 Biochemistry

Amino acids are considered the major contributors to the taste of fish sauce (Lopetcharat et al. 2001). The flavor and aroma of fish sauce is thought to arise in part from glutamic acid, histidine and proline (Saisithi et al. 1967, Raksakulthai and Haard 1992). For example, glutamic acid and lysine were found as predominant amino acids in bakasang, a fish sauce from Indonesia (Ijong and Ohta 1995). Similarly, glutamic acid was the predominant amino acid in momoni (Sanni et al. 2002). Likewise, the predominant free amino acids in feseekh were leucine, glutamic acid, lysine, alanine, valine, aspartic acid, isoleucine and citrulline (Rabie et al. 2009). Their concentrations accounted for 68 percent of the total concentration of amino acids after 60 d of fermentation due to the biogenic amine content.

Anti-oxidant activity has also been found in a number of fermented fishery products such as fermented blue mussels (Jung et al. 2005), fish sauces (Harada et al. 2003, Michihata 2003) and fermented shrimp paste (Peralta et al. 2005). In a recent study, the antioxidant activity and nutritional components of Philippine salt-fermented shrimp paste were improved through prolonged fermentation (90, 180, and 360 d). The antioxidant ability against 1, 1-diphenyl-2-picryhydrazyl (DPPH) radical, hydrogen peroxide, and lipid peroxidation increased significantly with prolonged fermentation. Polyunsaturated fatty acids like eicosapentaenoic acid and docosahexaenoic acid in the shrimp paste were not substantially damaged for 360 d, while free amino acid content dramatically increased at 90 d. These results suggest that properly prolonged fermentation would improve antioxidant ability and some nutritional value in the salt-fermented shrimp paste (Peralta et al. 2008). In another study, chemical composition and anti-oxidative activities of some Thai traditional fermented shrimp and krill products including Jaloo, Koong-Som and Kapi were studied. Water-soluble fraction from all products possessed DPPH and ABTS radical-scavenging activity, as well

as ferric reducing antioxidant power (FRAP) in a concentration-dependent manner. At the same concentration tested, the water-soluble fraction from Kapi exhibited the highest anti-oxidative activity (Faithong et al. 2010).

8 Health Hazards of Fermented Fish Consumption

A number of practices observed during processing of traditional fermented fish products constitute health hazards to consumers. These practices are related to the processing technique itself, the environment in which the fish is processed, the waste disposal of the fish, the unhygienic nature of processing materials and improper packaging of the product as well (Paludan-Müller et al. 2002, Zeng et al. 2013a). During the dressing step, the fish may be held under the foot on the ground. This practice can lead to microbial contamination of fresh fish with *Salmonella* and *Vibrio* species (Bernbom et al. 2009). The lack of potable water constitutes a problem during the washing step. Thus, water from lagoons, rivers, lakes or sea is generally used to wash the fish (Diop 2008). These water bodies are often polluted by all kind of wastes making them a possible source of chemical and microbial contamination. The traditional fermented fish products are often dried on the ground or on dirty materials. Drying fish in such conditions is source of contamination with sand and microorganisms (Zeng et al. 2013b). The fish, especially if unsalted, is subjected to blowflies and other types of insect attack. These types of problems lead to the illegal use of substances such as petroleum and insecticides. Processors usually package the products in various types of traditional containers or recycled containers during fermentation, storage and when transporting the product to the market. The unhygienic nature of these materials could be potential sources of microbial or other types of contamination. Consequently, in the artisanal fish industry where technology and standard are very low, fermented fish products could be considered as potential vehicles for transmission of foodborne diseases (Abu Hassan and Sulieman 2011).

Investigations on various fermented fish products confirmed high levels of biogenic amines, mainly histamine (Anihouvi et al. 2005). For instance, histamine content and numbers of histamine-forming and histamine-decomposing bacteria in 10 fish-nukazuke (salted and fermented fish with rice bran) samples were determined. Two mackerel-nukazuke and two sardine-nukazuke products showed high content of histamine from 12.6 to 30.5 mg/100 g. Both, the number of halophilic histamine-forming and histamine-decomposing bacteria were various in the fish-nukazuke products. The histamine content tended to be low in the product containing high number of halophilic histamine-decomposing bacteria. These results suggest that accumulation of histamine in fish-nukazuke may be affected by histidine content and halophilic histamine-related bacteria (Kuda et al. 2007,

2012, Tapingkae et al. 2010). Similarly, *Paenibacillus tyraminigenes* sp. nov., isolated from Myeolchi-jeotgal, a traditional Korean salted and fermented anchovy showed high ability to produce tyramine from tyrosine (Mah et al. 2008). Tsai et al. (2006) reported *Bacillus coagulans* and *B. megaterium* identified from Taiwan fermented fish products produced histamine in the range of 13.7 and 8.1 µg/g (dry weight, DW). In a recent study with Egyptian fermented fish product feseekh the total contents of biogenic amines ranged from 84 to 1633 mg/kg (DW) during the investigated period. Cadaverine was the major amine detected in feseekh at all sampling stages and its concentration varied between 21 and 997 mg/kg. The histamine content (211 mg/kg) only exceeded the maximum tolerance level (200 mg/kg) after 60 d. It could be concluded that feseekh can be consumed without any health risks between 20 and 40 d but can be hazardous after 60 d (Rabie et al. 2009).

When the foods are contaminated with bacteria containing decarboxylase enzymes, these free amino acids undergo decarboxylation to produce biogenic amines (Kim et al. 2002). For instance, histidine is decarboxylated to produce histamine; lysine is decarboxylated to produce cadaverine and putrescine. In general, most amines are heat stable and some decarboxylases remain active even after pasteurization. This implies that the amount of amine once formed will not be reduced during cooking. Thus, despite a lack of information on food poisoning caused by traditional fermented fish products, there is a potential for sporadic amine poisoning. Ingestion of food containing small amounts of histamine has little effect on humans, but in larger amounts histamine can be toxic. The incubation period of histamine poisoning is short; poisoning effects can occur within several minutes to a few hours following ingestion of a meal containing high levels of histamine. The duration of illness is usually short and, in most cases, symptoms such as flushing, oral burning or a blistering sensation and perspiration pass within a few hours. Less frequent symptoms include vomiting, diarrhoea, stomach pain, headaches, swelling of the tongue, facial swelling and dizziness (Taylor and Bush 1988). In conclusion, despite a lack of official reports on food poisoning caused by the consumption of fermented fish products, there is a potential for more than just sporadic amine poisoning, since many food poisoning cases in African countries for example do not reach official channels. The formation of biogenic amines in fermented fish products has been reported by various authors (Anihouvi et al. 2009). In this respect, the use of amine-negative starter cultures to prevent biogenic amines formation has been suggested by Holzapfel (1997). However, there is a controversy about the inhibitor effect of starter culture on biogenic amine formation. Bauer et al. (1994) reported that the addition of starter culture did not affect the formation of biogenic amines.

9 Conclusions and Future Perspectives

For many socio-economic and technical reasons, fermentation is one of most important fish preservation methods used in many parts of the world. The fermentation processes applied are generally indigenous and adaptable to the culture of the people. It has been observed that fermented fish processing is an artisanal activity and the processes differ from one country to another. Three basic methods are identified: fermentation with salting and drying, fermentation and drying without salting, and fermentation with salting but no drying. Fermented fishery products contribute to the protein intake of the people, especially those in the rural hinterland where fresh fish is not readily available. Curing by fermentation is found to be an important method of preservation, particularly because poor quality fish or unpopular species of fish are usually processed in this way. For this reason, fermentation helps to salvage fish which would otherwise have been thrown away. Post harvest losses in artisanal fisheries, especially in African countries, may thus be lower than often assumed.

Traditional fermented fish products are produced by spontaneous and largely uncontrolled fermentation. Like other Asian/African fermented food products, fermented fish are currently produced largely on a traditional small-scale basis under highly variable conditions. The quality of the product is unpredictable and shelf-life is short. With increasing urbanization and demand for high quality traditional products, there is a need for controlled fermentation process and to minimize the variation in product quality encountered during the spontaneous fermentation at cottage industry level. In this respect, the use of starter cultures to promote the processing of fermented fish products is necessary. It is expected that the use of starter cultures for fish fermentation may also reduce the fermentation time, enhance inhibition or elimination of foodborne pathogens, and improve shelf life and sensory quality of products in terms of taste, aroma, appearance and texture.

Keywords: African fish products, Asian fish products, fermented dried/salted fish, Fish paste, Fish sauce, Health hazards, Latin American fish products, Starter cultures benefits, Traditional fermentation

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7

Fermented Meat Products

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1 Introduction

Fermented meats are unique products that are often represented as elements of culinary heritage and identity. Yet, their success has often been compromised throughout history because of health and safety issues. Moreover, contemporary industrialized meat products are sometimes perceived as of inferior quality due to health and safety issues. But now, the industrial attitude towards safety issues of fermented meats has been changing. Therefore, novel strategies are emerging to influence quality as well as healthiness. Within a context of innovation, "artisan" elements are employed in marketing stratagems of meat products. This contrasts with process alterations, highlighting the volatility of models for "quality", "safety", "tradition", and "innovation" in food approaches (Leroy et al. 2013).

Nowadays, the preservation role of meat fermentation has become largely obsolete due to the introduction of the cold chain, in particular in Western countries. Nevertheless, fermented meat products remain very

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popular and are still produced in large amounts, especially in Europe where they occur in overwhelming varieties. Meat fermentation is therefore to be considered more as a means of transformation and diversification rather than preservation of meat (Ordóñez and de la Hoz 2007). Reasons for the persistence of fermented meats probably relate to their unique and specific sensory properties, their convenience, and their alleged rootedness in culinary and cultural heritage (Liu et al. 2011).

Meat processing in Europe started centuries ago with the manufacture of shelf-stable fermented meat products, which are either air-dried or smoked to enhance product stability and diversity. Examples of raw-fermented hams, which are air-dried, are Parma hams (Italy) and Jamon Serrano (Spain) or the heavily cold-smoked Black Forest and Guestphalia hams of Germany. The examples of dry-fermented sausages are the Hungarian or Italian Salami or Spanish Chorizo containing pork only or other European dry fermented sausages made of lean beef, and pork mixes and pork fat. Similar to hams, dry fermented sausages from Southern Europe are mostly air-dried, while the ones from Central and Northern Europe are in many cases cold-smoked. The tradition of meat processing in Asia, especially in China, is much older than in Europe. From China, there is the manufacture of raw-fermented hams, also known as Jinhua hams (Liu et al. 2011), which are similar to the European raw-fermented hams of the Parma (Italy) or Serrano type (Spain). Apart from hams, other traditional Chinese or Asian processed meat products are completely different from the European ones. Most traditional Asian meat products are fermented for shelf-life extension and flavor development (Liu et al. 2011). In this chapter we will give an overview of the fermentation process in meat products and sausages, looking into the current safety and quality attribute of the products, the shelf-life and the trends involved in the manufacturing of the products.

2 Nutritional Role of Meat Products

Meat has traditionally been considered as an essential component of the human diet to ensure optimal growth and development. With a limited range of foods available in societies throughout history, meat was important as a concentrated source of a wide range of nutrients. It is perhaps due to the fact that meat has been eaten as much for enjoyment as for its nutritional qualities that consumption of meat and meat products has increased with the affluence of the consumer. The meat consumption and production figures published by the United States Department of Agriculture and the European Union do not distinguish between fresh meat and processed or fermented meat products. Therefore, only rough estimates can be provided concerning the size of production of fermented meat products.

Approximately 5 percent of the total meat production (carcass weight) is further processed by fermentation. The major producers of fermented meat products in the European Union are Germany, Italy, Spain, and France. In these countries, 20–40 percent of processed meat products can be classified as fermented meat products (Leroy et al. 2013).

The fat content of meat as consumed is around 2–5 percent, even though total fat content varies with species, feeding regimes, and age. The principal fatty acids in meat are saturated fatty acids, including palmitic acid (C16:0) and stearic acid (C18:0). Around 40 percent of the fat in meat is monounsaturated, of which oleic acid (C18:1) is one of the main contributors (Marianski and Marianski 2009).

Protein of high biological value and micro-nutrients such as iron, zinc, vitamin B1, niacin equivalents, and vitamin B12 significantly contribute to the nutritional value of meat (Mann 2000). Iron is one of the most difficult nutritional requirements for humans to be met, because iron deficiency is caused not only by a low intake of food but is also the result of low bioavailability. Increased iron requirements may result from physiological variables or clinical problems. Red meat contains 50 to 60 percent of its iron in the heme form (from hemoglobin and myoglobin), which is absorbed in humans by a more efficient mechanism than the non-heme iron, the source of iron in plant foods (SACN 2010).

An important role of meat and meat products in everyday food culture and consumer health may be questioned by the fact that the populations of vegetarians living in rich countries are characterized by lower rates of cancer and cardiovascular disease (Deipiu 2012). The analysis of dietary patterns, as a possible approach to examining diet–disease relations, identified two major eating patterns defined by factor analysis using dietary data collected from food frequency questionnaires. The first factor, the "prudent dietary pattern", is characterized by a high intake of vegetables, fruits, legumes, whole grains, and fish or other seafood, whereas the second factor, the "Western pattern," which shows a high intake of processed meat, red meat, butter, high-fat dairy products, eggs, and refined grains. A study has been published involving Seventh-Day Adventists, a well-characterized population, in which the effect of dietary intake of nutrients on biochemical parameters in blood and urine were compared to vegetarian and non-vegetarian subjects (Deipiu 2012). Remarkably, the dietary intake of cholesterol was higher in non-vegetarian subjects (560 to 710 mg/d) compared to vegetarians (<20 mg/d) and was associated with elevated serum cholesterol levels in the non-vegetarian population. These results demonstrated a correlation between dietary intake of certain food components (e.g., cholesterol) relevant for diseases (e.g., coronary heart disease) and their blood concentrations.

3 Fermented Meat as Probiotic Foods

Dry fermented meat products are usually not or only mildly heated, which is adequate for the carriage of probiotic bacteria (Ammor and Mayo 2007). In addition, there is reason to believe that the sausage matrix protects the survival of probiotic lactobacilli through the gastrointestinal tract (Klingberg and Budde 2006). However, the potential negative impact of the meat environment on cell viability must be taken into account, in particular with respect to its high content in curing salt and its low pH and water activity due to acidification and drying. In general, cell viability in a fermented meat environment will most likely be strain-dependent. Therefore, the choice of appropriate microorganisms to be applied as probiotic strains in a fermented meat matrix will be important. One obvious possibility is the use of bacteria that are commonly associated with the meat environment and that possess the appropriate physiological requirements and health promoting properties. Such bacteria can be obtained by screening natural sausage isolates (Rebucci et al. 2007) or existing commercial meat starter cultures (Erkkilä and Petäjä 2000) for probiotic properties. Several candidate strains have thus been obtained.

As an example, the commercial meat starter strains *Lactobacillus sakei* Lb3 and *Pediococcus acidilactici* PA-2 may be of interest because of their survival capacities under simulated gastrointestinal conditions (Erkkilä and Petäjä 2000). Likewise, isolates of *Lactobacillus casei/paracasei* from sausages fermented with *Lb. casei*, *Lb. paracasei*, *Lb. rhamnosus*, and *Lb. sakei* were screened for relevant properties (Rebucci et al. 2007). The latter properties included viability in artificial gastric juice and intestinal fluid, *in vitro* adhesion to human intestinal cell lines, organic acid production, and pathogen inactivation. In addition, several *Lactobacillus plantarum* sausage isolates were found to have appreciable adhesion rates towards Caco-2 cell lines and were considered as better adhesive bacteria than *Lactobacillus brevis* and *Lb. paracasei*-group sausage isolates (Pennacchia et al. 2006). However, the demonstration of such properties does not necessarily lead to the consolidation of health-promoting properties, in particular because human studies are generally missing.

Alternatively, the performance of strains with documented health-promoting properties may be investigated in a fermented meat environment. Since such strains are usually human intestinal isolates, they should be able to compete with the natural meat microbiota in an environment which is not their natural habitat. Thus, they need to be able to survive the fermentation and drying process as well as refrigeration and storage conditions, and, preferably, be able to grow to numbers that enable the display of health-promoting effects. In this way, several lactobacilli of human intestinal origin

have been shown to survive the sausage manufacturing process and can be detected in the end-product (De Vuyst et al. 2008).

Unfortunately, most research concerning this strategy focuses on the survival of the added species in the meat matrix and its influence on the technological and sensory characteristics of the final product and not on health effects as such. Also, focus was on pathogen inactivation, as probiotic strains with additional food safety assets could confer added value to healthy fermented meat products. For instance, *Lactobacillus reuteri* ATCC 55730 and *Bifidobacterium longum* ATCC 15708 increased inactivation of *Escherichia coli* O157:H7 during sausage manufacturing (Muthukumarasamy and Holley 2007). *Lactobacillus rhamnosus* FERM P-15120 and *Lb. paracasei* subsp. *paracasei* FERM P-15121 inhibited growth and enterotoxin production of *Staphylococcus aureus* to the same extent as a commercial *Lb. sakei* starter culture (Leroy et al. 2006). In contrast, *Lb. acidophilus* FERM P-15119 could not satisfactorily decrease *Staphylococcus aureus* load, indicating the importance of careful strain selection with respect to both health-promoting and food safety properties.

4 Sausages

4.1 Introduction

Sausages are popular fermented meat products enjoyed by millions of consumer worldwide with many varieties especially within Germany (Mendoza et al. 2001).

The "oryae" or sausage of ancient Greece was mentioned in Grecian literature, which dated back to 850 BCE and the play Orya written by Epicharmus about 500 BCE also mentions about it (Prajapati and Nair 2008). Several sausages that originated from Europe are named after the city of their origin (Francis 2000); for example, bologna (from Bologna, Italy), romano (from Rome), and frankfurter (from Frankfurt) (Merriam-Webster's Collegiate Encyclopedia 2000). In Italy, when the product is partially fermented and requires cooking before consumption, it is called 'salsicca' and dried sausages are generally called "salame" (Francesca et al. 2013). The acceptance of dry-cured sausages relies mainly on sensory quality, and aroma is the most important parameter (Mendonca et al. 2013). The traditional Thai fermented sausage is mainly made up of ground lean pork mixed with lard. This resulted in high fat content of the final product. Thungtakul (1988) reported that the content of fat in Thai fermented sausage was 38.98 percent (dry basis) after 144 hr of fermentation.

4.2 Classification

The USDA classified sausages as fresh, uncooked-smoked, cooked-smoked, cooked, dry and semi-dry, luncheon meats, loaves, jelly products (Francis 2000). Table 1 summarizes the characteristic of each sausage based on the USDA and other classification systems.

4.3 Ingredients and their Roles in Sausage Making

Meat (lean or fatty), fats, carbohydrates sources such as sugars, salt, spices are the common ingredients added in sausage making and variations exists due to regional preferences. Below is a list and a brief write-up of the major ingredients used in the processing of sausages.

4.3.1 Meats

Pork meat, either alone or in combination with beef, is the main ingredient of sausages. In other products like poultry sausages, the main ingredient is poultry. It is important to control the characteristics of all these raw meats, in particular hygienic quality (Marianski and Marianski 2009). Beef meat contributes to a better red color of the product. The same applies to the use of meat from older animals, which are richer in myoglobin (Toldrá 2006a).

4.3.2 Fat

The other main ingredient is fat which must be fresh or kept under frozen storage. It is convenient to avoid long frozen storages because endogenous lipases are active at low temperatures and can generate free fatty acids that are prone to oxidation and development of rancidity (Toldrá and Reig 2007). Soft fats can produce smearing during chopping. Fat unsaturation control through the iodine index and the amount of free fatty acids through the acid index (as an indicator of freshness) are recommended. Granulated fat has an important technological function by loosening the sausage mixture and this facilitates the release of moisture from the inner layer of the products, which is absolutely necessary for well-done fermentation and development of flavor and aroma (Marianski and Marianski 2009).

4.3.3 Carbohydrates

The purpose of added carbohydrates into the sausage formulation is to ensure the presence of sufficient fermentable substrates to enhance the growth of lactic acid bacteria, which will result in the production of organic

Table 1. Classification of sausages based on USDA.

Classification	Characteristics	Smoking	Example
Fresh sausage	Made of fresh or frozen meat, chopped (comminuted) meat that are not cured, no added of nitrite or nitrate, seasoned and usually stuffed into casing (natural or manufactured casing) or chubs. These sausages must be kept under refrigeration and thoroughly cooked before serving	Yes or no	Bockwurst, country-style pork sausage, Italian-style sausage, pork sausage roll, breakfast sausage, whole hog sausage
Uncooked-smoked sausages	Made of either cured or uncured meat, comminuted, seasoned, stuffed and smoked but not cook. These sausages required refrigeration for preservation and must be fully cooked before serving	Yes	Kielbasa, mettwurst, smoked country-style pork sausage
Cooked-smoked sausages	Made of cured meats, chopped or ground, seasoned and stuffed into casing. These sausages do not require further cooking before consuming but are often heated before serving	Lightly smoked	Berlinger, bologna, cotto salami, frankfurters, smoke links, wieners
Dry and semi-dry sausages (sometimes called 'summer sausages')	Made of cured meats, comminuted seasoned and stuffed and air-dried under controlled of time, temperature and humidity conditions. It may or may not be smoked before drying. These sausages utilize bacteria to ferment sugars into lactic acid	Yes or no	Cappicola, chorizo, farmer-cervelat, frizzes, german salami, Italian salami, Lebanon bologna, pepperoni, thuringer
Luncheon, loaves, and jellied products	Made of cured or uncured meats, chopped (comminuted), seasoned and often made as loaves. These products are usually cooked or baked rather than smoked and generally sold in sliced and served cold	Yes or no	Chopped ham loaf, condiment loaf, head cheese, jellied corn beef, luncheon meat, minced ham, peppered load, scrapple, pimento loaf, olive loaf, honey loaf, jellies tongue, head cheese, and souse

Source: Vanichpun 2003

acid in amounts that are necessary for adequate pH drop (Marianski and Marianski 2009). Glucose or saccharose favors a faster pH drop. If an excessive amount of carbohydrates is added, pH can drop to values below 4.7 and most of the enzymes responsible for the generation of flavor compounds can be inhibited and the sensory quality is seriously affected. However, the addition of low amounts or long-chain carbohydrates may result in a deficient pH reduction that will allow the growth of undesirable microorganisms (Toldrá 2006b).

4.3.4 Salt

Salt is an essential ingredient of sausages, usually added at the rate of 2–3 percent. It contributes to the technological and sensory quality of meat through (Toldrá and Reig 2007):

1. Selection of the microbial flora by inhibition of the growth of undesirable microorganisms;
2. A characteristic salty taste of the sausage;
3. An increase of myofibrillar protein (actin and myosin) solubility; and
4. Control of enzyme activity.

4.3.5 Spices and flavorings

There is a wide variety of spices and flavorings agents that can be used for seasoning the sausages. Spices can also contribute to a specific flavor or color. There is a great variety of seasoning formulations for different types of sausages. This includes mustard, oregano, rosemary, garlic, onion, pepper, or paprika. Spices can be used either in a natural form (whole or ground) or as flavoring extracts (essential oils and oleoresins). Flavoring agents and flavor enhancers may be used to accentuate a specific flavor. Smoke flavoring may be applied on the surface as an oil or water solution to give a smoke flavor (Marianski and Marianski 2009).

4.4 Additives Use in Sausage Making

4.4.1 Phosphates

Phosphates and nitrites/nitrates are classified as additives since their incorporations are regulated (Hammes 2012). Phosphates have a wide application in the meat processing industry and improve binding and texture in processed meat products. They directly increase the water-holding capacity by raising the pH as their own pH is alkaline (above 7.0). Phosphates also stabilize the texture of meat products by increasing protein

solubility in connection with salt and reduce lipid oxidation/rancidity and hence the occurrence of negative flavors. Phosphates have also shown the ability to reduce microbial growth. The most common phosphates used in meat processing are [sodium tripoly-phosphate (pH 9.8) and sodium di-phosphate (pH 7.3)]. For meat preparations such as sausage mixes, where phosphates are added as dry powder, phosphates with moderate alkaline effect are preferred, in particular di-phosphates. The usual dose is 0.03 percent. Di-phosphates are the most effective form for increasing water binding. However, di-phosphates have low water solubility. Thus, for meat curing brines containing phosphates, more soluble poly-phosphates can be used (Heinz and Hautzinger 2007).

4.4.2 Nitrite/Nitrate

Nitrite constitutes an essential additive for sausage preservation. Sodium nitrite is formulated in a curing salt. In some cases, potassium nitrate can also be added in the formulation when sausages are ripened for a long time. In this case, nitrate acts as a reservoir of nitrite. Nitrate is reduced to nitrite by nitrate reductase activity from bacteria either naturally present in the mix or added as starter cultures (such as *Micrococcaceae*). Nitrite is very reactive and interacts rapidly with proteins, such as myoglobin, and inhibits the growth of undesirable microorganisms, in particular *Clostridium botulinum*. Despite its effectiveness against pathogens, the amounts of nitrate and/or nitrite added to the initial mixture are kept at a low-level to keep residues in the finished product as low as possible. The reason for this is that nitrites can react with secondary amines, producing nitrosamines, which can exert toxic (i.e., carcinogen) effects. Ascorbic and erythorbic acids or their sodium salts are used to accelerate the reduction of nitrite to nitric oxide, assuring the absence of residual nitrite in the products and thus avoiding the risk of nitrosamine formation (Marianski and Marianski 2009).

4.5 Fermentation of Sausages

Meat fermentation processes practice today are fundamentally little different from the processes used in ancient times. The manufacture of fermented sausages contains several steps such as (1) selecting, weighing and mixing the ingredients; (2) stuffing the mixture into casings; (3) fermentation under controlled conditions (i.e., temperature and humidity) and (4) post-fermentation maturation. Comminuted lean meat and its fat are mixed with salt, a source of fermentable carbohydrate, spices and other flavors, and extruded into casings. These are held at a controlled temperature, typically 30°C. Air is largely excluded from the interior of the casing so that aerobic

metabolism is inhibited. Rather than the generation of carbon dioxide and water, the carbohydrate is converted to lactic acid. In the presence of salt and absence of air, the lactic acid bacteria proliferate at the expense of other microbes and lower the pH as lactic acid accumulates over a period of days. Fermented sausages are usually partially dried or/and smoked during or after fermentation (Wang 2012). The microorganisms that are present at the beginning of sausage fermentation usually include yeast and anaerobes, psychrotropic and mesophilic bacteria, out of which some are responsible for spoilage, and some of which can be pathogenic (Cocolin et al. 2004). Mendonca et al. (2013) isolated 353 yeast strains from artisanal country style and industrial style in Spanish dry-cured sausages; the majority of strains identified belonged to the genus *Debaryomyces*, followed by *Candida*, *Rodoturala*, *Trichosporon*, *Yarrowia* and *Pichia*. Based on the analysis carried out, they have concluded that these yeasts may contribute to the development of the sensory characteristics of final products.

During fermentation, both chemical and biochemical changes, as well as sensory changes take place in the meat mixture. A distinct gel-like texture is developed as a result of the cumulative effects of bacterial acidification of the protein components, the solubilization of the salt-soluble proteins leading to the emulsification of the fat globules (Ravyts et al. 2012). Starter cultures are used to control nitrate reduction in the fermentation process (Hammes 2012). A very comprehensive tabulation of the processing features of fermented sausages was compiled by Vanichpun (2003) and shown in Table 2.

4.5.1 Fermentation microflora of sausages

The microbiology of fermented sausage is varied and complex. Sausage with a short ripening time have more lactobacilli right from the early stages of fermentation, and will give an acidic flavor. Those with longer ripening times contain higher numbers of *Micrococcaceae* in the early stages of fermentation, and these sausages will have a low rate of acid production, while the protease and lipase activities release various aromatic substances including organic acids (Cocolin et al. 2001). Kröckel (2013) reported that the flora found in fermented meat products include *Lactobacillius sakei*, *Lb. curvatus*, and *Lb. plantarum,* which exceed 10^6 cfu (colony forming units)/g at the end of fermentation period, whether the products are inoculated or not. *Kocuria varians* (formerly *Micrococcus varians*), *Kocuria kristinae* (formerly *Micrococcus kristinae*), and especially *Staphylococcus warneri, S. saprophyticus, S. carnosus,* and *S. xylosus* could be present as well. In general, lactic acid bacteria (LAB) are commonly isolated from meat and constitute a part of the initial microorganisms that can develop easily after meat is processed to fermented sausages (Olaoye and Ntuen 2011).

Table 2. Processing features of fermented sausages.

Type		Process	Product	Process Feature
Spanish sausage	Fermented	Smoked/ dried	Chorizo	Made of minced meat, beef or beef and pork or pork fat, mixed with salt, paprika, other spices and additives before inserted into natural or artificial casing, heavily smoked, ripening and drying are carried out under natural climatic conditions
Spanish sausage	Fermented	Smoked/ dried	Androlla	Manufacture in Galicia. It is made from low-quality pork, salt, sweet and spice paprika, garlic, and sometimes onion is added. Smoke-heating process for 1–10 d before drying-ripening process for 1–2 mon, consumed after cooking
Spanish sausage	Fermented	Cured/ dried	Salchichón	Manufactured from mixture of chopped pork and/or beef, lard, curing agents (salt, spices, nitrate, nitrite, sugar, and ascorbates), and whole or ground black pepper (piper nigrum). After mixing and casing, sausage is subjected to fermentation (20–24°C, 48 hr, relative humidity 90–95 percent), and ripening (12–15°C) for about 1 to 8 mon
Spanish sausage	Fermented	Smoked/ dried	Botillo	Made from pieces of ribs, vertebrae and other pork bones with their freshly parts, skin and lard. Meat mixed with salt, sweet and spicy paprika, garlic and marjoram. Left to stand for 48 hr before stuffed into pork caceum. Undergoes heating-smoking process for 7–15 d and left to dry and ripen for a rapid of up to 3 mon and consumed after cooking
Germanic sausage	Fermented	Smoked/ dried	Salami	Made from cured meat, fermentation takes place at 20–32°C under humidity 85–95 percent for 18–48 hr if a starter culture is added, or 5–9 d if not. It is usually hot-smoked to an internal temperatures of 55–63°C, dried slowly at 15–24°C for several weeks or months
Italian sausage	Fermented	Smoked/ dried	Naples-type salami	Made of coarsely minced lean pork, with fat, cut into small pieces, addition pepper, salt and skimmed milk powder, stuffed into natural or artificial casings, stewed for some hours at around 30°C, smoking, ripened at low temperature and relative humidity for 30 d
Italian sausage	Fermented	Cured/ dried	Milano salami	Made of fresh meat and fat mixed with ingredients (e.g., Skimmed milk powder), additives (e.g., nitrates, nitrite, antioxidants, spices, etc.), and sometimes starter cultures. After stuffing and casing, sausage was ripened at 15–25°C for several days, then changed to ripen at temperature 9–13°C up to 3 mon

Source: Coppola et al. 2000, Vanichpun 2003

Pereira et al. (2001) reported that *Lactobacillus* sp. is the lactic acid bacteria most commonly found in fermented meat products that depends on the

Table 3. Lactic acid bacteria and other organisms isolated from fermented sausages.

Fermented sausages	**Isolated strains**
French dry sausage	*Lb. sakei, Pd. pentosaceus, Pd. acidilactici, Staph. camosus, Staph. wameri, Staph. saprophyticus*
Spanish fermented sausages (Salchichón, Fuet, and Chorizo)	*Lb. sakei, Lb. plantarum, Lb. curvatus, Staph. xylosus, Staph. saprophyticus, Micrococcus varians*
Sucuk (Turkish style sausage)	*Lb. sakei, Lb. plantarum, Lb. curvatus, Lb. brevis*
Greek dry salami	*Lb. sakei, Lb. curvatus, Staph. saprophyticus, Staph. epidermidis, Staph. aureus, Staph. xylosus, Staph. cohnii, Staph. gallinarum*
Fermented Italian sausage	*Staph. xylosus*
Nham (fermented pork sausage in Thailand)	*Lactobacillus* sp., *Pediococcus* sp.
Isan sausage (fermented sausage in Thailand)	*Lb. plantarum, Pd. cerevisiae, Lb. brevis, Pd. halophilus*
Portugese dry fermented sausage	*Lactobacillus* sp., *Lb. homohiochii, Lb. curvatus*
Naples-type salami (italian)	*Lb. sakei, Lb. bavaricus, Debarymyces* sp., *Staph. xylosus, Staph. saprophyticus*

Source: Vanichpun 2003, Liu et al. 2011
Lb.= Lactobacillus; *Pd.= Pediococcus*, *Staph.= Staphylococcus*

availability of an adequate nitrogen supply. Table 3 shows the lactic acid bacteria and other organisms isolated from fermented sausages.

Lactic acid bacteria (LAB)

The LAB are usually the predominant component of the microbial population in fermented sausages (Bruna et al. 2001). The bacteria have two major functions in fermented foods. Firstly, they generate certain beneficial physiochemical changes in the food ingredients, e.g., acidification, curdling, and production of flavor compounds. Secondly, they inhibit the growth of microbial pathogens and spoilage organisms. Both functions are mainly accomplished by the production of microbial metabolites (Vereecken and Van Impe 2002).

The growth of LAB is dominant during sausage fermentation because bacteria enjoy the advantage of selection in the environment of fermented sausages, and their growth cause a breakdown of sugar, which results in the formation of organic acid, mainly lactic acid and other acids including acetic acid (Liu et al. 2011). Generally, lactic acid is present in two forms,

which are L and D, depending on the nature of microorganisms responsible for the fermentation and environmental conditions. Montel (2000) reported that *Lactobacillus* sp. and *Pediococcus* sp., which are homo-fermentative LAB mainly contribute to L- and D-lactic acid from carbohydrate catabolism, while the hetero-fermentative LAB species of *Lactobacillus* and *Leuconostoc* sp. produce acetate, CO_2, and ethanol. The ability of LAB species to produce L(+)-, D(–)-, or DL-lactic acid is determined by the type of enzyme lactate dehydrogenase (L, D or mixture of both) it contains (Ray and Joshi, chapter 1 in this book). Bello et al. (1997) reported that the ratio between D- and L-lactate varies somewhat depending on the species of LAB present in sausages, but approximately equal amounts of D- and L-lactate are present in the final products.

Homo-fermentative and hetero-fermentative lactic acid bacteria

The LAB can be divided into two groups depending on whether their main pathway of glucose fermentation is the Embden-Meyerhof-Parnas (EMP) pathway (Homo-fermentative) or a combination of the hexose monophosphate (HMP) followed by the phosphoketolase pathways (PKP) (Hetero-fermentative) (Ray and Joshi, Chapter 1 in this book). The LAB that use the EMP pathways have phosphoenol pyruvate-dependent sugar transporting phosphotrasferase systems (PEP-PTS), while those that use PKP do not. Indeed, the PTS has been reported to be widespread in homofermentative lactic acid bacteria but absent from heterofermentative bacteria.

Lactic acid bacteria and acidification process

The production of acid is an important process and is considered as very important attribute in dry sausage manufacture (Kameník et al. 2013); for aroma and flavor formation. The particular acid flavor of these products is due to the production of lactic acid that provide mild flavor, while other acids such as acetic, pyruvic, formic, and butyric acid contribute to strong flavor (Tan et al. 2007). According to Liu et al. (2011), lactate and acetate are often suggested to be major contributors to acidic aroma and taste of fermented sausages, and the combinations of the two organic acids give the sausages their typical "tangy" taste. Kröckel (2013) suggested that the acidic taste in fermented meat products is possibly correlated to D-lactate content, which is an acid purely from bacterial origin, and that *Lactobacillus sakei, Lb. curvatus, Lb. plantarum, Pediococcus pentosaceus,* and *Pd. acidilactici* have a strong influence on the production of this acid.

The acidification process is also important in texture development (Montel 2000). As acidification progresses, the coagulation of the soluble protein gives the desired texture by helping to cement the pieces of meat and fat together, which results in a compact and firm texture that can be sliced (Garcia-Varona et al. 2000).

Starter cultures for sausage fermentation

During fermentation, the initial microbial load and type usually varies but is very similar to that of the starting material such as the raw meat or pork or chicken, which ranges from 10^5 to 10^6 cfu/g (Kuo and Chu 2003). Natural fermentation relies entirely on the presence of LAB in the raw meat and handling the meat in such a way that gives the process the chance for success, however, the LAB are invariably present and sometimes the initial load is very low and this leads to the failure of fermentation (Kuo and Chu 2003). Back-slopping inoculation is the process that provides more successful fermentation compared with natural fermentation. It occurs by the addition of sausages from the previous batch, which contain large number of lactobacilli, directly into the mixed ingredient of a new batch to encourage an establishment of the desirable microflora and ensure a rapid rate of acid formation (Liu et al. 2011).

The use of starter cultures is by far the most common method adopted because of greater control and more consistent results, reduced fermentation time, decreased opportunity of spoilage and improved food safety, enhanced color and flavor development (Leroy et al. 2006). The primary LAB successfully utilized as starter cultures are *Lactobacillus* sp., *Pediococcus* sp., and *Micrococcus/Staphylococcus* sp.

Culture-independent approach for the identification of microflora associated with the production of sausages

Cocolin et al. (2006b) evaluated the ability of a commercial starter culture to perform 28 d sausage fermentation, combining PCR and DGGE analysis. As was declared on the starter culture label, LAB isolated from the starter belonged to *Lb. plantarum* species. CNC strains of *Streptococcus xylosus*, together with *S. carnosus*, were also isolated and identified in disagreement with what was declared from the factory producing the starter. Recently, PCR-DGGE was also used to investigate the yeast populations in Italian fermented sausages (Cocolin et al. 2006a). The work highlighted the dominance of *Debaryomyces hansenii*, which was already present, as the major species, at the beginning of the fermentation. This species was usually accompanied by *Candida zeylanoides* and only at the end of the fermentation, by *Metschnikowia pulcherrima*. At the start of the fermentation, a high

biodiversity was observed as five species could be identified; apart from *D. hansenii*, the species *Pichia triangularis*, *Candida parapsilosis*, *Saccharomyces cerevisiae* and *Sterigmatomyces elviae* were found.

5 Flavor Development and Aroma Generation in Fermented Meat Products

Flavor development is a complex phenomenon caused by the combination of different types of acids, which is affected by the selection of ingredients, processing methods and fermentation processes. The flavor of meat products and sausages are attributed to both volatiles and non-volatile components generated during fermentation. Majority of reports linked this characteristic to mainly lactic acid and acetic acid that produce the "acid flavor" note, however, the contribution of specific yeast strains on the flavor and texture development have been also documented (Mendonca et al. 2013). This is directly due to the ability of some yeast species to ferment different sugars and produce ethanol, acetaldehyde, ethyl acetate and other compounds. Yeast also plays a synergistic role by metabolizing the lactic acid present in the fermented products, then causing a shift in pH towards neutrality and producing a sweeter end product (Dura et al. 2004). Olivares et al. (2009, 2011) determined the effect of fat levels on aroma generation in dry fermented sausages, they found that the aroma was generated by lipids of highest odor-activity values and that the reduction in fat content resulted in the decrease of lipid derived volatiles, and consumer preference was related to aroma compounds hexanal, 2-hexanal, 2-nonenal, 2,4-nonadienal, ethyl butanoate and 1-octen-3-ol which contributed to green, medicinal, tallow, fruity and mushroom notes.

6 Development, Quality and Safety Issues of Fermented Meat Products

Product quality and safety is probably the most important aspect of fermented meat and poultry because it addresses the question of consumer acceptance and public health safety. While a processor may produce a wonderful sausage, the product must ultimately satisfy the consumer in terms of color, texture, taste, flavor, packaging. In the current political and social climate, food safety is a high priority (Quintavalla and Barbuti 2007).

6.1 Development of Fermented Meat Products

In genetic engineering the use of starter cultures for bioamines (bioamine is biogenic substance with one or more amine groups) products and product

development is a new area. By the use of genetic engineering we can get better production and activity of microbial protease, lipase, catalase, nitrate reductase. By this technique we can give newest properties, or make stronger the popular ones already present in the microorganism. By the use of this technology it is also possible to transfer the new gene from one to other microorganisms so that it can generate aroma components, vitamin and certain desirable metabolites. In addition to investigation on the conventional methods of meat fermentation, there are new elegant techniques to produce superior cultures with stronger activity that favors a good fermentation process. A lot of studies have been conducted in the field of meat fermentation. However, we are yet away from the thoughtful entire interrelations involving the microbiology, the technology and external factors used in fermentation and ripening procedures (Singh et al. 2012).

6.2 Shelf life and Effects of Processing Techniques on the Microbiology of Fermented Meat Products

Meat and meat products are consumed in almost all communities of the world. However, it gets easily contaminated by pathogenic microorganisms present in animals prior to being slaughtered. Fermentation of meat sausages, for example using selected LAB strains, strongly inhibit the spoilage bacterial growth but leave the organoleptic properties of the products intact (Aderiye et al. 2006). Thus, LAB strains can be effectively used to preserve meat products for quality purposes. Fermented meats are an archetype of what has been described as the hurdle or multiple barrier concept of food preservation in which the overall antimicrobial effects is the aggregate result of a number of antimicrobial factors. In the case of fermented meats, keeping quality and safety depends on the reduced water activity/salt, nitrite, the antimicrobial effect of herbs, and the activity of the LAB. These hurdles do not all apply at the start of processing but accumulate sequentially as processing proceeds (Leistner and Gould 2002). Of these, the LAB has attracted considerable attention since their acceptability as food additives would make any antimicrobial effect they achieve acceptable too. Generally, the finished product should conform to the microbiological guidelines, as previously described by Gilbert et al. (2000), with particular emphasis on faecal indicator organisms and pathogens as detailed (Table 4). In addition, the US guidelines have also recommended the detection of thermonuclease and enterotoxin as *Staphylococcus aureus* may initially be present and produce toxin but is gradually killed off during the fermentation process (Schelin et al. 2011).

Table 4. Guidelines for microbiological quality of fermented meats.

Criterion	Microbiological quality (CFU/g unless stated)			
	Satisfactory	**Acceptable**	**Unsatisfactory**	**Unacceptable/potentially hazardous**
(i) Indicator organisms				
Enterobacteriaceae	<100	100 to $<10^4$	$\geq 10^4$	N/A
E. coli (total)	<20	20 to <100	≥100	N/A
Listeria sp. (total)	<20	20 to <100	≥100	N/A
(ii) Pathogens				
Salmonella sp.	Not detected in 25 g			Detected in 25 g
Campylobacter sp.	Not detected in 25 g			Detected in 25 g
E. coli O157:H7 and other	Not detected in 25 g			Detected in 25 g
VTEC				
L. monocytogenes	<20	20 to <100	N/A	≥100
S. aureus	<20	20 to <100	100 to $<10^4$	$\geq 10^4$
C. perfringens	<20	20 to <100	100 to $<10^4$	$\geq 10^4$
Bacillus cereus and other pathogenic *Bacillus* sp.	$<10^3$	10^3 to $<10^4$	10^4 to $<10^5$	$\geq 10^5$

Source: Gilbert et al. 2000, modified

6.3 Microbial Contamination and Sanitation of Fermented Meat Products

Although a few microbial species are beneficial for meat in the context of human consumption, the unattractive changes in meat due to microbial spoilage can still occur (Rovira and Puszczewicz 2008). Figure 1 explains the possibility of microbial contamination in the meat processing chain and how to prevent it. The recognizable symptoms due to identified microbial activities are listed in Table 5.

Table 6 shows microorganisms associated with meat deterioration and their optimal growth temperature. Obviously, either cold or heat treatments can create an unfavorable environment to the growth or survival of spoilage microorganisms.

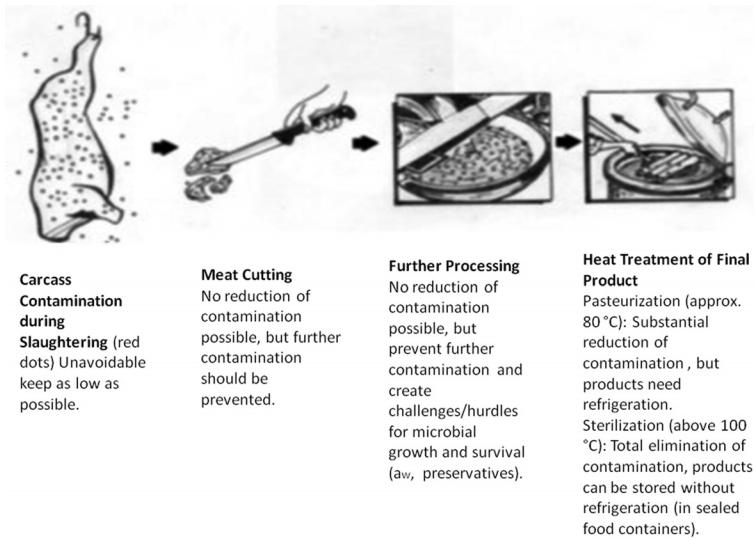

Fig. 1. The possibility of microbial contamination in the meat processing chain and how to prevent it.

Color image of this figure appears in the color plate section at the end of the book.

6.4 Safety Issues of Fermented Meat Products

According to the EC Regulation 2073/2005 (European Commission 2005), the growth of *Listeria monocytogenes* is not favored in ready-to-eat foods with pH ≤4.4 or water activity less or equal to 0.92 or pH ≤5.0 and water activity less or equal to 0.94. For foods that accomplished these conditions, a food safety criterion of 100 cfu/g of *Listeria monocytogenes* along shelf life has been

Table 5. Superficially recognizable symptoms of microbial spoilage of meat.

Oxygen status	Type of microorganisms	Symptoms of spoilage
Present	Bacteria	- Slime on meat surface - Gas production and off-odors and taints - Discoloration by destruction of meat pigments or growth of colonies organisms - Fat decomposition
Absent	Bacteria	- Purification accompanied by foul odors - Gas production - Souring
Present	Yeasts	- Yeast smile - Off-odors and tastes and - Discoloration - Fat decomposition
Present	Moulds	- Surface stickiness and "whiskers" - Odors and taints - Discoloration - Fat decomposition

Source: Wang 2012, modified

Table 6. Microorganisms associated with meat deterioration.

	Growth interval	Microorganism
Psychrophiles	–5 to 35°C	*Pseudomonas* sp., *Achromobacter*
Mesophiles	15–to 45°C	*Escherichia coli, Bacillus subtilis*
Facultative thermophiles	24 to 54°C	*Streptococcus thermophiles, Clostridium perfringens*
Thermophiles	45 to 75°C	*Clostridium thermosaccharolyticum, Bacillus stearothermophilus*

Source: Hernández-Macedo et al. 2011, modified

established (Hospital et al. 2012). In the same study, they have concluded that nitrate and nitrite exerted a significant effect on the typical microbiota of dry fermented sausages and effectively contributed to the control of *Listeria*. The application of E-beam radiation at ≤2 kGy had negligible effect on the sensory attributes; however, the treatment resulted in the achievement of 'zero tolerance' criterion for 5 strains of *L. monocytogenes*, *Salmonella enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium (Cabeza et al. 2009).

6.4.1 Formation of biogenic amines

Despite the popularity of low-acid fermented meat sausages, the presence of high salt and fat is a major public concern (Muguerza et al. 2004), and they may contain high levels of biogenic amines (Suzzi and Gardini 2003).

Papavergou et al. (2012) reported that the retail fermented meat products in Greece sampled in their studies showed high concentrations of tyramine, putrescine, histamine and cadaverine, which ranged from 0 to 510 mg/kg sample. Twenty-eight percent of the samples contained histamine that exceeded the toxicity level of 100 mg/kg used as the threshold in fish.

6.4.2 Food poisoning from fermented meats and sausage consumption

More importantly, there have been several recent reports which highlight the significance of fermented meats being a source of food-poisoning organisms, resulting in outbreaks (Table 7). However, it should be remembered that outbreak cases represent the minority of all laboratory-confirmed cases of gastrointestinal infection and compared to other meat products, the number of outbreaks due to fermented meat consumption is relatively small. Of concern has been the appearance of outbreaks of verocytotoxigenic *Escherichia coli* associated with fermented meats. However, it is difficult to estimate the true incidence of outbreaks and indeed sporadic cases of gastrointestinal infection associated with fermented meats, as reliance on

Table 7. Food-poisoning outbreaks and foodborne illness associated with the consumption of fermented meats.

Organism	**Food type involved**	**Number of people affected**	**Country**
(i) Confirmed outbreaks			
E. coli O157:H7	Genoa salami	39	Ontario, Canada
E. coli O157:H7	Dry cured salami	23	Washington state and Northern California
E. coli O111:H$^-$	Mettwurst	21	Adelaide, S. Australia
S. typhimurium DT124	Salami sticks	101	England
S. typhimurium	Fermented pork, Bologna-style sausage	17	Netherlands
S. typhimurium PT193	Salami	83	Italy
C. botulinum	- Fermented beaver tail and paw - Fermented trout - Fermented meats	14 2	- Southwest Alaska, USA - Hedmark, Norway - Canada
(ii) Epidemiological association			
Toxoplasma gondii	Salami	-	Poland
Salmonella and *Staphylococcal* food-poisoning	Dry fermented sausage	-	Netherlands
L. monocytogenes	Salami	-	Philadelphia, USA

Source: Schjønsby 2002, modified

the peer-reviewed literature alone might underestimate the true position since by definition, data published in the literature should be original and may not accurately reflect all actual outbreaks. The production of fermented meat gives rise to several possible hazards, including mainly: (a) use of contaminated raw meat with food-poisoning organisms, especially the verocytotoxigenic *E. coli* and (b) slow and inefficient fermentation process, i.e., failure to lower pH significantly and quickly, an anti-lactic acid bacteriophage activity (Quintavalla and Barbuti 2007).

6.4.3 Nitrite concern

Over consumption of high salt and high fat foods such as processed and fermented products like sausages have increase the risk of many populations around the world to heart-related diseases and alternative methods of preservation continue to be sought. Throughout the industrialized world, a growing concern for health has increased the interest in protein sources that are low in fat and cholesterol (Liu et al. 2011). Currently, health-conscious consumers try to reduce dietary fat intake by consuming low fat, reduced fat or fat-free foods. This has in turn increased consumer demand for low fat content meat products (Muguruma et al. 2003) and resulted in the development of new formulations or modification of traditional food products to contain less fat (Mendoza et al. 2001).

On the other hand, nitrite can cause a harmful effect on humans if added in the wrong dose or inadvertently confused with common salt. Katan (2009) reported that nitrate and nitrite could exert toxic effects and contribute to the total body burden of N-nitroso compounds. Nitrite is lethal to humans in a dose of approximately 1 g, while nitric oxide is an active nitrosating agent, which can react with secondary amines to form carcinogenic nitrosamines. Since the early 1900s, the USDA allowed sodium nitrite to be added maximum of 156 ppm (1/4 ounce or 7 g per 100 pounds of meat) (Marianski and Marianski 2009).

7 Conclusions

Although the manufacturing of fermented meat products and sausages were well established decades ago, due to the different handling requirement and procedures, industrial production practices, globalization of trade and acceptance of non-ethnic foods into new cultures has prompted the need to revisit the whole industry where the concerns on safety and more rapid/mass production is required while still maintaining the originality of the product.

Keywords: Fermented meat, Food safety, Lactic acid bacteria, Meat processing, Microbial population, Probiotics, Sausage

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8

African Fermented Foods: Historical Roots and Real Benefits

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1 Introduction

Traditional fermented foods are an important part of the diet in Africa. Although these have been in existence for centuries, scientific knowledge about them was available only in the latter part of the twentieth century. These foods were as important centuries ago as they are today. Some of the well-known ones include gari, a cassava-based product; ogi, a corn-based product from Nigeria; and mahewu, another corn-based product from South Africa. Much of the developments have been concentrated on the processing technology including the machinery for producing these foods (Olasupo et al. 2010).

The importance of fermented foods in the African food culture can be described as follows: they provide variety in the flavor of existing staples—basically cereals and root crops, they serve as a cheap way of preservation, and they enhance the nutritional quality and digestibility of the raw food

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materials. In addition, some foods that are normally inedible are made edible by fermentation. Recent knowledge has also shown that some of the food products have anti-cholesterolemic effects (Dike et al. 2001), viricidal effects (Gilbert et al. 1983) and anti-tumor and anti-leukemia effects (Esser et al. 1983). Decreased toxicity has also been reported for some products (Odunfa 1985). Some of these foods have been found to provide useful probiotic effects when they are directly consumed (Mathara et al. 2008).

This chapter highlights the importance of the fermented foods as a part of the cultural and traditional norm among the indigenous communities in Africa with special reference to nutritional values, safety and commercial potential.

2 Types of Fermented Foods in Africa

Classification of fermented products is useful when studying African foods, since the many native languages and localities make it difficult to differentiate the products into specific groups. For the purpose of convenience, fermented foods in Africa can be classified as follows on the basis of the substrates used for their preparations or the nature of the finished products (Olasupo 2006):

- Cereal-based fermented foods;
- Root and tuber-based fermented foods;
- Fermented vegetables;
- Fermented milk products;
- Fermented meat products;
- Fermented fish products;
- Alcoholic, non-alcoholic beverages.

2.1 Fermented Cereal-based Foods

Information on details of non-alcoholic cereal-based foods of Africa is shown in Table 1 and Fig. 1.

2.1.1 Kenkey

Kenkey is one of the principal fermented foods consumed in Ghana. It is prepared from fermented ground white corn (maize). To prepare kenkey, the corn has to be ground first into flour and mixed with warm water, followed by fermentation (for 2 to 3 d) into dough. The fermented dough is divided into two equal parts. One part is partially cooked in a large pot of water for about 10 min, stirring constantly and vigorously, after which it is combined with the remaining uncooked dough and mixed well. The

Table 1. African fermented non-alcoholic cereal-based foods.

Food	Area of Production	Substrate	Microorganisms	Textural characteristics
Kenkey	Ghana, Botswana	Maize, sorghum, or millet	*Lactobacillus* sp. and yeasts	Dough (solid)
Kisra	Sudan, Ethiopia	Sorghum	Lactic acid bacteria, yeasts	Dough (solid)
Injera	Ethiopia, Sudan	Sorghum, teff, corn, finger millet	Yeasts, *Lactobacillus* sp.	Dough (solid)
Hussuwa	Sudan	Sorghum	*Lactobacillus saccharolyticum, Gluconobacter oxydans, Acetobacter xylinum, Saccharomyces cerevisiae*	Dough (semi-solid)

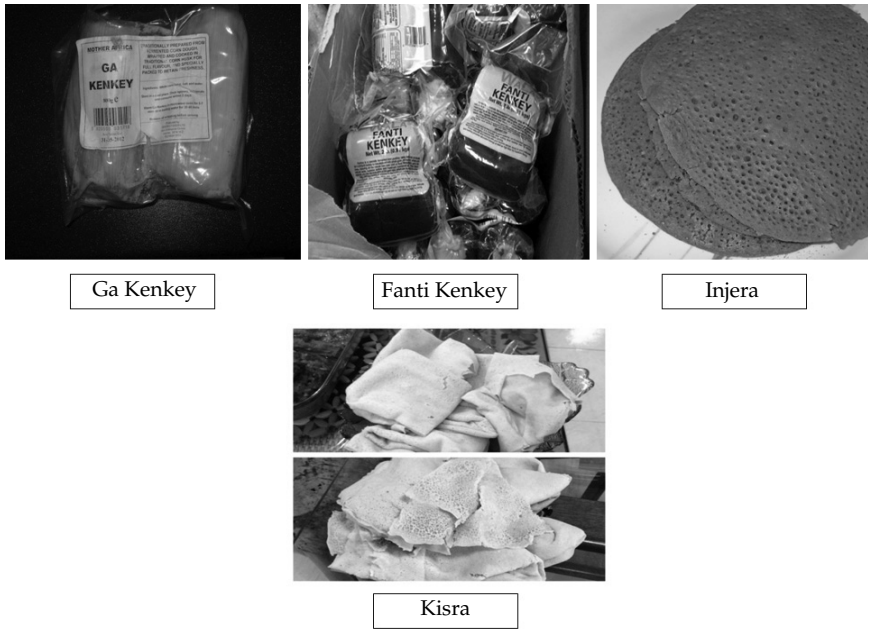

Fig. 1. Some non-alcoholic cereal-based foods of Africa.

Color image of this figure appears in the color plate section at the end of the book.

cooked half of the dough is called "aflata". The aflata-dough mixture is divided into serving-sized pieces and wrapped tightly in banana leaves, cornhusks, or foil. The wrapped dough packets are placed on a wire rack above water in a large pot and allowed to boil for 1–3 hr. The final product, kenkey is served with a sauce or any fish or meat dish. There are several versions of kenkey, such as ga and fante kenkey (Nyanzi and Jooste 2012).

2.1.2 Kisra

Kisra is a naturally lactic acid bacteria (LAB) and yeast-fermented sorghum pancake-like flatbread. It is baked in round, thin sheets, approx. 1–1.5 mm thick and 30–45 cm in diameter. Ideally it should be supple, soft and moist in texture, but not spongy (Asmahan and Muna 2009). Kisra appears to have considerable potential as the basis for development of a gluten-free sandwich wrap (AwadElkareem and Taylor 2011).

Kisra fermentation is a traditional process whereby sorghum or millet flour is mixed with water in a ratio of about 1:2 (w/v) (Rahman et al. 2010), usually a starter is added by a back-slopping using mother dough from previous fermentation at a level of about 10 percent. Fermentation is completed in about 12–19 hr by which time the pH drops from about 6.0 to less than 4.0 (Asmahan and Muna 2009). The fermented dough is baked into thin sheets and it is eaten with certain types of stew prepared from vegetables and meat (Kohajdová 2010).

2.1.3 Injera

Injera is a leavened, flat round Ethiopian traditional bread made from cereals such as teff and sorghum (Anyango et al. 2011) or from different cereal mixtures: teff and white sorghum, wheat and red sorghum and barley and wheat (Baye et al. 2013).

In making injera, teff flour is mixed with water and allowed to ferment for several days, as with sourdough starter. As a result of this process, injera has a mildly sour taste. The injera is then ready to be baked into large flat pancakes, either on a specialized electric stove or, more commonly, on a clay plate locally called Amharic mittad, Tigrinya mogogo that is placed over a fire. In terms of shape, injera compares to the French crêpe and the South Indian dosa as a circle shaped flatbread and used as a base for other foods. The bottom surface of the injera, which touches the heating surface, will have a relatively smooth texture, while the top will become porous. This porous structure allows the injera to be a good bread to scoop up sauces and dishes (Abiyu et al. 2013).

2.1.4 Hussuwa

Hussuwa is one of the important traditional fermented foods in Sudan. In some parts of the country; it is considered a solid food while in other areas it is a drink (Lei 2006).

Traditionally, hussuwa production relies on spontaneous fermentation by the autochthonous microflora. It is produced from semi-solid paste sorghum flour and sorghum malt in 1:0.5. The paste is left to ferment for

12 hr, after which it is cooked lightly in the form of dough or as pancakes. Both lactic and ethanolic fermentation take place during this process, which, finally results in a sweet-sour product (El Nour et al. 1999, Yousif et al. 2005).

2.1.5 Microbiology of fermented cereal-based foods

Apart from *Lactobacillus* sp., the fermented Ghanaian cereal product kenkey have been found to involve *Pediococcus cerevisiae*, *Leuconostoc mesenteroides*, and *Leuc. fermentum*, which are thought to play a major role in "doughing". *Lactobacillus fermentum* and *Lactobacillus amylovorus* have been suggested to be the predominant microorganisms during kisra fermentation (Asmahan and Muna 2009). Other microorganisms such as *Lactobacillus brevis*, *Pediococcus pentosaceas*, *Acetobacters* sp. and *Saccharomyces cerevisiae* were also identified from kisra (Rahman et al. 2010). The microorganisms involved in fermentation of injera are mainly yeasts, some fungi including *Pullaria* sp., *Aspergillus* sp., *Penicillium* sp., *Rhodotorula* sp., *Hormodendrum* sp., *Candida* sp. and number of unidentified bacteria (Kohajdová 2010). The fermentation of sorghum grain to produce hussuwa was accomplished by bacteria and yeast only. El Nour et al. (1999) found that bacteria *Lactobacilus saccharolyticum*, *Gluconobacter oxydans* and *Acetobacter xylinum* and the yeast *Saccharomyces cerevisiae* appear to be important in the fermentation of hussuwa.

2.2 Root and Tuber-based Fermented Foods

The fermented foods prepared from root and tuber crops such as cassava and yams have been extensively reviewed (Ray and Palaniswami 2008, Ray et al. 2010). A brief description of important items prepared from cassava is given below.

2.2.1 Gari

Gari is a fermented dry granular product obtained by spontaneous fermentation of cassava by LAB, yeasts and other bacteria such as *Bacillus* sp. Sanni et al. (2002) studied the use of *Lactobacillus plantarum* culture in the cassava fermentation as a starter for gari production. The final product (gari) had a lower pH and a greater production of lactic acid (50 g/kg) (dry weight basis) (Fig. 2). *Aspergillus niger* could also be used as starter culture for gari production (Evans et al. 2013).

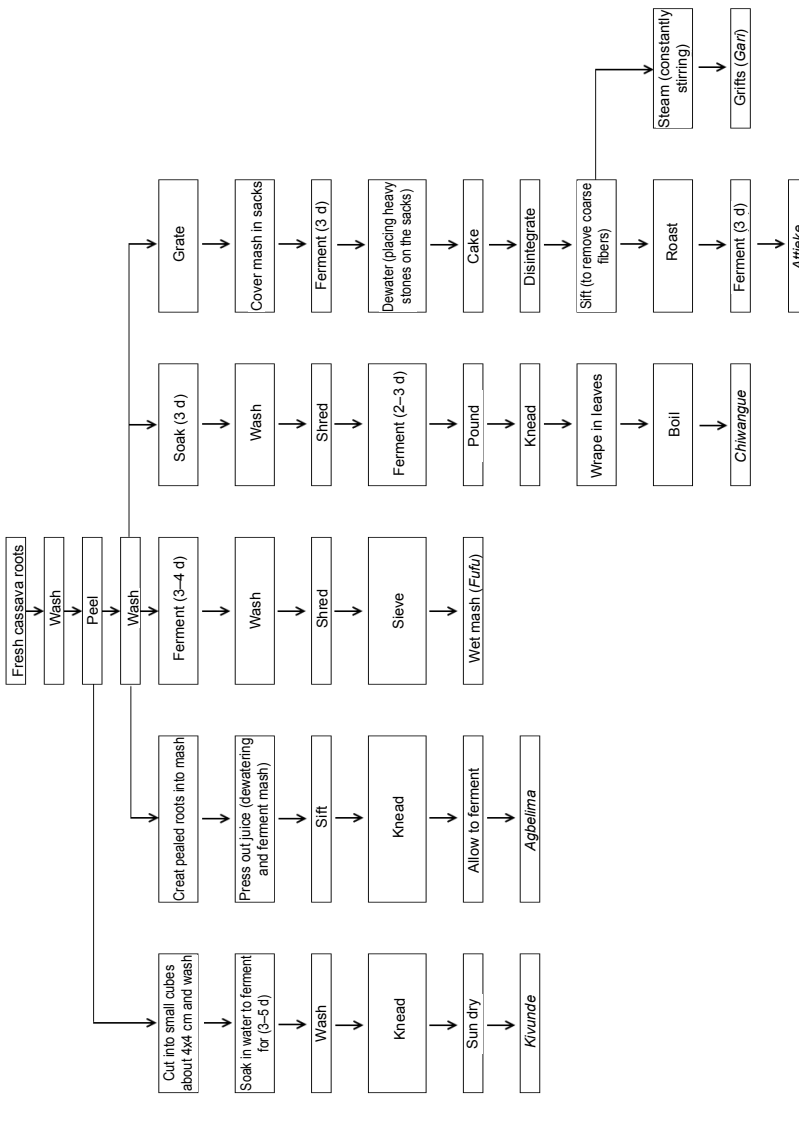

Fig. 2. Flowchart of cassava processing in African fermented products.

2.2.2 Fufu

Fufu is a fermented wet paste product processed from cassava (Evans et al. 2013) (Fig. 2). Fufu can be enriched with protein (from 1.85 to 5.5–8.2 percent) by co-fermentation with different proportion of cowpea and soybean. Improved cassava fufu, "akpu" could be produced by using starter culture (*Citrobacter freundii*, *Geotrichum* sp., *Candida* sp. and *Saccharomyces* sp.), which had a higher protein content than traditionally fermented fufu (Achi and Akomas 2006).

Fufu is traditionally sold in the wet form (moisture content about 50 percent) which renders it highly perishable. One approach to improving its shelf-life and marketability is to produce a dried product. Achi and Akomas (2006) reported that a good quality dried product was produced when wet fufu was air-dried at 65°C at a relative humidity of 60 percent and air velocity of 2 m/S. These conditions reduced the concentrations of butanoic acid but increased the concentration of other volatile constituents (butanol, dimethyl-N-N-formamide, acetic acid, propionic acid, etc.) in the wet fufu.

2.2.3 Lafun

Lafun is a fine powdery cassava product that is prepared by fermentation and is commonly consumed in the western states of Nigeria. The whole or peeled roots are immersed in a stream, in stationary water, or in an earthenware vessel for 3–4 d and fermented until they become soft. The fermented roots are then taken out and the pulp broken into small crumbs and sundried. The dried crumbs are milled into flour. The flour is added into boiling water with constant stirring until a smooth thick paste is formed. The paste is cooled to about 35°C and is then served with soups. The fermented and dried cassava pulp, lafun, is similar to "cossettes" in Zaire and Rwanda, "kanyanga" and "mapanga" in Malawi and "makopa" in Tanzania (Evans et al. 2013).

2.2.4 Kivunde

Kivunde is a fermented food in Tanzania (Fig. 2). Cassava fermentation methods (spontaneous fermentation, back-slopping and the use of starter culture) for the production of kivunde were compared in terms of cyanide level production, microbiology and product quality improvement. *Lactobacillus plantarum* strains were isolated from all types of fermentation based on their enzymatic activities and acid production (Ray et al. 2010).

2.2.5 Chickwanghe (kwanga)

Chickwanghe is the most popular processed food form of cassava in Zaire. Myondo and bobolo in Cameroon, mboung in Gabon and mangbele in Central African Republic also belong to this group. Preparation is similar to fufu preparation (Fig. 2) but it is a very viscous paste, much thicker than fufu (Ray and Palaniswami 2008).

2.2.6 Agbelima

Agbelima is a fermented product popular in the Ivory Coast that is similar to fufu (Fig. 2). The difference from fufu preparation is the steaming step in the final stages (Ray et al. 2010).

2.2.7 Attieke

Attieke is cassava-fermented product consumed in Ivory Coast and in other parts of West Africa. Daouda et al. (2012) reported that the best conditions for the attieke fermentations included an 8 percent v/v inoculum, temperature controlled at 28–30°C, a pH of 3.9–4.0 and 59–62 percent moisture (Fig. 2). Fermentation resulted in an increase in protein content with concomitant increase in amino acids such as aspartic and glutamic acids, followed by phenylalanine and alanine.

2.2.8 Microbiology of root and tuber-based fermented foods

The early stage of fermentation of cassava to produce gari is dominated by *Corynebacterium manihot*. However, several authors have shown that the major role in detoxification of the cyanogenic glucosides is by LAB including *Streptococcus* sp., *Lactobacillus plantarum* and *Leuconostoc* sp. Although other fungi have been isolated, *Geotrichum candida* is the dominant strain in the second stage of fermentation, and is responsible for the characteristic taste and aroma of gari which are a result of production of esters and aldehydes (Sanni et al. 2002).

The special role of LAB in the fermentation of cassava into fufu has been well elucidated. The retting process leading to the softening of the cassava roots during fufu production is known to be due to some pectinolytic microorganisms (Achi and Akomas 2006).

Five major microbial groups, similar to the spectrum of those implicated to other fermented cassava foods, were consistently isolated during lafun preparation. These included species of *Bacillus*, *Klebsiella*, *Leuconostoc*, *Corynebacterium*, *Candida* and *Lactobacillus*. The microbial succession

culminating in the dominance of yeasts and LAB after 48 hr of fermentation was also elucidated (Treche and Massamba 1995). Nwachukwu and Edwards (1987) have isolated five yeasts, two molds, and three bacteria during the fermentative production of lafun, essentially stressing the role of LAB.

Apart from LAB, unique organisms such as *Bacillus* sp., *Candida tropicalis*, and *Penicillium* are important flora in the fermentation of cassava to produce Ghanaian agbelima (Lei 2006). Other cassava products such as chikawgue from Zaire, kivunde from Tanzania, and kocho from Ethiopia are dominated mainly by LAB and yeasts.

2.3 Fermented Vegetables and Fruits

Traditional preservation technologies of vegetables and fruits products have long been practiced in North African areas to make these wholesome foods available throughout the year. Fermentation, pickling, cooking, and/or drying have been the main traditional techniques used to preserve many ripe products available only at given periods of the year such as olives, lemons, onions, green peppers, carrots and so on (Benkerroum 2013). Some important fermented vegetables of Africa are listed in Table 2.

Table 2. Some fermented vegetable-based foods of Africa.

Food	**Area of production/ Consumption**	**Raw material**	**Microorganisms**
Dawadawa or iru	Most of West Africa especially northern parts	African locust bean, soybean	*Bacillus subtilis*, *B. licheniformis*
Ogiri	Southwestern Nigeria	Melon (*Citrullus vulgaris*)	*Bacillus* sp. (predominant), *Proteus*, *Pediococcus*
Ogiri-nwan	Southeastern Nigeria	Fluted pumpkin bean	*Bacillus* sp.
Ogiri-igbo (ogiri-agbor)	Southeastern Nigeria	Castor oil seed (*Ricinus communis*)	*Bacillus subtilis*, *B. megaterium*, *B. firmus*
Ogiri-saro (sigda)	Sierra Leone, Sudan	Sesame seed	*Bacillus* sp.
Ogiri-okpec/okpehe	Middle belt of Nigeria	Mesquite (*Prosopsis africana*)	*Bacillus* sp.
Ugba (Apara)	Eastern Nigeria (Ogun State)	African oil bean (*Pentaclethra macrophylla*)	*Bacillus subtilis*, *Micrococcus* sp.
Owoh	Midwestern Nigeria	Cotton seeds	*Bacillus* sp.
Bukalga	Niger, Mali, Sudan, Burkina Faso	Kartade, red sorrel (*Hibiscus sabradiffa*)	*Bacillus subtilis*

Source: Olasupo et al. 2010

2.3.1 Dawadawa (Iru)

Dawadawa is a fermented product from soybean (*Glycine max*) or African locust bean (*Parkia biglobosa*). It is the most important food flavoring condiment in West Africa and Nigeria (Onyenekwe et al. 2012). Apart from its flavoring attribute, it contributes significantly to the intake of protein, essential fatty acids and B vitamins, particularly riboflavin (Achi 2005b). The flow sheet for the traditional product of dawadawa is shown in Fig. 3a.

2.3.2 Ugba and ogiri

Ugba is gotten traditionally from the fermentation of oil bean seed. It contains up to 44 percent protein, which comprise of at least 17 of the 20 amino acids, and protein digestibility and utilization increases with fermentation (Okechukwu et al. 2012). The oil bean seeds are boiled for 3 hr, de-hauled and cooked, the cooked seeds are then sliced (0.5 to 1 mm thickness) and boiled again for 2 hr, drained, rinsed thrice in water and steeped in cold water for 4 hr so as to eliminate the bitter taste. The sliced beans are wrapped with enough banana leaves (*Musca sapientum*), packed in a clean container and covered to ferment at room temperature.

Ogiri is a condiment obtained from the fermentation of castor oil seeds. The raw castor oil seed are boiled for 2 hr until the seed changes color to brown. The seeds are de-hauled, rinsed in clean water. The boiled seeds are boiled again for 1 hr more. It is then cooled and wrapped with enough banana leaves, which is then packed in a clean container with a cover to ferment at room temperature (Evans et al. 2013).

2.3.3 Pickled fruits

Pickled fruits, made in households or small factories, have been popular in Egypt for centuries (Mheen et al. 1983). They are used as appetizers and served with practically every meal.

Olives

A typical North African traditional olive pickling procedure is presented in Fig. 3b. Pickled olives may be seasoned before consumption by addition of different spices flavoring ingredients including rosemary, coriander leaves, grated garlic, oregano, chopped onion, hot red pepper and/or lemon juice and lemon pieces (Benkerroum 2013). The main organisms causing fermentation are *Lactobacillus plantarum*, *Lb. casei* and *Leuconostoc mesenteroides*.

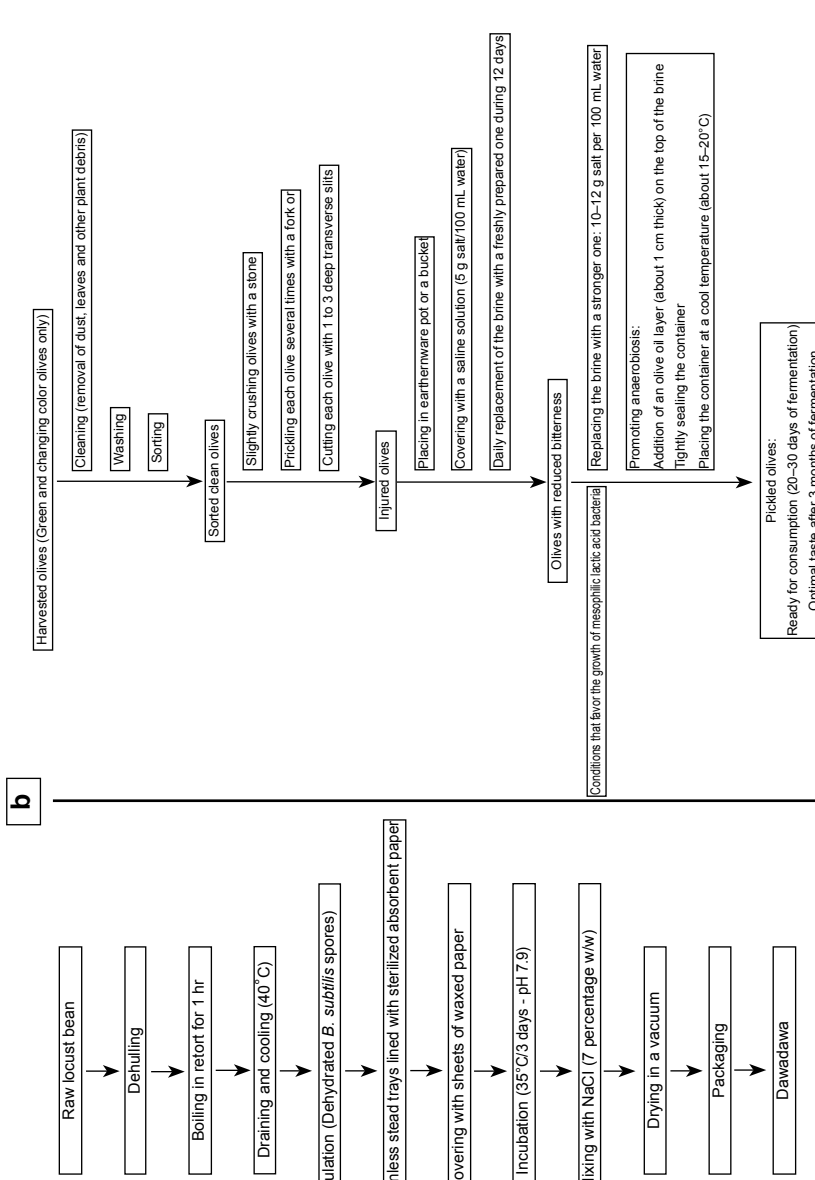

Fig. 3. Flow diagrams for the traditional procedures of (a) Dawadawa (Source: Achi 2005b); and (b) Pickling olives (Source: Benkerroum 2013).

2.3.4 Microbiology of fermented vegetables

The organisms involved in the fermentation of traditional fermented vegetable products are mainly the *Bacillus subtilis* group, which includes *B. subtilis*, *B. licheniformis* and *B. pumilus* (Ouoba et al. 2004, 2007). In iru, ogiri-nwan, ogiri-igbo, and ugba, *B. subtilis* plays a major role. Other notable species of *Bacillus* are *B. licheniformis*, *B. megaterium* and *B. firmus*. *Escherichia* sp., *Proteus* and *Pediococcus* play a minor role in ogiri (Odunfa 1981) while *Staphylococcus* sp. and *Micrococcus* sp. play a subsidiary role in iru (Odunfa and Komolafe 1989) and ugba fermentations. Also, *Staphylococcus saprophyticus* is almost always associated with the fermentation. Others found irregularly are LAB and *Enterobacteriaceae* (Odunfa and Oyewole 1998). The bacterial isolates from ogiri were *Bacillus megatarium*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Eschereichia coli*, *Proteus* sp., *Pseudomonas* sp., *Lactobacillus brevis*, *Lactobacillus plantarum* and *Lactobacillus casei*. They were all used for starter culture fermentation (Falegan 2011).

2.4 Dairy Products

African dairy tradition is indeed a remarkable wealth and carries a lot of know-how and products: fresh milk, cream, sweet or sour fermented milks, solid butter, butter oil, whey, concentrated milk, cheese lean, boiled milk, etc. (Duteurtre 2004).

2.4.1 Fermented dairy products

Fermented milk and butter are subject to a relatively large local trade and long distance. It is estimated, for example, in East Africa, 71 percent of the total milk production of cows is converted into fermented milk (Dieye et al. 2002). Half of this fermented milk is then churned to give butter, produced especially to be sold. The quantities of milk marketed in the form of solid or melted butter represent nearly 25 percent of the total milk production of cows in this sub-region. In Ethiopia, farm butter represents 65 percent of the value of dairy products consumed in Addis Ababa. In West Africa, the fermented milk has the greatest economic importance (Duteurtre 1998).

Table 3 and Fig. 4 present selected traditional fermented African dairy products with a brief description of their technologies.

Table 3. Traditional fermented African dairy products and a brief description of their technologies.

Vernacular name	Description	Main microorganisms involved in fermentation process	References
Leben or lben (Maghreb), or Laban khad/Laban Kherbah (in Egypt)	Fermented milk (buttermilk) obtained by churning spontaneously soured milk to remove butter. A common dairy product in all north African countries through with different names	Lactic acid bacteria (LAB): *L. lactis* subsp. *lactis*, *S. salivarius* subsp. *thermophiles*, *Lb. delbrueckii* subsp. *Bulgaricus*, *plantarum*, yeasts: *Saccharomyces cervisiae*, *Kluyveromyces marxianus*	Tantaoui-Elaraki and El Marrakchi 1987, Benkerroum and Tamime 2004
Wara	Traditional cheese-making process was developed by the nomadic Fulani and is based on the milk-coagulating properties of juice from the leaves of the Sodom apple plant or pawpaw leaves. The juice, obtained by crushing Sodom apple leaves, is mixed with fermented cow's milk gently heated in a pot over a wood fire. After the coagulation, the loose cured pieces are poured into small raffia baskets and allowed to drain	*Lactobacillus* sp., *Leuconostoc* sp., Pediococcus sp., *Lactococcus* sp., yeasts	Aworh 2008, Osuntoki and Korie 2010
Biruni	Cow/camel milk, acidic, semi-liquid drink in Sudan	LAB	Tamang 2010
Butter milk	Cow milk, acidic, drink in Egypt and Ethiopia	LAB	Tamang 2010
Gariss	Camel milk, acidic, Sudanese liquid	LAB	Tamang 2010
Kefir or kefyr	Goat, sheep, or cow milk, kefyr grain, acidic, mildly alcoholic, liquid, effervescent milk in North Africa	LAB, yeasts	Tamang 2010
Mish	Cow/camel milk, acidic, semi-liquid, refreshing beverage in Sudan and Egypt	LAB	Tamang 2010
Rob	Cow, goat, and sheep milk, Mildly acidic savory in Sudan	LAB	Tamang 2010

Amasi	Traditional preparation of *amasi* is similar in both countries where raw milk is poured into calabashes made of gourd or into stone jars. It is then left to ferment for several days. It is now available in South Africa with a shelf life of 21 d at 4°C	*Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *Cremoris*	Gadaga et al. 1999, Mufandaedza et al. 2006
Maziwa lala	Maziwa lala was produced in East Africa. It can be made by use lactic starter cultures	*Streptococcus lactis, S. thermophilus*	Olasupo et al. 2010
Ergo	Ergo is traditional Ethiopian fermented milk produced by spontaneous fermentation using traditional	*Lactobacillus* sp., *Lactococcus* sp.	Amenu 2013
Nono (delicious and refreshing beverage)	Traditionally, nono is prepared by inoculating freshly drawn cow milk with a little of the leftover as starter then is allowed to ferment for 24 hr at room temperature. During fermentation, some lactose is converted to lactic acid. In end of the fermentation, milk butter is removed by churning	*Lactobacilli (L. acidophilus* and *L. bulgaris), Lactococci* sp., *(L. cremoni*, and *L. lactis), Streptococcus thermophilus, Leuconostoc* sp. and *Saccharomyces* sp.	Evans et al. 2013

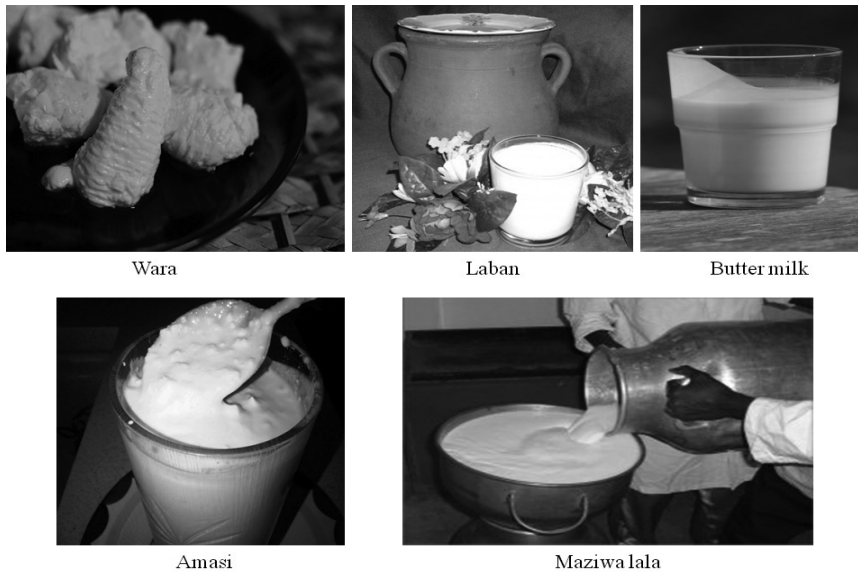

Fig. 4. Selected traditional fermented African dairy products.

Color image of this figure appears in the color plate section at the end of the book.

2.5 Fermented Meat and Meat products

Historically, cured, fermented and dried meat products are regarded as microbially safe, ready-to-eat foods because of their low water activity (a_w) and low pH as well as the presence of curing salts (Lücke 2000). These products have been consumed throughout history, and often have strong cultural associations.

2.5.1 Ndariko

Ndariko (Fulfulde/Peul) is a dried meat product found in the North-Eastern part of Nigeria. It is prepared from the meat of all ruminant animals including camel meat. According to Farouk and Bekhit (2012), ndariko is prepared by cutting meat into long strips about 2 cm thick, the strips are then hung or spread on papyrus mats to dry, which usually takes about 6–7 d. The strips may or may not be salted or spiced (Fig. 5a). The dried finished product is stored in earthen pots, metal containers or sacks made of natural fibers. A similar product to ndariko made from camel meat, which was cut into strips, salted and dried at ambient temperature for about 1 mon, is consumed in Ethiopia (Zegaye 1999).

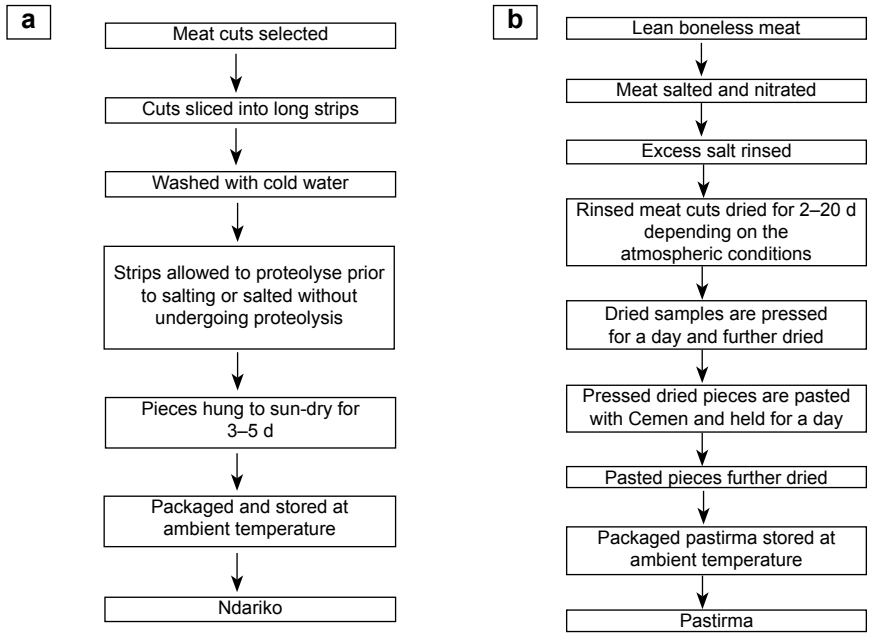

Fig. 5. Flow charts for the traditional manufacturing of (a) Ndariko; and (b) Pastirma (Source: Farouk and Bekhit 2012).

2.5.2 Merguez

Merguez is a typical Maghreb raw sausage with a small diameter (18 to 22 mm) and which does not undergo maturation or drying (Toldrá and Reig 2007), contrary to msrana or sujuk (Fig. 6a). Poultry merguez made from turkey or chicken is being increasingly made in a similar way as the beef variety, but without addition of paprika or other food color additives to keep the typical grayish color (Fig. 6b). Merguez is a highly perishable product and should therefore be consumed within 2 d after preparation. It is usually fried or barbecued to prepare sandwiches. However, in some countries, such as Tunisia and Algeria, it is commonly added as an ingredient in "couscous" (Benkerroum 2013).

2.5.3 Pastirma

Pastirma or basturma is a traditional intermediate-moisture meat product, most popular in Egypt and consists of cured and dried meat strips encased in a mixture of garlic, fenugreek, and various spices (Fig. 6c). It is believed that pastirma has been brought to Egypt from Turkey, most likely during

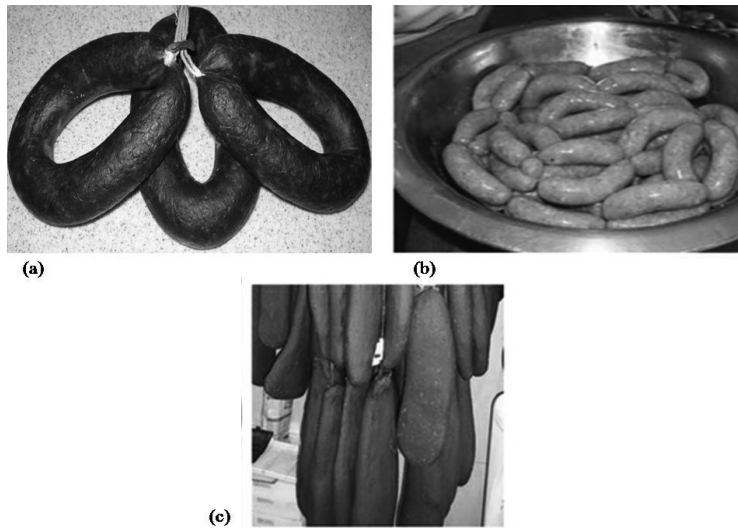

Fig. 6. Some African fermented meat products. (a) Sujuk. (b) Poultry merguez. (c) Pastirma.

Color image of this figure appears in the color plate section at the end of the book.

the Ottoman domination in Middle Eastern and North African areas in the 15th and 16th centuries. In fact, the term "bastirma" means "strong pressing action" in Turkish as pressure is a crucial step in the preparation of the product. The traditional pastirma process is summarized in (Fig. 5b). The finished product should have a pH of 4.5 to 5.8, water activity of 0.85 to 0.90, salt content of around 4.5–6.0 percent, and moisture of 35 to 52 percent (Obuz et al. 2012).

2.5.4 Kitoza

Kitoza is a salted/dried and spiced meat. It is a traditional product of Madagascar but similar products could be found in some other African countries. Depending on the process conditions, the fermentation can be spontaneous. Sometimes, it is smoked in order to improve organoleptic and shelf-stability properties. The first step of the process is the dry salting with coarse salt mixed with spices during a few hours at room temperature. The second step is the air drying in which meat is held in cool, dry, draughty ambient conditions during several weeks. The end product is a salted dried kitoza with a moisture content of 20–45 percent, a salt content of 2–8 percent, a pH of 5–6 and an a_w of 0.65–0.90.

2.6 Fermented Fish and Fish Products

In Africa, fish preservation is accompanied by partial fermentation within a few days during which flavor can be developed in the fish. Essuman (2001) noted that in Africa, fermented fish products are used as condiments especially in the rural areas. In some African countries such as Ghana, Gambia, Uganda, Sierra Leone, Chad, Ivory Coast, Mali and Sudan, fermented fish products are popular (Zakhia and Cuq 1991).

2.6.1 Terkin

Terkin is a processed small-young fish namely Kass (Tiger fish; *Hydrocynus* sp.) and Kawara (*Alestes* sp.) (Abu-Hassan and Adam Sulieman 2011).

Traditional methods of terkin fermentation involve two main techniques:

- Jebel Al-aulia terkin preparation
  Production of terkin uses whole unwashed fish (un-gutted) which is placed in plastic sac and sprayed with little salt, closed tightly and left for 1–2 d until fermentation signs appear. Little boiled-water is added and left till it ripens. The product is stirred continuously until a completely pasted product is achieved and cooled in steel-vessel. After cooling, 10 percent salt is be added and allowed to ferment for 3–4 d. Shelf life of Jebel Al-aulia terkin product is usually 6 mon.
- Wadi Halfa town terkin preparation
  The same fish species are used as in Jebel Al-aulia Dam, but larger in its size. The large fish of Kass and Kawara are collected from fishermen, eviscerated, packed and treated with little salt. The fish are placed in boiled water and stirred continuously until the fish are completely pasted, and left for cooling. Shelf-life of this product is usually 6 mon.

2.6.2 Salanga

The total annual fish production in Chad is estimated at 110,000 t. As much as 52,000 t (47 percent) is processed into partially fermented, sun-dried fish (Essuman 2001).

Salanga is a Chadian fermented and dried fish product, light brown in color, firm texture and has a very strong odor. Usually, salanga is made with fish of poor quality or those not suitable for smoking. Salanga processing method is shown in Fig. 7a. Generally, the raw fish (*Alestes* sp.) is dressed and washed. Larger species of fish are split dorsally and opened up. For the first variant of processing, the dressed fish is dried immediately after

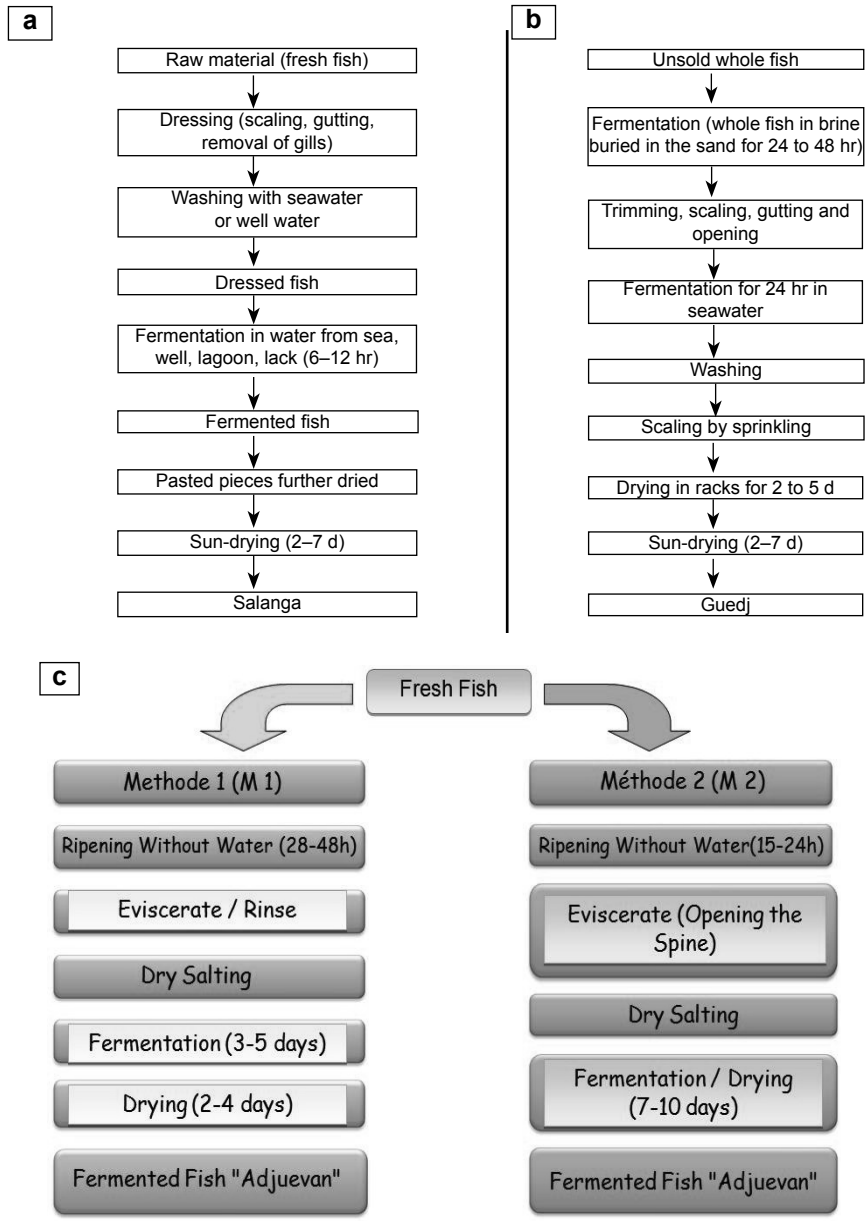

Fig. 7. Flow sheets for the traditional production of (a) Salanga (Source: Anihouvi et al. 2012); (b) Guedj (GRET et al. 1993); and (c) Adjuevan (Kouakou et al. 2012a).

washing and fermentation takes place during drying 6 hr. Regarding the second processing variant, the fish is left to ferment for 12–24 hr before drying. In salanga processing, salt is not used due to its scarcity and high cost (Anihouvi et al. 2012).

2.6.3 Guedj

Guedj is a semi-dry product with a strong pungent smell and a light brown color. However, since the raw fish is derived from poor quality or underutilized species which are cheap, product prices are affordable to many low income consumers. Some types of fermented fish, processed from high-value species and considered a delicacy (e.g., guedj) is however expensive. About 50 percent of the annual fish production in the Gambia is processed into salted, partially fermented and dried products mainly for export to Ivory Coast, Ghana and Mali. Gambians, however, prefer guedj as fermented, salted and sun-dried fish (Essuman 2001).

2.6.4 Adjuevan/Adjonfa

Adjuevan is a traditional Ivorian naturally fermented fish prepared from the Atlantic bumper *Chloroscombrus chrysurus* (Fig. 7c). This product is widely used and appreciated as a condiment in many types of flavorings and cuisines to season sauces for the consumption of yam, plantain, attieke, etc. and not eaten as food fish because of the strong smell (Koffi-Nevry and Koussémon 2012).

The occurrence of lactic acid bacteria flora (LAB) was investigated on Ivorian fermented fish adjuevan products produced with sea fish at different salt concentration (10, 15, 20, 25, 30 percent) (Kouakou et al. 2012b) (Fig. 8). The culture-independent method PCR-DGGE carried out in this study allowed us to describe the adjuevan biodiversity.

2.7 Alcoholic and Non-Alcoholic Beverages

Indigenous fermented foods prepared from major cereals are common in many parts of Africa; some are used as alcoholic and some as non-alcoholic beverages (Table 4, Fig. 9).

2.7.1 Bouza

Bouza is a fermented alcoholic wheat beverage known since the times of the Pharaohs. It is a light yellow thick, sour drink with an agreeable taste and produces a sensation of heat when consumed (Fig. 9a).

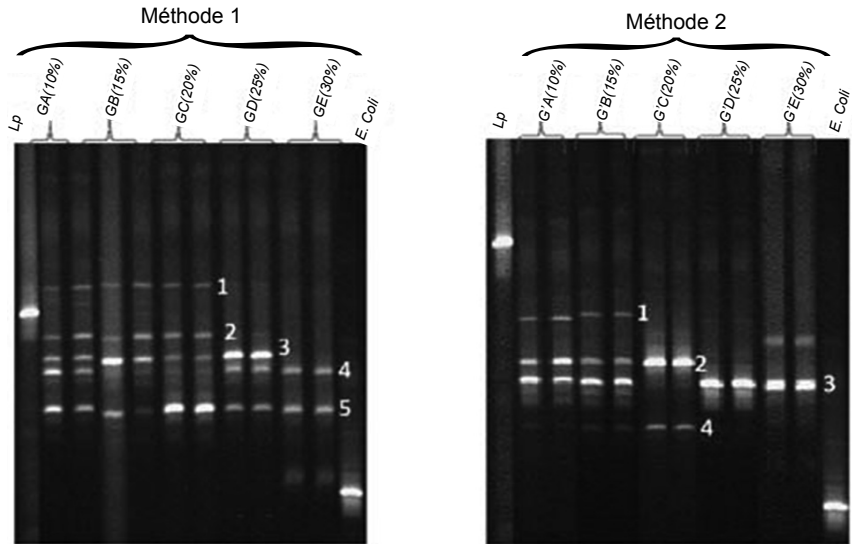

Fig. 8. PCR-DGGE of 16S rDNA profiles of lactic acid bacteria from five samples from adjuevan produced with two processing methods. Bands indicated by numbers were excised and, after re-amplification, subjected to sequencing. Method 1: 1. *Lactobacillus fermentum*. 2. *Lactobacillus delbrekii* subsp. *Bulgaricus*. 3. *Leuconostoc lactis* subsp. *Lactis*. 4. *Lactobacillus helveticus*. 5. *Pediococcus pentosaceus*. Method 2: 1. *Leuconostoc lactis*. 2. *Pediococcus pentosaceus*. 3. *Lactococcus reffinolactis*. 4. *Lactococcus garviae* (Kouakou et al. 2012b).

It is prepared by coarsely grinding wheat grains, placing a portion of them in a wooden basin and kneading them with the water into dough. The dough is cut into thick loaves, which are lightly baked. The remainder of the grains is moistened with water, germinated for 3–5 d, sun-dried, ground and mixed with the loaves of bread, which are soaked in water in a wooden barrel. Bouza from a previous brew is added to serve as an inoculum. The mixture is allowed to ferment at room temperature for a 24 hr period, following which the product is sieved to remove large particles and diluted with water to a desired consistency. Bouza has a very short shelf-life and is expected to be consumed within a day. Its pH decreases to 3.9 and 4.0 and its alcoholic content is usually between 3.8–4.2 percent within a 24 hr period.

2.7.2 Keribo

Keribo is an indigenous traditional, non-alcoholic, dark brown colored fermented beverage commonly consumed in rural and urban areas of Jimma zone, southwestern of Ethiopia, with some similarity to Boza of Bulgaria, Albania and Romania (Blandino et al. 2003). It is produced by an over-night

Table 4. Most common fermented beverages produced from cereals in Africa.

Product	Regions	Substrate	Starter
Bouza	Egypt	Wheat	Unknown
Keribo	Ethiopia	Barley	Lactic acid bacteria (LAB), Aerobic mesophilic bacteria (AMB), Aerobic spore-formers (ASF) and yeasts
Talla	Ethiopia	Sorghum	Unknown
Pito	Nigeria and Ghana	Maize, sorghum or a combination of both	*Candida tropicalis*, *Kloeckera apiculata*, *Wickerhamomyces anomalus*, *Torulaspora delbrueckii*, *Schizosaccharomyces pombe*, *Kluyveromyces africanus*, *Lactobacillus* sp., *Leuconostoc* sp.
Burukutu	Nigeria, Benin, Ghana	Sorghum	*Saccharomyces cerevisiae*, *S. chavelieri*, *Leuconostoc mesenteroides*, *Candida*, *Acetobacter*
Koko	Northern Ghana	Maize, sorghum, or millet	*Lactobacillus* sp. and yeasts
Ting	Botswana	Sorghum	LAB
Busa	Egypt	Rice or millet	*Lactobacillus*, *Saccharomyces*
Busaa	Nigeria, Ghana	Maize	*Lactobacillus helveticus*, *L. salivarus*, *L. casei*, *L. brevis*, *L. plantarum*, *L. buchneri*, *Saccharomyces cerevisiae*, *Penicillium damnosus*
Chikokivana	Zimbabwe	Maize and millet	*Saccharomyces cerevisiae*
Doro	Zimbabwe	Finger millet malt	Yeasts and bacteria
Kachasu	Zimbabwe	Maize	Yeasts
Mangisi	Zimbabwe	Millet	Unknown
Tobwa	Zimbabwe	Maize	LAB
Masvusvu	Zimbabwe	Maize	LAB
Otika	Nigeria	Sorghum	Unknown
Seketeh	Nigeria	Maize	*Saccharomyces cerevisiae*, *S. chevalieri*, *S. elegans*, *Lactobacillus plantarum*, *L. lactis*, *Bacillus subtilis*, *Aspergillus niger*, *A. flavus*, *Mucor rouxii*

Table 4. contd....

Table 4. contd.

Product	Regions	Substrate	Starter
Kaffir beer	South Africa	Kaffir corn	Yeasts, LAB
Sorghum beer	South Africa	Sorghum, maize	Yeasts, LAB
Maheu	South Africa	Maize, sorghum, millet	LAB
Mahewu	South Africa	Maize	LAB
Merissa	Sudan	Sorghum and millet	*Saccharomyces*
Hulumur	Sudan	Sorghum, rice, millet	LAB
Munkoyo	Zambia	Kaffir corn, millet or maize plus roots of munkoyo	Unknown
Aliha	Ghana, Togo, Benin	Maize, sorghum	LAB
Mawè	Benin, Togo	Maize	LAB, yeasts
Kishk	Egypt	Wheat, milk	LAB, yeasts
Tchapalo	Côte d'Ivoire	Sorghum	*L. plantarum, L. fermentum, L. cellobiosis, L. brevis, L. coprophilus, S. cerevisiae, C. tropicalis*
Uji	East Africa	Maize, sorghum, or millet	*Lactobacillus* sp.
Obusera	Uganda	Millet	LAB
Kunu-Zaki	Nigeria	Millet, sorghum	LAB
Bushera	Uganda	Sorghum, millet	*Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus plantarum Lactobacillus paracasei*
Togwa	East Africa and Tanzania	sorghum, maize, millet or maize + sorghum	*Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus cellobiosus, Lactobacillus fermentum, Weissella confusa, Pediococcus pentosaseus, Sacharomyces cerevisiae, Candida tropicalis*
Bogobe	Botswana	Sorghum	Unknown

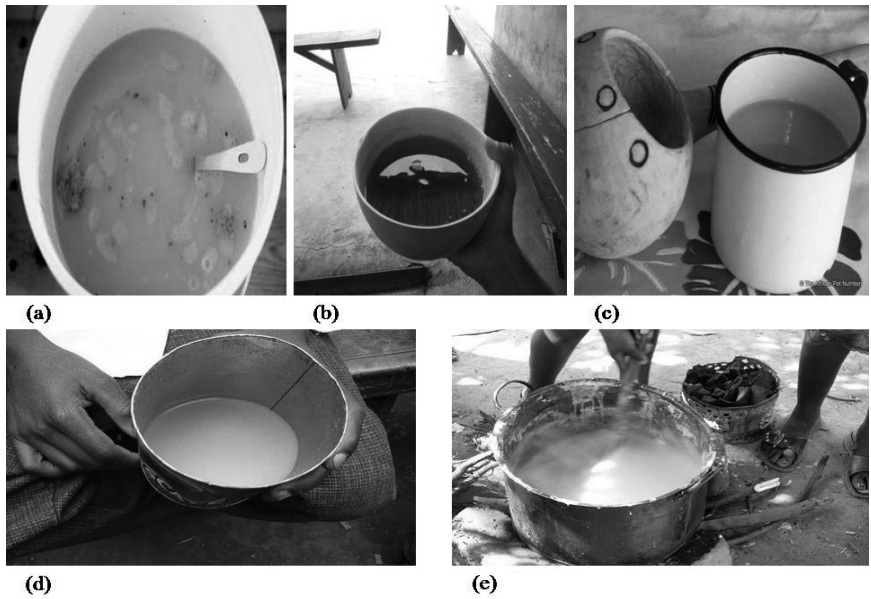

Fig. 9. Some fermented beverages produced from cereals in Africa. (a) Bouza. (b) Pito. (c) Mahewu. (d) Busaa. (e) Munkoyo.

Color image of this figure appears in the color plate section at the end of the book.

fermentation of cereal (barley) predominantly by activities of LAB like the fermentation of shamita (Bacha et al. 1999). High count of LAB could account for acidification of the product with extension of fermentation periods. It has poor keeping quality with shelf-life of not more than a day or two and it has a pronounced characteristic of the deteriorating beverage at the end of 48 hr of fermentation (Abawari 2013).

2.7.3 Pito

Pito is an indigenous Nigerian alcoholic drink produced from local grains such as guinea corn (*Sorghum vulgare*) and sorghum (*Sorghum bicolor*).

The preparation of pito involves soaking cereal grains (maize, sorghum or a combination of both) in water for 2 d, followed by malting, and allowing them to sit for 5 d in baskets lined with moistened banana leaves. The malted grains are ground mixed with water and boiled. The resulting mash is allowed to cool and later filtered through a fine mesh basket. The filtrate thus obtained is allowed to stand overnight, or until it assumes a slightly sour flavor, following which it is boiled to a concentrate. A starter from the previous brew is added to the cooled concentrate which is again allowed to ferment overnight. Pito, the product thus obtained, is a dark brown liquid

which varies in taste from sweet to bitter (Fig. 9b). It contains lactic acid, sugars, amino acids and has an alcohol content of 3 percent. Organisms responsible for souring include *Geotrichum candidum* and *Lactobacillus* sp. while *Candida* sp. are responsible for the alcoholic fermentation. The local method of pito production is similar to methods used to produce other local alcoholic drinks like burukutu except increased pH from 4.2–5.2 within 24 hr of fermentation and sharp decrease of pH to 3.7 after 48 hr (Asiedu 1989). Kolawole et al. (2007) have reported the chemical composition and microbial quality of pito. It has been observed that there was a variation in the nutrient content of pito produced using varying processing methods. The protein content of fermented and unfermented pito showed 2.5 percent and an undetected level respectively. Due to consumers demand for the locally fermented beverages like pito, the bacteriocin producing organisms are considered as a potential source of biological preservatives for such local drinks. Okoro et al. (2011) have reported that bacteriocin increases the protein content of such drinks and does not have the risks associated with common antibiotics and other chemical additives.

2.7.4 Tchapalo

Sorghum grains are malted and used to produce a traditional beverage called "tchapalo" (N'Guessan et al. 2010). The traditional processing of tchapalo is complex and involves milling of malted sorghum, mashing, acidification, cooking, cooling and the alcoholic fermentation of the final wort by dried yeast taken from a previous fermentation. The final product is a beverage that is an opaque sour beer.

3 Fermentation: Safety Valve in African Foods

The nutritional values, safety and shelf-life of the fermented foods are discussed in the following sections.

3.1 Nutritional Values

A large percentage of Africa's over 500 million people live below the poverty line, who consume diets poor in protein and other essential nutrients. Niba (2003) pointed out that protein and quality in grain cereals are improved *via* fermentation as a result of the fact that trypsin inhibitors are depleted, increasing digestibility of various amino acids. It is of interest that raw materials for the production of fermented plant protein product such as iru and ogiri are not normally consumed in their unfermented form. It has been found that fermentation markedly improves the digestibility,

nutritive value, and flavor of raw seeds (Oboh 2006). The organisms involved in these fermentation processes, particularly *Bacillus* sp., produce proteolytic enzymes, which hydrolyze proteins to amino acids and peptides (Leejeerajumnean et al. 2001). *Bacillus* strains from fermenting locust beans have been found to produce glutamic acid and extracellular proteinases (Ogbadu et al. 1990).

The major change in the fermentation of proteins is their hydrolysis to free amino acids and other soluble nitrogen compounds. The amino acids produced vary with each type of seed. The peptides and amino acids are important in the evolution of the flavor. An important flavoring component, glutamic acid, has been reported in the fermentation of iru or dawadawa (Beaumont 2002). There are many reports that confirm that vitamin levels are higher in fermented vegetable protein foods than in their raw materials, specifically for riboflavin, thiamine, niacin, and vitamin C (Olasupo 2006).

It has also been established that African fermented beverages, which contain a mixture of acid and alcohol, are more nutritious in that they contain vitamins and other essential growth factors (Odunfa and Oyewole 1998). Pito, for instance, contains lactic acids, sugars, and amino acids and alcohol content of only 3 percent (Kolawole et al. 2007). Kishk prepared from wheat and milk is a highly nutritious food, having a protein content of about 25.3 percent. When milk is fermented to wara, lactose is reduced from 4.6 percent to 0.2 percent, protein increased from 3.7 percent to 14.8 percent, and fat increased from 4.3 percent to 13.5 percent (Ogundiwin and Oke 1981). The increases in these parameters could be due to a reduction in moisture level. Substantial nutrient losses occur during the various steps of ogi processing, because much of the protein in cereal grains is located in the testa and germ, which are usually sifted off during processing (Odunfa 1999). Home-made mawe has more crude protein, crude fiber, crude fat, and ash content than the commercial mawe because more hulls and germs are retained during the home production (Hounhouigan et al. 1993). The reduction in nutrient value of ogi during production has prompted research in this area to improve nutritive value (Kohajdová 2010).

3.2 Safety and Improvement of Shelf Life

Safety is particularly an important issue with most African fermented foods because their production is still largely a traditional art at the household or cottage level, dependent on spontaneous and back-slopping fermentation and without starter cultures (Olasupo et al. 2001). Furthermore, drug-resistant strains of some microorganisms of public health concern such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* sp., and *Salmonella* sp. have been reported from fermented dairy products like wara (Gadaga et al. 2004). Notwithstanding this, research findings have shown that most traditionally

fermented African foods are either of acceptable safety quality or offer great potential for improving safety quality *via* introduction of starter cultures or improved process control (Ogunshe et al. 2006). Cooked ogi was found to exhibit antibacterial activity against *Escherichia coli* (Odugbemi et al. 1991). Ogi called "dogik" produced using starter cultures *Lactobacillus acidoplilus* DK 77 and *Lb. pentosus* DK 99 has antimicrobial activity against diarrheagenic bacteria. Although high coliform counts have been reported from locally produced kunu-zaki (Lei 2006), it has been shown that the shelf-life and safety of kunu-zaki can be improved by pasteurization, sterilization, and addition of sodium benzoate or a combination of these without noticeable effects on the organoleptic properties of the beverage (Lei 2006). A number of bacteriocin-producing LAB have been isolated from fermented milk products from Nigeria (Leroy and De Vuyst 2004). Nisin produced by *Lactococcus lactis* isolated from wara has been found to inhibit pathogenic species including *Listeria monocytogenes*, *L. innocua*, *Clostridium butyricum*, *Cl. perfringens*, and *Bacillus cereus* (Aworh 2008). Antibacterial activity against bacteria causing diarrhea has been linked with lactic acid bacteria involved in the fermentation of uji (Lei 2006). Also, lactic acid production during fermentation of teff to produce injera has been found to lower the population of potential pathogenic organisms (Kohajdová 2010).

The microorganisms involved in the fermentation of fermented vegetable-based foods also affect the safety of the fermented products. Most of the raw materials for fermented vegetable products are not edible in their unfermented state. During fermentation, the safety and nutritional levels are enhanced. Degradation of oligosaccharides such as galactomannan, arabinogalactan, stachyose, and raffinose (Ouoba et al. 2007) was also observed. One of the goals of fermentation is to improve the shelf-life of food. The primary role of LAB in fermentation is the conversion of sugars to organic acids which lower the pH. Preservation is enhanced by removal of carbohydrate as a source of nutrient and by production of anti-microbials such as hydrogen peroxide, bacteriocins, diacetyls, and secondary reaction products. Similarly, it was shown recently that LAB exhibited a bio-preservative role in a traditional cassava product, fufu, and inhibited some common food-borne pathogens (Obadina et al. 2006). More importantly, however, recent reports have shown that microorganisms in the genera from which African fermented foods are made such as *Lactococcus*, *Geotrichum*, *Debaromyces* and so on belong to the category of those "generally regarded as safe" (Ogier et al. 2008). Antimicrobial activities have also been reported in cottage cheese, maasai fermented milk, and Ethiopian sour milk. Also, fermentation helps to reduce bacterial toxinogens and aflatoxins, such as those produced by *Staphylococcus* sp., clostridia, bacilli, and aspergilli (Olasupo 2006).

3.3 Detoxification of Natural Toxins and Anti-Nutritive Factors

The role of fermentation in the removal or reduction of natural toxins and anti-nutritive factors in many African fermented foods has been well documented. Malu et al. (2007) reported a 36 percent reduction in cyanide content between the third and fourth day of fermentation for gari production in Nigeria. Microbial growth is central to the efficient removal of cyanogen in soaked cassava (Westby and Choo 1994). Amoa-Awua (1996) reported complete removal of cyanogenic glucosides from cassava (119.3 mg/kg) during the fermentation process to produce agbelima. Equally important is the grating step preceding fermentation that has been found to enhance linamarase (the endogenous enzyme responsible for detoxification) release (Olasupo 2006). According to Kimaryo et al. (2000), selected strains of *Lb. plantarum* were used for controlled submerged fermentation of cassava into the typical Tanzanian kivunde. The approach was found to significantly enhance detoxification of cyanogenic glucosides in cassava to a level below 10 mg/kg of dry weight. A combination of cooking and fermentation was found to improve the nutrient quality of sorghum seeds and reduce the contact of anti-nutritional factors to a safe level in comparison with other methods of processing (Obizoba and Atii 1991). In bambara (*Voandzeia subterrenea*) nut milk (Obizoba and Egbuna 1992), tannin content could be reduced by fermentation.

Phytates that are components of cereals, legumes, and tubers used in African fermented foods like ogi, iru, ogiri, and fufu, and which may reduce bioavailability of essential minerals and digestibility of proteins, have been found to be hydrolyzed during fermentation (Evans et al. 2013). Factors responsible for flatulence, indigestion, and diarrhea are also reduced during fermentation.

3.4 Commercial Potential

African fermented foods are a group of foods that are produced at homes, villages, and cottage industries (Anukam and Reid 2009). Both in rural and urban areas, market for these products abound, but usually confined within certain geographical locations or ethno-cultural areas. Cereal products such as ogi, kenkey, koko, and mawe are very popular among the peoples of various ethnic nationalities. It is estimated that about 40 million people in Nigeria consume ogi at least once a week (FIIRO 2006). Kunu-zaki is popular among northern Nigerians because of its pleasantly sweet aroma and moldy sour aftertaste. It is consumed as an appetizer and for refreshment (Lei 2006). In recent years, this nonalcoholic beverage has gradually made inroads into the southern part of the country, particularly among middle- and low-income earners. This is of significance in a country

with a population of over 150 million. Cassava-based fermented foods are consumed by large populations across the continent. Globally, more than 500 million people depend on cassava as a major energy source; of this 200 million live in sub-Saharan Africa. Gari, for example, is consumed in almost all parts of Nigeria and in many West African countries such as Ghana, Republic of Benin and Togo (Oboh et al. 2002). A lot of research input has been made into improving gari production and meeting the ever-expanding market of this very important staple food, particularly in the area of use of starter cultures and mechanization of the process (FIIRO 2006). With such improvements, there is hope that this energy-rich source and other cassava-based products such as agbelima (Ray et al. 2010) and kivunde (Ray et al. 2010) will leave the realm of localities to become very important on the global market as transnational staple foods.

4 Future of African Fermented Foods

There is need to educate the African citizens on the need of consuming fermented foods and a need in food safety. Fermented foods are generally safe and certain anti-microbial factors are usually present. The greatest drawback in the development of fermented food products in Africa is that many products are produced under primitive conditions, resulting in low yield and poor quality, including short shelf-life (Achi 2005a). However, lack of standardization in the methods used, the environment and the hygiene of the people that prepare them, will determine the quality of the product. Safety is of paramount importance. Personal hygiene should be practiced to complement the overall benefits of fermented foods. Other problems include the lack of appeal in the presentation and marketing of the food products, as well as the fact that the processes are often laborious and time consuming. The technology needs to be improved through research to advance its potential for food safety and nutritional value. The challenge is to ensure that technology is used to add value to such products, such as increased shelf-life, flavor and appealing packaging and labeling (Chelule et al. 2010).

Traditional starter cultures have to be preserved. Micro-encapsulation technology is a new technique which can be used to preserve and propagate LAB cultures for mass production of fermented foods (Solanki et al. 2013). This is hoped to preserve the cultures for future use as starter and as a base to extend the product-range of fermented foods. It is also important to document these traditional indigenous technologies in order to preserve them for future generations, as the old practices keep changing from time to time. This will also create a reference database for future generations of food research scientists, nutritionists and food regulatory bodies and policy makers in different ladders of government.

Keywords: Africa, Beverages, Dairy products, Fermented food future, Fermented food roles, Fermented food safety, Fermented food types, Fish products, Historical roots, Meat products, Vegetables

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9

Oriental Fermented Functional (Probiotic) Foods

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1 Introduction

Traditional fermented foods are the products of biotechnological processes those are produced by taking advantage of the natural microbiota associated with fresh food materials. It is one of the most practical, economic, and widely applied empirical methods for preserving and often enhancing organoleptic and nutritional quality of fresh food (Klayraung et al. 2008). Particularly in developing countries, where refrigeration is not always an option, the fermentation process is widely used and of crucial importance, since fermentation prolongs the shelf-life of foods in addition to improving the nutritional value and reducing the risk for food-borne illness. Fermented foods can even have beneficial health effects, when the fermenting microorganisms possess probiotic activity (Lei 2006).

Mixed-culture fermentations in the preparation of foods are fairly common in the Western world, but noticeably more so in the Far East.

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Oriental fermented foods mean the traditional fermented foods made in the orient, including for example Japan, Indonesia, India, Pakistan, Thailand, Philippines, Taiwan, China, Korea, and the encompassing areas. These foods were produced long before the written history; some of these processes are so little known that even today one can only guess as to the organisms used (Foroutan 2012).

Recent research has revealed that some of the fermented foods are rich in probiotics and other functional elements such as dietary fiber, minerals, vitamins, and antioxidants, while lacking the dairy allergens that might prevent consumption by certain segments of the population and surely the oriental fermented foods are no exception. This chapter reviews the probiotic and functional attributes of oriental fermented foods.

2 Functional Foods

2.1 Definition

Many definitions exist worldwide for functional foods, but there is no official or commonly accepted definition. One view is that any food is indeed functional because it provides nutrients and has a physiological effect. So, functional food should be considered a marketing term for a food whose attraction lies in its health claims and the way the product is perceived. Some even believe that, any food, if marketed appropriately, particularly with an accompanying health claim, is a functional food. Some foods considered to be functional are actually natural whole foods where new scientific information about their health qualities can be used to proclaim benefits. Many, if not most, fruits, vegetables, grains, fish, and dairy and meat products contain several natural components that deliver benefits beyond basic nutrition. Most definitions also suggest that a functional food should be, or looks like, a traditional food and must be part of our normal diet. A functional food can be targeted at the whole population or at particular groups, which may be defined, for example, by age or genetic constitution (European Commission 2010).

The EC Concerted Action on Functional Food Science in Europe (FUFOSE) proposed a working definition of functional food: a food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. It is consumed as part of a normal food pattern. It is not a pill, a capsule or any form of dietary supplement (European Commission 2010).

2.2 Criteria of Functional Foods

There is a wide variety of claims currently used in the labeling and advertising of foods relating to substances that have not been shown to be beneficial or for which there is not sufficient scientific agreement. Thus, it is necessary to ensure that the substances for which a claim is made have been shown to have a beneficial nutritional or physiological effect.

Table 1 summarized some criteria that are supposed to be present in functional foods.

Table 1. Some criteria of functional foods.

Criteria	Explanation
Criterion 1: The food or food component to which the claimed effect is attributed should be characterized	Functional ingredients are a diverse class of compounds and may be represented by single component ingredients, or complex herbal extracts or products derived from novel sources or processes; the compositional analysis for each of these types of products is a critical determinant of the approach to the determination of safety for the ingredient
Criterion 2: The efficacy of food or food component to which the claimed effect is attributed should be demonstrated without ambiguities	Building a strong scientific basis for functional food claims relies on the ability to demonstrate the efficacy of functional food components. It is complex and costly task, but it is essential to acceptance of functional food. Because of the number of bioactive compounds and the diversity of likely biological effects, numerous and diverse experimental approaches must be taken to increase the understanding of the biology of bioactive compounds
Criterion 3: The safety of food or food component should be demonstrated at efficacious levels	Although a functional ingredient is intended to produce a positive health benefit through physiologic or pharmacologic activity in the body, similar to a drug, there is a risk for a lifetime exposure and unsupervised consumption, as is the case for nutrients and food additives. Once food components are identified may help to prevent risk of disease, it is required that solid evidence is obtained on the safety and efficacy of these compounds when taken daily and on a long period
Criterion 4: The impact of food matrices on the activity and bioavailability of food component should be addressed	However, foods are mostly complex mixtures of macro and micro components that can trap active compound, modulate its release or inhibit its activity. Most of dietary active compounds are sensitive to conditions encountered during food processing such as temperature (vitamin), oxygen (antioxydant compound), light or in the gut such as acidic pH of stomach (probiotic), digestives enzymes (active peptides/proteins), presence of other nutrients
Criterion 5: Foods containing probiotics should be under-control, safe, stable and good manufactured	**Probiotics must be Safe**: Probiotic strains such as *Lactobacillus* species, *Bifidobacterium* species and *Streptococcus* species have long history of safe use and are Generally Recognized As Safe (GRAS). Probiotics must have safety data to be considered as probiotics

Table 1. contd....

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Criteria	Explanation
	Probiotic Stability: Probiotics must stay viable in food, feed, and dietary supplements (powder, capsules and tablets). Poor stability discredits the entire probiotic category. Probiotic stability is affected by high temperature, oxygen, humidity and high water activity. The probiotic products do not contain the same microorganisms with same viability, they will not offer same consistent good result **Good Manufacturing Practices (GMP)**: Good manufacturing Practices must be applied in the manufacture of probiotic foods and supplements with quality assurance and shelf-life conditions established. Manufacturing has great impact on probiotic stability **Quality Control**: The criteria and procedures for quality control must be determined and implemented; Genes, species, strains must be identified and viable microorganisms must be expressed (CFU/g) for every batch of probiotics manufactured; Test the viability of microorganisms at manufacturing and at expiration date; Test for pathogens and heavy metals in the probiotic culture and the finished product(s); Contamination of probiotic products with undesirable microorganisms is possible in uncontrolled fermentation; Other ingredients mixed with probiotic products may add contamination; Blending and packaging equipments may contribute to probiotic product contamination (follow good manufacturing practice)

3 Probiotic Foods as Functional Foods

3.1 Probiotics Definitions

Probiotics represent probably the archetypal functional food, and are defined as a live microbial supplement, which beneficially affect the host by improving its intestinal microbial balance. Kollath (1953) first defined the term "probiotic", when he suggested the term to denote all organic and inorganic food complexes as "probiotics" in contrast to harmful antibiotics, for the purpose of upgrading such food complexes as supplements. The largest segment of functional food market in Europe, Japan and Australia comprises foods containing probiotics, prebiotics and synbiotics.

The term probiotic was technically defined by an Expert Committee of FAO as "live microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition" (FAO/WHO 2002). This means that the microorganisms must be alive and present in high numbers, generally more than 109 cells per daily ingested dose. Each product should

indicate the minimum daily amount required for it to confer specific health benefits in the host (FAO/WHO 2002). Prebiotics are non-digestible food ingredients that beneficially affect the host by stimulating growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health. The term synbiotic is used when referring to a product that uses prebiotic and probiotic in combination (Stanton et al. 2005). Microorganisms might also indirectly impart health-promoting characteristics in food through the production of bioactive metabolites (referred to as biogenics) during fermentation (Takano 2002).

3.2 Microorganisms Considered as Food Probiotics

Lactic acid bacteria (LAB) are the most common type of microorganisms used as probiotics (Table 2). Strains of the genera *Lactobacillus*, *Bifidobacterium* (Yateem et al. 2008) and *Enterococcus* (Ljungh and Wadström 2006) are the most widely used and commonly studied probiotic bacteria. The yeast *Saccharomyces boulardii* has also been studied as probiotics (Ljungh and Wadström 2006).

Table 2. Common microorganisms used as probiotics.

***Lactobacillus* sp.**	***Bifidobacterium* sp.**	**Others**
Lb. acidophilus	*B. adolescentis*	*Bacillus cereus*
Lb. amylovorus	*B. animalis*	*Enterococcus faecium*
Lb. brevis	*B. brevis*	*Propinobacterium freudenreichii*
Lb. casei	*B. bifidum*	*Lactococcus lactis* subsp. *cremoriss*
Lb. rhamnosus	*B. infantis*	*Lactococcus lactis* subsp. *lactis*
Lb. crispatus	*B. lactis*	*Leuconostoc mesenteroides* subsp. *dextranicum*
Lb. delbrueckii subsp. *Bulgaricus*	*B. longum*	*Pediococcus acidilactici, P. halophilus*
Lb. fermentum	*B. thermophilum*	*Saccharomyces boulardii*
Lb. gasseri		*Streptococcus salivarius* subsp. *thermophilus*
Lb. helveticus		*Streptococcus lactis, St. equinus*
Lb. johnsonii		*Streptococcus cremoris, St. faecium*
Lb. lactis		*Streptococcus diacetilactis* *Sporolactobacillus inulinus*
Lb. paracasei		
Lb. plantarum		
Lb. reuteri		
Lb. salivarius		

Source: Blandino et al. 2003, Prado et al. 2008, Leroy et al. 2008

3.3 Probiotics in Food: Rules and Actions

The probiotics have been used for food fermentation since the ancient time; can serve a dual function by acting as food fermenting agent and potentially health benefits provider. LAB as probiotics are GRAS with no pathogenic, or virulence properties having been reported. For the use of LAB, some desirable characteristics such as low cost, maintaining its viability during the processing and storage, facility of the application in the products, resistance to the physicochemical processing must be considered (Song et al. 2012).

The beneficial effects of food with added live microbes (probiotics) on human health, and in particular on children and other high-risk populations, are being increasingly promoted by health professionals. It has been reported that probiotics can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating infectious disease in children. However, some health benefits, e.g., immune modulation, may be achieved even with dead bacteria (Kalliomaki et al. 2001). Figure 1 shows the major health benefits conferred by probiotics.

4 Probiotics and Gut Microflora

4.1 Definition of a 'Healthy' Gut Microbiome

Data have recently emerged regarding the composition and function of the healthy gut microbiome. Stool specimens from 242 healthy, young adults were analyzed using 16S rRNA gene pyrosequencing and whole metagenomic sequencing to assess the composition and function of the microbiome, respectively (The Human Microbiome Project Consortium 2012a,b). The biological diversity and richness of the distal intestine easily surpassed the relative richness of microbiomes, in terms of microbial taxa and genes, at other body sites such as human skin or the oral cavity. The predominant taxa varied in different body sites and, as expected, the phyla *Bacteroidetes* and *Firmicutes* represented the predominant phyla in the human intestine. The relative predominance of bacterial genera and species varied. For example, *Bacteroides fragilis* was present in a quantity of at least 0.1 percent of sequence reads in 16 percent of specimens from different individuals. Moreover, the prevalence of *B. thetaiotaomicron* was greater with quantities of at least 0.1 percent of sequence reads in 46 percent of individuals. Both *Bacteroides* species are known as commensal intestinal taxa that have been cultured and studied in the laboratory for their immunomodulatory and metabolic features.

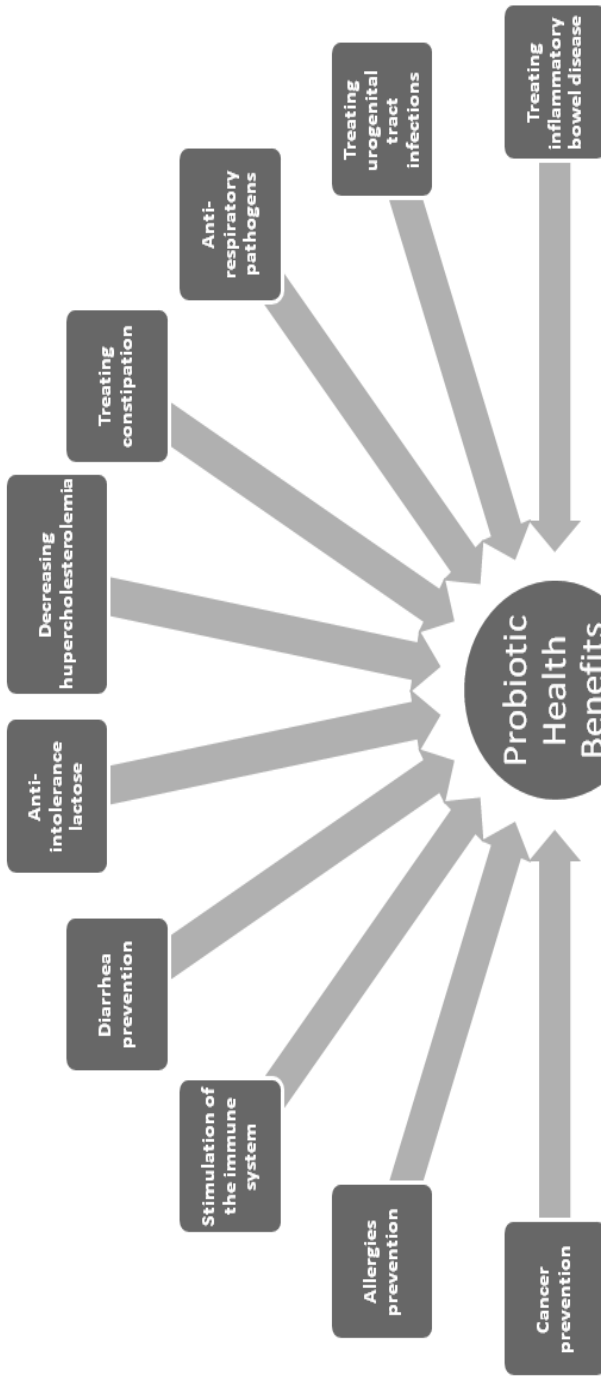

Fig. 1. Major health benefits conferred by probiotics.

In contrast to microbial composition, whole genome metagenomics data demonstrated relatively even distribution and prevalence of metabolic pathways across body sites and individuals (The Human Microbiome Project Consortium 2012a). Predominant metabolic modules such as central carbohydrate metabolism represent major functional categories in the distal intestine as well as other body sites. However, 86 percent of identified families of genes from the gut metagenomes have not yet been functionally characterized or mapped to complete pathways (The Human Microbiome Project Consortium 2012a).

4.2 Probiotic Survival during Gastric Transit

Gastrointestinal passage, which involves exposure to stomach acid, bile salts and enzymes, represents a major survival hurdle for probiotic bacteria, which should reach the intestine in viable form. In this respect, there is little point in putting huge effort into optimizing culture viability in the product, if the vast majority of cell viability is lost during gastric exposure. It is not surprising, therefore, that acid and bile tolerances are among the criteria for selection of probiotic strains. In addition, several studies have demonstrated that the food matrix can have a significant effect on the survival of bacteria during gastric transit. For example, Cheddar cheese was shown to elicit a protective effect on probiotic bacteria upon exposure to gastric juice when compared with yogurt (Gardiner et al. 1999). The composition of the environment can also influence microbial viability; for example, the presence of fermentable sugars (e.g., glucose) in the acidic environment can help to maintain the viability of some species of lactobacilli (Charalampopoulos et al. 2003). Furthermore, it has been suggested that exopolysaccharide-producing strains of LAB might be naturally protected in hostile environments (Stanton et al. 2005).

4.3 Probiotics Alter the Intestinal Microbiota

Probiotics may introduce beneficial functions into the gastrointestinal tract or enhance the functionality of existing microbial communities. Probiotics may also affect the composition and function of microbial communities by competition for nutrients, production of growth substrates or inhibitors and modulation of intestinal immunity (O'Toole and Cooney 2008). This concept is supported by results from randomized controlled clinical trials showing the benefits of probiotics during the treatment of gastrointestinal diseases (Preidis and Versalovic 2009, Thomas and Versalovic 2010). Probiotics may induce changes in the intestinal microbiota and stabilize microbial communities. However, further studies in humans are needed to assess

whether probiotics can make the same impact on the human intestinal microbiome and whether the changes are associated with clinical benefits in the host (Hemarajata and Versalovic 2013).

In addition to directly affecting the composition of the intestinal microbiota, probiotics may also modulate the global metabolic function of intestinal microbiomes. Fermented milk products containing several probiotics did not alter the composition of intestinal bacterial communities in gnotobiotic mice and monozygotic twins (McNulty et al. 2011). However, fecal metatranscriptomic analysis of probiotics-treated animals demonstrated significant changes in expression of microbial enzymes, especially enzymes involved in carbohydrate metabolism. Moreover, mass spectrometric analysis of urinary metabolites revealed altered abundance of several carbohydrate metabolites. These observations suggested that probiotics may affect the global metabolic function of the intestinal microbiome.

5 Oriental Fermented Foods as Sources for Probiotics

With the increased data about benefits of probiotics for human health and treatment and since most isolated and patented strains are of western origin, it is greatly inviting to try and isolate such probiotics from the untapped source exemplified by a wide variety of different fermented foods of the orient. Some representative probiotic foods from various Asian countries are described below.

5.1 Fermented Functional Foods of Korea

5.1.1 Kimchi

Kimchi is the name given to various traditional fermented vegetables, which are emblematic of the Korean culture. Kimchi is mainly manufactured with Chinese cabbages (*Brassica pekinensis*) and radish, but other seasonings ingredients such as garlic, green onion, ginger, red pepper, mustard, parsley, fermented seafood (jeotgal), carrot and salt may be used (Lee 2001, Jung et al. 2011).

South Koreans consume 40 pounds of kimchi per person annually, and many credit their nation's rapid economic growth in part to eating the dish. Kimchi is made of various vegetables and contains a high concentration of dietary fiber, while being low in calories. One serving also provides over 50 percent of the daily recommended amount of vitamin C and carotene. Most types of kimchi contain onions, garlic, and chilli peppers, all of which are salutary. The vegetables used in kimchi also contribute to its overall nutritional value as it is rich in vitamin A, thiamine (B1), riboflavin (B2),

calcium, and iron, and contains a number of lactic acid bacteria (LAB), among those the typical species being *Lactobacillus kimchii* (Leea et al. 2005, Kim and Chun 2005). Due to its nutritional properties, kimchi was recently included in the list of the top five "World's Healthiest Foods" (http://eating.health.com/2008/02/01/worlds-healthiest-foods-kimchi-korea/). These beneficial effects are attributed either to functional components (vitamins, minerals, fiber and phytochemicals) or to fermentation by LAB (Lee et al. 2011).

A 2005 South Korean study found, however, that when eaten in large quantities, kimchi may increase the risk of gastric cancer, particularly among people with certain genetic traits (Nan et al. 2005). One study conducted by Seoul National University claimed that chickens infected with the H5N1 virus, also called avian flu, recovered after eating food containing the same bacteria found in kimchi. During the 2003 SARS (Severe Acute Respiratory Syndrome) outbreak in Asia many people believed that kimchi could protect against infection although there was no scientific evidence to support this belief, and kimchi sales rose by 40 percent. However, in May 2009, the Korea Food Research Institute, Korea's state food research organization, said they had conducted a larger study on 200 chickens, which supported the theory that it boosts chickens' immunity to the virus (http://tasteofasia.ro/en/kimchi-top-5-worls-best-food/).

5.1.2 Sauerkraut

Present-day sauerkraut is a product resulting from lactic acid fermentation of shredded, salted white cabbage (*Brassica oleracea* var. *capitata* for. *alba* L.). There is no doubt that the preservation of plant material by fermentation dates back to prehistoric times. Plinius the Elder, in the first century A.D., is said to have been the first who described the production of sauerkraut by preservation of so-called salt cabbage in earthen vessels. It can be assumed that under the conditions described, the cabbage was fermented by microorganisms, some of which were typically associated with the plant phylloplane, but most of which were located in the pores of the fermentation vessels or originated from a former fermentation. Outside Europe there are two other regions with a significant production of sauerkraut: Korea, China, and other Far Eastern countries on the one side, and the United States on the other. At the present day, sauerkraut is manufactured in all European countries by small, medium, and large companies. Consequently, the production procedures differ within a wide range (Holzapfel et al. 2008).

Sauerkraut is considered to be a healthy product as it is an important source of vitamins (especially vitamin C), mineral salts, and dietary fibers. In our modern Western diet, however, sauerkraut no longer plays an essential role as source of vitamin C. A health-promoting effect of sauerkraut may be

linked to the high content of glucosinolates (up to 1 percent of dry weight) of the white cabbage. Glucosinolates undergo hydrolysis during fermentation by the enzyme myrosinase. Some of the resulting metabolic products, including indoles and isothiocyanates are highly reactive compounds and were shown to be powerful inhibitors of carcinogenesis in laboratory animals. Isothiocyanates are able to inhibit mitosis and stimulate apoptosis in human tumor cells, and influence phase I and phase II biotransformation enzyme activities, thereby possibly influencing several processes related to chemical carcinogenesis, e.g., the metabolism, DNA-binding, and mutagenic activity of promutagens. One of the major underlying mechanisms appears to be the selective inhibition of cytochrome P450 enzymes involved in carcinogenic metabolic activation. Another health-promoting property of sauerkraut may result from the LAB involved in sauerkraut fermentation. As with other fermented products, unpasteurized sauerkraut contains high numbers of viable LAB, which may include organisms showing a beneficial effect on the intestinal ecosystem of the consumer. Up to now, however, there are no reports on the probiotic efficacy of typical sauerkraut LAB, and the health effects of these organisms still have to be demonstrated (Holzapfel et al. 2008).

5.2 Fermented Functional Foods of China

5.2.1 Koumiss

Koumiss, also named "airag" or "chigee" is a traditional fermented milk product originating in the Central Asia and created by spontaneous fermentation of lactose to lactic acid and alcohol (Kücükcetin et al. 2003). It is a very popular fermented dairy product, which has been consumed for thousands of years by the inhabitants of Mongolia and Xinjiang provinces (Liu et al. 2011). The product season is between end of May and autumn. Traditional koumiss is made from fresh mare or camel milk by mixing it with prepared fermented koumiss. The mixtures are kept in a suitable bag which is made of animal skin. During the fermentation, a regular beating and storage temperature maintained at 20–30°C are required in order to develop flavor and control the process. When strong foam and special sour flavor are achieved, koumiss is ready to consume. Fermentation results in up to 2 percent alcohol content and low pH (4.0). The final product is a kind of homogeneous liquid with a milky or light yellow color. In previous studies, lactobacilli and yeast have been shown to be the predominant microbes in koumiss (Wu et al. 2009). During the fermentation, lactobacilli acidify the milk and yeasts change the raw material into a carbonated, mildly alcoholic drink. *Lactobacillus* genus has been used safely in food for a long time. Its roles in the fermentation affect aroma, texture, and

acidity of the product with additional benefits to human health (Danova et al. 2005). The main LAB strains in koumiss are usually lactobacilli, such as *Lactobacillus plantarum*, *Lb. helveticus*, *Lb. casei* and *Lb. kefiri* due to their higher acid tolerance (Wu et al. 2009).

The health value of koumiss has been recognized since the ancient times and it is considered as the best beverage for health care therapy. It is generally accepted that mare milk is an adequate source of nourishment for infants and effective in prevention of some human pathologies (Di Cagno et al. 2004). Numerous studies have shown that koumiss has therapeutic effects in cardiovascular disease, neurological disease, tuberculosis and diabetes (Li et al. 2006). Koumiss was consumed to nourish blood, sooth the mind and improve digestion. Even today, koumiss has not lost its therapeutic value. The "Koumiss therapy method" and "Koumiss therapy center" have been developed in China to assist in the treatment of hepatitis, chronic ulcers, and tuberculosis (Wu et al. 2009).

5.2.3 Stinky tofu

It also known as stinky soybean curd and is a famous and popular traditional fermented Chinese snack. Some consumers develop an increased appetite for it despite the strong odor. The historical records of the existance of stinky tofu have been found in the period from the Wei Dynasty to the Qing Dynasty. To produce stinky tofu, soybeans are made into milk which then forms tofu at the first step. When the brine medium (the aromatic fermentation liquid of stinky tofu) develops a strong odor after an open fermentation process taking place over several days, tofu squares are immersed in the medium for a few hours before further manufacture steps. The actions of microorganisms impart unique odors and form a sponge like structure inside the tofu. Traditionally, the stinky tofu can be consumed cold, fried or steamed. Stinky brine fermentation is an alkaline fermentation process since ammonia is produced and increases the pH of the product. The microbial strains found in stinky brine vary according to the specific ingredients of the manufacturers' adaptation. In general, there are two dominant types of strains in stinky tofu: *Bacillus* genus and LAB. As a nutrient-rich environment, stinky brine is an ideal habitat for diverse LAB growth. Huang and colleagues have isolated *Lactobacillus* genus and *Bacillus* genus from the fermented brine (Huang et al. 2009). Chao and co-workers have identified *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Weissella* belonging to 7 genera and 32 species in indigenous fermented stinky brine (Chao et al. 2008). Although it is not clear which particular microbes are involved in the fermentation of this product, it is believed that LAB are in part responsible for the change in pH and flavor development of stinky tofu brines (Chao et al. 2008).

Soybean (*Glycine max* L.) is rich in protein with more than 10 different kinds of amino acids. Due to the activity of microbes, proteins in the fermented stinky tofu are further decomposed. The amino acids content, especially the essential amino acids, are enriched. For instance, vitamin B12 in stinky tofu is abundant when compared to that of other common daily foods (Guo et al. 2004).

5.2.4 Chinese-style sausage

Chinese-style fermented sausage is a traditional meat-based indigenous fermented food consumed in many regions of China. It is convenient in meal preparation and provides flavor for the dishes. Fresh porcine boneless ham was manufactured by removing subcutaneous fat and grounding. Then pork is cut into 5 mm cubes. All curing ingredients and the meat blocks are then mixed thoroughly for several minutes. The sausage is then cured for several days before being stuffed into natural hog casings and dried. Curing is an ancient method for preserving meat products and many flavor components are normally formed in the curing stage. Varieties of Chinese-style fermented sausages are produced in this way, such as Cantonese sausage, Harbin sausage, and Jinhua ham. Nowadays, other than pork and back fat, many additional ingredients, such as yam, konjac, and chitosan have been incorporated into meat formulas for manufacturing novel Chinese-style sausage, especially, for consumers requesting reduced-fat, low calorie meat products (Tan et al. 2007).

LAB have been shown to be part of the natural microbiota of Chinese-style sausages, especially, *Lactobacillus*, which dominates in vacuum packaged meat (Kuo and Chu 2003). They play a leading role in decomposing carbohydrates and producing lactic acid, which decreases the pH value in meat. Their presence effectively prevents the growth of food spoilage and food-borne bacterial pathogens as well as production of enterotoxin. Their antagonistic actions in meat at refrigeration temperature have been reported by Castellano and colleagues (Castellano et al. 2010). In addition, some LAB can utilize H_2O_2 by reducing it to H_2O and O_2, contributing to desirable coloration (Tian and Zhang 2001).

5.3 Fermented Functional Foods of Japan

A variety of fermentation products, such as foods containing probiotic bacteria, black rice vinegar (kurosu), soy sauce (shoyu), soybean-barley paste (miso), natto and tempeh, are sold in food stores in Japan (Murooka and Yamashita 2008). These fermented food products are produced by traditional methods that exploit mixed cultures of various non-toxic

microorganisms. These microorganisms include LAB, acetic acid bacteria, sake yeast, koji molds and natto bacteria. Many traditional fermented foods have been studied and their effects on metabolism and/or immune system have been demonstrated in animal and/or human cells. In the following section, the scientifc basis for the effects of these traditional food products, which are currently produced commercially in Japan are described.

5.3.1 Black rice vinegars, komesu and kurosu

Two Japanese traditional rice vinegars, komesu, which is a polished amber rice vinegar, and kurosu, which is unpolished black rice vinegar, are both produced by a traditional static fermentation process. These vinegars are known for their health benefits *via* the prevention of inflammation and hypertension. Komesu and kurosu are produced by the same process, namely, saccharifcation of rice starch by a koji mold, *Aspergillus oryzae*; alcohol fermentation by a sake yeast, *Saccharomyces cerevisiae*; and oxidation of ethanol by acetic acid bacteria to yield acetic acid. For the static fermentation process, moromi, which is a mixture of alcoholic liquid media that contains acetic acid and acetic acid bacteria, is fermented in large covered containers. During fermentation, no strict sterilization measures are employed and no purified strains are introduced (Shibayama et al. 2010). After a few days, a crepe-like skin of acetic acid bacteria covers the surface of the moromi, at which time the fermentation process begins and then continues for about a month. The surface layer of acetic acid bacteria that covers the surface of the moromi is removed by scooping it up in a meshed strainer, and it is then gently floated on a new batch of moromi. These acetic acid bacteria were identified as *Acetobacter pasteurianus* on the basis of sequences of 16S rDNA, physiological characteristics, and patterns of DNA fragments after analysis by the polymerase chain reaction (Murooka and Yamashita 2008).

Since kurosu is produced from unpolished rice, it is characterized by higher levels of amino acids and organic acids than other vinegars, with the exception of balsamic vinegars. Kurosu was recently shown to suppress lipid peroxidation more effectively than extracts of other vinegars since the former has stronger anti-oxidative activity in a radical-scavenging system. Kurosu also exhibited anti-tumor activity in a mouse skin model of carcinogenesis and it had a suppressive effect on the growth of a variety of lines of cultured tumor cells. Vinegars produced from sweet shochu (Japanese whisky) post-distillation slurry by *Acetobacter aceti* had antitumor activity when administered orally to mice in a mouse model. They inhibited the activity of angiotensin I-converting enzyme and repressed the formation

of advanced end products of glycation. At present, kurosu vinegar is experiencing a burst of popularity as a health drink in Japan (Shizuma et al. 2012).

5.3.2 Fermented soybean-barley paste, miso

Miso is a common Japanese food or seasoning, and the first reference to miso appeared on the ancient Chinese text 'Syurai' around 700 BC. Miso is produced by fermentation of soybeans, rice or barley with koji mold (*A. oryzae*), which is cultivated on steamed rice or barley under solid-state conditions, and then mixed with salt. In some cases, *Saccharomyces cerevisiae* and LAB are used in addition to koji mold. Although the most common type of miso is made from soybeans, many kinds of miso are produced, with variations of the ingredients, the temperature and duration of fermentation (from one week to 20 mon), the salt content (5–13 percent), the variety of koji, and the fermentation vessel. The typical product is a thick paste that is used in miso soup, a Japanese culinary staple, which is prepared with vegetables, tofu (pressed protein curds from steamed soybeans), and dried sardines, seaweed or shellfish. Since miso contains a high level of protein and is rich in vitamins, amino acids, organic acids and minerals, it has played an important nutritional role in Japan. More than 95 percent of the Japanese population enjoyed miso and miso is part of the daily diet of much of the Japanese population (Shurtleff and Aoyagi 2009).

An epidemiological survey suggests that people who eat miso soup every day reduce their risk of gastric cancer. The estrogenic effects of isoflavones in soybean products might limit growth of tumors in the mouse liver. A study of human pulmonary adenomatosis cells and human gastric cancer cells revealed that fatty acids, such as oleic and linoleaic acids and their esters, which are found in miso, inhibit the proliferation of these cancer cells. Since miso contains vitamin E, saponin, isoflavones, lecithin, choline, and dietary fiber from soybeans; vitamin B2 from koji mold; and vitamin B12 from lactic or propionic acid bacteria, Japanese peoples believe that it is good for their health and, indeed, miso does lower cholesterol levels; it might even have anti-aging effects and prevent arteriosclerosis (Murooka and Yamashita 2008).

5.3.3 Natto

Natto is a fermented product made from soybeans cultured with *Bacillus subtilis* (synonym, *B. subtilis* subsp. *subtilis*; former name, *Bacillus natto*). Natto has been popular in Japan for more than 400 yr. Natto has a high nutritive value and is easily digested. In addition, natto has antibacterial

effects. To prepare natto soybeans are immersed in water and then steamed. The steamed soybeans are inoculated with spores of *B. subtilis*. The original source of the natto bacterium was rice straw. During the approximately 20 hr fermentation, the starch and proteins of the soybeans are converted to a mixture that contains amino acids, vitamins, and enzymes (Murooka and Yamashita 2008).

Natto contains saponin and isoflavones, which come from soybeans, as well as the fibrinolytic enzyme, vitamin K_2 and dipicolinic acid, which are generated by natto bacteria. The concentration of vitamin K_2 increases to 124 times that in the soybeans at the start of the fermentation by natto bacteria (Yanagisawa and Sumi 2005). Vitamin K_2 stimulates the formation of bone. After the growth of bacteria ceases (after approximately 15 hr-fermentation), natto becomes sticky as a result of formation of poly-γ-glutamic acid, which also stimulates absorption of calcium. These effects of natto might help to prevent osteoporosis in older women (Murooka and Yamashita 2008).

5.4 Fermented Functional Foods of India and Sri Lanka

5.4.1 Idli

A fermented, thick suspension made of a blend of rice (*Oryza sativum*) and de-hulled black gram (*Phaseolus mungo*) is used in several traditional foods in Southeast Asian countries. Among them, idli and dosa are very popular in India and Sri Lanka (Blandino et al. 2003). Traditionally, for idli preparation the rice and black gram are soaked separately. After draining the water, rice and black gram are grinded independently, with occasional addition of water during the process. The rice is coarsely ground and the black gram is finely ground. Then the rice and the black gram batters are mixed together (2:1 ratio) with addition of a little salt and are allowed to ferment overnight at room temperature (about 30°C). Finally, the fermented batter is placed in special idli pans and steamed for 5–8 min. The LAB *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Lactobacillus delbrueckii*, *Lb. fermenti*, *Lb. lactis* and *Pediococcus cerevisiae* have been found to be responsible for the fermentation process, although *Lc. mesenteroides* and *S. faecalis* are considered to be the microorganism essential for leavening of the batter and for acid production in idli (Ramakrishnan 1993). The yeasts *Geotrichum candidum*, *Torulopsis holmii*, *Torulopsis candida* and *Trichosporon pullulans* have also been identified in idli fermentation. Fermentation of idli batter appears to have a significant effect on the increase of all essential amino acids and in the reduction of anti-nutrients (such as phytic acid), enzyme inhibitors and flatus sugars (Blandino et al. 2003). Idli is a

low calorie, starchy and nutritious food, which is consumed as breakfast or snack. Steamed idli contains about 3.4 percent protein, 20.3 percent carbohydrate and 70 percent moisture (Blandino et al. 2003).

5.4.2 Dosa

It is very similar to idli batter except that the rice and black gram are finely ground and that the fermented suspension instead of being steamed is heated with a little oil, on a flat plate. A dosa suspension is prepared by grinding wet rice and black gram separately with water. The two suspensions are then mixed and allowed to undergo natural fermentation, usually for 8–20 hr.

The microbiological, physical and biochemical changes of dosa during fermentation and its nutritive value are quite similar to idli (Ramakrishnan 1993).

5.4.3 Dahi

Dahi (Sanskrit: dadhi) is a popular Indian fermented milk product that is quite analogous to plain yogurt in appearance and consistency. It is popular with consumers due to its distinctive flavor and because it is believed to have good nutritional and therapeutic values. It is utilized in various forms in many Indian culinary preparations.

Dahi is obtained by lactic fermentation through the action of single or mixed strains of LAB or by lactic fermentation accompanied by alcoholic fermentation by yeast from milk. The bacteria used to make dahi are known as "dahi cultures". Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give dahi its texture and its characteristic tang. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures, such as in dahi or as dietary supplements. *Lactobacillus acidophilus* and *Streptococcus thermophilus* are found in all plain dahi brands. Probiotic dahi brands claim that they have more beneficial bacteria that is good for health, however it is found in a recent study that plain dahi also has good bacteria in significant quantity (Consumer Voice 2012). The quality of dahi depends on the starter culture used, initial quality of milk and other ingredients added. Dahi has a host of benefits: as milk is an important source of proteins and calcium, so is dahi. But it has an added benefit of having hundreds of thousands of probiotics (live bacteria) which are excellent for overall health (Consumer Voice 2012).

5.4.4 Hawaijar

Among the fermented foods consumed in India, fermented soybean product locally known as hawaijar is traditionally prepared and consumed in Manipur. It plays an economical, social and cultural role in Manipur. Hawaijar making provides income to the rural masses in Manipur and bears deep attachment with socio cultural lives of the people (Das and Deka 2012). Hawaijar is consumed commonly in the local diet as a low cost source of high protein food (Devi and Kumar 2012). It has been reported in many studies for its various health benefits. They are known for their anti-cancer, anti-osteoporosis and hypo-cholesterolemic effects (Premarani and Chhetry 2011). In the traditional method of preparation of hawaijar, small and medium sized soybean seeds are cleaned and sorted (Devi and Kumar 2012). Although there are many varieties of soybean, two varieties namely, the local variety with small and bigger, round seeds and "JS355" are especially used in the preparation of hawaijar. However, hawaijar prepared from the local variety is more preferred because of its unique taste (Premarani and Chhetry 2008).

The fermentation process takes place naturally. In the hawaijar preparation whole soya beans are utilized. They are prepared at home without requiring much complicated equipments rather by using the rudimentary utensils available in every household. The seeds are soaked overnight/12–24 hr and washed thoroughly with tap water and boiled/pressure cooked till the seeds are soft. After the excess water is drained out the cooked soyas are washed with hot water, wrapped in clean cotton cloth/healthy fig leaves (*Ficus hispada*)/banana leaves (*Musa* sp.) and packed tightly in a small bamboo basket with a lid locally known as lubak. The base and sides of the basket are layered and lined with fig or banana leaves. The basket is then wrapped with cloth and kept in the sun or near a stove or buried in paddy straw for the fermentation process to take place. The whole process takes about 4 to 5 d. The final product is brown in color with sticky slimy white appearance that emits light ammoniacal aroma. The finished products are wrapped in banana leaves (Premarani and Chhetry 2010, General et al. 2011, Tamang et al. 2012).

The nutritional properties of hawaijar have been analyzed, examined and reported by many. The crude fiber content of fresh hawaijar was higher than the raw soybeans and then decreased during storage period (General et al. 2011). Fermented soya is known for their quality and richness in protein. The most significant biochemical changes that occur during hawaijar fermentation is protein hydrolysis (General et al. 2011). The percentage of soluble protein in hawaijar increases after fermentation with slight

increase during storage (Premarani and Chhetry 2010). Free amino acids are released by hydrolysis of protein with protease enzymes produced by micro-organism during fermentation (Dajanta et al. 2011). *Bacillus subtilis* found predominantly in fermented soya product gives rise to high proteolytic activity and markedly increases the free amino acid contents. Dajanta et al. (2011) reported that after fermentation, essential amino acids also increased extensively (6–9 times) with respect to their original amount in unfermented soybean in thaunao which is similar to hawaijar.

5.5 Fermented Functional Foods of Indonesia

There are many kinds of indigenous fermented foods in Indonesia that require the use of soybeans, cassava, and glutinous rice. The main ones are tempe, kecap (soy sauce), tauco (fermented whole soybean, similar to miso), tape ketela (cassava) and tape ketan (glutinous rice).

5.5.1 Tempe

Traditional tempe is a fermented food in which fungi, particularly *Rhizopus oligosporus*, plays an essential role. Fresh tempe is defined as a compact and sliceable mass of hydrated precooked bean cotyledons bound together by mold mycelium. The major desirable characteristics of tempe are its flavor, texture, and nutritional properties. In Indonesia, tempe is consumed as a protein-rich meat substitute by all economic groups. Yellow soybeans are most commonly used, but black-coated varieties are used in some areas. The best-quality tempe is made with soybeans, but lower-cost, lower-quality tempe is made with other kinds of beans, such as wing, pigeon, and velvet beans (Barus et al. 2008).

Tempe is a good source of protein, essential fatty acids, vitamin B, especially B12, and minerals, including iron. Tempe also contains antibacterial substances that can protect against infectious diseases, such as diarrohoea. Tempe contains potent antioxidants due to the conversion of isoflavonoid compounds present in soybeans into the 6,7,4′ trihydroxy isoflavon known as factor 2 and the more active antioxidant enzyme superoxide dismutase. The antioxidant activity of tempe may be related to its potential activity to retard the aging process. Tempe has been assumed to have hypocholesterolemic properties (Surya and Rahayu 2012).

5.5.2 Kecap (Soy Sauce)

It is a condiment used to add flavor and color to other foods, such as soybean curd, rice, fish, meat, and vegetables. It is consumed in several

grades or levels of quality by all societal levels in urban and rural areas. The quality consumed depends on the consumer's income level. Most of the manufacturers are small-scale producers with limited capital who cannot afford to invest in modern equipment and facilities. However, kecap is part of the Indonesian fermented food industry, and demand is on the rise. There are two types of Indonesian kecap. Kecap manis is sweetened soy sauce, and kecap asin is salty soy sauce. The sweetened variety is most popular. It is thicker and dark brown because of the addition of palm sugar. The dominant flavor of kecap is derived from spices, such as *Alpinia galanga*, *Carum roxburghianum*, leaves of *Citrus hystrix*, *Eugena polyantha*, *Foeniculum volagare* (fennel), or aniseeds, *Andropogen ceriferus*, *Coriandrum sativum* (coriander), garlic (*Allium sativum* L.), and *Polianthes tuberosa* (Shurtleff and Aoyagi 2012).

5.5.4 Tape ketan

Indonesian tape ketan is a sweet/sour rice-based alcohol in which an amylolytic mold of the *Amylomyces rouxii* type and at least one yeast of *Endomycopsis burtonii* type, hydrolyze steamed rice (starch) to maltose and glucose and then ferment the sugar to ethanol and organic acids, which produces an attractive flavor and aroma. The most common tape is tape ketela (cassava) or peuyem (West Java). By fermenting cassava to tape ketela, it is possible to raise the protein content to 4 percent or higher. When consumed in this form, it imparts important protein and amino acids. During the processing of tape ketela, 40–70 percent of cyanide in cassava is lost during steaming; on the other hand, 9 to 35 percent is lost during the 3 d of fermentation (Gandjar 2003).

5.6 Fermented Functional Foods of Nepal and Bhutan

The most popular fermented foods of Nepal are kinema (fermented soyabean), gundruk (*Brassica compestris* leaves), sinki (*Raphanus sativus*), tama (succulent bamboo shoot), selroti (deep fried preparation from rice flour), jand (local beer forms rice/maize millet), and tumba (fermented millet drink).

Fermented milk products are other popular food products in this mountainous region. Very popular fermented foods of milk are dahi (similar to yogurt), mohi (butter milk), sher, sher ghum (soft cheese from buttermilk), and chhurpi (dried very hard cheese form buttermilk), yak cherso. Other cheeses such as sher, shergum, shosis, churtsi chlauga or chhurpi are produced form nak (yak) milk in the higher altitude region of Bhutan (Joshi 2014).

5.6.1 Kinema

It is a non-salted, solid-substrate fermented, flavorsome, alkaline food, traditionally consumed mainly by the Nepalis, Lepchas and Bhutanese. It is a low-cost protein complement to rice. In the traditional method of kinema preparation, yellow seeded soybeans are cleaned, washed, soaked overnight (12–20 hr) at ambient temperature (10–25°C), cooked by boiling (90–95°C) in spring water for about 90 min, crushed lightly to grit, wrapped in fern banana leaves and sackcloth, and left to stand (25–35°C) for 1–3 d. The desired state of fermentation is indicated by the formation of a typical kinema flavor dominated by ammonia as well as a white viscous fluid on the beans. Fresh kinema is briefly fried in oil and added with vegetables, spices, salt and water to prepare a thick curry. *Bacillus subtilis* is the most dominant bacterium on raw soybeans (Tamang 2010); kinema contains *B. subtilis* as well as *Enterococcus faecium*. In addition to these two types of bacteria, *Candida parapsilosis* and *Geotrichum candidum* occur in many of the samples of kinema (Tamang 2010). *Bacillus* is the sole micro-organism carrying out the fermentation; the members of the accompanying flora are merely opportunists (Tamang 2010). As natural fermentation results in products of variable quality, development of controlled fermentation is essential for the manufacture of products of reproducible quality.

5.6.2 Gundruk

This fermented leafy green vegetable is a popular food in Nepal and is claimed to be one of the national dishes. It is popular not only in Nepal but also in the every household of Nepalese people worldwide. The annual production of gundruk in Nepal is estimated at 2,000 tons and most of the production is carried out at the household level. Gundruk is obtained from the fermentation of leafy vegetables. It is served as a side dish with the main meal and is also used as an appetizer. Gundruk is an important source of minerals particularly during the off-season when the diet consists of mostly starchy tubers and maize, which tend to be low in minerals.

It is commonly prepared during winter, i.e., October to December, when perishable leafy vegetables are plenty. These vegetables are mainly leaves of mustard (*Brasicca juncea*), rayo sag (*Brasicca rapa*), cauliflowers (*Brasicca oleracea*), radish (*Raphanus sativus*) and some other locally grown vegetables (Tamang 2010). The microorganism predominantly occurring in gundruk have been identified as *Lactobacillus brevis, Lb. plantarum, Lb. paracasei, Pediococcus pentosaceus, P. acidilactici* and *Leuconostoc fallax* (Tamang et al. 2005). For its fermentation, fresh leaves of the selected vegetables are first wilted and shredded using a sickle or knife. These are then crushed mildly and pressed into an earthen pot. The container is then made airtight and

left to ferment naturally at room temperature for about 7 to 10 d. After the incubation period the leaves take a mild acidic taste which indicates the completion of fermentation. The gundruk is then removed and sun dried for 3 to 4 d, which helps in storage.

5.6.3 Sinki

This is a form of fermented radish (*Raphanus sativus* L.) tap root and is consumed by the Nepalis in Darjeeling, Sikkim and Nepal. It is prepared during the months of winter when weather is least humid and there is ample supply of this vegetable (Tamang 2010). The microbes associated with its fermentation have been identified as *Lactobacillus plantarum, Lb. brevis* and *Lb. fermentum* (Tamang et al. 2012). Fresh tap roots of radish are cleaned by washing, wilted by sun-drying for 1–2 d until they become soft. They are then shredded, dipped in lukewarm water, squeezed and placed tightly into an earthen jar with the help of a heavy wooden pestle. The jar is sealed with an earthen lid and is covered with radish leaves. It is then kept in a warm and dry place for 15–30 d. Alternatively, a pit of about 1 m depth and diameter is dug in a dry place. This is cleaned, and dried by lighting a fire. The ashes are removed and the sides are plastered with mud while still hot. It is then covered on all sides with dried leaves of bamboo, banana or radish. The shredded roots are pressed tightly into this pit, then covered with dried leaves and weighed with heavy stones or wooden planks. The top is then plastered with mud or cow dung and left to ferment for a period of 30–40 d. After this the fermented mass is taken out, cut into small pieces and sundried for 3–5 d. This product can be kept for 2 yr or more at room temperature by exposing to it sunlight periodically (Tamang et al. 2012).

Sinki has a pH of 4.4, and a protein and fat content 14.6 g and 2.5 g, respectively on dry weight basis. It has a highly acidic flavor, and is used as a base for soup and pickle. It is said to be a good appetizer, and is used as a remedy for indigestion (Tamang and Sarkar 1993).

5.6.4 Mesu

Mesu is a fermented bamboo shoot product which is indigenous to the people of Himalayan regions of Darjeeling hills and Sikkim. It is prepared only during the months of June to September when Bamboo shoots sprout. The species of bamboo used are the locally available choya bans (*Dendrocalamus hamiltonii* Nees and Arnott), bhalu bans (*D. sikkimensis* Gamble) and karati bans (*Bambusa tulda* Roxb). Microbial analysis of young bamboo shoots demonstrated the presence of *Lactobacillus plantarum,*

Lb. brevis and *Lb. pentosaceus*. It was found that *Lb. pentosaceus* was the initiator of fermentation, followed by *Lb. brevis* and finally dominated by *Lb. plantarum* (Tamang and Sarkar 1996).

5.7 Fermented Functional Foods of Thailand and Phillippines

5.7.1 Miang

Miang (fermented tea leaves) is produced in the northern part of Thailand. The steamed tea leaves are wrapped tightly in individual bundles and packed into containers (small baskets for miang made from young tea leaves, or large underground cement wells for miang made from mature tea leaves). The tea leaves are pressed tightly, weighted down, covered with banana leaves or plastic sheets, and spontaneously fermented for 3–4 mon. *Lactobacillus*, *Pediococcus* and *Enterococcus* are dominant in the miang product (Tanasupawat and Visessanguan 2008).

5.7.2 Tapuy

Philippine rice wine, popularly known as tapuy, is an alcoholic rice drink. The name is derived from tapai, a fermented rice dish found in most of Southeast Asia. It is a traditional beverage that originated from Banaue and the Mountain Province, where it is used for important occasions such as weddings, rice harvesting ceremonies, fiestas and cultural fairs. It is produced from either pure glutinous rice or a combination of glutinous and non-glutinous rice together with onuad roots, ginger extract, and a powdered starter culture locally known as bubod (Philippine Rice Research Institute 2011).

The characteristics of tapuy, as in many other rice wines, depend on the process and ingredients used by each manufacturer. However, in general, tapuy is a clear full-bodied wine with a strong alcoholic flavor, moderately sweet and often leaves a lingering taste. The alcohol content is 28 proof or about 14 percent. It has no sulfites (which are preservatives found in other wines) that sometimes cause adverse reactions like hang-over and allergies. Tapuy is also not diluted with water and has no sugar added.

6 Biogenic Microbial Metabolites Derived from Fermentation

During fermentation, LAB produce a range of secondary metabolites, some of which have been associated with health-promoting properties. The most notable of these are the B vitamins and bioactive peptides released from food proteins, as outlined below. The (over)production of vitamins by LAB

provides a very attractive approach to improve the nutritional composition of fermented foods. Folacin (folic acid and related compounds; vitamin B11) is an essential vitamin for growth and reproduction in all vertebrates, and has a preventative role against several disorders affecting man including the development of neural tube defects, risk of coronary heart disease, some types of cancer and neuropsychiatric disorders (Finglas et al. 2003). Folic acid is an essential cofactor in bacterial metabolism and many bacteria used in food fermentations possess the biosynthetic capability to produce folate. The genes involved in folate biosynthesis have been identified in a folate gene cluster in *Lactococcus lactis* MG1363 and increased production of folate by metabolic engineering of this strain has been reported (Sybesma et al. 2003). Similar results have been achieved for vitamin B2 (riboflavin) and for a combination of vitamins B2 and B11, thereby opening up possibilities for multivitamin production in fermented foods by a single strain (Burgess et al. 2004). In nature, vitamin B12 (cobalamin) is exclusively of microbial origin, and is present in foods such as red meat and milk as a result of rumen microbial action. Vitamin B12 is an essential cofactor in fatty acid, amino acid, carbohydrate and nucleic acid metabolism. In addition to dietary sources, intestinal microflora contributes to vitamin B12 status in humans. Propionic acid bacteria are well known as efficient producers of this vitamin. The biosynthetic steps involved in vitamin B12 production are currently under investigation and appear to involve a complex pathway of at least 25 enzymatic reactions. Several fermented functional foods derive their activity from the release of bioactive peptides that are encrypted within the amino acid sequences of food proteins and released after enzymatic digestion. Peptides with inhibitory properties against angiotensin-I-converting enzyme (ACE) have an anti-hypertensive effect and have been isolated from enzymatic digests of various food proteins, while other properties ascribed to bioactive peptides include antimicrobial, opiate, cholesterol-lowering, immunostimulatory and mineral-utilizing properties (Stanton et al. 2005). Some food-grade bacteria, in particular *Lactobacillus helveticus*, have been used to generate bioactive peptides that exhibit anti-hypertensive, antimicrobial and immunomodulatory properties during milk fermentation (LeBlanc et al. 2002). In addition, it has been reported that *Lb. helveticus* fermented milk whey contains bioactive components that increase osteoblastic bone formation *in vitro*. Indeed, ACE inhibitory peptides produced during fermentation of milk are already the basis for health claims associated with some functional foods on the market such as CALPIS, a fermented milk manufactured by the Japanese company of the same name, and Evolus, launched by Valio (Stanton et al. 2005).

7 Conclusions and Future Perspectives

With the current technologies, it should be possible to be innovative about many of the foods produced using fermentation and indigenous knowledge systems. The challenge is to ensure that technology is used to add value to such products, such as increased shelf-life, flavor and appealing packaging and labeling. Old ferments are not an efficient way of preserving the LAB probiotic organisms as poor survival has been reported in these products. Ongoing basic research will continue to identify and characterize existing strains of probiotics, identifying strain-specific outcomes, determine optimal doses needed for certain results and assess their stability through processing and digestion. Gene technology will certainly play a role in developing new strains, with gene sequencing allowing for an increased understanding of mechanisms and functionality of probiotics.

Over time, the oriental fermented foods forms containing probiotics will emerge such as energy bars, cereals, juices, infant formula and cheese, as well as disease-specific medical foods. The establishment of standards of identity for probiotic containing food products will serve to accelerate the development and availability of these food products.

Keywords: Idli, Dosa, Hawaijar, Kimci, Lactic acid bacteria, Miso, Natto, Oriental foods, Probiotics, Tempe, Traditional foods

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10

Indigenous Fermented Foods of Latin America

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1 Introduction

In October 2012, The State of Food Insecurity in the World 2012 (SOFI) jointly published with the UN Food and Agriculture Organization (FAO), the International Fund for Agricultural Development (IFAD) and the World Food Programme (WFP), estimates of chronic undernourishment based on an improved methodology and data for the last two decades (FAO 2012). This report stated that nearly 870 million people, or one in eight, were suffering from chronic undernourishment in 2010–2012. The vast majority of the hungry, 852 million live in developing countries—around 15 percent of their population—while 16 million people are undernourished in developed countries. Latin America and the Caribbean had 49 million hungry in 2010–2012, while the prevalence of undernourishment dipped was 8.3 percent (FAO 2012).

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Recently, SOFI 2013 presented updated estimates of undernourishment and progress towards the Millennium Development Goal (MDG) and World Food Summit (WFS) hunger targets (FAO 2013). The latest assessment shows a total of 842 million people in 2011–13 were estimated to be suffering from chronic hunger, regularly not getting enough food to conduct an active life. This figure is lower than the 868 million reported with reference to 2010–12. The total number of undernourished has fallen by 17 percent since 1990–92 (FAO 2013). Although some progress has been made towards the 2015 MDG target, considerable and immediate additional efforts are needed.

It is important to state that in some countries, under-nutrition rates, as indicated by the proportion of stunted children, are considerably higher than the prevalence of under-nourishment, as indicated by inadequacy of dietary energy supply. In these countries, nutrition-enhancing interventions are crucial to improve the nutritional aspects of food security. Improvements require a range of food security and nutrition-enhancing interventions in agriculture, health, hygiene, water supply and education, particularly targeting women (FAO 2013). These people lack some or all nutritional elements required for human health, principally due to protein-energy malnutrition and micronutrient (vitamin and mineral) deficiency. UNICEF describes malnutrition as a term that not only contains under-nutrition, but also refers to all eating disorders including over-nutrition. People are considered malnourished if they do not acquire adequate amounts of calories and proteins through their diet which are essential for growth and maintenance. This can be caused by economic or social factors, i.e., not having access to adequate amounts of foods or also due to illness where nutrient absorption can be altered. Using this broader definition, malnutrition does not only affect people who live in developing countries, where food deprivation is constant, but also all populations, regardless of age, sex, income, or geographic origin. The only cause of malnutrition is bad eating habits (except for certain physiological disorders) and since these can cause a wide range of health problems (i.e., under development, avitaminosis, obesity, cardiac diseases, diabetes, etc.), there is an increased demand, principally in wealthier nations, for "healthier" foods that not only provide basic nutritional values but also provide health promoting properties, also known as functional foods. Due to this increased interest, some large companies, such as Unilever which has over 174,000 employees working in 20 different countries, are now studying the diets of ancient settlers such as cavemen in order to see what secrets these may contain (Halliday 2010).

Household fermentation of foods has a long and very important tradition in Latin American countries. The objective of this work is to shine a light on current knowledge of the biotechnological processes involved in the production of indigenous fermented foods from Latin America.

There is little scientific publications on the beneficial properties and/or production of indigenous fermented foods (LeBlanc et al. 2012), reason for which in this Chapter, a special emphasis will be placed on foods that are not currently mass-produced. The use of modern scientific tools and techniques now allow us to mine knowledge from the past and allow us to use it to produce novel beneficial foods in the future. The identification and isolation of microorganisms from these indigenous fermented foods of Latin America could help in the fight to prevent world hunger or at the very least improve the food safety of these foods that have been consumed for thousands of years without being adequately studied.

2 Microorganisms Involved in the Fermentation Process

Fermentation is one of the oldest forms of biotechnology and is one of the earliest methods used to preserve and improve the sensorial quality of foods. Fermentation consists of using microorganisms, or their enzymes, to modify the chemical composition of foods through a complex series of biochemical reactions. These microorganisms cause changes in the composition, flavor, and texture of raw food materials making them more enjoyable to eat and more importantly preventing the spoilage of highly perishable items.

There is a wide range of fermentative microorganisms; the most common are species from the *Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Weissella, Vagococcus, Pediococcus, Bifidobacterium, Streptococcus, Enterococcus* and *Enterobacterium* genera. These bacteria convert food carbohydrates to organic acid such as lactic acid that decrease the pH of the food matrix, which inhibits the growth of spoiling and pathogenic microorganisms. In acidic conditions, some food proteins, such as casein from milk, denature and coagulate causing physical changes in the raw food material. For example, the acidification of milk allows the formation of curd that can be consumed directly or pressed, molded, ripened and maturated to produce semi-hard or hard cheeses. Besides organic acids, lactic acid bacteria (LAB) also produce: (i) aromatic compounds such as acetaldehyde that is a major contributor to the flavor of fermented milk products such a yogurt, sour milk and fresh cheeses; (ii) exo-polysaccharides that are implicated in the firmness of yogurts and cheeses (Van der Meulen et al. 2007); (iii) vitamins, such as B group vitamins (especially riboflavin, folate, vitamin B_{12}, thiamin) whose concentrations are normally increased in fermented foods compared to the raw starting materials (LeBlanc et al. 2010a); (iv) bacteriocins that can prevent the growth of undesirable pathogens; (v) digestive enzymes that: (a) liberate peptides (some are bioactive) and free amino acids from food proteins and thus facilitating absorption, (b) hydrolyze non-digestible fibers

found in some plant materials (LeBlanc et al. 2008), (c) degrade lactose and other sugar that can cause digestive disorders in sensitive individuals, etc. Because of the numerous beneficial properties that have been attributed to LAB, these are the most commonly used probiotic microorganisms that can be defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2001). Some of the health claims attributed to probiotics include the following: improvement of the normal microbiota and stabilization of the gut mucosal barrier (del Carmen et al. 2011), prevention of infectious diseases and food allergies, hypocholesterolemic, anti-mutagenic and anti-carcinogenic properties (de Moreno de LeBlanc et al. 2007), immune system modulation (Galdeano et al. 2007, LeBlanc et al. 2004a), prevention of inflammatory bowel diseases (LeBlanc et al. 2011), and alleviation of lactose intolerance. They have also been shown to provide essential compounds such as vitamins (LeBlanc et al. 2005a, 2006, 2010a,b), hydrolytic enzymes (LeBlanc et al. 2004b,c,d, 2005b, 2008), bacteriocins (Todorov et al. 2008), bioactive compounds (LeBlanc et al. 2002), among others. Genetically modified probiotics are also emerging in order to increase their effectiveness or deliver novel compounds not normally produced by native strains (LeBlanc et al. 2010c).

Alternatively, yeasts, often of the *Saccharomyces* or *Kluyveromyces* genera, have allowed the production of a wide range of fermented beverages, such as beers, wines, and fermented grains that are more easily stored and transported. Yeasts can convert glucose to carbon dioxide in leavened breads, or the sugars in grain or fruit beverages to ethanol. Besides their obvious uses in food fermentation, some strains of *Saccharomyces* have also been shown to exert probiotic properties. For example, it was recently shown that *Saccharomyces cerevisiae* strain UFMG 905, an indigenous yeast isolated from the production of the traditional cachaça in Brazil, protects against bacterial translocation, preserves gut barrier integrity and stimulates the immune system in a murine intestinal obstruction model (Generoso et al. 2010).

Molds belonging to the genus *Penicillium, Aspergillus*, and *Mucor* are also very active in certain fermentations. Molds are useful since they possess both proteolytic and lipolytic activities that can produce a wide range and variety of compounds from either proteins or lipids, respectively. Some molds, such as *Penicillium,* responsible for the distinctive flavor of blue-cheeses, can produce the important antibiotic penicillin of common use in modern medicine to combat bacterial infections. The molds, *Aspergillus oryzae* and *Aspergillus sojae* are used in the production of soy sauce, but some strains of *Aspergillus* can be dangerous since they can produce toxic compounds such as aflatoxins (LeBlanc 2010).

3 Indigenous Fermented Foods

Indigenous fermented foods such as bread, cheese, beer and wine, have been prepared and consumed for thousands of years. Archeologists have demonstrated the use of fermentation processes by ancient cultures in Egypt, Mesopotamia and America as a method to preserve the food products. Locally available materials and more importantly the methods of preparation are strongly linked to culture and tradition; the preparation of these foods still remains a household art in many isolated regions and are normally passed down from generation to generation. In Latin America, there are regions where fermented products are still manufactured traditionally using very simple equipments. In specialized markets throughout the world, these fermented products are highly appreciated and are considered to be of premium value because of their flavor characteristics and uniqueness. This is not the case when fermented products are obtained using modern large scale technologies since selected starter cultures are normally employed in order to standardize output, i.e., all the fermented foods produced commercially are exactly the same regardless of where they were produced.

Indigenous fermented foods have special organoleptic qualities and some even possess health promoting properties (Table 1). This is principally due to the presence of a biologically diverse microbiota present in the raw material or as a contaminant from the producers or instruments used to prepare them (Table 2). These microorganisms are important genetic reservoirs and hold great biotechnological and health improving potentials that should be exploited. Many have evolved in harsh environmental conditions in order to survive whereas others have done so in order to produce enzymes and compounds necessary for their growth on raw food materials. In the following sections, traditional fermented foods from Latin America will be described, taking into account their history, production methods and the microbial diversity necessary for their elaboration. The diversity of the raw materials used as substrates and methodologies used will also be helpful in understanding the cultural habits of various and often geographically isolated cultures. The isolation and conservation of the native microbiota found in these products is essential in order to prevent the loss of technologically and physiologically important microbial species.

3.1 Fermented Beverages

A recent study isolated and characterized lactic acid bacteria (LAB) from Brazilian food products searching by their probiotic properties (Ramos et al. 2013). The results showed the probiotic potential of certain strains of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis*

Table 1. Beneficial effects attributed to some indigenous fermented foods.

Fermented food	**Health benefits**	**References**
Chicha de jora	Source of riboflavin, thiamine, and a wide variety of B-vitamins	Steinkraus 2003
	Probiotic effect (decrease diarrheal episodes)	Traditional claim#
	Reduction of cholesterol and triglyceride; decrease of body weight	Traditional claims#
Pozol	It is richer in protein, niacin, riboflavin, lysine, tryptophan, and some other nutrients than unfermented maize(corn)	
	Decrease of diarrhea and fever Prevention or treatment for skin infections and wounds	Phister et al. 2004
	Bacillus sp. strain CS93 isolated from pozol produces antimicrobial compounds against several Gram-positive and Gram-negative bacteria, yeasts and molds	Phister et al. 2004
	Bacillus sp. strain CS93 produces bioactive lipopeptides	Moran et al. 2010
	Agrobacterium azotophilum, possesses bacteriocidal, bacteriolytic, bacteriostatic and fungistatic activities against a wide range of pathogenic microorganisms	Ulloa and Herrera 1972
Aloja	Carob flour shows activities against ulcers, childhood diarrhoeas and intestinal infections	Sahin et al. 2009
	Algarroba pods contain fibers that can maintain a beneficial intestinal microbiota and also have been shown to inhibit the growth of colon cancer cells	Klenow et al. 2008
	Algarrob pods contain polyphenols that possess antioxidant, anti-inflammatory, anti-rheumatic properties in addition to being beneficial for the heart and kidneys	Makris and Kefalas 2004
	Carob pods have been used as a treatment for rehydration following extreme diarrheas, and for the treatment of acute-onset diarrhea	Aksit et al. 1998, Loeb et al. 1989
Pulque	The isolated microorganisms were proposed to confer a beneficial effect on the digestive system	Cervantes-Contreras and Pedroza-Rodriguez 2007
Tocosh	It was used to treat many different illnesses such as stomach ulcers, colds, pneumonia, and hemorrhoids, to prevent gastrointestinal infections, used in childbirth (postpartum) and as a curative agent to treat wounds	Traditional claim#
	Its probiotic potential was demonstrated using an experimental animal model and compared with a recognized probiotic *Lactobacillus acidophilus*	Prentice and Milka 2005

#No scientific articles supporting these claims were found

Table 2. Microorganisms isolated from indigenous fermented foods of Latin America.

Fermented food	Region of origin	Isolated Microorganisms	Reference
Chicha de jora	Andean región (Argentina, Chile, Peru, Bolivia, etc.)	*Saccharomyces cerevisiae*	Quillama 1993, Vallejo et al. 2013
		Lactobacillus plantarum and *Lb. fermentum*	Quillama et al. 1995
Caxiri	Brazil (Northernmost state of Amapá, bordering French Guiana)	*S. cerevisiae, Bacillus* spp., *Sphingomonas* sp., *Pediococcus acidilactici*	Santos et al. 2012
Cauim	Coast of Brazil	*Lb. pentosus, Lb. plantarum, Corynebacteriu xerosis, C. amylocolatum, C. vitarumen, Bacillus cereus, B. licheniformis, B. pumilus, B. circulans Paenibacillus macerans*	Almeida et al. 2007
		Candida tropicalis. C. intermedia, C. parapsilosis, Pichia guilliermondii, S. Cerevisiae, Trichosporon asahii	Schwan et al. 2007
		Lb. plantarum, Lb. fermentum, Lb. paracasei and *Lb. brevis, Pichia guilliermondii, Kluyveromyces lactis, Candida* sp., *Rhodotorula toruloides* and *S. cerevisiae*.	Ramos et al. 2010, 2013
Cachaça	Brazil	*S. cerevisiaeis, Rhodotorula glutinis* and *Candida maltose* are predominant. Other yeasts also present were species from *Kluyveromyces, Pichia, Hanseniaspora,* and *Debaryomyces*	Schwan et al. 2001
		Lb. plantarum and *Lb. casei*	Gomes et al. 2010
Pozol	Southeastern Mexico and other Central American countries	*Lb. plantarum* and *Lb. fermentum*, together with members of the genera *Leuconostoc, Weissella* and the genus *Streptococcus*	Ampe et al. 1999, ben Omar and Ampe 2000
		Bifidobacterium, *Enterococcus*, and Enterobacteria	ben Omar and Ampe 2000
Aloja	Great Chaco forest of South America (Argentina, Bolivia, Peru, etc.)	*Bullera variabilis, Candida famata, Cryptococcus species, Debaryomyces hansenii, Pichia angusta, P. ciferrii, P. Farinose, Torulaspora delbrueckii*, and other *Candida, Kluyveromyces* and *Pichia* species	Spencer et al. 1995

Pulque	Mexico	*Zymomonas* sp., *Lactobacillus* sp. and *Saccharomyces* sp.	Cervantes-Contreras and Pedroza-Rodriguez 2007
		Species within the alpha- gamma-Proteobacteria and *Firmicutes*	Escalante et al. 2008
		Pediococcus parvulus, Lactobacillus brevis, Lb. composti, Lb. parabuchneri, and *Lb. plantarum* Genera such as *Weissella* and *Bacillus* were also present	Narvaez-Zapata et al. 2010
Tocosh	Peru and other regions of South America	Preliminary studies showed that bacteria and yeasts act during the initial transformation process, and lactobacilli are predominant in the final product	Yamamoto 1988
Cacao fermentation	Brazil	*S. cerevisiae, Hanseniaspora* sp., *Lb. fermentum*, *Lb. plantarum*; *Acetobacter tropicalis*, and *Bacillus subtilis*	de Melo Pereira et al. 2013
Kefir	Brazil	*S. cerevisiae*, *Lactobacillus kefiranofaciens* and *Lb. kefir*	Leite et al. 2012

(Ramos et al. 2013). This is a clear example that important biodiversity is present in traditional fermented foods and these are thus important reservoirs for health promoting and even biotechnologically important microorganisms. The important indigenous fermented beverages consumed in Latin American countries are discussed below.

3.1.1 Chicha de jora

Chicha de jora is one of the oldest fermented beverages in South America. It is believed that its origin dates back to the 15th century from the times of the 10th *Sapa Inca* (Inca ruler) Túpac Inca Yupanqui. Folk legend tells the story of how torrential rains deteriorated corn silos which caused the germination of the grains. The Inca Yupanqui ordered the distribution of the resulting malt throughout the Inca Empire so that it could be consumed as *mote* (grains cooked in water) but these were not well received because of unfamiliarity with its organoleptic characteristics. Legend has it that one hungry man ate these grains and became extremely intoxicated, thus discovering the alcoholic value of maize. During the Inca Empire, *Aklya Kona* ("Virgins of the Sun") were taught the techniques of brewing chicha in *Acllahuasis* (factories where young women worked for the benefit of the Empire). Maize was first chewed to pulp since saliva helps to convert starches to fermentable sugars. This pulp was then sun dried, mixed with warm water and left to ferment for a few days, converting the corn into a mildly alcoholic drink. Chicha de jora has been prepared and consumed in communities of Andes for millennia. The Inca used Chicha in rituals and during religious festivals. Currently it is produced in the Andean regions and sometimes in the lower altitude regions of countries such as Argentina, Bolivia, Brazil, Colombia, Ecuador and Peru and it is consumed during religious and agricultural festivities and during important family and social events.

Chicha de jora is traditionally prepared from specific yellow maize (jora) as a family activity where women and children sit in a circle chewing maize (*Zea mays*) kernels. The pulp mixture from each family member is then spit into a vat of warm water and allowed to ferment with yeasts present in the environment. The result is a yellow cloudy liquid with a slightly milky appearance that contains a relatively small amount of alcohol (as much as 6% ethanol). Chicha obtained in this manner is called "chichanuqueada" and is now banned in many countries because of the anti-hygienic production method and potential health hazards to the consumers. Although there are more modern ways to ferment maize, it is commonly believed that the unique sour taste of chicha is lost if no contact with human saliva is made, a property that may be conferred by the presence of *Lactobacillus* bacteria within the sputum of donating women (Nguyen and Wong 2006).

Chicha de Jora is now prepared by first soaking the maize grains in water for 24–48 hr on dampened plant leaves and put in a large pot lined with those leaves that will be placed in the sun to germinate for 8–15 days in order to produce the highest concentration of sugars. During the germination process, the grain's starches are converted into fermentable sugars (mono- and di-saccharides) by the action of amylases and proteins are broken down by proteases, both activities being necessary for yeast growth. The germinated maize is then boiled for 6–24 hr during which time different herbs and spices are added such as cinnamon, allspice, cloves, etc. The liquid is allowed to cool, strained through a clean cloth and unrefined sugar is added. It can be drunk either young and sweet or mature and strong when the mixture is allowed to ferment during 1–2 weeks in clay pots. The final fermented product has a final pH of 4.0–4.5 and alcohol content of 4–6%.

Very few studies have been carried out on the fermentation of Chicha de jora. It was shown that *Saccharomyces cerevisiae* is the predominant native yeast species present (Quillama 1993). In this sense, a recent study showed that *S. cerevisiae* strains found in the Chicha have a phenotypic profile that differed in more than 40% with the current *S. cerevisiae* strains (Vallejo et al. 2013). Lactic acid bacteria such as *Lactobacillus plantarum* and *Lb. fermentum* are also present in large quantities (Quillama et al. 1995). It was shown that *Lb. plantarum* E2, a strain isolated from Chicha de jora, is capable of producing a bacteriocin that limits the growth of *Lb. fermentum* Chj4C, another strain isolated from the native beverage (Quillama 1998).

Chicha has great cultural and medicinal value. In the fermentation, the growth of the yeasts provide riboflavin (its concentration is doubled), thiamine, and a wide variety of B-vitamins (Steinkraus 2003).

The presence of lactobacilli have been related with beneficial effects (as probiotics), especially at the gut microbiota level. In this sense there are documents that claim people who consume Chicha suffer fewer diarrheal episodes; however there are no scientific articles that document these findings.

Other claims traditionally (without scientific reports) attributed to fermented Chicha are the reduction of cholesterol and triglycerides, that help in digestion and decreased body weight.

3.1.2 Caxiri/Cachiri/Caciri/Cassiri

Caxiri is a traditional fermented alcoholic beverage produced from cassava (*Manihot esculenta* Crantz or it is sometimes referred to as manioc, mandioca or yucca root) and sweet potatoes (*Ipomoea batatas* L.) by the Wajapi Indians that live in the northernmost state of Amapá, Brazil bordering French Guiana and by the neighboring indigenous communities (such as Palikur, Yudja, etc.). Cassava fermentation processes are adequate for reducing its

toxic cyanogenic glucosides, for preservation and improving product flavor and aroma (Holzapfel 1997). This cassava based beer is always prepared by women in individual households during special festivities such as the Turé. Caxiri is made by all of the women living in a particular residence, usually surrounded by groups of children and pets but far away from the gazes of men. The cassava roots are baked, peeled, grated and pressed into a mash that are placed in a large pot along with water, sugar and/or other ingredients (such a pineapple juice, honey, etc.). The women then fill their mouths with cassava, chew on it for a while and then place the masticated roots inside the liquid, their saliva degrading the starches into more fermentable sugars and this chewed dough beings the primary source of fermenting microorganisms. The mixture is then cooked over an open fire after which the women gather around the pot, sing songs before allowing the mixture to ferment over 2 or 3 days. Sometimes an overturned bowl is placed at the bottom of the mixture; the carbon dioxide produced during the fermentation process causes the bowl to rise giving the women a sign that the caxiri will be good for consumption. It is said that caixiri gives these Indians access to the supernatural world; both the brewing pot and the drink itself are transformed into "supernatural entities" that are present in the indigenous cosmology. Several tens of liters are prepared by each family giving a total production of several hundreds of liters by each indigenous community. Caxiri is served in small bowls and binge-drinking to the point of disequilibrium and unconsciousness is common (Seale et al. 2002); the partiers even induce vomiting to be able to drink more of the alcoholic beverage and justify this action as being a means to cleanse their intestines. Recently, microbiological and physicochemical characteristics of caxiri were analyzed (Santos et al. 2012). Caxiri fermentation was associated with an increase in the total microbial population, with yeast being predominant. The bacteria were mainly represented by endospore-forming Gram-positive bacilli (*Bacillus* spp.; 61.5% of the isolates). *Sphingomonas* sp. and *Pediococcus acidilactici* were also described in this fermented product. The dominant yeast identified was *Saccharomyces cerevisiae*. Other characteristics of caxiri were a decrease in reducing sugars, and a high content of ethanol and a high concentration of lactic acid.

3.1.3 Cauim

Cauim is a non-alcoholic beverage produced by the Tapirapé Amerindians who probably descended from the Tupinamba tribe that populated part of the coast of Brazil in 1500. Unlike caxiri that is consumed only during special festivities, cauim is consumed in daily meals by adult Tapirapé Indians and is the main staple food for infants under the age of two years old (Almeida et al. 2007). This beverage was first described by Jean de

Léry in an account of his trip to Brazil in the 16th century (de Léry 1577). Cauim is principally produced using cassava, but several other substrates are also used such as rice, corn and peanuts. The preparation of cauim was recently described (Almeida et al. 2007). Briefly, cassava roots are allowed to ferment for 3 to 5 days in running water to soften the skin. After this time the cassava tubers are peeled, cut in small pieces and sun dried. The dried pieces are then grated into flour. This flour is then mixed with water and cooked for about 2 hr and then cooled at room temperature. When the porridge is cold an inoculum, consisting of masticated cassava root, is added to initiate the fermentative process, which usually takes 24 to 48 hr. During this fermentation, LAB numbers increased from the beginning of fermentation and become the pre-dominant microorganisms throughout the fermentation (Almeida et al. 2007). Bacterial populations belonging to *Lactobacillus pentosus*, *Lb. plantarum*, *Corynebacter iumxerosis*, *C. amylocolatum*, *C. vitarumen*, *Bacillus cereus*, *B. licheniformis*, *B. pumilus*, *B. circulans* and *Paenibacillus macerans* were identified (Almeida et al. 2007). Most of these strains were able to efficiently hydrolyze soluble starch and to secrete proteases, both activities being important in cassava fermentation since it can increase the availability of carbon sources for other non-amylolytic microbial groups. The predominant yeast species found in a rice cassava fermentation was *Candida tropicalis. Candida intermedia*, *Candida parapsilosis*, *Pichiaguilliermondii*, *Saccharomyces cerevisiae* and *Trichosporon asahii* were also found in high numbers during the fermentation (Schwan et al. 2007). The role of yeasts during cauim fermentation needs additional investigation but it has been suggested that they might be involved in the degradation of starch, flavor production, increasing levels of vitamin B, and production of free amino acids which would increase the nutritional value of the beverage for infants. *Exophialadermatidis*, often associated with blastomycosis, was found in the mash before inoculation and during the initial stages of the fermentation (Schwan et al. 2007). The presence of this pathogen might be associated with unhygienic conditions during production of the beverage and its disappearance might be due to the fermentative microbiota that can produce acids and other antimicrobial components during fermentation and could promote or improve the microbiological safety and stability of the product (Holzapfel 1997).

Yeast such as *Candida tropicalis, Candida intermedia*, *Candida parapsilosis*, *Pichia guilliermondii* and *Trichosporon asahii* may possibly be present and pose a potentially health hazard. Similar observation about yeast population was recorded in other traditional fermented products in Europe such as boza (Botes et al. 2007). Boza is traditional fermented beverage produced using different cereals and is greatly consumed in the Balkan Peninsula region of Europe. *Candida* spp. were isolated from this drink, which was shown

to be a reach source of probiotic LAB (Todorov et al. 2008) and was highly recommended by the traditional medicine in treatment of diarrhea in infants.

Another report about cauim was made using peanut and rice fermentation. This work studied the microorganisms associated to this fermented beverage using a combination of culture-dependent and -independent methods (Ramos et al. 2010, 2013). The results showed that microbial community changed during the fermentation process, with the predominance of bacterial species such as *Lactobacillus plantarum, Lb. fermentum, Lb. paracasei* and *Lb. brevis*, and the yeast species *P. guilliermondii*, *Kluyveromyces lactis*, *Candida* sp., *Rhodotorula toruloides* and *Saccharomyces cerevisiae*.

Yakupa is a traditional non-alcoholic cassava beverage produced by indigenous Juruna people, who inhabit the Indigenous Xingu Park in Mato Grosso (Brazil) and it is classified within refreshing cauims. A recently analysis of microbial diversity showed for the first time that LAB population was higher than yeast in the beginning of fermentation and after 36 hr both population increased reaching 7 log CFU/ml (Freire et al. 2013). This study also described that maltose, ethanol and lactic acid were the most abundant compounds identified in this beverage.

3.1.4 Cachaça/Aguardente De Cana/Pinga/Caninha

Cachaça is a fermented spirit obtained from the distillation of fermented sugar cane juice and is used to prepare the Brazilian world renowned cocktail "caipirinha". Although the exact origin of this beverage is unknown, it has been produced in Brazil since the 16th century and is now produced by 30,000 producers at an annual volume of 1.3 billion liters (Campos et al. 2010). It is no surprise that Brazil is the largest producer and consumer of chachaça since it is the world's largest producer of sugar-cane (Silva et al. 2010). Unlike rum, which is produced from the sugar refining by-product molasses, cachaça is produced from crushing freshly-cut sugar canes. The released juice is fermented by microbial inoculum (*pé de cuba*) that is prepared by a method known as *fermentocaipira* consisting of making a mash with undiluted juice, rice, maize flour, salt biscuits with the addition of lemon or orange juice to decrease the pH. This starter culture consists of wild yeasts with good fermenting properties and in most cases *Saccharomyces cerevisiae* is the predominating species along with *Rhodotorula glutinis* and *Candida maltose*; however, other yeasts are also present such as species from *Kluyveromyces, Pichia, Hanseniaspora,* and *Debaryomyces* (Schwan et al. 2001). Currently, there is no standardized method for the production of the starter culture thus cachaça production varies significantly in terms of yield and quality of the beverage from region to region and between each individual producer (Schwan et al. 2001). In this sense, de Souza et al. (2012)

demonstrated the importance to develop strategies for the selection of yeast strains to be used as starters in "cachaça" production in order to improve the quality of the final product. These strategies use the co-inoculation of *S. cerevisiae* and *Lb. fermentum* (Duarte et al. 2011) or the co-inoculation of different yeasts (Duarte et al. 2013). The starter cultures are used to inoculate the main vat (fermentation vessel), during 24 hr. The fermented sugar-cane juice is then decanted (to remove the yeast that has sedimented to the bottom) to a copper still (*alambique*) which is heated over wood fire and water bath (at 80ºC). The distillate, which contains between 48 to 56% ethanol (v/v), is often allowed to mature in wooden barrels for several years giving rise to aged (gold) cachaça that is drunk straight whereas unaged (white) chacaça is used to prepare caipirinha and other beverages.

The identification of populations of LAB present during cachaça fermentation was conducted in two distilleries located in the state of Minas Gerais showing that LAB were isolated in high frequencies during all of the fermentative processes (Gomes et al. 2010). *Lb. plantarum* and *Lb. casei* were the most prevalent species. Another more recent study also showed that the majority of clones obtained from cachaça samples during the fermentation of sugar cane juice were from the genus *Lactobacillus* (Lacerda et al. 2011).

The negative effect associated with cachaca consumption can be related to the presence of ethyl carbamate and polycyclic aromatic hydrocarbons (PAHs) in this beverage (Riachi et al. 2014). Ethyl carbamate is a carcinogen demonstrated in experimental animals and probably is carcinogenic to humans when present in quantities exceeding the recommended safety limit in cachaça (Lachenmeier et al. 2010). On the other hands, a recent study showed that the polyethylene tank is a source for PAHs in cachaça (de R. Machado et al. 2014).

3.1.5 Pozol

Pozol is a non-alcoholic beverage made from fermented maize that has been produced by the Maya Indians in southeastern Mexico and other Central American countries (such as Guatemala) since pre-Hispanic times (Wacher et al. 2000). Today it still forms part of the basic daily nutrition of urban and rural populations of different ethnic groups that reside in this region and is not only produced by Mayan descendents, but also by Mestizo (people of mixed European and Amerindian heritage) populations (Jiménez Vera et al. 2010). The first step in pozol preparation is called nixtamalization, where the kernels obtained by shelling cobs of maize (white, yellow or black) are boiled for 1.5 hr in a pot containing 1 to 2 liters of a 10 per cent (w/v) calcium hydroxide solution. These are then de-hulled and rinsed using tap water (the discard is denominated nexayote). The maize is then coarsely ground to make dough (called nixtamal) that is shaped into

balls, wrapped in banana leaves to prevent desiccation, and normally left to ferment at ambient temperatures for 2–7 days but sometimes up to a month. The fermented dough is then suspended in water and drunk during meals, at work or anytime during the days as a refreshing beverage. Traditionally, pozol was produced primarily for family consumption, the drink being consumed by adults, children and infants (Ulloa et al. 1987) but sometimes slightly larger-scale producers make pozol for sale. Some fibrous components are not completely solubilized by nixtamalization and sediment is present in the beverage when the dough is suspended in water. The Mestizo population has apparently modified the Indian process by adding a second boiling of the nixtamal grains in water before grinding to reduce sediment formation (Wacher et al. 2000). In the state of Tabasco, a modification of the traditional pozol is obtained by adding ground cacao beans to the dough prior to fermentation and this fermented product is called chorote. In the state of Yucatan, ground coconut is also be added to pozol (Cañas-Urbina et al. 1993). However, an evaluation of microbiological and sensory qualities of fermented white pozol, with cacao and coconut showed that the addition of these ingredients did not improve acceptability between consumers (Jiménez Vera et al. 2010).

In the first complete study of the microbiota of pozol, it was shown that freshly prepared pozol contained 10^4 to 10^6 CFU (Colony forming Units)/g of LAB; l0^4 to 10^5 aerobic mesophiles; 10 to 10^3 *Enterobacteriaceae*; 10 to 10^4 yeasts; and $<10^3$ mould propagules (Wacher et al. 1993). After 30 hr at 28°C the numbers were: 10^9 LAB, 7 x 10^6 aerobic mesophiles, 5 x 10^5 *Enterobacteriaceae*, 10^6 yeasts and 10^4 molds. Soaking alkali-treated grains overnight allowed LAB, aerobic mesophiles and *Enterobacteriaceae* to grow and these then constituted the primary microbial flora of the pozol dough. It was shown that although the additional cooking stage added in the Mestizo process significantly modified the physical properties of the dough, no significant differences were observed in the microbial composition of Mestizo compared to traditionally prepared pozol (Wacher et al. 2000). The presence of yeast and molds were associated to samples wrapped in banana leaf and no fungi were found in samples stored in plastic bags (Nuraida et al. 1995, Wacher et al. 1993, 2000).

The main soluble sugar of maize is sucrose which is present at a concentration of 2% (w/w) of the whole kernel on a dry weight basis but this concentration is reduced to 0.1–0.7% (w/w) of dry dough after alkaline cooking, soaking, and washing to produce nixtamal (Diaz-Ruiz and Wacher 2003). This concentration would be insufficient in order to maintain microbial diversity and the high bacterial concentration reported in pozol (Diaz-Ruiz et al. 2003). Although LAB are the dominant group during all stages of pozol fermentation, as has been shown previously by classical culture methods (Nuraida et al. 1995, Wacher et al. 1993, 2000) and culture-

independent methods (Ampe et al. 1999, Escalante et al. 2001), significant changes of population dynamics occur throughout pozol production. For example, it was shown that high concentrations of amylolytic LAB (ALAB) were detected at the beginning of the fermentation process and that a relatively high number of non-amylolytic LAB were observed at the end of fermentation (Diaz-Ruiz and Wacher 2003). These results suggest a symbiosis in that ALAB would first degrade starch at the beginning of the fermentation, decreasing the pH of the dough and liberating sugars that could be used for the growth of non-amylolytic microorganisms.

LAB (such as *Lb. plantarum* and *Lb. fermentum*, together with members of the genera *Leuconostoc* and *Weissella*) accounted for 90 to 97% of the total active microflora of pozol; no streptococci were isolated, although members of the genus *Streptococcus* accounted for 25 to 50% of the microflora (Ampe et al. 1999). The presence of *Bifidobacterium*, *Enterococcus*, and Enterobacteria suggests a fecal origin of some important pozol microorganisms (ben Omar and Ampe 2000).

A very complete microbial community dynamics study during the production of pozol was performed using traditional and culture-independent techniques (ben Omar and Ampe 2000). It was shown that *Streptococcus* species dominated the fermentation and accounted for between 25 and 75% of the total flora throughout the process. The initial aerobic microflora was replaced in the first 2 days by hetero-fermentative LAB (closely related to *Lb. fermentum*); this heterolactic flora was then progressively replaced by homo-fermentative LAB (mainly by genetically close relatives of *Lb. plantarum*, *Lb. casei*, and *Lb. delbrueckii*), which continued acidification of the maize dough. At the same time, a very diverse community of yeasts and fungi developed, mainly at the periphery of the dough. This study demonstrated that a relatively high number of species, at least six to eight, are needed to perform pozol fermentation. Overall, the results obtained with different culture-dependent or -independent techniques clearly confirmed the importance of developing a polyphasic approach to study the ecology of fermented foods.

Since little sanitary measures are taken during pozol preparation, microbial contamination of the maize dough is inevitable. Potentially pathogenic fungi such as *Candida parapsilosis*, *Trichosporon cutaneum*, *Geotrichum candidum*, *Aspergillus flavus* have been recovered from pozol during the first few hours of fermentation (Ulloa et al. 1987). Although most of these pathogens are killed off by the decrease in pH during the fermentation process, serious health problems can still arise. A major health risk associated with pozol and other maize-based foods is the consumption of mycotoxins, because even when the fungi are destroyed by the nixtamalization process, the toxins can remain in the dough. It was recently shown that 19% of pozol samples were contaminated with aflatoxin

B2 (AFB2) and traces of aflatoxin B1 (AFB1), but only 1 sample contained aflatoxin concentrations above 20 ppb (Mendez-Albores et al. 2004). In the same study it was also shown that pozol prepared with white maize and ones prepared with cacao showed the highest ranges of contamination whereas when yellow kernels were used, the presence of aflotoxins was not detected. Amerindians consume equal amounts of pozol prepared using white, yellow and black maize, although popular belief was that the yellow and black varieties contained higher vitamin contents. Mestizos normally utilize white maize for pozol preparation and also consume chorote, a pozol derivative where ground cocoa beans are added to the maize mixture. The former population would thus be less likely to consume aflotoxin containing pozol that the latter, however in Mestizo preparation the additional boiling step would be helpful in eliminating aflotoxin producing microbes.

Another important consideration is the improvement in the nutritive value of pozol over that of maize kernels. In this sense, people that consume mainly maize, as in Mexico or Peru, normally have a problem with niacin intakes that can lead to diseases such as pellagra. Pozol is richer in protein, niacin, riboflavin, lysine, tryptophane, and some other nutrients than maize. In addition, on the basis of its essential amino acid composition and growth-promoting efficiency in albino rats, the protein quality of pozol was found to be better than that of maize. The nitrogen content of pozol was determined to be higher than that of unfermented maize dough. The early Mayans, not only used pozol as a source of nutrients and in ceremonies promoting the growth and harvest of maize, they also used this fermented beverage as a medicine to control diarrhea, to reduce fever, to cure intestinal infections and to prevent or treat skin infections and wounds (Phister et al. 2004). Increased scientific evidences are now confirming these popular beliefs. It has been stated earlier that LAB are well known for their health promoting capabilities, and since they are the dominant microbial species in pozol, it is not surprising that this refreshing beverage can induce health promoting properties. The antagonistic effect of pozol on several species of bacteria and fungi, some of them pathogenic to man were documented in vitro (Herrera and Ulloa 1975). Three antimicrobial compounds produce by *Bacillus* sp. strain CS93 isolated from pozol were identified as iturin, bacilysin, and chlorotetaine, and exhibited activities against several Gram-positive and Gram-negative bacteria, yeasts and molds (Phister et al. 2004). In addition to these antimicrobial compounds, it was shown that this strain produces other bioactive lipopeptides, which might account for some of the medicinal properties of Pozol (Moran et al. 2010). It was also shown that *Agrobacterium azotophilum*, present in pozol, possesses bacteriocidal, bacteriolytic, bacteriostatic and fungistatic activities against a wide range of pathogenic microorganisms (Ulloa and Herrera 1972). Production of these

compounds could be related with the medicinal properties attributed to pozol. All these findings, along with future studies will surely help in the promotion of consuming this interesting Mayan drink.

3.1.6 Aloja/Piquillí

Aloja is a crystal clear foamy alcoholic beverage that is prepared by the fermentation of carob beans (*Prosopis alba, P. chilensis, P. nigra*) and is consumed in the Great Chaco forest of South America that stretches across four countries: Argentina, Paraguay, Bolivia and Brazil. In Chile, this "algarroba beer" is called piquillí and was originally produced by the Tobas Indians who had a custom of drinking it in the skulls of the enemies they defeated in battle.

Aloja is prepared by grinding the mature carob pods ("vainas" or "algarroba") using wooden or stone mortars, adding water and allowing the mixture to ferment in leather sacs ("noque") or in large wooden or clay pots ("bilqui"). These recipients are covered and kept in cool, dark areas and left to ferment during days after which the sediment is hand removed and the remaining mixture filtered through a cloth and placed in bottles for immediate consumption (aloja has a very short shelf life). If the fermentation is too extensive, giving rise to a very strong nauseating drink, ground pods and water are added, the resulting drink being both sweet and spicy.

Carob pods have elevated concentrations of sugars (40–50% w/w, composed principally of fructose, glucose and sucrose), proteins (5%), and minerals (such as calcium, iron, magnesium, phosphate, zinc, selenium and potassium) (Ayaz et al. 2009). Because of its high nutritional value and after adding water, contaminating yeasts quickly start producing carbon dioxide and ethanol after only a few hours of fermentation. Sometimes, yeasts and the sediment ("concho") from other batches of aloja are added to accelerate fermentation. Although little is known about the microflora in aloja, different yeasts have been isolated from exudates of algarrobo (Carob) trees and from carob pods from northwestern Argentina. Most of the yeasts were identified as *Bullera variabilis, Candida famata, Cryptococcus albidus* and other *Cryptococcus* species, *Debaryomyces hansenii, Pichia angusta* (*Hansenula polymorpha*), *Pichia ciferrii, Pichia farinosa* and *Torulaspora delbrueckii,* however, other, *Candida, Kluyveromyces* and *Pichia* species were also found (Spencer et al. 1995). It is assumed that these yeasts would be the principal cultures responsible for aloja fermentation.

Although aloja production is not very diffuse because of its artisanal preparation, this beverage has been used for medicinal purposes for centuries. Recent studies have shown that carob flour shows activities against ulcers, childhood diarrheas and intestinal infections (Sahin et al. 2009). The algarroba pods contain fibers (principally pectin and lignin)

that not only can maintain a beneficial intestinal microbiota, but also have been shown to inhibit the growth of colon cancer cells (Klenow et al. 2008). Pectin, commonly used as a thickening agent and stabilizer in food has been shown to provide different beneficial properties such as: laxative, coagulant, bacteriacidal, prevention of cancer, reduces cholesterol, helps in the formation of cellular membranes, eliminates heavy metals and other toxins from the body and helps to protect the intestinal mucosa (Saura-Calixto et al. 2007, Pérez-Jiménez et al. 2008). These pods also contain polyphenols that possess antioxidant, anti-inflammatory, and anti-rheumatic properties in addition to being beneficial for the heart and kidneys (Makris and Kefalas 2004). The isolation and structure elucidation of the major individual polyphenols in carob fiber have also been described (Owen et al. 2003). Carob pods have also been used as a treatment for rehydration following extreme diarrheas (Aksit et al. 1998), and for the treatment of acute-onset diarrhea (Loeb et al. 1989).

Unfortunately, in the last 200 years, the number "algarroba" (*Prosopis* sp.) trees have been drastically reduced since it was one of the only sources of wood in these dry and hostile regions and as such were used in the construction of houses, post for the growth of grapevines or burnt as a fuel source. These trees also provided shade to the Indians and were an excellent food source since the carob seeds were either consumed directly or ground to flour and used in the elaboration of breads and sweets and the pods used to feed farm animals. Because of this unsustainable wood harvesting culture, aloja production has dramatically been converted from a popular drink that was sold in parks and "plazas" of many cities to a relatively unknown and forgotten beverage.

3.1.7 Pulque and Mezcal

Pulque is an alcoholic drink that is prepared in Mexico by fermenting the juice of different *Agave* species. It is either consumed directly or distilled to produce Mezcal that depending on the *Agave* species used and the geographic location of production, this distilled beverage is called Tequila, bacanora and/or raicilla.

Pulque is made from fermenting the sap of the agave plant that is extracted with a fat wooden tube placed in the heart of 8–12 yr old plants. Each tapped plant is able to release sap 3 times a day constantly over 3 to 6 months. The sap, or *aguamiel,* is left to ferment producing a milky and slightly sour tasting drink that contains between 2 to 8% alcohol. Sometimes fruit or nuts are added to change the flavor. In pre-Hispanic times, only chosen Aztec men (priests, nobles and the elderly) were allowed to consume pulque during religious ceremonies. In colonial times, pulque was widely consumed and became an important source of revenue for the Mexican

government. There were establishments called *pulquerias* where this drink was served and a whole popular culture grew up around these *pulquerias* that were almost exclusively frequented by men.

Mezcal can be made from different varieties of agave, though most mezcals in the market are made with *Agave espadin*. With the arrival of the Spanish, the methodology to prepare pulque was modified, the agave leaves were cut leaving the inside of the plant (that is described as looking like a pineapple or *piña*) that is then roasted and mashed to extract the juices that are then fermented and distilled, producing mezcal. Tequila is a type of mezcal that is made exclusively from a specific agave plant, the blue agave or *Agave Tequilana Weber*. It is only produced in the region of Western Mexico near the town of Tequila, Jalisco where over 90,000 acres of blue agave are under cultivation, which is now a UNESCO World Heritage Site.

The microbiology of pulque and mezcal production has been extensively studied. In one study, samples were taken at different production stages and different morphological groups of microorganisms were identified as being Gram-negative coccobacilli, regular non-sporing Gram-positive rods and yeasts (Cervantes-Contreras and Pedroza-Rodriguez 2007). Strains of *Zymomonas* sp. (4.1×10^8 CFU/ml)), *Lactobacillus* sp. (3.4×10^7 CFU/ml) and *Saccharomyces* sp. (4.5×10^9 CFU/ml) were identified and shown to be responsible for the alcoholic, acid and viscous fermentation that are characteristic of this Mexican beverage (Cervantes-Contreras and Pedroza-Rodriguez 2007). The end product contained high contents of proteins (1 g/l) and reduced sugars (4.75 g/l) making it interesting from a nutritional point of view and the isolated microorganisms were proposed to confer a beneficial effect on the digestive system (Cervantes-Contreras and Pedroza-Rodriguez 2007). Another analysis of bacterial community during the fermentation of pulque allowed the detection of several new and previously reported species within the alpha-, gamma-Proteobacteria and *Firmicutes* (Escalante et al. 2008).

In a more recent study, a culture-independent analysis of the lactic acid bacterial diversity and metabolite accumulation during the fermentation of a typical agave must was evaluated (Narvaez-Zapata et al. 2010). The analysis of metabolite production indicated a short but important malolactic fermentation stage not previously described for mezcal. The denaturing gradient gel electrophoresis (DGGE) analysis of the 16S rRNA genes showed a distinctive LAB community composed mainly of *Pediococcus parvulus, Lactobacillus brevis, Lb. composti, Lb. parabuchneri*, and *Lb. plantarum*. Some atypical genera such as *Weissella* and *Bacillus* were also found in the residual must (Narvaez-Zapata et al. 2010). These results suggest that LAB could be implicated in the organoleptic attributes of this traditional Mexican distilled beverage.

A public health risk for pulque consumers is the possible contamination with *Escherichia coli* O157:H7 because this pathogen can survive by developing acid and alcohol tolerance in pulque (Gomez-Aldapa et al. 2012). However, another study suggested that the potential risk to consumers of contracting any of the five tested pathogenic bacterial strains (*Salmonella Typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Shigella flexneri* and *Shigella sonnei*) from pulque was low (Gomez-Aldapa et al. 2011).

3.2 Fermented Foods

3.2.1 Tocosh/Fute

The potato originated approximately 8,000 years ago in the Andes region of South America. Early Spanish explorers brought it to Europe in the 16th century where it quickly spread across the globe. Although all cultivated potatoes belong to just one botanical species, *Solanum tuberosum*, they come in thousands of varieties with great differences in size, shape, color, texture, cooking characteristics and taste. The International Potato Center (with its main offices in Lima, Peru) currently holds 7,500 different varieties of potatos (1,950 of them wild). More than 5,000 native varieties still grow in the Andes in South America. Here, numerous ingenious ways of preserving potatoes in order to maintain adequate stocks for survival such as sun-drying or natural freeze-drying to obtain white or black chuño (*papa seca*) or fermentation to obtain tocosh have been developed. When stored under appropriate conditions, these potato products can be kept for years, which is especially important during the heavy rainfall season when potato stocks are very prone to rot.

Tocosh has been prepared for hundreds of years since Inca times in South America and is still prepared in many small communities in Peru. In the south of Colombia, this fermented product is called *fute.* The traditional method of preparation consists of digging a hole (until water started to appear) in the ground near a river, a large amount of potatoes (normally discarded potatoes) are placed between straw layers (*shicshi*) and rocks used to cover the pile in order to prevent that the tubercles be carried away by the slight river current that passed through the ditch. The potatoes are left to ferment in this running water for 1–12 months. After this time, the potato package, now blackened due to putrification, is placed in a dry shaded area to allow the water to drain. Once dried, the tocosh is then placed in a new straw package and kept for sale or consumption. Most commonly, tocosh is then sun-dried and ground to obtain a fine flour-type product that is used to prepare different broths, stews and "mazamorra de tocosh" that is prepared by boiling a mixture of tocosh, water, sugar, cinnamon, and cloves and served cold.

Tocosh contains high concentrations of carbohydrates (80% w/w) and proteins (3.9%) and very little fatty matter, making it an ideal high caloric food (343,4 cal/w). From a microbiological point of view, only preliminary studies have been performed demonstrating that the product results from microbial fermentation and bacterial putrification (Yamamoto 1988). Bacteria and yeasts act during the initial transformation process, and lactobacilli represent the predominant microorganisms in the final product. These latter beneficial microorganisms are thought to be responsible for the large variety of medicinal properties that have been attributed to this product.

Tocosh is sometimes referred to as the "antibiotic of the Incas" because penicillin is produced during the fermentation process. It was used to treat many different illnesses such as stomach ulcers, colds, pneumonia, and hemorrhoids, to prevent gastrointestinal infections, used in childbirth (postpartum) and as a curative agent to treat wounds. This beneficial product was accepted by all populations groups and used without contraindications. It was shown to have antimicrobial properties, stimulate the immune system and contains steroids, alkaloids, amino acids and is ideal to treat fever because it can regulate blood pressure. This last property also makes it useful in treating altitude sickness. A study presented in the V World Congress of Traditional Medicine in Lima (Peru) showed the potential probiotic of tocosh using an experimental animal model and compared it with a recognized probiotic *Lactobacillus acidophilus* (Prentice and Milka 2005).

Today, Tocosh can be found in specialty stores that sell natural products as a flour or even in capsule form to facilitate its consumption. Since little microbial studies are performed, especially in artisanal preparations, consumers should be aware of the origins and sanitation certificates of the products they intend to consume in order to avoid health complications due to unsanitary conditions used during manufacture.

3.2.2 Cacao Fermentation

The cacao tree is native to the Americas and before the Spanish conquest, cocoa beans were very common (Wood and Lass 2001). Cacao beans must be fermented before they are used in making chocolate, and their commercial value is related to this procedure. The microbial succession in the fermentation process has been defined by many studies. Yeasts dominate the fermentation for the first 24 hr. Different yeast were isolated during the fermentation (Martelli and Dittmar 1961, Schwan and Wheals 2004). The growth of yeasts is then followed by LAB, but as the pulp disappears, oxygen penetrates the box and acetic acid bacteria start to dominate, producing acetic acid. It was also reported that the successful use of a

defined starter culture is necessary to obtain more reproducible chocolate and with better quality (Schwan 1998, Pereira et al. 2012). Traditionally, wooden boxes are used to carry out the fermentation. A recent study described the use of a novel-design stainless steel tank and compared it with the traditional Brazilian methods of fermentation in wooden boxes (de Melo Pereira et al. 2013). Both fermentation processes revealed the same dominant species: *Saccharomyces cerevisiae* and *Hanseniaspor*a sp., between the yeasts; *Lb. fermentum* and *Lb. plantarum* between the lactic acid bacteria; *Acetobacter tropicalis*, and *Bacillus subtilis.* However, the physical-chemical properties showed that the stainless steel tank model may be useful in designing novel bioreactors for the optimization of cocoa fermentation.

3.2.3 Brazilian Kefir

Kefir is a fermented milk drink made with kefir "grains" that contain a mix of yeast and bacteria, and has its origins in the north Caucasus Mountains. However, Kefir is currently a food taken in different parts of the world. So the composition of kefir grains was modified as much as the characteristics of the final product, which also depends on the type of milk used in its preparation. In this sense, microbial diversity and chemical composition of Brazilian kefir beverage was evaluated (Magalhaes et al. 2011). A recent study, using culture-independent methods showed *Lactobacillus kefiranofaciens* and *Lb. kefir* as the major bacterial populations in three different kefir grains, and *S. cerevisiae* being the dominant yeast (Leite et al. 2012). It was also reported that different diversity of microorganisms and chemical changes are associated with sugary the Brazilian kefir beverage (Magalhaes et al. 2010).

4 Conclusion

Although mass-production of these special indigenous fermented foods is limited by the availability of raw materials and that their short shelf-life greatly limits their introduction into foreign markets, increased knowledge of the processes and microorganisms involved will be useful in improving and standardizing their production and increase consumer acceptance and safety.

In this Chapter, it was demonstrated that without even knowing the basic theories of microbiology, the Inca, Aztecs, Mayans and other pre-Hispanic populations were able to optimize the biotechnological aspects of fermentation, giving rise to foods and beverages that were not only useful because of their nutritive and social aspects, but also provided health-promoting and curative properties, many of which are just recently

being described by concrete scientific research. These foods thus merit further scientific investigations in order to understand exactly which microorganisms could be useful to be used as biotechnological tools for the development of new and improved foods.

Keywords: Indigenous fermented foods, Traditional foods, Lactic acid bacteria, Yeasts, Biotechnology

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11

Food Safety Challenges Associated with Traditional Fermented Foods

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1 Introduction

Traditions are customs or beliefs taught by one generation to the next, often by word of mouth, and they play an important role in cultural identification. Each culture, ethnic group or region has specific traditions. Some traditions, such as religious customs, overlap different cultures, ethnic groups or regions.

Specific eating habits play an important role in the traditional habits of many cultures. The use of particular food ingredients and food preparation methods has been passed on from one generation to the next, and are now-a-days referred to as 'traditional foods'. Traditional foods have played a major role in traditions of different cultures and regions for thousands of years. They include foods that have been consumed locally and regionally for an extended time period. Preparation methods of traditional foods are part of the folklore of a country or a region. Unfortunately, many traditional

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foods are at risk of disappearing due to altered lifestyles. Therefore, it is important to study and document traditional foods to sustain important elements of cultures.

Foods are the basic survival needs for human being. Since ancient time, various methods have been used to process and preserve foods. Fermentation is one of the oldest and widely used food preservation methods in households, small-scale food industries as well as in large enterprise. Fermentation can be defined as the biochemical modification of primary food products brought about by the action of microorganisms and their enzymes. Fermentation is intentionally carried out to enhance the taste, aroma, shelf-life, texture, nutritional value, and other properties of food. Several classifications had been used to categorize the wide spectrum of fermented foods including the diversification of microorganisms, different food groups and types of fermentation involved (Steinkraus 2002, Benkerroum 2013). The different categories of fermented foods, i.e., cereals, vegetables, milk, fish and meat, are elaborately discussed in Chapters 3 to 7, respectively in this book.

Fermented foods are mainly lactic acid-fermented cereals (e.g., maize, sorghum, millet), root crops (e.g., cassava, sweet potato), milk, and, to a lesser extent, fish, meat, or vegetables. Alcoholic fermentation involving the production of ethanol is generally yeast fermentation. Fermented foods are usually produced from plant or animal-based raw materials in combination with fungi or bacteria, which are either present in the natural environment, or added intentionally by humans to obtain the desirable end products. Indigenous fermented foods such as bread, cheese and wine, have been prepared and consumed for thousands of years and are strongly linked to culture and tradition, especially in rural households and village communities.

Eating cooked or fermented fish and vegetables is one of a typical traditional culture shared among the peoples living in South-East Asia, Africa and Europe. The high level of safety and quality of these foods rely on the production methods and environments based on the extensive experience in each of the manufactures for long period. In this chapter, some common and widely used traditional fermented foods in different countries are outlined. The food safety aspects of these traditional fermented foods and possible control measures were presented and discussed in this chapter.

2 Popular Traditional Foods in Different Countries

Fermented foods are typically unique and differ according to the regions due to the variation in climate, social patterns, consumption practices and most importantly the availability of raw materials. Availability of raw materials brings about the conversion of the raw materials to different forms of

fermented food products in order to increase the food varieties as well as to maintain food security. Most widely known traditional foods are fermented milk (i.e., kefir) and milk products (i.e., yoghurt, cheese). In the Southeast Asian region, fermentation of cereal grains to produce a wide variety of foods has been a practice for a long time. Rice wine is one of the popular alcoholic beverages in some Asian countries. Consumption of wines from the inflorescences of palm such as coconut and talipot palm, is derived from the landscape of Indo-China and Sri Lanka (Law et al. 2011). "Gundruk" which is a fermented and dried vegetable product is very important for ensuring food security for many Nepali communities, especially in remote areas (Dahal et al. 2005, Tamang et al. 2005). It is served as a side dish with the main meal and is also used as an appetizer in the bland, starchy diet. Gundruk is an important source of minerals particularly during the off-season when the diet consists primarily of starchy tubers and maize, which tend to be low in minerals.

Cassava roots are normally consumed as a staple food, and used for starch processing or as carbohydrate-rich animal feed (Ray and Ward 2006) in the South-East Asian region. In countries such as Malaysia and Indonesia, cassava roots are fermented to produce the popular sweet and sour snack namely tapai/tape/ tempeh (Law et al. 2011). Bitter varieties of cassava roots contain potentially poisonous compounds such as cyanogenic glucosides (linamarin and lotaustralin) that can be detoxified *via* lactic acid bacteria, as in gari and fufu—the fermented cassava products of Africa (Ray and Ward 2006). The major groups of global fermented food products are briefly given in Table 1.

Kimchi is a traditional fermented Korean food whose preparation involves a series of processes, including pretreatment of Chinese cabbage (or radish), brining, blending with various spices and other ingredients, and fermentation. There are many (>50) types of kimchi, depending on the raw ingredients and preparation methods used. Of these types of kimchi, baechu kimchi (Chinese cabbage kimchi) is the most typical and is often referred to simply as ''kimchi'' (Cheigh and Park 1994, Cagno et al. 2013, Montet et al., Chapter 4 in this book). Similalry, lightly salted and/or fermented vegetables known as ''Tsukemono'' is a popular fermented food in Japan (Murooka and Yamshita 2008). Cabbage leaves are washed and cut into small pieces and then mixed with dry salt and then soaked in salt solution and kept as such at 10^0C for 8 days under pressure for salt curing. This kind of lightly fermented pickle prepared with cucumber, radish and other vegetables are popular traditional foods in Japan and in the broad area of South-East Asian countries. Salted (or fermented) sea foods ("Shiokara", "Karashi Mentaiko", and "Narezushi"), fermented vegetables ("Tsukemono") are also popular Japanese traditional foods (Inatsu 2005a).

Table 1. Major groups of fermented foods in the world.

Product group (raw materials)	**Examples**	**Region**
Dairy Products	Cheese, fresh cheese (quark), wara, yoghurt, kefir, etc.	Europe, North America, Middle East, Africa, South-East Asia
Beverages	Beer, sorghum beer, wines, sake, schnaps, arak, coffee, cocoa, pito, pulque	Worldwide
Cereals	Bread, pan cakes, tape ketan, kenkey, injera, ogi, Uirou, Ang-kak, idli, dosa	Europe, East Africa, Indonesia, India
Meat	Raw ham (bacon), fermented meat products (e.g., salami)	Europe, North America, Thailand, Middle East
Fish	Fish-sauces, Bagoong, Prahoc, Katsuobushi, Kamaboko	East Asia, Sout-East Asia
Seafoods	Shiokara, Karashi Mentaiko, Narezushi	Japan
Legumes	Soy sauce, miso, doenjang, natto, kinema, soumbala, tempeh, douche, dawadawa	China, South-East Asia, East Asia, Middle East; Oceania
Roots and tubers	Gari, lafun, fufu, merissa, lao-cha, chichwangue	Africa
Miscellaneous	Edible mushrooms (champignons), nata, ragi, asyn, Oncom (ontjom, lont jom)	India, South-East Asia
Products rich in starch	Ketella, poi, bwiru	Africa, Asia
Fruit and Vegetables	Pickles, olives, kimchi, hum-choy, Sauerkraut, Tsukemono, pickled garlic, pickled beets, pickled radish	Worldwide

Other widely-eaten types of health-boosting substances include fermented soybean products found in Asia (Liu et al. 2011). Antioxidant and other properties are reported to exist in foods such as natto, a sticky dish that is high in protein and popular at breakfast in Japan (Inastu et al. 2006, Kokuba et al. 2007, Murooka and Yamshita 2008), that may help prevent people from having brain haemorrhages (Murooka and Yamshita 2008). Natto is also rich in vitamin K2 (Yanagisawa and Sumi 2005), which stimulates the formation of bones and might help to prevent osteoporosis in older people (Inastu et al. 2006, Ray et al., Chapter 9 in this book). Similarly, consumption of Indonesia's tempeh reduces cholesterol levels (Law et al. 2011) and, like China's douche (Liu et al. 2011), lowers high blood pressure (Fujita et al. 2003).

The traditional fermented foods in North Africa are: roots and tubers (gari, lafun, and fufu), cereals (ogi), legumes (dawadawa and iru), milk (local

cheeses), and beverages (palm wine and pito) (Benkerraum 2013, El Sheikha and Montet, Chapter 8 in this book). Although the North African diet is typically low in foods of animal origin compared to foods of plant origin, mainly cereals and olives (Padilla et al. 2005, Alexandratos 2006), a variety of centuries-old dairy products are known and are still highly appreciated by consumers in these countries (El Sheikha and Montet, Chapter 8 in this book). Pulque, one of the oldest alcoholic beverages prepared from the juices of cactus plants in Mexico, is rich in vitamins such as thiamine, riboflavin, niacin and biotin (Steinkraus 2009, LeBlabc et al. Chapter 10 in this book).

Kefir is a viscous, acidic, mildly alcoholic milk beverage produced by fermentation of milk with a particular grain (kefir grain) that is consumed in Eastern European and Middle Eastern countries (El Sheikha and Montet, Chapter 8 in this book). There are other non-dairy fermented foods including sauerkraut, pickled cucumbers, pickled garlic, pickled beets, pickled radish, pickled corn relish, Korean kimchi, natto, miso, tempeh, soy sauce, tofu and naturally fermented and unpasteurized beers.

3 Importance of Food Fermentation in Public Health

Fermented grains and starchy roots form the most important part of diets of people in parts of Africa, especially West Africa. However, several factors are responsible for their continued popularity which includes: inaccessibility to commercially processed foods, inconsistency in electricity supply to encourage refrigeration and ultimately the fact that, very high populations of the consumers are low income earners. As such, their reliance upon fermentation to provide a variety of diets consisting of grains is inevitable. Lately, fermentation processes have caught the attention of food scientists and food microbiologists due to the subtle changes that occur in the foods as a result of the growth of beneficial microorganisms such as lactic acid bacteria (LAB) and yeasts in them. These changes may be beneficial or otherwise. The beneficial properties documented are: enhancement of the nutrient content of foods through the biosynthesis of vitamins, essential amino acids and proteins; improvement of protein and fiber digestibility; enhancement of micronutrient bioavailability; degradation of various anti-nutritional factors such as cyanogenic compounds present in cassava; enhancement of food safety by reducing toxic compounds such as aflatoxins and production of antimicrobial compounds like lactic acid, bacteriocins, carbon dioxide, hydrogen peroxide and ethanol which facilitates the inhibition or elimination of foodborne pathogens (Ross et al. 2002, Ray and Joshi, Chapter 1 in this book). In addition to its nutritive, safety and preservative effects, fermentation is known to improve the shelf-life of

foods, adding value to agricultural raw materials thus providing income and generating employment (FAO 2006).

Despite fermented foods being popular, foodborne diseases are a major public health problem. Developing countries, however, bear most of the brunt of the problem. Although statistics on the incidence of foodborne diseases are not available, the high prevalence of diarrhoeal diseases, particularly in infants and young children in these parts of the world, is an indication of an underlying safety problem. The etiological agents responsible for foodborne diseases are broad and include bacteria, viruses and parasites. Some of the principal pathogens responsible for diarrhoeal diseases are pathogenic strains of *Escherichia coli*, *Shigella* spp., *Salmonellae*, *Vibrio cholerae* O1, *Listeria monocytogenes, Staphyloccous aureus, Clostridium botulinum,* and *Campylobacter jejuni*; protozoa such as *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium* spp. and viruses such as Hepatitis A and E, and Rotavirus (Motarjemi 2002). Sources of food contamination are diverse and include polluted water, night soil, dust, flies, domestic animals, dirty utensils and food handlers. Raw foods may also be a source of contaminants as many foods harbour pathogens or originate from infected animals. Moreover, during food preparation, there is an added risk of cross contamination. One major factor leading to food contamination during food preparation and storage is time-temperature abuse, which results in the survival, growth and production of toxins by pathogens (Fig. 1).

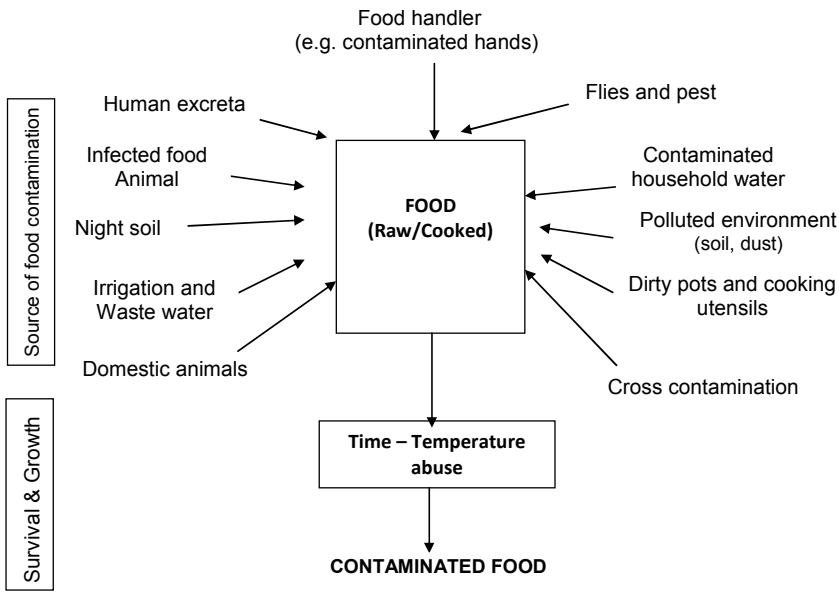

Fig. 1. Sources of food contamination during food preparation (Beuchat 2012).

In addition to being an agent for diarrhoeal diseases, food may also be a vehicle for chemical hazards, whether naturally present in the food (e.g., cyanide) or contaminating the food as a result of poor agricultural practices (e.g., pesticide residues) or environmental pollution (heavy metals, dioxins). Depending on the dose, chemical hazards may lead to acute intoxication or long-term health problems such as cancers and other chronic diseases. Food may also contain anti-nutritional factors such as enzyme inhibitors, phytates, lectins and polyphenols, which interfere with digestion, absorption or other aspects of nutrient metabolism (Holzapfel 2002).

Food processing technologies are applied to increase digestibility, enhance the edibility of food, intensify sensory quality, increase shelf-life, improve nutritional quality, and/or render food safe. Foods processing technologies whether carried out at the household level, based on experience, or at the industrial level are designed to optimize all of these properties in the final product. All of the above objectives can rarely be achieved using a single operation. Often, a combination of different operations is required, as is the case for fermentation. Fermentation is often part of a sequence of food processing operations, which may include unit operations such as cleaning, grinding, soaking, salting, cooking, packaging, and distribution. The potential of fermentation for improving the nutritional quality and safety of foods should therefore be viewed within the context of the complete food processing operation (Ray and Panda 2007).

4 Risk Associated with Fermentation

Irrespective of the origin of traditional foods (plant or animal), the raw materials used for their manufacture are nutritionally rich and provide an adequate environment for the growth of various microorganisms including those of health and spoilage significance. In addition, the raw materials generally host an abundant and complex microbial flora whose microbial groups would potentially grow and compete for nutrients. Therefore, the main purpose of traditional technologies is the alteration of ecological parameters of the raw material in a way to select for specific beneficial groups of microorganisms that will govern the processing steps and eventually predominate (Steinkraus 2002). The product may then be considered safe while having the desired and unique nutritional and gustatory qualities. On the other hand, some traditional technologies aim to inhibit or inactivate as many of the microorganisms as possible that are initially present in the food to allow the least microbiological changes during storage, thereby extending the shelf-life of the food for as long as possible. This is usually achieved by heat treatment (scalding or boiling), dehydration, and/or use of high salt or sugar concentration. Some traditional meat products, such as gueddid and khlii, dairy products

including kishk, domiati, tallaga, and aoules, and vegetable products such as pickled lemon, dried figs, prickly pears, and raisins, are examples of such foods. Nonetheless, survival or adaptation of microbial strains/groups to extreme conditions is well documented (Beales 2004, Allen et al. 2007), and these products may still be at risk to consumers.

Simple, fermentation can result in undesirable products that are sometimes even risky or dangerous. The reasons being that the final quality and safety of the fermented products are dependent on factors such as: (i) quality of the raw material; (ii) initial level of contamination (which in turn depends on local conditions); (iii) levels of hygiene and sanitation; (iv) quality of the starter culture; (v) conditions of fermentation (e.g., temperature and pH); and (vi) degree of acidity achieved.

These parameters are sometimes very difficult to control, particularly when processing is carried out under the rudimentary conditions of some small-scale industries or under household conditions. Some examples of documented outbreaks of foodborne diseases, associated with fermented products, are listed in Table 2. There are reasons to believe that the frequency of improper handling and unhygienic conditions during the production of fermented foods is greater than that reported in the literature. Weaknesses in foodborne disease surveillance systems do not provide for identification and reporting of these outbreaks. A number of foodborne hazards are not affected by lactic fermentation and thus fermentation should not be relied upon for the elimination or reduction of these hazards. In order to ensure food safety, fermentation should therefore be combined with a number of other processing operations, such as cooking and soaking. A number of foodborne hazards are capable of surviving during fermentation processing. Enteropathogens, such as enterohaemorrhagic *Escherichia coli*, show some patterns of acid resistance and may survive certain fermentation processes (Carlos et al. 2012). Yoghurt and fermented meat have been recognized as potential vehicles of enterohaemorrhagic *E. coli* infection. In 2001, a widespread outbreak of *E. coli* O157:H7 in the Kanto area of Japan occurred, and epidemiological investigation, reported locally made lightly fermented cabbage and cucumber were the incriminated food items, raising special concerns about the transmission of *E. coli* O157:H7 in fermented foods (Ozaki et al. 2003a,b). Another outbreak caused by lightly fermented cucumber occurred in 2002 in Fukuoka (Oda et al. 2004). Clavero and Beuchat (1996) reported that pathogenic bacteria like *E. coli* O157:H7 were able to survive under acidic conditions (at pHs of ≥4.0) for up to 54 days but were affected by acidulants and temperature. Other serotypes of *E. coli* have been shown to survive for at least 2 days in traditional lactic acid–fermented foods (Sainz et al. 2001). *Salmonella* and *Listeria* also have systems

Table 2. Examples of food borne disease outbreaks associated with fermented foods (modified from Motarjemi 2002).

Implicated food	Causative agent	Cases	References
Vegetables			
Paste of soybeans and wax gourds	*Clostridium butyricum*	6	Meng et al. (1997)
Sauerkraut	Histamine poisoning		Mayer and Pause (1972)
Canned Hot dog chili sauce	*C. botulinum* type A	8	Patricia et al. (2013)
Milk products			
Yoghurt	*C. perfringens*	167	MOH (1993)
Hazelnut yoghurt (hazelnut puree was contaminated)	*C. botulinum*	27	O'Mahony and Mitchell (1990)
Sour milk	*C. botulinum*	11	Smith et al. (1979)
Yoghurt	*Escherichia coli O157*	16	Morgan et al. (1993)
Meat products			
Semi-dry sausages	*E. coli O111:NM*	23	CDC (1995a)
Pork (labh-raw and nahm-fermented)	*Trichinella*	27	Khamboonruang and Nateewatana (1975)
Salami-stick	*Salmonella typhimurium*	85 (including 13 secondary cases)	Cowden et al. (1989)
Salami	*E. coli O157*	23	CDC (1995b)
Fermented beaver tail and paw	*Clostridium botulinum*	14	CDC (2001)
Sausages (Lebanon bologna)	*S. typhimurium*	26	Sauer et al. (1997)
Fish			
Fish (seal flipper)	*C. botulinum*	1	Shaffer et al. (1990)
Fish (beaver tails)	*C. botulinum*	7	Shaffer et al. (1990)
Fish (salmon fish heads)	*C. botulinum*	8	Shaffer et al. (1990)
Salmon eggs	*C. botulinum*	15	Hauschild and Gauvreau (1985)
Cheeses			
Soft cheese	*Salmonella berta*	82 (including three secondary cases	Ellis et al. (1998)
Cheese	*S. enteridis*	700	CCDR (1999)
Soft cheese	*S. dublin*	42	Maguire et al. (1992)
Goats milk cheese	*S. paratyphi*	273	Desenclos et al. (1996)
Cheddar cheese	*S. heidelberg*	339	Fontaine et al. (1980)

Table 2. contd....

Table 2. contd.

Implicated food	Causative agent	Cases	References
Cheeses			
Mozzarella cheese	*S. typhimurium*	321	Altekruse et al. (1998)
Cheese	*E. coli O157*	22	The Pennington Group (1997)
Cheese (Brie, Camembert, Coulommiers)	*E. coli O124 B17*	387	Marier et al. (1973)
Cheese (Brie, Camembert)	*E. coli O27 H20*	170	Altekruse et al. (1998)
Cheese (Brie, Camembert)	*C. botulinum*	27	Pourshafie et al. (1998)
Mexican-style soft cheese	*Brucella melitensis*	31	Altekruse et al. (1998)
Hand-pressed direct set cheese	*Staphylococcus aureus*	16	Altekruse et al. (1998)
Cheese	*S. sonnei*	50	Sharp (1987)
Swiss cheese	Histamine poisoning	6	Taylor et al. (1982)

that allow adaptation to low pHs (3.0 to 4.0) and survival at even lower pHs (Foster 2000, Tiganitas et al. 2009). A study by Simango and Rukure (1991) on mahewu and sour porridge (traditional fermented foods in Zimbabwe) showed that all strains of enteric pathogens except campylobacters survived for 24 hr after inoculation.

Foodborne viruses are recognized as a cause of gastroenteritis, and rotavirus is one of the most common causes of childhood diarrhoea. Simian rotavirus has been shown to survive high levels of acidity during 24 hr of storage in model fermented foods. There is little information on the effect of fermentation on parasites, such as *Cryptosporidium*, *Giardia lamblia* and foodborne trematodes. The cysts or metacercariea of these organisms often show resistance to adverse conditions, but are believed to be destroyed by adequate cooking.

Certain algae, bacteria, and moulds produce toxins that may be transmitted by food. Risk associated with mycotoxin contamination, biogenic amines and the lactic acid isomers in fermented foods are covered elsewhere (Holzapfel 2002). Similarly, there is no evidence that bacterial toxins can be degraded by fermentation alone. Bacterial toxins, such as *Clostridium botulinum* toxin for example, are heat labile and may be destroyed by adequate heat treatment, whereas others such as those produced by *Staphylococcus aureus* are heat stable (Nipa et al. 2009). Lactic

fermentation has a limited effect on anti-nutritional factors, such as protease inhibitor and lectins.

5 Fermentation and Pathogen Control: A Risk Assessment Approach

Most fermented foods owe their origin to the fact that processes used in their production are inhibitory to many microorganisms (Adams and Nicolaides 2008). As a result, fermented products generally have a longer shelf-life than their original substrate and their ultimate spoilage is different in character. The antimicrobial effects of fermentation are not confined to spoilage organisms alone and can also affect pathogens that might be present. Thus, traditional food fermentations can take potentially hazardous raw materials, such as raw meat and milk, and transform them into products with both improved keeping qualities and a reduced risk of causing illness. The extent to which fermented foods are safe and how fermentation processes should be conducted to achieve a required level of safety are key questions that are not simple to answer (Bidlack et al. 2009). All approaches to this depend critically upon the quality of the data available. In the past, we have had to rely largely on expert judgement to interpret the available information, but modern microbiological risk assessment (MRA) techniques will enable us to achieve food safety objectives related to fermented foods in a more reliable and consistent fashion. Microbiological risk assessment is essentially a tool for applying our knowledge of microbiological food safety in a logical, systematic, consistent and transparent way to assess food safety risks (Adams and Mitchell 2002). It aims to tell us what is the chance that a certain food will cause illness, who will be affected, and what those effects will be. It provides a scientific basis for the management and control of the microbiological risks posed by foods. A generally agreed approach on how this is done has evolved over the last few years and has been well described in a number of recent monographs (FAO/WHO 1996, Voysey 2000, Benford 2001, Martin and Mitchell 2002, Bidlack et al. 2009). Essentially, the process consists of the stages outlined in Fig. 2. The first requirement is to formulate the problem in a statement of purpose—what is your objective in doing the risk assessment. Once this is done, it is necessary to decide which hazardous organisms will be of concern in the product (Hazard Identification); what will be the intake of hazard as a result of food consumption (Exposure Assessment); what will the effect be on people (Hazard Characterization); and, finally, what is the overall risk to a given population (Risk Characterization).

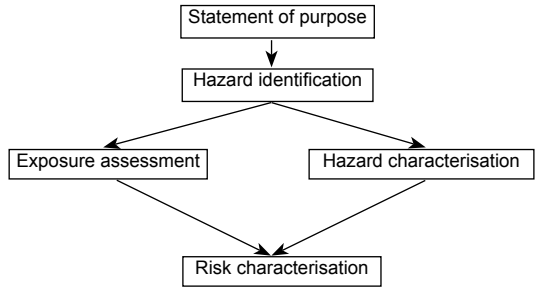

Fig. 2. Microbiological risk assesment.

6 Interventions for Improving the Safety and Nutritional Value of Fermented Foods

Since fermentation is typically one part of a multistep food-processing operation, assessing the safety and nutritional value of fermented foods and determining appropriate interventions to improve these should be considered in the context of the entire process transforming foodstuffs from raw materials to end-products. In the light of present practices for the preparation of fermented weaning foods, three areas of concern can be identified: safety, nutrition, and socio-cultural factors.

The time necessary for food processing in traditional household fermentation processes is a constraint on the hours available to food preparers who may also be engaged in child care and other household activities. The lack of sufficient time can have significant implications for the safety and nutritional quality of fermented foods, as any time saved by shortening the period of fermentation can jeopardize the effectiveness of acidification or the degradation of anti-nutritional factors. Some reduction in time expenditure may however be acceptably achieved by accelerating the fermentation process (e.g., through the use of starter culture) or by a different distribution of labour (e.g., through food production by an entrepreneur). Care should in any case be taken that no short cuts are used that might make the food unsafe for consumption. Food safety problems can also arise from processing or handling food under unhygienic conditions. Improvement in food quality and safety can be achieved by implementing or promoting principles of good manufacturing and good hygienic practice, and by using the Hazard Analysis Critical Control Point system (HACCP). The HACCP system constitutes a food-safety assurance tool that can be used for the identification, assessment, and control of hazards at all points in the process from raw material to packaging and consumption.

The application of the HACCP system to several fermented African foods was carried out to illustrate the use of this approach in order to identify the necessary measures required to improve the safety of such foods (Holzapfel 2002) and to determine the practices and behaviours that should be the subject of health education for food handlers. Application of the HACCP system also demonstrated the relatively high risks associated with the preparation of certain fermented foods and the importance of assessing the safety of each product and each preparation individually.

From a nutritional point of view, it is possible to improve the nutrient density of fermented foods while maintaining their desirable semi-liquid consistency by the addition of amylase-rich flour, provided that the starchy cereal or roots intended for fermentation is cooked. Likewise, it is possible to improve the protein content of fermented porridges by enriching them with legumes, e.g., soybeans or cowpeas (Panda et al. 2008).

6.1 Interventions to Improve the Safety of Fermented Vegetables

Fermented vegetables are an integral part of people's diet in East to South-East Asia (Steinkraus 2002). Normally, leafy vegetables harbors 2–7 log CFU/g of mesophilic bacteria including coliform or spoilage bacteria such as *Pseudomonas* spp. as their normal microflora and lactic acid bacteria (LAB) on leaves play an important role in fermentation. The organic acids and other metabolites produced by LAB during fermentation process give raw vegetables a desirable taste, flavor and texture (Buckenhuuskes 2001). In addition, fermentation enriches food substances biologically with vitamins, proteins, essential amino acids and fatty acids (Cheigh and Park 1994).

Traditionally more than 5% salt concentration has been applied for a week for vegetable fermentation. The growth of spontaneously contaminated pathogenic or spoilage bacteria will be suppressed by high salt concentration and organic compounds (such as organic acid and bacteriocin) produced by the co-existing LAB (Adams and Nicolaides 2008). However, lightly (less than 3%) salted and/or lightly (overnight to one day) fermented vegetables are becoming more preferable to most of the consumers because a trend toward convenient yet healthy foods is seen as part of modern lifestyle. This salted (lightly fermented) vegetable is consumed in raw state within 2 to 4 days of preparation. Therefore, several food borne outbreaks have been reported with lightly fermented (salted) vegetables (Ozaki et al. 2003a,b, Uehara 2000). For example, the presence of enterohaemoragic *E. coli* O157:H7 in fermented turnip (Uehara 2000), lightly fermented kimchi (Ozaki et al. 2003b) was found responsible for these outbreaks in Japan. In addition, *L. momocytogenes* contamination in fermented vegetables can be a potential hazard of foodborne illness because of its salt resistance and ability to grow in low temperature (FAO 2006).

The fate of *E. coli* O157:H7, *Salmonella* Enteritidis, *Staphylococcus aureus*, and *L. momocytogenes* contamination into Korean and Japanese styled kimchi at 10°C was studied (Inatsu et al. 2004, Kim et al. 2005). Irrespective of the initial inoculums size, no significant reductions of *E. coli* O157:H7, *S*. Enteritidis, *S. aureus* and *L. momocytogenes* were observed after 7 days storage at 10°C, in commercial Japanese kimchi (Table 3). In 2007, Sunahara et al. (2007) examined *E. coli* O157:H7 outbreak associated with lightly fermented leafy vegetables and suggested the possibility of the growth of *E. coli* and other bacteria during the salting process and the author stressed the need of temperature control and surface sanitation of vegetables before starting the fermentation process.

Although washing vegetables with tap water may remove some soil and other debris, it cannot be relied upon to remove microorganisms completely and may result in cross-contamination of food preparation surfaces, utensils and other food items (Beuchat 1998). To reduce the risk of food poisoning caused by light-fermented vegetables, effective sanitization of the raw produce may be required. Many sanitizers, including sodium hypochlorite (NaClO) (Kondo et al. 2006), electrolyzed water (Bari et al. 2003, Deza et al. 2003, Rahman et al. 2010, Al-Haq and Gomez 2012), ozonated water (Selma et al. 2008, Inatsu et al. 2011), hydrogen peroxide (Huang and Chen 2011), acidified sodium chlorite (ASC) (Inatsu et al. 2005a) and calcinated calcium (Bari et al. 2002) have been evaluated for their effectiveness in killing microorganisms on different fresh produce (Keskinen et al. 2009, Isshiki et al. 2009, Gómez-López et al. 2007), and the experimental reports revealed that only 1.0 to 2.0 log CFU/g reduction of surface attached pathogens on vegetables was achieved by these sanitizers (Zhang and Farber 1996, Sapers 2005).

Table 3. Recovery of inoculated pathogens into Japanese style kimchi.

E. coli (log CFU/g)		*S. Enteritidis* (log CFU/g)	
Day 0	Day 7	Day 0	Day 7
5.3 ± 0.1	5.1 ± 0.1	5.6 ± 0.1	5.3 ± 0.1
4.3 ± 0.1	4.2 ± 0.0	4.6 ± 0.1	4.5 ± 0.1
3.3 ± 0.0	3.2 ± 0.0	3.7 ± 0.1	3.5 ± 0.1
2.5 ± 0.1	2.5 ± 0.2	2.7 ± 0.1	2.5 ± 0.3
***S. aureus* (log CFU/g)**		***L. monocytogenes* (log CFU/g)**	
Day 0	Day 7	Day 0	Day 7
5.3 ± 0.0	5.1 ± 0.1	5.3 ± 0.0	5.0 ± 0.0
4.3 ± 0.0	4.1 ± 0.0	4.3 ± 0.0	3.9 ± 0.0
3.3 ± 0.0	3.1 ± 0.1	3.3 ± 0.0	2.9 ± 0.2
2.6 ± 0.2	2.1 ± 0.3	2.5 ± 0.5	2.3 ± 0.3

Four pathogens were separately inoculated into commercially purchased Japanese style kimchi and stored at 10°C. Viable cells were enumerated by agar plate method and MPN method. Average value and standard variation of duplicated 4 times examinations were shown. No significant reduction of viable cells after storage was observed for all strains and initial inoculums size ($P>0.05$).

Mild heating (65–75ºC for 10–20 min) of final product (120–150 g) is commonly applied for manufacturing of long term fermented vegetables, for example, fermented red turnip ("*Sunki*" or "Suguki") (Miyao 2004). Mild heating is effective not only in reducing Gram-negative pathogens and spoilage bacteria (such as *Pseudomonas* sp. and *Flavobacterium* sp.) but also selected LAB useful for long time fermentation. The short-term exposure of superheated steam (SHS) is shown to be applicable for preserving some fleshy vegetables. Ono et al. (2006) examined the effectiveness of SHS on the surface of Chinese cabbage contaminated with *E. coli* O157:H7 and *S. aureus,* used for preparing lightly fermented vegetable. SHS treatment at 110ºC for 10 sec reduced the dip-inoculated pathogens (5.0 log CFU/g) to near the detectable limit (2.0 log CFU/g) (Table 4). No significant loss of quality parameters such as taste and flavor was observed in Chinese cabbage (Ono et al. 2006).

The natural antimicrobial compounds including chitosan and allyl isothiocyanate (AIT), Japanese horseradish (*Eutrema wasabi* Maxim), hop extract (*Humulus lupulus* L.), etc. have shown (Fig. 3) potentials to control the growth of contaminated pathogens or spoilage bacteria in lightly fermented vegetables (Inatsu et al. 2005b).

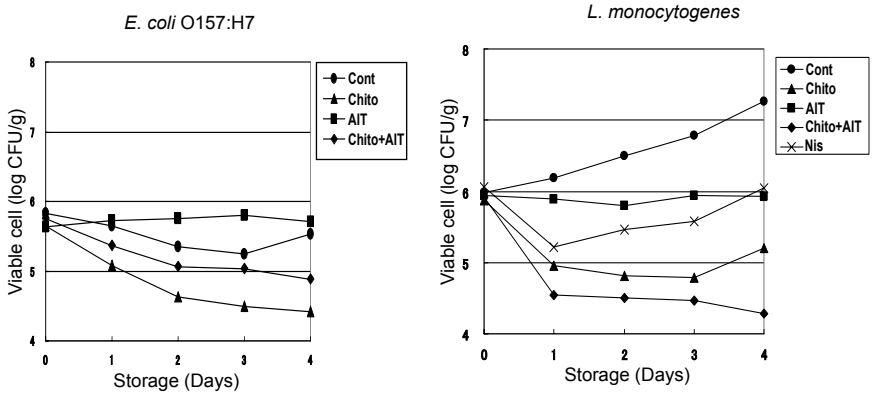

Fig. 3. Effectiveness of several natural antimicrobial compounds to pathogenic bacteria inoculated into lightly fermented Chinese cabbage. ● Control, ▲ 1.0 g/L Chitosan, ■ 2.0 g/L WASAORO EXT®, × 0.05mg/g Nisin A.

6.2 Interventions to Improve the Safety of Salted or Fermented Fish Foods

Until the early 1950s, raw fish in diet was distributed only to restricted areas that were nearby a sea or by a river side, because of the lack of appropriate temperature control for long distance transportation (Imada

Table 4. Comparison of sterilization effect of SHS to *Eshecichia coli* O157:H7 and *Staphylococcus aureus* [1)]Distilled water or sodium hypochlorite (1.0 mg/L as active chlorine) washing were performed for 1 min. SHS treatment was performed for 10 sec in each temperature. Means values with the same letter were not significantly different (P>0.05).

	Recovery Medium	Population of *E. coli* (O157 : H7) and *Staph. aureus* (log CFU/g)[1)]					
		Before treatment	After distilling water washing	After NaClO washing	After SHS treatment		
					110°C	130°C	150°C
E. coli O157 : H 7	TSA-Rif	4.8±0.1^{A}	4.1±0.1^{B}	3.7±0.1^{C}	2.7±0.2^{D}	<2.5±0.0^{D}	<2.5±0.0^{D}
	SMAC-Rif	4.7±0.1^{A}	4.1±0.1^{B}	3.5±0.1^{C}	2.5±0.1^{D}	<2.5±0.0^{D}	<2.5±0.0^{D}
Staph. aureus	TSA-Rif	5.1±0.1^{A}	4.8±0.1^{B}	4.2±0.2^{C}	2.6±0.1^{D}	<2.5±0.0^{D}	<2.5±0.0^{D}
	MSA-Rif	4.9±0.0^{A}	4.6±0.0^{B}	3.8±0.0^{C}	2.5±0.0^{D}	<2.5±0.0^{D}	<2.5±0.0^{D}

and Fujita 2003). Fermentation technology of fish has been developed and is commonly used to preserve and distribute fish foods worldwide. With these developments, many fish related food poisoning cases have ben reported. In 1999, a large outbreak (1,505 patients) of *Salmonella* Oranienburg and *S. chester* was caused by sun-dried salt squid snacks (Yoda 1999). In 2007, *Vibrio Parahaemolyticus* O3:K6 (620 patients) outbreak was found associated with squid guts pickle. Contamination of *Listeria momocytogenes* in Ready-to-Eat (RTE) sea foods was confirmed. Surveillance study of commercially purchased 394 samples of sea foods during 1999 to 2001 in Tokyo revealed that fillet tuna, fillet trout, minced tuna, smoked salmon, salted egg of salmon ("*Sujiko*"), salted cod roe ("*Tarako*"), red pepper seasoned salted cod roe ("*Karashi-Mentaiko*") and boiled octopus harbored *L. momocytogenes* (Hara et al. 2003). However, the ministry of health, welfare and labor (MHWL) in Japan declared decreased trends of food borne pathogens in sea foods from 36.7% in 1975 to 9 % in 2007 or less in recent years (Kato et al. 2008).

Salting of fish has been widely used to preserve fish and fish products. The traditional range (15–20%) of salt concentration is critical for the growth of many bacteria concerned with fermentation. Under salting conditions, fermentation and/or the enzymatic auto digestion of fish meat or guts occurred gradually to give complex sensory characteristics. However, there is a recent trend to produce short length fermented fish foods that are kept in lower NaCl concentration because of the changes of consumers preference based on the interest of healthy food life style. It has become a common challenge to deal with the increasing risk of food poisoning caused by *Vibrio* spp. (Codex Alimentarius Commission 2006), *L. monocytogenes* (FAO 2006) or other pathogenic bacteria in salted and/or fermented fish foods in many countries.

Several intervention methods including, control of pH and water activity (aW), storage and processing temperature, addition of different food additives or biocontrol agents, along with good hygiene practice and temperature control could be used to improve the safety of salted or fermented fish. For example, a challenge tests containing different concentration of NaCl (3.4 to 9.8%) was able to reduce 2.0 log CFU-MPN/100g of *S*. Oranienburg, *S*. Chester and *S*. Typhymurium in squid guts pickle (Iwano et al. 2001). *L. momocytogenes* contamination in red pepper seasoned salted cod roe could be reduced by dipping in seasoned salt water (NaCl 0.9–1.0%), or addition of sodium acetate (1%-w/w) or nisin (0.15%-w/w) at refrigeration temperature (Hiwaki et al. 2007). The fate of *L. momocytogenes* in salted cod roe during storage at 6ºC with or without food additives has been shown in Fig. 4.

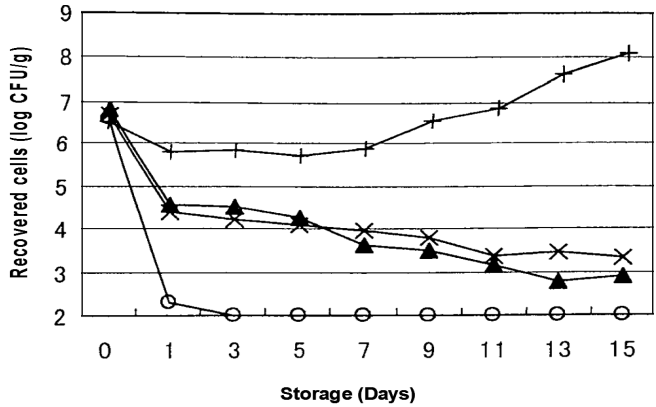

Fig. 4. Fate of *Listeria momocytogenes* serover 1/2b inoculated into salted cod roe during storage at 6°C with or without food additives (Hiwaki et al. 2007). + control, ○ Nisin, ▲ sodium acetate product (KTN), × sodium acetate product (NSK).

6.3 Interventions to Improve the Safety of Fermented Soybean without Salt (Natto) and Soybean Curd (Tofu)

Fermented soybean with/without salt or soybean curd is eaten commonly in East or South-East Asian countries (Inatsu et al. 2006, Inatu et al. 2002). Traditionally, fermented soybean without salt (Natto) was prepared by packing boiled or steamed soybean into a case made from hot (boiling water) sterile straw of rice. *Bacillus subtilis* attached on rice straw grew rapidly during 18 to 48 hr of fermentation at 40°C in 90% relative humidity. Control of the growth of undesirable bacteria depends on the pre-sterillization of soybean and packing. These foods are manufactured and sold in United States and European countries at a commercial scale (Ashraf et al. 1999). Health-promoting effect and risk of consumption of soybean foods including these traditional soybean foods have been studied (Kokubo et al. 2007, Murooka and Yamashita 2008, Velentzis et al. 2008).

Despite all these health benefits, several large-scale outbreaks of *Salmonella* were documented (In October 1949, 621 patients and 30 died in Fukushima prefecture; In October 1952, 46 patients and 3 died in Kyoto prefecture. In March 1953, 621 patients and 3 died in Chiba prefecture). All these outbreak cases were caused by *Salmonella* in the rice straw that was contaminated from rat feces. As a result of these incidents, the use of pure culture of *B. subtilis* strain is strongly recommended and improvement of general hygiene practices during manufacturing process is promoted by the industry and governments. Major large facilities have introduced the HACCP based manufacturing system and aseptic filling system. Now, heat sterile (80–90°C for 40–60 min) packed soybean curd is manufactured with

a short shelf-life and marketed all over the world. However, non-sterile raw soybean curd is much preferred by some consumers (i.e., Japanese) and is prepared by solidification of boiled soymilk by adding salts (magnesium chloride, calcium sulfate) or gluconic acid at warm temperature. Usually the shelf-life of soybean curd produced by small manufacturing companies is commonly 3 to 6 days, but increased shelf-life up to 12 days was achieved by introducing "closed line automation production system" and GHP/HACCP based hygiene control. No food additive has been permitted to use for controlling the growth of contaminated pathogenic or spoilage bacteria for Tofu production. However, recent studies have demonstrated that application of chitosan (Kim and Son 2004, No and Mayers 2004, No et al. 2002) or nisin (Cha et al. 2003, Schillinger et al. 2001) to extend the shelf-life of Tofu and reduce the risk of *L. momocytogenes* in Tofu production occurs. In addition, disinfecting the facility by using ozone gas (0.5–3.0 mg/L) for 5 to 8 hr everyday for upto 6 mon effectively reduced the contamination of *L. mesenteroides* and *Tricospron pullulans*, major spoilage bacteria of soybean curd (Naito 2008).

7 Constraint of Fermentation in Developing Countries

Application of the basic rules of food hygiene (as shown in Fig. 5) will help prevent contamination, growth and survival of pathogens in foods and will reduce the incidence of diarrhoeal diseases. Socio-economic constraints such as an inadequacy of supplies of safe water, lack of facilities for safe preparation and storage of food (e.g., refrigeration, fuel for hot holding or thorough reheating) and time constraints for the proper preparation of food prior to each meal can, however, interfere with the application of these rules. As a result, low income households are simply not able to apply certain essential food safety principles, such as feeding infants with freshly prepared foods, refrigeration, hot storage, and re-heating of stored foods. Fermentation provides an economic means of preserving food and inhibiting the growth of pathogenic bacteria even under conditions where refrigeration or other means of safe storage are not available. At the same time, it enhances the nutritional quality of certain foods. In many parts of the world, particularly in Asia and in Africa, the technology has been traditionally used as a method of preservation, and to ensure food safety. In some African countries, the technology is applied in the preparation of weaning foods. In Kenya, Nigeria, the United Republic of Tanzania and Uganda, feeding infants with either fermented cereals, or fermented root-crop products is a customary practice. Fermentation has also been used in the production of beverages. In areas where the safety of water supplies cannot be assured, the technology contributes to reducing the risk of waterborne diseases (El-Diasty and El-Kaseh 2007).

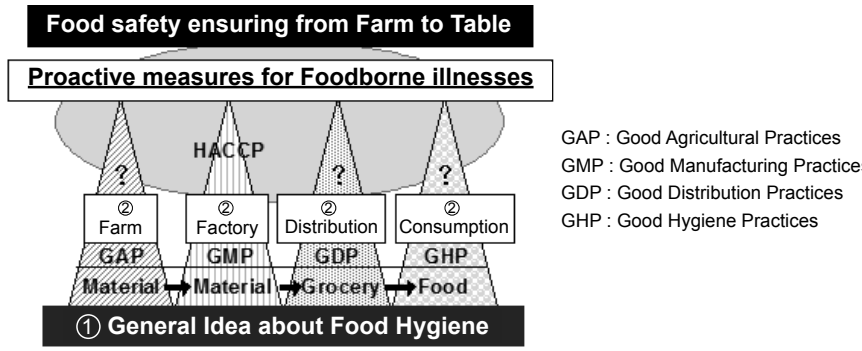

Fig. 5. General idea about Food Hygiene (Partially modified from Tanaka et al. 2003).

8 Recent Trends in General Hygiene Practices Applicable for Manufactures

The implementation of good hygiene practices (GHP) into food facility is strongly required to improve the safety level of the products. The HACCP system may work well as the base of prerequisite programs (PRPs) and the GHP is a most important part of PRPs. The main purpose of GHP is hygienic control of the environment of the food factory, especially the controlling of the "microbial level" cleanness. Based on the traditional "5S" quality control (QC) activities performed in each of the facilities, Komemushi et al. (2008) proposed the introduction of "Food Safety 7S" for voluntary activities of GHP, which consisted of 7 components; shifting (*Seiri*), systematic arrangement (*Seiton*), sweep (*Seisou*), scrub (*Senjyou*), sanitation (*Sakkin*), self-discipline (*Shitsuke*) and cleanness (*Seiketsu*) (Fig. 6). The original "5S" activity, which focused on improving the quality of manufacture and the operation efficiency of business activity, also included "clean up (*Seisou*)" as one of the five components. To clarify the procedural steps, Komemushi et al. (2008) proposed dividing the cleaning steps into 3 components in the "food hygiene 7S"; sweeping (*Seisou*) under dry condition to remove large garbage, scrubbing (*Senjyo*) under wet condition to remove rather small dusts and sanitize (*Sakkin*) to remove and/or disinfect spoilage or pathogenic bacteria in the processing environment. The objective of "shifting (*Seiri*)" is to distinguish ones required or not for work. The identified required ones should be systematically arranged (*Seiton*). These two components are a prerequisite for the latter 3 steps ("sweeping" to "sanitize") to increase the effectiveness of workability. Another important point is to divide the objective (cleanness or *Seiketsu*) and 5 actual practices ("shifting (*Seiri*)" to "sanitize (*Sakkin*)"). The self-discipline (*Shitsuke*) is

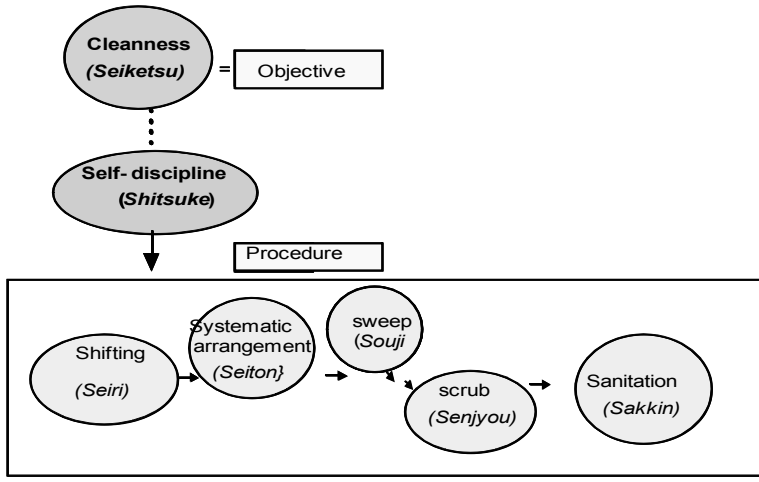

Fig. 6. Basic concept of "Food safety 7S".

Color image of this figure appears in the color plate section at the end of the book.

seated between the objective and actual practices. The *Shituke* include the meaning of conventionalizing of good practices and its objective is to enhance labors' skills by training and education.

Many practices to insure and improve the hygiene of the processing environment have been conducted in the food-manufacturing facilities. The ATP measurement method can be a candidate to confirm hygiene conditions but it is costly and rather complicated to apply in routine checking in smaller facilities, which are common in traditional food producing companies. Simple and rapid detection methods of indicating bacteria, which reflect the level of contamination of spoilage or pathogenic bacteria, have been explored to monitor hygienic condition of lines and instruments. The low initial costs and low running costs are strictly required for daily voluntary check of the hygiene condition in food manufacturing units. Kawasaki et al. (2006) compared three different detection methods for microbial control in food processing plants including the "protein-wiping test method", "microbial test", and "visual observation by the site foreman. The objective of this study was to confirm if the easy and cheap protein-wiping inspection could be an alternative to bacterial counting by the conventional plating method in the actual food-processing lines. The principle of protein-wiping method is to detect residual protein (within 40 to 80 µg) by confirming the change of color caused by chemical reaction. This detection limit of protein is too high to detect bacterial cells directory, yet it may be sufficient to detect residual protein, which can be a nutrient of residual bacteria. One hundred and fifty-two samples taken from processing lines of facilities (which

producing commercially supplied omelets, Japanese style confectionery, chicken, side-dishes, and vegetables and so on) were tested. The coincidence rate between the results of the protein-wiping testing and those of the other two methods was 94.7%. Only three samples (2.0%) were found false negative, however, microbial contamination level in these samples were relatively low (less than 2.3 log CFU/100 cm^2). The visual observation by the site foreman missed to find about 40% cases of residual protein and bacteria. These results suggested that the protein-wiping method could be a simple and rapid voluntary inspection method for confirming residual microbial contamination after washing and cleaning without requiring special skills.

To find out a contamination source or identify the routes of contaminated bacteria in facilities is essential to effectively control the contamination of bacteria in the final product. The PCR based method or DNA probe method can be a candidate to examine bacterial flora of processing environmental samples or the ones in the processing foods. However, these methods are impractical to apply for daily bacterial quality control or in food hygiene practices because of its high cost. Tomioka et al. (2008) developed a novel easy and cheap method to estimate major contamination routes by using a set of agar plates. This "Rapicom" (Rapidly Detect a Contaminated Origin of Microflora) system consists of four kinds of agar media, which can distinguish different groups of coliforms. Similarity of bacterial flora based on the ratio of enumerated cells on each of the four agar media is used in clustering assay to compare the similarity of bacterial flora. This clustering result may not directly suggest the actual contamination routes in all cases; however, the irregular changing of the pattern will suggest the suspected point of contamination. For that purpose, an accumulation of daily data for each of facility is required. Tomioka et al. (2008) confirmed the reliability of this method in a soybean curd facility and had a good result; however, the accumulation of more practical application data is required. Especially, the selected 4-agar plate may not necessarily be suitable to apply in all kind of food processing facility because the micro flora of samples will have wide diversity depending on the processing foods and facility. Even this Rapicom system does not detect spoilage or pathogenic bacterial species specifically; its application for daily voluntary hygiene inspection could be a choice of a practical way of good management practice.

9 Conclusion

Because of the changing of consumers' preference worldwide, it is getting difficult to control the risk of foodborne illness and spoilage of traditional foods. Especially, reducing the use of salt or sugar decreased the hurdle and growth of bacteria due to the increase of water activity of foods. Shortened fermentation times also increases the risk because of insufficient

lactic acidic fermentation. It is important to maintain safety and quality of any foods by preventing contamination and suppressing the growth of bacteria. Keeping suitable temperature during distribution and storage is also important. In addition, introduction of good hygiene practices (GHP) in each steps of the food processing chain and application of suitable microbial controls including using sanitation processes and/or some food additives may contribute to the achievement the objective. In addition, over 100 organizations (on March 2009) have been certified ISO22000:2005 in food safety management systems since May 2007. However, introducing advanced technologies into most food processing facilities may not be an easy task because of lack of financial and human resources. It may be an important effort to increase the level of sanitation condition in all the small enterprises. Developing and encouraging the broad use of a cost effective and easy way to find out and control bacteria will be required.

Keywords: Food Safety, Traditional Fermented Foods, Risk factor, Pathogens and Food Processing Technology

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Color Plate Section

Chapter 4

Fig. 1. Fermented gherkin (Ray and Panda 2007).

Fig. 2. Lacto-pickle from orange fleshed sweet potato (Panda et al. 2007).

Fig. 7. Manufacturing aseptic form, fill and seal packaging machinery for cups (Reproduced with courtesy of Oystar Hassia Holding GmbH, Stutensee, Germany).

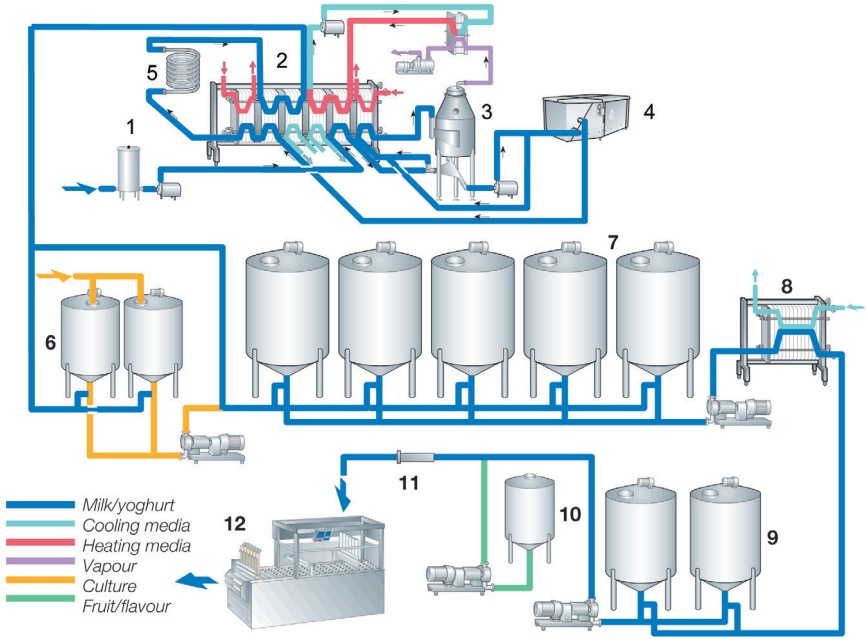

Fig. 8. Scheme of a stirred yogurt production line (Reproduced with courtesy of Tetra Pak, Processing Systems Division A/B, Lund, Sweden). 1: balance tank; 2: plate heat exchanger; 3: deaerator; 4: homogenizer; 5: holding tube; 6: bulk starter tank; 7: incubation tanks; 8: plate cooler; 9: buffer tanks; 10: fruit/flavors; 11: mixer; 12: packaging.

Chapter 6

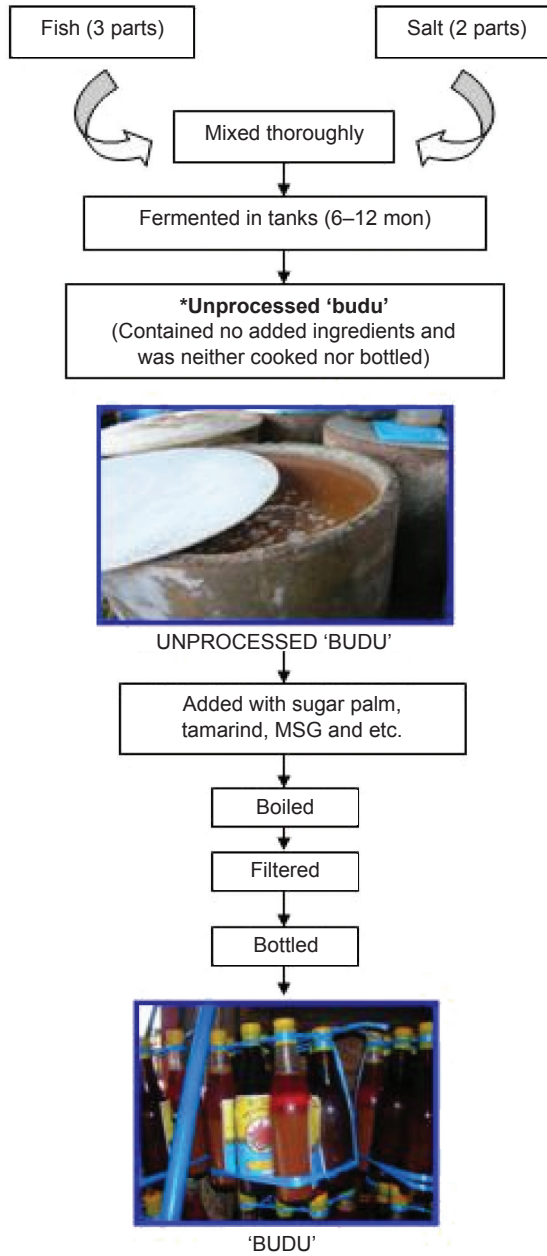

Fig. 1. Flow chart for production of budu (Source: Rosma et al. 2009).

Fig. 2. Fish paste "bagoong".

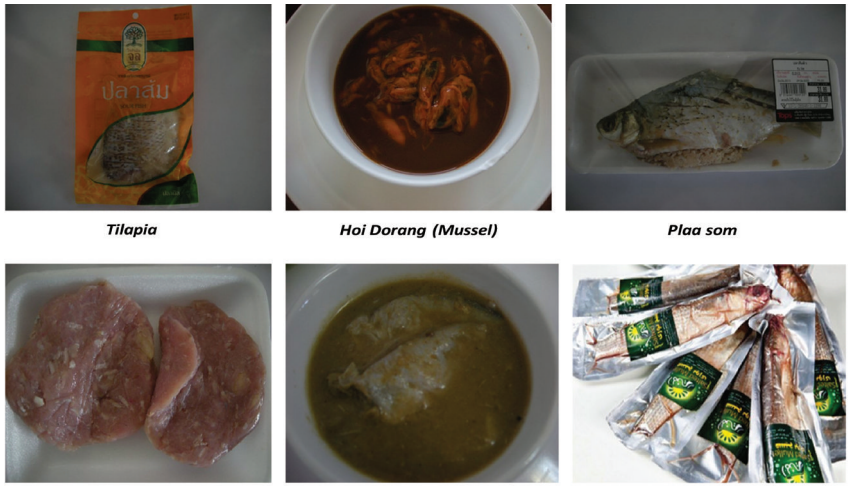

Fig. 4. Some fermented fish products.

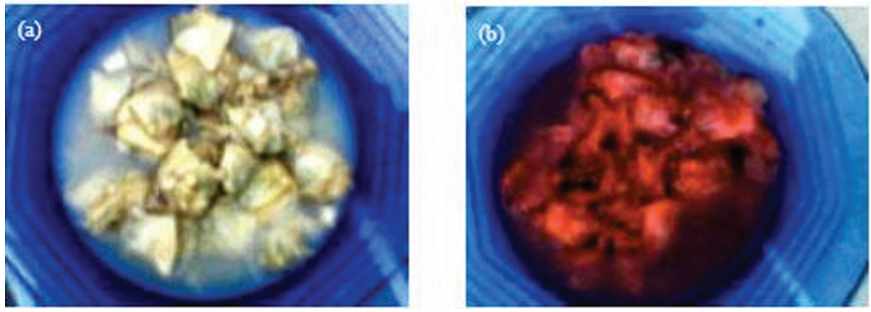

Fig. 5. Fermented ale-ale. (a) White fermented ale-ale. (b) Red fermented ale-ale.

Chapter 7

Carcass Contamination during Slaughtering (red dots) Unavoidable keep as low as possible.

Meat Cutting No reduction of contamination possible, but further contamination should be prevented.

Further Processing No reduction of contamination possible, but prevent further contamination and create challenges/hurdles for microbial growth and survival (a_w, preservatives).

Heat Treatment of Final Product Pasteurization (approx. 80 °C): Substantial reduction of contamination , but products need refrigeration. Sterilization (above 100 °C): Total elimination of contamination, products can be stored without refrigeration (in sealed food containers).

Fig. 1. The possibility of microbial contamination in the meat processing chain and how to prevent it.

Chapter 8

Ga Kenkey | Fanti Kenkey | Injera

Kisra

Fig. 1. Some non-alcoholic cereal-based foods of Africa.

Fig. 4. Selected traditional fermented African dairy products.

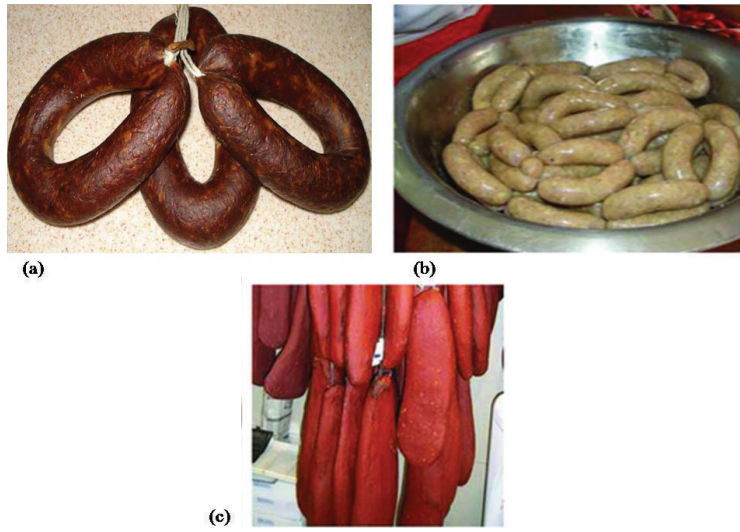

Fig. 6. Some African fermented meat products. (a) Sujuk. (b) Poultry merguez. (c) Pastirma.

Fig. 9. Some fermented beverages produced from cereals in Africa. (a) Bouza. (b) Pito. (c) Mahewu. (d) Busaa. (e) Munkoyo.

Chapter 11

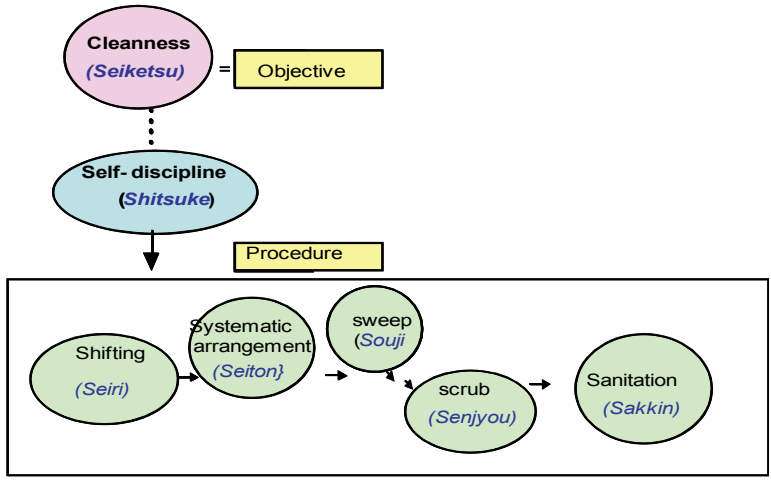

Fig. 6. Basic concept of "Food safety 7S".

The first volume in a series covering the latest information in microbiology, biotechnology, and food safety aspects, this book is divided into two parts. Part I focuses on fermentation of traditional foods and beverages, such as cereal and milk products from the Orient, Africa, Latin America, and other areas. Part II addresses fermentation biology, discussing specific topics including microbiology and biotechnology of wine and beer, lactic fermented fruits and vegetables, coffee and cocoa fermentation, probiotics, bio-valorization of food wastes, and solid state fermentation in food processing industries.

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