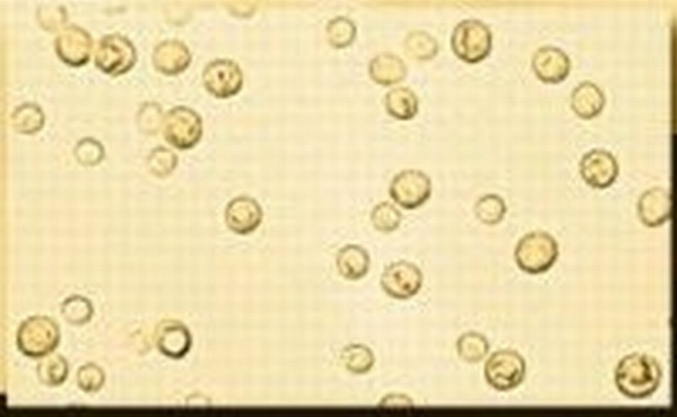
Fermented Foods and Beverages of the World



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Preface

Fermented foods and beverages are one of the indispensable components of the dietary culture of every community in the world. Traditional fermentation, and smoking, drying, and salting processes were developed by ancient people to preserve foods for consumption, a remarkable step in the food culture history of human beings. Wine was believed to be made in the Caucasus and Mesopotamia as early as 6000 BC, and the colonization by Romans spread winemaking all over the Mediterranean. The antiquity of Chinese foods and cuisines has been documented by several historians as far back as 4000 BC based on historical evidence. The ancient monuments of Nepal indicate that Himalayan ethnic foods have been consumed in the region for more than 2500 years. Food prepared by different communities is unique and distinct due to the geographical location, environmental factors, food preference, and the availability of plant or animal sources. Approximately 50–400 g per capita of fermented foods and alcoholic beverages are consumed daily worldwide, representing about 5%–40% of the total daily food intake. Low-cost, high-value, and socially and culturally acceptable fermented foods and beverages are consumed as staple foods, currries, stews, side dishes, fried foods, cooked foods, pastes, seasonings, condiments, pickles, confectioneries, salads, soups, desserts, savories, drinks, candied foods, masticators, colorants, tastemakers, and alcoholic and nonalcoholic beverages. About 5000 varieties of unlisted major and minor fermented foods and beverages are prepared and consumed by billions of people belonging to different communities and ethnicities across the world.

Fermented foods and beverages harbor diverse microorganisms from the environment, which include mycelial molds; yeasts; and bacteria, mostly lactic acid bacteria, bacilli, and micrococci. Microorganisms transform the chemical constituents of raw materials during fermentation and enhance the nutritive value of the products; enrich bland diets with improved flavor and texture; preserve perishable foods; fortify products with essential amino acids, health-promoting bioactive compounds, vitamins, and minerals; degrade undesirable compounds and antinutritive factors; impart antioxidant and antimicrobial properties; improve digestibility; and stimulate probiotic functions. Most of the ethnic fermented foods and beverages are produced by natural fermentation, except the alcoholic beverages in Asia, which are produced by using a consortium of microorganisms in the form of a dry, cereal-based starter. Diversity within the species or strains of several functional genera of dominant microorganisms has created ethnic foods with different sensory characteristics.

This book has 16 chapters covering the description of products; culinary practices; and the microbiology, biochemistry, nutrition, and functional properties of different categories of fermented foods and beverages of the world: fermented vegetables, fermented soybeans and non-soybean legumes, fermented cereals, fermented milks, fermented/dried/smoked fish, fermented/dried/smoked meats, fermented root/tuber products, fermented beverages and alcoholic drinks, and miscellaneous fermented products including vinegar, *nata*, *pidan*, tea, coffee, cacao, etc. There is

a complete chapter devoted to the dietary culture and antiquity of fermented foods and beverages of the world.

We attempted to update and collate information and research carried out on various aspects of major as well as minor fermented foods and beverages of the world. We are grateful to all the contributing authors who accepted our invitation to write this book. Many are well-recognized scientists and researchers with vast experience in the field of fermented foods and beverages. We are happy to bring all of them onto a same platform that helped in bringing out this book, and thanks to Martin Adams, Jean-Pierre Guyot, M.J. Robert Nout, Kofi E. Aidoo, Delwen Samuel, Ulrich Schillinger, Charles M.A.P. Franz, Carmen Wacher, N.A. Olasupo, S.A. Odunfa, Toshirou Nagai, Etsuko Sugawara, Baltasar Mayo, Mohammed Salim Ammor, Junus Salampessy, Namrata Thapa, O.S. Obayori, Louis Ban-Koffi, Mariam Farhad, Gloria Díaz-Ruiz, Ángel Alegría, and Susana Delgado. We are also grateful to Taylor & Francis for publishing this comprehensive book on important topics. We hope this book will be utilized by researchers, students, teachers, food entrepreneurs, agriculturalists, government policy makers, anthropologists, historians, geographers, ethnologists, sociologists, and media persons who are interested in fermented foods and beverages. Though there are hundreds of research articles, review papers, and quite a few books on fermented foods and beverages, *Fermented Foods and Beverages of the World* is the latest compilation of various aspects of fermented foods and beverages including many undocumented, minor, or lesser-known ethnic fermented products of the world.

We dedicate this book to Dr. C.W. Hesseltine, Prof. K.H. Steinkraus, Prof. Michio Kozaki, and Dr. Sayuki Nikkuni, all deceased, whose contributions to the field of fermented foods and beverages of the world is immeasurable; their memories will remain in this book forever. We salute them for creating a forum of knowledge and a base for research to study in depth the culture and science involved in the production of fermented foods and beverages and also to validate the worthy knowledge of ethnic people.

Jyoti Prakash Tamang
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Editors

Dr. Jyoti Prakash Tamang has been one of leading researchers on fermented foods and beverages for the past 23 years. He obtained his PhD from North Bengal University, and completed his postdoctoral research with the National Food Research Institute (Japan) and the Institute of Hygiene and Toxicology (Germany). Dr. Tamang won the National Bioscience Award of the Department of Biotechnology, Ministry of Science and Technology, Government of India, in 2005, one of the prestigious awards, and became a fellow of the Biotech Research Society of India in 2006. He has published more than 90 research papers in peer-reviewed international and national journals, filed a patent, and guided several PhD students. He has presented his work in different countries and is a member of many prestigious national and international academic and scientific organizations. He is a regular reviewer of many peer-reviewed international scientific journals in microbiology and food sciences, and of books. Dr. Tamang recently authored *Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values* published by CRC Press, Taylor & Francis. Prof. Tamang is a Head of Food Microbiology Laboratory and was the first academic coordinator of Sikkim University at Gangtok, India.

Dr. Kasipathy Kailasapathy is an associate professor in the School of Natural Sciences, University of Western Sydney, Hawkesbury, New South Wales, Australia. He has a bachelor's degree in agricultural science from the University of Peradeniya, Peradeniya, Sri Lanka, and graduate degrees from Obihiro Chickusan University, Obihiro, Japan (MSc in agricultural science), Penn State University, University Park, Pennsylvania (PhD in food science), and University of Western Sydney, Australia (MBA). He is the research leader for the Probiotics and Encapsulated Foods Unit at the Centre for Plant and Food Science, University of Western Sydney, Australia. His research has focused on dairy fermented foods incorporating probiotic bacteria and prebiotic substances, health benefits, microencapsulation, immobilization of enzymes, active packaging, and bioactive peptides, among others. In the past 10 years, he has authored and coauthored more than 75 papers in scientific journals, several chapters in books, has supervised over 20 higher degree students, and has given more than 50 presentations in national and international conferences.

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Dietary Cultures and Antiquity of Fermented Foods and Beverages

Jyoti Prakash Tamang and Delwen Samuel

CONTENTS

1.1 Cultural Foods

Each and every community has a distinct food culture or dietary culture that symbolizes its heritage and the sociocultural aspects of its ethnicity. Food prepared by different communities is unique and distinct due to the geographical location, environmental factors, food preference, and the availability of plant or animal sources. Religions, dietary laws, customary beliefs, and social groupings are some of the characteristics contributing to the description of a culture, while ethnicity is the affiliation with a race, people, or cultural group (McWilliams 2007). Religions and beliefs exert a strong influence on dietary habits, particularly through dietary laws such as taboos imposed on the consumption of certain food items. Fermented foods and beverages

are one of the integral components of cultural foods. Some cultural foods have been mentioned in holy books such as the Bhagavad Gita, the Bible, and the Koran, and as a result, most of the traditional or ethnic foods are influenced by religion and taboo.

1.1.1 Hindu Foods

In the Bhagavad Gita, the sacred book of the Hindus, foods are classified into three different types based on property, quality, and sanctity: these are *sattvika*, *raajasika*, and *taamasika*. The *sattvika* food denotes food for prosperity, longevity, intelligence, strength, health, and happiness. This type includes fruits, vegetables, legumes, cereals, and sweets. The *raajasika* food signifies activity, passion, and restlessness, and this type includes hot, sour, spicy, and salty foods. The *taamasika* food is intoxicating and unhealthy, which generally causes dullness and inertia. Hindu foods follow the concept of purity and pollution that determines interpersonal and intercaste relationships (Kilara and Iya 1992). The kitchens of the Brahmin Hindu produce two types of meals: *kaccha* meaning uncooked and unripe meals, and *pakka* meaning ripe and cooked meals. *Kaccha* foods are highly vulnerable to contamination and, therefore, there are strict codes for cooking, serving, and eating these foods. *Pakka* foods are fried, therefore not vulnerable to contamination (Misra 1986). Hindus are traditionally vegetarians, but many non-Brahmins are nonvegetarians. Since the cow is considered to be sacred, beef is not eaten by Hindus. Fish seem to be more acceptable than other flesh foods. Brahmin Hindus do not eat garlic, onion, nor consume intoxicants. Foods are offered at shrines/temples for worshipping gods, to free oneself from the possession of spirits, and feeding domestic and some wild animals including birds on religious occasions. Ethnic foods have social importance for celebrations particularly during festivals and other social occasions. Cooking is usually done by daughter-in-laws, daughters, or mothers. The Vedic Indians took their meals in the sitting posture (Prakash 1987). Traditionally orthodox Hindu men avoid taking meals with their wives. Women generally take their meals in absence of male members. A custom of serving meals first to the elder male members in the family is prevalent in the Hindu food culture. Traditionally, Hindu female family members eat afterward.

1.1.2 Buddhist Foods

Strict Buddhists avoid eating meat and fish out of respect for living things (Hinnells 1997). However, nonvegetarian foods are not strictly forbidden. According to the Buddhist religious dietary code, if animal flesh is eaten, the animal should be killed by non-Buddhists. Monks are likely to be more restricted in their dietary practices than lay Buddhists, and they may avoid eating meats and fish. Monks do not eat anything solid in the afternoon. Fasting for the entire day is expected on the new moon and the full moon each month. Buddhists usually eat together at home with their families. Tibetan Buddhists usually eat noodles in soup, *skiu* or *momo* (small dumplings of wheat flour with meats), baked potatoes, *tsampa* (ground roasted barley grains), *chhurpi* (cottage cheese), *kargyong* (sausage), and *chyang* (alcoholic beverage) (Tamang 2010). Tibetan Buddhism does not restrict the consumption of animal flesh and alcoholic beverages. In Tibet, by virtue of its location in high altitudes and cold climate, vegetables and plant sources are not abundant; people have to depend on meat. Tibetans do not eat small animals such as chickens, ducks, goats, and pigs;

they believe taking the lives of many small animals is more sinful than killing a single large animal (yak, cow), which is more practical. Fish eating is uncommon among the Tibetan Buddhists, since they worship fish for longevity and prosperity. Nepali Buddhists also do not follow the dietary rules of Buddhism. Except the Tamang and the Sherpa, other Nepali communities do not eat beef and yak. Nepali Buddhism is the fusion of Tibetan Buddhism and Hinduism with a blend of nature and ancestor worship. Buddhists in Southeast Asia eat fish and soybean products.

Buddhist influence in traditional Japan led to various restrictions on the consumption of animal products other than fish. The introduction of soybean as a food along with its fermented products into Japanese cuisine was due to the introduction of Buddhism in the AD sixth century (Hamamo 2001). The introduction of Buddhism to the Korean peninsula in the Koguryo Kingdom in AD 372 and in the Silla Kingdom in AD 528, respectively, changed the food culture from animal-based foods to vegetable-based foods (Lee and Kwon 2005). The people of the unified Silla Kingdom of Korea during the Koryo dynasty (AD 918–1392) were orthodox Buddhists who prohibited meat consumption, and vegetables were preferred (Park and Rhee 2005).

The religion in China for the majority of people is a blend of Confucianism, Taoism, and Buddhism (Hinnells 1997). Confucianism underlines the morality and behavior of people, including rites of passage, and Taoism provides for the needs and healing of the sick and is a basis for regulating festivals. Though Confucianism and Taoism do not provide guidelines for daily diet, the ancient food culture in China was mostly vegetarian diets. The Lunar New Year (festival of spring) is celebrated by a big gathering of family and relatives where they share a banquet. Before eating, the Chinese celebrate the family's ancestors by offering sets of chopsticks, cooked rice in bowls, alcoholic drinks, and tea, which are placed at the family altar. This combination of Confucianism and Taoism influences the Chinese dietary culture. Shintoism is the religion of early Japan and is still in practice today, a blend of both Shintoism and Buddhism. In Shintoism, the ancestors are revered. Some Japanese homes still maintain two altars, a *kami* (ancestor) altar for life and its activities, and a Buddha altar for death and ancestral worship. Both altars are provided with fresh foods and *saké* by the Japanese for a good beginning of the day.

1.1.3 Christian Foods

Certain foods are symbolically used at the Eucharist or communion by Christians. A wafer or bread is placed on the tongue (or in the hand) to represent the body of Jesus, and wine is drunk symbolizing His blood (Hinnells 1997). The apostle Paul is credited with freeing Christian laws from the diet laws practiced by the Jews, which thus served as a means of distancing the new Christian religion from Jewish origins. In fact, the symbolic drinking of wine as a representation of the blood of Christ was a significant departure from the strong avoidance of blood, which was proscribed in the Jewish dietary laws. *Paska* is a special Easter bread that is prominent in Eastern Orthodox Church celebrations. The name of this bread reflects the fact that Jesus was crucified during the Jewish Passover. *Paska* is a sweet, yeast-leavened bread quite different from the unleavened matzo eaten during the Jewish Passover that symbolizes the exodus from Egypt (Hinnells 1997). In Eastern Europe, women bring their baskets containing foods to church for the Easter dinner so that priests can bless them. Eggs are considered to be symbolic of the Resurrection of Christ, and are usually

decorated and featured by Christians throughout North America and Northern Europe (McWilliams 2007). In Christian food culture, all family members sit together at the table and eat together after family prayers. Varieties of ethnic foods such as loaf, cheese, and sausage constitute the cultural foods of the majority of Christians, mostly in Europe, America, and Australia.

1.1.4 Muslim Foods

Consumption of foods is governed by the strict dietary laws for Muslims developed by Muhammad (Hussaini 1993). Food prohibition includes avoiding eating swine, the flesh of carrion (dead animals), blood in any form, food previously offered to gods, and alcohol and other intoxicants. As per the dietary laws, Muslims foods are prepared accordingly without any alcoholic beverages. Traditionally, Muslim women and children may eat separately after the male members finish their meals. During Ramadan, a month-long fasting, family members, friends, and relatives share common meals after sunset. In Sudan, traditionally at the time of Ramadan, *hulu mur*, a traditional fermented sorghum bread drink, is made by soaking the sheets of leavened bread in a glass of water (Bärwald 1994). The drink is freshly prepared and drunk within 1 h so that no measurable amounts of ethanol can be produced, making the product nonalcoholic, which is permitted during Ramadan (Agab 1985).

1.2 World Dietary Culture

World dietary culture has three distinct traditional food habits based on staple cereal diets: (1) cooked-rice eaters of Eastern food culture, (2) wheat/barley-based breads/loaves of Western and Australian food culture, and (3) sorghum/maize porridges of African and South American food culture. Rice is a staple food for millions of people in China, Japan, Korea, Taiwan, Philippines, Malaysia, Singapore, Thailand, Laos, Cambodia, Myanmar, Bangladesh, Bhutan, Nepal, Mongolia, east and south India, and Sri Lanka; whereas wheat or barley is a staple food in north and west India, Pakistan, Afghanistan, Iran, Iraq, and all of mid-Asia, Europe, North America, and Australia; while sorghum, maize, millets, and cassava are the staple crops of Africa, maize is the staple food in South America. A typical diet of the Eastern World consists of boiled rice with many side dishes containing fermented and nonfermented soybean products, vegetables, pickles, fish, meat, and alcoholic beverages. The Western including Australian food culture has wheat or barley as the staple food, followed by milk and fermented milks (cheese, yoghurt, curd), meat and meat products (sausages, hams), and wine. The African dietary culture includes both fermented and nonfermented sorghum, maize, millets, cassava products, wild legume seeds, tubers, meat, milk products, and alcoholic beverages. The South American dietary culture includes both fermented and nonfermented maize products, meat, milk products, and alcoholic beverages. In Europe, America, and Australia, fruits, mostly grapes, are fermented into wine, whereas in Asia and Africa grapes are eaten fresh without processing into wine. Interestingly, the Himalayan dietary culture has both rice and wheat or barley or maize as the staple food along with varieties of ethnic, fermented and nonfermented foods prepared from soybeans, vegetables, bamboo, milk, meat, fish, alcoholic beverages, and wild edible plants (Tamang 2010). Drinking animal milk is not part of

the food culture of ethnic Chinese, Koreans, Japanese, and many Mongolian-origin races despite an abundance of cows in their regions, whereas, the Indians, Europeans, Semites, and the nomadic tribesmen of North Central Asia are traditionally milk drinkers (Laufer 1914–1915). In Far East Asia, the soybean, called as the "cow of China" (Hymowitz 1970), is processed to make soy milk, *tofu* (soya curd), and fermented into a number of ethnic, fermented soybean products such as *miso*, *shoyu*, *natto*, *kinema*, *thua nau*, *douchi*, *chungkokjang*, *tempe*, and *sufu*. The Himalayan dietary culture is a fusion of the Hindu–Aryan culture and the Tibetan–Mongolian culture influenced by ancient Chinese cuisines with modifications based on ethnical and sensory preferences over a period of time (Tamang 2010). Countries bordering other countries have closed cultural affinities, which has influenced the dietary cultures of many regions.

Indian food is spicy, and salt is added directly while cooking; seasonings such as soy sauce and monosodium glutamate (MSG) are never used. Chinese, Korean, and Japanese foods are not spicy, and use soy sauce for seasoning and other taste enhancers such as MSG. European and American foods are grilled, fried, roasted, or baked. In North America, sweet tomato-based ketchup is widely used as a condiment, while pickled vegetables such as cucumbers and onions, and relishes based on fruits, are common European accompaniments. African foods are also grilled or steamed, and hot. Eastern foods are more salty, spicy, and hotter than Western foods, which are less salty, more sweet, oily, nonspicy, and crispy.

People have developed different methods of eating foods in the course of the history of dietary cultures. The three major methods of eating foods are using hands/fingers, chopsticks, and cutlery, which have remained the tradition among consumers worldwide.

1.2.1 Use of Hands

The use of hands for eating must have existed since ancient times. Using hands to eat remains a tradition of Hindu and Muslim dietary cultures, and is also practiced by many Buddhists of Southeast Asia. Asians of the Indian subcontinent and non-Chinese people of Southeast Asia and mid-Asia use hands for eating. The practice of washing hands and mouths before and after eating meals was common during the Vedic period (1500–800 BC) (Prakash 1987), which is a part and parcel of dietary rules and etiquette of the Hindus. Eating foods with hands has been mentioned in Nepalese history during the Lichchhavi dynasty in AD 100–880 (Bajracharya and Shrestha 1973). There is no mention of use of cutlery or chopsticks in the ancient dietary culture of the Hindus. Traditionally, Africans also use hands for eating.

1.2.2 Use of Chopsticks

Oriental Asians, mostly Chinese, Tibetans, Mongolians, Koreans, and Japanese, use chopsticks for eating. Though the exact date of the origin of chopsticks has not been recorded, archaeological evidence of chopsticks unearthed at Yin Xu, the capital of China's Shang dynasty (1300–1046 BC), indicated that they were at least 3400 years old (Warrant and Honten 2008). Besides their use as feeding tools, chopsticks were also used as a tool in rituals for making offerings of food items to the spirits of ancestors. Probably the use of chopsticks as a more hygienic way of eating and a convenient way of handling hot foods might have prompted the invention of chopsticks. The origin of chopsticks is described in the *Classic of Rites*, one of the five classics

in the Confucian canon, that mentions the social forms, ancient rituals, and court ceremonies of the Zhou dynasty (1122–256 BC) (Warrant and Honten 2008). In the beginning, chopsticks were used by royal and aristocratic families during feasts, until around 100 BC during the Han dynasty (206 BC–AD 9) when chopsticks were used by commoners, and has remained a part of the food culture of many Orientals.

1.2.3 Use of Cutlery

One attribute of Western cultural cuisine is the use of cutlery to cut food on the plate and to transfer food from the plate to the mouth (Young 1999). This culture has diversified over time so that the manipulation of knives and forks differs in Europe and the United States (Childe 1956:113). The English word "cutlery" is derived from "cutler," a craftsman skilled in the art of making knives. This reflects the central importance of the knife in ancient British culture. In the pre-Christian era, burials of wealthy and powerful people included their knives, showing the significance and value of this tool (Moore 1999). Spoons have been in use since ancient times, especially to manipulate food during preparation, and to feed those less able to assist themselves, such as babies and the sick.

The fork as a widespread tool for eating is a much more recent development (Elias 1994). Perhaps the earliest known forks date to ancient China, where they were mainly made of bone. Artistic depictions show them associated with dining scenes, so forks may have been used for eating in ancient China, despite their later absence (Huang 2000). Examples of Western forks are known from Roman archaeology, and there is a fine example of a fork as part of a Roman multi-use tool in the Fitzwilliam Museum, Cambridge, United Kingdom (Vassilika 1998, and see www.fitzmuseum.cam.ac.uk/opac/search/search.html, then enter "GR.1.1991"). These metal utensils are rare. Since forks of worked bone or wood are unlikely to survive, and metal was generally recycled in the ancient world, it is hard to know just how early and widespread the fork may have been, and how it was used.

The first documented use of the fork in Europe comes from about AD 1060, when a Byzantine princess married the heir to the Doge of Venice and brought a case of forks with her (Giblin 1987). Although this "new" technology was greeted with suspicion and distrust, and even as late as the seventeenth century Louis XIV of France (1638–1715) refused to use a fork, eventually its use spread from Italy and throughout Europe. By the early seventeenth century, the fork had arrived in Britain, but was in far from common use over the next hundred years. As the cuisine of the upper classes became more elaborate through the eighteenth and nineteenth centuries, forks together with spoons and knives became widely differentiated for use with different courses and types of food. In modern times, these protracted and complex meals have largely disappeared, and cutlery has simplified. Specific types, such as the fish knife and fork, still persist amongst some diners. Today, a set of table cutlery is convenient for eating across the world regardless of ethnic origins.

1.2.4 Evolution of Dietary Culture

Dietary culture has evolved as a result of traditional knowledge and experiences of generations over time. Cuisine is based on a wide range of factors, including environment, availability of edible substrates, sensory properties, ethnic preferences, customary beliefs, religions, socioeconomy, regional politics, cultural practices, and dietary

laws. Though the food cultures of the Western and the Eastern worlds are not parallel, frequent movement as well as migration of people carrying their dietary cultures and food habits along with their ethnic cuisines from one region to other regions within the same or different countries might have influenced the settlers, and thus resulted in the amalgamation or fusion of dietary cultures over time. The ethnic food culture of many indigenous people is being lost due to changes in climate, global economy, the process of rapid urbanization and development, and the increasing availability of commercial ready-to-eat fast foods in the markets. Today, there is a rapid transformation in food habits due to health consciousness and also due to rising prices.

1.3 Antiquity and Cultural Aspects

Dietary culture carries the cultural history of ethnic communities (Tamang 2001a). Traditional fermentation, smoking, drying, and salting processes were developed by ancient people to preserve foods for consumption and to improve their nutritional value, a remarkable step in the food culture history of human beings. Fermented foods and beverages are socially and culturally widely acceptable food items in local cuisines. Records of historical food cultural practices in many parts of the world can be rich and detailed. Documentary sources are patchy, however, leaving vast regions and historical periods unknown.

Our knowledge of more ancient times is even more fragmentary. Archaeology is our only source of information, and many areas have yet to be studied in any detail. Archaeological recovery relies on the survival of material artifacts. Materials impervious to decay, such as pottery fragments, stone tools, and stone hearths provide insights into the tools used to prepare food. Food itself, though, is highly transient and ephemeral in nature. Despite this, ancient remains of food ingredients are often preserved by virtue of their robust structure, such as bones and teeth, or by transformation processes that render them resistant to decay. Archaeobotany, the study of ancient macroremains such as seeds and chaff, largely relies on the fact that plant material charred through contact with fire is inert to biological processes. When ancient cooking methods exposed dense food items to burning, plant remains can be abundant. Sappy, leafy, and water-rich plant foods are rarely recovered archaeologically. Where archaeological research has incorporated the study of ancient plant and animal remains, food resources are often well understood. More challenging by far is the recovery, recognition, and analysis of prepared foods. Even here, improved awareness and the development of new techniques have allowed archaeologists to investigate food preparation, including ancient fermentation.

The antiquity of Chinese foods and cuisines has been documented by several historians since 4000 BC based on historical evidence (Lee 1984, Yoon 1993, Huang 2000). Indian foods have been well documented from before 3000 BC based on historical documents and archaeological evidence (Yegna Narayan Aiyar 1953, Prajapati and Nair 2003). The ancient historical monuments of Nepal indicate that the Himalayan ethnic foods have been consumed in the region for more than 2500 years (Tamang 2010). Ancient Chinese books such as *Jeijeon* written by Yangseu and *Dongijeon* written by Namsa described how the Kokurye people, the Koreans' ancestors (37 BC–AD 668), developed various fermented foods such as fermented soybeans, vegetables, fish, and alcoholic beverages (Kim 1997).

In the ancient Near East, the earliest historical Sumerians and Egyptian cultures generally did not record cooking methods, but some clues can be gleaned from written records. In some cases, archaeology is able to provide new data about ancient food practices. European historical documents pertaining to food practices generally start with the Romans. As a result, writers such as Pliny can be heavily and perhaps inappropriately relied upon for insights, when the cultural practices of interest were not related to the Roman world. Past food cultural practices in regions such as Africa and South America are poorly documented or have only recently been recorded. For example, northern and western African traditions were recorded from the eighth century onward by Arab travelers, when fermented foods were staples in the African diet, but elsewhere traditions were not recorded until much later (Odunfa 1988). These regions retain a rich traditional cuisine up to the present day, particularly in more rural areas, where domestic fermented and other foods are often prepared as they have always been. The rich dietary culture and the history of diverse ethnic, fermented foods and beverages of the Himalayas have been recently documented by Tamang (2010); otherwise such important information was unknown to rest of the world. The native skills of food fermentation have been passed from mothers to daughters, and fathers to sons through the traditional knowledge of elders; which include grandmothers/grandfathers, mothers/fathers, and village elders; self practice, family; community; and neighbors. Although this chapter is not comprehensive, it aims to document a global overview of ancient and traditional cultural aspects of ethnic, fermented foods and beverages.

1.3.1 Fermented Vegetables

Traditional techniques of fermentation or pickling vegetables seem to have developed independently, for example, in Asia (Pederson 1979), the Mediterranean (Hulse 2004), and possibly in Europe. The product we know today as sauerkraut is a European foodstuff made from dry, salted cabbage. The process evolved over time, and was not fully developed in its present form until the seventeenth century. Sauerkraut or *sauerkohl* is a German word meaning sour cabbage, which is generally prepared from shredded white cabbage through spontaneous lactobacilli fermentation (Steinkraus 1983). It can be markedly acidic if the salt concentration or the fermentation temperature is higher than usual (Pederson and Albury 1969). Although sauerkraut fermentation is generally thought to have started in Germany or North Europe, one historian suggests this method of preserving vegetables originated in China and was brought into Eastern Europe by the Tartars (Toomre 1992).

The first description of a naturally acidified product, sauerkraut, or *choucroute* in French, can be found in *Le Tresor de santi* (1607), which describes it as a German product (Davidson 1999). Sauerkraut was traditionally very popular in Poland and remains so today (Kowalska-Lewicka 1988). Valued not only for preserving fresh vegetables, this fermentation technique was important as it provided an intense flavor to a largely bland diet. Large-scale production was carried out in special pits lined with wooden planks, or in barrels. The flavor could be varied through various additives. For example, otherwise inedible apples, or sometimes pears, were included, or herbs such as caraway seeds or dill were layered with the cabbage. Even oak or cherry leaves were sometimes used. Other vegetables are also traditionally fermented in Europe, including onions, cucumbers, and beets. In the past, edible mushrooms were

very commonly pickled and were esteemed in Poland. In past centuries, barrelsful were prepared by villagers for their landlords. This food is now rare because wild mushrooms have become scarce (Kowalska-Lewicka 1988).

Olives are native to the eastern Mediterranean. The precise date and place of olive domestication is unknown, but early to late fourth millennium sites excavated in the Jordan Valley show evidence of olive use. The beginnings of olive fruit preservation are unknown, and may be difficult to establish. Certainly, by Roman times, olives were preserved by various methods, as described by Columella and other Roman authors (Sealey and Tyers 1989). That olives were widely traded is attested by the occasional finds of pottery jars filled with olive stones found on Roman shipwrecks, and, unusually, from a Roman-period Spanish jar dated between AD 50 and 150 dredged up from a sand bank on the Thames estuary (Sealey and Tyers 1989). Not all olive fruits are preserved by fermentation, but olives require some form of processing because the bitter glucoside, oleuropein, renders the raw fruit inedible.

In Spain, olives were widely processed by hand for home use, using methods and ingredients typical for each region (March and Rios 1988). This traditional knowledge includes seasoning ingredients, precise timings for each stage of the processing, and using specific containers. Only green olives are fermented. The optimum salt concentration is determined by dissolving coarse salt in water and placing an egg in the brine. When the narrow end of the egg floats uppermost, the salt concentration is correct. During the time the olives are fermented in the brine, flavoring agents such as herbs, lemon, and garlic are added, according to traditional regional and family recipes. The olives may be ready in as little as 10 days or take as long as 9 months to mature. Olives are a symbol of hospitality in Spain and in rural areas are offered to guests upon their arrival.

In China, the laborers engaged in the construction of the Great Wall in the third century BC ate acid-fermented mixed vegetables (Pederson 1979). One of the most important early sources of information about ancient Chinese agriculture and cooking is the text *Chhi Min Yao Shu* (Essential Arts for the People's Welfare) (Sabban 1988, Huang 2000). It was written between AD 533 and 544 by Chia Ssu-Hsieh, a middle-ranking official in northern China, and contains some of the most comprehensive and detailed descriptions of culinary processes. This text details ingredients and provides 41 recipes for the preparation of vegetables pickled in salt, or salt and vinegar. Amongst the vegetables preserved in this way were Chinese cabbage, mallow, and mustard greens. Some fruits were also pickled, such as melons and pears. By the time of the Song dynasty (960–1279), sophisticated techniques for pickling vegetables were well established and have remained more or less the same to the present day.

Salted and preserved vegetables have been consumed in Korea since 2000 years (Kim 1997). The antiquity of *kimchi* processing in Korea can be traced back to AD 3–4 (Chang 1975). The word *kimchi* originated from *chimchae*, meaning pickled vegetables with salt in Chinese (Park and Rhee 2005). One of the historical documents from Korea, *Samkuksaki* (published in AD 1145), indicated that fermented vegetables were prepared using stone pickle jars in the Bupju temple at Mt. Sokri during the Shinla dynasty (AD 720) (Cheigh 2002). The description of *kimchi* preparation from turnip by adding salt was found in an ancient Korean historical document, *Dongkukisangkukjip*, written by Lee Kyubo during AD 1168–1241 (Cheigh 2002). Use of garlic, Chinese pepper, ginger, and tangerine peels during the preparation of *kimchi* is mentioned in this historical document. One of the oldest Korean

cookbooks, *Umsikdimibang*, written by Chang (AD 1598–1680), described the processing methods of seven types of vegetable pickles. The most important classic literature concerning *kimchi* processing is *Imwonsibyukji*, written by Suh Yu-Geo (1764–1845). *Kyuhapchongseo* written by Lee (1759–1829) described in detail the processing methods of three types of *kimchi* including *sukbakji*. *Baechu* (Chinese cabbage) and white radish became the main ingredients of *kimchi* after AD 1600 during the mid-Chosun dynasty. *Jibongyusol* (AD 1613) showed the first records of red peppers, and their use in *kimchi* preparation was recorded in *Sallimkyongje* in AD 1715 (Cheigh 2002). Red pepper was introduced to Korea in the AD seventeenth century when the salt stocks were in short supply (Park and Rhee 2005). The addition of red pepper during *kimchi* preparation provided a harmonious taste, good color, and antimicrobial activity (Cheigh and Park 1994). Koreans believe that red color due to the addition of red pepper could protect them from evil spirits (Lee and Kwon 2005). Radish and cucumber were the most important ingredients for *kimchi* making until the nineteenth century, since Korean cabbage was not available then (Lee 1994). The number of *kimchi* varieties increased remarkably from 11 to 36, in the course of its 200 year evolution (Lee 1994). In modern Korea, *baechu-kimchi*, made from Korean cabbage, is the most important variety, followed by *kkakdugi* and *dongchimi kimchi*, made from radish (Yu and Chung 1974, Park and Rhee 2005). *Kimchi* has a unique sour and carbonated taste and is traditionally served cold (Mheen et al. 1981).

In the Himalayas, fermented vegetables such as *gundruk*, *sinki*, and *goyang* have cultural significance for Nepalis. An oral history on the invention of *gundruk* and *sinki* has been documented in detail by Tamang (2010). In ancient Nepal, farmers were forced to flee villages due to war, leaving their agricultural lands cultivated with plenty of leafy vegetables, radish, and paddy. However, a wise king at that time thought of preserving food stocks for his soldiers and farmers on return and of depriving food to the enemy. The king ordered his subjects to cut ripening paddy and uproot radish crop from the fields and directed them to dig pits, make beds of hay, and bury the prematurely harvested crops in separate pits by covering them with hay and mud. The farmers and soldiers dug the pits and buried all the agricultural products including radish, leafy vegetables, and paddy and whatever they could hide from the attackers. The king and his subjects returned to their homes after a few months, and dug out the pits where paddy was buried; however, the rice obtained from the paddy was stinky while the radish and leafy vegetables had developed a acidic and sour taste, which was different from that of fresh vegetables. Large amounts of acidified vegetables, after their removal from the pits, lay on the fields in the open. Somehow after a few days, the freshly fermented vegetables were sun dried and were fully preserved. People liked the acidic taste and flavor and termed these products as *gundruk* (leafy vegetables) and *sinki* (radish tap roots). They made the products into soups and pickles using their culinary skills. The word *gundruk* might have originated from the Newar (one of the major ethnic groups of Nepal) word *gunnu*, meaning dried taro stalk (Tamang 2010). The invention of the pit fermentation method for the acidification and preservation of perishable vegetables is unique to the Himalayas. Unlike the Korean *kimchi*, the Chinese *suan cai*, the Japanese *sunki*, the Indonesian *sayur asin*, and the German sauerkraut, *gundruk* and *sinki* are dried acidified products. Fresh, wet fermented vegetables are rarely or not eaten by Nepalis. Sun drying of freshly prepared *gundruk* and *sinki* is a traditional preservation method by which the shelf life of the products is prolonged. Dry, fermented vegetables are comparatively

lighter than fresh substrates and can, therefore, be carried easily while traveling long distances in the difficult terrains of the Himalayas. The mountain people of Nepal might have included the extra technique of sun drying even after the completion of the fermentation of perishable vegetables so that they could feed themselves during the scarcity of vegetables during long monsoons (rainy season) in the Himalayas.

Pickled vegetables are also consumed in other parts of the world. For example, Malaysians pickle foods such as cucumbers, ginger, leeks, chillies, and bamboo shoots, as well as unripe fruits including mangoes, papaya, and lime (Merican 1983). In the Near East, grape leaves are preserved by pickling in brine. The salt concentration is adjusted in the same way as the brine made for olive making in Spain: by floating an egg in the salt solution (Dagher 1991). Pickled vegetables are very popular in Egypt, where large amounts of pickled carrots, cucumber, turnips, cauliflowers, and other vegetables are consumed (Mahmoud et al. 1972). It is probable that the practice of pickling in these countries was imported from earlier times. The African practice of pickling seeds is likely an indigenous practice. Some examples of pickled West African seeds include the locust bean (*Parkia biglobosa*), the oil bean (*Pentacletha macrophylla*), and the poisonous castor oil seed (*Ricinus communis*) (Odunfa 1985). The latter loses its toxicity during fermentation. A variety of little-known vegetable fermentations are prepared by different peoples of Sudan, often as accompaniments, famine foods, or as meat substitutes for the very poor (Dirar 1993).

Because of the acidic taste, *gundruk*, *sinki*, *kimchi*, and sauerkraut have been known from ancient times as good appetizers, and people use these foods as a remedy for indigestion. *Gundruk* and *sinki* signify the food culture of every Nepali across the world. *Kimchi* is eaten on every social and religious occasion by Koreans, which signifies its importance as a cultural or heritage food. A Chinese meal may be incomplete without *suan cai* as a side dish, and is consumed on all social occasions as a cultural food. Several ethnic, fermented bamboo shoot products are preferred during social and religious ceremonies by ethnic Indian people such as the Meitis of Manipur, the Nagas of Nagland, the Apatanis of Arunachal Pradesh, and also by Thais and Malays.

1.3.2 Fermented Soybeans and Non-Soybean Legumes

Wild soybeans may have first been domesticated in the eastern half of North China around eleventh century BC during the Shang dynasty (ca. 1700–1100 BC), or perhaps earlier (Singh and Hymowitz 1999). This area is generally considered the primary soybean gene pool (Hymowitz 1970). The spread of soybeans from the primary gene pool to central and south China and peninsular Korea perhaps took place during the expansion of the Zhou dynasty (first millennium BC), and had happened by the AD first century. Soybean then spread to Japan, and throughout Southeast Asia and northern India over the course of the succeeding centuries. In these new areas, landraces were established, which created a secondary center of diversification for soybeans (Hymowitz 1990). The transformation of soybean cuisines from China was mostly because of the migration of some ethnic Chinese to other countries and the rapid acceptance of soybeans as a cultural food by non-Chinese communities. New evidence has very recently emerged suggesting more complex soybean origins, but this has yet to be fully published.

In China, *chi* (*shi*) and *jiang* (*chiang*), the two major types of fermented protein-based foods, have been popular since 2000 (Bo 1982a, 1984a). *Chi* refers to

exclusively soybean-based foods cultured with microorganisms, while *jiang* refers to proteinaceous plant or animal foods mixed with mold-cultured cereals or *chu* and salt (Bo 1982b, 1984b, Sabban 1988). The most popular seasoning was *chi*, known as *douchi*, which was next to salt use in China and which was mentioned in an ancient Chinese historical document called *Shi-ji* written by Si-Ma-Qian in second century BC (Wang and Suang 1986). The preparation of 3 different types of *chi* and 14 *jiang* recipes is described in detail in a AD sixth century book *Chii Min Yao Shu* (Sabban 1988, Yokotsuka 1991a). Today, soy sauce is an integral part of Chinese cooking, but it was not an ancient and deliberate preparation. It developed as a by-product of some *jiang* relishes, and did not become of culinary importance until the Sung dynasty (960–1279) (Sabban 1988).

Although Japanese cuisine was heavily influenced by Chinese cultural interchanges, the preparation of fermented savory sauces made from pickled fish, shellfish, and meat (*hishio*) was probably an earlier, independent Japanese development (Huang 2000). The Taiho Laws of Japan, enforced in AD 701, mention an Imperial court that dealt with several fermented soybean foods including *douchi*, *miso*, and *jiang* or *hishio* (Yokotsuka 1991b). According to the book *Engishiki* (AD 906), 360 mL of *doujiang* and 180 mL of *douchi* constituted a part of the monthly allowance supplied to government officials along with rice, soybeans, and red beans, among others (Yokotsuka 1991b). Salted *douchi* has its origins in central Japan and is known by different names such as *hama-natto* and *daitokuji-natto*; in Taiwan it is known as *in-si* (Yokotsuka 1991b).

Many historians claim that the ethnic, fermented soybean foods of Asia might have originated from *douchi* or *tau-shi*, one of the oldest ethnic, fermented soybean foods of China during the Han dynasty in southern China around 206 BC (Bo 1984b, Zhang and Liu 2000). Production and consumption of *douchi* expanded northward to northern China, westward to eastern Nepal, southward to Indonesia, and eastward to Japan during the Han dynasty (Yoshida 1993). Fermentation of soybeans into various food products might have originated only after eleventh century BC. *Natto*, a fermented, sticky soybean, was introduced to Japan from China by Buddhist priests during the Nara period around AD 710–794 (Ito et al. 1996, Kiuchi 2001).

The production of *shoyu* and *miso* in China was recorded around 1000 BC, with the transfer of the indigenous knowledge to Japan happening at around AD 600 (Yokotsuka 1985). The *kanji* script combination used for the word *shoyu* (soy sauce) is said to have first appeared in Japan during the mid-Muromachi period (AD 1336–1573); however, the method of preparation of *shoyu* using soybeans and wheat was introduced only during the Edo period (AD 1603–1867) (Hamamo 2001). *Tempe* made in present day Indonesia was originally introduced by the ethnic Chinese centuries ago during the rapid expansion of trade and also by the migration of people from mainland China. The earliest record of the word *tempe* can be found in *Serat Centini* (around AD 1815), which indicated that *tempe* had been produced in early seventeenth century in Indonesia (Astuli 1999). *Kinema*, a fermented, sticky soybean prepared by Kirat Nepalis, might have originated in east Nepal around 600 BC–AD 100 during the Kirat dynasty (Tamang 2001b). The word *kinema* was derived from the word *kinamba* of the Limboo language of the Kirat race, *ki* meaning "fermented" and *namba* meaning "flavor" (Tamang 2010).

The plasmid from the *Bacillus subtilis* (*natto*) strain resembles that of *B. subtilis* isolated from *thua-nao* and *kinema* (Hara et al. 1995). On the basis of phylogenetic

analysis, similarity among strains of *B. subtilis* isolated from common sticky, fermented soybean foods of Asia (*kinema*, *natto*, and *chungkokjang*) was observed indicating that *B. subtilis* strains might have originated from a common stock of fermented soybeans (Tamang et al. 2002). If hypothetical lines are drawn on a map, the lines join in the form of a triangle, the lines starting from Japan (*natto*); touching Korea (*chungkokjang*); South China (*douchi*); eastern Nepal, Darjeeling hills, Sikkim, and South Bhutan (*kinema*); Northeast India (*tungrymbai*, *hawaijar*, *bekang*, *aakhone*, *peruyaan*); Myanmar (*pepok*); northern Thailand (*thua-nau*); and Cambodia (*sieng*). Tamang (2010) calls this hypothetical triangle as the "*kinema-natto-thua nao* triangle" (KNT triangle) based on the original concept of the "*natto* triangle" proposed by Nakao (1972). Within the proposed KNT-triangle-bound countries, a variety of *Bacillus*-fermented sticky and nonsalty soybean foods are consumed by the different ethnic tribes in Japan, Korea, south China, Cambodia, Laos, Vietnam, north Thailand, Myanmar, Northeast India and the Darjeeling hills in India, Bhutan, and east Nepal). The proposed KNT triangle does not include nonsticky and non-bacilli fermented soybean products such as *tempe*, *miso*, *sufu*, *shoyu*, etc.

Fermented sticky soybean could also have been an accidental discovery. Cooked soybeans could have been leftover after a meal and the next morning viscous stringy threads that emanated a typical flavor were noticed on the cooked beans. People might have tasted this product and started liking its flavor and sticky texture, and named the product accordingly. Further improvements to the production and culinary practices occurred over a period of time, depending on consumers' preferences. It has been observed that mildly alkaline flavored sticky fermented soybean foods are popular among the Mongolian-origin races. This may be due to development of a typical flavor called *umami* (Kawamura and Kara 1987) during the proteolysis of soybean proteins to amino acids during fermentation, which enriches the sensory property of the product. *Umami*-flavored products are popular with the Mongolian races all over the world. Non-Mongoloid-origin Indians do not prefer soy milk due to its bean flavor and also due to cultural acceptance of consuming animal milk. Due to the practice of using salt and spices while cooking, seasonings like soy sauce and MSG do not find a place in Indian, Pakistani, Sri Lankan, Bengali, and Nepali cuisines.

Fermented soybeans are prepared and consumed exclusively by the Mongolian races mainly in South, North, and East Asia. Though soybean was not grown traditionally in Africa, a similar product called *dawadawa* or *iru* or *sumbara* is prepared from wild locust bean, and is the most important condiment of West and Central Africa. The bean is inedible raw, but provides an important source of protein, especially for poor families who can afford little meat (Odunfa 1988). *Tempe* of Indonesia is now more popular in the Netherlands.

1.3.3 Fermented Cereals

The preeminent staple food of Europe, west Asia, and the Near East is bread; as such it is deeply embedded with social and symbolic activities (Jaine 1999). Bread is an important part of the diet in many other cultures. Although bread can be unleavened—notably documented in the Biblical account of Jews fleeing Egypt (Exodus 13:3–10)—most types of bread are leavened with yeast or lactobacilli (sourdough). In modern times, gluten-rich grains (bread wheat, *Triticum aestivum*; spelt wheat, *Triticum spelta*; and rye, *Secale cereale*) are often considered essential for satisfactory

baking. The first cereal domesticates, barley (*Hordeum vulgare*), einkorn (*T. monococcum*), and especially emmer wheat (*T. dicoccum*), were also used for bread making in earlier times despite their lower gluten content. The mention of bread in the Bible is one indication of the antiquity of baking, but bread making goes much further back in time than the Bronze Age. Bread is mentioned in ration lists and offerings to deities in surviving documents from ancient Mesopotamia and Egypt. There are ample archaeological remains of tools and installations that were used to make bread in these ancient times. The origin of leavened bread is unknown, but is likely to have arisen after the domestication of cereals, about 10,000 years ago, because bread making requires a substantial quantity of grain. Prior to this, people may have made a type of bread from a variety of starch-rich sources, akin to the cycad "loaves" of the Gidjingalis from northern Australia (Jones and Meehan 1989), but this is an unleavened food.

Although many artifacts associated with cereal processing have been recovered from archaeological sites (Curtis 2001), the most direct evidence for ancient baking are preserved bread loaves. Charred ancient loaves or loaf fragments are extremely rare, but have been found in European archaeological deposits. Finds from such diverse times and locations such as Neolithic (ca. fourth millennium BC) Swiss lake villages, Roman Pompeii, and medieval (Viking) Swedish graves are summarized in Samuel (2002), and many other ancient breads are described by Währen (2000). It is likely that some fragmented specimens have been lost because their true nature was unrecognized at the time of excavation. Due to the arid environment of Egypt and the practice of interring food for the dead, there are numerous examples of desiccated ancient Egyptian bread loaves recovered from tombs, and these are now in museums throughout the world (Samuel 2000).

Analysis of ancient bread began early but was sporadic. For example, a Medieval Swedish bread fragment was shown to consist of field pea and Scots Pine (*Pinus sylvestris*) inner bark—a combination not normally associated with bread making (Rosendahl 1912). The use of Scots Pine inner bark as a bread ingredient among the Medieval Vikings was later confirmed by the work of Hansson (1995). More recently, analyses of bread have widened, especially that of the magisterial research undertaken by Währen (2000).

Ancient Egyptian loaves are particularly well-suited to analysis and investigation because of their excellent preservation. As early as 1932, a thorough investigation of an ancient loaf integrated archaeological evidence for baking with modern rural Egyptian baking techniques, Egyptian artistic depictions of bread making, and macro- and microscopic analyses of the loaf itself (Borchardt 1932, Grüss 1932). More recently, Samuel has undertaken an extensive study of ancient Egyptian baking, including analysis using low-power light microscopy (Samuel 2000).

Egyptian loaves range in form from a 9 cm long bread in the shape of a fish, to domed oval loaves over 20 cm in length (Sist 1987, Fig. 61). Some are representational, such as the fish-shaped example, but many are disk shaped, often with a central crater or depression in the center, perhaps designed to hold a moist accompanying food. The surface may be plain, or embellished with indented decorations. In general, Egyptian loaves are milled to a medium consistency, in which grain fragments of between 0.5 and 1.5 mm are the norm. Numerous large fragments of grain are common, but these were probably deliberate additions, much like the modern "granary" or "multigrain" loaves of today with the addition of whole or cracked grains.

The microstructure of these desiccated loaves is remarkably well preserved. Scanning electron microscopy has revealed in some cases the presence of pitted and channeled starch granules, indicating that malt (sprouted grain) was an ingredient of the loaves. The details of leavening are harder to ascertain. Nearly all the analyzed Egyptian breads were made of emmer wheat, which produces a dense crumb. Air pockets within the loaves are small. Detecting yeast cells or possibly lactobacilli cells, which would have been present in low concentrations, is challenging. Nevertheless, yeast cells were detected in a few loaves, showing that these specimens at least were likely to have been leavened (Samuel 2000).

Bread was very important in Roman times. The loaves were usually round and scored into six or eight wedges. Fancy breads were made in pans in the shape of animals such as hares and pigs. There are descriptions of baking in Roman texts, as well as some detailed reliefs on Roman tombs (Curtis 2001). There were several methods of leavening the dough: by saving some dough from the previous day's batch, by using foam from the brewing process, and by making a starter from grape juice and wheat bran. There were 250 bread bakeries operating in Ancient Rome around 100 BC (Pederson 1979).

With the conquest of the Middle-Russian territory by the Mongolians, *kvass*, the bread drink, has become firmly established in Slavonic customs and from the Saint Vladimir, the *kvass* was offered to the poor people of Kiev (Bärwald 1994). Many types of bread, especially in European countries, are still produced by traditional processes where no commercial strains of baker's yeast (*Saccharomyces cerevisiae*) are added (Hammes and Ganzle 1998). *Trahanas*, known as *kapestoes* or *zamplaricos* in Greek and *tarhanocirv* in Turkish, is a fermented food made from crushed wheat and fermented sheep milk, which are boiled together, dried, and stored in the form of biscuits (Economidou 1975). *Trahanas* is a cultural food consumed mainly during the winter and is widely used for feeding weaned infants and young children (Economidou and Steinkraus 1996).

Although not a cereal crop, African cassava is mentioned here because it is a starch-rich food. In the tropics, cassava is the most abundant starch source (Odunfa 1985). It is used to make *gari*, the staple food of many West Africans, but it is not indigenous. Cassava originates from South Africa, and the production of *gari* is thought to have been introduced by freed slaves from Brazil in the nineteenth century. The most important African fermented foods are made from cereals. They are based on sorghum, maize, and millets. Sour porridge made from maize, sorghum, or millet, known as *ogi*, *koko*, or *akasa*, respectively, is one very popular fermented food of West Africa (Odunfa 1985). Other similar porridges are prepared in other African regions (Odunfa 1985, Rombouts and Nout 1995). The extensive steeping and sieving used in the manufacture of *ogi* leads to significant nutrient losses (Odunfa 1985). Nevertheless, phytic acid is removed (Oke 1967) and protein quality is improved (Hamad and Fields 1979). Although traditionally these fermented starch foods have been fed to weaning infants, this use has sometimes been discouraged on the grounds of hygiene. However, studies have shown that traditional fermentation reduces contamination by harmful bacteria and is of considerable benefit to infant health and nutrition (Mensah et al. 1990, Watson et al. 1996, Oyewole 1997).

Jalebi, a fermented cereal-based in pretzel-like product, has been known in northern India since AD 1450 and is probably of Arabic or Persian origin (Gode 1943). Records of the Indian *dosa* and *idli* go back to AD 1100 (Gode 1943). The poet Chavundaraya

of South India described the preparation of *idli* in AD 1025 (Iyengar 1950). In a book, *Manasollasa*, written in Sanskrit about AD 1130, there is a description of *idli* preparation from fine wheat flour and spices such as pepper powder, cumin, and asafetida (Shrigondekar 1939). *Dosa*, a traditional fermented pancake food made from rice and black gram, was first mentioned in Tamil Sangam literature in India about AD sixth century (Srinivasa 1930). *Dhokla*, a fermented mixture of wheat and Bengal gram of western India, was first mentioned in AD 1066 (Prajapati and Nair 2003). *Idli* and *dosa* are the cultural foods of Tamil, Telugu, Malayalam, and Kannada people of Dravidian origin in India, Sri Lanka, Malaysia, Singapore, and many other Asian countries. Besides being consumed for breakfast, these cultural foods are relished in every religious and social occasion by south Indians. Today these foods can be claimed to be the heritage foods of south India. *Dhokla* and *khaman* form a part of the food culture of every Gujarati in India and elsewhere. In the northern part of India too, some fermented cereal foods have ethnic values. *Siddu*, a fermented wheat food of Himachal Pradesh in India, is served hot with *ghee* (clarified butter) or *chutney* (pickle) in rural areas as a special dish on customary occasions (Tamang 2010). *Chilra* or *lwar* is an ethnic, fermented buckwheat or barley food product, which is traditionally prepared during marriage ceremonies and festivals in Himachal Pradesh. *Marchu*, a traditional fermented wheat flour product in the form of a flat bread, is eaten during local festivals (*phagli*, *halda*) and religious and marriage ceremonies in Lahaul, Himachal Pradesh. It is a customary for daughters to eat *marchu* whenever they visit their maternal homes and when they return back to their in-laws' in Himachal Pradesh (Thakur et al. 2004). Celebration of festivals with *selroti*, an ethnic, fermented rice food, is a cultural aspect of Nepalis in the Himalayas (Tamang 2010). *Selroti* is prepared in almost all functions and festivals, particularly on *bhai-tika*, a day to wish and honor the brothers by their sisters for their longevity and prosperity during *tihar* or *diwali* festival. It is customary among Nepalis to hand over a basket full of freshly fried *selroti* to the bride's parents by the groom during marriage. Traditionally, a newly married Nepali bride visits her parent's house once in a year (Yonzan and Tamang 2009). When she returns back to her husband's house she should carry a *thumsey* (local name for bamboo basket) containing freshly fried *selroti* as a gift.

1.3.4 Fermented Milks

Depictions of cow worship and cows being milked are found in rock drawings (9000 BC) discovered in the Libyan desert (Pederson 1979). It is apparent from writings, drawings, and friezes dating back to 3000 BC in Mesopotamia that dairying was highly developed. A sculptured relief, which dates back to 2900–2460 BC found at Teil Ubaid in mid-East Asia, in the territory of ancient Babylonia, shows the development of a system for processing milk (Prajapati and Nair 2003). Production of fermented milks is mentioned in early Sanskrit and Christian works, while recipes of both sweet and savory fermented milks date back to Roman times, around AD 200 (Oberman 1985). Today, in the Near East and North Africa, there are many traditional fermented milk foods that are variations of yoghurt and cheese. Sour milk products of the type consumed in Africa today were mentioned by medieval Arab travelers, but were not always to their taste (Odunfa 1988).

Sudan has a particularly rich tradition of fermented dairy products (Dirar 1993). The most widespread is *rob*, usually made from cow's milk but also from goat's

and sheep's milk. *Gariss* is made by pouring fresh camel's milk into skin bags, attaching the bags to saddles on the camels, and allowing the jerking motion of the camel's gait to constantly agitate the liquid. The resulting fermented liquid is consumed exclusively by men. *Biruni* and *mish*, two fermented milk products, are semiliquid foods. *Mish* takes about 1 month to mature, whereas *biruni* is generally consumed after 3–4 years. Whether the Sudanese *mish* has any connection to the Egyptian *mish* is uncertain. The Egyptian soft pickled cheese *mish*, which has been claimed to have originated with the ancient Egyptians, is usually prepared with any of a range of added ingredients, such as chilli peppers, black pepper, caraway, and fennel (Dagher 1991).

In Hinduism, the cow is regarded as a sacred animal, and its milk and milk products are used in every religious and cultural function. The development of the dairy system in ancient India has been mentioned in some of the historical records. Cows and the importance of milk products are referred in the Rig Veda, the oldest sacred book of the Hindus (Prajapati and Nair 2003). The Vedas and the Upanishads mention the origin of *dahi*, one of the oldest fermented milk products of the Hindus, and fermented milk products during 6000–4000 BC (Yegna Narayan Aiyar 1953).

The preparation and consumption of *dahi* has been recorded since 2000 BC in India (Prakash 1961). It is well known in ancient Indian history that *dahi*, buttermilk, and ghee (clarified butter) were widely consumed milk products during Lord Krishna's time around 3000 BC (Prajapati and Nair 2003). *Dahi* plays an important part in the socio-religious practices of the Indian subcontinent and is considered as a sacred item in many festivals and religious ceremonies both by Hindus and Buddhists. Many Indian ethnic, fermented milk products have cultural aspects in the dietary system, and have been consumed for more than 3000 years. *Lassi*, buttermilk, is a by-product obtained during the preparation of clarified butter (*ghee*) from *dahi* by traditional methods, and is the most common nonalcoholic refreshing beverage for the hot climates in India. *Misti dahi* (sweetened *dahi*, *mishti doi*, *lal dahi*, or *payodhi*) is a sweetened fermented milk product prepared by Bengalis in India and Bangladesh. *Shrikhand* is an ethnic, concentrated sweetened fermented milk product made by Gujaratis and Rajasthanis in India. *Rabadi*, an ethnic, fermented milk-based, thick slurry-like product is prepared by fermenting cereals and pulses including wheat, barley, maize, and pearl millet in the north and western parts of India.

Cattle (cow) rearing was one of the important pastoral systems during the Gopala dynasty in Nepal, one of the earliest kingdoms in ancient Nepal in 900–700 BC (Adhikari and Ghimirey 2000). However, the history of ancient Nepal records the main pastoral system during the Mahishapala dynasty (700–625 BC) in Nepal was buffalo rearing instead of cow rearing (Bista 1967). In modern Nepal, both cow and buffalo rearing is common practice among farmers. The Tibetans in high altitudes have been using yak milk and its different fermented products such as *chhurpi*, *chhu*, *philu*, etc., unlike the Chinese who do not traditionally consume milk and milk products. *Shyow* (curd) is served exclusively during the *shoton* festival of Tibetans.

For both Hindus and Buddhists, *gheu* or butter is a sacred item in all their religious ceremonies and is used in ceremonies associated with birth, marriage, death, as well as in other prayers as sacred offerings. *Gheu* is given along with honey to a new born baby by the father to protect from diseases and is a tradition among the Hindus. *Gheu* is also used for lighting the lamps of gods and goddesses in Hindu temples and Buddhist monasteries. *Somar*, an ethnic, fermented milk product made from cow or

yak milk, is generally consumed by the Sherpas of the highlands in the Himalayas to increase appetite and to cure digestive problems (Tamang 2005).

Historical evidence proves that the present day fermented milk products were originally developed by the west Asian nomads who used to rear and breed cattle for milk and milk products. In the AD sixth century text *Chii Min Yao Shu*, the author details methods for making a yoghurt-like product from cow's and sheep's milk in China (Sabban 1986, Huang 2000). Fermented alcoholic drinks made from mare's milk were also known in medieval China. This indicates that dairy products were once a part of the Chinese diet, at least for the northern part of the country, no doubt influenced by the pastoralist traditions of the north. Traditionally, milk is not consumed in China, Mongolia, Korea, and Japan. The Aryan–Hindu pastoral system has influenced the preparation and consumption of milk and dairy products in the early settlements in the Himalayas. The milk and milk products of the Himalayas might have originated from the main Indian Hindu culture.

It is believed that the ancient Turkish people in Asia, who lived as nomads, were the first to make yoghurt and named it as "yoghurut" (Rasic and Kurmann 1978); however, according to Chomakow (1973) yoghurt originated from the Balkans. The inhabitants of Thrace used to make soured milks called *prokish* from sheep's milk, which later became yoghurt (Chomakow 1973).

There is a mention of the importance of milk and cheese in the dietary cultures of Greece (1500 BC) and Rome (750 BC) (Scott 1986). Impressions of baskets found at Windmill Hill in Dorset, England (1800 BC), indicate that cheese was made in England well before the arrival of the Romans (Scott 1986). It is reported that the first cooperative cheese factory was started at Voralberg in the Balkans around AD 1380 (Scott 1986). Cheddar cheese originated from Cheddar village in Somerset, England, and was popular during the reign of Queen Elizabeth I (AD 1558–1603) (Prajapati and Nair 2003). In 1851, the first cheese factory was established in Oneida County, New York, and it proved so successful that within a few years several other factories were established (Prajapati and Nair 2003).

The ancient Turks, who were Buddhists, used to offer yoghurt to the angels and stars that protected them (Rasic and Kurmann 1978). Cheese was offered to gods by the ancient Greeks at Mount Olympus (Scott 1986). Bulgarian or bulgaricus buttermilk, sour milk prepared from boiled goat's or cow's milk inoculated with a portion of previously fermented milk, might have originated from the Trak's tradition, that is, from the tradition of the sheep breeders who came to Asia from Bulgaria in the fifteenth century (Oberman 1985). *Kishk* is a fermented milk–wheat mixture stored in the form of dried balls in Egypt (Abd-ed-Malek and Demerdash 1977), and is popular among the rural populations of Egypt, Syria, Lebanon, Jordan, Iraq, and North Africa (Basson 1981). *Kishk* is a cultural as well as a traditional functional food with excellent keeping quality, richer in B vitamins than either wheat or milk, well adapted to hot climates because of its lactic acid content, and has a therapeutic value (Morcos et al. 1973a).

1.3.5 Fermented Fish

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 BC–AD 200) learned of it when they expanded 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 (Ruddle 1986). Southeast Asia has a wide variety of ethnic, fermented fish products that are deeply associated with the food culture of ethnic Asians (Ishige 1986a). In this region, the coastal deltas have been settled relatively recently. In earlier times, the settlement was limited to those areas most suited for the cultivation of irrigated rice, and as a consequence freshwater fish species were those mainly used for fermentation. Freshwater fermented fish products are best developed in the area from the west of the Annamite Mountains to Lower Myanmar, where the main populations are Thai–Lao, Burmese, and Khmer (Ishige 1986a). Thus, it is unlikely that the tradition of preparing fermented fish products existed among those ethnic people prior to the time of their southward migration. The Thai–Laos originated in the Yunnan province of China, where the only reports on fermented fish products concern *narezushi* (Ishige 1986b). There are no Chinese historical documents related to the preparation of fermented fish products among the ethnic groups who lived south of the Yangzte River. Many of these people were Thai–Laos. There is a likelihood that these people adopted the use of fermented fish products from the earlier settlers after they entered the Indo-Chinese peninsula, and that fermented fish products did not originate in China (Ishige 1993).

Preparation of fermented fish might have originated by accident when a batch of old or improperly prepared salted fish fermented, and the resulting *umami* taste was found acceptable (Mizutani et al. 1988). Origins might have taken place independently in many different locations, but they would have only developed and been deliberately elaborated where the taste was culturally acceptable and where the products were found complementary to the established cuisine (Mizutani et al. 1992). When cooked rice is added to the fish and salt mixture, the product is called *narezushi* and if no vegetables are added, the salt–fish mixture yields fish sauce, which is commonly used as a condiment, and if the product of fish and salt preserves the whole or partial shape of the original fish, it is called *shiokara*, which is then made into a paste (Ishige and Ruddle 1987). There is, however, philological evidence that *narezushi* was known in the area, and the Han Chinese (200 BC–AD 200) learned of it when they expanded south of the Yangtze (Ishige 1986b). It appears that production of *narezushi* was associated with rice cultivation of Southeast Asia, and later on *narezushi* was taken from there to China. *Narezushi* remains common in the Mekong basin areas of Laos, Cambodia, and Thailand. Ishige (1993) and Ruddle (1993) advocated that the Mekong and associated basins of southwest China, Laos, and northern and northwest Thailand were the most probable place of origin of fermented fish products. Fermented fish products are prepared from freshwater and marine finfish, shellfish, and crustaceans that are processed with salt to cause fermentation and thereby to prevent putrefaction (Ishige 1993). *Shiokara* paste is known by many names in Southeast Asia; in Myanmar it is known as *nga-pi*, in Cambodia as *pra-hoc*. *Shiokara* is used mostly as a side dish, and is important in the diet of the people of Cambodia; Laos; North and Northeast Thailand; lower Myanmar; Luzon and the Visayas in the Philippines; and Korea. Squid *skiokara* is the most popular fermented sea food in Japan (Fujii et al. 1999).

Fish sauce was made by the ancient Greeks, but we know little about the method by which it was prepared. They distinguished two types: *garum* and *muria*. The Romans had four different products: as well as the Greek forms, they distinguished *liquamen* and *allec*. These may have been differentiated by the type of fish used, the parts of

the fish used, and whether the resulting product was a solid or liquid. It is not until the Roman period that documentary sources record how fish sauces were made. The methods may have been learned from the Carthaginians or the Greeks, and some types of fish processing may have been indigenous to southern Italy by the fourth century BC (Curtis 2001). Fish sauces were ubiquitous condiments for the Romans (Badham 1854, Wilkins and Hill 2006).

There are several literary records describing preparation, which seems to have remained more or less unchanged through antiquity (Curtis 2001). The basic procedure was to salt the fish, pack it into a vessel, and leave it in the sun for up to 3 months. It was stirred or shaken during this time, and at the end of the sitting period, the solids were separated from the liquid. If the sauce were required quickly, a brine solution was prepared. The method of gauging the salt concentration by floating an egg, as is still done today for some vegetable fermentations (see above), is recorded in ancient sources. The fish was placed in the brine and boiled. When the mixture cooled, it was sieved. The sauce could be varied through the addition of wine, herbs, and spices (Curtis 2001).

Today, Southeast and East Asia are the main regions of production of fermented fish products; however, these are of very minor importance outside Asia. Fish sauces called *botargue*, *ootarides*, and *aimeteon* were produced in Italy and Greece in the nineteenth century (Beddows 1985). Norwegian *gravlaks*, or buried salmon, is a traditional, relatively mild tasting product; more heavily fermented products are *rakefisk* or *surfisk*, the most popular varieties of which are *rakörret*, fermented trout, in Norway; and *surströmming*, made from herring in Sweden (Riddervold 1990, Kobayashi et al. 2000). A limited number of fermented fish products are prepared and consumed in the Near East. The most widespread product is *feseekh*, made by salting partially fermented mullet or other fish (Dagher 1991). The same product is made in Sudan and is thought to be a recent introduction (Dirar 1993). A little-known fermented fish paste, which is considered indigenous to Sudan, is *terkin*, prepared by salting the whole fish, curing it for about 2 weeks, and then placing the container on hot sand in full sun for a few days in summer, and longer in winter. This procedure is like that used by the ancient Romans for *garum*. Perhaps the shorter exposure stage is due to the much higher summer temperatures in Sudan compared to the Mediterranean. Before eating, it is diluted and strained to remove the bones and scales, and then other ingredients such as raw or fried chopped onions are mixed (Dirar 1993). A few fermented fish foods are produced on a small scale in West Africa (Odunfa 1985).

Lactic fermented fish products are often associated with inland areas such as the Central Luzon region of the Philippines and Northeast Thailand where the freshwater fish are the usual raw material and their microorganisms tend to reflect the local environment more than that of marine species (Adams 1998). In Northeast Thailand, it was found that several different sources were used for fermenting fish—the flooded rice fields, paddy ponds beside rice fields used for collecting fish when the field has dried up, and large local freshwater reservoirs (Dhanmitta et al. 1989). Culturally, in hot countries, fermented fish products continue to play a vital role in adding protein, flavor, and variety to rice-based diets (Campbell-Platt 1987).

Fish is popular in places nearby rivers and their tributaries, lakes, and ponds in the Himalayas (Tamang 2010). Nonconsumption of fish products by the Tibetans may be due to religious taboo as fish is worshiped for longevity, and Buddhists strongly believe that releasing captured fish into the rivers may prolong their lives. Moreover, lakes are regarded as sacred by the Buddhists in the Himalayas, barring them from

angling in the lakes. Another reason for not consuming fish may be due to the preference for animal meats and dairy products, and the difficulty in picking out the small bones and scales of fish. Unlike Chinese-type fermented fish, the Himalayan fish products are slightly different and are mostly dominated by dried and smoking processes. Fermentation fish products include *ngari* and *hentak* in Manipur and *tungtap* in Meghalaya in India; the rest of the fish products are dried or smoked that include *sidra*, *sukuti*, and *gnuchi* in Nepal, Darjeeling hills, and Sikkim, and *karati*, *lashim*, and *bordia* in Assam, India (Thapa et al. 2004, 2006, 2007). Fermented fish foods are associated with the food culture of the Meiteis in Manipur; the products are prepared and eaten on every festival and religious occasion. Consumption of fish products in the Himalayas, though constituting an important part of the diet, is comparatively less than other fermented products such as vegetables and dairy products. This may be due to the adoption of the pastoral system of agriculture and the consumption of dairy products in these regions.

1.3.6 Fermented Meats

Sausage was prepared and consumed by ancient Babylonians as early as 1500 BC and by the ancient Chinese (Pederson 1979). Southern and Central Europe, dating back to Roman times, is the original home of many cured and fermented meat products made from pork and beef (Pederson 1979). The recorded history of sausage manufacture begins in the ninth century BC as established by statements in Homer's *Odyssey* (Prajapati and Nair 2003). Grecian literature after Homer's time makes frequent mention of sausage. The name *salami* is believed to have originated from the city of Salamis located on the east coast of Cyprus, which was destroyed in 449 BC (Lücke 1985).

Much later, European emigrants carried the knowledge of meat processing and introduced it to North America and Australia (Campbell-Platt 1987). These regions now share a range of whole-meat bacon, to be cooked before eating, ready-to-eat country ham, and chopped semidry *cerevalat*, dry German salami and pepperoni, which are smoked, and dry Italian *salame* and *chorizo*. Cooked fermented meat products such as *mortadello*, *kochsalami*, and *thüringer* are less common (Campbell-Platt and Cook 1995). Manufacturing of fermented smoked sausages was recorded in Germany only 150 years ago while air-dried spicy sausages are predominant in Mediterranean countries, France, Hungary, and the Balkan countries (Lücke 2003). Sudan has over 10 different fermented meat products made from all the tissues of the animal carcass. Dirar (1993) provides details of all these foods, which are variously fermented during drying, or wet fermented and dried, or wet fermented and stored wet.

In Asia, fermentation, smoking, and drying of animals meats are restricted to a few countries. Some common fermented meat products of Thailand are *nham* (fermented beef or pork sausage), *naang* (fermented pork or beef), *nang-khem* (fermented buffalo skin), and *sai-krork-prieo* (fermented sausage) (Phithakpol et al. 1995, Visessanguan et al. 2006), and *lup cheong* of China (Leistner 1995).

A sizable number of people in the Himalayas are meat eaters; however, regular consumption of meat is expensive for a majority of the poor people. People slaughter domestic animals (goats, pigs, cow, yaks, and sheep) usually on special occasions like festivals and marriages. During festivals, goats are ritually sacrificed after the ceremony; the fresh meat is cooked and eaten as part of a family feast; the remaining

meat is smoked above an earthen oven to make *suka ko masu* for future consumption. Tibetans, Bhutias, Drukpas, and Lepchas slaughter yaks occasionally and consume the fresh meat, and the remaining flesh of the meat is smoked or preserved in open air called, the product being called *satchu*. The making of *kargyong*, ethnic, fermented sausages made from minced yak meats where the intestines are used as a natural casing, might have started long time before Buddhism was embraced by the Tibetans probably 3000 years (Tamang 2010). The ethnic people of the Western Himalayas in Uttarakhand, Himachal Pradesh, and Ladakh prepare *chartayshya*, a fermented meat product, especially during festivals and offer it to ancestors before eating (Rai et al. 2009).

1.3.7 Fermented Beverages and Alcoholic Drinks

More than any other fermented foodstuff, fermented beverages have served to delineate social relations between family and group members as well as among the elite and commoners, and to express a relationship between humans and deities (Dietler 2006). Here we provide a brief overview of some of these beverages and their role in ancient and traditional societies, as well as an indication of how such drinks were made.

Despite some suggestions to the contrary (e.g., Johnson 1989), reliable archaeological evidence of grape domestication dates to the late Chalcolithic and early Bronze Age in a region known as the Levant, that is, the eastern Mediterranean coastal region and adjacent inland areas (Zohary and Hopf 2000). Ancient domesticated grape remains are notoriously difficult to distinguish from wild remains, and where wild grapes are indigenous, it is impossible to be certain which is which. This is unfortunate, as the distribution of wild grapes is broad (Zohary and Hopf 2000), and encompasses many of the regions where ancient civilizations developed and flourished or with which they had close contacts. Because grape fermentation is a spontaneous, natural process, the origins of wine making are equally impossible to ascertain. There are about 60 species of grapes (*Vitis*), and many produce fruits which can be made into wine. It was the wild species *Vitis sylvestris* that gave rise to the cultivated form, *Vitis vinifera*.

Notwithstanding this, each of the great wine-loving cultures has its own myth about the origins of wine. Archaeological finds and chemical analyses of residues recovered from the Neolithic (sixth millennium BC) Hajji Firuz Tepe, and Early Bronze Age (fourth millennium BC) Godin Tepe, both in western Iran, have been commonly reported to represent the earliest evidence of wine making (e.g., Renfrew 1999, Wilson 1999, Curtis 2001). The chemical analyses used to reach these conclusions, however, have not yet been sufficiently robust to show convincingly that these finds were linked to wine making (Boulton and Heron 2000). Furthermore, occasional archaeological finds of grape pips, stems, and skins do not necessarily equate to wine making, since other products such as fruit syrup and fruit leathers involve grape pressing. Particularly in the earliest times, this type of preparation would have preserved the fruit for longer and have provided a rare but rich source of sugars. These would have been important considerations for managing food supplies. The early archaeological evidence for wine making must, therefore, be treated with some caution.

Documented evidence of wines in Mesopotamia appeared by the mid-third millennium BC, but was rare before the first millennium BC (Curtis 2001). There are also some

artistic depictions from earlier periods that appear to show wine drinking. The region is unsuitable for viticulture, and wine supplied to the south came from the north and northwest. In central Anatolia, there are records of grape cultivation dating to the early second millennium. Later Hittite records show that wine was a very important commodity for the palace and was mostly mentioned in religious contexts. Here, in common with most regions prior to the Classical period, wine was a beverage of the elite. Some large Iron Age wine making installations have been excavated in the Levant (Curtis 2001).

Wine was a component of ancient medicines in the Near East, but its efficacy for specific complaints was doubtful. The oldest known medical handbook, a Sumerian pharmacopoeia written on a clay tablet dated approximately to 2200–2100 BC and excavated at Nippur in AD 1910, recommended the use of wine with various drugs as treatments for various ailments, such as sweet wine with honey to treat a cough (Bang 1973). *Tabatu* was a Babylonian medical drink made from water and small amounts of fermented fruit juice or wine (Norrie 2003). Wine was also a common vehicle for medicinal preparations in ancient Egypt, although there is no evidence that it was used other than to provide a pleasant taste to unpleasant flavors from other ingredients, or to act as a mild intoxicant to alleviate discomfort (Nunn 1996).

The earliest evidence for the grape in Egypt is seeds in jars imported from the Levant, dating to about 3150 BC (Murray 2000a). That wine was possibly produced in Egypt itself by 3000 BC is suggested by the depiction of possible wine presses on seals. The main source of evidence for wine making thereafter comes mainly from funerary practices: wine jars placed in tombs, artistic depictions on tomb walls, and offering lists for the dead. Wine was drunk by the elite throughout Pharaonic times; the drink of the commoner was beer.

On the basis of the artistic evidence, the broad outline of wine making seems fairly clear. Harvested grapes were carried from the vineyard in baskets and emptied into large vats. The grapes were trodden by foot so as not to crush the seeds and stems, and thus release unwanted tannins and other undesirable compounds. The juice was then drained off and collected. The crushed mass of fruit, stem, and seeds remaining in the vats were wrapped in cloth or sacks and twisted until no further juice could be extracted. Fermentation would have been easy to initiate, as yeasts naturally present on the grape skins would immediately come in contact with the sugar-rich grape juice. When the primary fermentation was complete, the wine was decanted into jars. The jars would have been sealed within a few days to prevent the wine turning into vinegar. There is some archaeological evidence for the release of gas from the sealed jars, but not all jars and stoppers show a release mechanism (Murray 2000a). The types of wine were mainly distinguished by their place of production, and it is hard to know exactly how they may have differed in terms of color, flavor, and strength (Curtis 2001).

The culture of wine drinking according to particular customs was a key part of the Classical Greek identity (Curtis 2001). Unlike the drinking culture of Egypt, the Near East, Anatolia, and indeed in earlier Bronze Age Greece, wine was considered as a basic food rather than a drink solely reserved for the elite. It was drunk diluted with water, another way in which wine drinking in Greece differed from that of surrounding cultures (Wilkins and Hill 2006). A proportion of small-holding Greek farmers specialized in cultivating grapes (Curtis 2001). Wine was an important trading commodity and was therefore a major part of the Greek economy. Many different types were distinguished. It also was vital for religious ceremonies, was used for medical purposes, and played a central role in the male drinking party called the "symposium."

In Greece, wine was drunk after dinner at a symposium, with men reclining on couches, propped up on an elbow, sipping wine from a shallow two-handled cup (Johnson 1989). Women, if present, were of low status or were prostitutes, and sat on the edge of a couch or on a chair. The symposium had a chairman whose job it was to stimulate conversation. Sometimes those present at a symposium were entertained by girls playing flutes or harps, or dancing. A light-hearted drinking game called *kattabos* was developed about 600 BC (Hyams 1987) and was extremely popular for about 300 years (Renfrew 2003). Drinkers at a symposium competed with each other to extinguish a lamp on the top of a tall stand by throwing the dregs of their wine cups at it.

From earlier Minoan and Mycenean Bronze Age periods, the main evidence for wine comes from the presence of specific drinking cups and pottery jars called "amphoras" (Wilkins and Hill 2006), as well as the remains of wine presses (Zohary and Hopf 2000). There is little literary or archaeological evidence for wine making from Classical times, but there is a rich artistic record particularly from very finely made Attic (Athenian) pottery jars and bowls, painted in a distinctive black and red style. These date between 540 and 430 BC (Curtis 2001). They include many representations of the grape vine, drinking, and drunkenness (Wilkins and Hill 2006).

Viticulture probably spread into the Western Mediterranean with the Phoenicians and certainly spread along with the Greeks as they founded colonies along the Mediterranean coast (Wilson 1999, Curtis 2001). Viticulture subsequently spread through much of Europe from southern France and, in due course, from Rome under the influence and spread of Roman culture (Wilson 1999). Once introduced into Rome, wine became as popular as it was in Greece. It was similarly used in all aspects of life, for pleasure, for medicine, and for religious purposes (Curtis 2001). Like the ancient Egyptians, the grapes destined for wine making were trodden in vats. The pressing stage was more sophisticated: squashed grapes were pressed with a mechanical press in a basket. The selected juices were set aside to ferment, during which time various additives might be added such as calcitic material in the form of chalk or marble dusk for deacidification, and herbs or resins to create specialty wines or mask poor quality ones. Once fermented, the wine was separated from the settled solids and transferred to amphoras for aging or transportation.

Alcoholic drinks have continued to be widely consumed in India since pre-Vedic times, and specific reference to their consumption among the tribal people was mentioned in the *Ramayana* (300–75 BC) (Prakash 1961). During the Vedic period of Indian history (2500–200 BC), based originally around the Indus River system, wine was worshipped as the liquid god *Soma* because of its medicinal attributes (Bose 1922). In the Vedas, *Soma* was credited with great medicinal powers (Sarma 1939). *Soma* is originally thought to have been the fermented juice of an East Indian leafless vine (*Sarcostemma acidum*) and other wild indigenous grapevines (Sarma 1939). *Vitis vinifera* was introduced into China by Chang Ch'ien during the second century BC after he had learnt winemaking in Persia (Ackerman 1945). Wine making eventually came to Northern India and China in 1 BC (Robinson 1999b, Pretorius 2000).

The other main alcoholic drink of ancient times was beer. The evidence for ancient Mesopotamian beer brewing is based on documentary sources rather than clear archaeological evidence. As a result, it is somewhat controversial, as scholars disagree about the interpretation of various terms used in the ancient written records (Curtis 2001). Many have used traditional ideas on ancient Egyptian brewing as comparanda

for ancient Mesopotamian practice. However, Egyptologists interested in brewing have referred to Mesopotamian procedures, so the arguments may be dangerously circular. A general summary of the current broad consensus is provided by Curtis (2001). In this interpretation, early Mesopotamian beer was based on barley malt, that is, sprouted and dried grain. The malt may have been crushed, mixed with flavoring ingredients such as dates, honey, and herbs, formed into dough with the addition of water, and then baked into a type of bread called *bappir.* This product may not have been a bread as recipes seem to describe it as a measurable loose product. Whatever its nature, *bappir* was mixed with green malt and hulled barley together with water to produce a liquid that was gently heated and stirred. The mixture was cooled and then fermented, before being decanted into storage or transport jars.

For many years, Egyptologists relied on the rich artistic records from Egyptian tombs, textual evidence including a late Roman account by Zozimos of Panopolis (see below), modern-day traditional *bouza* making based on bread, and the interpretation of ancient Mesopotamian beer to interpret Pharaonic Egyptian brewing. The interpretations published in the literature are inconsistent on many points. Broadly speaking, the Egyptians were thought to have crushed and pounded grain, most often barley, and mixed the resulting coarse grouts with yeast and water to form dough. The dough was formed into loaves and lightly baked. These "beer loaves" were then broken up and washed through a sieve with water, where the mixture was left to ferment. Dates are often said to have been added, but there is disagreement on the stage at which this occurred. Once the beer was ready, it was decanted into pottery vessels for transport or storage (Samuel 2000).

More recently, research has focused on the analysis of ancient beer residues, together with a comparison of preserved bread loaves using scanning electron microscopy (Samuel 1996, 2000). This is possible because the excellent arid conditions in Egypt have preserved the microstructure of organic remains as well as the macrostructure. The microstructure of beer residues showed extensively gelatinized starch granules, in which undistorted granules were embedded, showing the typical pitting and channeling caused by amylase enzyme breakdown. The pattern of starch microstructure is incompatible with the use of bread or lightly baked bread in the brewing process. Some but not all of the residues contained yeast cells and probably lactobacilli. No tissues derived from dates were detected, nor are date stones common in Pharaonic Egyptian sites (Murray 2000b).

This rich archaeological evidence has led to a new interpretation of ancient Egyptian brewing. The evidence of residues points to a two-part process. One batch of cereal grain may or may not have been malted. It was coarsely ground without prior removal of the chaff, and then heated well or boiled in water, perhaps to the consistency of a thick porridge. The second cereal batch was malted and gently dried before being partly ground and mixed with cool water. The hot "porridge" and cool paste were mixed, and the gelatinized starch from the cooked grain would have rapidly been broken down by the enzymes present in the malt. The whole starch remaining in the unheated malt would have been broken down more slowly. Overall, this would have been a satisfactory system to produce large quantities of sugar for fermentation, without the need to control temperatures and moisture content too closely (Samuel 2000). The source of fermentation is uncertain. As with ancient Mesopotamian beer, fermentation may have been spontaneous, but a more controlled process would have been achieved by inoculation with microorganisms in the brewing vat, or using a

starter from a previous batch (Hartman and Oppenheim 1950). This proposed two-part process is very similar to some of the traditional African beers brewed today (e.g., Rooney et al. 1986). As with traditional African beers (Dirar 1993), it is likely that elaborations and variations on this basic procedure were used, depending on individual brewers and regional practices.

Although the Classical Greeks considered beer unfit for drinking, there is some evidence to show that beer drinking did take place in peripheral parts of Greece such as the North and Crete (Wilkins and Hill 2006). Beer was prepared and drunk in some regions of the Italian peninsula in early Roman times. Wine was always favored by the Romans but by the time of the Roman Empire cultural distinctions were less clear-cut than in Greek times, because the population included Europeans, Egyptians, and Babylonians, where the practice of beer drinking was deep-rooted. In ancient Rome, the habit of beer drinking was sometimes sneered upon as a "barbarian" practice, but sometimes also equated with a "purity" or "simplicity" of culture remote from the sophisticated but corrupt center of the empire (Wilkins and Hill 2006).

There is little documentary evidence for how beer was made during Roman times. The best known and most detailed text was written in the late third or early AD fourth century, by an Egyptian called Zozimos of Panopolis (Helck 1971). His recipe involved partially baked yeasted bread loaves made from incompletely malted grain, which were soaked in water and left to ferment. Whether this account is applicable to all regional brewing practices during the Roman Empire is doubtful.

After the collapse of the Roman Empire in the early AD fifth century, brewing was carried out on a small domestic scale in northern Europe, probably much as had been done in the preceding centuries. Monasteries began to emerge as relatively sizable regional institutions in the eighth and ninth centuries AD. The rise of the monasteries was the catalyst for the development of European beer, eventually into the beverage brewed today. Monastic beer improved and evolved through the use of new and better equipment, the application of new techniques, and the work of skilled specialist artisans (Unger 2004). Archaeobotanical evidence from northern Germany and Scandinavia shows that hops and sweet gale (*Myrica gale*) became important beer flavorings in early Medieval times (AD ninth to tenth century). Sweet gale was once more common than hops as a beer additive in this region, but by the thirteenth century, hops began to become dominant, until sweet gale was outlawed in the eighteenth century (Behre 1984).

A northern European alcoholic drink which is now rare is mead, made from fermented honey. The identification of large quantities of *Tilia* (lime tree) pollen together with pollen from meadowsweet in a deposit within a Scottish Bronze Age burial has led to the suggestion that it came from ancient mead (Dickson 1978). The drink was popular in early medieval northern Europe but its consumption seems to have declined over time and was largely replaced by beer. It was still valued for medicinal purposes in later times (Unger 2004). In Britain, mead was associated with medieval monasteries, as bees were reared for candle wax and the excess honey was used for the drink. Mead virtually disappeared at the time of the dissolution of the monasteries (1536–1540) at the command of Henry VIII (Robinson 1999a).

In Asia, the malting process is rarely used in traditional fermentation processes, instead amylolytic starters prepared from the growth of molds and yeasts on raw or cooked cereals are more commonly used (Haard et al. 1999). The use of mixed starters, common to the Himalayas and Southeast Asia, might have its origins during the time of Euchok, the daughter of the legendary king of Woo of China, known as

the Goddess of Rice-Wine in Chinese culture, about 4000 BC (Lee 1984). The first documentation of *chu*, the Chinese starter, similar to the *marcha* of the Himalayas (Tamang 2010), was found in the Shu-Ching document written during the Chou dynasty (1121–256 BC), in which it is stated that *chu* is essential for making alcoholic beverages (Haard et al. 1999). According to the text *Chhi Min Yao Shu*, written by Chia Ssu-Hsieh of the Late Wei kingdom between AD 533 and 544, many methods of preparation of *chu* were described (Yoon 1993, Huang 2000). The use of *chu* for rice-wine production was common in spring and fall and in the Warrior Periods of China during sixth to seventh centuries BC and the beginning of the Three Nations' Periods in Korea during first century BC to AD second century (Lee 1995). It might have transferred from Korea to Japan in the AD third century according to Kojiki, or Chin, whose memorial document is kept in a shrine at Matsuo or Matsunoo Taisha, Kyoto, Japan (Lee 1995).

The process of cereal alcohol fermentation using mold starters was well established by 1000 BC, and 43 different types of cereal wines and beers were described with detailed processing procedures in *Chhi Min Yao Shu* (Haard et al. 1999). According to this document, *chu* was prepared from barley, rice, and wheat (Yoon 1993). Ten different types of *chu* were described in *Chhi Min Yao Shu* (Yoon 1993, Huang 2000), all of which were used for the fermentation of alcoholic beverages in China. Cake type *ping-chu* is identical to *nuruk* prepared in Korea, and granular type *san-chu* is similar to Japanese *koji* (Yoon 1993). According to Yokotsuka (1985), *chu* may either be yellow (*huang*), possibly due to *Aspergillus oryzae*, or white probably due to *Rhizopus* and *Mucor. Nu-chu* is prepared from cooked rice, which is shaped into a cake and then cultured with molds (Yokotsuka 1985). Wheat *chu* originated in Northern China and the Korean peninsula, while rice *chu* originated in South China (Haard et al. 1999). The word *ragi* was first noted on an ancient inscription called the Kembang Arum, near Yogyakarta in Java, Indonesia, around AD 903 (Astuli 1999).

There is a historical document on the consumption of alcoholic beverages with wine, rice, and other food items including fermented soybean, dried meat, and fish sauces during a wedding ceremony in the royal family of the Sill kingdom of Korea in AD 683 (Lee 1984). The ancient Japanese history book, *Kojiki*, mentions that a man from Baekje taught them how to make an alcoholic drink from rice, *saké*. According to old records from Japan, virgin women chewed cooked rice until it was sweet so it could be fermented into *saké* (Yokotsuka 1991a). The memorial tablet of a man called Chin of Sila is kept in a shrine Matsuo or Matsunoo Taisha in Kyoto (Japan) which is regarded as the God of alcoholic beverage (Lee 1995). The first author (Tamang) personally visited Matsuo/Matsunoo Taishi shrine at Kyoto and observed these historical documents. Today *saké* producers in Japan attend an annual worship ceremony for him, in order to pray for success in their brewing factories, which has become a ritual in Japanese food culture.

Jaanr was mentioned in the history of Nepal during the Kirat dynasty in 625 BC–AD 100 (Adhikari and Ghimirey 2000). The Newar community of Nepalis used to ferment alcoholic beverages from rice during the Malla dynasty in AD 880 (Khatri 1987). There are brief descriptions of ethnic alcoholic drinks in historical documents during British rule in Darjeeling hills, and the then Sikkim kingdom in India (Hooker 1854, Risley 1928, Gorer 1938). The mixed starter cultures of the Himalayas, *marcha* or *phab*, might have originated from south China during the migration of Mongoloid races to the Himalayas (Tamang 2010). The *nu-chu*- and *marcha*-making processes

are very identical, and, moreover, mycelial molds consisting of mucorales are dominant in *marcha* along with alcohol-producing yeasts.

In Asia, the production technique of ethnic starter cultures to make alcoholic beverages is usually kept secret and the indigenous knowledge of processing is not easily passed on. However, the protected hereditary right of making ethnic mixed starters is passed to daughters by mothers, and they carry the indigenous knowledge to their in-laws' house after marriage. Traditionally, preparation of ethnic mixed starters is done exclusively by women; *marcha* is prepared by the Limboo and Rai castes of Nepal, *ragi* by Indonesians, *loogpang* by ethnic Thais, *nuruk* by ethnic Koreans, and *bubod* by Filipinos. Marital status is a strong determinant in the preparation of *marcha* by the Rai castes of Nepal who allow only widows or spinsters to make *marcha* (Tamang et al. 1996). *Marcha* and *ragi* producers believe that addition of wild herbs gives more sweetness to the product, and they also believe that adding chillies and ginger get rid of devils that may spoil the product during preparation. This is actually to check the growth of undesirable microorganisms that may inhibit the growth of native microorganisms of ethnic starters (Soedarsono 1972), and the addition of sweet herbs is to supplement the carbon source for growing organisms in *marcha*. It is interesting to observe that the Asians developed the technology of culturing essential microorganisms (mycelial fungi, amylolytic alcohol-producing yeasts, and lactic acid bacteria) for the production of alcoholic beverages and drinks that were adapted to coexist in a dry rice base supplemented with carbon-rich herbs as ethnic starters in contrast to the monoculture starters of the Europeans.

Pulque is a fermented beverage prepared from *Agave* juice, and is the national drink of Mexico where it was inherited from the Aztecs (Goncalves de Lima 1975). At the time of European contact, there were two important types of fermented agave drink: one made from the sap of the flower stalk, and another made from pit-roasted stems and leaf bases (Zizumbo-Villareal et al. 2009). Centuries ago, *pulque* was traditionally offered to Mayahuel, the Aztec goddess as a part of religious ceremony. However, it lost its preeminence for religious rituals after the fall of the Aztec empire, but it remains a traditional and popular drink in Mexico (Goncalves de Lima 1977).

Chicha is a clear, yellowish alcoholic beverage prepared from maize for centuries by the Andes Indians in the lower altitude regions of Ecuador, Brazil, Peru, Bolivia, Colombia, and Argentina (Escobar 1977). During its traditional preparation, human saliva serves as the source of amylase for the conversion of starch to fermentation sugars. Frequently, salivation is combined with malting to yield *chicha*. Today, *chicha* is consumed both as an everyday drink and as a ceremonial beverage. Chewing is not always a part of the process, and malted maize is generally used (Hayashida 2008).

In the days of the Incas, *chicha* was considered to be the vehicle that linked man to his gods through the fecundity of the earth (Nicholson 1960). In those days, the emperor held office only as long as he could supply the required amounts of *chicha* for his people, in exchange for which the people carried out economic activities such as building an extraordinary network of roads and installing vast areas of irrigated terraces for agriculture. Maize in this region has always had a profound religious and magical significance, and *chicha* played a role in fertility rites. It was used to induce the Thunder God to send rain and was also used in sun and harvest festivals (Maxwell 1956). *Chicha* also played a key role in economic and social life, acting as

the lynchpin of reciprocal exchanges (Morris 1979). Archaeological evidence shows that maize was a prestige crop ill-suited to the Inca territories, but highly valued because of its conversion into *chicha*. Great efforts were undertaken for cultivating maize, and *chicha* was brewed in great quantities, apparently under direct state control. Even today, *chicha* manufacture is a significant household or communal activity, and the beverage is consumed mainly by Indians during religious and agricultural festivities and during important family and social events (Escobar et al. 1996). A brief overview of selected Latin American fermented beverages prepared on a domestic scale is given by Quevedo (1997).

Palm "wine" is a common fermented beverage made in West Africa (Odunfa 1985). The sugar-rich sap of various palms is tapped and spontaneously fermented through the action of yeasts. The sap or wine can be consumed at various stages, from the fresh alcohol-free stage to a rather sour product containing about 7% alcohol. It has a very short shelflife but can be preserved to some extent by using the bark of a particular tree. Diluted honey is also used to make an expensive type of African wine (Odunfa 1985).

There is a rich and diverse range of traditional African cereal-based alcoholic drinks. Their use goes back at least to Medieval times, as recorded by Arab travelers and merchants (Odunfa 1988), and probably much earlier. *Pito* is a light brown, alcoholic beverage made from malted maize or sorghum in Nigeria and Ghana (Ekundayo 1996). Traditionally, *pito* is considered to be a source of instant energy for work by many Nigerian tribes. It is also offered to ancestors by pouring it on the ground, a ritual practiced by Nigerians. *Talla* or *tella*, a home-based beer from Ethiopia, is traditionally served in wedding ceremonies (Vogel and Gobezie 1996). *Busaa*, an alcoholic beverage made from maize or finger millet or a mixture of finger millet and sorghum, is traditionally drunk from a common pot using straws by the Luo, Abuluhya, and Maragoli tribes on special cultural occasions in Kenya (Harkishor 1996). It is usually consumed in large quantities in festivals, social gatherings, and funerals, and is used for ritual purposes in Kenya. The similarly named *bouza* is an alcoholic wheat drink made in Egypt (Morcos et al. 1973b). Although it is often thought to be similar to ancient Egyptian beer, this is not the case (see above). Some African fermented beverages are a mixture of cereals and bananas. For example, the Haya of northwest Tanzania brew *orubisi*, an effervescent, slightly sour alcoholic drink from bananas and sorghum. The drink is especially important to accompany celebrations and rituals, and the manner in which it is consumed is governed by tradition (Shayo et al. 2000).

Sprouted sorghum is used to make *burukutu* in West Africa, and *kaffir* beer in South Africa (Odunfa 1985), as well as *merissa* in Sudan (Dirar 1993). *Burukutu* is made with cassava powder; prior to the introduction of cassava to Africa it must have been prepared with a different substrate. *Kaffir* beer has been made for centuries in villages and has now been developed into a modern industrial process (Odunfa 1985, Ridgely 1994). Although the sorghum is malted, the breakdown of starch into sugars is not carried out by grain enzymes but by fungal action (Platt 1964). The finished product is unhopped with a sour flavor resembling that of yoghurt. It is consumed in large quantities in South Africa and is of great nutritional and social importance. A laborer can consume between 3 and 5 L/day, gaining significant calories and vitamins. Beer parties bring together villagers to work collectively on important major projects (Platt 1964).

Sorghum is the staple crop of Sudan and has a long pedigree of use there. It is made into a wide variety of fermented foods, of which the most complex and diverse is the alcoholic beverage *merissa* (Dirar 1993). It involves three separate stages of production, and the final products vary considerably in taste depending on the individual skills of the brewers who make it. It also varies regionally, as somewhat different procedures and ingredients are used in different areas. *Merissa* is highly nutritious and plays a central role in traditional Sudanese belief systems. An unusual alcoholic drink from Sudan is *kanyu-moro*, fermented from sesame seeds. It has a low alcoholic content and is mainly consumed by women (Dirar 1993).

Drinking of alcohol is a part of the social provision for a majority of the ethnic people of the Himalayas except for the Brahmin Hindu and Muslims for whom alcohol is taboo (Tamang 2010). *Jaanr* and *raksi* are essential to solemnize the marriage ceremony of non-Brahmin Hindu Nepalis and Buddhist tribes. Eloping is a common practice in the Himalayas. Traditionally, relatives of the boy usually visit the girl's parents after 3 days with bottles of locally prepared ethnic, distilled liquor *raksi* to respect the verdict of her parents, and pay the penalty for elopement. Once the consent is granted by the girl's parents, freshly prepared *raksi* is served to signify the union of the two families, and the marriage is thus solemnized. Such a practice of bridging between two families by a bottle of alcoholic drink is common only among the Himalayan people, mostly among the non-Brahmin Nepalis. Ethnic alcoholic beverages have a strong ritual importance. Distilled alcoholic liquors are offered to family gods and ancestors and also have uses in spirit possession by many Mongolian-origin Asians. Those who come to offer condolences gathered at a funeral or a memorial service for the deceased are served with alcoholic beverages, a culture which is followed by many Asians. Distilled liquor is offered during the construction of a new house preferably when the first foundation pillar is laid by the Japanese, which is also common among non-Brahmin Nepalis and Tibetans. Every official or family gathering is initiated by serving and drinking *saké* in Japan; *yakju*, *takju*, *soju* in Korea; *chi* in China; and *raksi* in India and Nepal.

1.4 Conclusion

Fermented foods and beverages are one of the integral components of dietary culture. Every community in this world has its distinct food or dietary culture that symbolizes the heritage and sociocultural aspects of the ethnicity. Religions and beliefs exert a strong influence on dietary habits, particularly through dietary laws such as taboos imposed on the consumption of certain food items. Several ancient foods are now the cultural foods as well as national foods of many countries. Food prepared by different communities is unique and distinct due to the geographical location, environmental factors, food preference, and the availability of plant or animal sources. What we see is the relationship between humans and microorganisms. In the process, both have benefited; microorganisms flourish on the substrates and produce bioactive compounds that enrich the human diet and benefit the health of human beings. The word "culture" in relation to food denotes food habits or dietary habits of ethnicity, and another meaning for the word is a cluster of microbial cells or inoculum, an essential biota for fermentation. On the basis of dietary culture and antiquity of fermented food and beverages, the following conclusions are drawn:

1. The dietary cultures of the world have three distinct food habits based on staple cereal foods: (a) cooked rice eaters of Eastern food culture, (b) wheat/barley-based breads/loaves of Western food culture, and (c) millet sorghum/maize porridges and flat loaves of African and South American food culture.
2. Soybean and its fermented products are prepared and consumed in Japan, Korea, China, Taiwan, Thailand, Laos, Cambodia, Vietnam, the Philippines, Indonesia, Malaysia, Singapore, Myanmar, Northeast India including Darjeeling hills, Bhutan, Nepal, and Mongolia.
3. Alcoholic drinks, besides being consumed for pleasure, are used to worship gods and goddesses, family ancestors, spirit possessions, and are used in offerings to shrines/temples and nature in Southeast Asia, Africa, in some Latin American countries, and in many ancient cultures.
4. Pit fermentation for acidification of perishable vegetables is unique to the Himalayas; however, such methods of pit fermentation are also seen in Oceania.
5. In Africa cassava is washed and fermented naturally into non-toxic edible product as staples food.
6. In the Indian subcontinent and mid-Asia, various spices are used as seasoning, whereas in the Orient, use of fermented seasonings such as soy sauce are part of food culture.
7. Traditionally, Chinese, Japanese, Koreans and other Mongoloid-origin races are nonmilk drinkers, and are soybean eaters.
8. Fruits are fermented into wine in Europe, North and South America, and Australia, whereas fruits are eaten fresh without fermenting into wine in Asia and Africa.
9. In the East, rice is fermented into a number of alcoholic beverages.
10. India has a fusion of both milk-drinking and soybean food cultures with a variety of ethnic sausages, cheese-like products, fermented soybean foods, alcoholic drinks, and fish products, mostly seen among the Mongolian-origin Indians.

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2

Diversity of Fermented Foods

Jyoti Prakash Tamang

CONTENTS

2.1 Introduction

Since the evolution of humans, the gathering of edible ingredients has become necessary for our survival and to maintain our physiological functions. Various substrates for edible products have evolved along with culinary practices as a result of the traditional wisdom and empirical experiences of generations over time, based on climate change, topography, ethnic preference, societal pattern, regional economy, geopolitics, demography, ethnicity, religions, customary beliefs, and cultural practices. The preservation of perishable raw materials of plant and animal sources as foods, the diversification of their products, and the innovation in food production and culinary practices have also evolved along with the development of human civilization. A variety of fermented foods and alcoholic beverages are produced naturally (by indigenous microorganisms) or by using microorganisms (starter cultures) and are consumed across the world, and every community has its fermented foods that are specific to it. Campbell-Platt (1994) claimed that around one-third of our food intake comprises fermented foods. Kwon (1994) estimated that around 20% of the total food consumed in the world is fermented foods. Data on the consumption and frequency of fermented foods are not widely available and are not very accurate. We conducted a survey on the consumption of fermented foods in Sikkim during 2003–2005. The data show that the per capita consumption of ethnic, fermented foods and beverages in Sikkim is 163.8 g/day, and the proportion of daily consumption of ethnic, fermented foods and beverages to the consumption of total food is 12.6% (Tamang et al. 2007a). It may be projected that 50–400 g per capita of fermented foods and alcoholic beverages are consumed daily worldwide, representing about 5%–40% of the total daily food consumption. Low-cost, high-value, and socially and culturally acceptable ethnic, fermented foods are consumed in diverse forms of cuisines such as staple diets, curries, stews, side dishes, fried foods, cooked foods, pastes, seasonings, condiments, pickles, confectionaries, salads, soups, desserts, savories, drinks, candied foods, masticators, colorants, taste makers, and as alcoholic and nonalcoholic beverages. In Asia and Africa, and in some countries in Europe and Latin America, ethnic women more are actively involved in the preparation of foods using their native knowledge of food fermentation technology than men and also supplement culinary practices. There are about 5000 varieties of major and minor unlisted fermented foods and beverages in the world prepared and consumed by billions of people belonging to different communities and ethnicities. However, the consumption of some less known and uncommon ethnic, fermented foods is declining due to changes in lifestyle and the shift from cultural foods to commercial foodstuffs and fast foods, and also due to climate change in some places which affects traditional culinary practices drastically. Chinese, Indians (several ethnic groups), and Africans (several tribes) have the largest varieties of ethnic, fermented foods and beverages.

2.1.1 Definitions of Fermented Foods

Many food researchers have defined the fermented foods according to their own interpretation; some of them are noted herein. van Veen (1957) defined fermented foods as foods that are fermented till at least one of the constituents has been subjected to the action of microorganisms for a period, so that the final products have often undergone

considerable changes in chemical composition and other aspects due to microbial and enzymatic changes.

Hesseltine (1965, 1979) defined traditional fermented foods as those that have been used for centuries, even predating written historical records, and that are essential for the well-being of many people of the world, especially people of the Near East, Southeast Asia, India, Far East, and Africa. Hesseltine and Wang (1967) further added typically that microorganisms are those that are present in or on the ingredients and are selected by adjusting the fermentation conditions. The late Dr. Hesseltine used the word "traditional" before "fermented food" in his publications.

Steinkraus (1994, 1996) defined indigenous fermented foods as foods where microorganisms bring about some biochemical changes in the substrates during fermentation, such as the enrichment of the human diet through the development of a wide variety of flavors, aromas, and textures in foods; the preservation of foods through lactic acid, and alcoholic, acetic acid, and alkaline fermentations; the enrichment of food substrates biologically with proteins, essential amino acids, essential fatty acids, and vitamins; the detoxification of undesirable compounds; and the decrease in cooking times and fuel requirements. Late Prof. Steinkraus used the word "indigenous" for every fermented food he mentioned in his books and publications.

Campbell-Platt (1987, 1994) defined fermented foods as those foods that have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification to the food and the direct consumption of fungal fruit bodies or mushrooms. Fermented foods may have originated as natural processes in which nutrient availability and environmental conditions selected particular microorganisms which modified and preserved the food.

Holzapfel (1997) described fermented foods as palatable and wholesome foods prepared from raw or heated raw materials by microbial fermentation. Traditionally, by trial and error, skills have been developed to control technical parameters during fermentation. Inoculation of raw materials with a residue of a previous batch, that is, a backslopping, accelerated the initial fermentation phase and controlled desirable changes.

Tamang (2010) defined ethnic, fermented foods as foods produced by ethnic people using their native knowledge from locally available raw materials of plant or animal sources either naturally or by adding starter culture(s) containing functional microorganisms that modify the substrates biochemically and organoleptically into edible products that are culturally and socially acceptable to the consumers. He used the term "ethnic" to denote community-based fermented foods and beverages prepared by different ethnic people using their native or traditional knowledge.

Biochemically, fermentation is defined as the process that does not require O_2 and the use of organic molecules as electron acceptors and is performed only by active living cells of microorganisms (Mansi et al. 2003).

2.1.2 A^3 for Fermented Foods

Literally, fermented foods and beverages bear the 3A or the A^3 connotation that denotes acidic, alkaline, and alcoholic properties. The major sensory and physicochemical properties of fermented foods are as follows: some of them are acidic in taste (low pH) such as lactic fermented foods (*gundruk*, *kimchi*, yoghurt); some foods are alkaline in nature (high pH) such as *kinema*, *dawadawa*, and *pidan*; some are

alcoholic—beer, wine, *saké*, and *pulque*. In lactic fermentation, the substrates are kept in an airtight container (less or no oxygen or anaerobic conditions) to allow lactic acid bacteria (LAB) to grow on starchy materials to obtain the acidic product. In alkaline fermentation, semi-anaerobic or aerobic conditions should be maintained to facilitate the growth of aerobic bacilli (mostly *Bacillus subtilis*) as in *kinema* and *natto*. After saccharification (starch to glucose) and glycolysis (glucose to alcohol and) CO_2 is obtained during the production of alcoholic beverages. Traditionally, the producers (ethnic people) know how to obtain desirable products for consumption using their indigenous knowledge. The scientific explanation behind the processes and the nature of the functional microorganisms involved were unknown to them. The essential objective of food fermentation is to carry over food supplies from times of plenty to those of deficit.

2.2 Microbial Diversity of Fermented Foods

Fermented foods harbor diverse microorganisms from the environment that include mycelial or filamaneotus molds, yeasts, and bacteria. Microorganisms are present in or on the ingredients, plant or animal sources, utensils, containers, and the environment, and are selected through adaptation to the substrates (Hesseltine 1983, Steinkraus 1997, Tamang 1998). Microorganisms transform the chemical constituents of raw materials during food fermentation and enhance the nutritive value of the products; enrich bland diets with improved flavor and texture; preserve perishable foods; fortify products with essential amino acids, omega-3 fatty acids, isoflavones, health-promoting bioactive compounds, and vitamins and minerals; degrade undesirable compounds and anti-nutritive factors; produce antioxidant components such as α-tocopherol, β-carotene, selenium or phenolic compounds, and antimicrobial compounds; improve digestibility; and stimulate probiotic functions. A study of the microbiology of fermented foods mainly focuses on the following parameters such as the determination of microbial loads (colony-forming unit per gram or liter of sample); isolation, enrichment, and purification of microorganisms; phenotypic (morphological, biochemical, and physiological tests) and genotypic characterization; and the proper identification of functional microorganisms following the standard norm of the International Code of Botanical Nomenclature (ICBN) and well-authenticated taxonomical keys. Proper identities of isolated microorganisms associated with the production of final edible products are an important aspect of microbial taxonomy as these microorganisms determine the quality of the product (Tamang and Holzapfel 1999). After the authentic identification and assignment of nomenclature for genera and species, identified strains of microorganisms shall be preserved in 15% glycerol at below −20°C and deposited at authorized microbial gene banks or microbial culture collection centers. Three major groups of microorganisms are associated with ethnic, fermented foods: bacteria, yeasts, and fungi.

2.2.1 Bacteria

Bacteria play dominant roles in the production of many fermented foods. Among bacteria, LAB are widely encountered in fermented foods; bacilli, micrococcaceae, etc., are also involved in the fermentation of foods.

2.2.1.1 Lactic Acid Bacteria

LAB are non-spore-forming, gram-positive, catalase-negative without cytochromes, non-aerobic or aerotolerant, fastidious, acid-tolerant, and strictly fermentative bacteria with lactic acid as the major end-product during sugar fermentation (Axelsson 1998). LAB genera isolated from various fermented foods are *Lactobacillus*, *Pediococcus*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Streptococcus*, *Tetragenococcus*, *Carnobacterium*, *Vagococcus*, and *Weissella* (Stiles and Holzapfel 1997, Carr et al. 2002, Salminen et al. 2004). *Propionibacterium* and *Bifidobacterium* species, commonly present in fermented milks, are also considered among LAB (Parente and Cogan 2004). Among the genera of LAB, *Lactobacillus* (both hereto- and homolactic) is the most dominant genus in fermented foods, mostly followed by species of *Pediococcus*. LAB produce organic acids during fermentation, mostly lactic acid which is the characteristic fermentative product and which reduces the pH of the substrate to a level where the growth of pathogenic, putrefactive, and toxinogenic bacteria are inhibited (Holzapfel et al. 1995). LAB are conferred the GRAS (generally recognized as safe) status in foods (Donohue and Salminen 1996). Many species of LAB can also act as "biopreservers" (Holzapfel et al. 2003) and some of them are exploited commercially. LAB modify the flavor of the original ingredients and improve the nutritional value of foods during fermentation (Nout and Ngoddy 1997).

2.2.1.2 Bacillus

Bacillus is a Gram-positive, endospore-forming, rod-shaped, catalase-positive, motile, and aerobic to semi-anaerobic bacterium (Gordon et al. 1973). The common species of *Bacillus* present in fermented soybean foods are *B. subtilis*, *B. natto*, *B. licheniformis*, *B. thuringiensis*, *B. coagulans*, *B. megaterium*, etc. (Kiers et al. 2000). Some strains of *B. subtilis* produce λ-polyglutamic acid (PGA), which is an amino acid polymer commonly present in Asian fermented soybean foods, giving the characteristic sticky texture to the product (Urushibata et al. 2002).

2.2.1.3 Micrococcaceae

Micrococcaceae are Gram-positive cocci, aerobic, non-spore-forming, nonmotile, and catalase-positive in the shape of irregular clusters or packets (Schleifer 1986). Among four genera of micrococcaceae, only species of *Staphylococcus* and *Micrococcus* are reported in fermented meats (Villar et al. 2000) and fish products (Wu et al. 2000). Micrococcaceae species are used to enrich fermentative microorganisms during ageing of the products in order to enhance the color stability of the cured meat and prevent rancidity (Papamanoli et al. 2002).

2.2.1.4 Other Bacteria

Klebsiella pneumoniae, *K. pneumoniae* subsp. *ozaenae*, *Enterobacter cloacae*, *Haloanaerobium*, *Halobacterium*, *Halococcus*, *Pseudomonas*, etc., have also been reported in many ethnic fermented foods.

2.2.2 Yeasts

Yeasts dominate the microbial composition of many fermented beverages and few fermented foods of the world (Tamang and Fleet 2009). About 21 yeast genera with several species of yeasts have been reported from fermented foods and beverages that include *Brettanomyces* (its perfect stage, *Dekkera*), *Candida*, *Cryptococcus*, *Debaryomyces*, *Galactomyces*, *Geotrichum*, *Hansenula*, *Hanseniaspora* (its asexual counterpart *Kloeckera*), *Hyphopichia*, *Kluyveromyces*, *Metschnikowia*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Saccharomycodes*, *Saccharomycopsis*, *Schizosaccharomyces*, *Torulopsis*, *Trichosporon*, *Yarrowia*, and *Zygosaccharomyces* (Kurtzman and Fell 1998, Pretorius 2000, Romano et al. 2006, Tamang and Fleet 2009). Yeasts food fermentation is practiced in nearly all the countries, along with bacterial and fungal fermentation, or in combination with them. In the fermentation of any substrate, *Saccharomyces* ferments sugar, produces secondary metabolites, inhibits the growth of mycotoxin-producing molds, and has several enzymatic activities such as lipolytic, proteolytic, pectinolytic, glycosidasic, and urease activities (Tamang and Fleet 2009). *Debaryomyces* contributes to sugar fermentation, increases the pH of the substrates, and produces growth factors for bacteria. *Hanseniaspora* and *Candida* contribute to sugar fermentation, produce secondary metabolites, and have enzymatic activities. *Yarrowia lipolytica* plays a role in sugar fermentation; has lipolytic, proteolytic, and urease activities; and reduces fat rancidity in the product (Tamang and Fleet 2009).

2.2.3 Mycelial Fungi

Fungi in fermented foods are relatively limited. Some common genera of mycelial or filamentous fungi associated with fermented foods and beverages of the world are *Actinomucor*, *Amylomyces*, *Aspergillus*, *Monascus*, *Mucor*, *Neurospora*, *Penicillium*, *Rhizopus*, and *Ustilago* (Hesseltine 1983, 1991, Samson 1993, Nout and Aidoo 2002). Mycelial fungi are mostly present in Asian fermented foods and beverages, as well as European cheese and sausages. Functional properties of the fungi in fermented foods are mainly production of enzymes such as maltase, invertase, pectinase, α-amylase, β-galactosidase, amyloglucosidase, cellulase, hemi-cellulase, and acid and alkaline proteases, lipases, and also the degradation of anti-nutritive factors, thus improving the bioavailability of minerals (Nout and Aidoo 2002).

2.3 Types of Fermented Foods

On the basis of the fermentation pattern, there are three major types of fermentation: solid-state, liquid-state and semisolid-state fermentations. In solid-state fermentation, the product is solid, e.g., *tempe*. In liquid-state fermentation, it is submerged, e.g., *shoyu* or soy sauce, and in semisolid-state fermentation, it is moist, e.g., *kinema*. On the basis of the use of microorganism(s), there are two types of fermented foods: (1) spontaneous or natural fermentation, that is, raw materials get fermented by natural microflora present on the raw ingredients or in the environment, without addition of starter culture(s), e.g., *gundruk*, and (2) controlled fermentation using starter culture(s). Again, controlled fermentation is of two types: (a) monoculture fermentation using a single, pure culture strain of microorganism, e.g., *natto*, and

(b) multicultural fermentation using a biculture or multicultural strains of microorganisms, or mixed inocula, e.g., cheese, sausages. Most of the traditional/indigenous/ethnic, fermented foods are prepared by processes of solid-substrate fermentation in which the substrate is allowed to ferment either naturally or by adding starter cultures. In East and Southeast Asia, filamentous or mycelial molds are predominant organisms in the fermentation processes, whereas in Africa, Europe, Australia, and America, fermented products using bacteria or a combination of bacteria–yeasts mixture are predominant; mycelial molds are seldom used. The Himalayan ethnic fermented foods involve all the three major groups of microorganisms: molds, yeasts, and bacteria (Tamang 2010).

On the basis of substrates, nonalcoholic fermented foods are categorized into eight major groups: (1) fermented vegetables; (2) fermented soybean and non-soybean legumes; (3) fermented cereals; (4) fermented milks; (5) fermented fish; (6) fermented meats; (7) fermented root/tuber products; and (8) miscellaneous fermented products. The preparation and culinary, microbiology, nutrition, and functional properties of all categories of fermented foods are described in Chapters 5 through 13.

2.3.1 Fermented Vegetables

Almost everyone consumes vegetables including those who eat eggs, meat, and fish. Vegetables are grown and eaten in every part of the world as salads, curries, pickles, soups, and side dishes. Common vegetables are cabbage, cauliflower, leafy mustard, radish, carrot, beats, young tendrils of pumpkins and squash, brinjal, chilly, cucumber, ladies finger, sponge gourd, tomato, tree tomato, lemon, spinach, asparagus, and lettuce. Tender shoots of bamboo are also eaten as a delicacy in many parts of the world. Wild edible plants including ferns, stinging nettles, and their parts are commonly eaten in many parts of Asia, Africa, and South America. However, leafy and green vegetables are easily perishable. Refrigeration, freezing, and canning techniques have been developed in the twentieth century for preserving and prolonging the shelflife of foodstuffs. Even today, a majority of people living in underdeveloped and developing countries cannot afford canned or frozen foods, and are preserving foods by natural fermentation. Acid fermentation combined with salting remains one of the most practical methods of preserving and often enhancing the organoleptic and nutritional quality of fresh vegetables. The art of pickling vegetables or lactic fermentation has developed to preserve the vast amount of perishable, green and leafy vegetables without refrigeration, for future consumption. Fermented vegetable products are acidic in nature, that is, they are produced by purely lactic fermentation. Varieties of fermented vegetable products are prepared and eaten in different parts of the world (Table 2.1). Pit fermentation of *sinki* is an unique type of biopreservation of perishable radish by lactic acid fermentation in the Himalayas (Tamang 2010). Pit fermentation has been practiced in the South Pacific and Ethiopia for the preservation of breadfruit, taro, banana, and cassava (Steinkraus 1996).

Fermentation of vegetables are mostly dominated by species of *Lactobacillus* and *Pediococcus*, followed by *Leuconostoc*, *Weisella*, *Tetragenococcus*, and *Lactococcus*. Species of LAB strains isolated from several fermented vegetable products have antimicrobial activities including bacteriocins and nisin production as reported in fermented olives (Rubia-Soria et al. 2006), sauerkraut (Niksic et al. 2005), fermented carrots (Uhlman et al. 1992), fermented cucumbers (Breidt 2006), and *inziangsang*

TABLE 2.1

Some Fermented Vegetable Products of the World

Fermented Food	Substrate	Sensory and Product Nature	Culinary	Microorganisms	Country
Anishi	Taro leaves	Acidic, wet	Curry	LAB	India
Bastanga	Bamboo shoot	Acidic, soft	Curry	LAB	India
Burong mustala	Mustard	Acidic, wet	Salad	LAB	Philippines
Cucumber pickle	Cucumber	Acidic, wet	Salad	LAB	Europe, United States, Canada
Dhamuoi	Cabbage	Acidic, wet	Salad	LAB	Vietnam
Dakguadong	Mustard leaf	Acidic, wet	Salad	LAB	Thailand
Ekung	Bamboo shoot	Acidic, sour, soft	Curry, soup	LAB	India
Eup	Bamboo shoot	Acidic, sour, dry	Curry, soup	LAB	India
Fu-tsai	Mustard	Acidic, sour	Soup, stew	LAB	Taiwan
Goyang	Wild vegetable	Acidic, sour, wet	Condiment, soup	LAB	India, Nepal
Gundruk	Leafy vegetable	Acidic, sour, dry	Soup, pickle	LAB	India, Nepal, Bhutan
Hirring	Bamboo shoot tips	Acidic, sour, wet	Curry, soup	LAB	India
Inziangsang	Mustard leaves	Acidic, sour, dry	Curry, soup	LAB	India
Inziangdui	Mustard leaves	Acidic, sour, liquid	Condiment	LAB	India
Jeruk	Fruits and vegetables	Acidic, wet	Salad	LAB	Malaysia
Khalpi	Cucumber	Acidic, sour, wet	Pickle	LAB	India, Nepal

Kimchi	Cabbage, radish	Acidic, mild-sour, wet	Salad, side dish	LAB	Korea, China
Lung-siej	Bamboo shoot	Sour-acidic, soft	Curry	LAB	India
Naw-mai-dong	Bamboo shoot	Acidic, wet	Side dish	LAB	Thailand
Mesu	Bamboo shoot	Acidic, sour, wet	Pickle	LAB	India, Nepal, Bhutan
Olives (fermented)	Olive	Acidic, wet	Salad, side dish	LAB	United States, Spain, Portugal, Peru, Chile
Pak-gard-dong	Leafy vegetable	Acidic, wet	Side dish	LAB	Thailand
Pak-sian-dong	Leaves of *Gynandropis pentaphylla*	Acidic, wet	Side dish	LAB	Thailand
Poi	Taro corms	Acidic, semisolid	Side dish	LAB, yeast	Hawaii
Sauerkraut	Cabbage	Acidic, sour, wet	Salad, side dish	LAB	Europe, United States, Canada, Australia
Sayur asin	Mustard leaves, cabbage	Acidic, sour, wet	Salad, side dish	LAB	Indonesia
Sinnamani	Radish	Acidic, sour, wet	Pickle	LAB	Nepal
Soibum	Bamboo shoot	Acidic, sour, soft	Curry	LAB	India
Soidon	Bamboo shoot tips	Acidic, sour, soft	Curry	LAB	India
Soijim	Bamboo shoot	Acidic, liquid	Condiment	LAB	India
Sinki	Radish tap root	Acidic, sour, dry	Soup, pickle	LAB	India, Nepal, Bhutan
Suan-cai	Vegetables	Acidic, sour, wet	Pickle	LAB	China
Sunki	Turnip	Acidic, sour, wet	Pickle	LAB	Japan
Suan-tsai	Mustard	Acidic, sour, dry	Soup, stew	LAB	Taiwan

(Tamang et al. 2009b). Leafy vegetables usually contain low levels of biogenic amines but they may increase during fermentation due to the decarboxylase activity of microorganisms (Silla Santos 1996). In foods, biogenic amines are mainly generated by the decarboxylation of corresponding amino acids through substrate-specific enzymes of the microorganisms present in foods (Straub et al. 1995). Among the fermented vegetable products of the world, the most widely and extensively studied products are sauerkraut (Eom et al. 2007, Johanningsmeier et al. 2007, Plengvidhya et al. 2007) and *kimchi* (Chang et al. 2008, Yim et al. 2008, Nam et al. 2009). Many ethnic, fermented vegetables as well as bamboo shoot products from the Himalayas have been documented recently (Tamang 2010).

2.3.2 Fermented Soybeans and Non-Soybean Legumes

Legumes are high-protein plant foods in the human dietary system. Some common legumes grown and eaten through the world are soybean, garden pea, black gram, green gram, black lentil, French bean, etc. As far as the fermentation of legumes is concerned, 90% of fermented legumes are soybean-based foods, and the rest are non-soybean foods (Table 2.2). Fermentation of the soybean is an ancient practice for many Asians mostly Chinese, Nepalis, Japanese, Thais, Koreans, Indonesians, and many minor ethnic groups. Consumption of ethnic, fermented soybeans and other legumes is not part of the traditional food culture of non-Mongoloid races. However, various non-soybean legume-based fermented foods are consumed in Africa. Fermented legumes are alkaline in nature because of alkaline fermentation. *B. subtilis* is a dominant bacterium used as a starter culture for many Asian and African fermented legume foods (Kiers et al. 2000, Kimura and Itoh 2007). Fermented soybeans that are exclusively fermented by *Bacillus* spp. (mostly *B. subtilis*) are *natto* of Japan; *kinema* of India, Nepal, and Bhutan; *thua nao* of Thailand; *chungkokjang* of Korea, and all have a characteristic stickiness (Tamang 2001, Hosoi and Kiuchi 2003). Fermented soybeans that are mostly fermented by molds (spp. of *Rhizopus*, *Aspergillus*) are *tempe* of Indonesia, *douchi* of China, *miso* and *shoyu* of Japan, and *sufu* of China. Among the common non-soybean fermented legumes of the world are *dawadawa* or *iru* and *ugba* of Africa; *papad*, *dhokla*, and *wari* of India; and *ontjom* of Indonesia.

2.3.3 Fermented Cereals

Cereals are the staple food of billions of populace around the world. The major cereal crops are rice, maize, wheat, rye, millets, barley, buckwheat, sorghum, etc., that are cultivated in various agro-climatic zones all over the world. In most of the Asian countries, rice is fermented either by using mixed culture(s) into alcoholic beverages, or food beverages, whereas in Europe, America, and Australia, cereals, mostly wheat, rye, barley, or maize, are fermented by natural fermentation or by adding commercial baker's yeast into leavened batter, forming dough breads or loaves, which are usually baked or steamed. In Africa, fermented cereal foods are traditionally used as a staple food as well as complementary and weaning foods for infants and young children (Nout 2001, Blandino et al. 2003, Tou et al. 2007). Two major types of cereal-based fermented foods are produced: (1) alcoholic food beverages and (2) nonalcoholic foods. The majority of alcoholic food beverages are prepared using mixed cultures

TABLE 2.2

Some Fermented Legume Products of the World

Fermented Food	Substrate	Sensory Property and Nature	Culinary	Microorganisms	Country
Aakhone	Soybean	Alkaline, sticky, paste	Side dish	*Bacillus* spp.	India
Bhallae	Black gram	Mildly acidic	Fried patties, snack	LAB, yeasts	India
Bekang	Soybean	Alkaline, sticky, paste	Side dish	*Bacillus* spp.	India
Chee-fan	Soybean whey curd	Cheese-like, solid	Salad	Molds	China
Chiang	Soybean	Alkaline, paste	Soup	Mold	China
Chungkokjang	Soybean	Alkaline, sticky	Condiment, soup	*Bacillus* spp.	Korea
Dauchi	Soybean	Alkaline, paste	Condiment, soup	*Bacillus* spp., molds	China, Taiwan
Dawadawa	Locust bean	Alkaline, sticky	Condiment, soup	*Bacillus* spp.	Africa
Dawadawa	Locust bean	Alkaline, sticky	Condiment, soup	*Bacillus* spp.	Ghana
Dhokla	Bengal gram	Mild acidic, spongy	Steamed, snack	LAB, yeasts	India
Doenjang	Soybean	Alkaline, paste	Soup	Mold	Korea
Furu	Soybean curd	Mild acidic	Savory	Mold	China
Hawaijar	Soybean	Alkaline, sticky	Side dish	*Bacillus* spp.	India
Iru	Locust bean	Alkaline, sticky	Condiment, soup	*Bacillus* spp.	Nigeria, Benin
Kawal	Leaves of legumes (*Cassia* spp.)	Alkaline, strong flavored, dried balls	Soup, stew	*Bacillus* spp., *Propionibacterium* spp.	Sudan
Kecap	Soybean, wheat	Liquid	Condiment, seasoning	LAB, yeasts	Indonesia
Ketjap	Soybean (black)	Syrup	Seasoning agent	Mold	Indonesia
Kinda	Locust bean	Alkaline, sticky	Condiment, soup	*Bacillus* spp.	Sierra, Leone
Kinema	Soybean	Alkaline, sticky	Curry, soup	*Bacillus subtilis*, *Enterococcus faecium*	India, Nepal, Bhutan
Khaman	Bengal gram	Mild acidic, spongy	Breakfast food	LAB	India
Maseura	Black gram	Dry, ball-like, brittle	Condiment	Bacilli, LAB, yeasts	Nepal, India

(*continued*)

TABLE 2.2 (continued)

Some Fermented Legume Products of the World

Fermented Food	Substrate	Sensory Property and Nature	Culinary	Microorganisms	Country
Meitauza	Soybean	Liquid	Drink	Mold	China, Taiwan
Meju	Soybean	Alkaline, paste	Seasoning agent	Mold	Korea
Miso	Soybean	Alkaline, paste	Soup	Mold	Japan
Natto	Soybean	Alkaline, sticky	Side dish, breakfast	*Bacillus natto*	Japan
Ontjom	Peanut	Alkaline, solid cake	Roasted or fried	Mold	Indonesia
Papad	Black gram	Circular wafers	Snack	LAB, yeasts	India, Nepal
Pepok	Soybean	Alkaline, sticky	Side dish	*Bacillus* spp.	Myanmar
Peruyaan	Soybean	Alkaline, sticky	Side dish	*Bacillus* spp.	India
Sieng	Soybean	Alkaline, sticky	Side dish	*Bacillus* spp.	Cambodia, Laos
Shoyu	Soybean	Alkaline, liquid	Seasoning	Mold	Japan, Korea, China
Soumbala	Locust bean	Alkaline, sticky	Condiment, soup	*Bacillus* spp.	Burkina, Faso
Soy sauce	Soybean	Alkaline, liquid	Seasoning	Mold	Worldwide
Sufu	Soybean curd	Mild-acidic, soft	Side dish	Mold	China, Taiwan
Tauco	Soybean	Alkaline, paste	Soup	Mold	Indonesia
Tempe	Soybean	Alkaline, solid	Fried cake	*Rhizopus oligosporus*, *Klebsiella pneumonia*	Indonesia (origin), the Netherlands, Japan, United States
Thua nao	Soybean	Alkaline, paste, dry	Soup	*Bacillus* spp.	Thailand
Tofu si	Soybean	Alkaline, liquid	Seasoning	*Bacillus* spp.	China, Japan
Tungrymbai	Soybean	Alkaline, sticky	Curry, soup	*Bacillus* spp.	India
Ugba	African oil bean (*Pentaclethra macrophylla*)	Alkaline, flat, glossy, brown in color	Side dish	*Bacillus* spp.	Nigeria
Uri	Locust bean	Alkaline, sticky	Condiment, soup	*Bacillus* spp.	West Africa
Vadai	Black gram	Paste	Fried patties, snack	LAB, yeasts, bacilli	India
Wari	Black gram	Ball-like, brittle	Condiment	LAB, yeasts	India

with the coexistence of filamentous molds, yeast, and LAB in the form of a flattened ball and dry-cake-like starter, mostly in Asia (Tamang and Fleet 2009). Popular as well as less familiar fermented cereals of the world are listed Table 2.3.

In Europe, people still practice the traditional method of preparation of breads or loaves without using any commercial strains of baker's yeast (Hammes and Ganzle 1998). Dough fermentation, mostly San Francisco sourdough, is conducted by yeasts and LAB, and the resultant products are generally called sourdough breads because they have higher contents of lactic acid and acetic acid due to bacterial growth (Rehman et al. 2006). During cereal fermentation, the nutritional and mineral contents of raw cereals are always enhanced (Umeta et al. 2005). The well-documented fermented cereal foods are *sourdough* from Europe, America, and Australia (Brandt 2007, De Vuyst 2009), *idli* from India (Mukherjee et al. 1965, Steinkraus et al. 1967), *dosa* from India (Soni et al. 1985, 1986), *jalebi* from India and Pakistan (Batra 1986), *rabadi* from India (Gupta et al. 1992), *selroti* from India and Nepal (Yonzan and Tamang 2009), *masa* from South Africa (Efiuvwevwere and Ezeama 1996), *mawé* or *ogi* from Benin (Hounhouigan et al. 1993), *kisra* from Sudan (Mohammed et al. 1991), *ben-saalga* from Burkina Faso (Tou et al. 2007), *kenkey* from Ghana (Nout et al. 1996), *togwa* from Tanzania (Mugula et al. 2003), and *tarhana* from Turkey (Erbas et al. 2006).

2.3.4 Fermented Milks

Milk is a global drink that is a polyphasic emulsion having physical, chemical, and biological properties (Huria 2002). Fermented cow-milk products are prepared from whole milk, or partially or fully skimmed milk, or concentrated milk by microbial fermentation mainly by LAB (Robinson and Tamime 2006). Milk of buffaloes, yaks, goats, camels, donkeys, and horses is also fermented into a number of minor ethnic milk products. Varieties of fermented milks are produced and consumed throughout the world (Table 2.4). The wide acceptability of fermented milks by consumers is attributed to their taste and extended shelflife due to the production of diacetyl and other desirable flavors by LAB. Lactic acid bacteria largely convert the lactose of milk into more digestible lactate, and proteins into free amino acids, imparting digestibility to fermented milks (Tamang and Holzapfel 1999). Fermented milks are generally classified into four types: (1) acid/alcohol-type such as *kefir* and *koumiss*, (2) high-acid-type such as Bulgarian sour milk, (3) medium-acid-type such as acidophilus milk and yoghurt, and (4) low-acid-type such as cultured buttermilk and cultured cream (Kosikowski 1977, 1997). Cheese and cheese products derived from the fermentation of milk are of major nutritional and commercial importance throughout the world (Galloway and Crawford 1985, de Ramesh et al. 2006). Yoghurt and yoghurt-like products, buttermilk, and butter are also commercially available fermented milk products throughout the world (Tamime and Robinson 2007).

2.3.5 Fermented, Dried, and Smoked Fish Products

Culturally, fish is a main dish for people residing near coasts, rivers, streams, lakes, and ponds where fish is available and is 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 (Table 2.5). The term "fermented fish" covers two categories of fish products: (1) fish–salt formulations,

TABLE 2.3

Some Fermented Cereal Products of the World

Fermented Cereals	Substrate	Sensory Property and Nature	Culinary	Microorganisms	Country
Aliha	Maize, sorghum	Mild-acidic, liquid	Nonalcoholic drink	LAB	Ghana, Togo, Benin
Ambali	Millet, rice	Acidic, pan cake	Shallow-fried, staple	LAB	India
Ang-kak	Red rice	Powder	Colorant	Mold	China
Banku	Maize and cassava	Solid	Staple food	LAB, yeasts	Ghana
Bahtura	Wheat flour	Bread	Deep-fried bread	LAB, yeasts	India
Bongkrek	Coconut press cake	Solid	Roasted or fried in oil	Mold	Indonesia
Burukutu	Sorghum and cassava	Creamy, liquid	Drink	LAB, yeasts	Nigeria
Ben-saalga	Pearl millet	Gruel	Weaning food	LAB, yeasts	Burkina Faso, Ghana
Chilra	Wheat, barley, buckwheat	Like *dosa*	Staple	LAB, yeasts	India
Dosa	Rice and black gram	Thin, crisp pancake	Shallow-fried, staple	LAB, yeasts	India, Sri Lanka, Malaysia, Singapore
Enjera	*Tef* flour, wheat	Acidic, sour, leavened	Pancake-like bread, staple	LAB	Ethiopia
Hopper	Rice, coconut water	Steam baked	Pancake, staple	Yeasts, LAB	Sri Lanka
Hussuwa	Sorghum	Cooked dough	Staple	Yeasts, LAB	Sudan
Hulumur	Sorghum, rice, millet	Mildly acidic, liquid	Nonalcoholic drink	LAB	Sudan, Turkey
Idli	Rice and black gram	Mildly acidic, soft, moist, spongy	Breakfast food	LAB, yeasts	India, Sri Lanka, Malaysia, Singapore
Jalebi	Wheat flour	Crispy, sweet, donut-like	Deep-fried, snacks	Yeasts, LAB	India, Nepal, Pakistan
Kenkey	Maize	Acidic, solid	Steamed dumpling, staple	LAB, yeasts	Ghana
Khanom-jeen	Rice	Noodle	Staple	LAB	Thailand
Kichudok	Rice	Steamed cake	Side dish	Unknown	Korea
Kisra	Sorghum	Thin pancake bread	Staple	LAB, yeasts	Sudan
Kishk	Wheat, milk	Dried balls	Refreshing beverage	LAB, yeasts	Egypt
Maheu	Maize, sorghum, millet	Sour, nonalcoholic	Refreshing beverage	LAB	South Africa
Mahewu	Maize	Sour, nonalcoholic	Refreshing beverage	LAB	South Africa

Mawè	Maize	Sour, nonalcoholic	Intermediate product used to prepare beverages, porridges	LAB, yeasts	Benin, Togo
Masvusvu	Maize	Sour, nonalcoholic	Refreshing beverage	LAB	Zimbabwe
Marchu	Wheat flour	Baked bread	Staple	Unknown	India, Pakistan
Me	Rice	Acidic, sour	Condiment	LAB	Vietnam
Minchin	Wheat gluten	Solid	Condiment	Molds	China
Naan	Wheat flour	Leavened bread, baked	Staple	Yeasts, LAB	India, Pakistan, Afghanistan
Ogi	Maize, sorghum, millet	Mildly acidic, viscous	Porridge, staple	LAB, Yeasts	Nigeria
Perkarnaya	Rye	Acidic, aerated bread	Breakfast	Yeasts, LAB	Russia
Pizza dough	Wheat	Leavened dough	Pizza base	Baker's yeast	Worldwide
Poto poto	Maize	Slurry	Gruel	LAB, Yeasts	Congo
Pozol	Maize	Mildly acidic, thick viscous	Porridge, staple	LAB, yeasts, molds	Mexico
Puda/Pudla	Maize, Bengal gram	Solid food, pancake	Snack food	LAB, yeasts	India
Pumpernickel	Rye	Acidic, aerated bread	Breakfast	Yeasts, LAB	Switzerland, Germany
Puto	Rice	Steamed cake	Breakfast or snack	LAB, Yeasts	Philippines
Rabadi	Buffalo or cow milk and cereals, pulses	Mildly acidic, thick slurry-like product	Drink	LAB, yeasts	India, Pakistan
Rye bread	Rye	Sandwich, bread	Breakfast	LAB	Denmark
San Francisco bread	Rye, wheat	Mildly acidic, leavened bread	Breakfast	Yeasts, LAB	United States
Seera	Wheat grains	Dried	Sweet dish	Unknown	India, Pakistan
Selroti	Rice, wheat flour, milk	Pretzel-like	Deep fried bread, staple	Yeasts, LAB	India, Nepal, Bhutan
Siddu	Wheat flour, opium seeds, walnut	Steamed bread, oval-shaped	Staple	unknown	India
Shamsy bread	Wheat flour	Spongy bread	Staple	Yeasts	Egypt
Sourdough	Rye, wheat	Mildly acidic, leavened bread	Breakfast	Yeasts, LAB	America, Europe, Australia
Trahana	Sheep milk, wheat	Mildly acidic, sweet and sour	Soup or biscuit	LAB, yeasts	Cyprus, Greece, Turkey
Taotjo	Wheat, rice, soybeans	Semisolid food	Condiment	Yeasts, LAB	East Indies
Uji	Maize, sorghum, millet, cassava flour	Acidic, sour	Porridge, staple	LAB	Kenya, Uganda, Tanzania

TABLE 2.4

Some Fermented Milk Products of the World

Fermented Milks	Substrate	Sensory Property and Nature	Culinary	Microorganisms	Country
Acidophilus milk	Cow milk	Acidic, sour	Drink	LAB	Russia, East Europe, Greece, Turkey, North America, Scandinavia
Airag	Mare or camel milk	Acidic, Sour, mild acoholic	Drink	LAB, yeasts	Mongolia
Biruni	Cow/camel milk	Acidic, semiliquid	Drink	LAB	Sudan
Bulgarian buttermilk	Cow milk	Acidic, sour	Drink	LAB	Yugoslavia, Bulgaria, Greece, Turkey, Albania, Romania
Butter	Animal milk	Soft paste	Butter	LAB	All parts of the world
Butter milk	Cow milk	Acidic, sour	Drink	LAB	United States, Canada, Russia, Scandinavia, Middle East, Egypt, Ethiopia, India, Australia, New Zealand
Cheese	Animal milk	Soft or hard, solid	Side dish, salad	LAB, yeasts, mold	Worldwide
Chhurpi (soft)	Cow milk	Mildly acidic, soft, cheese-like	Curry, pickle	LAB, yeasts	India, Nepal, Bhutan
Chhurpi (hard)	Yak milk	Hard mass	Masticator, gum-like	LAB, yeasts	China (Tibet), India, Nepal, Bhutan
Chhu or *sheden*	Cow or yak milk	Acidic, soft, strong flavored	Curry, soup	LAB, yeasts	China (Tibet), India, Bhutan
Chur yuupa	Yak milk	Mildly acidic, soft, flavored	Curry, soup	LAB	China (Tibet), India, Bhutan
Dahi	Cow milk	Acidic, viscous	Curd, savory	LAB, yeasts	India, Nepal, Pakistan, Sri Lanka, Bangladesh, Bhutan
Dachi	Cow or yak milk	Soft, cheese-like, strong flavored	Hot curry	LAB, yeasts	Bhutan
Dudh chhurpi	Cow milk	Hard mass	Masticator, chewing gum-like	LAB, yeasts	India, Nepal, Bhutan
Ergo	Milk	Acidic, semithick	Yoghurt-like	LAB	Ethiopia
Filmjölk	Cow milk	Less sour than yoghurt	Yoghurt-like	LAB	Sweden
Gariss	Camel milk	Acidic, liquid	Refreshing beverage	LAB	Sudan
Gheu/ghee	Cow milk	Soft, oily mass, solid	Butter	LAB, yeasts	India, Nepal, Bhutan, Bangladesh, Pakistan
Kefir or *kefyr*	Goat, sheep, or cow milk, *kefyr* grain	Acidic, mildly alcoholic, liquid	Effervescent milk	LAB, yeasts	Russia, Europe, Middle East, North Africa

Kishk	Sheep milk, wheat	Mildly acidic, dried balls	Drink	LAB, yeasts	Greece, Turkey, Egypt, Libya, Middle East, Iran
Koumiss or *kumiss*	Horse, donkey, or camel milk	Acidic, mildly alcoholic, liquid	Drink	LAB, yeasts	Kazakhstan, Russia, Scandinavia, Mongolia, China
Laban	Animal milk	Acidic, viscous	Yoghurt-like	LAB, yeasts	Egypt, Turkey
Lassi	Cow milk	Acidic, buttermilk	Refreshing beverage	LAB, yeasts	India, Nepal, Bhutan, Bangladesh, Pakistan, Middle East
Långfil	Cow milk	Elastic texture, sour	Yoghurt-like	LAB	Sweden
Maa	Yak milk	Mildly acidic, viscous	Butter	LAB, yeasts	China (Tibet), India, Bhutan
Mohi	Cow milk	Acidic, buttermilk	Refreshing beverage	LAB, yeasts	Nepal, India, Bhutan
Mish	Cow/camel milk	Acidic, semiliquid	Refreshing beverage	LAB	Sudan, Egypt
Misti dahi	Buffalo/cow milk	Mildly acidic, thick gel, sweet	Sweet curd, savory	LAB, yeasts	India, Bangladesh
Phrung	Yak milk	Mildly acidic, hard-mass-like *chhurpi*	Masticator	Unknown	India, China (Tibet)
Philu	Cow/yak milk	Cream	Curry	LAB	India, China (Tibet), Bhutan
Pheuja or suja	Tea, yak butter, salt	Salty with buttery flavor, liquid	Refreshing tea	unknown	India, China (Tibet), Bhutan, Nepal
Paneer	Buffalo or cow milk	Whey, soft, cheese-like product	Fried snacks	LAB	India, Nepal, Pakistan, Bangladesh, Middle East
Rob	Cow, goat, and sheep milk	Mildly acidic	Savory	LAB	Sudan
Shrikhand	Cow, buffalo milk	Acidic, concentrated, sweetened, viscous	Savory	LAB	India
Somar	Cow or yak milk	Mildly bitter, strong flavored, paste	Condiment	LAB	India, Nepal
Shyow	Yak milk	Acidic, thick-gel viscous	Curd-like, savory	LAB, yeasts	China (Tibet), Bhutan, India
Tarag	Cow, yak, goat milk	Acidic, sour	Drink	LAB, yeasts	Mongolia
Viili	Cow milk	Thick and sticky, sweet taste	Breakfast	LAB, yeasts	Finland
Yakult	Cow milk	Mildly acidic, sweet, savory	Probiotic yoghurt	*Lactobacillus casei*, *Bifidobacterium breve*	Japan
Yoghurt	Animal milk	Acidic, thick-gel viscous	Curd-like product, savory	LAB, yeasts	Europe, Australia, America

TABLE 2.5

Some Fermented, Dry and Smoked Fish Products of the World

Fish Product	Substrate	Sensory Property and Nature	Culinary	Microorganisms	Country
Ayaiba	Fish	Smoked fish	Pickle, curry	Unknown	India
Bagoong	Fish, shrimp	Paste	Condiment	Micrococci, LAB	Philippines
Balao balao	Shrimp	Fermented shrimp	Condiment	Micrococci, LAB	Philippines
Belacan	Shrimp	Paste	Condiment	Micrococci, LAB	Malaysia
Bordia	Fish	Dried, salted	Curry	LAB, yeasts	India
Budu	Anchovies	Fish sauce	Condiment	Micrococci, LAB	Malaysia
Burong isda	Fish-rice	Fermented rice-fish mixture	Sauce, staple	Micrococci, LAB	Philippines
Gnuchi	Hill river fish	Smoked fish	Curry	LAB, *Bacillus*, yeasts	India
Gulbi	Shell-fish	Salted and dried	Side dish	Bacilli, Micrococci, LAB	Korea
Hákarl	Shark flesh	Fermented	Side dish	LAB	Iceland
Hentak	Fish and petioles of arid plants	Fermented fish paste	Curry	LAB, yeasts	India
Jaadi	Marine fish	Fermented fish paste	Curry, condiment	LAB	Sri Lanka
Jeot kal	Fish	High-salt fermented	Staple	LAB	Korea
Karati	Fish	Dried, salted	Curry	LAB, yeasts	India
Kapi	Small fish	Paste	Condiment	Micrococci, LAB	Thailand
Kecap ikan	Fish	Liquid, sauce	Seasoning	Micrococci, LAB	Indonesia
Kung chao	Shrimp, salt, sweetened rice	Paste	Side dish	LAB	Thailand
Lashim	Fish	Dried, salted	Curry	LAB, yeasts	India

Mehiawah	Marine fish	Fermented paste	Side dish	LAB, yeasts	Middle–East Asia
Mio	Fish	Dried	Curry	Unknown	India
Naakangba	Fish	Dried	Pickle, curry	Unknown	India
Nga pi	Fish	Fermented paste	Condiment	LAB	Myanmar
Nam-pla	Anchovies	Fish sauce	Condiment	LAB	Thailand
Narezushi	Sea fish, cooked millet	Fermented paste	Side dish	LAB	Japan
Ngan pyaye	Fish	Fish sauce	Condiment	LAB	Myanmar
Ngari	Fish	Fermented fish	Curry	LAB, yeasts	India
Nuoc mam	Marine fish	Fish sauce	Condiment	LAB	Vietnam
Patis	Marine fish	Fish sauce	Condiment	LAB	Philippines
Pla ra	Fish, rice	Fermented paste	Condiment, staple	LAB	Thailand
Pedah	Mackerel	Partly dried and salted	Side dish	LAB	Indonesia
Pekasam	Freshwater fish-rice	Fermented fish	Side dish	LAB	Malaysia
Pindang	Fish	Dried, salted	Side dish	LAB	Indonesia
Shiokara	Squid	Fermented; side-dish	Side dish	LAB	Japan
Shottsuru	Marine fish	Fermented fish	Condiment	LAB	Japan
Sidra	Fish	Dried fish	Curry	LAB, yeasts	Nepal, India
Sikhae	Fish-cereals	Low-salt fermented	Sauce	LAB	Korea
Suka ko maacha	River fish	Smoked, dried	Curry	LAB, *Bacillus*, yeasts	Nepal, India
Sukuti	Fish	Dried fish	Curry	LAB, yeasts	Nepal, India
Surströmming	Herring	Fermented	Side dish	*Haloanaerobium praevalens*	Sweden
Trassi	Shrimps/fish	Fermented paste	Side dish	LAB	Indonesia
Tungtap	Fish	Fermented fish, paste	Pickle	LAB, yeasts	India

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% 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 (Adams 1998). Fermented fish products contribute significantly to the diet by increasing protein intake for a large population of the world (Beddows 1985). 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.

Fermented fish products are prepared from freshwater and marine finfish, shellfish, and crustaceans that are processed with salt to cause fermentation and thereby to prevent putrefaction (Ishige 1993). Preserved fish products are largely confined to East and Southeast Asia where the traditional processes are still followed (Adams 1998). Among the fermented fish products, the most widely used are fish sauces and pastes (van Veen 1965). The fish sauce of Asia is a nutritious condiment made from a traditionally fermented fish and salt mixture (Thongthai and Gildberg 2005). Species of *Lactobacillus*, *Pediococcus*, *Micrococcus*, *Bacillus*, and yeast including species of *Candida* and *Saccharomyces* are reported from *nam pla* and *kapi*, fermented fish products from Thailand (Watanaputi et al. 1983). *Micrococcus* and *Staphylococcus* are dominant microorganisms during the ripening of *shiokara* (Tanasupawat et al. 1991, Wu et al. 2000). *Haloanaerobium praevalens* has been reported from *surströmming*, fermented herrings from Sweden, and *Haloanaerobium fermentans* from Japanese puffer fish ovaries (Kobayashi et al. 2000a,b). *Tetragenococcus muriaticus* and *T. halophilus* have been isolated from Japanese fermented puffer fish ovaries (Kobayashi et al. 2000c). Species of *Bacillus*, mostly *Bacillus stearothermophilus*, *B. shaercus*, *B. circulans*, etc., are predominant microflora in *nga pi*, a fermented fish paste from Myanmar (Tyn 1993). Species of *Halobacterium* and *Halococcus* spp. have been isolated from *nam pla* (Thongthai and Suntinanalert 1991).

2.3.6 Fermented, Dried, and Smoked Meat Products

Animal flesh is consumed all over the world except by a majority of Hindus and a few other communities because of religious taboo. A variety of traditionally processed meat products are consumed throughout the world (Table 2.6). In developed countries, a wet-curing process for meat has been evolved, which involves use of a solution of salt, sodium nitrate/nitrite, whereas in under-developed and developing countries, preservation of meat is done by curing with salt followed by drying or smoking or fermentation (Romano et al. 2006). Fermented meat products are divided into two categories: those made from whole meat pieces or slices, such as dried meat and jerky, and those made by chopping or comminuting the meat, usually called sausages. Meat processing is the combination of chemical curing, fermentation, and drying, which together give stable, safe, and ready-to-eat products (Bacus 1984). Cooked fermented meat products such as *mortadello*, *kochsalami*, and *thüringer* are less common (Campbell-Platt and Cook 1995). *Salsiccia* and *soppressata* are traditional, dry fermented sausages produced in Basilicata in Southern Italy (Parente et al. 2001). The hot climatic regions of Africa and Asia are the home of relatively few fermented meat

TABLE 2.6

Some Fermented, Dry and Smoked Meat Products of the World

Meat Products	Substrate	Sensory Property and Nature	Culinary	Microorganisms	Country
Alheira	Pork or beef	Dry, semi-dry	Sausage	LAB, micrococci, yeast	North of Portugal
Androlla	Ground lean pork	Dry	Sausage	LAB, micrococci, yeast	Spain
Arjia	Large intestine of chevon	Sausage	Curry	LAB, bacilli, micrococci, yeasts	India, Nepal
Bacon	Slices of cured pig, beef	Dry, semi-dry	Staple	LAB, yeast, micrococci	Germany, Belgium, Spain
Bagjinam	Pork	Fermented pork	Curry	Unknown	India
Chartayshya	Chevon	Dried, smoked meat	Curry	LAB, bacilli, micrococci, yeasts	India
Chorizo	Pork	Dry	Sausage	LAB	Spain
Faak kargyong	Pork	Sausage, soft or hard, brownish	Curry	LAB	India, Nepal, China (Tibet), Bhutan
Ham	Cured pork	Semi-dry	Breakfast	LAB, yeasts, micrococci	Spain, Italy
Jamma	Intestine of chevon, finger millet	Sausage, soft	Curry	LAB, bacilli, micrococci, yeasts	India
Jerky	Beef	Dry, semi-dry	Side dish	LAB, yeast, molds, micrococci	South America
Kochsalami	Beef, pork	Semi-dry, fermented	Sausage	Micrococci, LAB	Germany, United States
Lang kargyong	Beef	Sausage-soft or hard	Curry	LAB, micrococci	India, Nepal, China (Tibet), Bhutan
Lang satchu	Beef	Dried, smoked meat, hard	Curry	LAB	India, Nepal, China (Tibet), Bhutan
Lang chilu	Beef fat	Hard, used as an edible oil		LAB	India, China (Tibet), Bhutan
Luk chilu	Sheep fat	Hard, solid, oily	Edible oil	LAB	India, China (Tibet), Bhutan
Lang kheuri	Beef	Chopped intestine of beef	Curry	LAB	India, Nepal, China (Tibet), Bhutan
Mortadello	Pork	Unsmoked chopped meat	Sausage	LAB, micrococci	Italy, France, United States

(*continued*)

TABLE 2.6 (continued)

Some Fermented, Dry and Smoked Meat Products of the World

Meat Products	Substrate	Sensory Property and Nature	Culinary	Microorganisms	Country
Nham	Pork	Dry, semi-dry	Sausage	LAB, micrococci, yeast	Thailand
Nem chua	Pork, salt cooked rice	Hard, salty	Sausage	LAB	Vietnam
Pastirma	Chopped beef	Dry, semi-dry sausage	Sausage	LAB, micrococci	Turkey, Iraq
Peperoni	Pork, beef	Dried meat, smoked	Sausage	LAB, micrococci	Europe, America, Australia
Sai-krok-prieo	Pork, rice	Sausage	Sausage	LAB	Thailand
Salami	Pork	Sausage	Sausage	LAB, micrococci	Europe
Salchichon	Pork or beef	Dry	Sausage	LAB, yeast, micrococci, molds	Spain
Salsiccia	Chopped pork	Dry, semi-dry	Sausage	LAB, yeast, staphylococci, micrococci	Italy
Soppressata	Chopped lean pork	Dry, semi-dry	Sausage	LAB, yeast, staphylococci, micrococci	Italy
Sucuk	Chopped pork or beef	Dry	Sausage	LAB, micrococci	Turkey
Suka ko masu	Buffalo meat	Dried, smoked	Curry	LAB	India, Nepal
Sukula	Buffalo	Dried, smoked	Curry	LAB	Nepal
Thuringer	Beef, pork	Semi-dry, fermented	Sausage	LAB	Germany, United States
Yak chilu	Yak fat	Hard, oily	Edible oil	LAB	India, China (Tibet), Bhutan
Yak kargyong	Yak	Sausage, soft	Curry	LAB	India, Nepal, China (Tibet), Bhutan
Yak kheuri	Yak	Chopped intestine of yak	Curry	LAB	India, China (Tibet), Bhutan
Yak satchu	Yak meat	Dried, smoked meat	Curry	LAB	India, China (Tibet), Bhutan

products, although whole-meat dry uncooked jerky is produced in Africa as well as America (Klettner and Baumgartner 1980). Ethnic meat products of many countries have been well documented and studied, such as fermented sausages, salami in Europe (Toldra 2007), ham (Simoncini et al. 2007), *alheira* in Portugal (Ferreira et al. 2006), *androlla* in Spain (Garcia Fontán et al. 2007), jerky in America and Africa (Baruzzi et al. 2006), and *nham* in Thailand (Visessanguan et al. 2006, Chokesajjawatee 2009).

The main microbial groups involved are LAB and coagulase-negative cocci and, in addition, depending on the product, other groups may play a role, such as yeasts and enterococci (Rantsiou and Cocolin 2006). LAB exert an important effect on the production and quality of various fermented meat products (Schillinger and Lücke 1987, Hammes and Hertel 1998). *Pediococcus* and *Lactobacillus* species are active in producing lactic acid and thus help in lowering pH, which helps preserve meat (Bacus 1986). Dominant microorganisms in fermented meat products are mostly *Lb. sakei*, *Lb. curvatus*, *Lb. plantarum*, *Pediococcus pentosaceus*, *Enterococcus faecium*, *Leuc. carnosum*, *Leuc. gelidium*, *Leuc. pseudomesenteroides*, *Weissella*, etc. (Collins et al. 1993, Parente et al. 2001), and also coagulase-negative staphylococci (Hugas et al. 2003). *Micrococcus* and *Staphylococcus* species help reduce nitrate if added to nitrite in fermented sausages (Lücke 2003). Some yeasts and molds may develop on the surface of dry fermented sausages during ripening (Lücke 1985, Tamang and Fleet 2009). Yeast species like *Debaryomyces*, *Candida*, *Cryptococcus*, and *Trichosporon* have been reported in traditional Greek dry salami (Metaxopoulos et al. 1996). *Penicillium* species constituted the surface mycoflora of *chorizo*, a Spanish variety of fermented sausage (López-Díaz et al. 2001). Bacteriocinogenic enterococci can be used to enhance preservation in meat products (Hugas et al. 2003). Bioprotective LAB have been found to contribute to the safety of dry sausages by producing bacteriocins and other low-molecular-mass compounds (Tyopponen et al. 2002). Enterocins could be considered as an extra biopreservative against listeria in dry fermented sausage (Aymerich et al. 2000). The Himalayan people have a variety of traditionally processed smoked, sun dried, air dried, or fermented meat products including ethnic sausages from yak, beef, pork, sheep, and goats (Tamang 2010). Some common as well as some less known traditionally processed ethnic meat products of the Himalayas are *kargyong*, *satchu*, *suka ko masu*, *kheuri*, *chilu*, *chartayshya*, *jamma*, and *arjia* (Rai et al. 2009). These are naturally cured without starter cultures or the addition of nitrites/nitrates.

2.3.7 Fermented Root/Tuber Products

Roots and tubers of various plants are a staple food as they are a source of calories in many parts of Africa and Asia. Cassava (*Manihot esculenta*) is one of the most important tropical root crops. In Africa, cassava root is traditionally fermented into many products such as *gari* in Nigeria; *fufu* in Togo, Burkina Faso, Benin, and Nigeria; *agbelima* in Ghana; *chikawgue* in Zaire; *kivunde* in Tanzania; *kocho* in Ethiopia; *foo foo* in Nigeria, Benin, Togo, and Ghana, which are all staple foods (Table 2.7). Processing of *gari* involves several stages including fermentation, dextrinization, partial gelatinization, and retrogradation (Oyewole et al. 2004, Abimbola 2007). In the initial stage of fermentation of cassava into *gari*, *Corynebacterium manihot* dominates (Oyewole and Odunfa 1991). *Streptococcus* spp., *Lb. plantarum*, and *Leuconostoc* sp. have a major role in the detoxification of the cyanogenic glucosides during *gari* fermentation

TABLE 2.7

Some Fermented Root/Tuber Products of the World

Food	Substrate	Sensory Property and Nature	Culinary	Microorganisms	Country
Agbelima	Cassava roots	Paste	Staple	LAB	Ghana, Togo
Chickwangue	Cassava roots	Paste	Staple	LAB	Congo
Foo foo	Cassava roots	Paste	Staple	LAB	Nigeria, Benin, Togo, Ghana
Fufu	Cassava roots	Paste (acid soaked)	Staple	LAB	Togo, Burkina Faso, Benin, Nigeria
Gari	Cassava roots	Paste (acid grated)	Staple	LAB	Africa
Kivunde	Cassava roots	Paste	Staple	LAB	Tanzania
Kocho	Cassava roots	Paste	Staple	LAB	Ethiopia
Lafun	Cassava roots	Paste	Staple	LAB, yeast	Benin, Togo, Nigeria
Peujeum	Cassava roots	Acidic, solid	Eaten after baking	Yeasts, mold	Indonesia

(Ngaba and Lee 1979). *Geotrichum candida* is the dominant strain in the second stage of fermentation and is responsible for the characteristic taste and aroma of *gari*, as a result of production of esters and aldehydes (Okafor and Ejiofor 1990). The retting process leading to the softening of the cassava roots during *fufu* production is known to be due to some pectinolytic microorganisms (Oyewole and Odunfa 1992). Along with LAB, *Bacillus* sp., *Candida tropicalis*, and *Penicillium* also play important roles in the fermentation of cassava into *agbelima* in Ghana (Amoa-Awua et al. 1996).

2.3.8 Miscellaneous Fermented Products

A few essential fermented food products that do not fall under the general categories of fermented foods on the basis of substrates are placed under miscellaneous fermented products (Table 2.8). These miscellaneous fermented products are vinegar, *nata*, *pidan*, tea, coffee, cacao, etc. Vinegar has been used as a condiment, a preservative, and a medicine since ancient times. Vinegar can be prepared from any sugar-containing substrate and hydrolyzed starchy materials through alcoholic fermentation followed by acetic acid fermentation (Yokotsuka 1991). Various types of vinegars are produced worldwide including wine vinegar, malt vinegar, fruit vinegar, grain vinegar, spirit vinegar, whey vinegar, honey vinegar, and others. Typical vinegar fermented with *koji* mold is rice vinegar, which is popular in China, Japan, and Thailand, and other oriental countries where rice wine is produced with the *koji* mold. *Acetobacter* species like *A. pasteurianus*, *A. aceti*, *A. xylinum*, and *A. polyxygenes* are the dominant bacteria for vinegar fermentation (Entani and Masai 1985). *Lb. fructivorans*, *Lb. acetotolerans*, and *Moniliella acetobutans* also supplement the fermentation (Entani and Masai 1985). Recent studies have shown that a large number of yeast species are involved in the fermentation of vinegar; these are *Zygosaccharomyces bailii*, *Z. rouxii*, *Z. pseudorouxii*, *Z. mellis*, *Z. bisporus*, *Z. lentus*, *Hanseniaspora valbyensis*, *H. osmophila*, *Candida lactis-condensi*, *C. stellata*, *Saccharomycodes ludwigii*, and

TABLE 2.8

Some Miscellaneous Fermented Products of the World

Fermented Products	Substrate	Sensory Property and Nature	Culinary	Microorganisms	Country
Achar/chatney	Fruits, vegetables, oil, salt	Acidic, hot and sour	Pickles	LAB	India, Nepal, Pakistan, Bangladesh
Crabs	Crabs	Flavored, solid	Side dish	Unknown	Thailand, India
Chuk	Fruits	Sour, dark-brown paste	Therapeutic uses	Unknown	Nepal, India
Cacao	Cacao beans in pods of tree *Theobroma cocao*	Chocolate	Confectionery	Yeasts, bacteria	Worldwide
Coffee	Coffee	Flavored coffee	Refreshing drink	Yeasts	Worldwide
Fuzhuan brick	Tea	Fermented tea	Drink	*Aspergillus*, *Penicillium*, *Eurotium*	China
Hakua	Rice	Strong off-flavor	Therapeutic uses	unknown	Nepal, India
Huitlacoche or 'maize mushroom'	cobs of pre-harvest maize	Large fruiting body edible	Condiment	*Ustilago maydis*	Mexico
Kawal	*Cassia obtufalia* leaves	Alkaline	Condiment	LAB, *Bacillus* spp.	Sudan
Kombucha or Tea fungus	Tea liquor	Flavored	Drink	LAB, Yeasts	China (Tibet), India
Miang	Tea	Fermented tea, flavored	Drink	LAB	Thailand
Nata de coco	Coconut water or coconut skim milk	Thick white or cream-colored, candied	Ice cream, fruit salads	*Acetobacter* spp.	Philippines
Nata de piña	Juice from pineapple	Thick white or cream-colored, insoluble gelatinous film of polysaccharides	Ice cream, fruit salads	*Acetobacter* spp.	Philippines
Ogiri	Melon seeds	Alkaline	Condiment	LAB, *Bacillus* spp.	Nigeria
Owoh	Cotton seeds	Alkaline	Condiment	LAB, *Bacillus*	Nigeria
Pidan	Duck egg	Alkaline	Side dish	*Staphylococcus*, *Bacillus*	China
Puer	Tea	Fermented tea, brownish red, and a fragrance produced	Drink	*Aspergillus niger*, yeasts	China
Ugba	Oil-bean seeds	Alkaline	Side dish	LAB, *Bacillus* spp.	Sierra Leone, Nigeria
Vinegar	Sugar containing substrates	Acetic acid flavored, liquid	Condiment, seasoning	*Acetobacter* spp.	Worldwide

Saccharomyces cerevisiae (Solieri and Giudici 2008). Fungal denaturing gradient gel electrophoresis (DGGE) profile indicated that the transition from *Aspergillus oryzae* to *Saccharomyces* sp. took place at the initial stage of vinegar fermentation at which alcohol production was observed (Haruta et al. 2006). The early stage was characterized by the coexistence of *Saccharomyces* sp. and LAB, and almost all of the LAB DGGE bands were replaced by bands derived from *Lactobacillus acetotolerance* and *Acetobacter pasteurianus* at the stage at which acetic acid started to accumulate (Haruta et al. 2006).

Pidan is made from alkali-treated fresh duck eggs, and has a strong hydrogen sulfide and ammonia smell; *pidan* is consumed by the Chinese (Wang and Fung 1996). Instead of using microorganisms, *pidan* is made using alkali-treated fermentation. The main alkaline chemical reagent used for making *pidan* is sodium hydroxide, which is produced by the reaction of sodium carbonate, water, and the calcium oxide of pickle or coating mud. *Pidan* was originally produced in mainland China, dating back to AD 1640 (Liu and Zhang 1989). It is also consumed in Taiwan, Japan, South Korea, and some Southeast Asian countries. *Staphylococcus cohnii*, *Staphy. epidermidis*, *Staphy. haemolyticus*, *Staphy. warneri*, *Bacillus cereus*, and *B. macerans* are predominant in *pidan* (Wang and Fung 1996). Compared with fresh duck egg, *pidan* has a higher protein content and lower carbohydrate content (Hou 1981). Some reports claimed that *pidan* may have a therapeutic effect, especially benefiting people with high blood pressure or coronary problems (Wang and Fung 1996).

Nata is a delicacy from the Philippines, which when candied resembles gum drops in texture and flavor. *Nata* is the thick white or cream-colored, insoluble gelatinous film of cells and polysaccharides, a delicacy from the Philippines (Steinkraus 1996). Fermentation is due to *Acetobacter xylinum* that forms on the surface of an acidified medium containing sugar, ethyl alcohol, and other nutrients (Kozaki 1976). Two types of *nata* are well known: *nata de piña*, produced using juice from pineapple trimmings, and *nata de coco*, produced using coconut water or coconut skim milk. It is often eaten with ice cream and in fruit salads. The process of cellulose formation in a *nata de coco* culture system has been investigated, and it was found that the process of cellulose formation or the consumption of glucose is controlled by the diffusion of atmospheric oxygen (Budhiono et al. 1999).

Tea is generally produced by a natural oxidation process without microorganisms; however, there are also microbial fermented teas such as *puer* tea, *fuzuan brick* tea, *kombucha* from China (Mo et al. 2008), and *miang* from Thailand (Tanasupawat et al. 2007). Coffee seeds are harvested from green coffee trees and are processed by either wet or dry methods to remove the pulp and mucilaginous materials that surround the seeds (Silva et al. 2008). Various species of yeasts and bacteria grow throughout these processes, producing an array of pectinolytic, hemicellulolytic, and other enzymes that facilitate the pulp and mucilage degradation (Masoud et al. 2004). *E. cloacae*, *Klebsiella oxytoca*, and *Hafnia alvei* are isolated from coffee berries in Ethiopia (Holzapfel and Müller 2007). Cacao beans are contained in pods of the *Theobroma cocao* tree, mostly cultivated in the tropical equatorial regions of the world. Cacao beans are the raw material for manufacturing chocolates, and require fermentation as one of the first stages in the chocolate production chain (Schwan and Wheals 2004) for the development of flavor and aroma precursors (Bopaiah 1990). Cacao is obtained by grinding the fermented, dried, roasted, and peeled cocoa seeds. After harvesting, the beans are removed from the pods and placed as large masses

in wooden boxes, on trays or as heaps covered with plantain leaves. They undergo a spontaneous, indigenous fermentation consisting of filamentous fungi, yeasts, LAB, acetic acid bacteria, and *Bacillus* species (Kostinek et al. 2008). Yeasts have prominent roles during the fermentation of cacao for the development of chemical precursors that render a chocolate flavor, the flavor developing through a succession of *Hanseniaspora uvarum*, *H. quilliermundii*, *Saccharomyces cerevisiae*, *Pichia membranifaciens*, *Issatchenkia orientalis* (*Candida krusei*), and *Kluyveromyces* species (Ardhana and Fleet 2003).

2.4 Biological Importance of Fermented Foods

The most remarkable aspect of ethnic, fermented foods is that they have biological functions enhancing several health-promoting benefits for the consumers due to the functional microorganisms associated with them. Biopreservation of perishable foods, bioenrichment of nutritional value, protective properties, bioavailability of minerals, production of antioxidants and omega-3 polyunsaturated fatty acids, therapeutic value, and immunological effects are some of the biological functions of fermented foods (Tamang 2007, Liong 2008). Today, some of these fermented foods are commercialized and marketed globally as health foods, functional foods, therapeutic foods, or nutraceutical foods or biofoods or medico-foods.

2.4.1 Biotransformation into Tasty Food

The biological transformation of a bland vegetable protein into meat-flavored amino acid sauces and pastes by mold fermentation is common in the Japanese *miso* and *shoyu*, the Chinese soy sauce, and the Indonesian *tauco* (Steinkraus 1996). In *angkak*, an ethnic, fermented rice food of Southeast Asia, *Monascus purpureus* produces a purple-red water-soluble color in the product, which is used as a colorant (Beuchat 1978). Halophilic microorganisms contribute flavor and quality to the fermented fish products of Southeast Asia (Itoh et al. 1993). Fermentation improves the taste of otherwise bland foods, and imparts typical flavor and texture to fermented products such as *kinema* (Tamang 2001). In fermented milks, LAB produce diacetyl and other desirable flavors (Kosikowski 1977). During *tempe* fermentation, the mycelia of *Rhizopus oligosporus* knit the soybean cotyledons into a compact cake, which when sliced resemble nontextured bacons (Steinkraus 1994). Similarly, in *ontjom*, an Indonesian fermented peanut product, *Neurospora sitophila* knits the particles into firm cakes, imparting a meat-like texture (Steinkraus 1996).

2.4.2 Biological Preservation

Biological preservation refers to extended storage life and implies a significant approach to improve the microbiological safety of foods without refrigeration by lactic acid fermentation. Species of LAB during fermentation produce organic acids that reduce the pH of the substrate thereby inhibiting the growth of pathogenic microorganisms; thus, LAB can exert a biopreservative effect (Holzapfel et al. 1995). During the fermentation of Himalayan ethnic, fermented vegetable products, *Lb. plantarum*, *Lb. brevis*, *Pediococcus pentosaceus*, and *Leuconostoc fallax* produce lactic acid and

acetic acid and lower the pH of the substrates, making the products more acidic in nature (Tamang et al. 2005, 2008). Due to the low pH, high acid content, and the sun-drying process of freshly fermented vegetables in the Himalayas, perishable vegetables can be preserved without refrigeration and addition of any synthetic preservative for several years. This is a good example of biopreservation of perishable vegetables, which are plenty in the winter season in the Himalayan regions. Several fermented vegetable products preserved by lactic acid fermentation are *kimchi* in Korea, sauerkraut in Germany and Switzerland, etc. Pickled vegetables, cucumbers, radishes, carrots, and even some green fruits such as olives, papaya, and mango are acid fermented in the presence of salt.

2.4.3 Biological Enhancement of Nutritional Value

During fermentation, biological enrichment of food substrates with essential amino acids, vitamins, and various bioactive compounds occur spontaneously. In *tempe*, the levels of niacin, nicotinamide, riboflavin, and pyridoxine are increased by *Rhizopus oligosporus*, whereas cyanocobalamin or vitamin B_{12} is synthesized by nonpathogenic strains of *Klebsiella pneumoniae* and *Citrobacter freundii* during fermentation (Liem et al. 1977, Keuth and Bisping 1994). Thiamine, riboflavin, and methionine contents in *idli* are increased during fermentation (Rajalakshmi and Vanaja 1967, Steinkraus et al. 1967). *Pulque*, produced by lactic acid fermentation of juices of the cactus plant, is rich in vitamins such as thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, and biotin, and serves as an important diet for children of low economic status in Mexico (Steinkraus 1996). During *tempe* fermentation, isoflavone, particularly factor-II and aglycone contents, are found to increase (Pawiroharsono 2002). During the production of *kinema*, proteolytic enzymes produced by *Bacillus subtilis* break proteins into peptides and amino acids, enhancing digestibility (Tamang and Nikkuni 1998). In fermented milks, LAB largely convert lactose into a more digestible lactate and proteins into free amino acids, thus imparting digestibility to the product (Campbell-Platt 1994). *Kimchi* has been selected as one of the world's healthiest foods in 2006 by the *Health* magazine due to its many beneficial properties (Nam et al. 2009).

2.4.4 Biodegradation of Undesirable Compounds

Enzymes produced by functional microorganisms present in the fermented foods degrade unsatisfactory or anti-nutritive compounds and thereby convert the substrates into consumable products with enhanced flavor and aroma. Bitter varieties of cassava tubers contain the cyanogenic glycoside linamarin, which can be detoxified by species of *Leuconostoc*, *Lactobacillus*, and *Streptococcus* in *gari* and *fufu*, and thereby rendering these products safe to eat (Westby and Twiddy 1991). Trypsin inhibitor is inactivated during *tempe* fermentation by *Rhizopus oligosporus*, which eliminates flatulence-causing indigestible oligosaccharides such as stachyose and verbascose into absorbable monosaccharides and disaccharides (Hesseltine 1983). Microorganisms associated with *idli* batter fermentation reduce the phytic acid content of the substrates (Reddy and Salunkhe 1980). Raw soybeans contain some allergenic proteins, the major one being Gly m Bd 30K (Ogawa et al. 1991), which is also found in nonfermented soybean foods (Tsuji et al. 1995). *B. subtilis* (*natto*) is found

to degrade Gly m Bd 30K of raw soybeans during fermentation and makes *natto* a suitable food for persons allergic to raw soybeans (Yamanishi et al. 1995).

2.4.5 Bioimprovement in Lactose Metabolism

Some people suffer from lactose intolerance or lactose malabsorption, a condition in which lactose, the principal carbohydrate of milk, is not completely digested into glucose and galactose (Onwulata et al. 1989). Since lactose is cleaved into its constituent monosaccharides by β-D-galactosidase, lactose malabsorption results from a deficiency of this enzyme (Shah and Jelen 1991). *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, the cultures used in making yoghurt, contain substantial quantities of β-D-galactosidase, and it has been observed that the consumption of yoghurt may assist in alleviating the symptoms of lactose malabsorption (Shah 1993). Yoghurt or probiotic yoghurt is tolerated well by lactose-intolerant consumers, may be due to some lactose hydrolyzed by lactics and bifids during fermentation; the bacterial enzyme autodigests lactose intracellularly before it reaches the intestine, and it may be due to a slower oral-cecal transit time (Shah et al. 1992). Yoghurt as viscous foods may delay gastric emptying and thus may be effective in alleviating lactose intolerant symptoms (Shah 1994). As a result, fermented acidophilus milk may be better tolerated than sweet acidophilus milk, as coagulated milk, because of its viscous nature, may pass more slowly through the gut than unfermented milk (Shah 2005). The consumption of *kefir* minimizes symptoms of lactose intolerance by providing an extra source of β-galactosidase (Hertzler and Clancy 2003).

2.4.6 Probiotic Properties of Fermented Foods

Probiotic foods are defined as foods containing live microorganisms, which actively enhance the health of consumers by improving the balance of the gut microflora when live microorganisms are ingested in sufficient viable numbers (Lee and Salminen 2009). Probiotic cultures are considered to provide health-promoting benefits by means of stabilizing the gastrointestinal tract (Lee and Salminen 2009). Probiotics have been added to drinks and marketed as supplements including tablets, capsules, and freeze-dried preparations, and more than 70 bifidus- and acidophilus-containing products are produced worldwide, including sour cream, buttermilk, yoghurt, powdered milk, and frozen desserts (Shah 2007). In Japan, more than 53 different types of probiotic milk products are marketed, whereas in Europe their use is largely restricted to the yoghurt sector (Hilliam 2000). Some strains of LAB, primarily species of *Lactobacillus* and *Enterococcus*, and species of *Bifidobacterium*, are used as probiotic adjuncts and as biotherapeutic agents for protection against diarrhea, stimulation of the immune system, alleviation of lactose intolerance symptoms, and reduction of serum cholesterol (Shah 2005). Species of LAB are normal residents of the complex ecosystem of the gastrointestinal tract (Holzapfel et al. 1997, 1998). The Himalayan fermented yak milks have probiotic properties (Tamang et al. 2000, Dewan and Tamang 2007). *Lactobacillus johnsonii* (=*Lb. acidophilus*), which is used in the production of acidophilus milk, is considered as important representative of probiotic bacteria used in the production of novel yoghurt-like products (Stiles and Holzapfel 1997). Some common probiotic cultures used in the production of fermented functional foods are *Lb. acidophilus* La2, La5, Johnsonii; *Lb. bulgaricus* Lb12;

Lb. lactis L1a; *Lb. plantarum* 299v, Lp01; *Lb. rhamnosus* GG, GR-1; *Lb. reuteri* MM2; *Lb. casei* Shirota; *Lb. paracasei* CRL 431; *Lb. fermentum* RC-14; *Lb. helveticus* B02; *Bifidobacterium adolescentis*; *B. longum* BB536; *B. breve* Yakult; *B. bifidus* Bb-11; *B. essensis* Danone; *B. lactis* Bb-12; *B. infantis* Shirota; *B. laterosporus* CRL 431; *B. lactis* DR-10; and *B. longum* UCC35624 (Krishnakumar and Gordon 2001, Shah 2004). Ingestion of probiotic yoghurt has been reported to stimulate cytokine production in blood cells and enhance the activities of macrophages (Solis and Lemonnier 1996). Translocation of a small number of *Lb. acidophilus* and bifidobacteria via M cells to Payer's patches of the gut-associated lymphoid tissue in the small intestine is responsible for enhancing immunity (Marteau et al. 1997). The probiotic strain of *Lb. acidophilus* La-5 produces conjugated linoleic acid (CLA), an anticarcinogenic agent (Macouzet et al. 2009). The probiotic strain of *Lb. plantarum*, isolated from *kimchi*, inhibits the growth and adherence of *Helicobacter pylori* in MKN-45 cell lines, with small peptides acting as possible inhibitors (Lee and Lee 2006). *Yakult* is a Japanese commercial probiotic milk product that has several health-promoting benefits such as modulation of the immune system, maintenance of gut flora, regulation of bowel habits, alleviation of constipation, and curing of gastrointestinal infections (Kiwaki and Nomoto 2009).

2.4.7 Bioproduction of Enzymes

During fermentation, indigenous microorganisms or starter cultures produce enzymes that breakdown complex compounds in substrates to simple biomolecules, which perform several biological activities. Functional microorganisms in fermented foods show a wide spectrum of enzymatic activities such as amylase, glucoamylase, protease, and lipase activities. Some of these strains produce a high amount of enzymes, which may be exploited for commercial production. *Bacillus subtilis* produces enzymes such as proteinase, amylase, mannase, cellulase, and catalase during *natto* and *kinema* fermentation (Ueda 1989, Tamang and Nikkuni 1996). Species of *Actinomucor*, *Amylomyces*, *Mucor*, *Rhizopus*, *Monascus*, *Neurospora*, and *Aspergillus* produce various carbohydrases (enzymes) such as α-amylase, amyloglucosidase, maltase, invertase, pectinase, β-galactosidase, cellulase, hemicellulase, pentosan-degrading enzymes, acid and alkaline proteases, and lipases (Nout and Aidoo 2002). Taka-amylase A (TAA), a major enzyme produced by *A. oryzae* (present in *koji*), is well-known worldwide to be a leading enzyme for industrial utilization (Suganuma et al. 2007). *Saccharomycopsis fibuligera*, *Sm. Capsularis*, and *Pichia burtonii* have high amylolytic activities as shown in *marcha*, an ethnic amylolytic starter for alcohol production in the Himalayas (Tsuyoshi et al. 2005, Tamang et al. 2007a,b). Considerable amounts of glucoamylase are produced by *Rhizopus* spp. (Ueda and Kano 1975) and by *Sm. fibuligera* (Ueda and Saha 1983).

2.4.8 Antimicrobial Properties

The protective properties of LAB due to antimicrobial activities are useful in food fermentation, making foods safe to eat. The consumption of LAB in fermented foods without any adverse health effects confers a GRAS status and, therefore, their bacteriocins might have potential as biopreservatives (Adams 1999). LAB compete with other microbes by screening antagonistic compounds and modify the

micro-environment by their metabolism (Lindgren and Dobrogosz 1990). *Kimchi* has strong antimicrobial activities against *Listeria monocytogenes*, *Staphy. aureus*, *E. coli*, and *Salmonella typhimurium* (Kim et al. 2008a, Lee et al. 2009). Many strains of LAB isolated from *kimchi* produce antimicrobial compounds, such as leuconocin J by *Leuconostoc* sp. J2 (Choi et al. 1999), bacteriocin by *Lc. lactis* BH5 (Hur et al. 2000), and *Leuc. citreum* GJ7 (Chang et al. 2008), and pediocin by *P. pentosaceus* (Shin et al. 2008). The effect of nisin-producing LAB has been shown to inhibit the growth of *L. monocytogenes* in Camembert cheese (Maisnier-Patin et al. 1992). Bacteriocins inhibit *L. monocytogenes* in fermented sausages, cottage cheese, and smoked salmon (McAuliffe et al. 1999).

2.4.9 Medicinal Values

Many fermented foods have medicinal values. Fermented products produced by lactics and bifids have potential anticarcinogenic activity (Goldin and Gorbach 1977). The consumption of fermented foods containing viable cells of *Lb. acidophilus* decrease β-glucuronidase, azoreductase, and nitroreductase (which catalyze the conversion of procarcinogens to carcinogens), thus possibly removing procarcinogens and activating the immune system of consumers (Goldin and Gorbach 1984). The removal of procarcinogens by probiotic bacteria might involve a reduction in the rate at which nitrosamines are produced, due to the fact that certain species of *B. breve* have high absorbing properties for carcinogens, such as those produced upon charring of meat products (Mitsuoka 1989). The consumption of fermented milks containing very large populations of probiotic bacteria (~10^9 bacteria/g) by hypercholesterolemic persons has resulted in lowering cholesterol levels from 3.0 to 1.5 g/L (Homma 1988). *Kimchi* has large amounts of ascorbic acid, carotene, and dietary fiber, which have anticarcinogenic effects (Cheigh and Park 1994, Park 1995). Lactic acid produced by *kimchi* is found to prevent fat accumulation and to improve obesity-induced cardiovascular diseases (Park et al. 2008). Antioxidants are found in *kimchi* (Sim and Han 2008, Sun et al. 2009). Glycoprotein antimutagenic substances have been isolated from *Lb. plantarum* isolated from *kimchi* (Rhee and Park 2001). Several health benefits of *kimchi* have been reported such as prevention of constipation and colon cancer, and the reduction of serum cholesterol (Park et al. 2006), and *kimchi* possesses antistress properties (Lee and Lee 2009). *S*-Adenosyl-L-methionine, a bioactive material used in the treatment of depression, osteoarthritis, and liver disease (Lee et al. 2008), having antiobesity effects (Kong et al. 2008) and inhibiting atherosclerosis (Kim et al. 2008b), is also found in *kimchi*. Dietary fiber is especially important to help prevent chronic diseases, as its effects include reducing blood cholesterol, stabilizing blood sugar, regulating bowel movements, among others, and is present in *kimchi* (Lee et al. 2008). Antioxidant activities have been reported in many ethnic, fermented soybean foods such as *chungkokjang* (Shon et al. 2007), *kinema* (Tamang et al. 2009a), *natto* (Iwai et al. 2002), *douchi* (Wang et al. 2007), and *tempe* (Horii 2008). *Kefir* has antitumor activity due to its antioxidative properties (Güven et al. 2003).

Puer tea extract is known to prevent cardiovascular disease and thus mortality (Mo et al. 2008). Tea has several medicinal properties (Banerjee and Chaudhuri 2005). Acidophilus milk is used therapeutically (Kosikowski 1977). *Koumiss* is considered therapeutic, particularly in the treatment of pulmonary tuberculosis (Auclair and Accolas 1974). *Kvass* prepared in Ukraine provides protection to the digestive tract

against cancer (Wood and Hodge 1985). *Natto* prevents hemorrhage caused by vitamin K deficiency in infants (Ueda 1989). Intake of *natto* increases the serum levels of MK-7 and γ-carboxylated osteocalcin in normal individuals (Tsukamoto et al. 2000). Consumption of *tempe* reduces cholesterol levels, which is due to the inhibition of hydroxymethylglutaryl coenzyme A reductase, a key enzyme in cholesterol biosynthesis, by oleic acid and linoleic acid formed during the fermentation (Hermosilla et al. 1993). The content of γ-aminobutyric acid in *tempe* can improve the blood flow to the brain and control high blood pressure (Aoki et al. 2003). Thrombolytic activity (average 450 IU/g dry weight) has been observed in *tempe* (Sumi and Okamoto 2003). *Douchi* produces angiotensin-I-converting enzyme inhibitors having the potential to lower blood pressure (Zhang et al. 2006). In the Himalayas, ailing persons and postnatal women consume the extract of the ethnic, fermented rice product *bhaati jaanr* as it has high calorie content to regain strength (Tamang and Thapa 2006).

2.5 Conclusion

The diversity of fermented foods in the world is directly related to the food culture of each and every community and also the availability of raw materials. A survey on the consumption and production of ethnic, fermented foods and beverages in every country and the calculation of per capita consumption are urgent needs to be addressed by food policy makers of governments. The diversity of functional microorganisms ranging from mycelia fungi to enzyme-producing to alcohol-producing yeasts and both gram-positive and a few gram-negative bacteria in fermented foods has several novel properties. Ethnic, fermented foods have been prepared and consumed by the people for centuries for nutritional supplements, stability, taste, aroma, and flavor, and also for therapeutic purposes. Fermented foods are biologically important because of some of these properties.

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3

Diversity of Fermented Beverages and Alcoholic Drinks

Jyoti Prakash Tamang

CONTENTS

3.1 Introduction

Fermented beverages and alcoholic drinks are culturally and socially accepted products for consumption, drinking, entertainment, customary practices, and religious purposes. Making and drinking of alcoholic beverages are widespread interest enhancing the nutritional significance as well as imparting pleasure of drinking (Darby 1979). Wine was believed to be made in the Caucasus and Mesopotamia as early as 6000 BC, and the colonization by the Romans spread wine making all around the Mediterranean and eventually came to Northern India and China in 100 BC (Robinson 1994, Pretorius 2000). Consumption of alcoholic drinks in India has been mentioned in the *Ramayana* during 300–75 BC (Prakash 1961). Platt (1964) referred to a traditional fermented beverage as the primary example of "biological ennoblement" due to the bioenrichment with essential nutrients through fermentation. Alcoholic beverages represent a vast diversity of products ranging from ethnic, fermented beverages, alcoholic drinks, and distilled alcoholic products to wine and beer (Stewart 1987). Ethnic, alcoholic beverages have a strong ritualistic importance among the ethnic people of Asia and Africa where social activities require the provision and consumption of appreciable quantities of alcohol. Ethnic alcoholic brewing is a home-based industry mostly practiced by rural women of Asia and Africa using their native skills of alcohol fermentation. Similarly, wine has a deep-rooted cultural history for the European as well as Mediterranean ethnic people.

Alcoholic foods and beverages are prepared by starch hydrolysis, and fermentation is accomplished by amylolytic molds and yeasts, followed by alcohol-producing yeasts,

and also flavor-enhancing lactic acid bacteria. Mycelial fungi present in fermented beverages are mostly species of *Actinomucor*, *Amylomyces*, *Mucor*, and *Rhizopus* (Hesseltine 1991, Lee and Lee 2002, Nout and Aidoo 2002, Samson and Hoekstra 2002). Yeasts associated with fermented beverages are species of *Saccharomyces*, *Saccharomycopsis*, *Schizosaccharomyces*, *Pichia*, *Hansenula*, *Candida*, *Kluyveromyces*, *Debaryomyces*, *Torulopsis*, and *Zygosaccharomyces* (Pretorius 2000, Tamang and Fleet 2009). Major yeasts, which ferment saccharified cereal starch to alcohol, are *Saccharomycopsis fibuligera*, *Sm. burtonii*, *Saccharomyces cerevisiae*, and *Candida lactosa* (Dung et al. 2005). Species of *Pediococcus* and *Lactobacillus* are frequently found in some ethnic amylolytic starters and fermented beverages (Tamang et al. 2007). Yeasts are involved with the production of beer and wine, which depends on the ability of some yeast species to rapidly and efficiently ferment sugar into ethanol and their ability to tolerate an ethanol concentration of 15%–20% v/v (Kodama 1993). Unique strains of *S. cerevisiae* have evolved to conduct these fermentations, generating products with high ethanol content (12%–20%), attractive flavors, and aroma (Dung et al. 2005, 2006).

Distilled alcoholic liquor forms a large part of the market for fermented beverages. Although a diverse range of alcoholic products are available, a general scheme for their production can be presented as (1) selection of the raw material, (2) processing of the raw material to give a fermentable extract, (3) alcoholic fermentation by yeast, principally by strains of *S. cerevisiae*, (4) distillation of the fermented material to give the distillate product, and (5) post-distillation processing (Watson 1993, Bluhm 1995). In Asia, the malting process for alcohol production is rare or unknown. Wine making is not a tradition in Asia since fruits are eaten directly without extraction into juices or fermentation into wines. Dry, mixed starters containing a consortia of microorganisms are traditionally used in the fermentation of alcoholic beverages in many countries in Asia. There are several types of fermented or alcoholic beverages in the world:

1. Nondistilled and unfiltered alcoholic beverage consumed as a food produced by amylolytic starters
2. Nondistilled and filtered alcoholic beverages produced by amylolytic starters
3. Distilled alcoholic beverages produced by amylolytic starters
4. Alcoholic beverages produced using human saliva
5. Alcoholic beverages produced by monofermentation
6. Alcoholic beverages produced from honey
7. Alcoholic beverages produced from plants
8. Alcoholic beverages produced by malting (germination)
9. Alcoholic beverages prepared from fruits without distillation
10. Distilled alcoholic beverages prepared from fruits and cereals

3.2 Ethnic Amylolytic Mixed Starters

Three types of amylolytic mixed cultures or inocula are traditionally used as starters to convert cereal starch to sugars and, subsequently, to alcohol and organic acids (Hesseltine et al. 1988, Fleet 1998, Tamang and Fleet 2009).

First type: A consortium of mycelial or filamentous molds, and amylolytic and alcohol-producing yeasts and lactic acid bacteria (LAB) along with rice or wheat as the base in the form of dry, flattened or round balls of various sizes forms the first type. The starter is inoculated with a previous starter. This mixed flora is allowed to develop for a short time, then dried, and used to make either alcohol or fermented foods from starchy materials. Ethnic starters have different vernacular names such as *marcha* in India and Nepal, *ragi* in Indonesia, *bubod* in Philippines, *chiu/chu* in China and Taiwan, *loogpang* in Thailand, *nuruk* in Korea, and *men* in Vietnam (Tamang et al. 1996, Dung et al. 2007), all of which are used as starters for a number of fermentations based on rice and cassava or other cereals in Asia. There are several major types of ethnic amylolytic mixed starters in the form of dry, ball-flattened discs sold in local markets in India, Nepal, Bhutan, China, Thailand, Myanmar, Cambodia, Laos, Malaysia, Indonesia, Korea, Japan, Singapore, and Taiwan (Table 3.1). Calmette (1892) was the first to report the presence of several wild yeast species accompanied by *Amylomyces*, *Mucor*, and *Aspergillus* and 30 different bacteria in starters used in China.

Second type: A combination of *Aspergillus oryzae* and *A. sojae* are used in the form of a starter called *koji* in Japan to produce alcoholic beverages including *saké*. *Koji* also produces amylases that convert starch to fermentable sugars, which are then used for the second stage yeast fermentation to make nonalcoholic fermented soybean products called *miso* and *shoyu*, while proteases are formed to breakdown the soybean protein.

Third type: Whole-wheat flour with its associated flora is moistened and made into large compact cakes, which are incubated to select certain desirable microorganisms. The cakes are used to inoculate large masses of starchy material, which is then fermented to produce alcohol. This type of starter contains yeasts and filamentous molds, and is mostly used in China for alcohol production.

3.2.1 *Koji*

Koji is a mold culture and is prepared from steamed-cooked cereals in Japan. The term *koji* is Japanese, meaning naturally, spontaneously, or artificially molded cereals and beans (Yokotsuka 1991). The Chinese call *chu*, *shi*, or *qu* for *koji*. Rice is steamed, cooled on bamboo-made trays, stacked with gaps of about 10 cm in between to allow air circulation, inoculated with 0.1% mold spores locally called *tane-koji*, and incubated at 23°C–25°C. The rise in temperature due to the growth of mold is kept within the range of 35°C–45°C by stirring and turning the *koji* from the top to bottom on trays at about 20–40 h, normally fermented for 3 days, when mold mycelium spread throughout mass before sporulation (Lotong 1985). *Aspergillus oryzae*, *A. sojae*, *A. kawachii*, *A. shirousamii*, and *A. awamori* have been widely used as starters in the preparation of *koji* in Japan for the production of *saké*, *shoyu*, *miso*, and *shochu* (Kitamoto 2002, Matsushita et al. 2009). Among these molds, *Aspergillus oryzae* is the most important and popular in Japan, and has been used as a yellow *koji* (Suganuma et al. 2007). *A. oryzae* is used for starch saccharification in *saké* manufacture (Inoue et al. 1992). Since *koji* is not cultivated in a closed system, it is a mixture of several microorganisms. At an early stage of cultivation, the yeast grows on steamed rice grains and after that, about 20 h after inoculation of seed *koji*, the mold begins to grow. *Koji*, besides being a saccharifying and diastatic agent, also

TABLE 3.1

Ethnic Mixed Amylolytic Starters of the World

Ethnic Starter	Substrate	Appearance	Products	Organisms	Country	Status
Balan	Wheat	Dry, ball-like starter	Alcoholic drinks	Molds, yeasts	India	Homemade[a]
Bakhar	Rice, herbs	Dry, ball-like starter	Alcoholic drinks	Yeasts	India	Homemade
Bubod	Rice, wild herbs	Dry, mixed starter	Basi	Molds, yeasts, LAB	Philippines	Homemade
Chiu-yueh	Rice, wild herbs	Gray-white, dry ball	*Lao-chao*	Molds, yeasts, LAB	China, Taiwan, Singapore	Homemade
Chou or *chu*	Rice, wheat, sorghum, or barley flour	Dry, ball, cake or brick shaped	Alcoholic drinks, vinegar	Molds, acetobacter, LAB	China	Homemade
Chuzo	Rice, wild herbs	Dry, ball-like starter	Alcoholic beverage	Molds, yeasts, LAB	Mongolia	Homemade
Dhehli	Barley, 36 wild Himalayan plants	Dry, brick shaped, starter	*Sura*	Molds, yeasts	India	Homemade
Emao	Rice, herbs	Mixed starter	Alcoholic drinks	Molds, yeasts	India	Homemade
Hamei	Rice, wild herbs	Dry, mixed starter	*Atingbai*	Molds, yeasts, LAB	India	Homemade
Ipoh/siye	Rice, wild herbs	Dry, mixed starter	Alcoholic drinks	Molds, yeasts, LAB	India	Homemade
Jui paing	Rice, wild herbs	Dry, ball-like starter	*Tapai*	Molds, yeasts, LAB	Malaysia	Homemade
Loogpang	Rice, wild herbs	Dry, mixed starter	*Khao-maak*, *krachae*, *nam khao*, *ou*, *sato*	Molds, yeasts, LAB	Thailand	Homemade
Khekhrii	Germinated rice	Dry starter	*Zutho*	Yeasts, LAB	India	Homemade
Koji	Rice, wheat	Dry, black-yellow colored, mold cultured	*Saké*, *miso*, *shoyu*	*Aspergillus oryzae*, *A. sojae*, yeasts	Japan	Industrialized[b]

(*continued*)

TABLE 3.1 (continued)

Ethnic Mixed Amylolytic Starters of the World

Ethnic Starter	Substrate	Appearance	Products	Organisms	Country	Status
Marcha	Rice, wild herbs, spices	Dry, mixed starter	*Kodo ko jaanr*, *bhaati jaanr*, *gahoon ko jaanr*, *makai ko jaanr*, *raksi*	Molds, yeasts, LAB	India, Nepal	Homemade
Mana	Wheat, herbs	Dry, granulated starter	Alcoholic drinks	*A. oryzae*	Nepal	Homemade
Manapu	Rice, wheat, herbs	Dry, mixed starter	*Poko*	Molds, yeasts	Nepal	Homemade
Men	Rice, wild herbs, spices	Dry, ball-like starter	*Ruou*	Molds, yeasts, LAB	Vietnam	Homemade
Nuruk	Rice, wild herbs	Dry, mixed starter	*Takju*, *sojo*, *yakju*	Molds, yeasts, LAB	Korea	Industrialized
Phab	Wheat, wild herbs	Dry, mixed starter	*Chyang*	Molds, yeasts, LAB	India, China (Tibet), Bhutan, Nepal	Homemade
Pham	Rice, herbs	Dry, mixed starter	Alcoholic beverages	Molds, yeasts	India	Homemade
Ragi	Rice, wild herbs	Dry, mixed starter	*Tapé*	Molds, yeasts, LAB	Indonesia	Homemade
Thiat	Rice, herbs	Dry, mixed starter	*Kiad-lieh*	Molds, yeasts, LAB	India	Homemade

[a] Homemade means the method of preparation is traditional and is prepared on a home scale.

[b] Industrialized means the method of preparation is upgraded, and the product is industrialized and commercialized.

contributes to the color, flavor, and aroma of the fermented foods that are important for their overall attributes (Kitamoto 2002). Tanaka (1982) studied enzyme activity of steamed or uncooked glutinous rice–*koji* inoculated with *A. oryzae* and *Rhizopus jawanicus* and found α-amylase to be 1527 U/g in *Aspergillus* and 100 U/g in *Rhizopus* in steamed rice *koji*, whereas 1255 U/g and 100 U/g in uncooked rice *koji*, respectively. Antioxidant activity has been observed in *koji* prepared with *Aspergillus awamori* (Lee et al. 2007) and with *Aspergillus candidus* (Yen et al. 2003).

3.2.2 *Marcha*

Marcha or *murcha* (the correct spelling is *marcha* however) is not a food but is a mixed dough inocula prepared as a dry, round to flattened, creamy white to dusty white, solid ball which is used as an amylolytic starter to produce ethnic alcoholic beverages in the Himalayan regions of India, Nepal, Bhutan, and Tibet in China (Tamang 2010). During *marcha* preparation, glutinous rice is soaked in water for 8–10 h, and soaked rice is crushed in a foot-driven heavy wooden mortar by a pestle. In ground rice, various ingredients are added which include roots of *Plumbago zeylanica*, leaves of *Buddleja asiatica*, flowers of *Vernonia cinerea*, ginger, red dry chilli, and 1%–2% of previously prepared *marcha* as the mother culture. The mixture is made into a paste by adding water and kneading it into flat cakes of varying sizes and shapes. These are then placed individually on the ceiling, above the kitchen, made up of bamboo strips inlaid with fresh fronds of ferns (*Glaphylopteriolopsis erubescens*), covered with dry ferns and jute bags, and are left to ferment for 1–3 days. A distinct alcoholic and ester aroma and puffy/swollen appearance of the *marcha* indicate completion of fermentation, and fresh cakes of *marcha* are sun dried for 2–3 days (Tamang et al. 1996). *Marcha* is stored at room temperature and in a dry place for more than a year. The Khampa community of Tibet (Bhatia et al. 1977) prepares *phab*, which is similar to *marcha*.

Marcha-making technology reflects the native skill of ethnic people on subculturing of desirable inocula (microorganisms consisting of filamentous molds, amylolytic, and alcohol-producing yeasts and species of LAB) from a previous batch to a new culture using rice or wheat as a starchy base or medium. This indigenous technique of "microbiology" preserves the functional microorganisms necessary for fermentation of starchy substrates to alcoholic beverages in the Himalayas. Kobayashi et al. (1961), who reported *Rhizopus oryzae*, *Mucor praini*, and *Absidia lichtheimi* in *marcha*, carried out the first preliminary study of the microbiology of *marcha* samples collected from Sikkim. Batra and Millner (1974) reported *Hansenula anomala* var. *schneggii* (=*Pichia anomala*) in *marcha* collected from Kalimpong in the Darjeeling hills. Hesseltine et al. (1988) isolated *Mucor* and *Rhizopus* spp. from *marcha* samples in Nepal. Uchimura et al. (1990) reported the dominant yeast to be *Saccharomycopsis*, and also molds *Penicillium* sp. and *Aspergillus* sp. in *chang-poo* or *phab*, a Bhutanese *marcha*. The population of filamentous molds in *marcha* was 10^6 cfu/g, whereas the yeast and lactic acid bacteria loads were 10^8 and 10^7 cfu/g, respectively.

Filamentous mold species present in *marcha* are *Mucor circinelloides* forma *circinelloides*, *Mucor* sp. close to *M. hiemalis* and *R. chinensis*, and *R. stolonifer* variety *lyococcus* (Tamang et al. 1988). *Mucor* spp. are more prevalent than species of *Rhizopus* in *marcha*. *Aspergillus*, *Penicillium*, *Amylomyces*, and *Actinomucor*

are not present in *marcha* prepared in the Himalayan regions. Yeasts present in *marcha* are *Saccharomycopsis fibuligera*, *Sm. capsularis*, *Saccharomyces cerevisiae*, *S. bayanus*, *Pichia anomala*, *P. burtonii*, and *Candida glabrata* (Tsuyoshi et al. 2005). *Sm. fibuligera*, *Sm. capsularis*, and *P. burtonii* have high amylolytic activities indicating that these may be amylolytic yeasts, whereas *S. bayanus*, *C. glabrata*, and *P. anomala* are alcohol-producing yeasts (Tsuyoshi et al. 2005, Tamang et al. 2007). *Sm. fibuligera* is the most dominant yeast in *marcha* (Tamang and Sarkar 1995), and is typically found growing on cereal products (Hesseltine and Kurtzman 1990). Saccharifying activities are mostly shown by *Rhizopus* spp. and *Sm. fibuligera* whereas liquefying activities are shown by *Sm. fibuligera* and *S. cerevisiae* (Thapa 2001). *Rhizopus* spp. and *Sm. fibuligera* degrade cereal starch and produce glucose, and then alcohol-producing yeasts species of *Saccharomyces* and *Pichia* rapidly grow on the resulting glucose to produce ethanol. A considerable amount of glucoamylase is produced by *Rhizopus* spp. (Ueda and Kano 1975) and by *Sm. fibuligera* (Ueda and Saha 1983). Among LAB, *P. pentosaceus*, *Lb. bifermentans*, and *Lb. brevis* are present in *marcha* (Hesseltine and Ray 1988, Tamang and Sarkar 1995, Tamang et al. 2007). Pediococci are more dominant LAB than lactobacilli in *marcha* (Tamang et al. 2007). LAB present in *marcha* play a role in imparting flavor, antagonism, and acidification of substrates.

3.2.3 *Ragi*

Ragi is an amylolytic starter from Indonesia in the form of dry and flat cakes (Saono et al. 1974). During the production of *ragi*, rice or millet or cassava or other starchy bases are milled, mixed with herbs and spices, roasted together, and then sieved. The mixture is added with water and 2%–4% powder of old *ragi*, mixed thoroughly, shaped into balls, and fermented at 25°C–30°C for 72 h in a humid environment. Fermented balls are sun dried and used as a starter for the production of alcoholic beverages and drinks in Indonesia. Went and Prinsen-Geerligs (1895) were the first to report *Monilia javanicus* (=*Pichia anomala*) and *Saccharomyces cerevisiae* as the principal yeasts in *ragi*. Dwidjoseputro and Wolf (1970) reported *Candida parapsilosis*, *C. melinii*, *C. lactosa*, *Hansenula subpelliculosa*, *H. anomala*, and *H. malanga* in *ragi*. The addition of spices to *ragi* contributes other microorganisms and also inhibits the growth of undesirable microorganisms (Soedarsono 1972). Saono et al. (1974) conducted studies on *rag*i and its fermented products such as *tape keté la*, *tapé ketan hitam*, *oncom hitam*, and *oncom mérah* from various places in West Java, Indonesia, and reported that *Candida* spp was dominant among yeasts, and *Mucor* spp. and *Rhizopus* spp were dominant among molds. Kato et al. (1976) studied the properties of glucoamylase of *Saccharomycopsis fibuligera* isolated from *ragi*. Saono and Basuki (1978) reported 13 species of *Candida* from *ragi*. Hadisepoetro et al. (1979) reported that the population of yeasts in three *ragi* samples was 10^6–10^7 cfu/g that of bacteria was 10^4–10^5 cfu/g, and that of mold was 10^4 cfu/g. Ardhana and Fleet (1989) found *C. pelliculosa* and *Amylomyces rouxii* in four samples of *ragi*. Ishimaru and Nakano (1960) isolated *Enterococcus faecalis*, *Lb. plantarum*, and *P. pentosaceus* in *ragi* in the range of 10^5–10^8 cfu/g. *P. pentosaceus* and *E. faecalis* are the dominant LAB in *ragi*, which may produce secondary products from the glucose formed by the amylolytic yeasts and

molds always found in the starters (Hesseltine and Ray 1988). *Bacillus coagulans*, *B. brevis*, *B. stearothermophilus*, and an unidentified species of *Acetobacter* were also reported from *ragi* at the level of 10^3–10^4 cfu/g (Ardhana and Fleet 1989). Improved *ragi* has been prepared by using pure strains of *A. rouxii* AU3 and CB3, *S. cerevisiae* RM-1 and K-3 and *Rhizopus formosaensis* FIRD (Saono et al. 1984). Elegado and Fujio (1993) isolated two polygalacturonase-producing strains of *Rhizopus* spp from *ragi* and studied the enzyme's stability in a wide range of pH from 2 to 11 and its tolerance at 50°C for 20 min. Uchimura et al. (1991b) revealed that there is a high variability rate of *P. pentosaceus* in older *ragi* than fresh *ragi*, and the result suggested that rod-shaped bacteria cannot survive for a long time under dry conditions in *ragi*. Although *S. bayanus* species has not been isolated from any other Asian amylolytic starter, the closely related *S. cerevisiae* species has been isolated from *ragi* (Hesseltine et al. 1988). Filamentous molds, amylolytic yeasts, and some LAB are coexited as dominant microorganisms, which include species of *Rhizopus*, *Mucor*, *Amylomyces*, *Aspergillus*, *Saccharomycopsis*, *Candida*, *Saccharomyces*, *Hansenula*, and *Pediococcus* (Yokotsuka 1991).

3.2.4 *Bubod*

Bubod is used as an ethnic amylolytic starter in the Philippines. During its production, rice and ginger are powdered, and mixed thoroughly with enough water to have a consistency that permits rolling the material into a ball and flattening it (Tanimura et al. 1977). The discs are coated with 1–3 month old *bubod* and incubated in rice straw for 36 h at room temperature and are sun dried. The population of molds in *bubod* was 10^3–10^5 cfu/g, yeasts 10^7–10^8 cfu/g, and LAB 10^5–10^7 cfu/g (Sanchez 1986). *Mucor circinelloides*, *M. grisecyanus*, *Rhizopus cohnii*, *Saccharomyces cerevisiae*, and *Saccharomycopsis fibuligera* have been reported from *bubod* (Kozaki and Uchimura 1990); however, *Sm. fibuligera* is the dominant amylolytic yeast in *bubod* (Hesseltine and Kurtzman 1990). The activated starter for production of *basi*, a mild alcoholic beverage consumed as a food in the Philippines, is called *binubudan* (Tanimura et al. 1978). Rice is cooked with water so that the grains remain separate, any lumps broken up, and the rice is cooled to 40°C–45°C. Then it is inoculated with 300 g powdered *bubod* for 4 kg rice. The rice and *bubod* are mixed in a clean basin and covered with banana leaves and then with a clean cheese cloth, and fermentation continues for 24 h to get a fresh activated starter called *binubudan*.

3.2.5 *Nuruk*

Nuruk is an ethnic amylolytic starter from Korea. Historically, the substrate for *nuruk* was rice but at present it is wheat (Park et al. 1977). Generally *nuruk* is prepared by natural inoculation of molds, bacteria, and yeasts; however, it can be prepared by inoculation with *Aspergillus usamii*. Traditionally, *nuruk* is prepared by moistening wheat flour, kneading and molding into balls, and fermented for 17 days at 30°C–45°C, dried for 2 weeks, and cured for 1–2 months at room temperature (Park et al. 1977). Kim (1968) isolated *Aspergillus oryzae* (10^7 cfu/g), *A. niger* (10^7 cfu/g), *Rhizopus* sp. (10^6 cfu/g), anaerobic bacteria (10^7 cfu/g), aerobic bacteria (10^6–10^7 cfu/g), and yeasts (10^5 cfu/g) from *nuruk*.

3.2.6 *Loogpang*

Loogpang is an ethnic amylolytic starter from Thailand, which is commonly used to prepare alcoholic drinks and vinegar. The main ingredient of *loogpang* is rice flour with the addition of different types of spices and microorganisms. The microorganisms originate from the inoculum, from the surroundings, or from the previous batch (Vachanavinich et al. 1994). Species of molds present in *loogpang* are *Amylomyces*, *Rhizopus*, *Aspergillus*, *Mucor*, and *Absidia* (Pichyangkura and Kulprecha 1977), and yeasts and LAB are *Saccharomycopsis fibuligera*, *Hansenula*, *Saccharomyces*, and *Pediococcus* (Dhamcharee 1982, Uchimura et al. 1991a). *Sm. fibuligera* isolated from *loogpang* has high glucoamylase activity (Sukhumavasi et al. 1975).

3.2.7 *Men* or *Banh*

Men or *banh men* is an ethnic amylolytic starter from Vietnam that is used to produce traditional alcoholic beverages and a drink called *ruou* (Dung 2004). During the preparation of *men*, uncooked rice flour is mixed with local herbs and spices and moistened with a little amount of water to form dough, which is then made into small balls or flattened discs. The dough is spread on a bamboo tray and mixed with powdered *men*. The inoculated dough discs are incubated at room temperature for a few days. Molds such as *Amylomyces rouxii* and *Rhizopus* spp.; yeasts such as *Saccharomycopsis fibuliger*, *Hyphopichia burtonii*, and *Saccharomyces cerevisiae*; and LAB are found in *men* (Dung et al. 2006, 2007). *S. bayanus* has not been isolated from *men*, but the closely related *S. cerevisiae* has been isolated from *banh men* (Lee and Fujio 1999). On the basis of a PCR-mediated DGGE system, several yeasts and LAB have been isolated from *banh men* that consist of amylase producers (*Rhizopus oryzae*, *R. microsporus*, *Absidia corymbifera*, *Amylomyces* sp., and *Saccharomycopsis fibuligera*), ethanol producers (*Saccharomyces cerevisiae*, *Issatchenkia* sp., *Pichia anomala*, *P. ranongensis*, *Candida tropicalis*, and *Clavispora lusitaniae*), yeast contaminants (*Xeromyces bisporus*, and *Botryobasidium subcoronatum*), LAB (*Pediococcus pentosaceus*, *Lactobacillus plantarum*, *Lb. brevis*, *Weissella confusa*, and *W. paramesenteroides*), amylase-producing bacilli (*Bacillus subtilis*, *B. circulans*, *B. amyloliquefaciens*, and *B. sporothermodurans*), acetic acid bacteria (*Acetobacter orientalis*, and *A. pasteurianus*), and environmental contaminants (*Burkholderia ubonensis*, *Ralstonia solanacearum*, and *Pelomonas puraquae*) (Thanh et al. 2008).

3.2.8 *Chiu-Yueh*

Chiu-yueh or *peh-yueh* is a Chinese amylolytic starter for *lao-chao*, a fermented rice product (Wang and Hesseltine 1970). It is a gray-white ball containing yeasts and fungi grown on rice flour and is closely related to the Indonesian *ragi*. *Rhizopus*, *Amylomyces*, *Torulopsis*, and *Hansenula* species are present in *chiu-yueh* (Wei and Jong 1983).

3.2.9 *Hamei*

Hamei is an ethnic amylolytic mixed dry, round or flattened starter from Manipur, India, that is very similar to *marcha* (Tamang 2010). *Hamei* is used to prepare a rice-based beverage locally called *atingba* and a distilled clear liquor called *yu* in

Manipur. During its production, local varieties of raw rice, without soaking or soaking for 30 min, and then dried, are crushed and mixed with the powdered bark of *yangli* (*Albizia myriophylla* Benth.) and a pinch of previously prepared powdered *hamei*. The mixture is handpressed into discs that determines the shape, sizes, and forms of *hamei* as per the choice of the *hamei* makers. The pressed cakes are kept over paddy husk or paddy straw in a bamboo basket, covered by a sackcloth for 2–3 days at room temperature (20°C–30°C), and then sun dried for 2–3 days. Yeast species present in *hamei* are *Pichia anomala*, *Saccharomyces cerevisiae*, and *Trichosporon* (Jeyaram et al. 2008). *Pediococcus pentosaceus* and *Lactobacillus plantarum* are present in *hamei* (Tamang et al. 2007). Pediococci are the more dominant lactobacilli in *hamei*. *P. pentosaceus* HS:B1 is found to produce bacteriocin against *Listeria innocua* and *L. monocytogenes* at levels of 128 and 32 AU/mL, respectively (Tamang et al. 2007). LAB strains of *hamei* do not produce biogenic amines, which is a good indicator for a starter culture. LAB in *hamei* impart flavor, exert antagonism, and acidify substrates (Tamang et al. 2007).

3.2.10 *Mana*

Mana is a granular-type starter prepared from wheat flakes in Nepal (Tamang 2010). During its production, wheat grains are soaked in water overnight, steamed for 30 min, and are transferred to a bamboo basket, drained, and ground into a lump. The floor is cleaned; straw is spread on the ground, and the wheat lump is placed over it, covered with paddy straw or straw mat, and fermented for 6–7 days. After 7 days, a green mold appears on the wheat grains and is dried in the sun to get *mana* and is stored. *Mana* contains 10^6 cfu/g of mucorales, 10^7 cfu/g of aspergilli, 10^3 cfu/g of yeasts, and 10^5 cfu/g of LAB (Nikkuni et al. 1996). *Aspergillus oryzae* and *Rhizopus* spp. are present in *mana* (Nikkuni et al. 1996, Shrestha et al. 2002). None of the amylolytic starters of the Himalayas have *Aspergillus* except *mana*, which is very significant.

3.2.11 *Manapu*

Manapu is an ethnic amylolytic starter from Nepal similar to *marcha* that is prepared from rice flour and millets (Tamang 2010). Rice or millet is milled to get flour, and is mixed with 20% old *manapu*, 5% *manawasha* (white flower of a wild plant), and 5% black pepper. It is then kneaded to prepare a cake and placed on straw, which is then covered by straw and fermented at 30°C–33°C for 5–7 days. Freshly fermented dough is sun dried to get *manapu*. The microorganisms present in *manapu* are *Saccharomyces cerevisiae*, *Candida versatilis*, *Rhizopus* spp., and *P. pentosaceus* (Shrestha et al. 2002). Yeast and LAB loads in *manapu* are 10^5–10^9 cfu/g, and the mold population is 10^7 cfu/g (Shrestha et al. 2002).

3.3 Nondistilled Mild Alcoholic Beverage Consumed as a Food Produced by Amylolytic Starters

Ethnic, fermented beverages with mild to desirable alcohol contents produced by ethnic amylolytic starters are consumed directly after liquefaction, saccharification, and alcohol fermentation without distillation and unfiltered. The biological process of

liquefaction and saccharification of cereal starch by aerobic molds and yeasts, supplemented from ethnic amylolytic starters, under solid-state fermentation is one of the two major stages of production of alcoholic beverages in Asia. These alcoholic beverages are mostly considered as a food and are consumed as a staple food in many parts of Asia as they provide high calories (Table 3.2). Some categories of alcoholic beverages are listed in the table.

3.3.1 *Kodo Ko Jaanr*

Kodo ko jaanr or *chyang* is one of the most common ethnic, fermented *kodo* or finger millet (*Eleusine coracana*) beverages of the Himalayan regions in India (Sikkim, Darjeeling hills, Himachal Pradesh, Ladakh, Arunachal Pradesh), and Nepal, Bhutan, and China (Tibet) with a mild alcoholic and sweet taste (Tamang 2005). The custom of alcohol drinking in Sikkim and Darjeeling hills in India has been described in a few historical documents (Hooker 1854, O'Malley 1907). *Chyang* or *chhang* or *lugri* is prepared from a huskless local variety of barley (*Hordeum nulum*) called *sherokh* in Ladakh, India (Bhatia et al. 1977). *Chiang* or *lugri*, a barley-based fermented beverage, is a mild alcoholic, thick, translucent, foamy drink with a sweet–sour taste and a somewhat aromatic flavor (Batra and Millner 1976, Batra 1986). During its production, finger millet seeds are cleaned, washed, and cooked for about 30 min; the excess water is drained off, and the cooked millets are spread on a bamboo mat for cooling. About 1%–2% of powdered *marcha* is sprinkled over the cooked seeds, mixed thoroughly, and packed in a bamboo basket lined with fresh fern (*Thelypteris erubescens*), and then covered with sackcloths, and fermented at room temperature for 2–4 days. The saccharified mass is transferred into an earthen pot or bamboo basket, made airtight, and fermented for 3–4 days during summer and 5–7 days in winter at room temperature for alcohol production. Freshly fermented *kodo ko jaanr* is filled into a bamboo-made vessel locally called *toongbaa*, and lukewarm water is added up to its brim and left for 10–15 min. Then, the milky white extract of *jaanr* is sipped through a narrow bamboo straw called *pipsing*, which has a hole on the side, near the bottom, to avoid passing of grits into the straw. Water is added twice or thrice to the vessel after sipping the extract. Consumption of fermented finger millet beverages in an exclusively decorated bamboo or wood-made vessel called *toongbaa* is unique in the Himalayas (Tamang 2010). *Kodo ko jaanr* liquor is believed to be a good tonic for ailing persons and postnatal women. After consumption, the residues or grits of *kodo ko jaanr* are used as fodder for pigs and cattle. This is a good example of complete utilization of a substrate as a food and a fodder as the discarded grits contain nutrients.

Marcha is used as an amylolytic starter supplement containing all functional microorganisms in *kodo ko jaanr* fermentation (Thapa 2001). Mycelial molds have roles only in the initial phase of fermentation, mostly in saccharification of the substrates. Yeasts such as *Pichia anomala*, *Saccharomyces cerevisiae*, *Candida glabrata*, *Saccharomycopsis fibuligera*; and LAB such as *Pediococcus pentosaceus* and *Lactobacillus bifermentans* have been recovered in *kodo ko jaanr* samples. There is no report of occurrence of enterobacteriaceae, *Bacillus cereus*, or *Staphylococcus aureus* in *kodo ko jaanr* (Thapa and Tamang 2004). The pH of *kodo ko jaanr* is 4.1, moisture content is 69.7%, acidity 0.3%, and alcohol content is 5.0% (Thapa and Tamang 2004). The population of filamentous molds, which originated from *marcha*, declines daily during *in situ* fermentation of *kodo ko jaanr* and finally disappears

TABLE 3.2

Some Alcoholic Beverages and Drinks of the World

Beverage	Substrate	Sensory Property and Nature	Use	Starter/ Organisms	Country	Status
Aara	Cereals	Clear distilled liquor	Alcoholic drink	Unknown	India	Homemade[a]
Aarak	Barley, millet	Distilled from *chyang*, clear liquor	Alcoholic drink	*Phab*	India, China (Tibet), Bhutan	Homemade
Atingba	Rice	Mildly alcoholic, sweet–sour	Beverage consumed as a food	*Hamei*	India	Homemade
Apong	Rice	Mildly alcoholic	Beverage consumed as a food	*Phab*	India	Homemade
Bagni	Millet	Liquid	Alcoholic drink	LAB, Yeasts	Russia	Homemade
Bantu beer	Sorghum, millet	Opaque appearance, sour flavor	Beer	LAB, Yeasts	South Africa	Industrialized[b]
Basi	Sugarcane	Clear or cloudy liquid	Alcoholic drink	*Bubod*, *binubudan*	Philippines	Industrialized
Bhaati jaanr	Rice	Mildly alcoholic, sweet–sour, paste	Beverage consumed as a food staple food	*Marcha*	India, Nepal	Homemade
Bhang-chyang	Maize, rice, barley	Extract of *mingri*	Alcoholic beverages	*Phab*	India	Homemade
Brandy	Fruit juice	Distillates of fermented fruit juices	High-alcohol drink	*S. cerevisiae*	Worldwide	Industrialized
Brem	Rice	Dried, sweet–sour, mild alcoholic product	Dried	*Ragi*	Indonesia	Homemade/ industrialized
Bouza	Wheat, malt	Alcoholic thin gruel	Alcoholic drink	LAB	Egypt	Homemade/ industrialized
Boza	Wheat, rye, millet, maize	Cooked slurry	Beverage consumed as a food	LAB, yeasts	Bulgaria, Romania, Turkey, Albania	Industrialized
Bussa	Maize, sorghum, finger millet	Alcoholic thin gruel	Refreshing drink	Yeasts, LAB	Kenya	Homemade
Bushera	Sorghum, millet	Slurry	Beverage consumed as a food	Yeasts, LAB	Uganda	Homemade
Buza	Barley	Thick liquor	Alcoholic drink	*Phab*	India	Homemade

(*continued*)

TABLE 3.2 (continued)

Some Alcoholic Beverages and Drinks of the World

Beverage	Substrate	Sensory Property and Nature	Use	Starter/ Organisms	Country	Status
Champus	Maize	Mild alcoholic beverage	Drink	Yeasts	Colombia	Homemade
Cider	Apple	Clear alcoholic drink	Drink	Yeasts	France, Spain, Ireland, Slovenia	Industrialized
Chyang/chee	Finger millet/barley	Mildly alcoholic, slightly sweet, acidic	Beverage consumed as a food	*Phab*	China (Tibet), Bhutan, Nepal, India	Homemade
Chulli	Apricot	Filtrate, clear	Alcoholic drink	Yeast	India	Homemade
Darassun	Millet	Liquid	Alcoholic drink	LAB, yeasts	Mongolia	Homemade
Daru	Cereal	Alcoholic beverages, filtrate	Jiggery	Yeast, LAB	India	Homemade
Duizou	Red rice	Fermented rice beverage	Alcoholic drink	Yeast, LAB	India	Homemade
Ennog	Rice, paddy husk	Black rice beer	Alcoholic drink	Yeast, LAB	India	Homemade
Ewhaju	Rice	Nondistilled, filtered and clarified, clear liquor	Alcoholic drink	*Nuruk*	Korea	Homemade/ industrialized
Faapar ko jaanr	Buck wheat	Mildly acidic, alcoholic	Beverage consumed as a food	*Marcha*	India, Nepal	Homemade
Feni	Cashew apple	Distilled wine from cashew apples, strong flavor	Alcoholic drink	*S. cerevisiae*	Worldwide	Industrialized
Gahoon ko jaanr	Wheat	Mildly acidic, alcoholic	Beverage consumed as a food	*Marcha*	India, Nepal	Homemade
Gin	Maize, rye, barley	Clear, high-alcohol distillate from fermented maize, flavored with juniper berries	Alcoholic drink	*S. cerevisiae*	Worldwide	Industrialized
Gowé (*Sifanu*)	Sorghum	Alcoholic drink, cooked slurry	Beverage	Yeasts, LAB	Benin	Homemade
Jao ko jaanr	Barley	Mildly acidic, alcoholic	Beverage consumed as a food	*Marcha*	India, Nepal	Homemade
Jou	Rice	Mild-alcoholic beverage	Alcoholic drink	Yeasts, LAB	India	Homemade
Kachasu	Wild fruit (*Ziziphus mauritiana*)	Distilled, high-alcohol-content drink	Distilled, clear alcoholic drink	Yeasts, LAB	Zimbabwe	Homemade

Kaffir beer (same as *Bantu* beer)	Sorghum, millet	Opaque appearance, sour flavor	Beer	LAB, yeasts	South Africa	Industrialized
Kanji	Carrot/beet roots	Strong flavored	Alcoholic drink	*Torani* contains LAB, yeasts	India	Homemade
Khao maak	Rice	Juicy, white colored, sweet taste, mildly alcoholic	Dessert	*Loogpang*	Thailand	Homemade
Kiad lieh	Rice	Distilled liquor, clear	Alcoholic drink	*Thiat*	India	Homemade
Krachae	Rice	Nondistilled and filtered liquor	Alcoholic drink	*Loogpang*	Thailand	Industrialized
Kodo ko jaanr	Finger millet	Mildly alcoholic, sweet, acidic	Alcoholic beverage	*Marcha*	India, Nepal	Homemade
Lao chao	Rice	Sweet–sour, mildly alcoholic, paste	Dessert	*Chiu yueh*	China	Homemade/ industrialized
Lohpani	Maize, rice, barley	Alcoholic liquor	Alcoholic beverage	Unknown	India	Homemade
Lugri	Barley	Sweet–sour, mildly alcoholic, thick liquid	Alcoholic beverage	*Phab*	India, China (Tibet)	Homemade
Madhu	Rice	Distilled liquor	Alcoholic drink	Yeast, mold	India	Homemade
Mangisi	Maize	Liquor	Alcoholic drink	Yeast, LAB	Zimbabwe	Homemade
Makai ko jaanr	Maize	Mildly alcoholic, sweet–sour	Beverage consumed as a food	*Marcha*	India, Nepal	Homemade
Mbege	Malted millet	Acidic, mildly alcoholic	Alcoholic drink	Yeast, LAB	Tanzania	Homemade
Merrisa	Millet, cassava	Turbid drink	Beer-like	Yeasts, LAB	Sudan	Homemade
Mingri	Maize, rice, barley	Sweet, mildly alcoholic, thick	Beverage consumed as a food	*Phab*	India	Homemade
Nam khao	Rice	Distilled liquor	Alcoholic drink	*Loogpang*	Thailand	Homemade
Nareli	Coconut palm	Sweet, milky, effervescent, mildly alcoholic	Alcoholic beverage	Yeasts, LAB	India	Homemade
Nchiangne	Red rice	Distilled liquor	Alcoholic drink	*Khekhrii*	India	Homemade
Oh	Rice, millet	Soft, mild-alcoholic beverage	Alcoholic drink	Unknown	India	Homemade
Ou	Rice	Distilled liquor	Alcoholic drink	*Loogpang*	Thailand	Homemade
Palm wine/toddy	Palm sap	Sweet, milky, effervescent, and mildly alcoholic	Alcoholic beverage	Yeasts, LAB	Palm-growing regions	Homemade/ industrialized
Poko	Rice	Sweet, acidic, mildly alcoholic	Beverage consumed as a food	*Manapu*	Nepal	Homemade

(*continued*)

TABLE 3.2 (continued)

Some Alcoholic Beverages and Drinks of the World

Beverage	Substrate	Sensory Property and Nature	Use	Starter/ Organisms	Country	Status
Pona	Rice	Mildly alcoholic, sweet–sour, paste	Beverage consumed as a food	Molds, yeast, LAB	India	Homemade
Pulque	Agave juice	White, viscous, acidic, alcoholic	Refreshing drink	Yeasts, LAB	Mexico	Industrialized
Raksi	Cereals	Clear distilled liquor	Alcoholic drink	*Marcha*	India, Nepal	Homemade
Rum	Molasses	Distilled liquor, clear	Alcoholic drink	*S. cerevisiae*	Worldwide	Industrialized
Ruou de	Rice	Distilled liquor, clear	Alcoholic drink	*Men*	Vietnam	Homemade
Ruou nep	Rice	Distilled liquor, clear	Alcoholic drink	*Men*	Vietnam	Homemade
Ruou nep than	Rice (purple)	Nondistilled fermented rice, viscous, thick	Beverage consumed as a food	*Men*	Vietnam	Homemade
Ruou nep chan	Rice, maize, cassava	Nondistilled fermented rice, viscous, thick, sometimes distilled	Alcoholic beverage	*Men*	Vietnam	Homemade
Ruhi	Rice	Distilled liquor	Alcoholic beverage	Yeasts	India	Homemade
Saké	Rice	Nondistilled, clarified, and filtered liquor	Alcoholic drink	*Koji*	Japan	Industrialized
Sato	Rice	Distilled liquor	Alcoholic drink	*Loogpang*	Thailand	Homemade
Shochu	Rice	Distilled spirit	Alcoholic drink	*Koji*	Japan	Industrialized
Shoto saké	Sugarcane	Liquor	Alcoholic drink	*Koji*	Japan	Industrialized/ homemade
Simal tarul ko jaanr	Cassava tuber	Mildly alcoholic, sweet–sour	Beverage consumed as a food	*Marcha*	India, Nepal	Homemade
Sing sing	Barley	Beverage	Alcoholic drink	Yeasts	India	Homemade
Soju	Rice	Distilled liquor	Alcoholic drink	*Nuruk*	Korea	Industrialized
Sparkling wine or champagne	Grapes	Clear and flavored	Alcoholic drink	*S. cerevisiae*	Worldwide	Industrialized

Sura	Finger millet	Alcoholic	Staple food	*Dhehli*	India	Homemade
Takju	Rice, wheat, barley, maize	Alcoholic	Beverage	*Nuruk*	Korea	Industrialized
Tapuy	Rice	Sweet, sour, mildly alcoholic	Dessert	*Bobod*	Philippines	Industrialized
Tapai pulut	Rice	Sweet, sour, mildly alcoholic	Dessert	*Ragi* or *jui-piang*	Malaysia	Industrialized
Tapai ubi	Cassava	Sweet, sour, mildly alcoholic	Dessert	*Ragi* or *jui-piang*	Malaysia	Industrialized
Tapé- kekan	Rice, cassava, maize, millet	Sweet–sour alcoholic paste	Dessert, Beverage consumed as a food	*Ragi*	Indonesia	Industrialized
Tari	Date palm	Sweet, alcoholic beverage	Beverage	Yeasts, LAB	India	Homemade
Tchoukoutou	Red sorghum	Effervescent, sweet	African beer	Yeast	Benin	Homemade
Tequila	Agave juice	Effervescent, sweet	Alcoholic drink	Yeast		Homemade
Themsing	Finger millet, barley	Mildly alcoholic, sweet	Alcoholic beverages	Molds, yeasts	India	Homemade
Tien-chiu-niang	Rice	Mildly alcoholic, sweet	Alcoholic beverage	*Chiu-yueh*	China, Taiwan	Industrialized
Tari	Palmyra and date palm sap	Sweet, milky, effervescent, and mildly alcoholic	Alcoholic beverage	Yeasts, LAB	India	Homemade
Togwa	Maize	Cooked slurry	Beverage	Yeasts, LAB	East Africa	Homemade
Vodka	Mashed potato	Clear, distillate, flavored, high-alcohol-content spirit	Alcoholic drink	*S. cerevisiae*	Russia, Poland, Finland	Industrialized
Whisky	Barley	Distillate, clear liquor from fermented malted barley	Alcoholic drink	*S. cerevisiae*	Worldwide	Industrialized
Wine	Grapes	Red, white, flavored, clear	Alcoholic drink	Yeasts	Worldwide	Industrialized
Yakju	Rice, wheat, barley, maize	Alcoholic	Beverages	*Nuruk*	Korea	Industrialized
Yu	Rice	Distilled from *atingba*, clear	Alcoholic drink	*Hamei*	India	Homemade
Zu	Rice	Distilled from fermented rice, clear liquor	Alcoholic drink	Yeasts, LAB	India	Homemade
Zutho/zhuchu	Rice	Milky white, sweet–sour, mildly alcoholic	Alcoholic beverage	*Khekhrii*	India	Homemade

[a] Home-made means prepared at home using indigenous knowledge of production.
[b] Industrialized means that the method of preparation is upgraded and commercialized.

after the fifth day (Thapa and Tamang 2006). The yeast load increases from 10^5 cfu/g to 10^7 cfu/g within the second day, indicating its role in amylase production during fermentation. *P. pentosaceus* and *Lb. bifermentans* loads increase significantly to 10^8 cfu/g on the first day and decrease to a level of 10^3 cfu/g at the end of the fermentation. Acidity increases during the fermentation with decrease in pH. The cause of increase in acidity and the consequent drop in pH during the fermentation of cereal is likely due to the utilization of free sugars of the substrate by yeasts and LAB, since all the strains of *marcha* were able to ferment glucose (Thapa and Tamang 2004). Alcohol and reducing sugar contents increase significantly till the third day followed by a decrease in the total sugar content. This is due to maximum breakdown of starch from substrates to reducing sugars by amylolytic enzymes, produced by molds and yeasts during fermentation. Maximum activities of saccharification (glucoamylase) and liquefaction (α-amylase) of finger millets are observed on the second day of fermentation. Saccharifying activities are mostly shown by *Rhizopus* spp. and *Sm. fibuligera* whereas liquefying activities are shown by *Sm. fibuligera* and *S. cerevisiae* (Thapa and Tamang 2006). *Sm. fibuligera* and *R. chinensis s*accharify and liquefy millet starch into glucose and produce alcohol during the *in situ* fermentation of *kodo ko jaanr.*

Kodo ko jaanr prepared by a combination of pure strains of *R. chinensis* MJ:R3 and *S. cerevisiae* MJ:YS2 has a mild alcoholic sweet flavor, which is acceptable to consumers (Thapa and Tamang 2006). *S. cerevisiae* possesses a strong tendency to ferment sugars into alcohol (Kozaki and Uchimura 1990). Although *jaanr* prepared by a mixture of *R. chinensis* MJ:R3 and *Sm. fibuligera* KJ:S5 has a sweet–sour taste due to low-alcohol content, the product has an unpleasant odor, which is unacceptable to consumers. *Sm. fibuligera* gives a high yield of biomass during the fermentation of cassava starch, which leads to a low ethanol yield (Reddy and Basappa 1996). To make good quality *kodo ko jaanr* or *chyang*, a consortium of a selected strain of mold (*Rhizopus*) and an amylolytic alcohol-producing yeast (*S. cerevisiae*) is recommended. The fermentation of finger millet enhances the bioenrichment of minerals such as Ca, Mg, Mn, Fe, K, and P, contributing to mineral intake in the daily diet of the rural people of the Himalayas (Thapa and Tamang 2004). The Himalayan fermented cereal beverages give more than 400 kcal (100 g/dry matter) of energy (Thapa and Tamang 2004), which is considerable for the maintenance of body functions (Wardlaw et al. 1994, Basappa 2002). Because of the high calorie content, ailing persons and postnatal women consume *kodo ko jaanr* extract to regain strength. *Chyang* contains more riboflavin, niacin, pantothenic acid, and folic acid than the substrate (Basappa 2002). The essential amino acids also increased during fermentation of *chyang* (Basappa et al. 1997).

3.3.2 *Bhaati Jaanr*

Bhaati jaanr is a Himalayan sweet–sour, mild alcoholic beverage consumed as a food, in the form of a paste, prepared from rice and consumed as a staple food (Tamang 2010). Glutinous rice is steamed, spread on a bamboo mat for cooling, and 2%–4% of powdered *marcha* is sprinkled over the cooked rice, mixed well, and kept in a vessel or an earthen pot for 1–2 days at room temperature. After saccharification, the vessel is made airtight and fermented for 2–3 days in summer and 7–8 days in winter. *Bhaati jaanr* is made into a thick paste by stirring the fermented mass with the help of a hand-driven wooden or bamboo stirrer. It is consumed directly as a food.

Occasionally, *bhaati jaanr* is stored in an earthenware crock for 6–9 days, and a thick yellowish-white supernatant liquor, locally called *nigaar*, is collected at the bottom of the vessel. *Nigaar* is drunk directly with or without the addition of water. *Bhaati jaanr* is an inexpensive, high-calorie staple food consumed as a beverage by postnatal women and old people in villages who believe that it helps to regain their strength.

The microbial analysis of *bhaati jaanr* shows the yeast population to be at the level of 10^7 cfu/g whereas that of the LAB load is found in the range of 10^4–10^6 cfu/g (Thapa 2001). The yeast population is higher than that of LAB in *bhaati jaanr.* Filamentous molds are absent in the final product (Thapa 2001). *Bhaati jaanr* is prepared using native *marcha*, following the traditional method (Tamang and Thapa 2006). The alcohol content increases up to 10% during the fermentation of *bhaati jaanr.* Maximum activities of saccharification and liquefaction of rice are observed on the third day of fermentation. *Saccharomycopsis fibuligera*, *Rhizopus* spp., and *Mucor* spp. contribute to the saccharification and liquefaction of glutinous rice, breaking the starch from substrates into glucose for alcohol production and also for aroma formation, during the preparation of *bhaati jaanr.* Increase in mineral contents, mostly calcium, iron, sodium, potassium, and phosphorus, is also observed in *bhaati jaanr* due to fermentation (Tamang and Thapa 2006).

3.3.3 *Lao-Chao*

Lao-chao is a popular ethnic, fermented rice food of the Cantonese in China. It has a sweet taste and a mild alcoholic flavor with a fruity aroma, and is made from rice by using *chiu-yueh* or *peh-yueh* as amylolytic starters (Wang and Hesseltine 1970, Wang 1980). It is served as a dessert and is also a traditional diet for new mothers who believe that it helps them regain their strength (Wei and Jong 1983). The production process is similar to that of the Indonesian *tapé keatan*. *Rhizopus*, *Amylomyces*, *Torulopsis*, and *Hansenula* are found in *lao-chao* (Wei and Jong 1983). A pure culture fermentation of *lao-chao* was developed by Wang and Hesseltine (1970), and it was showed that good fermented rice was made when a mold, *Rhizopus chinensis* NRRL 3671, and a yeast, *Saccharomycopsis* sp. NRRL Y7067, were used as inocula instead of a commercial starter.

3.3.4 *Tapé*

Tapé is a sweet and sour paste with an alcoholic flavor, prepared from glutinous rice or cassava or other cereals by using a starter, *ragi*, in Indonesia (Campbell-Platt 1994). It is eaten as a dessert or delicacy before meals in Indonesia. There are various starchy substrates used to prepare *tapé*, such as cassava (the product is *tapé ketala*), glutinous rice (*tapé ketan*), maize (*tapé jagung*), and millet (*tapé cantel*). During the preparation of *tapé*, glutinous rice is washed, soaked, steamed, cooled to room temperature on a woven bamboo tray, sprinkled with powdered *ragi*, packed in small banana leaves, and fermented for 2–3 days at room temperature. Thus a soft juicy mass of *tapé* is produced (Saono et al. 1977). *Tapé ketala* is deep-fried in coconut oil before consumption. It is sun dried and used later in soups or other Indonesian cuisines (Ardhana and Fleet 1989). The pH of *tapé ketan* is 4.0 (Ko 1972). The ethanol content ranged from 3% v/v (Tanuwidjaja 1972) to as high as 8.5% v/v (Cronk et al. 1977). A combination of *Aspergillus rouxii* and *Saccharomycopsis burtonii* reduced total

solids by 50% in 192 h at 30°C, which raised the crude protein content in *tapé ketan* by 16.5% on a dry weight basis (Cronk et al. 1977, 1979). Rice lipids are hydrolyzed during *tapé ketan* fermentation (Cronk et al. 1977). A mixed culture of *Streptococcus*, *Rhizopus*, and *Saccharomycopsis* produced a higher level of aroma in *tapé* (Suprianto et al. 1989), whereas *Sm. fibuligera* produced α-amylase and *Rhizopus* sp. produced glucoamylase (Suprianto et al. 1989).

3.3.5 *Tapai*

Tapai is a Malaysian fermented food commonly consumed as a dessert (Merican and Yeoh 1989). There are two main types of *tapai*, namely, *tapai pulut*, prepared from fermented glutinous rice, and *tapai ubi*, prepared from tapioca or cassava. *Tapai* tastes sweet, with a slightly alcoholic and pleasant aroma. The amylolytic starter used for the production of *tapai* is *ragi tapai* or *jui-paing* that originated from Indonesia and is mainly used for preparing dessert *tapai*, and *ragi samsu* that originated from China is mainly used for preparing an alcoholic drink from *tapai*. During the traditional method of *tapai pulut* preparation, the glutinous rice is washed, soaked, cooked, and cooled. Powdered *ragi tapai* or *jui-paing* is mixed with cooled glutinous rice on a woven bamboo tray and is covered in banana leaves and allowed to ferment at room temperature (28°C–30°C) for 1–3 days. The fermentation mass is stirred at least once a day to keep the surface moist. The resulting liquid turns clear yellow and tastes very sweet. *Candida* spp. *Saccharomycopsis fibuligera*, *Amylomyces rouxii*, *Mucor circinelloides*, *M. javanicus*, *Hansenula* spp, *Rhizopus arrhizus*, *R. oryzae*, and *R. chinensis* are found in *tapai ubi* and *tapai pulput* (Wang and Hesseltine 1970, Ko 1972, Merican and Yeoh 1982). *Lactobacillus casei* is present in tapai (Adnan and Tan 2007). The protein content of rice doubles to about 16% after fermentation as a result of losses of total solids and synthesis of proteins by the microorganisms (Cronk et al. 1977). Functional organisms necessary to produce a good *tapai pulut* consist of a mixture of *A. rouxii*, *Sm. fibuligera*, and *H. anomala*, and for production of good quality *tapai ubi* the essential microorganisms are *A. rouxii* and *Sm. fibuligera* (Merican and Norrijah 1983, 1985).

Tapai is also used as an alcoholic drink in Malaysia. After 1 week of *tapai* fermentation, if wine or brandy is added to the mash as a preservative and allowed to ferment for another 25 days, the resulting alcoholic *tapai*, which is pink red, is collected by immersing a strainer-like collection vessel into the mash (Wong and Jackson 1977).

3.3.6 *Tapuy*

Tapuy is an ethnic, mild alcoholic beverage with a sweet and sour taste prepared by the Ifugao people living in the hills of northern Philippines. *Tapuy* is prepared by washing, cooking, and cooling glutinous rice, and after placing in a clay pot, pulverized *bubod* is sprinkled over it. The pot is covered with cheesecloth and incubated in a cool place for 2 weeks (Tanimura et al. 1977). *Saccharomycopsis fibuligera*, *Rhodotorula glutinis*, *Debaromyces hansenii*, *Candida parapsilosis*, *Trichosporon fennicum*, and LAB including some species of *Leuconostoc* constitute the microbial consortia of *tapuy* (Uyenco and Gacutan (1977) mainly supplemented by *bubod*. Reducing sugars in *tapuy* are 4.1%–5.2%, pH is 3.3–4.9, and the ethanol content is 13.5%–19.1% (Tanimura et al. 1977).

3.3.7 *Poko*

Poko is an ethnic, mild alcoholic beverage prepared from rice in Nepal using *manapu*, which is similar to *bhaati jaanr* (Tamang 2010). It is widely consumed during celebrations, festivals, and ceremonies especially by the rural population in Nepal. People believe that *poko* promotes good health, nourishes the body, and provides vigor and stamina. In the traditional method of *poko* production, rice is soaked overnight, steamed until cooked and sticky, and spread to cool on the clean floor at room temperature. Powdered *manapu* is sprinkled on the cooked rice, mixed well, and packed in earthen vessels, and covered and allowed to ferment at room temperature for 2–5 days. The sticky rice is transformed to a creamy white, soft juicy mass that is sweet–sour in taste, has a mild alcoholic and aromatic flavor, and is ready for consumption as a dessert. *Rhizopus*, *Saccharomyces cerevisiae*, *Candia versatilis*, and *Pediococcus pentosaceus* are present in *poko* (Shrestha et al. 2002). Contents of vitamins, mostly thiamine, pyridoxine, vitamin B_{12}, folic acid, and niacin, increase during *poko* fermentation (Shrestha and Rati 2003). The nutritive value, including vitamin content, in *poko* is increased during the traditional fermentation process (Shrestha and Rati 2003, Dahal et al. 2005).

3.4 Nondistilled and Filtered Alcoholic Beverages Produced by Amylolytic Starters

This category of alcoholic beverages produced by amylolytic starters is not distilled but the extract of fermented cereals is filtered into a clarified high-alcohol-content liquor. Some common alcoholic drinks of these categories of liquor are described.

3.4.1 *Saké*

Saké is the national drink of Japan and is one of the most popular traditional nondistilled alcoholic drinks in the world. Japanese elderly people mostly, 61% of aged men and 18% women, prefer *saké* and beer according to a questionnaire survey (Jin et al. 2005). It is prepared from rice using *koji* and is a clear, pale yellow liquid containing 15%–20% alcohol. Polished rice is washed, steeped in water, and steamed for 30–60 min, and then cooled and mixed with *koji*, water, and a selected yeast starter culture for alcoholic fermentation. The main fermentation takes place in open tanks in cool conditions, starting at about 10°C, and the temperature increasing to about 15°C. After fermentation, a liquid material called *moromi* is separated from the solids to give clarified *saké*, which is settled, re-filtered, pasteurized, blended, and diluted with water before bottling (Yoshizawa and Ishikawa 1989). Unique strains of *S. cerevisiae* have evolved to conduct these fermentations, generating products with high ethanol content (12%–20%), attractive flavor, aroma, and odor (Kodama 1993). The first organisms that develop in the mash under traditional fermentation conditions are nitrate-reducing bacteria such as *Pseudomonas*, *Achromobacter*, *Flavobacterium*, or *Micrococcus* spp. (Murakami 1972). These are followed by *Leuconostoc mesenteroides* var *saké* and *Lactobacillus saké*, and yeasts (Kodama and Yoshizawa 1977). The highly refined *saké* brewed by the most skillful brewers using very highly polished rice at low temperatures of 9°C–11°C for 25–30 days is known as *gonjoshu* (Kodama

and Yoshizawa 1977). Most LAB that spoil *saké* are homofermentative rods and are more tolerant to ethanol and acid than nonspoilers (Inoue et al. 1992).

The difference in responses to osmotic stress between the laboratory and *saké*-brewing strains of *Saccharomyces cerevisiae* at the translational level was compared and found that enhancement of glycerol formation due to enhancement of the translation of the Hor2p protein is required for the growth of *S. cerevisiae* under high osmotic pressure conditions (Hirasawa et al. 2009). *Saccharomyces cerevisiae* strains with disrupted ubiquitin-related genes produced more ethanol than the parental strain during *saké* brewing (Wu et al. 2009). Several researchers have reported on improved strains of *Aspergillus oryzae* for *saké* production on an industrial scale (Hirooka et al. 2005, Kotaka et al. 2008, Hirasawa et al. 2009).

3.4.2 *Basi*

Basi is an ethnic, nondistilled alcoholic drink from the Philippines made by fermenting freshly extracted, boiled sugarcane juice (Tanimura et al. 1978). During its production, sugarcane is extracted into juice and is boiled with tree barks to give color and flavor. After 2–3 h of boiling, the concentrated juice is filtered and poured into earthenware jars, allowed to cool, and inoculated with a 24 h *binubudan* starter, covered with a lid, and fermented for a week before *bubod* is added to speed up the fermentation. After 1 month of fermentation, the earthenware jar is covered with a clean cloth or absorbent paper and is sealed. The product, *basi*, is then allowed to age for 1 year before consumption. In another method of preparation, the sugarcane juice is inoculated with organisms present on 1 year old dried fruit, leaves, or bark of *samac* (*Macharanga tanarius*, *M. gradifolia*) (Tanimura et al. 1978). In this process, fresh cane juice is boiled for 2 h to about three-quarters of the original volume, at which point the concentrate is transferred to an earthenware jug and cooled overnight. Milled rice, dried *samac* leaves, bark, and dried fruit are added and mixed thoroughly. The mouth of the jug is covered with banana leaves and fermentation continues for 3 months, and is aged for 1 year. The dominant yeasts in *basi* are *S. cerevisiae* as well as *Saccharomycopsis* spp. (Kozaki 1976, Sakai and Caldo 1985). An improved method for the preparation of *basi* has been developed, which is more acceptable to consumers (Sanchez and Kozaki 1984).

3.4.3 *Yakju* and *Takju*

Yakju and *takju* are ethnic Korean alcoholic beverages made from rice by using *nuruk* (Park et al. 1977). The lower or diluted concentration of *yakju* is known as *takju*. During *yakju* preparation, steamed and cooled rice is mixed with *nuruk*, fermented, and the resulting liquid is pressed from the fermenting mass, filtered under pressure, and is aged and bottled. Yeasts, *Bacillus* spp., *Lactobacillus* sp., and *Leuconostoc* spp. are present in these Korean beverages (Kim 1970, Lee and Rhee 1970, Shin and Cho 1970). *Saccharomyces cerevisiae* is the most important organism in alcohol production while *Hansenula* spp. play an important role in flavor development (Kim and Lee 1970, Kim and Kim 1993). Increase in thiamine content during fermentation of *yakju* and *takju* has been observed (Kim and Choi 1970). High-value *yakju* possessing the pharmaceutical functionality of *Ganoderma lucidum* was developed with antihypertensive property (Kim et al. 2004).

3.4.4 *Brem*

Brem is a nondistilled ethnic alcoholic drink from Indonesia prepared from rice. It is a dried sweet–sour rice starch extract and is eaten as a snack. *Brem* is of three types: *brem madiun*, which is yellowish-white in color, sweet–sour in flavor, and is prepared in blocks of 0.5 × 5–7 cm; *brem wonogiri*, which is sweet flavored, very soluble, white, and is prepared in thin circular blocks of 5 cm diameter; and *brem bali*, which is a famous alcoholic liquor produced in Bali, Indonesia (Basuki 1977). All three types of *brem* are made from the liquid portion of *tapé ketan*. During *brem* production, the filtrate of *tapé ketan* is boiled down, poured onto a table, covered with banana leaves, and left to cool to ambient temperature for over 8–12 h (*brem madiun*) or sun dried for 1 day to produce *brem wonogiri* (Campbell-Platt 1987). The liquid portion of *tapé ketan* is aged for 7 months during which solids precipitate, leaving a clarified *brem* known as *brem bali*, and is decanted and bottled (Basuki 1977). The alcohol content of *brem* is 6.13% (Winarno 1986). *Brem* produced by improved *ragi* has more desirable flavor than *brem* prepared by a conventional method (Saono et al. 1984).

3.4.5 *Krachae*

Krachae or *nam-khaao* or *sato* is a nondistilled ethnic alcoholic drink from Thailand prepared from Thai rice using *loogpang* as a starter (Vachanavinich et al. 1994). During *krachae* production, a local Thai rice variety is washed, soaked, cooked, cooled, and sprinkled with *loogpang* powder and mixed well. The mixture is placed in an earthenware jar and fermented at 30°C–35°C for 2 days to which water is added. The jar is again incubated for 3–4 days. The fermented mass is filtered and only the clarified supernatant is collected, which is yellowish-white in color, effervescent, and is now called *krachae*. Microorganisms in *krachae* are supplemented from the ethnic starter *loogpang*. Molds play an important role in the initial stage of fermentation for cleaving the sugar polymers present in rice to substantial sugars, which can be used as substrates for simultaneous fermentation by yeasts and LAB (Lotong 1985). Molds produce sugars from starch, and subsequently yeasts convert sugars to alcohol (Ko 1982). LAB help in the formation of flavor and taste in *krachae* (Lim 1991).

3.5 Distilled Alcoholic Beverages Produced by Amylolytic Starters

This category of alcoholic drinks are clear, distilled, high-alcohol-content drinks prepared from fermented cereal beverages by using amylolytic starters. These types of distilled liquors are mostly drunk in Asia, and the most popular type is *raksi* from the Himalayan regions of India, Nepal, and Bhutan.

3.5.1 *Raksi*

Raksi is an ethnic alcoholic drink from the Himalayan regions having a characteristic aroma, and is distilled from traditional fermented cereal beverages (Kozaki et al. 2000). *Raksi* is a common word in Nepali meaning alcoholic drink. *Bhaati jaanr*, *poko*, *makai ko jaanr*, *kodo ko jaanr*, *gahoon ko jaanr*, fermented masses of

buckwheat, potato, canna, and cassava roots are distilled in a large cylindrical metallic vessel measuring 40 cm × 30 cm × 25 cm for 2–3 h continuously over firewood in an earthen oven (Tamang 2010). Above the main cylindrical vessel, a perforated container called *phunga* is placed, inside which a small metallic collector called *poini* is kept on an iron tripod called *odhan* to collect the distillate called *raksi*. Another metallic vessel containing cold water is placed above the *phunga* and acts as a condenser. The bottom of the condenser attached with the tip of the *phunga* is plastered by mud to prevent excess ventilation during distillation. Water is replaced three to five times after it gets heated. Condensed *raksi* is collected in a small collecting metallic vessel called *poini*. *Raksi* prepared after replacing condensing water thrice is known as *theen pani raksi*; this contains a high amount of alcohol and is traditionally prepared for religious purposes. *Raksi* prepared after replacing the condensing water five times is known as *panch pani raksi*, which is a common alcoholic drink. The traditional distillation apparatus can distill 2–4 kg of *jaanr* to get 1–2 L of *raksi* after replacing condensing water thrice. *Raksi* is usually stored in bottles capped with a piece of dry corncob. Sometimes, petals of *Rhododendron* spp. are mixed during distillation to give a distinct aroma to *raksi*. This type of *raksi* is commonly prepared in the *Rhododendron*-growing regions of the Himalayas (Tamang et al. 1996). The alcohol content of *raksi* is 22%–27% (v/v) (Thapa 2001). *Raksi* is drunk directly without addition of water along with fried meat or any other curry. *Raksi* is commonly available in local liquor shops, restaurants, and hotels.

3.6 Alcoholic Beverages Produced Using Human Saliva as an Amylolytic Starter

This category of alcoholic beverage is unique in which, traditionally, saliva serves as the source of amylase for the conversion of cereal starch to fermentation sugars.

3.6.1 *Chicha*

Chicha is a unique ethnic, fermented alcoholic beverage prepared by the Indians living in the Andes in South America. *Chicha* is prepared from maize with the addition of human saliva (Escobar 1977). It is yellow in color, clear, effervescent, with a flavor resembling cider. *Chicha* is produced in the Andes regions and sometimes in the lower altitude regions of Ecuador, Brazil, Peru, Bolivia, Colombia, and Argentina. When made from pigmented maize varieties, the color can range from red to purple. During its production, starch from maize is converted to sugars traditionally by chewing, normally by women, who release amylases from their saliva to breakdown starch. This practice is now being replaced by malting (germination) of maize kernels to produce malt, known as *jora*; when germinated maize is dried, ground *jora* is called *pachoucho*. It is then boiled with water and then left warm up to 24 h to extract soluble materials, and filtered to give wort that is left to ferment in previously used containers at ambient temperature for 1–5 days. *Chicha* is ready to drink when the sweet taste disappears and the flavor turns semi-sharp. The alcoholic content varies from 2% to 12% v/v. *S. cerevisiae*, *S. apiculata*, *S. pastorianus*, and species of *Lactobacillus* and *Acetobacter* are present in *chicha* (Escobar 1977). There is an increase in vitamin content in *chicha* during fermentation (Escobar et al. 1996).

3.7 Alcoholic Beverages Produced by Monofermentation

Some alcoholic beverages are produced solely by one pure strain, that is, monofermentation. The most common example is beer, which is consumed worldwide.

3.7.1 Beer

Beer is the fermented extract of malted barley drunk throughout the world as a refreshing mild alcoholic beverage. Beer was produced by the Sumerians before 7000 BC (Dufour et al. 2003). Barley is germinated and kilned in a process called malting. The malted barley is extracted with water under carefully controlled conditions (mashing) to give the extract, termed wort, which contains fermentable carbohydrates and other nutrients for yeast metabolism. The wort is boiled with hops and clarified; the boiled wort is essentially sterile and brewers conduct the fermentation by inoculating (pitching) it with pure cultures of yeasts. After fermentation, the yeast cells are harvested and acid washed to decrease bacterial contamination, stored at 4°C–5°C, and then used to inoculate the next batch of wort. Generally, brewers maintain and propagate their own stocks of yeasts, principally strains of *Saccharomyces cerevisiae*. After fermentation, the beer is clarified, matured, and then packaged (Hammond 1993, Fleet 1998). Beer has an ethanol content of 2%–8%, and a distinctive flavor that originates from constituents of the malt extracts of hops and products of yeast metabolism (Tamang and Fleet 2009). There is a significant metabolic, physiological, and genetic diversity in the strains used, and these determine the final flavor and quality of the beer and the efficiency of the process (Dufour et al. 2003). The production of beer depends on the raw materials, the mashing, the fermentation and conditioning processes, and the strain of yeast. Strains within *Saccharomyces carlsbergensis* and *S. uvarum* have been merged into either *S. cerevisiae* or *S. pastorianus* (Kurtzman and Robnett 2003). Some key technological properties used by brewers in selecting strains for particular beers include the profile of aroma produced, the rate and extent of sugar content, higher (25°C–30°C) temperatures, flocculation and sedimentation characteristics, oxygen requirement, ethanol and osmotolerance, especially in relation to performance in high gravity brewing (Dufour et al. 2003). Brewer's yeast can be recycled about seven to eight times, which can lead to an increase in the populations of mutant strains and killer strains within the total yeast population and which can determine beer quality (Russel and Stewart 1995). Exposure of beer to air will encourage the growth of oxidative species comprising the following genera: *Pichia*, *Debaryomyces*, *Candida*, and *Hansenula* (Romano et al. 2006). Species of *Dekkera* produce high levels of acetic acid and esters; *Pichia* and *Hansenula* species produce excessive esters while others can give phenolic off-flavors and ferment residual dextrans in the beer (Fleet 1998, Dufour et al. 2003). Some beers, such as those produced in African countries and the limbic beers of Belgium, are still produced by traditional processes where mixtures of wild yeasts and bacteria conduct the fermentations.

3.8 Alcoholic Beverages Produced from Honey

Some alcoholic beverages are produced from honey whose sugars are used as fermentable carbohydrate sources. An example of this type of beverage is the Ethiopian *tej*.

3.8.1 *Tej*

Tej is a typical example of a fermented honey-based beverage from Ethiopia (Vogel and Gobezie 1977). It is a yellow, sweet, effervescent, and cloudy alcoholic beverage. The flavor of *tej* depends on the part of the country from where the bees collect their nectar and the climate. Hops and spices add a distinctive flavor to *tej*. Due to the high cost of honey, *tej* is consumed only on special occasions. Originally, it was found only in the homes of royalty and noblemen. A less expensive version of *tej* is made by replacing part of the honey with sugar and adding a natural yellow food coloring. During its production, honey is either collected from wild nests or produced in traditional barrel-type hives. The fermentation pot is smoked so that the *tej* has the desired smoky flavor. Honey, water, and hops (*Rhamnus prinoides*) along with ginger are added and put into the smoked pot covered with cloth and fermented naturally for 2–3 days. The proportions of honey to water can vary from 1:2 to 1:5 v/v. After 3 days, wax and top scum are removed, peeled hops are boiled and again put into the fermenting pot, and the pot covered and fermented for 8–20 days depending on the weather. After completion of desirable fermentation, the mass is stirred and filtered to obtain *tej*. The final alcohol content of *tej* is 7%–14% v/v (Vogel and Gobezie 1977). *Saccharomyces cerevisiae*, *Kluyvermyces bulgaricus*, *Debaromyces phaffi*, *K. veronae*, and LAB *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Pediococcus* species are responsible for the fermentation of *tej* (Bahiru et al. 2006).

3.9 Alcoholic Beverages Produced from Plants

This category of alcoholic beverages is produced from palm, cactus, and other plant parts without amylolytic starters. Examples of such alcoholic beverages are *pulque*, toddy, etc.

3.9.1 *Pulque*

Pulque is one of the oldest alcoholic beverages prepared from juices of the cactus (*Agave*) plant in Mexico (Steinkraus 1994). It is a white, viscous, and acidic–alcoholic refreshing drink and is considered as the national drink of Mexico where it was inherited from the Aztecs (Goncalves de Lima 1977). *Pulque* or *ocotli* or *huitztle* is industrialized and marketed under various trademarks in Mexico (Sanchez-Marroquin 1977). In the traditional method of production of *pulque*, *Agave* juice called *aguamiel* is poured into a large wooden tank. Uncontrolled natural inoculums from a previous batch of *pulque* fermentation is added to the tank and allowed to ferment for 8–30 days depending on temperature, weather, and other uncontrolled factors. After fermentation, a white, viscous, acidic–alcoholic beverage *pulque* is drawn out from tank. Mixed starter cultures were developed containing *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, *Zymomonas mobilis*, and *Leuconostoc mesenteroides* for upgrading *pulque* production under controlled conditions (Sanchez-Marroquin 1977). However, *Z. mobilis* is the principal microorganism, which transforms about 45% of the glucose to ethanol (4%–6% v/v) (Nout 2003). The bacterial community present

during the fermentation of *pulque* was studied by Escalante et al. (2004, 2008) who reported several species: *Leuc. citreum*, *Leuc. mesenteroides*, *Leuc. kimchi*, *Leuc. pseudomesenteroides*, *Lb. acidophilus*, *Lb. kefir*, *Lb. acetotolerans*, *Lb. hilgardii*, *Lb. plantarum*, *Lactococcus lactis* subsp. *lactis*, the γ-Proteobacteria *Erwinia rhapontici*, *Enterobacter* spp., *Acinetobacter radioresistens*, several α-Proteobacteria such as *Zymomonas mobilis* and *Acetobacter malorum*, *Acetobacter pomorium*, *Microbacterium arborescens*, *Flavobacterium johnsoniae*, *Gluconobacter oxydans*, and *Hafnia alvei*.

A pilot plant with a capacity of 1500 L/day followed by a commercial 50,000 L/day plant was built. Pasteurization of fresh *Agave* juice is accomplished in plate heat exchangers, and the juice then passes though presteamed lines to the fermentation tanks. The tanks are inoculated and fermentation continues 48–72 h or until the sugar has been mobilized. After fermentation, *pulque* is piped to settling, blending, and viscosity-adjusting tanks. The product is then bottled or canned. This process markedly shortens fermentation time, allows optimization of operational parameters, and yields a standardized product (Sanchez-Marroquin 1977). *Pulque* constitutes a good source of the B vitamins and calories (Goncalves de Lima 1977).

3.9.2 Palm Wine (Toddy)

Toddy is the European designation for the sweet fermented juice of tropical palms. Palm wine is fermented palm sap, which is sweet, milky, effervescent, and mildly alcoholic (Steinkraus 1996). During production, palm sap is collected in a calabash or clay pot, once or twice a day, and is poured into an earthen pot. Sometimes, sugar and water are also added, and are allowed to ferment naturally in palm-growing regions (temperature of about 25°C–35°C) vigorously for a few hours. The fermented sap is taken out and filtered and bottled and is drunk directly. The alcohol content of palm wine is 1.5%–2.1%. *Lactobacillus*, *Leuconostoc*, *Saccharomyces cerevisiae*, and *Zymomonas mobilis* are functional microorganisms naturally present in the palm sap as soon as it comes out of the palm plant (Faparusi 1973). *Saccharomyces cerevisiae* as the predominant yeast species along with other yeasts such as *Saccharomycodes ludwigii*, *Zygosaccharomyces bailii*, *Hanseniaspora uvarum*, *Candida parapsilopsis*, *C. fermentati*, and *Pichia fermentans* have been reported in palm wine from Cameroon (Stringini et al. 2009). 3-isobutyl-2-methoxypyrazine, an earthy-smelling compound; acetoin, a buttery-smelling compound; fruity compounds ethyl hexanoate and 3-methylbutyl acetate; and the popcorn-like-smelling compound 2-acetyl-1-pyrroline are important odorants of palm wine, with 3-isobutyl-2-methoxypyrazine, acetoin, and 2-acetyl-1-pyrroline being compounds that give it a distinct aroma (Lasekan et al. 2007).

In India, there are three types of toddy (Batra and Millner 1974): (1) *sendi*, from palm; (2) *tari*, from palmyra and date palms; and (3) *nareli*, from coconut palm. *Geotrichum*, *Saccharomyces*, and *Schizosacchoramyces* spp. of yeasts are responsible for fermentation (Batra and Millner 1974).

Microorganisms that are responsible in fermenting toddy are *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Acetobacter aceti*, *A. rancens*, *A. suboxydans*, *Leuconostoc dextranicum*, *Micrococcus* sp., *Pediococcus* sp., *Bacillus* sp., and *Sarcina* sp. (Shamala and Sreekantiah 1988).

3.9.3 *Kanji*

Kanji is an ethnic, strong-flavored, mild alcoholic beverage prepared from carrots by natural fermentation in India (Batra and Millner 1974). It is drunk as a mildly alcoholic refreshing drink in India. The alcohol content in *kanji* is 2.5% and the pH is 4.0, which indicates the product as mildly alcoholic having an acidic taste (Sura et al. 2001). During its preparation, carrots or beet are washed, shredded, and mixed with salt and mustard seeds, and placed in earthen pot and allowed to ferment naturally at 26°C–34°C for 4–7 days. Sometimes, the mixture is inoculated with a portion of a previous batch of *kanji*. After fermentation, a pink alcoholic liquor is drained off and bottled or drunk directly. In north India, it is prepared with purple or occasionally orange cultivars of carrots plus beets and spices, whereas in south India *torami*, an yeast-containing fermented rice gruel, is used as a starter for *kanji* production. *Hansenlu anomala*, *Candida guilliermondii*, *C. tropicalis*, and *Geotrichium candidum* are involved in *kanji* fermentation (Batra and Millner 1974). *Leuc. mesenteroides* is the typical LAB that initiates *kanji* fermentation (Sura et al. 2001).

3.10 Alcoholic Beverages Produced by Malting or Germination

In this type of alcoholic beverage, a malting or germination process allows amylase to breakdown cereal starch to sugars, which are used as substrates for alcohol fermentation. Some examples include *Bantu* beer or sorghum beer.

3.10.1 *Bantu* Beer/*Kaffir* Beer/*Sorghum* Beer

Bantu beer or *kaffir* beer or *sorghum* beer is an ethnic beer of the Bantu tribes of South Africa and is an alcoholic, effervescent, pinkish-brown beverage with a sour flavor, having the consistency of a thin gruel and an opaque appearance (Taylor 2003). The major part of the sorghum crop (*Sorghum caffrorum* or *S. vulgare*) is malted and is used for brewing beer. Maize is also used for *Bantu* beer production. In the traditional method of production of *Bantu* beer, beer is made in large drum-like pots in 115–180 L batches (Haggblade and Holzapfel 1989, Dirar 1993). Sorghum malt is produced by soaking the grain in water for 1–2 days, draining, and then allowing the seeds to germinate for 6–7 days. Sorghum requires a warm temperature (25°C–30°C) for the optimum production of amylases, and grains must be kept moist and aerated, and turned to prevent overheating. The sprouted grain is sun dried and allowed to mature for several months. The malt is pulverized, slurried to a thin gruel, boiled, and cooled, and a small amount of fresh, uncooked malt is added, probably both for its amylolytic action and also as a source of microorganisms. The mixture is kept for 1 day during which lactic acid fermentation occurs. On the second day, it is boiled in a cooking pot and returned to a brewing pot for the alcoholic fermentation. On the third and fourth days, more pulverized uncooked malt is added, and on the fifth day the brew is strained through a coarse basket to remove the husks. The beer is then ready to drink (Platt 1964). In traditional fermentations, the yeasts for the alcoholic fermentation are introduced with the malt. *Kaffir* beer is not hopped or pasteurized and is consumed while still actively fermenting.

Large-scale brewing process for *Bantu* beer production involves two distinct fermentations—a lactic acid fermentation and an alcoholic fermentation (Novellie 1968). Souring (lactic acid preparation) is achieved by holding a mixture of sorghum malt and water at 48°C–50°C for 8–16 h until the proper degree of acidity is attained. The souring step controls the course of the remaining fermentation, mashing, body, and alcoholic content of the beer; thus, it is very important. Although a pure culture inoculation of LAB is not a practice in *Bantu* beer production, about 10% of each batch of sour is used to inoculate the next batch. The soured malt mixture is pumped to a cooker, diluted with two volumes of water, and maize grits as an adjunct is added, and the whole mixture is boiled for 2 h. The thick, cooked sour mash is cooled to 60°C, and a small amount of malt may be added when the temperature reaches 75°C–80°C to reduce the viscosity. Conversion malt is added at 60°C for 2 h that makes the mash thinner and sweet. It is cooled to 30°C and inoculated with a top-fermenting yeast strain of *S. cerevisiae*. The yeast is produced locally and distributed as a dry yeast that is slurried before pitching, and the pitched mash is passed through coarse strainers to remove husks. The wort is fermented in tanks for 8–24 h at 30°C (Haggblade and Holzapfel 1989). An attempt has been made to increase the shelf life of sorghum beer by removing the second step of malt conversion with 20 min pasteurization at 80°C (Kutyauripo et al. 2009).

3.10.2 *Pito*

Pito is an ethnic light brown, alcoholic, slightly bitter, sweet–sour beverage from Nigeria with a fruity flavor made by fermentation of malted, mashed maize or sorghum (Ekundayo 1969). *Pito* is usually consumed as a nutritious beverage by Nigerians. During its production, maize and/or sorghum grains are soaked in water for 2 days, drained, and held in a moist chamber for germination/malting for 5 days. The sprouted grains are sun dried, mashed, mixed with water, boiled for 6–12 h, and then cooled and filtered. The filtrate is again fermented overnight and becomes slightly sour due to microbial action. It is then boiled for 12 h to concentrate it and is again cooled. The starter (sediment) from a previous brew is added to the cooled concentrate and is incubated for 12–24 h to get *pito.* Although germination (malting) is the essential step in producing amylases required to hydrolyze the starches to sugars for the alcoholic fermentation, the microorganisms present are species of *Leuconostoc*, *Lactobacillus*, *Saccharomyces*, *Candida*, and *Geotrichum* (Ekundayo 1977). Besides yeasts, several species of bacteria are also present in *pito. Acetobacter aceti*, *A. hansenii*, *A. pasteurianus*, *Alcaligenes*, *Flavobacterium*, *Lactobacillus plantarum*, and *Lb. brevis* are present in *pito* (Sanni et al. 1999). *Saccharomyces cerevisiae* is the predominant yeast used for the spontaneous fermentation of *pito* in Ghana (Glover et al. 2005). *Pito* is an excellent source of calories and also contributes valuable protein to consumers (Ekundayo 1969).

3.10.3 *Tchoukoutou*

Tchoukoutou is one of the ethnic African beers that is made from red sorghum and is consumed as a refreshing drink in Benin (Kayode et al. 2007a). *Tchoukoutou* beer is slightly alcoholic (3%–4% v/v), effervescent, and sweet. In the traditional preparation

method, sorghum grains are cleaned, soaked, and allowed to germinate; this is followed by sun drying in order to stabilize the obtained malt. Germinated grains are ground and mashed with water at gradually increasing temperatures until the final boiling, within a total period of 4–5 h. The wort obtained is decanted, cooled, and transferred to fermentation vessels, which contain active yeast sediments. *Saccharomyces cerevisiae* is used in the production of African beers including *tchoukoutou* (Jespersen et al. 2005). *Tchoukoutou* has a high iron content (Kayode et al. 2007b).

3.11 Alcoholic Beverages Produced from Fruits without Distillation

This category of alcoholic beverage is produced from fruits, mainly grapes to produce wine. Examples of such alcoholic beverages include wine and its varieties.

3.11.1 Wine

The word wine is derived from the Latin *vinum* that means fermented juice of grapes. Wine generally refers to the alcoholic fermentation of grape juice, or other fruits without distillation. Besides traditional wine drinkers from Europe, America, and Australia, wine is becoming popular among the non-wine drinkers of the urban populace in Asia and Africa as it is globally available. Red wine is made from red grapes and other dark-colored grapes. The pigment of these grapes causes the resulting red coloring. In the preparation of red wine, only mashing of the grapes is required in the preparation for fermentation. This allows the yeast to reach the pulp inside the grapes. White wine is produced by the fermentation of white grapes that have been mashed, and from dark-colored grapes that have been prepared by removing the skins, pulp, and seeds. This preparation method can result in fewer skins in the prepared vat as the fermentation proceeds. Red wines and white wines have markedly different flavors, and individual wines within the white or red classification may have noticeably different tastes as well (Robinson 1994). Traditional winemaking has been illustrated by Walker (1998). Grapes are collected from the vineyard, de-stemmed, crushed and macerated, and pressed and fermented by naturally present yeasts or by adding starters. After the desired fermentation, the mass is filtered to remove sediments and is stored in wooden casks for maturation for several months, and then bottled, matured, stabilized, and filtered into clear red or white wines (depending on color of grapes). Until 75–100 years ago, most wines were produced by a spontaneous or natural alcoholic fermentation of grape juice by indigenous yeast flora (Pretorius 2000). Müller-Thurgau in 1890 introduced the concept of inoculating wine fermentations with pure yeast cultures. As a result, the quality and quantity of wine production have improved (Pretorius 2000). The source of yeasts is from the surface of the grape berry; the surface of winery equipment that come in contact with the juice during crushing, pressing, pumping, and fermentation; and air (Raspor et al. 2006). Many years of research have identified various strains of *S. cerevisiae* and *S. bayanus* as the principal yeasts in wine fermentation. At present, many wine makers purchase commercial dried preparation of these yeasts for inoculation into grape juice and for initiation of the alcoholic fermentation (Fleet 1998). Wine fermentation is initiated by growth of various species

of non-*Saccharomyces* yeasts (e.g., *Hanseniaspora uvarum*, *Kloeckera apiculata*, *Candida stellata*, *Candida colliculosa*, *Metschnikowia pulcherrima*, *Kluyveromyces thermotolerans*) as well as *Saccharomyces* yeasts, which are generally limited to the first 2–4 days of fermentation, after which they die (Fleet 1993, Moreira et al. 2005). They achieve maximum populations of 10^6–10^7 cfu/mL before death, thereby influencing the metabolic behavior of the fermentation and the products released into the wine, and their death is attributed to an inability to tolerate the increasing concentrations of ethanol, which is largely produced by the *Saccharomyces* species. After 4 days or so, the fermentation is continued and completed by *Saccharomyces* species, especially strains of *S. cerevisiae*, *S. bayanus*, and in some cases *S. paradoxus* (Moreira et al. 2005). Wine fermentations reflect not only an ecological succession of different yeast species, but also a succession growth of strains within a species (Fleet 2003). Different species and different strains within a species can produce substantially different profiles of metabolic end products such as organic acids, higher alcohols, esters, and sulfur volatiles (Romano et al. 2003).

3.11.2 Cider

Cider is the fermented alcoholic wine prepared from apple juice. Like grape wines, it is also produced by traditional fermentation or by inoculation with selected strains of *Saccharomyces cerevisiae* or *S. bayanus* (Beech 1993). Initially, *Hanseniaspora uvarum* contributes to the fermentation along with species of *Metschnikowia*, *Candida*, and *Pichia* (Naumov et al. 2001), and gives way to predominant *S. cerevisiae* and *S. bayanus* to complete the fermentation (Morrissey et al. 2004). *Dekkera*, *Zygosaccharomyces*, *Saccharomycodes*, and *Hanseniaspora* species may develop during maturation and either enrich the product flavor or cause spoilage to cider (Valles et al. 2007).

3.12 Distilled Alcoholic Beverages without Amylolytic Starters

In this category of beverages, fermented fruits and cereals are prepared without amylolytic ethnic starters, and are distilled. The high-alcohol-content distillate from fermented cereals is whisky, that from fermented molasses is rum, and that from fermented grapes is brandy.

3.12.1 Whisky

Whisky is the clear liquor distillate obtained from fermented malted barley or cereal products. Specific flavors are added to the distillate to give particular products (Bluhm 1995). Strains of *Saccharomyces cerevisiae* are generally purchased from yeast-producing companies and are inoculated into the raw material extract at a load of 10^6–10^7 cfu/mL to initiate and conduct the fermentation. The key criteria for strain selection include efficient production of high concentrations of ethanol and the production of a desired profile of volatile flavor that carries over into the distillate (Watson 1993). Traditional processes are still being conducted, where indigenous yeasts make a significant contribution to the fermentation and product flavor.

3.12.2 Rum

Rum is made by distilling fermented sugarcane or molasses. Traditional Brazilian rum, called *cachucha*, is distilled from fermented cane juice where the successive growth of several yeast species occurs. A diversity of different strains of *Saccharomyces cerevisiae* eventually dominate the fermentation after the initial growth of other species within the genera *Pichia*, *Candida*, and *Kluyveromyces* (Schwan et al. 2001). Rum production from molasses fermentation may involve contributions from *Schizosaccharomyces pombe* as well as *S. cerevisiae* (Fahrasame and Ganow-Parfeit 1998).

3.12.3 Brandy

Brandy is prepared from distillates of fermented fruit juices such as grapes or other fruits. It is a high-alcohol spirit distillate consumed in all regions of the world. Wine from fermented grape juice or from other fermented fruit juices such as apricots, apples, cherries, plums, mirabelle, sloes, peaches, bilberries, raspberries, blackberries, blackcurrants, strawberries, rowan berries, and figs are distilled, usually twice (Hallgarten 1979). The first distillation gives a clear liquor called *brouillis*, which is again redistilled to give a spirit with about 70% ethanol and is called *eau de vie*. In some cases, such as in armagnac-type of brandy, only single distillation is used, giving a spirit with 50%–55% ethanol. Clear brandies such as kirsch are bottled directly, while others such as cognac and armagnac are aged for several years in wooden casks to give characteristic flavors and brown color. *Saccharomyces cerevisiae* is the functional yeast in the fermentation of fruits into wines.

Besides whisky, rum, and brandy, there are many flavored high-alcohol-content drinks such as gin, vodka, and ouzo. They are generally produced by alcoholic fermentation of extracts from cereals or their agricultural commodities and are then distilled.

3.13 Conclusion

Alcoholic beverages represent a vast diversity of products ranging from ethnic, fermented beverages, alcoholic drinks, and distilled alcoholic products to wine and beer. Fermented beverages and alcoholic drinks constitute an integral part of the dietary culture of different countries in the world. There are 10 different types of alcoholic beverages in the world: nondistilled and unfiltered alcoholic beverage consumed as a food produced by amylolytic starters; nondistilled and filtered alcoholic beverages produced by amylolytic starters; distilled alcoholic beverages produced by amylolytic starters; alcoholic beverages produced using human saliva; alcoholic beverages produced by monofermentation; alcoholic beverages produced from honey; alcoholic beverages produced from plants; alcoholic beverages produced by malting (germination); alcoholic beverages prepared from fruits without distillation; and distilled alcoholic beverages prepared from fruits and cereals. Crude subculturing of mixed inocula using rice or other cereals as base substrates for the preparation of amylolytic mixed starters in Asia is a remarkable and innovative technology that uses the native skills of ethnic people to maintain the essential consortia of microorganisms.

Ethnic amylolytic starters contain mycelia molds, enzyme-producing and alcohol-producing yeasts, and a few pediococci and lactobacilli. Distilled alcoholic liquor form a large part of the market for fermented beverages. The nutritional composition of alcoholic beverages and drinks indicates the calorie content.

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4

Functional Yeasts and Molds in Fermented Foods and Beverages

Kofi E. Aidoo and M. J. Robert Nout

CONTENTS

4.1 Introduction

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

TABLE 4.1

Main Functional Properties of Fungi in Fermented Foods

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

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

fermented foods and beverages across the world (Aidoo et al. 2006) that signify the food culture of the regions and the community (Tamang and Fleet 2009). About 21 major genera with several species of functional yeasts have been reported from fermented foods and beverages that include *Brettanomyces* (its perfect stage, *Dekkera*), *Candida*, *Cryptococcus*, *Debaryomyces*, *Galactomyces*, *Geotrichum*, *Hansenula*, *Hanseniaspora* (its asexual counterpart *Kloeckera*), *Hyphopichia*, *Kluyveromyces*, *Metschnikowia*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Saccharomycodes*, *Saccharomycopsis*, *Schizosaccharomyces*, *Torulopsis*, *Trichosporon*, *Yarrowia*, and *Zygosaccharomyces* (Kurtzman and Fell 1998, Pretorius 2000, Romano et al. 2006, Tamang and Fleet 2009). Molds in fermented foods and beverages are relatively limited, and include the genera *Actinomucor*, *Mucor*, *Rhizopus*, *Amylomyces*, *Monascus*, *Neurospora*, *Aspergillus*, and *Penicillium* (Hesseltine 1983, 1991, Samson 1993, Nout and Aidoo 2002).

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

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

4.2.1 Fermented Foods of Asian Origin

4.2.1.1 Furu

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

TABLE 4.2

Some Functional Yeasts and Molds in Fermented Foods of the World

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

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

(*continued*)

TABLE 4.2 (continued)

Some Functional Yeasts and Molds in Fermented Foods of the World

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

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

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

4.2.1.2 Idli

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

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

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

4.2.1.3 Ragi *and* Men

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

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

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

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

4.2.2 Fermented Foods of Australasian Origin

4.2.2.1 Bubod

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

4.2.2.2 Puto

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

4.2.2.3 Tapuy

Tapuy is a traditional alcoholic drink popular in the mountains of northern Philippines. *Tapuy* is produced by inoculating cooked rice with rice yeast, *bubod*, and allowed to ferment in a jar in a cool place for 2 weeks. The fermented mass is then pasteurized for 30 min and stored for a minimum of 6 weeks; after that the clear liquor is decanted. The liquor undergoes a final pasteurization before bottling. The alcohol content reaches about 5.6% after 48 h and increases with fermentation. One kilogram of glutinous rice may produce about a liter of rice wine. Yeasts, molds, and LAB bring about fermentation of *tapuy* with yeasts as the predominant organisms. Yeasts

isolated from *tapuy* include *Sm. fibuligera*, *Rhodotorula glutinis*, *D. hansenii*, *Pichia burtonii*, *Candida parapsilosis*, and *Tr. fennicum* (Sakai and Caldo 1985, Kozaki and Uchimura 1990). During the fermentation, yeasts like *Sm. fibuligera* and *P. burtonii*, which are also amylase producers, dominate the early stages. *Mucor* and *Rhizopus* spp. also produce amylolytic enzymes that hydrolyze the rice starch to sugars for conversion to alcohol. LAB, *Lactobacillus viridescens* and *Lb. brevis*, also proliferate during fermentation and contribute to the production of organic acids. In the Philippines, drinking *tapuy* is a part of traditional ritual.

4.2.3 Fermented Foods of African Origin

4.2.3.1 Mawè

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

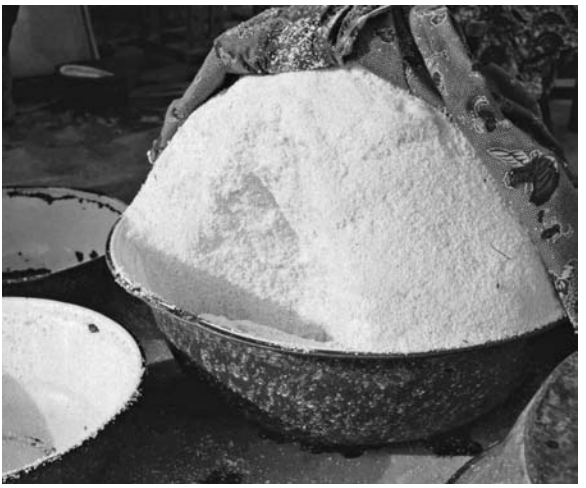

FIGURE 4.4 *Mawè* for sale at a market in Cotonou, Bénin. (Picture by Nout, M.J.R., 2002.)

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

4.2.3.2 Tchoukoutou

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

4.2.3.3 Kachasu

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

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

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

4.2.4 Fermented Foods of European Origin

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

4.2.4.1 Sourdough

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

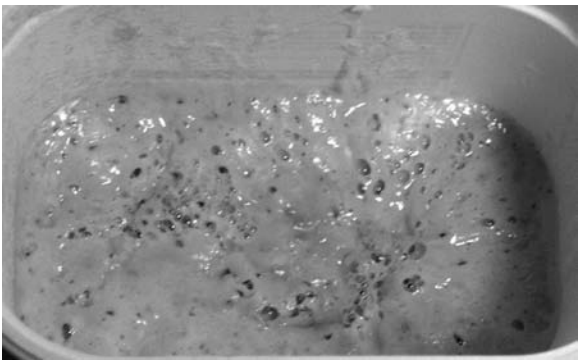

FIGURE 4.7 Sourdough. (From http://en.wikipedia.org/wiki/File:masa_madre.jpg, accessed March 20, 2009.)

4.2.4.2 Cheese

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

4.2.4.3 Fermented Meats

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

products. *Debaryomyces polymorphus*, *C. zeylanoides*, *P. membranifaciens*, *P. guilliermondii*, and *Cryptococcus* spp. are known to be responsible for the ripening of cured ham.

4.2.5 Fermented Foods of Middle East Origin

4.2.5.1 Tarhana

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

4.2.5.2 Kefyr

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

4.2.5.3 Koumiss

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

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

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

4.2.6 Fermented Foods of Latin American Origin

4.2.6.1 Pozol

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

4.2.6.2 Pulque

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

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

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

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

4.2.6.3 Huitlacoche

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

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

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

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

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

4.4 Conclusion

Yeasts and molds play a major role in the fermentation of foods during which there is bio-enrichment leading to the production of proteins, vitamins, minerals, aroma, alcohols, acids, esters, and also improvements in digestibility, preservation, and

organoleptic properties. The authors have presented some examples of fermented foods that have received much attention and are produced with a high degree of technological advancement and automation, as well as some lesser-known and less-developed products. Some of the problems associated with fungal-fermented foods have also been highlighted. The majority of these fermented food products is of plant origin and could fulfill the ever-increasing worldwide demand for healthy foods, naturally fermented products, protein-rich meat substitutes, and exotic foods of plant origin. Although fungal-fermented foods are now receiving more attention, further developments are necessary to scale up and/or improve some of the lesser-known products to maximize substrate utilization, process control, yields, and hygiene.

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5

Fermented Vegetable Products

Carmen Wacher, Gloria Díaz-Ruiz, and Jyoti Prakash Tamang

CONTENTS

5.1 Introduction

Canned or frozen foods are too expensive or not easily available for the majority of people living in underdeveloped and developing countries, where acid fermentation combined with salting remains one of the most practical methods of preserving and often enhancing the organoleptic and nutritional quality of fresh vegetables (Steinkraus 1996). The knowledge of the art of pickling vegetables (fermentation), a process of preservation of foods, is lost in antiquity (Battock and Azam-Ali 1998). It may have been developed in Asia as suggested by Pederson (1979) or in the Mediterranean (Hulse 2004), but until more evidence is available its origin will remain obscure (Steinkraus 1996). In any event, this method of food preservation has been used for many centuries and is one of the important methods of food preservation still in use for vegetables and fruits where production by canning, drying, or freezing is not the method of choice (Vaughn 1985). Biopreservation refers to extended storage life and enhanced safety of foods using the natural microflora and their antibacterial products (Stiles 1996, Tamang and Tamang 2009a). The women communities of different ethnic groups of the Himalayas have been practicing the lactic fermentation process to preserve perishable and seasonal vegetables in the absence of refrigeration and freezing (Tamang 2010). The preserved fermented vegetables are consumed during the long monsoon season when fresh leafy vegetables may not be available in plenty in the mountainous regions. Young bamboo shoots are consumed by people across the world besides their use as nonfood products such as house materials, handicrafts, etc. Young, edible tender bamboo shoots are traditionally fermented into food products in many Asian countries. Some fermented vegetable foods are sauerkraut, *kimchi*, *gundruk*, *sinki*, *khalpi*, etc., and some traditional fermented bamboo shoot products are *mesu*, *soibum*, *soidon*, *ekung*, *eup*, *hiring*, *naw-mai-dong*, etc. The fermented

vegetable products are categorized into three major groups: traditional fermented vegetables, pickled vegetables, and fermented bamboo shoot products.

5.2 Traditional Fermented Vegetables

5.2.1 Sauerkraut

Sauerkraut, meaning "sour cabbage" in German, is fermented white cabbage pickle (Figure 5.1).

5.2.1.1 History

Sauerkraut is a dish from central Europe. It found its first mention in American English in 1776, and the dish was long associated with the German communities living in the United States (Stradley 2004). The immigrants to America carried barrels of sauerkraut with them on their ships, as sauerkraut possessed some disease-fighting properties. Pennsylvania Dutch cooking is indigenous to those areas of southeastern Pennsylvania where the Mennonites and the Amish settled. With the encouragement provided by William Penn's (the founder of Pennsylvania) open invitation to persecuted religious groups, various sects of Christian Anabaptists–Mennonites and offshoots such as the Amish and the Brethren emigrated from Germany and Switzerland. The first large group arrived in America around 1730 and settled near Lancaster County, Pennsylvania. Chinese laborers who were building the Great Wall of China over 2000 years ago ate shredded cabbage fermented in rice wine. Genghis Khan substituted salt for the wine and carried this sauerkraut (as it is now called) to Eastern Europe. Germans and the Alsatians prepare sauerkraut as their national dish (Sauerkraut 2009).

5.2.1.2 Preparation and Culinary

To prepare sauerkraut, white cabbage is shredded finely and layered with salt in a large crock or wooden tub. It is covered with a heavy lid and left to ferment, below 15.5°C for at least a month. Spices such as caraway seeds, peppercorns, and juniper berries

FIGURE 5.1 Sauerkraut. (Image by Simone Voight 2010. Used under license from Shutterstock.com.)

are sometimes added to the cabbage during the fermentation. Sauerkraut is also traditionally produced in the Balkans using whole heads of cabbage instead of shredded cabbage. Fermentation time is longer, as it lasts several months (Niksic et al. 2005). Sauerkraut is served with other dishes such as smoked meats and sausages or on its own. It is sold in jars or cans in supermarkets, or in delicatessens where fresh sauerkraut is sold in plastic bags.

5.2.1.3 Microbiology

Sauerkraut fermentation has been reported to occur in two stages: an initial heterofermentation, which is followed by a homofermentation where *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lb. plantarum*, and *Pedicoccus pentosaceus* are the main microorganisms, but *Lb. curvatus*, *Lb. sakei*, *Lactococcus lactis* subsp. lactis, and *Leuc. fallax* have also been reported (Plengvidhya et al. 2007). The fermentation end products of *Leuc. fallax*, lactic and acetic acids and mannitol, but not malolactic enzyme, are similar to that of *Leuc. mesenteroides* (Schillinger et al. 1989, Barrangou et al. 2002a). Shredded cabbage fermentations start with 10^6 cfu/g aerobic microorganisms, 10^6 cfu/g enterobacteriaceae, and less than 10^2 cfu/g yeasts and molds. During the first 2 or 3 days of sauerkraut fermentation, less acid-tolerant lactic acid bacteria (LAB) dominate, but after this more acid-tolerant LAB predominate. Each of these populations reaches concentrations of 10^8–10^9 cfu/g. The fermentation is complete in 2 weeks and at this time the most acid-resistant *Lb. plantarum* predominates. Plengvidhya et al. (2007) studied isolates from the microbiota of commercial sauerkraut fermentations using the rRNA gene intergenic transcribed spacer method with 16S rRNA gene sequence analysis, and found new species that had not been reported in this fermentation: heterofermenters *Weissella* and *Leuc. citreum*, and the already reported *Lb. brevis* and *P. pentosaceus*. Bacteriophage contamination is a serious problem, as viruses infect LAB and are responsible for the failure of fermentations. Barrangou et al. (2002b) isolated six phages active exclusively against *Leuc. fallax*. Three of them were assigned to the family Siphoviridae and the other three to the family Myoviridae. Bacteriophages have the potential to control population levels and diversity in natural microbial communities. Lu et al. (2003a) studied bacteriophage ecology in commercial sauerkraut fermentations. They isolated 171 phages, which included 28 different ones. Host strains were *Leuconostoc*, *Weissella*, and *Lactobacillus*. There were two phage–host systems that corresponded to populations before and after the shift from heterofermentative to homofermentative strains, respectively. This suggests that phages play a role in microbial succession. The salt concentration controls the microbial succession in sauerkraut fermentation. Two percent salt is added to the traditional fermentation, and to reduce salt waste, 1% salt has been proposed. To ensure the initial stages of the fermentation are adequate, *Leuc. mesenteroides* as a starter was introduced (Plengvidhya et al. 2007). ITS-PCR database and 16S rRNA gene sequence comparison of two commercial processes revelaed *Leuc. mesenteroides* and *Lb. plantarum*. *Weissella* sp. and *Lb. curvatus* were the next most common species (Plengvidhya et al. 2007).

5.2.1.4 Starter Cultures

There is a need for commercial bacterial cultures to ferment vegetable substrates, and several starter cultures have been proposed for sauerkraut. In sauerkraut fermentations, *Lb. alimentarius* and *P. pentosaceus* have been used as starters. *Leuc. mesenteroides* and *P. dextranicus* produce good quality sauerkraut (Tolonen et al. 2002, Tamminen et al. 2004). Tamminen et al. (2004) isolated and identified (using carbohydrate profiling and PCR–ELISA) LAB from sauerkraut and found *Lb. brevis* and *Lb. plantarum*-related bacteria, which grow after *Leuc. mesenteroides*. On the other hand, when the salt concentration added to the shredded cabbage was reduced, the natural fermentation resulted in a soft product with off-flavor; however, after the addition of a *Leuc. mesenteroides* starter culture to a reduced salt concentration substrate, the product was firm and with acceptable sensory characteristics (Johanningsmeier et al. 2007). A psychrotrophic (mesophilic microorganisms capable of growing at refrigeration temperatures) *Leuconostoc* spp. strain with dextransucrase (enzyme that catalyzes dextran synthesis) activity was proposed as a starter culture for vegetable fermentations, as sauerkraut, besides lactic and acetic acids, alcohol, CO_2, and mannitol, which give flavor to the product, would produce prebiotic polysaccharides from its dextransucrase activity (Eom et al. 2007). Sucrose addition to the substrate resulted in an objectionable product with a sticky consistency, but the addition of maltose inhibited the synthesis of dextran, and if sucrose and maltose are added together, dextrin synthesis is reduced, as maltose is used as an acceptor and panose (a trisaccharide, composed by one maltose unit and one glucose unit linked by an α-1, 6-glycosidic bond) is formed. Harris et al. (1992) proposed the use of a starter culture with two strains: one of them was *Leuc. mesenteroides* that was resistant to nisin, and the second one, a nisin-producing *Lc. lactis* subsp. *lactis*. A nisin concentration of 100 AU/mL slightly suppresses the growth of *Lb. plantarum* and does not affect the growth of *Leuc. mesenteroides*, so its dominance is extended and the product's quality is improved (Harris et al. 1992).

5.2.1.5 Biochemistry

In a study of two commercial sauerkrauts, Plengvidhya et al. (2007) reported that glucose and fructose were the primary fermentable sugars in the cabbage, with concentrations of 1.5% and 2.2%, respectively, and sucrose concentration was less than 0.2%. Lactic acid, acetic acid, and mannitol were produced, and on the fourteenth day the pH value of all the tanks increased from 3.4 to 3.7. Sulfur compound profiles of sauerkraut fermentation with 0.5% NaCl inoculated with *Leuc. mesenteroides* revealed the presence of allyl isothiocyanate, dimethyl disulfide, dimethyl trisulfide, methyl methanethiosulfinate, and methyl methanethiosulfonate. The sulfur flavor is correlated with the presence of dimethyl trisulfide and methyl methanethiosulfonate (Johanningsmeier et al. 2005).

5.2.1.6 Food Safety

Traditional sauerkraut is a natural fermentation, and in some cases whole heads of cabbage (instead of shredded cabbage) are used. Whole heads of cabbage fermentation is longer (several months) and represents a higher risk. Pathogens can be present on raw cabbage, because of cattle manure contamination or because of the low microbiological quality of cabbage and its ingredients. A large outbreak of *Listeria*

monocytogenes occurred in Canada in 1983, because of the consumption of cabbage contaminated with sheep manure (Conner et al. 1986). The final pH of whole-head salads was lower than that of shredded cabbage. There was no significant effect of cabbage types, but more acid was produced in shredded cabbage fermentations because shredding releases carbohydrates and fermentation is faster. According to Niksic et al. (2005), cabbage shredding releases cabbage cellular components that establish a buffering system with a slightly higher pH. *Escherichia coli* O157:H7 is known for its extreme resistance to low pH. There was a significant interaction between the type of cabbage (whole or shredded) and the sampling day. Whole-head sauerkraut has higher microbial populations over time, as compared to the shredded one. This can be explained because its outer leaves are not removed and these are dirtier than the rest (Niksic et al. 2005). The traditional whole-cabbage fermentation, if done properly, eliminates both the pathogens, although variations in salt content and fermentation time affect survival. Shredded cabbage contained a higher level of titratable acidity, which reduced the risk associated with survival of both pathogenic bacteria (Niksic et al. 2005). Plant-derived molecules such as allyl-ITC (allyl isothiocyanate) possess antimicrobial activity, which is eliminated with heat. Sinigrin is relatively innocuous, but its hydrolysis products (isothiocyanates, thioyanates, and nitriles) inhibited growth (Tolonen et al. 2004). Biogenic amines form a group of natural biologically active compounds that occur in foods, where they are produced from decarboxylation reactions. An excessive intake of biogenic amines in foods may be desirable under some physiological conditions. Tyramine concentration was found to increase in sauerkraut during storage (Kalac et al. 2000a). Histamine, tryptamine, spermidine, and spermine concentrations in sauerkraut are below 10 mg/kg (Kalac et al. 2000b). Tyramine, putrescine, and cadaverine are in higher concentrations (450–780 mg/kg) but are suppressed by *Lb. plantarum* and Microsil (an antimicrobial nanosilver compound) (Kalac et al. 2000b).

5.2.1.7 Socioeconomy

Per capita consumption of sauerkraut in the United States has been declining since the 1960s and 1970s. To promote its consumption, new ingredients (garlic, onion, dill seed, and jalapeno peppers) have been added. Sauerkraut with garlic and sauerkraut with dill are those with the highest marketing potential (Uva et al. 2007).

5.2.2 *Kimchi*

Kimchi is a generic term used to denote a group of fermented cabbage, radish, and garlic foods in Korea. It is a spicy fermented pickle (Figure 5.2). The flavor of *kimchi* is dependent on the ingredients, fermentation conditions, and LAB involved in the fermentation process (Lee et al. 2005a). *Kimchi* is stored for several months, when a lactic fermentation occurs (Lee and Lee 2006).

5.2.2.1 History

Kimchi (the word is thought to come from *shimchae*, which means salting of vegetables) was created around the seventh century. Koreans liked cultivating and consuming vegetables, but as these were not available during winter, they developed

FIGURE 5.2 *Kimchi*. (Image by Stargazer 2010. Used under license from Shutterstock.com.)

a pickling method to preserve it (Korean Restaurant Guide 2009). *Kimchi* was at first just a salted vegetable; in the twelfth century they included spices and seasonings; in the eighteenth century hot red pepper became one of the major spices for *kimchi*, and in the nineteenth century Chinese cabbages were introduced, to produce the dish as it is known today (Korean Restaurant Guide 2009).

5.2.2.2 Preparation and Culinary

More than 100 types of vegetables can be used to prepare *kimchi* (Kim and Chun 2005). Chinese cabbage, radish, and cucumber, with a seasoning mixture made of red pepper powder, garlic, ginger, and green onion, are among them (Nam et al. 2009). Without starter cultures, *kimchi* is made through lactic acid fermentations of Chinese cabbage at low temperatures to ensure proper ripening and preservation. Since *kimchi* is representative of a typical open ecosystem, each batch of fermented product has a different composition of bacteria depending on fermentation conditions and ingredients, which can be highly variable.

Three types of *kimchi* can be prepared: whole-cabbage *kimchi* (*jeotgukji*), diced-radish *kimchi* (*kakdug*i), and water *kimchi* that was served to the kings of Joseon. *Kimchi* is prepared as follows: First, cut well-washed cabbage and radish into small chunks and salt them. Second, mix them with chopped hot red pepper, garlic, dropwort, leaf mustards, and some seaweed. Third, boil fermented fish in some water and cool it. Fourth, add it to the above blended stuff. Fifth, store them in a pot and wait till they are fermented (Korean Restaurant Guide 2009).

5.2.2.3 Microbiology

LAB are the most important microorganisms in *kimchi* fermentation. Using conventional methods of isolation and phenotypic identification, the following species are found to be responsible for *kimchi* fermentation: *Leuc. mesenteroides*,

Leuc. pseudomesenteroides, *Leuc. lactis*, *Lb. brevis*, and *Lb. plantarum* (Kim and Chun 2005). *Leuc. mesenteroides* was reported to predominate during the first hours of fermentation (Kim and Chun 2005). The pH value gradually falls to 4 and then *Lb. plantarum* becomes predominant (Kim and Chun 2005). It is well known that not all microorganisms are culturable and, therefore, it is important to use culture-independent methods. *Kimchi* is one of the fermented foods that have been more widely studied using novel microbial ecology methods. Kim and Chun (2005) used 16S rRNA gene PCR–DGGE and clone libraries of the same gene to study the microbial community structure of *kimchi* made of Chinese cabbage from five major manufacturers. Most of the clones were LAB, including several species of *Lactobacillus*, *Leuconostoc*, and *Weissella*. *Weissella koreensis* was present in all samples and predominated in three *kimchi* samples, while *Leuc. gelidum*, *Leuc. gasicomitatum*, and *Lb. sakei* were common in the other two samples. Two of the samples contained two species of LAB; another sample was diverse. The most abundant groups in the third sample were *Lb. sakei* and *Leuc. gelidum*, and were followed by *W. koreensis.*

Novel species of LAB have been isolated from *kimchi*: *Leuc. kimchii* (Kim et al. 2000), *Lb. kimchii* (Yoon et al. 2000), *W. koreensis* (Lee et al. 2002), *W. kimchi* (Choi et al. 2002), *Lc. inhae* (Kim et al. 2003), and *Tetragenococcus koreensis* (Lee et al. 2005b). Lee et al. (2005b) determined the microbial composition during *kimchi* fermentation at 10°C and 20°C, using PCR–DGGE. Chinese cabbage *kimchi* was prepared in the laboratory, incubated at 10°C and 20°C, and samples were taken during fermentation. The same evolution of pH during fermentation was obtained at 10°C and 20°C. The pH value started at 5.4 and decreased to 3.7 and 3.2 after 20 and 14 days fermentation, both at 10°C and 20°C. Likewise, the DGGE profiles were similar. *W. confusa*, *Leuc. citreum*, *Lb. sakei*, and *Lb. curvatus* are the main microorganisms during *kimchi* fermentation. The microbiota during the first hours of the fermentation was different than that during the end of the fermentation. *W. confusa* and *Leuc. citreum* are present throughout the fermentation. *Lb. sakei* and *Lb. curvatus* are also important during fermentation. *Leuc. lactis* ssp. *lactis* is present when the pH value is low, and *Leuc. gelidum* is only present in *kimchi* samples incubated at 10°C. Bae et al. (2005) used genomic probing microarrays to monitor the population dynamics of LAB during *kimchi* fermentation. Hybridization of the probes (genomic DNA isolated from 149 different strains) with fluorescently labeled bulk community DNA was assessed. More positive signals were detected in the late than in the early stages of the fermentation, with several *Weissella* species being the dominant microbiota. One hundred species were detected during fermentation, and as it progressed, more *Weissella* and *Leuconostoc* and some *Lactobacillus*, *Enterococcus*, *Pediococcus*, and *Lactococcus* species were detected. The structure of the bacterial community of *kimchi* has been elucidated with the studies presented previously especially with genomic probe microarrays; however, as they have been based on DNA, nonactive, together with active microorganisms, are included. Metatranscriptome (sequencing the expressed genetic information of an ecosystem) analyses, in which mRNA was obtained by removing 16S and 28S rRNAs from the extracted RNA (Nam et al. 2009), revealed significant differences in the relative compositions of LAB during fermentation. *Weissella* spp. also contribute to *kimchi* fermentation, and less abundant microorganisms, according to the metagenome, participated in the fermentation. Regarding microorganisms different than bacteria, Chang et al. (2008) detected haloarchaea comprising *Halococcus* spp., *Natronococcus* spp., *Natrialba* spp., and *Haloterrigena* spp. as the predominant

archaea, and *Lodderomyces* spp., *Trichosporon* spp., *Candida* spp., *Saccharomyces* spp., *Pichia* spp., *Sporisorium* spp., and *Kluyveromyces* spp. as the main yeasts in *kimchi*. Regarding the latter, they could be involved in the flavor and smell of the product.

5.2.2.4 Starter Cultures

In order to obtain a product of constant quality, a starter culture consisting of *Leuc. mesenteroides* and *Lb. plantarum* strains was used, and the product was not different from conventional *kimchi*. *Leuc. citreum* HJ-P4, a strain isolated for *kimchi* fermentation, with high dextransucrase activity and adapted to grow at low temperature, has been proposed to be used as a starter culture to produce *kimchi* commercially (Yim et al. 2008).

5.2.2.5 Food Safety

Kimchi is the result of a lactic fermentation, so it is expected to be protected, as pathogenic bacteria are inhibited by acids, hydrogen peroxide, diacetyl, and bacteriocins. Lee et al. (1995) studied the fate of *Listeria monocytogenes* during *kimchi* fermentation. The viable cell count of *Listeria* increased during the first 2 days of fermentation and after this time it decreased, but viable cells remained after 10 days at 35°C. The relationship between the concentration of LAB or the pH and growth of three gram-positive food-borne pathogens (*Bacillus cereus*, *L. monocytogenes*, and *Staphylococcus aureus*) was evaluated. Heat treatment (85°C for 15 min) or neutralization treatment (pH 7.0) was conducted on day 0 and day 3 of incubation and it was found that pathogenic bacteria were inhibited by *kimchi* (Kim et al. 2008a). Lee et al. (2009) investigated the antimicrobial activity of *kimchi* against *L. monocytogenes*, *Staphy. aureus*, *E. coli* O157:H7, and *Salmonella typhimurium*. The effect of different incubation temperatures (0°C, 4°C, 10°C and 20°C) on the antimicrobial activity of the fermented product was studied (Lee et al. 2009). Higher acidity values were obtained when the incubation temperature was higher. The greatest inactivation of *S. typhimurium* occurred in *kimchi* fermented at 20°C, while *L. monocytogenes* was inactivated in *kimchi* fermented at 0°C. Raw garlic showed strong antimicrobial activity against the pathogens.

Different methods have been assessed to preserve *kimchi*: high-dose gamma radiation (25 kGy) together with a pretreatment with calcium lactate and vitamin C to improve texture (Song et al. 2008), and freeze drying (Jung and Choi 2008). A number of bacteriocin-producing LAB have been isolated from *kimchi* that would make this natural, biological method of preservation possible. *Leuconoctoc* sp. J2 produces a bacteriocin called leuconocin J, which is active against some LAB and some food-borne pathogens (Choi et al. 1999). *Lc. lactis* BH5 produces a bacteriocin with a broad spectrum of activity against pathogenic and nonpathogenic microorganisms (Hur et al. 2000). A bacteriocin-producing *Lc. lactis* subsp. *lactis* from *kimchi* inhibited strains of *Clostridium perfringens*, *C. difficile*, *L. monocytogenes*, vancomycin-resistant *Enterococcus*, and one out of four *Staphy. aureus* strains resistant to the antibiotic methicillin, as well as some closely related LAB (Park et al. 2003). A pediocin from *P. pentosaceus* (Shin et al. 2008) would be another possibility. Microbial interactions have been found to be important, as LAB sensitive to a bacteriocin produced by *Leuc. citreum* GJ7 enhance its production (Chang et al. 2008).

5.2.2.6 Functional Properties

Kimchi was selected as one of the world's healthiest foods in 2006 by *Health* magazine due to its many beneficial properties (Nam et al. 2009). Among the best functional components are beta-carotene, chlorophyll, vitamin C, and dietary fiber (Park 1995). Treatment of lactic acid from *kimchi* was found by Park et al. (2008) to play a role in the prevention of fat accumulation and to improve obesity-induced cardiovascular disease, particularly atherosclerosis, by attenuating the TNF-α-induced changes of adipokines. Glycoprotein antimutagenic substances were isolated from a culture supernatant of *Lb. plantarum* isolated from *kimchi* (Rhee and Park 2001), and antioxidants were isolated from *kimchi* (Sim and Han 2008, Sun et al. 2009). It has been reported that the consumption of *kimchi* causes weight loss; prevents constipation and colon cancer; reduces serum cholesterol (Park et al. 2006); exerts antistress principles (Lee and Lee 2009); is beneficial because *kimchi* contains *S*-adenosyl-L-methionine, a bioactive material used in the treatment of depression, osteoarthritis, and liver disease (Lee et al. 2008a); has antiobesity effects (Kong et al. 2008); and inhibits atherosclerosis (Kim et al. 2008b). A potential probiotic strain of *Lb. plantarum* isolated from *kimchi* inhibited the growth and adherence of *Helicobacter pylori* in an MKN-45 cell line, with small peptides as the possible inhibitors (Lee and Lee 2006).

5.2.2.7 Biochemistry and Nutrition

The composition of *kimchi* depends on the variety of vegetables used, the process, and fermentation and preservation methods. Complex biochemical changes occur before, during, and after fermentation. These include especially changes in carbohydrates, vitamins, accumulation of organic acids, texture degradation, and softening. It is an important source of vitamins (especially ascorbic acid), minerals, dietary fiber, and other nutrients (Cheigh and Park 1994). Several organic acids, such as citric, malic, succinic, and lactic acids, are produced during *kimchi* fermentation. Kim et al. (2005) developed a sensor to follow *kimchi* fermentation. The pH value decreased during fermentation, and the lower the incubation temperature (25°C, 10°C, 4°C) the higher was the pH value obtained. After 8 days storage, the pH values of the fermented product were 3.8, 3.7, and 3.3 at 25°C, 10°C, and 4°C, respectively (Kim et al. 2005). Because of its high consumption, *kimchi* is included in a food composition database of Korean foods (Lee et al. 2008b). One hundred and fifty foods were selected to be evaluated and included in the table, and the criterion was that their per capita daily intake was higher than 1.0 g and that the total dietary fiber be 0.5 g/100 g or higher. Dietary fiber is especially important to help prevent chronic diseases, as its effects include reducing blood cholesterol, stabilizing blood sugar, regulating bowel movements, among others (Lee et al. 2008b). The contribution of the ingredients used for the preparation of *kimchi* to its dietary fiber content is important as shown in Table 5.1 (Lee et al. 2008b).

The role of amino acids as the major structural and functional components of the body is well known; the amino acid composition determines the protein quality of foods, and recent investigations have shown that a balance between essential and nonessential amino acids as well as the effect of amino acid supplements on muscle strength (Kim et al. 2009). One hundred and fifty high-protein foods were selected.

TABLE 5.1

Total, Insoluble and Soluble Dietary Fiber Contents of *Kimchi* Ingredients

Food	Total Dietary Fiber
Kimchi, Welsh onion	5.05
Kimchi, mustard leaves, raw	3.97
Kimchi, leafy radish	3.32
Kimchi, napa cabbage with red pepper[a]	2.98
Kimchi, small radish	2.89
Kimchi, *kkak du ki* (radish)	2.84
Kimchi, cucumber	2.50
Kimchi, *nabak*	1.54
Kimchi, napa cabbage without red pepper	1.43
Kimchi, *dong chi mi*	0.79

Source: Modified from Lee, Y. et al., *J. Food Compos. Anal*., 21, S35, 2008b.

Note: Foods were analyzed in the raw form.

[a] 2.8 g/100 g and 0.18 g/100 g edible portion insoluble and soluble dietary fiber, respectively.

TABLE 5.2

Amino Acid Composition of *Kimchi* Ingredients (mg/100 g Edible Portion)

Food	Thr	Trp	Val	His	Arg	Ala	Asp	Glu	Gly	Pro	Ser
Kimchi	58	17	128	102	64	200	121	120	21	108	45
Kimchi (young turnip greens)	61	11	69	83	74	79	168	298	54	53	50
Nabak kimchi	1	0	2	3	1	2	3	8	1	1	1
Kimchi (small radish)	32	103	45	114	32	68	85	184	30	38	28
Kimchi (radish in salt water)	15	24	18	23	39	24	60	111	13	8	14

Source: Modified from Kim, B.H. et al., *J. Food Compos. Anal*., 22, 44, 2009.

According to the 2001 National Health and Nutrition Survey, the major foods contributing to protein intake were rice, beef, pork, chicken, egg, milk, soybean curd, and *kimchi*. Some of these foods are low in protein content, but their contribution is important because they are consumed in large amounts. The average protein intake for Koreans was found to be 127% (Korean Nutrition Society 2000) and was even higher for children. Results for *kimchi* ingredients are shown in Table 5.2.

5.2.2.8 Socioeconomy

A typical adult Korean consumes an average of 50–200 g of *kimchi* per day and its market in Korea exceeded 130 million USD in 2002 (Kim and Chun 2005). Currently, more than 1.5–106 tons of *kimchi* is consumed each year in South Korea, a phenomenon which is rapidly growing and spreading to other countries in Asia. According to

Ahmed and Chon (1994), because South Koreans have become high-spending international travelers, American travel companies organize Korean-speaking groups and include Korean food, among which *kimchi* is important. Also, Korean immigrants to China, Russia, Hawaii, and Japan introduced *kimchi* abroad, and it is now also consumed by natives of these countries, so it is now a global food (Korean Restaurant Guide 2009). It is such an important food that with the first Korean astronaut going to space, dehydrated *kimchi* was prepared, to obtain what they call "space *kimchi*" (Song et al. 2009).

5.2.3 *Gundruk*

Gundruk is an ethnic fermented, dry, and acidic vegetable product of the Himalayas (Figure 5.3). The daily per capita consumption of *gundruk* is 1.4 g with an annual production of 3.2 kg/house in the Indian state of Sikkim (Tamang et al. 2007).

5.2.3.1 History

There is a myth regarding the invention of *gundruk* in the Himalayas that has been documented in detail by Tamang (2010). The word *gundruk* might have originated from *gunnu*, meaning dried taro stalk in the Newar dialect (Newar is one of castes of the Nepali).

5.2.3.2 Preparation and Culinary

Gundruk is prepared from fresh leaves of a local vegetable called *rayo-sag* (*Brassica rapa* subspecies *campestris* variety *cuneifolia*), mustard (*Brassica juncea*), and cauliflower (*Brassica oleracea* variety *botrytis*), which are wilted and shredded using a sickle. Wilted and shredded leaves are crushed mildly and pressed into an earthen jar or container, and made airtight by covering with dried bamboo bract sheaths and

FIGURE 5.3 *Gundruk*.

fern fronds, and weighted down by stones. The container is kept in a warm place and allowed to ferment naturally for about 7–10 days. Unlike *kimchi* and sauerkraut, freshly fermented *gundruk* is sun dried for 3–4 days before consumption, and dried *gundruk* is preserved for more than 2 years. *Gundruk* is eaten as a soup or pickle. The soup is made by soaking *gundruk* in water for 10 min, and then squeezing and frying in edible oil with chopped onions, tomatoes, chillies, turmeric powder, and salt. It is then boiled for 10–15 min, and served hot with steamed rice. *Gundruk* soup is a good appetizer in a bland and starchy diet. *Gundruk* is sold in all local markets.

5.2.3.3 Microbiology

Lb. fermentum, *Lb. plantarum*, *Lb. casei*, *Lb. casei* subsp. *pseudoplantarum*, and *P. pentosaceus* are present in *gundruk* (Tamang et al. 2005). During the *in situ* fermentation of *gundruk*, indigenous LAB change spontaneously, and at the end of the fermentation *Lb. plantarum* is involved (Tamang and Tamang 2010). The pH of the fermenting substrates decreases, and the titratable acidity increases as the *gundruk* fermentation progresses due to the growth of LAB, which convert fermentable sugars into lactic acid. *Saccharomyces* sp., *Pichia* sp., and *Zygosaccharomyces* sp. are found during the initial stage of *gundruk* fermentation, and then disappear during the fermentation (Tamang and Tamang 2010). Similarly, the population of pathogenic contaminants disappears during the fermentation because of the dominance of LAB. By averting the invasion of these contaminants, lactic acid fermentation imparts safety in a product like *gundruk*. There has been no report of any food poisoning or infectious disease infestation by consuming fermented vegetables and fermented bamboo shoots in the Himalayas. *Gundruk* fermentation is initiated by *Lb. fermentum* and is followed by *P. pentosaceus*, and finally by *Lb. plantarum*, *Lb. casei*, and *Lb. casei* subsp. *pseudoplantarum* (Karki et al. 1983d, Tamang and Holzapfel 2004). These bacteria produce lactic acid and acetic acid that lower the pH of the substrates, making the products more acidic in nature (Karki et al. 1983d, Dietz 1984, Dahal et al. 2005). Due to the low pH and high acid content, *gundruk* can be preserved for longer periods without refrigeration. This is cited as a practical example of biopreservation of perishable vegetables in the Himalayas.

5.2.3.4 Food Safety

Staphylococcus aureus and enterobacteriaceae have been isolated from some market samples of *gundruk* at below 10^2 cfu/g (Tamang 2006). These pathogenic bacteria might have been introduced during the handling of raw materials for the preparation when the pH is not low enough to inhibit their growth. Otherwise, no pathogenic bacteria such as *Listeria*, *Salmonella*, and *Shigella* have been detected in *gundruk*. The inability of LAB strains from *gundruk* to produce biogenic amines is a good indication of food safety (Tamang et al. 2009) and their acceptability for their possible development as a starter culture.

5.2.3.5 Functional Properties

Some LAB isolated from *gundruk* showed strong acidification properties by lowering the pH to less than 5, and also showed coagulating abilities (Tamang et al. 2009).

Though, these strains, although originating from plant sources and not from milk, appeared to be adapted to the milk ecology, they coagulated and acidified the skim milk used in the applied method. Most of the lactic acid bacterial strains isolated from *gundruk* showed high activity of peptidases (Tamang et al. 2009). Most of the LAB strains of *gundruk* have antimicrobial properties against a number of gram-positive and gram-negative bacteria. Some LAB strains showed strong arylamidase and phosphatase activities. Most of the LAB strains isolated from *gundruk* degraded anti-nutritive factors such as phytic acids, raffinose, and stachyose (Tamang et al. 2009). A few strains of LAB from *gundruk* showed more than 75% hydrophobicity and were able to adhere to mucus-secreting HT29 MTX cells, adhesion of most strains being in the same range as that of the reference strain *Lb. rhamnosus* GG (Tamang et al. 2009). This may advocate their probiotic character (Holzapfel et al. 1998), provided these strains are consumed in a viable state.

5.2.3.6 Starter Cultures

Sometimes, natural fermentation results in inferior quality and non-acceptability of the product due to fluctuating incubation temperatures, consortia of microorganisms, environmental conditions, etc. An attempt was made to upgrade the traditional processing of perishable vegetables using pure strains of LAB isolated from the traditionally fermented vegetable products of the Himalayas (Tamang and Tamang 2010). On the basis of superior technological properties of LAB strains (Tamang 2006) such as acidification ability, antimicrobial activities, nonproduction of biogenic amines, ability to degrade anti-nutritive factors, and even a high degree of hydrophobicity, *Lb. plantarum* GLn:R1 (MTCC 9483) and *P. pentosaceus* GLn:R2 (MTCC 9484) were selected as a starter culture for the production of *gundruk*. *Gundruk* was prepared using cell mixtures of *Lb. plantarum* GLn: R1and *P. pentosaceus* GLn:R2 at the level of 10^7 cfu/g (Tamang and Tamang 2010). Organoleptically 6-day-old *gundruk* fermented at 20°C scored the highest general acceptability (Tamang and Tamang 2010). *Gundruk* prepared by using a starter culture has advantages over the conventional method, such as shorter fermentation time, good quality, and flavor.

5.2.3.7 Nutrition

In *gundruk*, 90% of the organic acids consist of lactic and acetic acids and other organic acids are citric, malic, and acetic acids (Karki 1986). The level of palmitic, oleic, linoleic, and linolenic acids is much higher in mustard leaf *gundruk* compared to that in the unfermented vegetables (Karki et al. 1983c). In mustard *gundruk*, free amino acids, mostly glutamic acid, alanine, leucine, lysine, and threonine remarkably increase with a decrease in asparagine, glutamine, histidine, and arginine during the fermentation (Karki et al. 1983b). *Gundruk* contains cyanides and isothiocyanates as the main flavor components, followed by alcohols, esters, and phenyl acetaldehyde (Karki et al. 1983a). The levels of iron and calcium are high, while carotenoids are reduced by more than 90%, probably during sun drying (Dietz 1984). Due to the high content of organic acids, *gundruk* is considered as a good appetizer (Tamang 2010).

FIGURE 5.4 *Sinki.*

5.2.4 *Sinki*

Sinki is an ethnic, fermented radish tap root product of the Himalayas (Figure 5.4). It is prepared by pit fermentation in the Himalayas in India, including Darjeeling hills, Sikkim, a few regions in Uttarakhand, and Himachal Pradesh, and in Nepal and Bhutan.

5.2.4.1 Preparation and Culinary

A pit of about 2–3 ft with same diameter is dug in a dry field, cleaned, plastered with mud, and warmed by burning. After removing the ashes, the pit is lined with bamboo sheaths and paddy straw. Radish tap roots are wilted for 2–3 days, crushed, dipped in lukewarm water, squeezed, and pressed tightly into the pit, then covered with dry leaves and weighted down by heavy planks or stones. The top of the pit is plastered with mud and left to ferment naturally for 22–30 days. Freshly fermented *sinki* is removed, cut into small pieces, sun dried for 3–5 days, and stored at room temperature for future consumption. Dry *sinki* can be kept for 2 years and is stored in an airtight container. Pit fermentation of *sinki* preparation is an unique type of biopreservation of perishable radish by lactic acid fermentation in the Himalayas (Tamang 2010). *Sinki*, with a highly acidic flavor, is typically used as a base for soup and as a pickle. The soup is made by soaking *sinki* in water for about 10 min, squeezing, and frying along with chopped onions, tomatoes, green chillies, and salt. The soup is served hot along with meals. Sinki soup is more sour than that of *gundruk*, and people use it as a remedy for indigestion. The pickle is prepared by soaking *sinki* in water, squeezing it dry, and mixing it with salt, mustard oil, and chillies. *Sinki* pickle is more preferred by Nepalis than soup or curry. *Sinki* is also sold in all local markets, mostly by rural women for their livelihood.

5.2.4.2 Microbiology

Lb. plantarum, *Lb. brevis*, *Lb. casei*, and *Leuc. fallax* were isolated from *sinki* (Tamang and Sarkar 1993, Tamang et al. 2005). Recovery of *Leuc. fallax* in *sinki*

FIGURE 5.5 *Khalpi.*

(Tamang et al. 2005) is remarkable, since there are not many reports on the occurrence of *Leuc. fallax* in foods.

5.2.5 *Khalpi*

Khalpi is an ethnic, fermented cucumber product of the Nepal and Indian regions of Darjeeling hills, and Sikkim in the Himalayas (Figure 5.5).

5.2.5.1 Preparation and Culinary

During *khalpi* preparation, ripened cucumber is collected from the field, and cut into pieces and is sun dried for 2 days, and then put into a bamboo vessel and made airtight by covering with dried leaves. It is fermented naturally at room temperature for 3–5 days. Fermentation after 5 days makes the product more sour, which is not preferred by the consumers. *Khalpi* is consumed as a pickle by adding mustard oil, salt, and powdered chillies along with steamed rice. It is not sold in markets.

5.2.5.2 Microbiology

The predominant LAB in *khalpi* are *Leuc. fallax*, *P. pentosaceus*, *Lb. brevis*, and *Lb. plantarum* (Tamang et al. 2005). The population of LAB in raw cucumber is very small (10^3 cfu/g) during *in situ* fermentation that increases significantly ($p < 0.05$) to 10^8 cfu/g within 36 h, and then remains at the level of 10^7 cfu/g in the final product (Tamang and Tamang 2010). Heterofermentative LAB such as *Leuc. fallax*, *Lb. brevis*, and *P. pentosaceus* are isolated from the initial fermentation stage of *khalpi*. As the fermentation progresses, more acid-producing homofermentative lactobacilli mainly *Lb. plantarum* remain dominant. The microbial load of *Staphylococcus aureus* and enterobacteriaceae disappears during fermentation. The microbial load of yeasts in raw cucumber disappears after 48 h. The pH decreases from 5.6 to 3.2 and the percentage of acidity increases from 0.28% to 1.24% at the end of the fermentation. Acidity, pH, and buffer capacity greatly influence the growth of LAB during cucumber fermentation (McDonald et al. 1991).

5.2.5.3 Starter Cultures

Lb. plantarum KG:B1 (MTCC 9485), *Lb. brevis* KG:B2 (MTCC 9486), and *Leuc. fallax* KB:C1 (MTCC 9487) previously isolated from *khalpi* (Tamang et al. 2005) are selected, on the basis of superior technological properties such as acidifying capacity, antimicrobial activities, nonproduction of biogenic amines, and the ability to degrade anti-nutritive factors of the raw materials (Tamang and Tamang 2010).

5.2.6 Fermented Olives

The olive is the fruit of the olive tree (*Olea europea* L.), which is the most widespread plant in Mediterranean countries. The olive fruit has a bitter component (oleuropein), low sugar content, and high oil content (International Olive Oil Council 2009). These characteristics make it a fruit that cannot be consumed directly from the tree, and it has to undergo a series of processes (fermentation). Table olive is a very important fermented food obtained from it (Figure 5.6). It is highly appreciated for its good taste, as well as for its nutritional peculiarities. Table olives are different from other fermented foods (carrot, cabbage, pumpkin, etc.) in their chemical composition and because they are not edible without prior treatment.

5.2.6.1 History

The existence of the olive tree dates back to the twelfth millennium BC (International Olive Oil Council 2009). The wild olive tree originated in Asia Minor, where it is extremely abundant and grows in thick forests (International Olive Oil Council 2009). It appears to have spread from Syria to Greece, although other hypotheses point to Egypt, Nubia, Ethiopia, the Atlas Mountains, or certain areas of Europe as its source area. The Assyrians and Babylonians were the only ancient civilizations in the area who were not familiar with the olive tree. The Romans continued the expansion of the olive tree to the countries bordering the Mediterranean while they extended their empire. With the discovery of America (1492), olive farming spread beyond its Mediterranean limits. In more modern times, the olive tree has continued

FIGURE 5.6 Fermented olives. (Image by Sander Crombeen 2010. Used under license from Shutterstock.com.)

to spread outside the Mediterranean and today is farmed in places as far removed from its origins as Southern Africa, Australia, Japan, and China (International Olive Oil Council 2009).

5.2.6.2 Preparation and Culinary

Olives, as they come from the tree are too bitter to eat without some kind of curing, and hundreds of processing methods are used around the world. From an economical point of view, only three are relevant: The Spanish style, the most important for industrial preparations; the Californian style, for black oxidized olives; and the Greek style for naturally black olives. However, there are many other traditional table olive preparation recipes. In all cases, fruits undergo fermentation in brine solution, which preserves and increase palatability (Brenes 2004, Panagou et al. 2008). The preparation of Spanish style green olives includes an alkaline treatment, aiming at an efficient hydrolysis of the bitter glycoside oleuropein; a washing step used to eliminate the excess of lye accumulated over the olives; and finally the brining, where the typical lactic acid fermentation occurs (Chammem et al. 2005). Concentration of NaOH during the alkaline treatment, the period of immersion, and the degree of penetration achieved depend on various factors such as the variety, the degree of maturity of the fruits, and the temperature. Furthermore, the brining of olives takes place after washing them twice; this is an important influence in the pickling process (Chammem et al. 2005). A natural selection of microbiological strains during fermentation depends on the initial concentration of brine. Adequate alkaline treatment is also important because the use of low concentrations of lye leads to bitterness in the fruit due to the weak hydrolysis of oleuropein, recognized as one of the fermentation inhibitors (Brenes et al. 1992). Some authors have evaluated the effect of lye and sodium chloride on the microbiota and the quality of fermented olives for the Tunisian olive variety "meski" treated according to a Spanish style; the optimum alkali treatment/brine concentration ratio was 2/9 that allowed satisfactory fermentation and gave the best quality final product (Chammem et al. 2005).

To prepare Californian-style black oxidized olives, they are preserved in an aqueous solution (brine, acidic water, etc.) and darkened throughout the year. Darkening consists of several treatments with dilute NaOH solutions and water washes between them. During the oxidation process, air is passed throughout the suspension of the olives in the liquid. Once the olives obtain the proper color ring around the outer surface, they are fixed by immersion in lactate or gluconate iron solution. These olives are usually packed in light brine. Their commercial presentations are limited to plain, pitted, sliced, and, sometimes, olive paste (Arroyo-López et al. 2008). Naturally black olives in brine are obtained by directly brining olives without any prior debittering (a process for removing the bitter components) treatment. The final product is characterized by a fruity flavor and retains a slightly bitter taste. This kind of preparation is popular in Turkey, Greece, and other Northern African countries. Processing involves several steps, namely harvesting when the drupes are fully ripe or slightly before full ripeness (3/4 of the mesocarp has attained black color), transportation to the factory, sorting to exclude defective drupes, washing to remove superficial dirt, and finally brining in 8%–10% salt concentration where the olives undergo spontaneous fermentation by a mixed microbiota of gram-negative bacteria, LAB, and yeasts (Panagou et al. 2008).

5.2.6.3 Microbiology

The composition of the microbiota (bacteria, yeasts, and molds) of the olives before brine making is one of the factors that could affect the dynamics of the fermentation and the quality of the product. Olive fermentation is similar to that of sauerkraut except that it is slower, involves a lye treatment, and may require the addition of starters. LAB are recognized to play an important role in olive fermentation, and at the end of the process *Lactobacillus* species are involved (Ercolini et al. 2006). *Lb. plantarum*, *Lb. pentosus*, and *Lb. casei* are regarded as the main species leading this process (Randazzo et al. 2004, Ercolini et al. 2006). In natural olive fermentations, lactobacilli are usually detected by cultivation methods (Tassou et al. 2002, Randazzo et al. 2004); however, molecular methods are also used. PCR/restriction fragment length polymorphism (RFLP) of 16S rDNA and partial sequencing analysis of the 16S rDNA were used to characterize lactobacilli; isolates were identified as *Lb. brevis* and *Lb. casei*, while none of the strains were identified as *Lb. plantarum* (Randazzo et al. 2004). No *Lactobacillus* was found in raw olives by FISH (fluorescence *in situ* hybridization); *Leuconostoc*, *Pediococcus*, *Pseudomonas*, and *Raoultella* were identified by PCR–DGGE (Ercolini et al. 2006). Randazzo et al. (2004) revealed microbial communities of naturally fermented green olives by using a combination of classical and molecular techniques; *Lb. plantarum*, *Lb. casei*, *Lb. brevis*, and *Enterococcus faecium* were identified by conventional methods; however, on the basis of the restriction profiles and sequences it was possible to assign isolates to *Lb. brevis*, *Lb. casei*, and *Enterococcus* ssp., while none of the strains were identified as *Lb. plantarum*, in contrast with the results of the biochemical identification. In a normal fermentation, the prevailing microbial groups are LAB and yeasts, the relative population of which defines the characteristics of the final product. Thus, when LAB outgrow yeasts, the lactic acid fermentation is favored rendering a more acidic product with a lower pH, which is greatly desirable in naturally black olive fermentation. As a consequence of the alkaline treatment of Spanish-style green olives, brine pH values during first days after brining are not favorable for the growth of lactobacilli, whereas potential spoilage microorganisms such as enterobacteriaceae and butyric-acid-forming clostridia can proliferate and spoil the product (de Castro et al. 2002). Several studies have focused on the detection of yeasts adhered to the surface of olive fruits; however, Arroyo-López et al. (2008) reported low yeast count on the surface of fresh olive fruits (<1 log cfu/g); yeast populations ranging from 3 to 5 log cfu/g have been determined in the brine during the fermentation of different kinds of olives (de Castro et al. 2002). *Candida boidinii*, *Debaryomyces hansenii*, *Pichia anomala*, *P. membranifaciens*, *Rhodotorula glutinis*, and *S. cerevisiae* are species frequently isolated from olive fermentation processes (Arroyo-López et al. 2008). Yeasts can play a double role in table olive fermentation; they can produce compounds associated with desirable organoleptic attributes and can also spoil the product (Hernández et al. 2007, Arroyo-López et al. 2008).

5.2.6.4 Starter Cultures

Modern table olive industry is directed toward the use of pure starter cultures to achieve an improved and more predictable fermentation process, desirable organoleptic characteristics, as well as improved safety and reduced hygienic risks.

The selection of starters is based on diverse criteria including homo- and heterofermentation, acid production, salt tolerance, flavor development, temperature range, oeluropein-splitting capability, and bacteriocin production (Durán et al. 1999, Delgado et al. 2005). Panagou et al. (2008) studied 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; *Lb. pentosus* (commercial starter) and *Lb. plantarum* isolated from cassava were used as starters. Both starter cultures were effective in establishing an accelerated fermentation process and reduced the survival period of gram-negative bacteria.

5.2.6.5 Food Safety

Inhibition of *E. coli* O157:H7 was evident in all olive fermentation procedures regardless of the treatment used. The pathogen numbers declined but did not die out during fermentation. It was evident that the rate of death was higher in samples supplemented with starter cultures compared to natural fermentation (Spyropoulou et al. 2001).

5.2.6.6 Functional Properties

Olives and derivative products are recognized as functional foods because of their natural phenolic antioxidant content; these compounds also contribute to the color, taste, and texture of the product (Ben Othman et al. 2009). The consumption of 50 g of table olives provides about 152 mg of phenolic compounds (Ben Othman et al. 2009).

5.2.6.7 Biochemistry and Nutrition

The main constituents of the olive fruit when harvested are oil, water, sugars, proteins, anthocyanins, and oleuropein (Ünal and Nergiz, 2003) These compounds are influenced by the method of preparation of table olives (Table 5.3). The total phenolic

TABLE 5.3

Chemical Composition of Table Olives

	Green Table Olive		Black Table Olive	
Characteristics	**After Harvesting**	**After Fermentation**	**After Harvesting**	**After 8 Months Storage (10% NaCl Solution)**
Moisture (%)	64.8	73.4	55.4	55.3
Oil (%)	14.9	14.3	26.6	28.4
Protein (%)	1.4	1.3	1.3	1.1
Ash (%)	1.4	5.9	1.8	5.8
Acidity (% as lactic acid)	0.1	0.4	0.1	0.5
pH	4.6	4.7	5.1	5.0
NaCl (%)	ND	3.9	ND	4.4
Reducing sugar (%)	1.4	ND	1.9	ND
Total sugar (%)	2.9	ND	2.2	ND
Crude fiber (%)	5.1	4.6	4.8	3.9

Source: Modified from Ünal, K. and Nergiz, C., *Int. J. Fats Oils*, 54, 71, 2003.
Note: ND, not detected.

content and the distribution of the phenolic components are affected by the cultivar, growing, location, and the degree of ripeness (Ryan et al. 1999). The oil is characterized by a good content of total phenols, o-diphenols, tocopherols, and a good resistance to oxidation. Ben Othman et al. (2009) showed that olive processing induced an important loss in phenolic compounds, leading to a reduction in antioxidant value. Olive fruit normally does not contain sodium chloride when harvested; during the preparation and storage of table olives, NaCl is diffused into the flesh. Ünal and Nergiz (2003) concluded that water-soluble constituents and minerals were affected during processing and storage. The method of processing of table olives also affects the nutritional constituents of the olive fruit. Nutritional benefits of olive fruits are mainly related to α-tocopherol and fatty acid contents; during processing (Spanish style) of Tunisian olive fruits, both α-tocopherol and fatty acids amounts decreased; this decrease was more pronounced in the black fruit than in the green one for the same cultivar (Sakouhi et al. 2008).

5.2.6.8 Socioeconomy

Table olives are probably the most popular fermented vegetable in the Western world and a main part of the Mediterranean diet together with olive oil. Table olives are prepared from specifically cultivated fruit varieties harvested at the predetermined stage of maturation (Panagou et al. 2008). According to the latest estimates for the 2007–2008 crop year (International Olive Council 2009), the EU table olive production was expected to be 740,700 ton, and olive oil was 2,820,500 ton at the world level. The Spanish-style green olive is the most important industrial preparation with about 60% of the production (International Olive Council 2009).

5.2.7 *Goyang*

Goyang is an ethnic, fermented wild plant food of the Sherpas of Sikkim and Nepal (Tamang and Tamang 2007).

5.2.7.1 Preparation and Culinary

Goyang is prepared with the leaves of a wild plant locally called *magane-saag* (*Cardamine macrophylla* Willd), which are plenty in the Eastern Himalayas. Leaves of the wild edible plant are collected, washed and cut into pieces, squeezed to drain off excess water, and are tightly pressed into bamboo baskets lined with 2–3 layers of leaves of fig plants. The top of the baskets are then covered with fig plant leaves, and fermented naturally at room temperature (15°C–25°C) for 25–30 days. Freshly fermented *goyang* is transferred into an airtight container which can be stored for 2–3 months. The shelf life of *goyang* can be prolonged by making the freshly fermented *goyang* into balls and sun dried for 2–3 days. Sun-dried *goyang* can be kept for several months. *Goyang* is generally prepared at home by the Sherpa women in the high mountains of the Himalayas (Tamang 2010). *Goyang* is boiled in a soup along with yak or beef meat and noodles to make a thick *thukpa*, a common staple food of the Sherpa.

5.2.7.2 Microbiology

Lb. plantarum, *Lb. brevis*, *Lc. lactis*, *E. faecium*, *P. pentosaceus*, and yeasts like *Candida* spp. were isolated from *goyang* (Tamang and Tamang 2007).

5.2.8 *Inziangsang*

Inziangsang is an ethnic, fermented dry leafy vegetable product of Nagaland and Manipur in India (Tamang 2010). It is prepared from mustard leaves and is very similar to *gundruk*.

5.2.8.1 Preparation and Culinary

Withered mustard leaves, locally called *hangam* [*B. juncea* (L.) Czern], are crushed and soaked in warm water. The leaves are squeezed to remove excess water and pressed in to a container and made airtight to maintain anaerobic conditions. The container is kept at ambient temperature (20°C–30°C) and allowed to ferment for 7–10 days. Like *gundruk*, freshly prepared *inziangsang* is sun dried for 4–5 days and stored in a closed container for a year or more for consumption. Freshly fermented *inziangsang* juice is also extracted, instead of sun drying, squeezing by hand, and concentrated by boiling. The liquid form of the fermented extract is called *ziang dui* and the concentrated paste is called *ziangsang*. The extract concentrate, *ziangsang*, is stored in a traditional bamboo container for a year.

Inziangsang is consumed as a soup with steamed rice by Nagas. The fermented extract, *ziang dui*, is used as a condiment in local meals.

5.2.8.2 Microbiology

Lb. plantarum, *Lb. brevis*, and *P. acidilactici* were isolated from *inziangsang* (Tamang et al. 2005). *Lb. plantarum* IB2 isolated from *inziangsang* showed bacteriocin activity (Tamang et al. 2009). Bacteriocin activity shown by *Lb. plantarum* IB2 was quantified to be 32 AU/mL (Tamang et al. 2009).

5.2.9 *Jeruk*

Jeruk is a homemade fermented pickle indigenous to many races in Malaysia and is prepared from common fruits and vegetables (Merican 1996). Among the common vegetables used are gherkin and cucumber, ginger, onion, chilli, bamboo shoot, mustard leaves, etc. Low-income rural people consume large quantities of *jeruk* because pickling is an inexpensive way to preserve surplus food. Pickled vegetables are prepared like fresh vegetables; pickled fruit is eaten as a relish especially by children and expectant mothers because of the sweet–sour flavor.

5.2.9.1 Microbiology

Species of *Leuconostoc*, *Lactobacillus*, *Pediococcus*, and *Enterococcus* are present in *jeruk* (Merican 1996).

5.2.10 *Pak-Gard-Dong*

Pak-gard-dong is the fermented vegetable product of Thailand prepared from the leaf of mustard (Boon-Long 1986). During its preparation, leaves of black mustard are collected; defective leaves are removed, washed, and wilted in the sun, and 2.5% salt is added to the wilted leaves, packed into a container, and left for 12 h. After removing the salt water, 3% sugar is added and fermented at room temperature. Fermentation is completed in 3–5 days (Boon-Long 1986).

5.2.10.1 Microbiology

P. pentosaceus, *Lb. brevis*, and *Lb. plantarum* are the major LAB involved in the fermentation of *pak-gard-dong* (Mingmuang 1974).

5.2.11 *Pak-Sian-Dong*

Pak-sian-dong is an ethnic, fermented leafy vegetable product of Thailand (Dhavises 1972). It is prepared from the leaves of *pak-sian* (*Gynandropis pentaphylla* DC.). The fresh vegetable is thoroughly cleaned with water and then spread out in the air or under the sun to lose water until the sample is distinctly flaccid. It is then mixed with water, salt, and sugar and kept in a tightly covered container. To reduce bitter flavor, the leaves are sometimes soaked in water and salt overnight; the liquid portion is discarded and fresh water and sugar are added. Usually, raw cane sugar or palm sugar is preferred to refined sugar because it enhances the flavor of the finished product. *Pak-sian-dong* is ready in 72 h. At this stage, the pH of the liquor is 3.9 and the acidity between 0.7% and 0.8%.

5.2.11.1 Microbiology

Dominant LAB in *pak-sian-dong* are *Leuc. mesenteroides*, *Lb. fermentum*, *Lb. buchneri*, *Lb. plantarum*, *Lb. brevis*, and *P. pentosaceus* (Dhavises 1972).

5.2.12 *Sayur asin*

Sayur asin is an ethnic, fermented mustard cabbage leaf product of Indonesia (Puspito and Fleet 1985). Mustard cabbage leaves are wilted, rubbed, or squeezed with 2.5%–5% salt. Liquid from boiled rice is added to provide fermentable carbohydrates to ensure that sufficient acid is produced during the fermentation.

5.2.12.1 Microbiology

The fermentation is initiated by *Leuc. mesenteroides* and *Lb. confusus* and later dominated by *Lb. plantarum* and *P. pentosaceus*. The pH falls from 6.5 to 4.2 in 8 days of fermentation (Puspito and Fleet 1985).

5.2.13 *Suan cai*

Suan cai is an ethnic Chinese fermented vegetable product (Miyamoto et al. 2005). Taxonomical analysis of two genetically distinguished *Lactobacillus* strains isolated

from *suan cai*, which formed L-lactate from glucose, revealed facultative heterofermentative organisms that had a DNA G + C content of 53–54 mol% (Miyamoto et al. 2005). On the basis of DNA–DNA hybridization analysis, *Lb. harbinensis* was isolated from *suan cai* (Miyamoto et al. 2005).

5.2.14 *Sunki*

Sunki is a nonsalted and fermented vegetable product prepared from the leaves and stems of red of turnip in Japan (Battock and Azam-Ali 1998). During its preparation, turnip is boiled, inoculated with *zumi* (a small wild apple) and dried *sunki* from the previous year, and allowed to ferment for 1–2 months. *Sunki* is eaten with rice and *miso* (fermented soybean) soup. *Sunki* is produced under low temperatures (in the winter season).

5.2.14.1 Microbiology

The microorganisms involved include *Lb. plantarum*, *Lb. brevis*, *Lb. buchneri*, *Enterococcus faecalis*, *B. coagulans*, and *P. pentosaceus* (Itabashi 1986). Recently, Watanabe et al. (2009) reported four novel species of lactic acid bacteria isolated from *sunki* products of Japan, which included *Lactobacillus kisonensis* sp. nov. (type strain YIT 11168^T = NRIC 0741^T = JCM 15041^T = DSM 19906^T), *Lactobacillus* sp. nov. (type strain YIT 11163^T = NRIC 0742^T = JCM 15040^T = DSM 19908^T), *Lactobacillus rapi* sp. nov. (type strain YIT 11204^T = NRIC 0743^T = JCM 15042^T = DSM 19907^T), and *Lactobacillus sunkii* sp. nov. (type strain YIT 11161^T = NRIC 0744^T = JCM 15039^T = DSM 19904^T).

5.2.15 *Fu-tsai* and *Suan-tsai*

Fu-tsai and *suan-tsai* are ethnic fermented mustard products of Taiwan prepared by the Hakka tribes, and are commonly consumed as soup, or fried with shredded meat, or stewed with meat (Chao et al. 2009). In the traditional method of preparation, freshly harvested mustard is wilted, placed in a bucket in layers by alternating with 4% salt, sealed airtight, and left to ferment naturally for 7 days to 2 months at ambient temperature. If fermentation is continued for approximately 2 months, the resultant product is called *suan-tsai*. However, for the production of *fu-tsai*, partly fermented mustard is removed from the bucket after 7 days, cleaned with water, sun-dried for 1–2 days, and then again fermented for at least 2 days. This process of sun-drying during the daytime and fermentation during the night is repeated two or three times. The partly dried mustard is then divided into pieces and packed tightly into glass bottles, earthenware pots, or plastic containers, which are then sealed and, finally, placed upside down for maturation for a period of 3 months. The resultant product is called *fu-tsai*.

5.2.15.1 Microbiology

Lactic acid bacteria involved in the natural fermentation of *fu-tsai* and *suan-tsai*, the products of Taiwan, are *Pediococcus pentosaceus* and *Tetragenococcus halophilus* (Chen et al. 2006), *Lactobacillus farciminis*, *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides*, *Weissella cibaria*, and *Weissella paramesenteroides* (Chao et al. 2009).

5.3 Pickled Vegetables

Vegetables may be preserved by fermentation, direct acidification, or a combination of these along with other processing conditions and additives to yield products that are referred to as pickles. Although the term pickles in the United States generally refer to pickled cucumbers, the term is used in a broader sense to refer to all vegetables that are preserved by fermentation or direct acidification. Cucumbers, cabbages, olives, and peppers account for the largest volume of vegetables and fruits commercially pickled. Lesser quantities of onions, tomatoes, cauliflowers, carrots, melon rinds, okra, artichokes, beans, and other produce also are pickled (Fleming 1982). The combination of acid, spices, and sugar with cucumbers creates the acidic food product known as pickles (Schafer 1989). Pickled cucumbers are made in Africa, Asia, and Latin America. Cucumbers undergo typical lactic acid fermentation and change from a pale product to a darker green and more transparent product. Sauerkraut, which is made of cabbage, salt, and in some cases also spices, and fermented to obtain an acid product, is an ancient food which is now consumed mainly in Europe.

5.3.1 Pickled Cucumbers

5.3.1.1 History

The cucumber (*Cucumis sativus*) was brought from India to the Middle East about 4000 years ago (Harris 1998). Vegetable fermentation may have started in China. The pickling of cucumbers probably originated in Southeast Asia. Pickled vegetables, made in households or small factories, have been popular in Egypt for centuries (Prajapati and Nair 2003). Pickles were brought to the New World by Christopher Columbus, who is known to have grown cucumbers for the purpose of pickling on the island of Haiti. As early as 1606, pickles were being produced at home and commercially in Virginia (Terebelsky and Ralph 2003).

5.3.1.2 Preparation and Culinary

Pickle products are classified on the basis of ingredients used and the method of preparation. There are two general classes: (1) Fermented pickles or brined pickles (Figure 5.7) that undergo a curing process for several weeks in which fermentative bacteria produce acids necessary for the preservation process. These bacteria also generate flavor compounds which are associated with fermented pickles; (2) fresh-pack or quick process pickles (i.e., whole cucumber dills, crosscut cucumber slices, bread-and-butter pickles) are made by the addition of an acid such as vinegar and not by the natural fermentation of the vegetable. The flavor of these easily prepared products is due to the acetic acid in vinegar (Schafer 1989).

Cucumber pickles are preserved by fermentation (40% U.S. production), pasteurization (40%), or refrigeration (20%) (Fleming et al. (1995) referred by Harris (1998)). Cucumbers for pickling differ from market varieties in that they are selected for regular form and firm texture. Pickling cucumbers are harvested while still immature (Harris 1998). Cucumbers without bruising or damage are washed in potable cold water and drained. The cucumbers can be pickled whole or sliced (Battock and Azam-Ali 1998).

FIGURE 5.7 Fermented cucumber. (Image by AGphoto 2010. Used under license from Shutterstock.com.)

About 1 kg of salt is added to every 20 kg of small cucumbers and 15 kg of large cucumbers. The brine should be formed within 24 h by osmosis. If the brine formed by osmosis does not cover the cucumbers, 40° salometer brine is added to the desired level. A day or two after the tank is filled and closed, the brine should be stirred in order to help equalize the concentration of salt throughout the mass (Vaughn 1985). Brining has long been used for the storage of many vegetables. It is a low-energy means for the temporary storage of perishable produce (Fleming et al. 1983).

As soon as the brine is formed, fermentation starts and bubbles of carbon dioxide appear. Fermentation takes between 1 and 4 weeks depending on the ambient temperature. It is complete when no more bubbles appear (Battock and Azam-Ali 1998).

During fermentation the brine becomes cloudy for the first few days due to the growth of bacteria. Later if the brine is not covered, a filmy yeast growth will often occur on the surface (Pederson 1979). Cucumbers undergo lactic acid fermentation during storage. Fermentation offers the advantages of acid formation and removal of fermentable sugars, which serve to prevent the growth of pathogenic microorganisms, to stabilize the products and to enhance flavor in the products (Fleming et al. 1983). Successful fermentation of brined vegetables is influenced by numerous chemical and physical factors including the concentration and types of fermentable carbohydrate of the raw product, and buffering capacity of the vegetables (Fleming 1982). Etchells et al. (1973) added sodium acetate to brined cucumbers, which permitted complete fermentation of cucumber sugars by the added culture of *Lb. plantarum*. Neutralization of brine acid by addition of NaOH has been suggested as an inexpensive means of assuring complete fermentation of cucumbers (Lingle 1975). Cucumber fermented by *Lb. plantarum* with pH control were microbiologically stable during 12 months storage in hermetically sealed jars at 24°C, provided all fermentable sugars were removed during fermentation, and the products were stored at pH 3.8 or below (Fleming et al. 1983). Cucumber pickle is usually stored in clean capped jars. They keep well if stored in a cool place. Pasteurization of the fermented products prevents microbial growth in cucumber products, but did not prevent firmness loss and off-flavor changes (Fleming et al. 1983). Blanching prior to fermentation has been suggested by Fleming et al. (1983) and others and has been applied in controlled fermentation

to reduce the incidence of bloater formation and to increase firmness retention of the fermented products. Blanch treatment had little effect on sugar utilization, acid production, and terminal pH in cucumber fermentations (Lu et al. 2002).

5.3.1.3 Microbiology

The natural succession of microbial populations in cucumber fermentations begins with high levels of aerobic bacteria, sometimes including pathogenic and spoilage organisms (Breidt 2006). Relatively low numbers of LAB are naturally present initially, but are able to outcompete, to thrive in the presence of organisms that are competing for resources, early during the fermentation due to their ability to survive in extreme environments characterized by high acid and salt concentrations (Breidt 2006, Hutkins 2006). *Lb. plantarum* is the dominating species in natural cucumber fermentation and is detected in all phases of fermentation, as well as in the storage period (Etchells et al. 1975 referred by Tamminen et al. 2004). Tamminen et al. (2004) identified *Lb. plantarum*-related (*Lb. plantarum* and *Lb. pentosus*) and *Leuconostoc* species. Singh and Ramesh (2008) reported an analysis using culture-independent and -dependent methods to determine the dominant LAB genera in fermented cucumber and simultaneously monitor antagonism by detecting bacteriocin producers at various periods of the fermentation. During early hours of fermentation, *Lactobacillus* and *Leuconostoc* emerged as the dominant genera. Nucleic acid sequence of culture-independent clones confirmed the detection of *Pediococcus* as a dominant genera emerging during the late stages of the fermentation. PCR also revealed time-dependent emergence of mesentericin, pediocin, and plantaricin A producers and accounted for the LAB succession in the fermenting samples. Bacteriocin producers have been isolated from cucumber fermentation. A strain of *P. acidilactici* CFR K7 isolated from cucumber produced an antimicrobial peptide that acted against *Leuc. mesenteroides*, selected strains of *Lactobacillus* spp., *Pediococcus* spp., and *Enterococcus* spp. The bacteriocin possessed strong antilisterial activity and was susceptible to proteolytic enzymes (Halami et al. 2005). The pathogen of concern in anaerobic, acid, or acidified foods is *Clostridium botulinum*, which will not produce its neurotoxin when the pH is at or below 4.6 (Ito et al. 1976). Incidence of food-borne illness due to fermented or acidified vegetables is extremely rare due to the nature of these products (Breidt 2006).

The U.S. Food and Drug Administration has raised concerns about the safety of acidified vegetable products that are not heat processed. The minimum times and temperatures for heat processing acidified cucumbers to ensure safety have been determined (Breidt et al. 2005). However, acidified vegetable products with a pH of 3.3 or below may contain enough acid to ensure a 5-log reduction in bacterial pathogens without heat treatment. Breidt et al. (2007) show that in a representative acidified vegetable product (cucumbers) with a pH at or below 3.3, a 5-log reduction in viable cell counts for acid-resistant pathogens (*E. coli* 0157:H7, *Salmonella enterica*, and *L. monocytogenes*) occurred without heat treatment within 6 days. The utilization of starter cultures enables manufacturers to make food products with standard quality in a shorter time (Tamminen et al. 2004). Desai and Sheth (1997) have reported that the use of *Lc. lactis*, *P. pentosaceus*, *Lb. brevis*, *Lb. plantarum*, and *Leuc. mesenteroides* as starters in the fermentation of cucumbers significantly

increased acid production, when compared with spontaneously fermented cucumbers. In the manufacture of fermented cucumbers, LAB starter cultures are known to prevent economic losses due to pickle spoilage such as bloater, softness, and off colors. The formation of "bloaters" (hollow stock) in brined cucumbers has been attributed chiefly to gaseous fermentation produced by yeasts, coliforms, or heterofermentative LAB. To remove CO_2, manufacturers will often purge air into the tanks (Etchells et al. 1968, Fleming et al. 1975). Pickle softening occurs as a consequence of spoilage bacteria and fungi being present. These organisms produce enzymes that hydrolyze pectin in the cucumber cell wall during fermentation (Breidt et al. 2007). Spoilage by propionic and butyric acids has been documented in the fermentation of cucumbers. Occasional red-colored cucumber spoilage has been reported in the pickling industry. *Lb. casei* and *Lb. paracasei* were determined as the causative agents (Pérez-Díaz et al. 2007). Putrescine, spermine, and tyramine were found in packed fermented cucumbers. There are no regulations anywhere in the world regarding the concentration of histamine and total biogenic amines in pickles; however, several authors have indicated that a histamine content of less than 50 mg/kg or a total amine content of less than 100 mg/kg can be considered safe (Nout 1994, García-García et al. 2001). *Pediococcus* phages were detected in some fermented vegetables, such as cucumber pickle, sauerkraut, and *kimchi* (Yoon et al. 2002). *Lb. plantarum* bacteriophage ΦJL-1 was isolated and characterized from a cucumber fermentation (Lu et al. 2003b). Sung-Sik et al. (2007) detected and characterized a lytic *Pediococcus* bacteriophage from fermenting cucumber brine.

5.3.1.4 Biochemistry

The composition of cucumber varies with the fruit size and the cultivar. Cucumbers are high moisture foods (95%) with little protein (1%). The total sugar content in cucumbers is in the range of 1.7%–2.4%, depending on the fruit size. The principal fermentable carbohydrates found in pickling cucumbers are fructose and glucose (Lu et al. 2002, Hutkins 2006). Compositional factors such as pH, buffer capacity, and initial sugar content of the fruit may affect the extent of sugar utilization and terminal pH in cucumber fermentation. Larger fruits contain higher levels of sugar, which will require more added buffer to ensure complete sugar utilization. Smaller fruits, however, contain less sugar and a higher natural buffering capacity than larger ones (Lu et al. 2002).

5.3.1.5 Socioeconomy

Currently, about half of the total cucumber crop (1 billion kg) in the United States is used for pickles (Harris 1998).

5.4 Fermented Bamboo Shoots

5.4.1 *Mesu*

Mesu is an ethnic fermented bamboo shoot product of Nepalis living in the bamboo-growing Himalayan regions of India, Nepal, and Bhutan (Figure 5.8).

FIGURE 5.8 *Mesu*.

5.4.1.1 Preparation and Culinary

Tender shoots of species of bamboo such as *Dendrocalamus sikkimensis*, *Dendrocalamus hamiltonii*, and *Bambusa tulda* are defoliated, chopped finely, and pressed tightly into a green hollow bamboo stem. The opening of the vessel is covered tightly with leaves of bamboo or other wild plants and left to ferment under natural anaerobic conditions for 7–15 days. *Mesu* is eaten as a pickle. *Mesu* pickle is mixed with oil, chillies, and salt, and can be kept in a closed jar for several months. It is sold in the local markets when bamboo shoots are available plenty.

5.4.1.2 Microbiology

Functional LAB, such as *Lb. plantarum*, *Lb. brevis*, *Lb. curvatus*, *Leuc. citreum*, and *P. pentosaceus* have been isolated from *mesu* (Tamang and Sarkar 1996, Tamang et al. 2008). DAP-negative homofermentative strains isolated from *mesu* have been genotypically identified as *Lb. curvatus* (Tamang et al. 2008).

5.4.2 Soibum

Soibum is an ethnic, fermented tender bamboo shoot food popular in Manipur, India (Figure 5.9).

5.4.2.1 Preparation and Culinary

During *soibum* production, thin slices of the succulent bamboo shoots (*D. hamiltonii*, *D. sikkimensis*, *D. giganteus*, *Melacona bambusoide*, *B. tulda*, and *B. balcooa*) are packed compactly into a chamber. After filling the chamber with the slices to its capacity, the upper surface is sealed with polythene sheet and weights are then put on top for proper pressing. The bottom of the chamber is perforated for draining acidic fermented juice during fermentation. It is left for 6–12 months for fermentation. After fermentation, *soibum* is stored for 10–12 months. Different dishes are prepared from

FIGURE 5.9 *Soibum* and *soidon*.

soibum in Manipur, India, such as *ironba*, *athongba*, *kangou*, and *chagempomba* (Tamang 2010). *Soibum* is commonly sold in local vegetable markets in Manipur.

5.4.2.2 Microbiology

Lb. plantarum, *Lb. brevis*, *Leuc. fallax*, *Leuc. mesenteroides*, *Leuc. lactis*, and *Enterococcus durans* are the functional LAB in *soibum* (Tamang et al. 2008).

5.4.2.3 Nutrition

Increase in free amino acids has been observed during the fermentation of *soibum* (Giri and Janmejay 1994, 2000). Enhancement of nutritional value in *soibum* has been reported by Pravabati and Singh (1986). *B. subtilis*, *B. licheniformis*, *B. coagulans*, and *Micrococcus luteus* isolated from *soibum* are found to be involved in the microbial bioconversion of phytosterol (Sarangthem and Singh 2003).

5.4.3 *Soidon*

Soidon is the fermented tip of a matured bamboo shoot product popular in Manipur, India (Figure 5.9).

5.4.3.1 Preparation and Culinary

Tips of matured bamboo shoot are collected, and the outer casings and lower portions are removed. Whole tips are submerged in water in an earthen pot. The sour liquid (*soijim*) of the previous batch is added as a starter in 1:1 dilution, covered, and fermented for 3–7 days at room temperature. Leaves of *Garcinia pedunculata* Roxb., locally called *heibung*, may be added in the fermenting vessel during the fermentation to enhance the flavor of *soidon*. After fermentation, *soidon* is removed from the pot and is stored in a closed container at room temperature for a year. It is consumed as a curry as well as a pickle with steamed rice.

5.4.3.2 Microbiology

Lb. brevis, *Leuc. fallax*, and *Leuc. lactis* have been isolated from *soidon* (Tamang et al. 2008).

5.4.4 Soijim

Soijim is the liquid formed during the fermentation of *soidon*. It is acidic and sour in taste and is commonly used as a condiment in Manipur, India (Tamang 2010).

5.4.4.1 Preparation and Culinary

The acidic liquid portion formed during the fermentation of *soidon* is called *soijim*. It is stored in a bottle for a year or more. *Soijim* is used as a starter for the fermentation of *soidon* and also as a condiment to supplement the sour taste in local cuisine.

5.4.4.2 Microbiology

Lb. brevis, *Leuc. fallax*, and *Leuc. lactis* are the dominant LAB in soijim (Tamang et al. 2008).

5.4.5 Ekung

Ekung is an ethnic, fermented bamboo tender shoot product popular in Arunachal Pradesh, India.

5.4.5.1 Preparation and Culinary

Young bamboo tender shoots (*D. hamiltonii*, *D. giganteus*, *Phyllostachys assamica*, *B. tulda*, *B. balcooa*) are collected and outer leaf sheaths are removed. The edible portions are chopped into several pieces. A pit of about 3–4 ft. is dug in the forest usually in and around water sources to facilitate the washing of the bamboo shoot pieces. The bamboo baskets are laid into the pit and lined with leaves. Chopped bamboo shoot pieces are put into the basket. When the basket is full, it is covered with leaves and then sealed. Heavy stones are kept to give weight to drain excess water from the bamboo shoots and fermented for 1–3 months. *Ekung* can be kept for a year in an airtight container at room temperature. It is consumed raw or is cooked with meat, fish, and vegetables.

5.4.5.2 Microbiology

Lb. plantarum, *Lb. brevis*, *Lb. casei*, and *Tetragenococcus halophilus* were isolated from *ekung* (Tamang and Tamang 2009b).

5.4.6 Eup

Eup is a dry, fermented bamboo tender shoot food commonly prepared and consumed by different tribes of Arunachal Pradesh, India (Tamang 2010).

5.4.6.1 Preparation and Culinary

Bamboo shoots are chopped into small pieces and fermented in a similar manner as in *ekung*. Fermentation is completed in about 1–3 months. After fermentation, the fermented product, now *eup*, is again cut into smaller pieces and then dried in the sun for 5–10 days until its color changes from whitish to chocolate brown. *Eup* is consumed as a side dish with steamed rice, meat, fish, or vegetables.

5.4.6.2 Microbiology

Lb. plantarum and *Lb. fermentum* were isolated from *eup* (Tamang and Tamang 2009b).

5.4.7 *Hirring*

Hirring is a fermented topmost whole-bamboo shoot product, commonly prepared in Arunachal Pradesh, India.

5.4.7.1 Preparation and Culinary

The outer leaf sheaths of tender bamboo shoots are removed. The topmost tender edible portions are either cut longitudinally into 2–3 pieces or whole shoots are flattened by crushing, and are put into bamboo baskets lined with leaves. The baskets are placed into the pit, covered with leaves, sealed and weighted down with heavy stones, and fermented for 1–3 months. The baskets are taken out from the pits after the fermentation; *hirring* is ready for consumption. *Hirring* can be kept for 2–3 months at room temperature. It is consumed as a side dish mixed with vegetables, meat, and fish, along with steamed rice.

5.4.7.2 Microbiology

Lb. plantarum and *Lc. lactis* are the functional LAB in *hirring* (Tamang and Tamang 2009b).

5.4.8 *Naw-Mai-Dong*

Naw-mai-dong is a traditional fermented bamboo shoot (*Bambusa arundinacea*) product prepared in Thailand (Dhavises 1972). The sweeter species of bamboo such as *Bambusa burmanica* and *Dendrocalamus asper* are also used as raw materials (Boon-Long 1986). During preparation, young bamboo shoots are harvested; woody and defective portions are removed from the shoots by trimming. After washing, the shoots are sliced and mixed with 2% salt. The bamboo shoots are boiled in water, and the bitter liquor is discarded. These are then packed into an earthen jar, covered with plastic sheets, and weights kept at the top. The fermentation is complete in 3–4 weeks at room temperature.

5.4.8.1 Microbiology

The microorganisms present in *naw-mai-dong* are *Leuc. mesenteroides*, *Lb. fermentum*, *Lb. buchneri*, *Lb. brevis*, and *P. pentosaceus* (Dhavises 1972, Phithakpol et al. 1995).

5.5 Conclusion

LAB are the primary functional microorganisms in vegetable fermentation. Most of the traditional fermented vegetables as well as fermented bamboo products are through natural lactic acid fermentation. Species of *Lactobacillus*, *Leuconostoc*, *Enterococcus*, *Pediococcus*, *Lactococcus*, *Weissella*, and *Tetragenococcus* are associated with the biopreservation of perishable vegetables and bamboo shoots. It is interesting to note that most of the Himalayan fermented vegetable products are sun dried after fermentation, and consumers prefer the dry products instead of the wet. However, in other places, consumers prepare freshly fermented wet products such as *kimchi*, sauerkraut, etc. Strains of LAB play a complex and constructive role in the vegetable fermentation, enhancing the functional properties of the products. Most commercial starter cultures are used in dairy and meat fermentations. LAB of vegetable fermentations offer interesting characteristics too, so that some strains may be used as starter cultures for the *ex situ* fermentation of perishable vegetables.

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6

Fermented Legumes: Soybean and Non-Soybean Products

Toshirou Nagai and Jyoti Prakash Tamang

CONTENTS

6.1 Introduction

Among the legumes, soybeans are mostly fermented traditionally and made into recipes mostly by the ethnic people of Asia. The preparation and consumption of sticky, nonsalty, flavorsome fermented soybean foods are the traditional wisdom of the people from several South-East Asian countries, which have fostered a distinct food culture of the people (Tamang 2010). *Bacillus subtilis* is an important starter culture for many Asian and African fermented soybean foods (Kiers et al. 2000). Soybean is a major leguminous crop in the world, and its utilization as food is mostly confined to Asia. Some of the common ethnic, nonsalted sticky fermented soybean foods are *natto* (Japan); *kinema* (India, Nepal, and Bhutan); *tungrymbai*, *bekang*, *hawaijar*, *aakhuni*, and *peruyaan* (India); *thua nao* (Thailand); *chungkokjang* (Korea); *pepok* (Myanmar); and *sieng* (Cambodia). Nonsalted fermented soybean foods are concentrated in a region of the world represented by a triangle with three vertices, one each on Japan (*natto*), Nepal (*kinema*), and Indonesia (*tempe*). Nakao (1972) named this triangle the "*natto* triangle" and included all fermented soybean products including *miso*, soy sauce, and *tempe* and extended this triangle up to Indonesia (Figure 6.1). Tamang (2010) renamed this hypothetical triangle the "*kinema-natto-thua nao* triangle" (KNT triangle). Within the proposed triangle-bound countries, many fermented sticky nonsalty soybean foods are consumed by the different ethnic groups of people in Cambodia, Laos, Vietnam, Darjeeling hills and Northeast states in India, eastern part of Nepal, southern part of Bhutan, Myanmar, and southern parts of China (Figure 6.2). Beyond this hypothetical "KNT triangle," there is no report of *kinema*-like products that are sticky and ammonia-flavored fermented soybean foods, and the proposed "KNT triangle" does not include salted, nonsticky and non-bacilli fermented soybean products such as *tempe*, *miso*, *sufu*, soy sauce, etc. (Tamang 2010). Although the method of production and culinary practices vary from product to product, all bacilli-fermented Asian soybean foods have a characteristic stickiness and typical flavor. Hara et al. (1995) reported that the plasmid of *Bacillus subtilis* (*natto*) strain resembles that of *Bacillus subtilis* isolated from *thua nao* and *kinema*. A phylogenetic analysis showed the similarity among the strains of *B. subtilis* isolated from common sticky fermented soybean foods of Asia (Tamang et al. 2002, Meerak et al. 2007). This suggests that *B. subtilis* strains responsible for the fermentation of sticky soybean foods of Asia might have originated from the same stock.

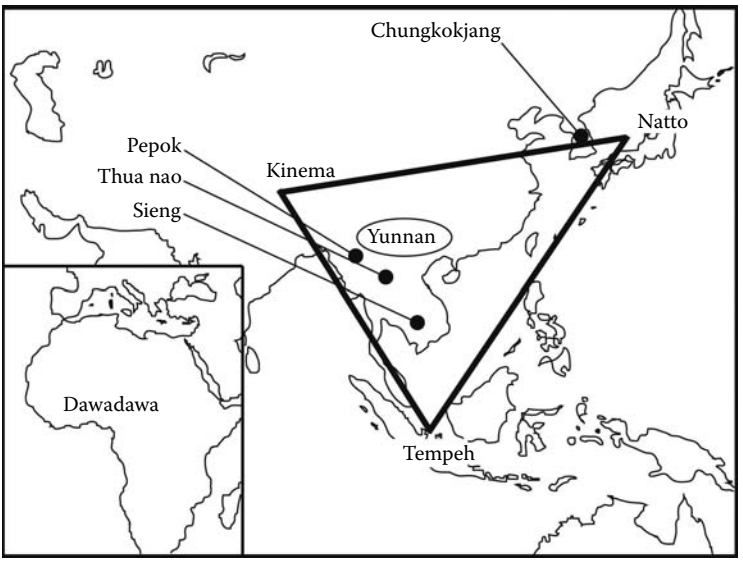


FIGURE 6.1 *Natto* triangle. (Based on Nakao, S., *Ryori no kigen*, Japan Broadcast Publishing (Japanese), Tokyo, Japan, pp. 115–126, 1972.)

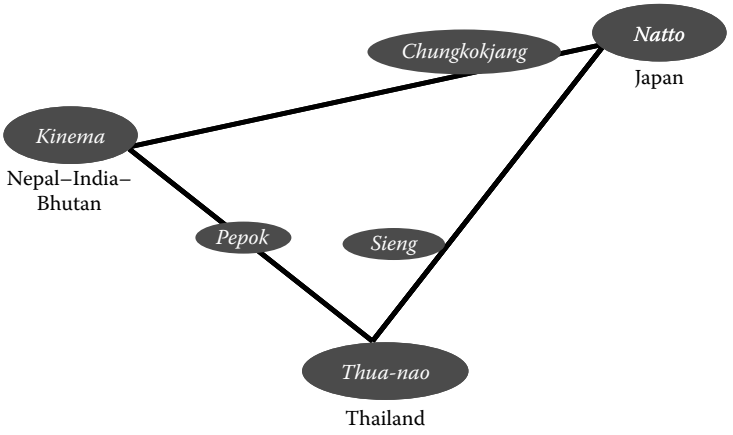


FIGURE 6.2 KNT (*kinema-natto-thua nao*) triangle. (Adapted from Tamang, J.P., *Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values*, Taylor & Francis Group, Boca Raton, FL, 2010.)

Another theory proposed was that nonsalted fermented soybean foods originated in the Yunnan province of China, which was the center of the hypothetical triangle. Therefore, the report of fermented non-soybean legumes (*dawadawa*, *iru*, *ugba*) from Africa caused an excitement among fermentation researchers (Odunfa 1985). The production processes of these legume foods in homes are very similar: boiled beans are wrapped in, for example, leaves or baskets and undergo fermentation for a long time. In this chapter, fermented legumes are described in two parts: fermented soybean foods and fermented non-soybean legume foods.

6.2 Fermented Soybean Foods

There are two types of fermented soybean products: fermented soybean foods that are solely fermented by *Bacillus* spp. (mostly *B. subtilis*), e.g., *natto*, *kinema*, *thua nao*, etc., and have characteristic stickiness, and the other type is foods fermented by molds, e.g., *tempe*, *miso*, *soy sauce*, etc. Again, fermented soybean can be classified into two types based on salty taste: nonsalted fermented soybeans, e.g., *kinema*, *natto*, *tempe*, etc., and salted fermented soybeans, e.g., *miso*, soy sauce, *douchi*, etc.

6.2.1 Bacilli-Fermented, Sticky and Nonsalty Soybean Foods

6.2.1.1 Natto

Natto is a Japanese fermented soybean food that has a characteristic ammonia odor, and contains fatty acids and viscous strands of a polymer of glutamate (Figure 6.3). In contrast to other salted fermented soybeans, *natto* is prepared in a very short time of 2 days (Hosoi and Kiuchi 2003).

6.2.1.1.1 History

The origin of *natto* is obscure or legendary. The most famous legend of the origin of *natto* is that of Yoshiie's. In 1083, Hachiman-taro-Yoshiie, the chief of a samurai legion, stayed at a house on the course of conquering the northern area of Japan. His retainer boiled soybeans for horses and wrapped the remaining soybeans with straws. The wrapped soybeans were fermented, after being tied to the back of a horse, that

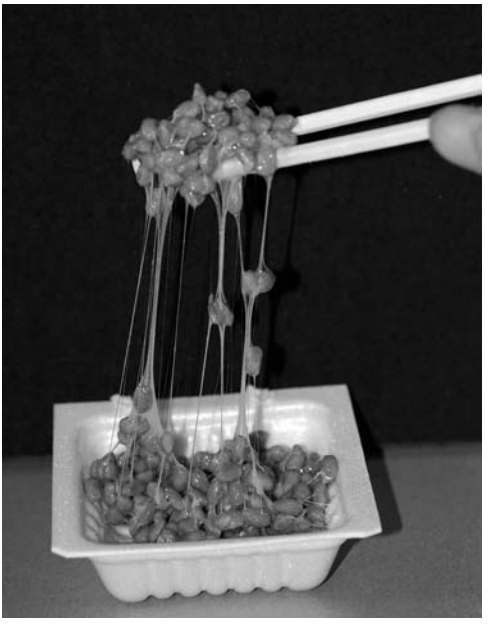


FIGURE 6.3 *Natto* in a polystyrene paper package. *B. subtilis* (*natto*) produces a polyglutamate, viscous material, which is called *ito*, meaning string in Japanese.

produced a viscous material on the soybeans. The retainer ate the soybeans and found it tasted very delicious. The fermented soybean food was *natto*. The first mention of *natto* appeared in *Shin-Sarugo-Ki* written around 1050 by Akihira Fujiwara. There is, however, a possibility that *natto* mentioned in the book was actually *shiokara-natto*, which is a salted fermented soybean food originating from ancient China. The second mention is found in *Shojin Gyorui Monogatari* written in the mid-fifteenth century. In this fairy tale, *natto* was personalized as a samurai warrier reflecting a characteristic of *natto*, i.e., the production of viscous strands (γ-polyglutamate) (Nagayama 1993).

6.2.1.1.2 Preparation and Culinary

In a classical method for preparing *natto*, soybeans are soaked in water overnight after cleaning them. Soon after boiling, the soybeans are wrapped with tied rice straws that have been soaked in boiling water to sterilize microorganisms other than heat-tolerant spores of *B. subtilis* (*natto*), which inhabit the surface of straws (Figure 6.4). *B. subtilis* (*natto*) ferments the soybeans after germination. A modern method to prepare *natto* is based on the classical one. After boiling, the soybeans are sprayed with a suspension of spores of *B. subtilis* (*natto*) and weighed out by 30–100 g in polystyrene paper packages (Figure 6.3) instead of wrapping in rice straws. The packages are transferred into incubators. After 16 h at around 40°C, the packages are cooled for 6–8 h for maturation. *Natto* is eaten directly without frying or cooking with boiled rice, usually mixed with *tare*, a kind of seasoning and some *yakumi*-like mustard, chopped cibol, dried bonito flake, or yolk depending on individual preferences. Recently, *natto* has been used as a cooking ingredient, for example, in pasta, *sushi*, and pizza.

6.2.1.1.3 Socioeconomy

Natto is mainly produced and consumed in Japan, though *natto*-like foods are prepared in other countries, especially in the Southeast Asian countries. People in the northern areas of Japan consume *natto* more than that of the western areas. In Japan, the total production of *natto* was 250,000 tons in 2004, which was equivalent to 139,000 ton of soybeans. The annual sales amounted to 111.4 billion yen in 2004 (Ministry of Agriculture, Forestry and Fisheries, Government of Japan 2006).

6.2.1.1.4 Microbiology and Food Safety

Bacillus natto was isolated from homemade *natto* by Sawamura (1906). The bacterium is different from *B. subtilis*, with regard to biotin requirement, production of polyglutamate, possession of 5.7 and 60 kb plasmids (Hara et al. 1983, Nagai et al. 1997)

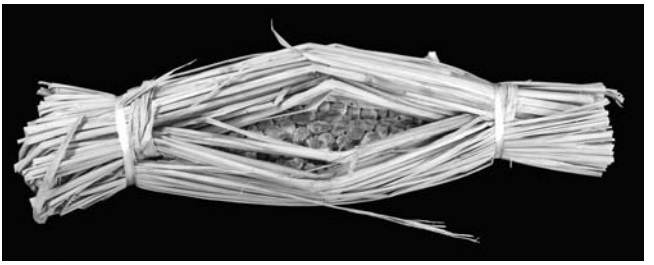


FIGURE 6.4 *Natto* in a classical package made of rice straws.

and insertion sequences (Nagai et al. 2000, Kimura and Itoh 2007), and infection with some kinds of bacteriophages (Fujii et al. 1975). The bacterium, however, was classified into *B. subtilis* in Bergey's Manual (Smith and Gordon 1957). At the result of genetic experiments on *B. subtilis* (*natto*) Asahikawa, it was reported that the regulatory gene for the polyglutamate synthesis was encoded on a 5.7 kb plasmid, pUH1 (Hara et al. 1983). Plasmids of this type, named "*natto* bacterium plasmids," are known to distribute not only among *B. subtilis* starters for *natto* production but also among *B. subtilis* strains isolated from nonsalted fermented soybeans in Asia. However, it was shown that the plasmid harbored no gene for polyglutamate production, because elimination of the plasmid did not affect the polyglutamate production and the plasmid did not confer the ability to produce polyglutamate on *B. subtilis* in transformation experiments (Koehler and Thorne 1987; Nagai et al. 1997). The genes for polyglutamate production, *pgsBCA*, were cloned, sequenced, and expressed in *Escherichia coli* clone cells (Ashiuchi et al. 1999).

Natto prepared naturally in straws was contaminated with bacteria occasionally, causing food poisoning. Since the early twentieth century, when *B. subtilis* (*natto*) was discovered, a pure culture of *B. subtilis* (*natto*) has been used to prepare *natto* in a clean environment. Contamination with bacteria has become very rare these days. *Natto* has been consumed by the Japanese for more than hundreds of years, proving that *natto* is a very safe food (Ohta 1986, Hosoi and Kiuchi 2003). However, patients who are treated with warfarin (also called coumadin) for anticoagulant therapy should not take *natto* because of the antagonistic actions of vitamin K_2 (menaquinone-7) produced by *B. subtilis* (*natto*) on warfarin (Kudo et al. 1978).

6.2.1.1.5 Biochemistry, Nutritional Composition, and Functional Properties

The nutritional composition of *natto* is summarized in Table 6.1. Compared to boiled soybeans, *natto* has a high vitamin K content. Vitamin K is known to be antagonistic to warfarin, while it plays an important role in blood coagulation and osteogenesis. *B. subtilis* (*natto*) produces nattokinase, which has a high fibrinolytic activity equal to that of urokinase and plasmin (Sumi et al. 1987). Capsulated nattokinase showed enhancement of fibrinolytic activity in plasma significantly after administration to adults (Sumi et al. 1990).

Soybeans contain some allergenic proteins, the major one being Gly m Bd 30K (Ogawa et al. 1991). Gly m Bd 30K was also detected in nonfermented soybean food products (soy milk, *tofu*, soybean flour, etc.) but not in fermented soybeans (*natto*, *miso*, *soysauce*) (Tsuji et al. 1995). Gly m Bd 30K from raw soybeans was degraded by *B. subtilis* (*natto*) in the course of fermentation (Yamanishi et al. 1995). Therefore, *natto* is a suitable soybean food for patients allergic to raw soybeans. Pyrazines are found to be the major flavoring agents contributing to the characteristic *natto* flavor (Sugawara et al. 1985), which are also commonly found in many Asian nonsalted, fermented soybeans, such as *kinema*, *thua nao*, and *pepok* (Sugawara et al. 1998).

6.2.1.2 Kinema

Kinema is an ethnic, bacilli-fermented, sticky soybean food with a slight ammonia flavor produced by natural fermentation (Figure 6.5). *Kinema* is one of the oldest cultural foods of the non-Brahmin Hindu Nepali (Tamang 2010).

TABLE 6.1

Nutritional Composition of Boiled Soybeans (as Control), *Natto*, and *Tempe*

	Boiled Soybeans	***Natto***	***Tempe***
Energy (kcal)	180	200	202
Water (g)	63.5	59.5	57.8
Protein (g)	16.0	16.5	15.8
Lipid (g)	9.0	10.0	9.0
Carbohydrate (g)	9.7	12.1	15.4
Ash (g)	1.8	1.9	2.0
Minerals			
Sodium (mg)	1	2	2
Potassium (mg)	570	660	730
Calcium (mg)	70	90	70
Magnesium (mg)	110	100	95
Phosphorus (mg)	190	190	250
Iron (mg)	2.0	3.3	2.4
Zinc (mg)	2.0	1.9	1.7
Copper (mg)	0.24	0.61	0.52
Manganese (mg)	—	—	0.80
Vitamins			
A, retinol (μg)	(0)	(0)	(0)
A, α-carotene (μg)	0	—	—
A, β-carotene (μg)	3	—	—
A, cryptoxanthin (μg)	0	—	—
D (μg)	(0)	(0)	(0)
E, α-tocopherol (mg)	0.8	0.5	0.8
E, β-tocopherol (mg)	0.3	0.2	0.2
E, γ-tocopherol (mg)	6.0	5.9	8.5
E, δ-tocopherol (mg)	3.4	3.3	4.0
K (μg)	7	600	11
B_1 (mg)	0.22	0.07	0.07
B_2 (mg)	0.09	0.56	0.09
Niacin (mg)	0.5	1.1	2.4
B_6 (mg)	0.11	0.24	0.23
B_{12} (μg)	(0)	Tr	0
Folate (μg)	39	120	49
Pantothenic acid(mg)	0.29	3.60	1.08
C (mg)	Tr	Tr	Tr
Fatty acids			
Saturated (g)	1.22	1.47	1.20
Monounsaturated (g)	1.73	1.90	1.61
Polyunsaturated (g)	4.93	5.39	4.69

(*continued*)

TABLE 6.1 (continued)

Nutritional Composition of Boiled Soybeans (as Control), *Natto*, and *Tempe*

	Boiled Soybeans	*Natto*	*Tempe*
Cholesterol			
Cholesterol (mg)	(Tr)	Tr	(0)
Dietary fibers			
Soluble (g)	0.9	2.3	2.1
Insoluble (g)	6.1	4.4	8.1

Source: Ministry of Education, Culture, Sports, Science and Technology, *Standard Tables of Food Composition in Japan*, 5th revised and enlarged edn., Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan, 2005. With permission.

Note: —, not determined; (), estimated; Tr, trace.

FIGURE 6.5 *Kinema*—freshly fermented.

6.2.1.2.1 Preparation and Culinary

During the traditional preparation of *kinema*, small-sized "yellow cultivar" dry soybean seeds are preferred (Tamang 2001), whereas in Nepal dark brown local varieties of soybean seeds are selected for making *kinema* (Nikkuni 1997). Soybeans are washed and soaked overnight, and soaked soybeans are removed and immersed into a container with fresh water, and boiled for 2–3 h until they become soft. Excess water is drained off, and the cooked soybean seeds are filled into a wooden mortar, locally called *okhli*, and are cracked lightly by a wooden pestle, locally called *muslo*, to split the cotyledons. This practice of cracking cooked seeds of soybeans is observed only during *kinema* production unlike that of *natto* and *chungkokjang*, probably to increase the surface area for a fast fermentation by aerobic spore-forming bacteria. About 1% of firewood ash is added directly to the cooked soybeans and mixed thoroughly to maintain the alkaline condition of the product. Soybean grits are placed in a bamboo basket lined with a locally grown fresh fern, *Glaphylopteriolopsis erubescens*, or

FIGURE 6.6 *Kinema* curry.

Ficus (fig plant) and banana leaves. The basket is covered in a jute bag and left to ferment naturally at ambient temperatures (25°C–40°C) for 1–3 days above an earthen oven. During summer, the fermentation may require 1–2 days while in winter it may require 2–3 days. The shelf life of freshly prepared *kinema* is 2–3 days in summer, and the maximum is a week in winter without refrigeration. It may be prolonged by drying in the sun for 2–3 days. Dried *kinema* is stored for several months at room temperature. Preparation of *kinema* varies from place to place and is still restricted to households. It is interesting to note mountain women using their indigenous knowledge of food production to prepare *kinema*. This unique knowledge of *kinema* making has been protected as a hereditary right and is passed from mother to daughter.

Some of the steps in *kinema* preparation do not resemble those of *natto*, and thus make *kinema* a unique nonsalted soybean fermented product. The cooked beans are lightly crushed to de-hull most of the seeds. But, fermentation is carried out with the kernels as well as the seed coats. Unlike *natto*, *kinema* is always fried in oil and made as a curry (Figure 6.6). Dried *kinema* is sometimes mixed with leafy vegetables to make a mixed curry.

6.2.1.2.2 Socioeconomy

The daily per capita consumption of *kinema* in Sikkim, India, is 2.3 g (Tamang et al. 2007). *Kinema* production is a source of marginal income generation in the Eastern Himalayas. *Kinema* is sold in all local periodical markets in eastern Nepal, Darjeeling hills, Sikkim, and southern parts of Bhutan by rural women. About 40% of profit is made by selling *kinema* by the marginal rural farmers in the Himalayas (Tamang 2010).

6.2.1.2.3 Microbiology and Food Safety

B. subtilis is the principal microorganism in *kinema* fermentation (Sarkar et al. 1994). A number of other species of *Bacillus* have been isolated from *kinema* that include *B. licheniformis*, *B. cereus*, *B. circulans*, *B. thuringiensis*, and *B. sphaericus* (Sarkar et al. 2002). However, *B. subtilis* is the dominant functional bacterium in *kinema* (Sarkar and Tamang 1994, Tamang and Nikkuni 1996). The traditional way of preparation of *kinema* and its use in curries is safe for consumption (Nout et al. 1998). Besides bacilli, the other lactic acid bacterium is *Enterococcus faecium*, and two types of yeasts, *Candida parapsilosis* and *Geotrichum candidum*, were also isolated from *kinema* samples (Sarkar et al. 1994). It has been observed that a rich microbial

diversity in various sources particularly soybean, the equipment used, and leaves used as wrapping materials harness microbiota for the spontaneous fermentation of *kinema* (Tamang 2000). Unclean mortars and pestles used during *kinema* production as well as fresh leaves used as wrapping materials supplement essential microorganisms for the natural fermentation of *kinema* without using starters (Tamang 2003). Strains of *Bacillus* isolated from *kinema* display a central to paracentral position of spores, with a few strains positive for the negative nitrate reduction test, whereas *B. subtilis* (*natto*) isolated from *natto* displays a central position of spores and all strains reduce nitrate (Tamang et al. 2002). The phylogenetic relationships among bacilli isolated from *kinema* (India), *chungkokjang* (Korea), *natto* (Japan), and other similar fermented sticky soybean foods of Asia on the basis of 16S rDNA sequence reveal that all bacilli strains belong to *B. subtilis* (Tamang et al. 2002). Although acidity increases at almost every 8 h interval, the fermentation is essentially alkaline, causing the pH of the beans to rise to about 8.5 (Sarkar and Tamang 1995). With the decline in protein nitrogen content, the nonprotein and soluble nitrogen contents increase during *kinema* fermentation. The protease produced by *B. subtilis* degrades soy proteins, which results in an increase of nonprotein nitrogen content. Due to the lipolytic activities of *Bacillus*, the fat content in soybeans is degraded to free fatty acids during fermentation.

6.2.1.2.4 Monoculture Fermentation of Kinema

Kinema is prepared by using a monoculture strain of *B. subtilis* KK2:B10 under optimized conditions in the laboratory (Tamang and Nikkuni 1998). During monoculture fermentation, the growth rate of viable cells of *B. subtilis* KK2:B10 is faster at 45°C till 16 h. *Kinema* prepared at 40°C shows a remarkable increase in relative viscosity from 16 to 20 h compared with *kinema* prepared at 35°C and 45°C (Tamang and Nikkuni 1998). The unique feature of *B. subtilis* KK2:B10 is the formation of sticky viscous materials, which starts from 8 to 12 h at all incubation temperatures. Both of water-soluble nitrogen and formol nitrogen contents of *kinema* increase rapidly during fermentation. This is due to the high proteolytic activity of *B. subtilis* KK2:B10 (Tamang 1995). Reducing sugars increase during the log phase and then decrease sharply during *kinema* fermentation (Tamang and Nikkuni 1996). The effect of temperature on the maturation of freshly prepared *kinema* has been studied, and a significant increase in the relative viscosity of *kinema* during maturation at 5°C and 10°C has been found (Tamang and Nikkuni 1998). However, no significant differences in water-soluble nitrogen and formol nitrogen content of *kinema* are observed during maturation at low temperatures compared with *kinema* kept at the control temperature. Maintaining freshly prepared *kinema* below 10°C for 1 day stabilizes the quality of the product by preventing further biological activity of microorganisms and shows better stickiness, which is a very important sensory property of *kinema* (Tamang 1995). Organoleptically, the monoculture fermentation of soybean by *B. subtilis* KK2:B10 (MTCC 2756) produces the best *kinema* because of a pleasant nutty flavor and highly sticky texture, minimizes the conventional fermentation time, maintains better hygienic conditions and consistency, and increases levels of soluble proteins (Tamang 1998).

6.2.1.2.5 Pulverized Starter for Kinema Production

Conventionally, crude soybean extract after cooking soybeans is discarded during *kinema* preparation. Inexpensive soybean extract broth, after adjusting the pH to 7, as a medium is prepared for the enrichment of *B. subtilis* spores instead of discarding

the soybean extract after autoclaving the soybeans (Tamang 1999). Moreover, nutrient broth, conventionally used for the enrichment of *B. subtilis* spores, is composed of expensive beef extract, which is not acceptable for the majority of the Hindu population in the Himalayas. *Kinema* prepared by using the *B. subtilis* KK2:B10 strain, which is harvested from soybean extract broth, is dried in an oven at 70°C for 10 h and ground aseptically to make a pulverized starter. One percent of the pulverized starter instead of *B. subtilis* is added aseptically to autoclaved soybeans and fermented to obtain *kinema*. The consumers' preference trials show that *kinema* prepared by using the pulverized starter under optimized conditions is more acceptable than that made using *kinema* from the market (Tamang 1999). Water-soluble nitrogen and formol nitrogen contents are higher in *kinema* prepared by using the pulverized starter than market *kinema* (Tamang 1999). Increased water-soluble nitrogen in *kinema* helps in digestion and high amounts of formol nitrogen, which contain free amino acids supplements, impart better taste to *kinema* (Nikkuni et al. 1995). Application of ready-to-use pulverized starters may appear appropriate in *kinema* production for marginal *kinema* producers in the Eastern Himalayas since it is cost-effective and easy to handle.

6.2.1.2.6 Biochemistry and Nutritional Composition

The nutritional composition of *kinema* is shown in Table 6.2. A marked decrease in the fat content of *kinema* compared to raw soybeans is due to the lipolytic activities of the microorganisms during *kinema* production, with concomitant increase in free fatty acidity (Sarkar et al. 1994). The higher ash content of market samples of *kinema* is likely due to the addition of firewood ash during production. Although the acidity in *kinema* increases by about 10-fold compared with raw soybeans, the product has a

TABLE 6.2

Nutritional Composition of *Kinema*

Parameters	Raw Soybean	*Kinema*
Moisture (%)	10.8	62.0
Ash (100 g/DM)	5.0	7.2
pH	6.7	7.9
Protein (100 g/DM)	47.1	47.7
Fat (100 g/DM)	22.1	17.0
Carbohydrate (100 g/DM)	25.8	28.1
Food value (kcal/100 g DM)	478	454
Fe (mg/100 g)	8.7	17.7
Mn (mg/100 g)	2.7	5.4
Zn (mg/100 g)	3.8	4.5
Na (mg/100 g)	1.7	27.7
Ca (mg/100 g)	186.0	432.0
Total amino acids (mg/100 g)	43,654	46,218
Free amino acids (mg/100 g)	472	5,129

Source: Adapted from Tamang, J.P., *Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values*, Taylor & Francis Group, Boca Raton, FL, 2010.

high pH value of 7.9. This is due to the high buffering capacity of the legume beans and the proteolytic characteristics of most vegetable protein fermentations (Hesseltine 1965). *Kinema* is the cheapest source of plant protein as compared to animal proteins. During the process of *kinema* production, soy proteins, which have been denatured by cooking, are hydrolyzed by proteolytic enzymes produced by *B. subtilis* into peptides and amino acids, which enhance digestibility (Tamang and Nikkuni 1998). A remarkable increase in water-soluble nitrogen and trichloroacetic acid (TCA)-soluble nitrogen content are observed during *kinema* fermentation (Sarkar and Tamang 1995). Total amino acids, free amino acids, and mineral contents increase during *kinema* fermentation (Table 6.2), and subsequently enrich the nutritional value of the product (Nikkuni et al. 1995, Sarkar and Tamang 1995, Sarkar et al. 1997a, Tamang and Nikkuni 1998). *Kinema* contains all essential amino acids and the quantity of essential amino acids is as high as that of egg and milk proteins (Sarkar et al. 1997a). Degradation of oligosaccharides has been reported in *kinema* (Sarkar et al. 1997b). *Kinema* is rich in linoleic acid, an essential fatty acid in foods (Sarkar et al. 1996). Phytosterols, which have a cholesterol-lowering effect, are increased during *kinema* fermentation (Sarkar et al. 1996). Traditionally prepared *kinema* contains 8 mg thiamine, 12 mg riboflavin, and 45 mg niacin per kg dry matter (Sarkar et al. 1998). The content of riboflavin and niacin increases in *kinema*, while that of thiamine decreases during fermentation (Sarkar et al. 1998). Increase in total phenol content from 0.42 mg GAE (gallic acid equivalent)/g in boiled soybeans to 2.3 mg GAE/g in *kinema* has been observed (Tamang et al. 2009). *Kinema* has many health-promoting benefits including antioxidants, digested proteins, essential amino acids, vitamin B complex, low-cholesterol content, etc. (Tamang 2010).

6.2.1.3 Thua nao

Thua nao is an ethnic, fermented nonsalty soybean food of northern Thailand. It is generally available as a dried paste, and is used as a flavoring agent in vegetable dishes (Figure 6.7).

FIGURE 6.7 *Thua nao* in a plastic bag.

6.2.1.3.1 Preparation and Culinary

In the traditional method of its preparation, dried whole soybeans are washed and boiled in excess water for 3–4 h till they can be crushed between fingers. The excess water is drained off, and the cooked beans are transferred to a bamboo basket lined with banana leaves. The basket is covered with banana leaves. The beans are left at room temperature for 3–4 days to undergo natural fermentation, and are considered properly fermented when they are covered with a sticky, viscous material, accompanied by a pungent odor of ammonia that replaces the beany flavor (Sundhagul et al. 1972). After fermentation, the raw *thua nao* is mashed lightly into a paste and mixed with salt, garlic, onion, and red pepper. The paste is wrapped in banana leaves and cooked by steaming before eating (Sundhagul et al. 1972). The protein contents were 16.9% and 36.8%, and the fat contents were 7.4% and 14.8% for the paste and chips, respectively (Sundhagul et al. 1972). A low cost, protein-rich food called "ferm-soy-mix" in powder form, which is ready to eat and having a long shelf life under normal conditions, has been developed by blending *thua nao* powder with flavoring agents and a small portion of high-grade fish. *Thua nao* is used as a seasoning agent and as an ingredient for soups, curries, and stir-fried vegetables (Okada 2008).

6.2.1.3.2 Microbiology

The gram-positive spore-forming rod-shaped bacterium *B. subtilis* is responsible for the fermentation of *thua nao* (Sundhagul et al. 1972). A initial bacterial load of 10^3 cells/g cooked beans increases to 10^{10} cells/g in *thua nao*. The increase is rapid during the first 2 days. During fermentation, the pH increases from 6.3 to 8.6 in the second day and remained relatively unchanged afterward. Inatsu et al. (2006) reported that *B. subtilis* produced protease, amylase, subtilisin NAT (nattokinase), and PGA in *thua nao*. It is said to serve as a substitute for fermented fish. *Thua nao* has been reported as a potential resource of food-processing enzymes and health-promoting compounds by Inatsu et al. (2006). Visessanguan et al. (2005) reported that *B. subtilis*-inoculated *thua nao* showed an increased proteolysis of soybean as *B. subtilis* released predominant active proteinases of molecular weights of 40,000 and 29,000 kDa. The γ-glutamyl hydrolase enzyme (28 kDa) purified from *thua nao* degraded γ-polyglutamic acid (PGA) to a hydrolyzed form of only about 20 kDa (with D- and L-glutamic acids in a ratio of 70:30), suggesting that the enzyme cleaves the γ-glutamyl linkage between L- and L-glutamic acid of PGA (Chunhachart et al. 2006). PGA-producing *Bacillus* strains, including *B. subtilis* IFO 3022, isolated from *thua nao* do not require biotin for growth, and the *thua nao* plasmids were found to be strongly hybridized with the *natto* plasmid, pUH1 (Hara et al. 1986). *B. subtilis* isolated from *thua nao* was shown to harbor a plasmid homologous to pUH1 (Hara et al. 1986).

6.2.1.4 Chungkokjang

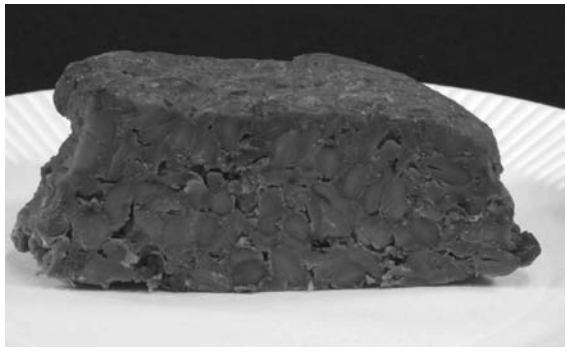
Chungkokjang (or *jeonkukjang*, *cheonggukjang*) is an ethnic, fermented soybean food of the Korean people (Figure 6.8a through c).

6.2.1.4.1 History

The first record of the production of *chungkokjang* appeared in the book written by Yoo Jung-Jim in 1765 (Lee 2008).

(a)

(b)



(c)

FIGURE 6.8 (a) Package of *chungkokjang* in Korea. As seen on the package, *chungkokjang* is used as an ingredient of a spicy soup, *chigae* in Korean. (b) Appearance of *chungkokjang*. (c) Cross section of *chungkokjang*.

6.2.1.4.2 Preparation and Culinary

Chungkokjang is prepared as follows: Boiled soybeans covered with matting made of straws are put on an *ondol*, a Korean underfloor heating system. After 3 days of fermentation, the soybeans are broken, mixed with soybean powder, ground with salt, and dried in the sun. In the modern method of the preparation of *chungkokjang*, a *Bacillus* starter is used (Lee 2008). The fermentation period of *chungkokjang* is longer than that of *natto*, which makes its color darker than that of *natto*. Such a longer fermentation period causes production of ammonia. *Chungkokjang* is consumed as a soup by Koreans.

6.2.1.4.3 Microbiology

B. subtilis isolated from *chungkokjang* produces a viscous polyglutamate and also shows fibrinolytic activity (Lee et al. 1991). Hara (1990) also isolated *B. subtilis* from *chungkokjang* and showed that the plasmid was homologous to pUH1. These studies indicate that the microorganism which contributes to *chungkokjang* fermentation is *B. subtilis* (*natto*). However, *B. licheniformis* B1, which was isolated from soil in Korea, is known to produce *chungkokjang* with good quality (Lee et al. 1999,

Choi et al. 2007). Super high molecular weight PGA has been reported to be synthesized by *B. subtilis* subsp. *chungkokjang* isolated from *chungkokjang* (Park et al. 2005). Increase in total phenol content has also been reported in *chungkokjang* (Shon et al. 2007). *B. megaterium* SMY-212 is a suitable fermenting strain that promotes antioxidant and free-radical scavenging activities in *chungkokjang* (Shon et al. 2007). An enzyme purified from the *Bacillus* sp. strain CK 11-4 shows thermophilic, hydrophilic, and strong fibrinolytic activities (Kim et al. 1996).

6.2.1.5 Hawaijar

Hawaijar is an ethnic, fermented soybean food of Manipur, India (Tamang 2010).

6.2.1.5.1 Preparation and Culinary

Small-sized soybean seeds are selected, washed, and boiled in an open cooker for 2–3 h. The excess water is drained off, cooled to ~40°C, and the soybean seeds are then packed in a small bamboo basket having a lid. The basket is lined with fresh fig leaves or banana leaves, and the cooked soybean seeds are placed inside the basket; the lid is closed loosely and kept nearby the kitchen for natural fermentation for 3–5 days. The shelf life of *hawaijar* is 7 days without refrigeration. Sometimes, it is sun dried for 2–3 days and stored for several weeks. Unlike *kinema* production, the practice of cracking and the addition of ash are not adopted by producers during *hawaijar* production. *Hawaijar* is eaten directly or used as a condiment or mixed with vegetables to make a curry.

6.2.1.5.2 Microbiology

B. subtilis, *B. licheniformis*, *B. cereus*, and other non-bacilli bacteria—*Staphylococcus aureus*, *S. sciuri*, *Alkaligenes* spp.—are found in *hawaijar* (Jeyaram et al. 2008, Tamang et al. 2009).

6.2.1.6 Tungrymbai

Tungrymbai is an ethnic, fermented soybean food popular in Meghalaya, India (Tamang 2010).

6.2.1.6.1 Preparation and Culinary

Soybean seeds are collected, cleaned, washed, and soaked in water for about 4–6 h. The seed coats of soybeans are normally removed before cooking by rubbing the soaked seeds gently. The soaked soybeans are cooked for about 1–2 h till all the water is absorbed. Cooked beans are allowed to cool, and are packed with fresh leaves of *Clinogyne dichotoma* and are placed inside a bamboo basket and covered by a thick cloth. The covered basket is kept over the fireplace, and fermentation occurs naturally for 3–5 days. *Tungrymbai* is mashed and put into a container with water and boiled till the water evaporates, and the container stirred continuously. It is mixed with fried onion, garlic, ginger, chilly, ground black sesame, and salt. A thick curry is made and is served as side dish with steamed rice. Pickles are also made from *tungrymbai*.

6.2.1.6.2 Microbiology

Many species of *Bacillus* are present in *tungrymbai* (Tamang et al. 2009).

6.2.1.7 Aakhone

Aakhone is an ethnic, fermented sticky soybean food prepared by people in Nagaland, India (Tamang 2010).

6.2.1.7.1 Preparation and Culinary

Soybean seeds are soaked and cooked, and the beans are wrapped in fresh leaves of banana or *Phrynium pubinerve* or *Macaranga indica* and kept above a fireplace for fermentation to occur naturally for 5–7 days. Fresh *aakhone* is made into cakes and dried above an earthen oven. Sometimes, each fermented bean is separated by hand, and is dried in the sun for 2–3 days. Such dried *aakhone* is stored in containers for future consumption. Pickle is made from freshly fermented *aakhone* by mixing with green chilly, tomato, and salt. The dried *aakhone* cakes are cooked with pork and are eaten as a side dish with steamed rice.

6.2.1.7.2 Microbiology

B. subtilis and other species of *Bacillus* are present in *aakhone* (Tamang et al. 2009).

6.2.1.8 Bekang

Bekang is an ethnic, fermented soybean food popular in Mizoram, India (Tamang 2010).

6.2.1.8.1 Preparation and Culinary

During the preparation of *bekang*, small-sized dry seeds of soybean are collected, cleaned, and soaked in water for 10–12 h. The excess water is dewatered and the beans are boiled for 2–3 h in an open cooker until the beans become soft. The excess water is drained off and wrapped in fresh leaves of *Calliparpa aroria* or leaves of *Phrynium* sp. The wrapped beans are kept inside a small bamboo basket. The basket is then placed near an earthen oven or a warm place and is allowed to ferment naturally for 3–4 days. *Bekang* is eaten as it is, or is made into a curry and consumed as a side dish with steamed rice.

6.2.1.8.2 Microbiology

Many species of *Bacillus* are present in *bekang* (Tamang et al. 2009).

6.2.1.9 Peruyaan

Peruyaan is an ethnic, fermented soybean food prepared in Arunachal Pradesh, India (Tamang 2010).

6.2.1.9.1 Preparation and Culinary

Dry seeds of soybeans are collected, washed, and cooked for 2–3 h till the beans become soft. The excess water is drained off and is cooled for some time. The cooked soybeans are kept in a bamboo basket (vessel) lined with fresh ginger leaves. The basket is loosely covered with ginger leaves and is kept on the wooden rack above a fire place for fermentation for 3–5 days. The stickiness of the product is checked, and if the product is sticky enough then the product is ready for consumption. *Peruyaan* is consumed mostly as a side dish. It is mixed with hot water, chillies, and salt, and is directly consumed without frying or cooking.

6.2.1.9.2 Microbiology

Many species of *Bacillus* are associated with *peruyaan* fermentation (Tamang et al. 2009).

6.2.1.10 Pepok

Pepok is an ethnic, fermented soybean food prepared in northern Myanmar (Tanaka 2008a).

6.2.1.10.1 Preparation and Culinary

Soybeans are soaked in water overnight, dewatered, boiled, and wrapped in leaves. After fermentation for 2–4 days, the fermented beans are mashed up with salt and hot pepper, rolled out into disks, and dried under the sun. *Pepok* is used as a seasoning agent or is consumed after roasting.

6.2.1.11 Sieng

Sieng is a traditional, fermented soybean food popular in Cambodia (Tanaka 2008b).

6.2.1.11.1 Preparation and Culinary

After boiling, soybeans are spread on bamboo baskets and fermented naturally by bacteria adhering to the baskets or suspended in the atmosphere. The soybeans are soaked in saltwater for 5–7 days immediately after 2 days of fermentation. Tree sap or enzymes are occasionally added to the saltwater. *Sieng* is used as a seasoning agent with salt and spices.

6.2.2 Mold-Fermented Soybean Foods

6.2.2.1 Tempe

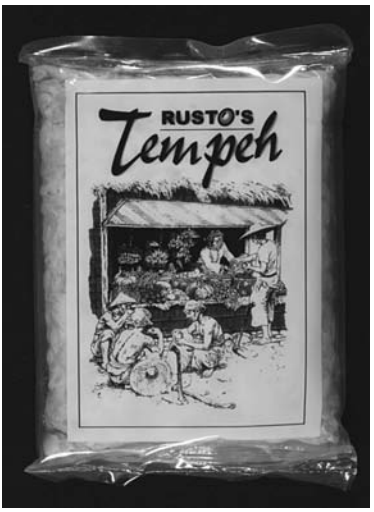
Tempe or *tempe kedelai* is a food prepared using nonsalted fermented soybeans, which are fermented by fungi unlike many other nonsalted fermented soybeans (Figure 6.9a through c). *Tempe* has an overwhelming advantage in terms of odor over other nonsalted fermented soybeans.

6.2.2.1.1 History

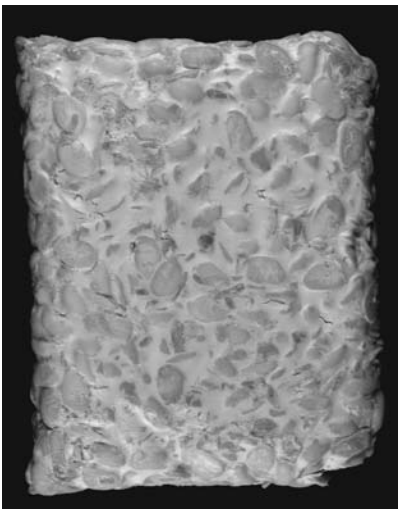
The origin of *tempe* is unknown, although it might have its root in ancient Indonesia. The first record of *tempe* appeared in the book *Serat Centini*, written around 1815, which indicated that *tempe* was produced in early seventeenth century (Okada 1988). There are two theories on the origin of *tempe*: The first theory describes that *tempe* originated from coconut *tempe*, called *tempeh bongkrek* in Indonesian, which is prepared currently, and the second theory describes that the method of *tempe* production originated from the method of production of Chinese fermented soybeans with *Aspergillus*, which was replaced by *Rhizopus oligosporus* in Indonesia because of its environmental suitability (Okada 1988).

6.2.2.1.2 Preparation and Culinary

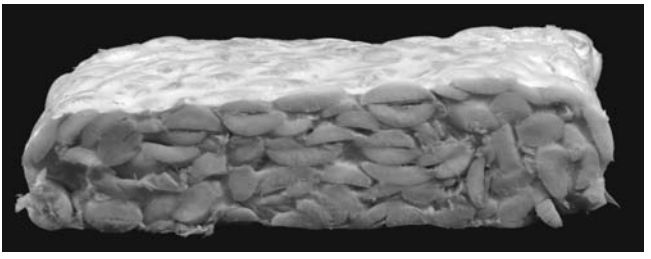
The method of *tempe* production in Indonesia is as follows: soybeans are soaked in water, where the pH of soybeans is lowered by LAB inhabiting the water. After dehulling and draining, the soybeans are inoculated with a *tempe* starter, locally called *usar*



(a)



(b)



(c)

FIGURE 6.9 (a) Package of *tempe*. (b) Appearance of *tempe*. (c) Cross section of *tempe*. Gaps between soybeans are crammed full of white mycelia of *R. oligosporus*.

(*Rhizopus* spores developed on hibiscus leaves) or *ragi tempe* (*Rhizopus* inoculated on starch pulp). The inoculated soybeans are packed in banana leaves or, recently, plastic bags, and fermented in 30°C for 24 h. In the modern procedure, the acidification process by LAB is replaced by the addition of organic acids (lactic acid, acetic acid or vinegar, or malic acid). A pure culture of *Rhizopus* as a starter is used in some *tempe* factories. Many Indonesians are fond of fried *tempe*, which is added to salads, soups, and *tempe goring* (a kind of fried *tempe* with seasonings). Recently, *tempe* has been used as an ingredient of pizzas, sandwiches, hamburgers, and *jiao-zi* (Chinese dumplings). Almost odorless and bland in taste, *tempe* goes with many foods as an ingredient.

6.2.2.1.3 Microbiology and Food Safety

R. oligosporus (*R. microsporus* variety *oligosporus*), a fungus, is a major microorganism for the fermentation of *tempe*, though some bacteria have been isolated from natural *tempe*. In addition, *Klebsiella pneumoniae*, *K. pneumoniae* subsp. *ozaenae*, *Enterobacter cloacae*, and gram-positive bacteria were isolated as vitamin B_{12}-producing bacteria in *tempe* (Okada et al. 1985). Bacterial contamination may be one reason for not eating raw *tempe*. In case a pure culture of *R. oligosporus* is

used for *tempe* production, vitamin B_{12} is not detectable in the products (Table 6.1). *Rhizopus stolonifer*, *R. arrhizus*, and *R. oryzae* can also be used to make *tempe*. In homemade *tempe* in Indonesia, the second important microorganism is LAB. The role of bacteria is to lower the pH of soybeans, both to prevent contaminated bacteria from growing dominantly and to provide *R. oligosporus* with a suitable environment to grow. *Lb. plantarum* and *Lb. lactis* were isolated from water in which soybeans were soaked (Okada 1988).

6.2.2.1.4 Biochemistry, Nutritional Composition, and Functional Properties

The nutritional composition of *tempe* is shown in Table 6.1. In *tempe*, some functional materials other than vitamin B_{12} (Liem et al. 1977) and components contained in soybeans by nature were detected and investigated. In *tempe* extract, strong thrombolytic activity (average 450 IU/g dry weight) was observed (Sumi and Okamoto 2003). The activity was attributed to an enzyme with a molecular weight of 30,000 and pH of 8.7. In laboratory-made *tempe*, the content of γ-aminobutyric acid, which improved blood flow to the brain and inhibited the elevation of blood pressure, increased when *tempe* was placed in an anaerobic environment after normal aerobic fermentation (Aoki et al. 2003). Processed *tempe* is expected to be available as an antihypertensive food. *Tempe* contains not only dietary fiber, saponins, and isoflavones of soybean origin but also superoxide dismutase, which eliminates active oxygen. It was also reported that a phytoestrogen, equol, derived from soybean isoflavone had a suppressive effect on prostate cancer (Horii 2008).

6.2.2.1.5 Socioeconomy

In Indonesia, 750,000 tons of soybeans per year are consumed as *tempe* (Ito 2002). In the United States, Europe, and Japan, some food companies produce *tempe* on a large scale (2–6 tons/week) (SoyInfo center, http://soyinfocenter.com/index.php). Among the nonsalted fermented soybeans, only *tempe* has been accepted worldwide.

6.2.2.2 Douchi

Douchi is a traditional, salt-fermented soybean product prepared in China.

6.2.2.2.1 History

Douchi has been used as a seasoning agent and for pharmaceutical purposes since the Han dynasty (206 BC). Even today, *douchi* is still added to some Chinese traditional medicines (Zhang et al. 2006). The first record of *douchi* is *Si Ji*, which was written by Si Maqian (about 104 BC). In *Ben Cao Gang Mu* (Chinese Materia Medica), which was written by Li Shizhen during the Min dynasty (AD 1368–1644), some health benefits of *douchi*, such as appetite enhancement, digestion promotion, and asthma prevention, have been recorded (Bao 1985).

6.2.2.2.2 Preparation and Culinary

Douchi is prepared from soybeans by pretreatment and a two-step fermentation process (primary and secondary fermentation) as described by Kang (2001). The soybeans are soaked in water and boiled for about 1 h but for bacterial-type *douchi*, the beans only need to be boiled for 30–40 min. The second step is *qu*-making, where the boiled beans are inoculated with *Aspergillus oryzae* (0.3%) or *Mucor* strain spores

(0.5%) or simply incubated at high temperature (over 25°C) for 3–4 days to harvest matured *qu*. In the case of *Aspergillus*-type *douchi* preparation, *koji* is washed with water to remove the spores, mycelium, and a part of the enzymes, in order to avoid a bitter and astringent flavor in the final product (Zhang et al. 2007). Then 18% (w/w, soybean base) salt, a little sugar, and flavor such as capsicum paste are mixed with *qu* in order to get a desirable flavor. The mixture is impacted into jars and sealed with a plastic film. *Aspergillus*-type *douchi* is fermented at 30°C–35°C for 7–40 days whereas *Mucor*-type *douchi* is fermented at about 20°C for 10–12 months. *Douchi* is consumed as a soup or side dish with bland foods, such as rice gruel. It can be used as a flavoring agent with vegetables, meat, and seafood (Hesseltine and Wang 1972).

6.2.2.2.3 Microbiology

Three types of *douchi* are fermented by *Mucor*, *Aspergillus*, and *Bacillus* strains, respectively. Among them *Aspergillus*-type *douchi* takes the shortest time for fermentation, and is the most popular type in China (Bao 1985).

The presence of two biogenic amines, namely, cadaverine and putrescine, leads to histamine toxicity in *douchi* by inhibiting histamine-metabolizing enzymes, such as diamine oxidase and histamine methyl transferase (Arnold and Brown 1978, Lehane and Olley 2000). *Douchi* produces angiotensin I-converting enzyme (ACE) inhibitors, having a potential to lower blood pressure (Zhang et al. 2006). Te Li et al. (2007) reported that anti-α-glucosidase activity was exhibited by *douchi qu* fermented with *A. oryzae*, and this activity became highest at 5.0% and 7.5% salt levels. *B. subtilis* isolated from Chinese fermented soybean seasoning produced bacteriocin (Zheng and Slavik 1999). Changes in isoflavone isomer distribution were found to be related to β-glucosidase activity during *douchi* fermentation, which was affected by NaCl supplementation (Wang et al. 2007a). Increase in total phenol content has also been reported in *douchi* (Wang et al. 2007b).

6.2.2.3 Sufu

Sufu is an ethnic, fermented soybean food prepared in China. It is a cheese-like product with a spreadable creamy consistency and a pronounced flavor (Han et al. 2001a).

6.2.2.3.1 History

Literally *sufu* (*furu*) means "molded milk" and *tosufu* (*dou-fu-ru*) means "molded soymilk." Due to numerous dialects used in China, *sufu* has appeared in the literature under many different names such as *sufu*, *fu-ru*, *dou-fu-ru*, *tou-fu-ru*, etc. It is indicated in the Chinese Materia Medica that *tofu* was invented by Liu An (179–122 BC), King of Weinan (Steinkraus 1996). The production of *sufu* started during the Han dynasty in China (Shi and Ren 1993). The first historical record mentions that *sufu* production was carried out during the Wei dynasty (AD 220–265) (Hong 1985).

6.2.2.3.2 Preparation and Culinary

Sufu is produced by various methods in different parts of China (Wang and Du 1998). Soybeans are washed and soaked overnight in water and ground into slurry. The slurry is pressed to obtain soymilk. It is then salted to coagulate; the excess water is removed by pressing with stones or wooden planks and, finally, a soft and firm cake-like *tofu* results. In the next step, *pehtze* (*pizi*) is prepared from fresh bean curd overgrown with

mold mycelia by natural fermentation. Cubes of *tofu* are placed in wooden trays made up of loosely woven bamboo strips and surrounded with straw for natural inoculation and fermentation at a temperature of 15°C–20°C for 5–15 days. The prepared *pehtze* has white or light yellow-white mycelium for an attractive appearance. It is then placed into a big earthen jar for salting, where salt is spread between the layers of *pehtze* for 6–12 days, after which the *pehtze* is removed from the jar, washed, and salted. Different dressing mixtures are then added for different types of *sufu*. The most common dressing mixture used consists of *angkak*, alcoholic beverages, salt, sugar, bean paste, and spices, and sometimes even essence for flavor. For ripening, alternate layers of *pehtze* and the dressing mixture are packed into jars in a 2:1 ratio. The mouth of the jar is wrapped with bamboo sheath leaves and sealed with clay and aged for 6 months for further maturation. Three types of *sufu*—red, white, and yellow—are prepared in Taiwan (Yuan 1994). *Sufu* is consumed as an appetizer or as a side dish mainly with rice or steamed bread.

6.2.2.3.3 Microbiology

The pure starter cultures consist of molds, that is, mucoraceae (*Actinomucor*, *Mucor*, and *Rhizopus*) or bacteria, that is, *Micrococcus* and *Bacillus* spp., which are used for the production of *sufu*. Most *sufu* contains considerable levels of antimicrobial NaCl (5%–15%) and ethanol (1%–7%) that could prevent the survival and growth of pathogens; it is also known that the endospore-forming rods such as *Bacillus* spp. and *Clostridium* spp. vary greatly in their salt tolerance (Brewer 2000). Chou and Hwan (1994) reported that addition of ethanol to the brine solution for ageing resulted in free fatty acids in the *sufu* product. *Actinomucor repens*, *A. taiwanensis*, *Mucor circinelloides*, *M. hiemalis*, *M. racemosus*, and *R. microsporus* variety *microsporus* have been isolated from starter cultures used in commercial *pehtze* fermentation for *sufu* production (Han 2003). A diversity of lactic acid bacteria was reported in fermented brines used to ferment *tofu* into *sufu*, which included *Enterococcus hermanniensis*, *Lactobacillus agilis*, *Lb. brevis*, *Lb. buchneri*, *Lb. Crispatus*, *Lb. curvatus*, *Lb. Delbrueckii*, *Lb. farciminis*, *Lb. fermentum*, *Lb. pantheris*, *Lb. salivarius*, *Lb. vaccinostercus*, *Lactococcus lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Leuconostoc carnosum*, *Leuc. citreum*, *Leuc. fallax*, *Leuc. lactis*, *Leuc. mesenteroides*, *Leuc. pseudomesenteroides*, *Pediococcus acidilactici*, *Streptococcus bovis*, *S. macedonicus*, *Weissella cibaria*, *W. confusa*, *W. paramesenteroides*, and *W. soli* (Chao et al. 2008). The microbiological composition of *sufu* indicates that its manufacturing processes and recipes prevent the growth of fungi and enterobacteriaceae (Han et al. 2001b).

6.2.2.4 Doenjang

Doenjang is a naturally fermented soybean paste made in Korea, similar to the Japanese *miso* (Min and Kim 1990). It is light grayish-brown and has a slightly chunky texture. Chinese *jiang* is believed to be the oldest form of *doenjang*. According to the ancient Chinese book, *Analects of Confucius*, *doenjang* has a history of more than 3000 years (Yokoysuka 1985).

6.2.2.4.1 Preparation and Culinary

During the preparation of *doenjang*, cooked soybeans are pounded and mashed in a mortar, shaped into balls, wrapped in rice straw, and hung under rafters until each ball is covered with a white bloom of natural mold. Next, the balls are crushed and

mixed with salt and water to form *meju*, sometimes with the addition of sesame seeds or leaves, and placed in an earthenware container of 1–10 gallon capacity. The fermentation period generally lasts for 6 months. It is used as a seasoning agent or consumed as a soup with boiled rice in Korea.

6.2.2.4.2 Microbiology

Several species of microorganisms have been reported from *doenjang*, LAB—*Leuconostoc mesenteroide*, *Tetragenococcus halophilus*, *E. faecium*; bacilli—*B. subtilis* and *B. licheniformis*; and fungi—*Mucor plumbeus*, *A. oryzae*, and *Debaryomyces hansenii* (Kim et al. 2009). Glutamic acid, glycine, lysine, and methionine are the main amino acids in *doejang* (Kim et al. 1968).

6.2.2.5 Miso

Miso is a semisolid fermented food made from soybeans, rice, or barley, and salt is added for preparing *miso* soup in Japan (Mullin 2005). The most popular *miso* in Japan is rice *miso*, which is prepared by mixing cooked soybeans with *koji* (steamed rice on which *A. oryzae* is cultured), salt, and a small amount of water, which is added to control the moisture level (Ebine 1989). The details of *miso* are mentioned in Chapter 7.

6.2.2.6 Shoyu/*Soy Sauce*

Shoyu or soy (soya) sauce is a fermented soybean liquid made from fungal fermentation and is commonly used as seasoning agent or condiment in Asian countries except the Indian subcontinent and Middle East Asia (Sasaki and Nunomura 2003). The details of soy sauce are mentioned in Chapter 7.

6.2.2.7 Tauco

Tauco is a traditional, fermented soybean product prepared in Indonesia, similar to *miso*. It is a yellow-colored saline paste with a meat-like flavor having a porridge-like consistency.

6.2.2.7.1 Preparation and Culinary

Tauco is prepared by mold fermentation for 3–5 days followed by brine (20%) fermentation for 20–30 days. The first stage of preparation consists of inoculating soybean with *R. oligosporus*, *R. oryzae*, and *A. oryzae*. Microorganisms that are active during brine fermentation are *Lactobacillus delbrueckii* and *Hansenula* sp. After the second phase of fermentation is completed, the brine is drained; palm sugar (25%) is added and the mixture is cooked and stored for 24 h or directly filled into bottles. *Tauco* is available in a viscous liquid form. It is also available in a semisolid form obtained by the sun drying of the liquid product to obtain a final moisture content of 25%. *Tauco* is used as a flavoring agent. It is produced mainly in western Java and is used for the preparation of soups and other side dishes.

6.2.2.7.2 Microbiology

Microorganisms present in *tauco* are *A. oryzae*, *R. oligosporus*, *R. oryzae*, *Lb. delbrueckii*, *Hansenula* sp., and *Zygosaccharomyces soyae* (Winarno et al. 1973).

6.3 Fermented Non-Soybean Legume Foods

6.3.1 *Dawadawa*

Dawadawa is an ethnic, nonsalted fermented food of West Africa prepared from African locust beans (*Parkia biglobosa*) (Kato 1990). Although *dawadawa* is used as a seasoning for soup and stew, it contributes to protein intake for African people. Dawada is common in Ghana; the similar product is called iru in Nigeria, *Kinda* in Sierra Leone and *soumbala* in Burkina Faso (Achi 2005, Azokpota et al. 2006).

6.3.1.1 Preparation and Culinary

For the preparation of *dawadawa* (Odunfa 1985), locust beans are boiled for 24 h, and the seed coats and testae are removed. After washing the cotyledons, the second boiling is carried out with the addition of a softening agent containing potash for 1 or 2 h. The beans are spread on a calabash tray and covered with a cloth. The beans are fermented naturally for 2–4 days. Recently, soybeans are replacing locust beans because of the shortage of African locust beans and the extended production of soybeans in the area. The process for the preparation from soybeans is somewhat different. Soybeans are fried and the seed coats are removed. Soybeans are boiled for 3 h and spread in a basket leaf with previously fermented *dawadawa*. The basket, which is covered with the same leaves, is kept in a warm place for 2–3 days. After fermentation, fresh *dawadawa* is sold in markets within a few days. *Dawadawa* is also salted in some areas and, most importantly, dried under the sun for long preservation after being mashed up and patted into a disk.

6.3.1.2 Microbiology

Microorganisms involved in the fermentation of *dawadawa/iru/soumbala* are mainly *Bacillus subtilis*, *B. licheniformis*, *B. pumilus*, and *B. megaterium* (Ouoba et al. 2004, Amoa-Awua et al. 2006, Azokpota et al. 2006), while *Staphylococcus xylosus*, *S. saprophyticus*, *S. hominis*, and *Micrococcus* spp. play a subsidiary role (Odunfa and Komolafe 1989). A plasmid of a size of 6.5 kb of *B. subtilis* isolated from *dawadawa* is homologous with a 5.8 kb plasmid, pUH1, of *B. subtilis* (*natto*) Asahikawa isolated from *natto* (Hara 1990). Meerak et al. (2008) reported PGA-producing *Bacillus* isolated from Ghanaian *dawadawa*, which included *B. subtilis*, *B. amyloliquefaciens*, *B. pumilus*, and *B. licheniformis*.

6.3.2 *Ugba*

Ugba is a Nigerian ethnic, fermented product prepared from African oil beans (Obeta 1983). It is flat, glossy, and brown in color.

6.3.2.1 Preparation and Culinary

During the preparation of *ugba*, leguminous seeds of African oil beans (*Pentaclethra macrophylla* Bentham) are boiled in water for 4–12 h to remove the fibrous seed coat. The cotyledons are sliced, washed, boiled for 1–2 h, and then soaked in water overnight to remove bitter components. It is then drained for 1 h in a basket lined with

banana leaves and wrapped on *ororompo* (*Mallotus oppositifolius* Mull) leaves and fermented naturally for 4–5 days. The longer the fermentation, a more strongly flavored *ugba* results. Less fermented (about 5 days) *ugba* is eaten directly. It is usually eaten as a side dish in Nigeria.

6.3.2.2 Microbiology

The bacteria isolated from the fermentation of *ugba* are *Bacillus*, *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Alcaligens*, and *Citrobacter* (Obeta 1983).

6.3.3 *Maseura*

Maseura is an ethnic, fermented black gram or green gram product prepared by Nepalis living in the Himalayas (Tamang 2010). It is a cone-shaped hollow, brittle, and friable product.

6.3.3.1 Preparation and Culinary

During the preparation of *maseura*, dry seeds of black gram (*Phaseolus mungo* Roxb.) or green gram (*Phaseolus aureus* Roxb.) are cleaned, washed, and soaked overnight. Soaked seeds are split by pressing through the hands and the hulls are removed, the split seeds being ground into a thick paste using a mortar and pestle. Water is added while grinding until the paste becomes sticky, which is then hand-molded into small balls or cones. If rice bean is used, then boiled potato or squash or yam is mixed with the paste to make it sticky. The mixture is then placed on a bamboo mat and left for natural fermentation for 2–3 days, and then sun-dried for 3–5 days depending upon the weather. *Maseura* can be stored in a dry container at room temperature for a year or more (Karki 1994, Dahal et al. 2003). *Maseura* is similar to the Indian *wari* or *dal bodi* and *sandige* (Soni and Sandhu 1990, Dahal et al. 2005). It is commonly used as a condiment or consumed along with vegetables in the Himalayas. *Maseura* is usually fried in edible oil with vegetables to make a curry or soup and served with rice.

6.3.3.2 Microbiology

Bacterial species present in *maseura* are *Lactobacillus fermentum*, *Lb. salivarius*, *Pediococcus pantosaceous*, *P. acidilactici*, *Enterococcus durans*, *B. subtilis*, *B. mycoides*, *B. pumilus*, and *B. laterosporous*; yeasts are *Saccharomyces cerevisiae*, *Pichia burtonii*, *Candida castellii*, and *C. versatilis*; molds are species of *Cladosporium*, *Penicillium*, and *Aspergillus niger* (Dahal et al. 2003, Chettri and Tamang 2008). The moisture content of dried *maseura* is about 8%–10%, protein content is 18%–20%, carbohydrate content is 67%–70%, and *maseura* also contains minerals (Dahal et al. 2003). An increase in soluble protein, amino nitrogen, nonprotein nitrogen, thiamine, and riboflavin has been observed in *maseura* (Dahal et al. 2003).

6.3.4 *Wari*

Wari is an ethnic, fermented black gram product of northern India and Pakistan. *Wari* is a dried, hollow, brittle, spicy, and friable ball, 3–8 cm in diameter and weighing 15–40 g (Batra 1986).

6.3.4.1 Preparation and Culinary

During the traditional method of *wari* preparation, black gram seeds are soaked in water for 6–12 h, dewatered, dehulled, and ground on a stone mortar into a smooth, mucilaginous paste. The dough is mixed with inoculum from a previous batch, and salt and spices including asafetida, caraway, cardamom, clove, fenugreek, ginger, and red pepper are added. The mixture is allowed to ferment at room temperature for 1–3 days and hand molded into balls. After air drying for 2–8 days on bamboo or palm mats, *wari* is turned over for further drying (Batra and Millner 1976). *Wari* is used as a condiment and is fried and mixed with vegetables as a side dish.

6.3.4.2 Microbiology

Yeasts like *Candida krusei*, *S. cerevisiae*, and *Hansenula* sp. along with LAB *Leuc. mesenteroides* have been isolated from samples of *wari* (Batra and Millner 1974, 1976, Batra 1981, 1986). Sandhu and Soni (1989) observed the occurrence of bacteria (10^9–10^{12} cfu/g) in all the market and laboratory-made samples of *wari*, but only 55% of the samples contained yeasts (0–10^7 cfu/g). *Leuc. mesenteroides* was the most abundant and present in all the market samples of *wari*, followed by *Candida vartiovaarai*, *Kluyveromyces marxianus*, *Trichosporon beigelii*, *C. krusei*, and *Hansenula anomala* (Sandhu and Soni 1989). *Leuc. mesenteroides*, *Lb. delbrueckii*, *Lb. fermentum*, *B. subtilis*, and *Flavobacter* spp., and yeasts like *Trichosporon beigelii*, *S. cerevisiae*, *C. krusei*, *P. membranaefaciens*, and *Hansenula anomala* predominated the initial stages of fermentation, and ultimately only *Leuc. mesenteroides*, *Lb. fermentum*, *S. cerevisiae*, and *Trichosporon beigelii* remained in the final product (Sandhu and Soni 1989).

6.3.4.3 Biochemistry

An increase in total acids from 0.50% to 1.50%, soluble solids from 7.8% to 14.7%, nonprotein nitrogen from 0.20% to 0.68%, soluble nitrogen from 0.95% to 1.50%, free amino acids from 9.8 to 45.2 mg/g, and proteolytic activity from 4.82 to 6.04 IU/g has been observed during *wari* fermentation (Soni and Sandhu 1990). On the other hand, the level of reducing sugars and soluble protein decreased from 13.7 to 4.3 mg/g and 50.5 to 17.4 mg/g, respectively. *Wari* fermentation also brought about an appreciable rise in water-soluble B vitamins including thiamine, riboflavin, and cyanocobalamin (Soni and Sandhu 1990).

6.3.5 *Oncom*

Oncom is an ethnic, fermented peanut or groundnut cake-like product of Indonesia, mostly produced in West Java.

6.3.5.1 Preparation and Culinary

During its preparation, peanut or groundnut seeds are soaked, and the press cakes are cooked along with solid wastes of tapioca and *tahu*, using a mixed culture of microorganisms that includes *Rhizopus* or *Neurospora* species (Winarno et al. 1973). Traditionally, two kinds of *oncom* are produced in Indonesia, *oncom hitam* (black

oncom) and *oncom merah* (orange *oncom*). When fermentation is carried out by strains of mold belonging to the genus *Neurospora*, the product is called red *oncom*; if *R. oligosporus* is used, the resulting product is called black *oncom*. *Neurospora intermedia*, *N. crassa*, and *N. sitophila* have been reported from *oncom* (Ho 1986). It is consumed as a side dish, either in the form of slices deep-fried in fat, in the form of small portions in soups, or in the other forms.

6.3.5.2 Microbiology

N. sitophila and *N. crassa* have been identified as typical molds in *oncom* (Winarno et al. 1973). *Oncom* is prepared by utilizing the same microorganism (*R. oligosporus*) as in *tempe*.

6.3.6 *Dhokla* and *Khaman*

Dhokla is an ethnic, fermented spongy-textured product made by Gujaratis in India that is prepared from Bengal gram and rice products, and is similar to *idli* except that dehulled Bengal gram *dhal* is used in place of black gram. *Khaman*, similar to *dhokla*, is also an ethnic, fermented spongy-textured product made by Gujaratis and is made solely using seeds of Bengal gram.

6.3.6.1 Preparation and Culinary

Dry seeds of Bengal gram and white polished rice are washed and soaked for 5–10 h. It is then ground, and salt and water are added to make a thick paste. The slurries are left for natural fermentation in a warm place (30°C–32°C) for 8–10 h. The spongy-textured product, *dhokla*, is ready. It is steamed open in a greased pie tin. After 10–15 min of steaming, *dhokla* is ready for consumption. *Dhokla* is mostly eaten for breakfast or as a snack. *Dhokla* and *khaman* are eaten commonly with pickle and *sambar*, a spiced soup prepared from *dhal* and vegetables, and are sold in almost all Indian sweet shops.

6.3.6.2 Microbiology

Lb. mesenteroides and *Enterococcus faecalis* are essential and responsible for the leavening of batter and acid production during *dhokla f*ermentation (Joshi et al. 1989). Acetoin and volatile fatty acids at their optimum concentration impart a characteristic flavor to *dhokla* (Joshi et al. 1989).

6.4 Conclusion

In Asia, a variety of ethnic, fermented soybean foods have been developed with the utilization of bacteria (mainly *Bacillus* spp. and lactic acid bacteria), fungi (*Rhizopus* spp. and *Aspergillus* spp.), and yeasts naturally occurring the environment. These foods are prepared using a common procedure: soybeans are soaked and boiled till the seeds become soft, are wrapped either in leaves of banana plants, fig trees, ferns, or dry paddy straws, and placed in bamboo baskets; microorganisms inhabiting the

wrapping materials or unclean utensils ferment the soybeans into flavorsome, textured, and appealing edible products. In commercial production processes, a seed culture is used for the fermentation because of quality control, hygiene, and mass production. The foods are important sources of proteins, peptides, and some vitamins and provide enough calories, especially for people who consume a limited amount of meat. Recently, fermented soybean foods have attracted attention as physiologically functional foods, which contain isoflavones, fibrinolytic enzymes, antioxidants, free-radical scavenging activities, angiotensin I-converting enzyme inhibitors, etc. Nowadays, fermented soybean foods are known to be not only nutritious but also healthy foods by people all over the world.

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7

Fermented Soybean Pastes Miso *and* Shoyu *with Reference to Aroma*

Etsuko Sugawara

CONTENTS

7.1 Introduction

Japanese soy sauce (*shoyu*) and fermented soybean paste (*miso*) are manufactured by almost the same fermentation process under a high concentration of salt and with many different microbial activities. *Shoyu* is indispensable for Japanese cooking, and *miso* is used as *miso* soup in Japan everyday. Rice is the principal food for the Japanese, and rice protein has low concentrations of some essential amino acids. It is thus necessary to make up for the nutritional deficiencies by consuming other plant protein foods, for example, *natto*, *tofu*, *miso*, and *shoyu*, all of which are soybean products. In Japan, large amounts of *miso* are consumed in the form of *miso* soup. Hirayama (1982) pointed out that daily intake of *miso* soup significantly reduces the standardized mortality rate from gastric cancer. The origin of *miso* is not clear; however, it is presumed to be the fermented product of a food named *hishio* that was prepared from soybean, *koji* (a fermenting agent made from foxtail millet), and salt around eleventh

century BC in China. Around the fifth century, *hishio* and its processing technology are presumed to have been carried into Japan by a Japanese envoy to the Tang dynasty of China. Around the fourteenth century, a large amount of soybeans were cultivated, and the processing method of *miso* had begun to spread throughout Japan. Around the fifteenth century, *miso* was consumed as *miso* soup by the general population and around the seventeenth century, the production of *miso* in factories began. Now, many types of *miso* are manufactured in various regions of Japan. It is said that *shoyu* originated from the juices collected in the bottom when *hishio* was carried from China to Japan around the fifth century. Around the sixteenth century, the character for *shoyu* appeared for the first time in a document, a national language dictionary, and *shoyu* was consumed by the general population. Around the seventeenth century, *shoyu* was manufactured in factories in various regions of Japan. Around the eighteenth century, the same product as the modern regular *shoyu* was being manufactured and in the nineteenth century, the export of *shoyu* to Europe started. Since World War II, *shoyu* has been manufactured in many countries around the world, and has been used as a seasoning throughout the world (Noshiro 1991). *Shoyu* is also becoming popular in Western cuisine, but *miso* is not as internationally popular as *shoyu*. The sterilization of *miso* is sufficiently difficult for its paste consistency, and its range of distribution is limited. However, *miso* has a common aroma and taste to that of *shoyu*, and a variety of *miso* products can be used as a new seasoning for ethnic dishes. Brewed and fermented foods especially have good taste and aroma, which are produced by microorganisms.

7.2 Aroma Compounds of *Miso* and *Shoyu*

The aroma compounds of brewed and fermented foods are composed of various compounds as follows:

1. Compounds that originate in the raw material
2. Compounds generated by heat treatment of the raw material
3. Compounds generated by malt and malt enzyme action
4. Compounds generated by microorganisms such as yeasts
5. Compounds generated by heat treatment, etc., after fermentation
6. Compounds generated by aminocarbonyl reactions, etc., and eluted from storage containers such as barrels

Figure 7.1 shows quantitatively the main aroma components (1–6) identified in *miso* aroma concentrates (Sugawara et al. 1990, Sugawara 1991a,b) and the key components (7, 8) of *shoyu* (Sasaki et al. 1991). One of the major aroma constituents of soybean, 1-hexanol (1, Figure 7.1), is a compound that originates from the raw material, and maltol (2), 2-furfural (3), and 4-hydroxy-2, 5-dimethyl-3(2*H*)-furanone (HDMF, 4), which has a sweet and caramel-like aroma, are compounds generated by heat treatment of the raw material, as well as by the aminocarbonyl reaction that occurs during aging (Sugawara 1991a,b). Maltol (2, Figure 7.1) is formed under usual cooking conditions of soybeans, and we think that, in addition to an aminocarbonyl reaction, the hydrolysis of a soybean saponin, which has a maltol structure as its aglycon (Kudou

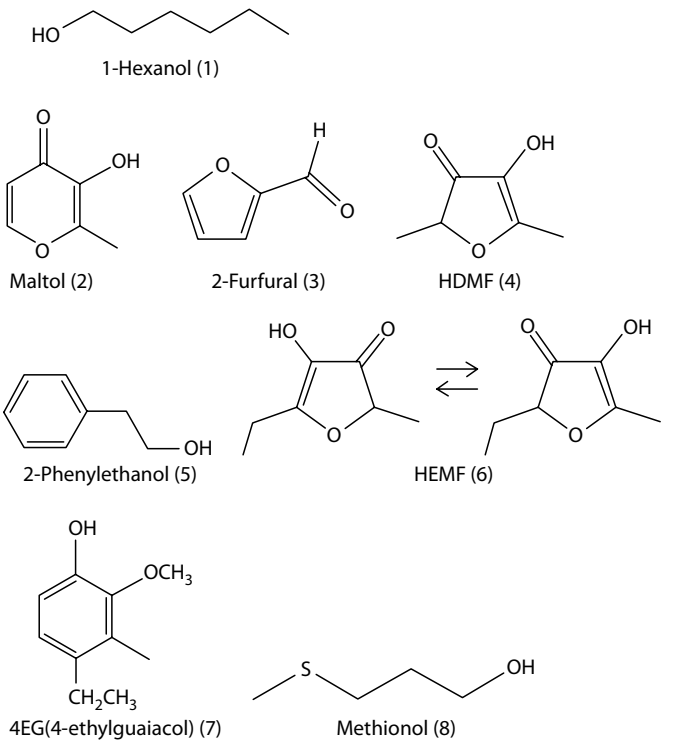

FIGURE 7.1 Quantitatively important aroma components in fermented soybean pastes *miso* and *shoyu*.

et al. 1992), is responsible for maltol formation. 2-Phenylethanol (5, Figure 7.1), which has a rose-like aroma, and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF, 6, Figure 7.1), which has a strong, sweet cake-like aroma with a threshold value of less than 20 ppb in water, were considered to be the compounds generated by microorganisms such as yeasts. 4-Ethylguaiacol (4EG, 7, Figure 7.1) has a characteristic smoky aroma and the threshold of this compound is lower than 1.3 ppb, and 3-methylthiopropanol (methionol, 8, Figure 7.1) has the lowest threshold value (0.3 ppb in water) (Guadagni et al. 1972).

7.3 Brewing of *Miso*

The original meaning of *miso* in Japanese is "immature *shoyu*." It is also a fermentation product of soybean and other cereals in the presence of salt. For the preparation of *miso* as defined by Japan Agricultural Standards (JAS), it is mandatory to use steamed soybeans, salt, and *miso koji*. *Miso koji* is made from a single cereal or soybean, that is, steamed rice, barley, or soybean itself, by inoculating with a *koji* starter of *Aspergillus oryzae*. There are various types of *miso* in Japan, and *miso* is classified into three types: rice *miso*, barley *miso*, and soybean *miso* depending on the kind of *koji*. The main

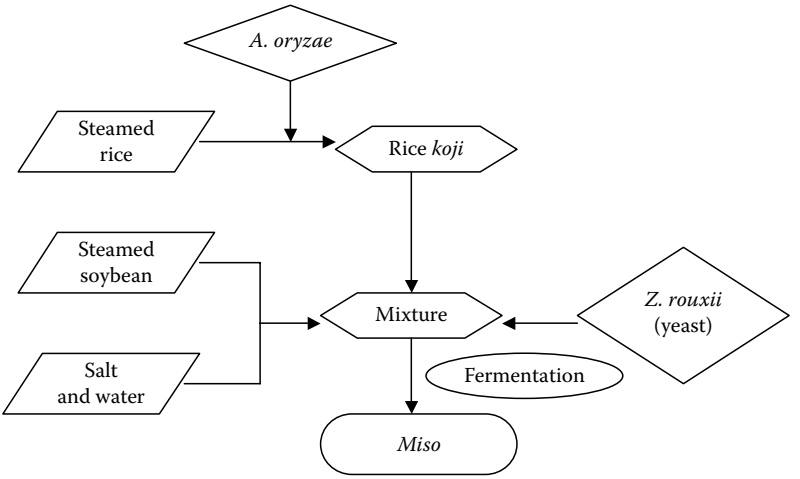

FIGURE 7.2 Simplified manufacturing process of a fermented soybean paste (red salty rice *miso*).

product is a red salty rice *miso* (*akakei-karakuti-komemiso*), whose typical composition at pH 5.0 is as follows: NaCl content, 12–13 g/100 g; total nitrogen, 1.6–2.0 g/100 g; total sugar, 13–14 g/100 mL; and alcohol content about 1.0 mL/100 g (Yoshii 1991). The simplified manufacturing process of red salty rice *miso* is shown in Figure 7.2. The manufacturing process basically consists of three stages: *koji* making; mixing of *koji*, steamed soybean, and salt; and fermentation. In the first process, steamed rice is inoculated with a starter culture of *A. oryzae*. After 40 h, a fungus grows over the culture mixture that is called "rice *koji*." In the second process, *miso* is prepared by mixing *koji*, steamed soybean, salt, and water, and then the halotolerant yeast *Zygosaccharomyces rouxii* and/or *Candida versatilis* is added to the mixture. Salt is then added to facilitate the fermentation by yeast and lactic acid bacteria, and to preclude any undesirable fermentation. In the third process, the fermentation of the mixture is continued for a longer period of 3–12 months. During this time, starch is changed to lactic acid, alcohol, and carbon dioxide by the conversion of simple sugars from starch as well as by enzymatic hydrolysis of protein to peptides and amino acids by yeast and lactic acid bacteria. Fungal, bacterial, and yeast species are almost the same as those for *shoyu*; however, the degree of hydrolysis is much lower in *miso* than in *shoyu*, and the product is a solid paste. At the same time, various kinds of volatile compounds are formed as fermented products. As *miso* is a paste, it cannot be pasteurized. Therefore, the flavor of *miso* is much more labile to heating. Honma (1987) applied steam distillation under reduced pressure and extracted the distillate with pentane and ethyl ether (1:2 v/v) to isolate the *miso* volatiles. They listed up to 208 compounds as *miso* volatiles, among which 27 hydrocarbons, 27 alcohols, 40 esters, 51 carbonyl compounds (aldehydes and ketones), 26 acids, 9 phenols, 10 pyrazines, 3 pyridines, 8 *S*-containing compounds, and 7 other compounds were described. Not all of these volatiles would contribute to the *miso* aroma; however, almost all aroma compounds are popular in fermented food products. The characteristic compound of *miso* flavor will be discussed later in comparison with the aroma impact compounds of *shoyu*.

As shown in Table 7.1, two types of red salty rice *miso* are compared, one with the pre-cultured *Z. rouxii* yeast (the strain was provided by the Experimental Station of

TABLE 7.1

Changes in General Constituents, the Number of Yeast Cells, and the Concentration of HEMF during *Miso* Aging

Aging (days)	Moisture (%)	NaCl (%)	WSN (%)	F.N. (%)	R.S. (%)	Ethanol (mg/100 g)	pH	No. of Yeast Cells (per g of *miso*)	HEMF (ppm)
A-0	46.4	12.7	0.58	0.086	12.0	3.5	5.84	4.0×10^5	0
A-7	45.8	12.6	0.74	0.178	15.3	69	5.67	1.6×10^6	0
A-14	45.5	12.8	0.85	0.241	15.5	259	5.57	3.5×10^6	3.35
A-21	45.3	12.7	0.87	0.275	14.6	253	5.55	2.6×10^6	9.30
A-30	46.9	12.9	0.97	0.290	14.2	759	5.46	7.2×10^6	7.95
A-60	47.8	13.1	1.09	0.350	11.8	1213	5.34	1.5×10^6	11.40
A-100	48.2	13.0	1.12	0.364	11.9	1121	5.18	1.0×10^4	11.95
N-0	46.1	13.2	0.58	0.086	12.5	0	5.84	3.0×10^3	0
N-7	45.6	12.4	0.74	0.184	15.0	0	5.69	4.0×10^2	0
N-14	45.5	12.4	0.80	0.249	16.9	0	5.61	2.0×10^2	0
N-21	45.2	12.5	0.85	0.287	17.0	17	5.59	2.2×10^3	0.30
N-30	45.4	12.7	0.94	0.292	17.3	45	5.55	2.2×10^5	4.35
N-60	45.2	12.6	1.06	0.344	16.3	242	5.29	2.0×10^6	12.35
N-100	45.8	0	1.09	0.364	16.1	374	5.20	4.7×10^5	15.70

Source: Sugawara, E. et al., *Biosci. Biotechnol. Biochem.*, 58, 1134, 1994. With permission.

A, *miso* with added yeast; N, *miso* without yeast; WSN, water-soluble nitrogen; F.N., formol-titrated nitrogen; R.S., reducing sugar.

the Miyagi *Miso-Shoyu* Industry Cooperative, Miyagi, Japan) and the other without the yeast, with regard to moisture, NaCl concentration, water-soluble nitrogen, formol-titrated nitrogen, reducing sugar, ethanol concentration, pH, number of yeast cells, and HEMF formation during aging (Sugawara et al. 1994). Both types of *miso* were aged at 30°C for 100 days. The indices of moisture, NaCl concentration, water-soluble nitrogen, and formol-titrated nitrogen changed at a similar rate for the two kinds of *miso*. The relationship between HEMF formation and the number of yeast cells is discussed in Section 7.6.

7.4 Brewing of *Shoyu*

There are many types of *shoyu* in Japan, although the main product is *koikuchi-shoyu*, a deep brown-colored *shoyu*, whose typical composition at pH 4.7 is as follows: NaCl content, 16.9 g/100 mL; total nitrogen, 1.57 g/100 mL; reducing sugar, 3.0 g/100 mL; and alcohol content about, 2–3 mL/100 mL (Mizunuma 1991). The manufacturing process basically consists of three stages (Figure 7.3): *koji* making, brine fermentation, and refining. In the first process, a mixture of steamed soybean and roasted wheat is inoculated with a starter culture of *A. oryzae* or *A. sojae*. After 3 or 4 days, a fungus grows over the culture mixture, which is called *koji*. Brine is then added to facilitate fermentation by the halotolerant yeast *Z. rouxii*, and then by *C. versatilis* and lactic acid bacteria, and to preclude any undesirable fermentation. This fermentation is continued for 6–8 months, during which starch is changed to alcohol, lactic acid, and carbon dioxide by the conversion of simple sugars from starch as well as

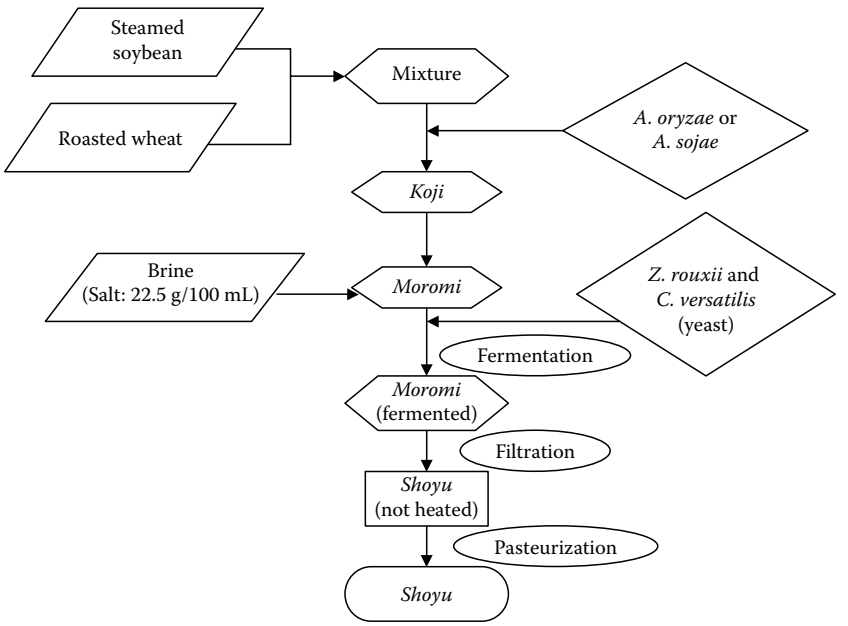

FIGURE 7.3 Simplified manufacturing process of *shoyu*.

by enzymatic hydrolysis of proteins to peptides and amino acids. The two starting materials, soybean and wheat, are generally used in equal amounts in the mixture. Therefore, the average amino acid composition of these two should correspond to that of *shoyu* if there are no chemical changes to amino acids during the fermentation process to hydrolyze the protein to water-soluble peptides and amino acids. The palatability of *shoyu* is greatly enhanced by the high concentration (approximately 7.5%) of amino acids and, particularly, by the presence of glutamic acid (Yamaguchi 1987), in spite of the 17% sodium chloride content in *shoyu*.

During the fermentation process, different types of volatile compounds are formed as fermented products. The refining process includes filtration and pasteurization, the latter involving heating to 70°C–80°C to develop the flavor of *shoyu*. Many flavor compounds appear as the result of the nonenzymatic or aminocarbonyl reaction at this stage, some of them being typically thermally generated aroma compounds. Yokotsuka et al. (1981) first applied steam distillation under reduced pressure and extracted the distillate with dichloromethane to isolate the *shoyu* volatiles. They listed up to 267 compounds as *shoyu* volatiles, among which 37 hydrocarbons, 29 alcohols, 40 esters, 15 aldehydes, 3 acetals, 17 ketones, 24 acids, 16 phenols, 16 furans, 4 lactones, 4 furanones, 5 pyrones, 25 pyrazines, 7 pyridines, 6 other *N*-compounds, 11 *S*-containing compounds, 3 thiazoles, 3 terpenes, and 2 other compounds were described. Not all of these volatiles would contribute to the *shoyu* aroma. They indicated that almost all aroma compounds are popular in fermented food products or in thermally generated aroma compounds; however, HEMF has been identified as the key compound of *shoyu* (Nunomura et al. 1976).

7.5 Identification of Important Aroma Components That Exist Together in *Miso* and *Shoyu*

We compared several extraction methods to avoid decomposition of the aroma components while extraction from *miso* and *shoyu*. The adsorption of aroma compounds from the aqueous phase by a porous polymer resin was thought to be most reasonable with respect to the recovery of fresh aroma and ease of sampling (Sugawara et al. 1990). Aroma components concentrated on a porous polymer were eluted with ether and analyzed by capillary gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). By these methods, the author identified the first HEMF as an aroma component of red salty rice *miso*. We have also found that HEMF was the most effective component in enhancing the aroma of red salty rice *miso* (Sugawara 1991a,b). HEMF has a strong, sweet, cake-like aroma with a threshold value of less than 20 ppb in water (Huber 1992), and is a unique compound and is abundant in red salty rice *miso* and *shoyu*. In recent years, HEMF has been detected in cheese (Milo and Reineccius 1997) and beer (Fritsch and Schieberle 2005); however, the contents of HEMF were lower than that in *miso* and *shoyu*. More recently, HEMF has been shown to be a strong antioxidant and exerted an anticarcinogenic effect on benzo(a) pyrene-induced mouse forestomach neoplasia (Nagahara et al. 1992). HEMF was also found to be effective in preventing radiation hazards, and has important physiological functions as well as being an aroma component.

The composition of aroma components, especially HEMF (6), 4EG (7), and methionol (8) (as shown in Figure 7.1), which were reported to be the key components of *shoyu* (Sasaki and Mori 1991), were compared in five types of *miso*: three types of rice *miso*, red salty rice *miso*, thin-colored salty rice *miso*, and weak-salty rice *miso*, which were produced from steamed soybeans and rice *koji*; barley *miso* produced from steamed soybeans and barley *koji*; and soybean *miso* produced from soybeans only. In addition, the influence of the difference in the materials and the manufacturing process of *miso* on the formation of aroma in five types of *miso* were discussed. Eight samples in each type of *miso* were collected from products evaluated highly at the 34th National *Miso* Competition in Tokyo, and their aroma concentrates were prepared (Kobayashi and Sugawara 1999). *Miso* (40 g) was finely ground and suspended in 160 mL of distilled water; the suspension was centrifuged at 3000 rpm for 15 min. The supernatant was passed through a Tenax TA (2.0 g) packed column. *Shoyu* volatiles were prepared by mixing 20 g of *shoyu* with 180 mL water. The adsorbed material was eluted with 50 mL of ether to which *n*-decanol was added as an internal standard. After drying and evaporating the ether solution, the concentrate was analyzed by GC and GC–MS. The amount of each compound was calculated from the ratio of the compound peak to that of the internal standard and was calculated to ppm (parts per million) of the original amount of *miso* or *shoyu* (Kobayashi and Sugawara 1999).

HEMF was identified in thin-colored salty rice *miso* and barley *miso*, which are usually aged after the fermentation process, as well as red salty rice *miso*. However, no HEMF was detected in weak salty rice *miso* and soybean *miso* as is shown in Table 7.2 (Sugawara and Yonekura 1998). 4EG (7), which was one of the characteristic aroma compounds of *shoyu*, was identified in barley *miso* and soybean *miso* as an important aroma component, but was not detected in *miso* produced from rice and soybeans. It is reported that quality improves when 4-EG is included at 1–3 ppm in *shoyu* (Sasaki et al. 1991). 4EG was detected only in barley *miso* and soybean *miso*, and was found to be an important aroma component that classifies whether *miso* was produced by rice *koji*. Among the aromatic compounds, phenols were predominantly present in *shoyu*. These have already been reported to be formed from the

TABLE 7.2

Comparison of the Concentration of the Character Impact Components (HEMF, 4EG, and Methionol) in Various Kinds of *Miso* and *Shoyu*[a]

Compound	Rice *Miso*, Red Salty	Rice *Miso*, Thin-Colored Salty	Rice *Miso*, Weak Salty	Barley *Miso*	Soybean *Miso*	*Shoyu*[b]
HEMF (6)	18.12	7.73	ND	8.71	ND	161.4
4EG (7)	ND	ND	ND	0.86	0.54	2.2
Methionol (8)	1.39	0.36	0.07	0.87	ND	3.6

Source: Sugawara, E. and Yonekura, Y., *Nippon Shokuhin Kogyo Gakkaishi*, 45, 323, 1998. With permission.

HEMF, 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone; 4EG, (4-ethylguaiacol); ND, not detected.

[a] ppm in five types of *miso* and *shoyu*, respectively.

[b] Unpublished data.

degradation of lignin glycosides during fermentation (Yokotsuka et al. 1981). Lignins are components of cereal bran, and whole wheat is used as *koji* from the early stage of *shoyu* fermentation; on the other hand, rice *koji* is made from polished white rice. The difference in the content of phenols in *shoyu* and *miso* is thought to be one of the factors differentiating the flavor character of each of these products. The threshold value of the sulfur-containing compound, methionol (8), is low, and its odor character is a conclusive factor determining the common characteristic aromas of *shoyu* and *miso*. Methionol is thought to be formed from methionine by yeasts (Aoki and Uchida 1991). The concentration of this component was higher in red salty rice *miso*, thin-colored salty rice *miso*, and barley *miso* than in weak-salty rice *miso* and soybean *miso*, which were manufactured without adding yeast. These results indicated that methionol in *miso* was formed by yeast.

7.6 Changes in the Concentration of HEMF during Fermentation

Table 7.3 shows the concentrations of maltol (2) and 3(2*H*)-furanones (4, 6) in steamed soybeans and following the fermentation processes of *miso*. The content of HEMF only increased during the later stages of the fermentation, and its yield was highest after 60 days. This means that the formation of HEMF is different from that of other components that are present in steamed soybean, and their content decreases during the fermentation (Sugawara 1991a,b). The biosynthetic route of HEMF was claimed to be through the pentose phosphate cycle performed by *shoyu* yeasts (Sasaki et al. 1991, Sasaki 1996). Therefore, its formation by yeasts via a pathway may occur during the fermentation of *miso*. However, this formation mechanism is not clear.

Two types of red salty rice *miso*, one with the pre-cultured *Z. rouxii* yeast (the strain was provided by the Experimental Station of the Miyagi *Miso-Shoyu* Industry Cooperative, Miyagi, Japan) and the other without the yeast, were compared with regard to HEMF formation and the number of yeast cells formed during aging (Sugawara et al. 1994). As shown in Table 7.1, HEMF was not detected from the sample immediately after the mixing and mashing of *miso*. In *miso* without added yeast, HEMF concentration increased with the increasing number of existing yeast cells. In *miso* without yeast aged for 21 days after mixing of the *miso* mash, 0.3 ppm HEMF was detected when the cell number was 2.0×10^3 cells/g. In yeast-added *miso*

TABLE 7.3

Changes in Flavor Compounds during *Miso* Fermentation[a]

Compound	Rice *Koji*	Steamed Soybean	Start	30 Days	60 Days	90 Days	120 Days
Maltol (2)	ND	14.9	7.2	3.9	2.9	3.3	1.2
HDMF (4)	ND	0.2	tr	tr	tr	tr	tr
HEMF (6)	ND	ND	ND	11.5	11.7	9.2	4.1

Source: Sugawara, E., *Nippon Shokuhin Kogyo Gakkaishi*, 38, 1093, 1991b. With permission.
tr, trace; ND, not detected.

[a] ppm in rice *koji*, steamed soybean, and *miso*.

aged for 7 days after mixing of the *miso* mash, no HEMF was detected, although the number of yeast cells 1.6×10^6 cell/g. In yeast-added *miso* and aged for 14 days after mixing of the *miso* mash, the HEMF was first detected. As a result, HEMF formation in red salty rice *miso* was found to be influenced by the growth phase of the yeast and the environmental conditions present. This is not a simple relationship because the number of yeast cells was not proportional to the amount of HEMF formed.

7.7 Formation Mechanism of HEMF by Yeast Cultivation in Model Media

The mechanism and factors involved in the formation of HEMF by yeast were examined in model systems. Blank and Fay (1996) have reported that the formation of HDMF and HEMF progressed in a mixture of an amino acid, glycine or alanine, and pentose in a pH 6.0 buffer solution by a 1 h reaction at 90°C. HEMF was detected in the pentose and alanine mixture, and HDMF, instead of HEMF, was detected in the pentose and glycine mixture. Blank et al. (1997) have reported that HEMF was formed by combining the five-carbon Amadori compound from pentose with acetaldehyde produced by the Strecker degradation of alanine; however, the amount of HEMF formed by the aminocarbonyl reaction was very small and did not reach the level detected in *miso* or *shoyu*.

I described here the study that was carried out in order to clarify the role of yeast in HEMF formation according to the mechanism involving the aminocarbonyl reaction proposed by Blank et al. (1997). I confirmed that HEMF formation was promoted by yeast cultivation in a medium including aminocarbonyl reaction products. The yeast strain used in this study was *Z. rouxii* 061 which was selected in order to produce the well-known red-brown *Sendai miso* by the Experimental Station of Miyagiken *Miso-Shoyu* Industry Cooperative (Miyagi Prefecture, Japan). *Z. rouxii* 061 was halophilic yeast commonly used in producing *Sendai miso*, and has been reported to be able to form a large amount of HEMF in *miso* (Sugawara 1991b). *Z. rouxii* 061 was cultivated in a heat-sterilized medium that included glucose, pentose, and nitrogenous compounds such as extract of *shoyu koji*, polypeptone, casamino acid (a mixture of amino acids obtained from hydrolysis of casein), or an amino acid such as glutamic acid, threonine, serine, or alanine. The composition of each medium is listed in Table 7.4.

All the media were adjusted to pH 5.2, before pouring 100 mL of each into a 200 mL flask and sterilizing at approximately 120°C for 15 min. During the heat sterilization process, an aminocarbonyl reaction would have occurred when the medium contained both sugar and amino acids. In order to investigate the effect of this aminocarbonyl reaction on the formation of HEMF, glucose, ribose, and amino acids were heat sterilized together and separately. With a type 1 medium, a solution containing all the components was heat sterilized in one flask. With a type 2 medium, the sugar solution was separated in advance from the solution containing the nitrogenous components and the other component, and was heat sterilized in a different flask. After the heat sterilization, the solutions were mixed under aseptic conditions immediately before their use as each type of medium. The subsequent experiments were conducted with 1 mL of the starter culture of *Z. rouxii* 061 inoculated into 100 mL of each medium. The inoculated media were incubated at 27°C

TABLE 7.4

Composition of Media Used for Studying Effects of HEMF Production by Nitrogenous Compounds

Parameters	A	B (1,2,3)	C (1,2,3,4,5,6)
Pentose[a] (g)	0	2.5	2.5
Shoyu koji extract (mL)	50	50	0
Nitrogenous compound[b] (g)	0	0	1.0
Glucose (g)	10	7.5	7.5
NaCl (g)	10	10	10
KH_2PO_4 (g)	1.0	1.0	1.0
$MgSO_4 \cdot 7H_2O$ (g)	0.5	0.5	0.5
Yeast extract (g)	0.5	0.5	0.5
Water (mL)	50	50	100

Source: Sugawara, E. and Sakurai, Y., *Biosci. Biotechnol. Biochem*., 63, 749, 1999. With permission.

[a] B1, ribose; B2, arabinose; B3, xylose; C1–C6, ribose.

[b] C1, polypeptone; C2, casamino acid; C3, glutamic acid; C4, threonine; C5, serine; C6, alanine.

for 3 weeks. The number of yeast cells in each culture, pH value, residual amount of glucose, and NaCl concentration in the medium were measured. Aroma concentrates were prepared and analyzed, the experimental and analytical conditions have been described in detail in the reference sugawara et al. (1990). The concentration of HEMF was based on the calibration curve for an authentic sample.

Table 7.5 shows that the results of yeast cultivation in media A and B. HEMF formation was promoted by cultivating an yeast (*Z. rouxii* 061) in a heat-sterilized medium (type 1) that included pentose, glucose, and a nitrogenous compound such as an extract of *shoyu koji*. HEMF formation in the type 1 medium varied according to the nitrogen source such as casamino acid, or an amino acid such as glutamic acid,

TABLE 7.5

Changes in Media Components after Yeast Cultivation of Media

Type of Medium	No. of Yeast Cells ($\times 10^7$/mL)	pH	Glucose (mg/mL)	NaCl (mg/mL)	HEMF (ppm)
A (type 1)	10.0	4.95	4.74	103.7	—
B1 (type 1)	4.7	4.80	0.78	100.6	10.5
B2 (type 1)	5.3	4.79	0.75	100.6	4.1
B3 (type 1)	5.3	4.83	0.89	108.2	4.3
B1 (type 2)	8.7	5.13	0.57	102.7	0.4

Source: Sugawara, E. and Sakurai, Y., *Biosci. Biotechnol. Biochem*., 63, 749, 1999. With permission.

Note: Compositions of the media are listed in Table 7.4.

Type 1: A solution containing all the components was heat sterilized in one flask.

Type 2: The sugar solution was separated in advance from the solution containing the nitrogenous components and the other component, and each of these solutions was heat sterilized in different flasks.

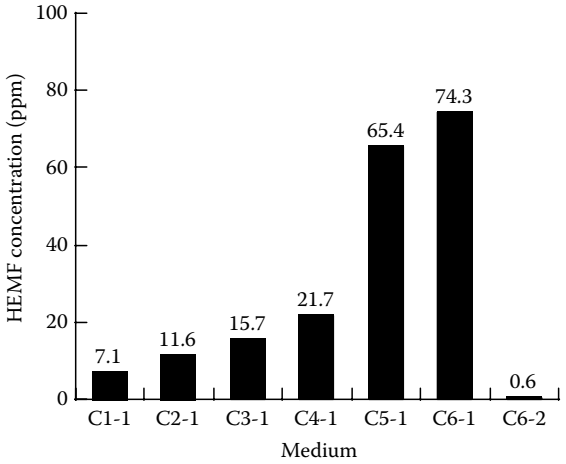

FIGURE 7.4 Effects of nitrogenous compounds in the media on the formation of HEMF by the yeast. C1, polypeptone; C2, casamino acid; C3, glutamic acid; C4, threonine; C5, serine; C6, alanine. Type 1: A solution containing all the components was heat sterilized in one flask. Type 2: The sugar solution was separated in advance from the solution containing the nitrogenous components and the other component, and each of these solutions was heat sterilized in a different flask. (From Sugawara, E. and Sakurai, Y., *Biosci. Biotechnol. Biochem*., 63, 749, 1999. With permission.)

threonine, serine, or alanine, as shown in Figure 7.4 (Sugawara and Sakurai 1999). It is suggested that HEMF was formed during the cultivation of yeast by using a precursor of HEMF, which may have been produced by the aminocarbonyl reaction of pentose with the amino acids during heating. We confirmed that HEMF formation was promoted by yeast cultivation in a medium including the aminocarbonyl reaction products based on ribose and glycine (Sugawara et al. 2007). Each medium was prepared as shown in Table 7.6. In order to investigate the effect of this aminocarbonyl reaction on the formation of HEMF, glucose, ribose, and glycine were heat sterilized together and separately. In the basic medium of type I-1, all of the constituents were heat sterilized within the same flask. The media of types II, III, IV, and V were prepared separately as shown in Table 7.6. After the heat sterilization, the solutions were mixed under aseptic conditions immediately before their use as each type of medium. The subsequent experiments were conducted with 1 mL of the starter culture of *Z. rouxii* 061 inoculated into 100 mL of each medium as shown in Table 7.6. Media types I-1, II, III, IV, and V were incubated at 27°C for 3 weeks.

HEMF formation in media types I-1, II, III, IV, and V is shown in Figure 7.5. The results for the media of types I-1 and II show that HEMF formation was promoted by yeast cultivation in a medium containing aminocarbonyl reaction products based on ribose and glycine. It was also found that much more HEMF formed when the yeast was cultivated in a model medium containing glycine instead of alanine or serine. The concentration of HEMF containing seven carbons formed from the media of types I-1 and III indicate that glucose did not affect the formation of the precursor of HEMF containing five carbons. The results for the media of types I-1, IV, and V suggest that the five-carbon precursor produced by the aminocarbonyl reaction of ribose with glycine was formed more stably in the buffer solution containing KH_2PO_4 and $MgSO_4 \cdot 7H_2O$.

TABLE 7.6

Composition of the Media Used for Studying the Effects of the Type of Yeast and Growth Conditions on the Formation of HEMF

	Basic					
Items	**I-1, II, III, IV, V**	**I-2**	**I-3**	**I-4**	**I-A**	**I-B**
Ribose (g)	2.5	2.5	2.5	2.5	2.5	2.5
Glycine (g)	1.0	1.0	1.0	1.0	1.0	1.0
Glucose (g)	7.5	7.5	7.5	7.5	3.0	0
NaCl (g)	10	0	5	15	10	10
KH_2PO_4 (g)	1.0	1.0	1.0	1.0	1.0	1.0
$MgSO_4 \cdot 7H_2O$ (g)	0.5	0.5	0.5	0.5	0.5	0.5
Yeast extract (g)	0.5	0.5	0.5	0.5	0.5	0.5
Water (mL)	100	100	100	100	100	100

Source: Sugawara, E. et al., *Biosci. Biotechnol. Biochem.*, 71, 1761, 2007. With permission.

Note: Type I-1, -2, -3, -4, -A, -B media, ribose, glycine, and other constituents were heat sterilized within the same flask.
Type II medium, ribose, and glucose were heat sterilized separately from glycine and the other media components.
Type III medium and glucose were heat sterilized separately from the other media components.
Type IV medium, ribose, and glycine were heat sterilized separately from the other media components.
Type V medium, ribose, glycine, KH_2PO_4, and $MgSO_4 \cdot 7H_2O$ were heat sterilized separately from the other media components.

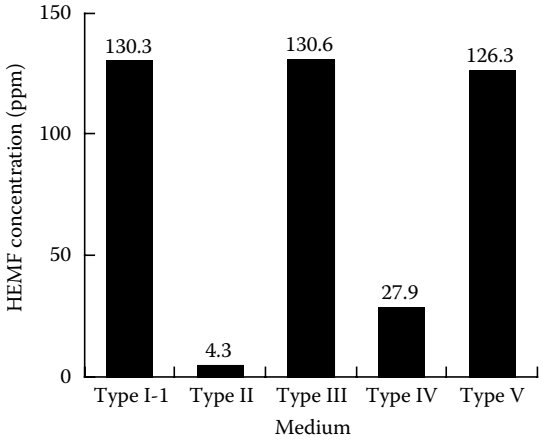

FIGURE 7.5 Effects of medium preparation on the formation of HEMF by *Z. rouxii* 061. The compositions and preparation methods for the media are shown in Table 7.5. (From Sugawara, E. et al., *Biosci. Biotechnol. Biochem.*, 71, 1761, 2007. With permission.)

7.8 Effects of the Type of Yeast and Growth Conditions on the Formation of HEMF

The effects of the type of yeast and growth conditions on the formation of HEMF were investigated by yeast cultivation for 5 weeks. The precursor of HEMF containing five carbons was suggested to be produced by the aminocarbonyl reaction of ribose with glycine. The rate of generation and the amount of the precursor of HEMF containing two carbons are considered to be influenced by the type of yeast and by the glucose concentration and NaCl concentration in the medium. Three types of yeasts, *Z. rouxii* 061, *C. versatilis*, and *Saccharomyces cerevisiae* K7, were used in this study. *C. versatilis* used for *shoyu* brewing was the halotolerant yeast, and *S. cerevisiae* K7 used for rice wine *sake* brewing was the halosensitive yeast. These latter two yeast strains were provided as stock cultures by Iwate Industrial Research Institute (Iwate Prefecture, Japan).

The composition of each medium is listed in Table 7.6. The preparation methods of all the media were described earlier. In the media of types I-1, I-2, I-3, I-4, I-A, and I-B, all of the constituents were heat sterilized in the same flask. The liquid medium for the starter culture of *Z. rouxii* 061 or *C. versatilis* was prepared. A starter culture of *S. cerevisiae* K7 was prepared in the same media as already described, from which only NaCl was excluded. The liquid medium (25 mL) was inoculated with a loopful of *S. cerevisiae* K7 from a slant culture, and incubated at 27°C for 3 days to obtain the starter culture. The subsequent experiments were conducted with 1 mL of the starter culture inoculated into 100 mL of each medium as shown in Table 7.6. The media of types I-1, I-2, I-3, I-4, I-A, and I-B inoculated with *Z. rouxii* 061 starter culture were incubated at 27°C for 1–5 weeks, while the media of types I-1 and I-2 were inoculated with starter cultures of *C. versatilis* and *S. cerevisiae* K7, respectively, and were incubated at 27°C for 1–5 weeks.

Z. rouxii 061 was incubated for 1–5 weeks with three different levels of glucose concentration in order to clarify the role of glucose in the medium. The amount of HEMF was negligible in the media of types I-1 (7.5% Glu), I-A (3.0% Glu), and I-B (no Glu) without yeast inoculation when incubated under the same conditions as those used for the yeast cultivation. The highest concentrations of HEMF were found after 3 weeks in the media of types I-1 (7.5% Glu) and I-A (3.0% Glu), the HEMF concentration in medium type I-A (3.0% Glu) being about 70% of that in medium type I-1 (7.5% Glu) after 3 weeks (Figure 7.6). The concentration of HEMF was very low in medium type I-B (no Glu). *Z. rouxii* 061 was incubated for 1–5 weeks under four different concentrations of NaCl. HEMF could not be detected with the media prepared without yeast inoculation after 3 or 5 weeks. Different NaCl concentrations of 5%, 10%, and 15% did not affect the level of HEMF concentration in the medium at the final stage (Figure 7.6). The medium excluding NaCl resulted in good growth of the yeast, but the activity of HEMF formation by the yeast was low. *C. versatilis* in the media of types I-1 (10% NaCl) and I-2 (no NaCl) and *S. cerevisiae* K7 in medium type I-2 (no NaCl) were confirmed to form HEMF. The amounts formed by *C. versatilis* and *S. cerevisiae* K7 were lower than that by *Z. rouxii*. HEMF formation by *S. cerevisiae* K7 in medium type I-1 (10% NaCl) was negligible because the yeast could not multiply in this medium.

These results suggest that the type of yeast and growth conditions, like glucose and NaCl concentrations in the medium, affected the generation rate and amount of

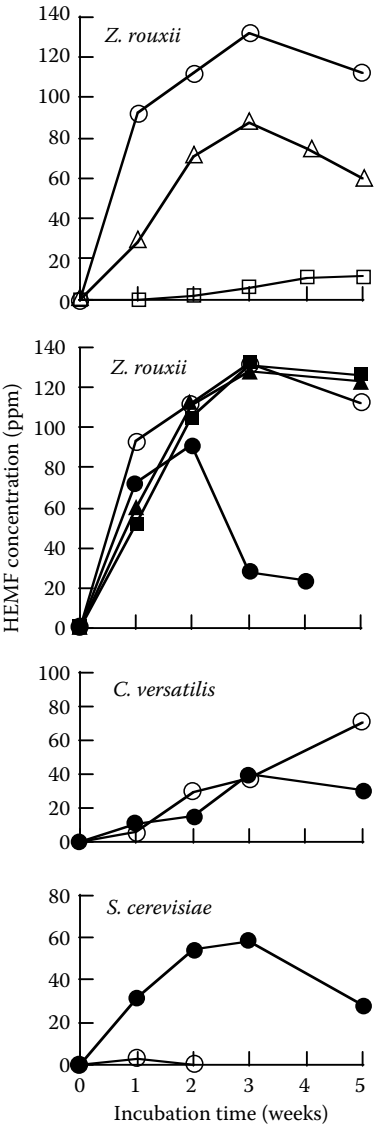

FIGURE 7.6 Effect of glucose concentration and NaCl concentration in the medium on the formation of HEMF by *Z. rouxii* 061, *C. versatilis*, and *S. cerevisiae*. Symbols: ○, type I-1 (7.5% Glucose, 10% NaCl) medium; △, type I-A (3% Glucose, 10% NaCl) medium; □, type I-B (no Glucose, 10% NaCl) medium; ●, type I-2 (7.5% Glucose, no NaCl) medium; ▲, type I-3 (7.5% Glucose, 5% NaCl) medium; ■, type I-4 (7.5% Glucose, 15% NaCl) medium. (From Sugawara, E. et al., *Biosci. Biotechnol. Biochem*., 71, 1761, 2007. With permission.)

the precursor of HEMF containing two carbons. One of the roles of yeast in HEMF formation is probably to provide glucose metabolites containing two carbons. HEMF in *miso* and *shoyu* is considered to be formed by the combination of the five-carbon compound produced through the aminocarbonyl reaction under mild fermentation conditions and the two-carbon chemical compound provided by the yeast.

7.9 HEMF Formation Pathway by Yeast in *Miso* and *Shoyu*

The amount of HEMF formed by the yeast was influenced by the glucose content in the medium (Figure 7.6). We assumed that the precursor of HEMF containing seven carbons is formed from the aminocarbonyl reaction product of five carbons (the C_5 precursor) formed by ribose and glycine, and from the two-carbon chemical compound (the C_2 precursor), which perhaps are formed by active glucose metabolism by the yeast, as in the case of acetaldehyde, as reported by Blank and Fay (1997). We clarified the hypothetical pathway of HEMF formation by the yeast. Stable isotopes were used to investigate how the sugar moiety and amino acids contribute to HEMF formation by the yeast (Ohata et al. 2007). The yeast was incubated in a medium containing the stable isotopes [U-^{13}C]-ribose, [U-^{13}C]-glycine, [U-^{13}C]-glucose, and [6-^{13}C]-glucose, and the incorporation of ^{13}C atoms into the skeleton of HEMF was investigated to confirm the roles of ribose, glycine, and glucose, which were in the medium. It is also investigated whether the C_2 precursor would be provided by the yeast.

The total volume of the experimental media with the stable isotope was 3.0 mL of the basic medium as shown in Table 7.6. A loopful of *Z. rouxii* 061 from a slant culture was incubated in the medium for the starter culture (50 mL) at 27°C for 2 days, before the medium was centrifuged (7000 rpm for 10 min at 4°C) and the yeast cells were collected. The collected cells were washed with a washing buffer containing 10 mL of 1 M Tris-hydrochloric acid (pH 7.4), 2.48 g of NaCl, and 0.123 g of $MgSO_4 \cdot 7H_2O$ dissolved in distilled water, and this solution was made up to 500 mL with distilled water, and sterilized. The supernatant was separated by centrifuging it twice under the same conditions to obtain intact cells. The intact cells were added to each experimental medium, and the final concentration was adjusted to 10^8cells/mL, before the medium was incubated at 27°C for 72 h. The aroma concentrates were prepared by extracting the supernatant (3 mL) of the yeast cells removed by centrifugation after incubation with two successive additions of 3 mL of dichloromethane in a 20 mL vial under magnetic stirring for 5 min each time. The two organic phases were collected, dried over anhydrous sodium sulfate, and concentrated under nitrogen. The aroma concentrate obtained was analyzed by GC–MS under the analysis conditions already described.

7.9.1 Contribution of Ribose and Glycine to HEMF Structure

A control experiment indicated that HEMF was effectively formed when yeast was incubated using normal glucose, normal ribose, and normal glycine in the basic medium. The main fragment ions of HEMF were assigned as follows: *m/z* 142, M^+; 127, $(M–CH_3)^+$; 114, $(M–CH_3CH_2)^+$; 99, $(M–COCH_3)^+$; 85, $(M–COCH_2CH_3)^+$; 71, $(M–CH_3COCHO)^+$; 57, $(CH_3CH_2CO)^+$; and 43, $(CH_3CO)^+$. [U-^{13}C]-ribose, instead of

normal ribose, was then incubated in the same medium. A fragment of the molecular ion was found at *m/z* 147, indicating that the HEMF formed contained five ^{13}C atoms. These ^{13}C atoms were not present in the ethyl group of the side chain, because they were found in the fragments $(M–CH_3)^+$ at *m/z* 132 and $(M–CH_3CH_2)^+$ at *m/z* 119. The MS result that all carbons included in the fragments of $(^{13}CH_3{}^{13}CO)^+$ at *m/z* 45 and $(M–{}^{13}COCH_2CH_3)^+$ at *m/z* 89 were ^{13}C atoms confirmed that all carbons of the five-ring and the methyl group of the side chain were ^{13}C atoms. In a further experiment, [U-^{13}C]-glycine was added to the medium instead of normal glycine, and the same incubation was conducted. In this case, the MS data for HEMF were the same as those in the control experiment. This result indicates that no carbons of glycine contributed to the structure of HEMF. These results reveal that the five-ring and the methyl group of the side chain of HEMF were formed from ribose, and that the ethyl group of the side chain was not formed from glycine. We assumed that the formation mechanism of HEMF by the yeast is based on the formation of the aminocarbonyl products of ribose and glycine, and that its subsequent decomposition (the elimination of the moiety from glycine) forms the C_5-1-deoxydiketose (the C_5 precursor), reported to be formed from pentose by Blank and Fay (1996). Further enolization of this compound can lead to the intermediate, which reacts with the C_2 precursor formed by the active metabolism of yeast.

7.9.2 Contribution of Glucose to the Ethyl Group of HEMF

No HEMF was detected when the control experiment was done without glucose. It appears that glucose had an important role in the formation of HEMF by the yeast. To determine the contribution of glucose, [U-^{13}C]-glucose was added to the medium instead of common glucose. After incubation, the fragment of the molecular ion at *m/z* 144 indicates that two ^{13}C atoms from glucose were incorporated into the structure of HEMF. The incorporation of two ^{13}C atoms is also indicated by fragments $(^{13}CH_3{}^{13}CH_2CO)^+$, $(M–CH_3COCHO)^+$, and $(M–COCH_3)^+$, at *m/z* 59, 73, and 101, respectively. Fragments $(CH_3CO)^+$ at *m/z* 43 and $(M–CO{}^{13}CH_2{}^{13}CH_3)^+$ at *m/z* 85 were the same as those obtained using common glucose. In addition, the daughter ions at *m/z* 128 $(M–{}^{13}CH_3)^+$ and *m/z* 114 $(M–{}^{13}CH_3{}^{13}CH_2)^+$ showed the presence of two ^{13}C atoms in the ethyl group of the side chain.

These results suggest that the C_2 precursor, in which all carbons were labeled, was initially generated from [U-^{13}C]-glucose by metabolism by the yeast. This C_2 precursor and the C_5 precursor from ribose then combined before the cyclization of the seven-carbon compound was obtained, resulting in HEMF finally being formed. To investigate the formation pathway of the ethyl group from glucose, a similar incubation was conducted using [6-^{13}C]-glucose instead of [U-^{13}C]-glucose. The intensity of the parent molecular ions at *m/z* 142 and 143 was about one to one, indicating that monolabeled HEMF and unlabeled HEMF formed in approximately a ratio of one to one. The daughter ions at *m/z* 58, 72, and 100 produced fragments at *m/z* 57, 71, and 99, respectively, by the fragmentation of monolabeled and unlabeled HEMF. These ions were of nearly equal intensity. The fragments at *m/z* 43 and *m/z* 85 were the same as those of unlabeled HEMF, while the daughter ions at *m/z* 127 $(M-{}^{13}CH_3)^+$ and *m/z* 114 $(M-{}^{13}CH_3CH_2)^+$ showed the presence of one ^{13}C atom in the methyl moiety of the ethyl group. These results indicate that the same amounts of the C_2 precursors,

one of which was labeled and the other unlabeled, were formed from one molecule of glucose, and reacted equally with the C_5 precursor. Hence we were able to confirm that the C_2 precursor was formed by the glucose metabolism of yeast, and that this formed C_2 precursor constituted the ethyl group of HEMF. The results of this study confirm that the five-ring and the methyl group of the side chain of HEMF were formed from the five-carbon chemical compound generated by the aminocarbonyl reaction of ribose and glycine (the C_5 precursor), and that the ethyl group of the side chain of HEMF was formed from the two-carbon chemical compound generated by the glucose metabolism of yeast (the C_2 precursor). Consequently, the formation mechanism of HEMF (Figure 7.7) was inferred from the results obtained in this study.

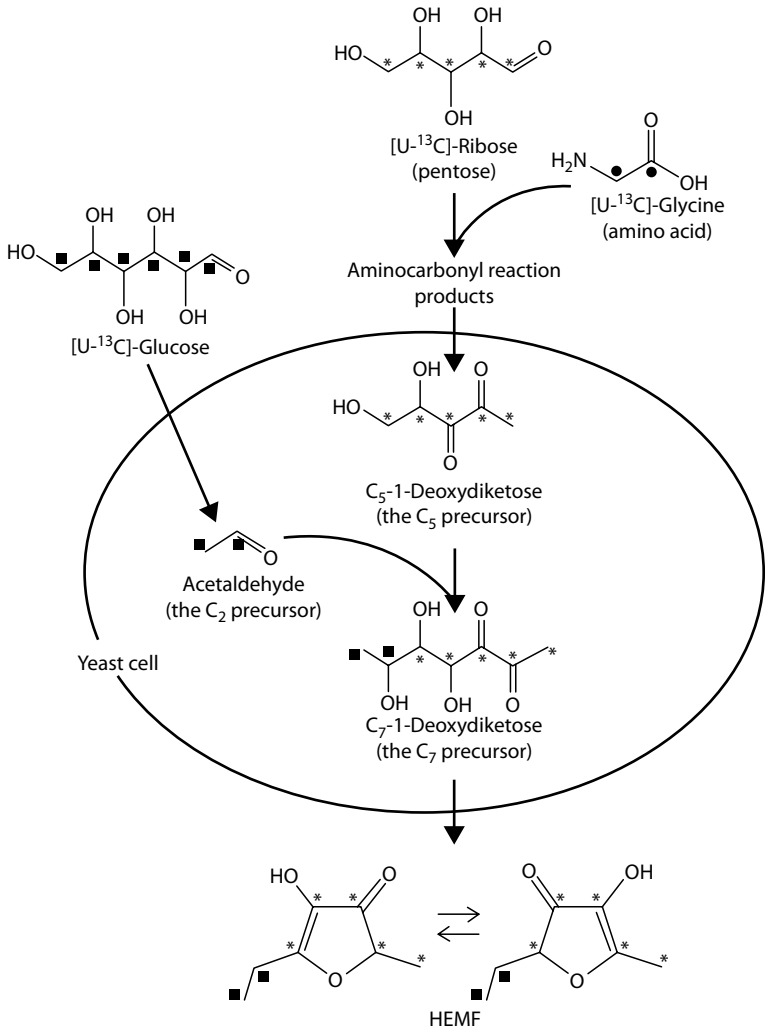

FIGURE 7.7 Hypothetical formation pathway of HEMF by yeast. *, ●, ■—^{13}C atom. (From Ohata, M. et al. *Biosci. Biotechnol. Biochem*., 71, 407, 2007. With permission.)

In *miso* and *shoyu*, HEMF was probably formed by the combination of the five-carbon compound generated by the aminocarbonyl reaction under mild conditions of fermentation and the two-carbon compound of the glucose metabolite formed by the yeast enzymatically. HEMF is the key compound in red salty rice *miso*, thin-colored salty rice *miso*, and barley *miso*, but has not been identified in weak salty rice *miso* and soybean *miso*. We, therefore, consider that there is likely to be another important compound in the unidentified odorant formed by the aminocarbonyl reaction and/or yeast, and that this compound would play an important role in the aroma of all types of *miso*. Further studies on the aroma impact compound of *miso* and *shoyu* should be taken up.

7.10 Conclusion

Miso and *shoyu* are important seasonings for the Japanese, and aroma is a significant factor for quality. More than 300 aroma components have been determined in *shoyu* and *miso*, and, in particular, the aroma components generated by yeasts or other microorganisms are the most important. 4-Hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF) has a strong, sweet, and caramel-like aroma, which is a very important characteristic component of *miso* and *shoyu*. It is well known that HEMF is formed by yeasts, but this formation mechanism was not yet clarified. By the cultivation of yeasts in model media containing glucose, ribose, and glycine, we confirm that the five-ring and the methyl group of the side chain of HEMF were formed from the five-carbon chemical compound generated by the aminocarbonyl reaction of ribose and glycine, and that the ethyl group of the side chain of HEMF was formed from the two-carbon chemical compound generated by the glucose metabolism of yeast. In *miso* and *shoyu*, HEMF was probably formed by the combination of the five-carbon compound generated by the aminocarbonyl reaction under mild fermentation conditions and the two-carbon compound of the glucose metabolite formed by the yeast enzymatically.

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8

Fermented Cereal Products

Jean-Pierre Guyot

CONTENTS

8.1 Introduction

Writing on cereal fermentations is a challenge considering all the reviews (Tamang et al. 1996, Beuchat 1997, Gadaga et al. 1999, Nout and Sarkar 1999, Blandino et al. 2003, Hammes et al. 2005, Kohajdová and Karovicova 2007, Humblot and Guyot 2008, Tamang and Fleet 2009) and books already published on this topic (Steinkraus 1996, Haard et al. 1999, Nout et al. 2005). Most of the cereal fermented foods involve a lactic acid fermentation step, which can be associated to an alcoholic fermentation step depending on the process used (e.g., brewing). It is now commonly known that lactic acid fermentation contributes to human welfare through the preservation of foods, the development of organoleptic characteristics and nutritional improvement of foods, and the health-promoting effects of lactic acid bacteria (probiotics) (Nout and Motarjemi 1997, Charalampopoulos et al. 2002, Kohajdová and Karovicova 2007). Fermented foods also contribute to cultural identity since they are associated with very ancient and traditional food habits and sometimes with religious practices.

In this chapter, we intend to derive some general characteristics from a few examples of cereal based fermented foods produced in developing and emerging countries, which roughly are referred as "tropical fermented foods." However, wheat and rye sourdoughs are the best examples to which the most complete studies were developed with regard to a category of cereal-based fermented foodstuff, using a combination of classical and molecular microbiological methods to depict the relationship between microbial diversity, cell physiology, and product characteristics (De Vuyst and Neysens 2005, Gobetti et al. 2005, Hammes et al. 2005, De Vuyst and Vancanneyt 2007). Therefore, research on this particular and economically important field in Western countries can contribute to provide some indications on further research needs for cereal-based tropical fermented foods which, unfortunately, do not benefit from such industrial backgrounds and economical support.

8.2 Diversity of Raw Materials and Traditional Processing Methods

Lactic acid cereal fermented foods and beverages are made from a great variety of cereals involving different processing methods (Table 8.1). Maize, millet, and sorghum grains in Africa are used to produce such lactic acid fermented foods like *ogi* (Benin, Nigeria), *togwa* (Tanzania), *bushera* (Uganda), *ben-saalga/koko* (Burkina Faso, Ghana), *gowé* (Benin), *mawé* (Benin), *kenkey* (Ghana), *hussuwa* (Sudan), *mahewu* (Zimbabwe), *poto poto* (People Republic of Congo) (Figure 8.1). In many African countries, the same cereals can be germinated to produce malt for their use in brewing of cereal slurries to make traditional beers of thick consistency, called under different vernacular names such as *dolo* in Burkina Faso; *bili bili* in Tchad; *burukutu* in Nigeria; *pito* in Ghana; and *kaffir*, a Bantu beer, in South Africa (for more details see the reviews of Steinkraus (1996) and Jespersen (2003)). In such processes that use malt to hydrolyze the cereal starch, an alcoholic fermentation develops spontaneously due to yeasts (mainly *Saccharomyces cerevisiae*) associated with lactic acid bacteria, which are responsible for the sour taste of the beer (Jespersen 2003, Maoura et al. 2005).

In Mexico and Guatemala, maize is used to produce *pozol* whereas rice is used to produce *selroti*, *bhaati jaanr*, *idli*, *dosa* in India, and *burong isda* in the Philippines. In the Himalayas, various cereals like finger millet, wheat, maize, and barley are used to produce different kinds of fermented beverages like *kodo ko jaanr* and *gahun to jaanr* and if we consider the particular case of the Balkan Peninsula, rye, wheat, millet, maize, rice, barley, and oat can be used alone or in mixtures to produce *boza*. Albeit limited, the list given here illustrates the diversity of cereals being used through different countries around the world. Furthermore, it is not an easy task to determine what cereal fermented foods are truly different, since products with the same name can be prepared from different cereals, for example, *ogi* from maize or sorghum and the different *boza* preparations, and that same or similar products can have different names according to the country and the ethnic groups who produce them. As an example, according to the different vernacular languages in Himalayan regions (in parenthesis), synonyms for *kodo ko jaanr* are *mandokpenaa thee* (Limboo), *sampicha ummaak* (Rai), *naarr paa* (Gurung), *saangla chi* (Tamang), *chirs shyaabu* (Sunwar), *paadaare haan* (Magar), *gyaar chyyaang* (Sherpa), *minchaa chhyaang* (Bhutia), and *mong chee* (Lepcha) (Tamang et al. 1996). It is, therefore, a challenge to identify what traditional processes and products are truly different together with the variations in traditional food processing and food consumption habits.

In such a context, there is a unique opportunity to link social sciences to food science through field surveys. In ideal cases, surveys at a population level should be the starting point of further research in the field of food science. This approach was used for the study of *togwa* in Tanzania (Kitabatake et al. 2003) and *ben-saalga* in Burkina Faso (Tou et al. 2006, Mouquet-Rivier et al. 2008), and to collect information on indigenous fermented food beverages of the Himalayas (Tamang 2010) and on *selroti*, an ethnic, fermented rice product of the Himalayas (Yonzan and Tamang 2009), allowing a description of food habits and processing methods based on interviews and data collection at the field level. Furthermore, such surveys can allow identifying a set of traditional producing units or homesteads for randomizing collection of food

TABLE 8.1

Traditional Lactic Acid Cereal-Based Fermented Foods and Beverages Consumed in Various Countries

Product	Country	Cereal	Malt	Nature of Fermented Product	Product Use
Banku	Ghana	Maize, cassava		Dough	Cooked dough
Ben-saalga, *koko*	Burkina Faso, Ghana	Pearl millet		Slurry	Gruel
Bhaati jaanr	Darjeeling hills and Sikkim (India), Nepal	Glutinous rice		Saccharified glutinous rice	Sweet–sour alcoholic beverage
Boza	Bulgaria, Romania, Turkey, Albania	Wheat, rye, millet, maize, etc.		Cooked slurry	Beverage
Bushera	Uganda	Sorghum, millet	+ (S, Mi)	Slurry	Beverage
Dosa	India	Rice and black gram		Batter	Pancake (fried)
Gahun to jaanr	Darjeeling hills and Sikkim (India)	Wheat		Cooked seeds	Sweet–sour alcoholic beverage from filtered fermented cooked seeds
Gowé (*Sifanu*)	Benin	Sorghum	+ (S)	Cooked slurry	Beverage
Hussuwa	Sudan	Sorghum	+ (S)	Dough	Dough-like food
Idli	India	Rice and black gram		Batter	Steamed cake
Injera	Ethiopia	Tef, sorghum, corn, finger millet, barley		Batter	Flat bread

(*continued*)

TABLE 8.1 (continued)

Traditional Lactic Acid Cereal-Based Fermented Foods and Beverages Consumed in Various Countries

Product	Country	Cereal	Malt	Nature of Fermented Product	Product Use
Kenkey	Ghana	Maize		Dough	Cooked/steamed dough
Kisra	Arabian Gulf, Sudan, Iraq	Sorghum, pearl millet		Dough to thick batter	Flat bread
Kodo ko jaanr	Darjeeling hills and Sikkim (India), Nepal	Finger millet		Cooked seeds	Thick, mildly alcoholic–acidic beverage from pressed fermented cooked seeds
Mahewu	South Africa, Zimbabwe	Maize	+ (S, Mi)	Slurry	Beverage
Mawè	Benin, Togo	Maize		Dough	Basis for ready-to-serve foods
Ogi	West Africa	Maize, millet, sorghum		Slurry	Basis for ready-to-serve foods
Poto poto	Congo	Maize		Slurry	Gruel
Pozol	Mexico, Guatemala	Maize		Dough[a]	Beverage
Selroti	Darjeeling hills and Sikkim (India), Nepal, Bhutan	Rice		Batter	Fritter (confectionery)
Togwa	East Africa	Maize	+ (Mi)	Cooked slurry	Beverage

Note: S, Sorghum malt; Mi, millet malt.

[a] Prepared from maize cooked in lime.

(a) (b)

FIGURE 8.1 (a) *Poto poto* at a retail outlet in a market at Pointe Noire (Congo). (b) Brewing red sorghum in a traditional processing unit to prepare *dolo* in Burkina Faso. (Courtesy of Claire Mouquet-Rivier.)

samples as done for *ben-saalga*, to describe fermentation kinetics in the traditional process, and to determine the nutritional quality of this fermented gruel (Mouquet-Rivier et al. 2008). Unfortunately, most often, surveys and sampling at the field level are difficult to perform and investigations based on such approaches are scarce; thus many studies are based on a very few samples, therefore, limiting their significance.

8.3 Microbiota of Tropical Cereal Fermented Foods

Investigation of the microbiota of fermented foods, despite the numerous works on this topic, is still an ongoing research field since the advent of culture-independent methods (Giraffa and Carminati 2008). The best approach would consist in using a combination of culture-dependent and -independent methods, particularly to investigate the dynamics of populations. However, most of the well-described tropical fermented foods rely on the use of classical culture-dependent methods through the isolation of microorganisms, and typing by phenotypic and/or genotypic techniques (Temmerman et al. 2004). Many ecological works conducted from the 1970s to the 1990s are mainly based on the identification of isolates using the commercial API 50CH system. Indeed, it is a very useful tool when it is necessary to investigate carbohydrate utilization of LAB isolates, but could lead to misleading identifications due to poor reproducibility and low taxonomic resolution (De Vuyst and Vancanneyt 2007, Giraffa and Carminati 2008). The use in the past of identification methods of low discriminating power, changes in the taxonomical status of some species, and the phenomenon of newly discovered LAB would necessitate to investigate again some already described traditional tropical fermented foods, using molecular tools for the unambiguous determination of microbial diversity.

Though it is not the intention here to give a full description of microbial diversity in fermented cereals, which would be like to give a fastidious enumeration, some general trends can be drawn from the existing literature. More exhaustive descriptions of LAB and yeast diversity will be found in the reviews of Blandino et al. (2003), De Vuyst and Neysens (2005), and De Vuyst and Vancanneyt (2007). Heterofermentative

and homofermentative LAB are commonly associated, and they belong to the genus *Lactobacillus*, *Leuconostoc*, *Weissella*, *Lactococcus*, *Pediococcus*, *Enterococcus*, and *Streptococcus*. Wheat and rye sourdoughs represent very particular food niches from which Hammes et al. (2005) reported a number of 46 LAB species associated to them, most of which belonging to the genus *Lactobacillus* with a dominance of heterofermentative LAB. The sourdough LAB associations will differ according to the sourdough type and to the traditional practices for their preparation. Typical sourdough lactobacilli are represented by *Lactobacillus sanfranciscensis*, *Lb. pontis*, *Lb. panis*, *Lb. paralimentarius*, *Lb. frumenti*, *Lb. spicheri*, *Lb. rossiae*, *Lb. zymae*, *Lb. acidifarinae*, *Lb. hammesii*, *Lb. nantensis*, and *Lb. mindensis*, but also more ubiquitous LAB often found in other cereal fermented foods can be isolated, such as *Lb. plantarum*, *Lb. fermentum*, *Lb. brevis*, and *Lb. amylovorus*. It is noteworthy that until recently Western sourdoughs have been a continuous source of new LAB species. In contrast, it is intriguing to observe that in spite of a great diversity of geographical situations and agroclimatic conditions, raw materials, processes and products, tropical cereal fermented foods have not been a very rich source for the discovery of new species. This leads to wonder about the reasons that underlie such a situation (e.g., use in the past of low discriminatory identification methods) and speaks in favor of carrying on thorough investigations on these foods by means of modern methods as mentioned above. The recent discovery of new LAB species in rye and wheat sourdoughs infers that new species could still be discovered in traditional tropical fermented foods.

To date, whatever the investigation methods used, it seems that there is a general consensus to state that LAB belonging to the above cited genus are commonly isolated, and that *Lb. plantarum* and/or *Lb. fermentum* are frequently the dominant species. Either species were regularly reported as dominant in African fermented foods such as *bushera* in Uganda (Muyanja et al. 2002), *togwa* in Tanzania (Mugula et al. 2003), *mawè*, *ogi*, and *gowé* in Benin (Hounhouigan et al. 1993, Nago et al. 1998, Vieira-Dalodé et al. 2007), *ogi* in Nigeria (Johansson et al. 1995), *poto poto* in the Republic of the Congo (Abriouel et al. 2006), and *koko* in Ghana (Lei and Jakobsen 2004) with a high degree of intraspecies diversity (Johansson et al. 1995, Hayford et al. 1999, Lei and Jakobsen 2004). In contrast, in mild-alcoholic beverages like *bhaati jaanr* made from glutinous rice and *kodo ko jaanr* made from seeds of finger millets in the Eastern Himalayas, *Pediococcus pentosaceus* and *Lb. bifermentans* were reported as dominant in association with yeasts such as *Saccharomycopsis fibuligera*, *Rhizopus* spp., *S. cerevisiae*, and *Candida glabrata* (Thapa and Tamang 2004, Tamang and Thapa 2006). More generally, yeasts are frequently associated with LAB (Jespersen 2003), particularly when the process leads to alcoholic–acidic beverages like African beers or *kodo ko jaanr.* In *gowé* and *togwa*, which share some similarities in the processing method by the addition of sorghum malt to a cooked cereal slurry, *Kluyveromyces marxianus*, *Pichia anomala*, *C. krusei*, and *C. tropicalis* were reported for *gowé* and *S. cerevisiae*, *C. pelliculosa*, and *C. tropicalis* were reported for *togwa*. However, in other fermented foods that do not involve malt addition, yeasts such as *Candida* spp., *Saccharomyces* spp., *Trichosporon* spp., *Kluyveromyces* spp., and *Debaryomyces* spp. are also associated with LAB in *kenkey*, a fermented maize dough from Ghana (Jespersen 2003), or *Candida* and *Geotrichum* species in the Beninese *ogi* (Nago et al. 1998). A wide diversity of yeasts belonging to the genus *Saccharomyces* and *Candida* were also found in *boza* from Bulgaria or Turkey (Hancioğlu and Karapinar 1997, Gotcheva et al. 2001). As for LABs in *boza*,

Gotcheva et al. (2001) reported that *Lb. plantarum*, *Lb. fermentum*, and *Lb. acidophilus* were dominant in the Bulgarian *boza*, but Hancioğlu and Karapinar (1997) found that *Leuconostoc paramesenteroides* and *Lb. sanfranciscensis* were dominant in the Turkish *boza*. These examples illustrate how variable some results can be regarding similar foods bearing the same name but prepared in different geographical areas. However, in these studies, not the same cereals and inoculation methods were used to produce these *bozas*; in addition, in the Bulgarian study sucrose was added to the wheat slurry before fermentation. Therefore, it is difficult to draw conclusive comparisons between these different *bozas* preparations. Since it is a widespread fermented beverage in the Balkans, a study covering different *boza* preparations in different Balkan countries would probably lead to interesting results.

In all examples cited here, except for *kodo ko jaanr*, species of the genus *Lactobacillus* form the dominant group in association with different cocci or coccobacilli belonging mainly to the genus *Weissella*, *Pediococcus*, and *Lactococcus*. However, there are a few fermented cereal-based foods, in which lactic acid cocci are dominant or form a significant part of the food microbiota. In *pozol*, a refreshing beverage produced from fermented maize dough, maize is first cooked in lime (nixtamalization), thereafter dehulled, washed, and finally ground to produce a dough called *masa*, which will be fermented for several days. In the fermented *masa*, diverse microorganisms, that is, LAB, yeasts, and fungi, were characterized (Ampe et al. 1999). In contrast to the above-described products, strains identified as *Streptococcus bovis* dominate the LAB population (Ben Omar and Ampe 2000) and were found predominant among forty amylolytic LAB isolated from *pozol* (Diaz-Ruiz et al. 2003). *S. bovis* comprises strains capable of growth at pH 9.6, and some strains are thermoduric. Considering these properties, the alkaline cooking of maize would have contributed in selecting these bacteria (Diaz-Ruiz et al. 2003). In *kisra*, a Sudanese sorghum sourdough, at an intermediate stage of the fermentation, the dominant bacteria were *Enterococcus faecalis*, *Lactococcus lactis*, and *Lb. fermentum*. At the end of the fermentation, only *Lb. fermentum* and *Lb. reuteri* remained as the dominant flora (Hamad et al. 1997). This illustrates also that dominance is a transitory state due to a succession of different LAB populations, notwithstanding numerous works are based on end point samplings or do not characterize population dynamics, giving therefore a "static picture" of the microbiota structure.

In *hussuwa*, another Sudanese semisolid, dough-like food, enterococci constituted about 10% of the LAB population (Yousif et al. 2005). Yousif et al. (2005) showed that a few *hussuwa* enterococci strains bore virulence determinants and 50% of the strains presented resistance to at least one antibiotic. These studies point out that enterococci may play a role in the fermentation of cereals, but also that their occurrence should deserve specific attention since some strains are opportunistic human pathogens.

8.4 Processes, Substrate Availability, and Microbial Diversity

Scheirlinck et al. (2008), using both culture-dependent and culture-independent methods, investigated the bacterial community in 39 Belgian sourdoughs sampled from 11 bakeries which were visited twice at 1-year intervals. Their conclusion was that the microbial composition of the sourdoughs was mainly affected by the bakery environment irrespective of the type of flour used (wheat, rye, spelt) to prepare the sourdough,

and the instability could be linked with variations in technological parameters. This study points out the importance of the environment in the production units, and such a conclusion is of general importance when microbial diversity has to be investigated. However, in tropical areas, the complexity of the problem and the difficulty in tackling it are higher due to the diversity in geographical, agroclimatic conditions, processing methods, and environmental conditions of the processing units. Unfortunately, one regrets that there is a lack of investigations similar to that of Scheirlinck et al. (2008) on such a topic to address the following question: is the structure of the microbiota of different tropical cereal fermented foods significantly different and what factors would affect its composition? As we briefly evoked in a simplified view, some general trends can be drawn with some recurrent actors being present (e.g., *Lb. plantarum*, *Lb. fermentum*). In reality, compared to soil, sediments, anaerobic digester sledges, or to gastrointestinal microbiota, species diversity in cereal fermented foods is rather limited in spite of an elevated concentration of microorganisms, which usually range from 10^7 to 10^9 bacteria/mL (or gram), and which should make estimates of diversity easier. Undoubtedly, unit operations in a process can affect the type of dominant microorganism by establishing a selection pressure like nixtamalization as in the production of *pozol* (Diaz-Ruiz et al. 2003). Furthermore, by decreasing or increasing the availability of readily fermentable substrates the process can determine the "driving force" for fermentation. For wheat and rye sourdoughs, maltose is the main fermentable substrate, and the use of external electron acceptors such as fructose, which is reduced to mannitol, together with acid tolerance and adaptability to the sourdough environment, explain the competitiveness of the sourdough heterofermentative LAB (De Vuyst and Neysens 2005, Gobetti et al. 2005, Vrancken et al. 2008). Readily available substrates in tropical cereals, in the absence of malt addition, are mainly sucrose, fructose, glucose, and, to a lesser extent, α-galactooligosaccharides (mainly raffinose and stachyose). These saccharides represent approximately 1%–3% of the cereal composition and can easily be fermented. Their availability in the food matrix can be increased by size reduction of the grains (e.g., grinding and pounding), which will improve their accessibility to fermenting microorganisms. In contrast, their availability can be decreased by operations like soaking (i.e., diffusion of low molecular weight sugar into the soaking water), washing out during sieving, and cooking under alkaline conditions. The effect of soaking on substrate availability in the grains has been poorly investigated. Investigating the production of *ben-saalga* (Figure 8.2) in 24 traditional production units in Ouagadougou (Burkina Faso), Tou et al. (2006) showed that the concentration of sucrose, the main disaccharide in pearl millet grains, decreased from 1.1 g/100 g dry matter (DM) before soaking the grains to 0.27 g/100 g DM after soaking. Further decrease in sucrose concentration occurred after grinding of the soaked grains and wet sieving of the dough, so that at the beginning of the settling step sucrose concentration was as low as 0.03 g/100 g DM. Lactic acid fermentation occurred at the settling step; however, under such conditions, it appeared that lactic acid concentration produced during fermentation was higher than expected on the basis of the initial concentration of readily fermentable sugars. This suggests the use of an additional carbon source, that is, starch. This hypothesis is consistent with the presence of amylolytic lactic acid bacteria (ALAB), which accounted for 12% of the LAB population in fermented pearl millet slurry (Tou et al. 2006).

As for *pozol* production in Mexico, the main soluble sugar of maize is sucrose, which is present at a concentration of 2 g/100 g of the whole kernel on a dry weight

(a) (b)

FIGURE 8.2 Some steps leading to substrate loss during *ben-saalga* production in a traditional processing unit (Ouagadougou, Burkina Faso) (a) soaking of pearl millet. (b) Sieving of pearl millet slurry obtained after grinding. (Courtesy of Claire Mouquet-Rivier.)

basis (Boyer and Shannon 1987). It is reduced to 0.1–0.7 g/100 g of dry dough, after alkaline cooking, soaking, and washing (Diaz-Ruiz et al. 2003), and the same observations and reasoning as above apply to *pozol*. 40% of LAB present in the initial dough was amylolytic, and this ratio decreased to 3% after 72 h of fermentation. The high initial concentration of ALAB indicates their importance during *pozol* fermentation, mainly during the first 24 h. Furthermore, species identified as *Streptoccocci* spp. were shown to be the dominant ALAB, consistent with their ability to survive drastic conditions (nixtamalization) (Figure 8.3). However, in spite of a high specific growth rate (0.94/h) and an efficient energy conversion yield to bacterial cell biomass (0.31 g of biomass of substrate) for the *pozol* amylolytic *Streptococcus* spp. strain 25124, its poor tolerance to low pH rapidly limits its growth. However, its ability to transiently produce maltooligosaccharides during fermentation can serve as energy

(a) (b)

FIGURE 8.3 (a) Nixtamalization (alkaline cooking) of kernels of maize in a traditional processing unit in Mexico. (b) Mixing the fermented masa with water to produce the mild acidic and refreshing beverage *pozol* at a retail outlet at Villahermosa (Mexico). (Photo by Guyot, J.P.)

sources for non-amylolytic species in *pozol* fermentation, explaining the observed diversity and the dominance of non-amylolytic LAB at the end of fermentation.

ALAB have been isolated from other fermented starchy foods made from cereals or cassava in different countries around the world (Reddy et al. 2008). Strains of either *Lb. fermentum* or *Lb. plantarum* with a high amylase activity were isolated from Nigerian traditional fermented foods (*fufu*, *burukutu*, *ogi*, and *kunu-zakki*) (Johansson et al. 1995, Sanni et al. 2002), from the Beninese *ogi* and *mawé* (Agati et al. 1998), from *burong isda* in the Philippines (a fermented food made from fish and rice) (Olympia et al. 1995), but also from retted cassava in the Popular Republic of Congo (Giraud et al. 1991); *Lactobacillus manihotivorans* was isolated from sour starch cassava in Colombia (Morlon-Guyot et al. 1998). Furthermore, *Lc. lactis* subsp. *lactis* B84, capable of utilizing starch as a sole carbon source and producing L(+)− lactate, was isolated from spontaneously fermented rye sourdough in Bulgaria (Petrov et al. 2008) (Figure 8.4).

The presence of ALAB during the fermentation of cereals can allow replenishing the microbiota with an extra energy source through the hydrolysis of starch and in some extreme cases (e.g., *ben-saalga* or *pozol*) could be a determining factor that would allow the growth of non-amylolytic LAB, contributing therefore to microbial diversity and abundance in starchy fermented foods. In general, the energetics of microbial growth in relation to the use of readily fermentable sugars and starch by LAB from tropical cereal fermented foods is still to be elucidated clearly, emphasizing the gap between current knowledge on the physiology and biochemistry of wheat and rye sourdough LAB (see review by Gobetti et al. 2005) and the lack of physiological data on LAB from tropical cereal fermented foods. In an attempt to understand the relation between growth energetics and α-amylase production, Calderon et al. (2001, 2003a,b) investigated the physiology of *Lb. fermentum* Ogi E1 isolated from *ogi* in Benin. Similarly to the heterofermentative LAB from sourdough (Gobetti et al. 2005,

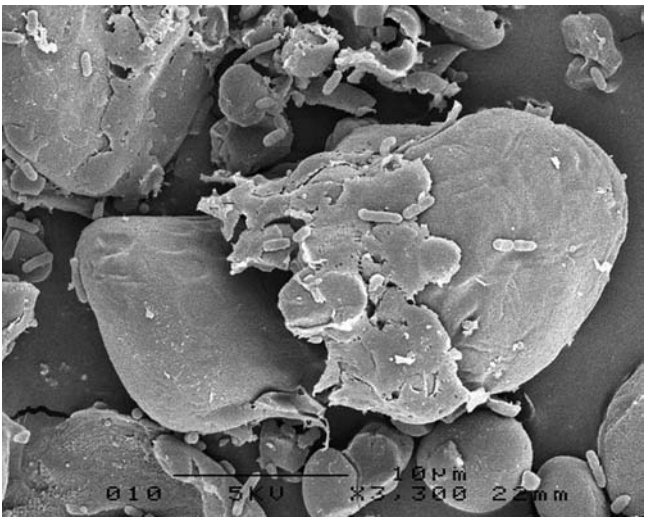

FIGURE 8.4 Hydrolysis of wheat starch granules by an amylolytic lactic acid bacterium (*Lb. plantarum* A6). (Photo by Jean-Pierre Guyot.)

Vrancken et al. 2008), this strain was able to grow under acidic conditions and to use free fructose or the fructose moiety of sucrose as an electron acceptor to produce mannitol and generate an extra gain of ATP through acetate formation instead of ethanol. It was also shown that *Lb. fermentum* Ogi E1 was able to simultaneously use sucrose, fructose, and glucose to produce α-amylase in such a mixture of substrates for starch hydrolysis (Calderon et al. 2003a), indicating that such a strain can have an active role in replenishing the pool of energetic substrates for the microbial community in *ogi*. In contrast, studies of the cell physiology of other ALAB isolated from cassava fermented foods, such as *Lb. plantarum* A6 and *Lb. manihotivorans* 18010^T, showed that α-amylase is not produced in the presence of glucose or sucrose (Guyot et al. 2000), suggesting catabolic repression of amylase synthesis by these strains, and indicating that ALAB can have different physiological responses to their growth environment.

8.5 Conclusion

Many other points could have been discussed in this chapter, such as the relation between processes, the protein composition of cereals, the nitrogen availability for cereal fermentations, or the probiotics potential of LAB isolated from cereal fermentations. However, one purpose of this overview was to point out that numerous exciting research questions are still to be addressed in the field of tropical cereal fermentations. The use of culture-independent approaches enables to investigate more samples at a same time than it was possible before, but these approaches cannot be dissociated from culture-dependent methods and the necessity to isolate and investigate the physiology of representative strains. Investigating the physiology of LAB isolates from tropical foods combined with "omics" approaches would allow a better understanding of how cell behavior is tuned by biotic and abiotic factors. In addition, research on intraspecies diversity of representative species and comparing isolates from different fermented foods and geographical areas would probably shed new light on fermented food microbial ecology by bringing more subtle knowledge on the functional role of this intraspecies diversity.

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9

Fermented Milk Products

Baltasar Mayo, Mohammed Salim Ammor, Susana Delgado, and Ángel Alegría

CONTENTS

9.1 Introduction

Milk is a highly nutritive secretion of mammals that serves to nourish offspring during the first periods of life. The high nutritive nature of milk has encouraged its use for human consumption from the very beginning of human societies. It probably started

when the first animals were domesticated, some 10,000–15,000 years ago, most likely under Sumerian, Babylonian, Egyptian, or Indian civilizations, as supported by archaeological evidence. Rock drawings discovered in the Libyan desert, believed to have been made about 9000 BC, depict cow worship and cows being milked. India's Ayurvedic writings dating back to 6000 BC expound the virtues of regular dairy product consumption and its contribution to a long, healthy life. It is apparent from writings, drawings, and friezes from Mesopotamia's Sumerians, dated around 6000 BC, that agriculture and husbandry were highly developed (Robinson and Tamime 2006). The actual role of milk and milk products in the diet of human communities varies greatly in different regions of the world (Table 9.1). Tropical countries have not been traditionally milk consumers, whereas the more Northern regions of the world, Europe (especially Scandinavia) and North America, consume far more milk and milk products. Nowadays, cow's milk is widely available all over the world and is the basis for most dairy fermented products; thought originally milk from other mammals, such as sheep, goat, camel, mare, buffalo, and yak was probably more important, and still is in certain regions of the developing and undeveloped world. Yak milk and its fermented products are popular dairy items in the Himalayan regions of India, Nepal, Bhutan, and China (Tamang 2010). Global milk production continues to grow, in particular, in the developing markets with a tendency toward production of milk types with reduced fat content (IDF 2008). Milk of various mammal species differs in chemical composition, including significant differences in parameters such as total solids, fat, protein, and mineral contents (Table 9.2). Differences in the chemical and sensorial properties of the raw material are conserved or even amplified in the transformed products, giving rise to products with characteristic and distinctive rheological and organoleptical properties. Besides majority components (proteins, fats, lactose), milk also contains minority compounds, such as vitamins, diverse oligosaccharides, free amino acids, assimilable peptides, and growth factors. The rich nutritive nature of milk, together with the fact that antimicrobial substances (such as lactenins, immunoglobulins, and the lactoperoxidase system) are scarce and rather inefficient, makes it an excellent growth medium for the development of many microbial types, including pathogens and spoilage microorganisms. Thus, milk is a highly perishable foodstuff that cannot be stored for long periods without spoiling. Through the ages, man has first realized and then fostered changes occurring naturally in milk, which preserve its nutritive components while being appealing and attractive for consumption.

9.2 Fermented Milks

Fermentation is among the oldest techniques of food preservation, and an easy way to derive new products. Indeed, milk can be fermented into a variety of hundreds of different products. Spontaneous fermentation occurs by developing in milk many types of lactic acid bacteria (LAB) species. Growth of LAB promotes a rapid lowering of the pH (acidification), which in turn inhibits spoiling microorganisms and pathogens. Bacterial growth further plays a part in the digestion of milk constituents increasing their bioavailability and in the formation of desirable flavor compounds. In modern industry, spontaneous fermentations have been replaced by the addition of well-characterized bacterial strains (starters), which bring about fermentations in

TABLE 9.1

Production (1000 Tons) and Per Capita Consumption (kg per Person) of Milk and Fermented Milk Products in Selected Countries

	Production				Consumption			
Country	**Liquid Milk**	**Fermented Milks**	**Cheese**	**Butter**	**Liquid Milk**	**Fermented Milks**	**Cheese**	**Butter**
European Union (25)	141,416	9770	8129	2065	93.5	20.9	18.4	4.2
Germany	28,403	2796	2019	443.0	94.7	30.8	22.2	6.4
France	24,385[a]	2202	1900	411.0	89.4	29.1	24.3	7.9
United Kingdom	14,066	—	374.6	120.5	142.0	—	18.4	3.2
Poland	12,088	620.0	570.0	172.0	46.1	7.8	10.7	4.2
Netherlands	11,134	658.8	732.3	129.2	123.3	45.0	17.3	3.3
Italy	12,164[a]	295.4	1143	115.9	128.4	8.3	20.5	2.2
Spain	6,075[b]	777.2	312.0	38.6	112.0	29.1	7.3	0.5
Switzerland	3,992	236.2	176.3	36.9	79.0	31.4	15.4	5.7
United States	84,188	1577	4745	693.1	83.0	8.2	16.0	2.2
Mexico	10,599	678.0	154.2	12.9	38.3[d]	5.3	2.2	0.2[b]
Canada	8,096	252.0	442.6	82.4	94.3	7.6	12.6	2.8
Brazil	25,377	—	580.0	82.0	73.2[b]	—	—	—
Argentina	9,800	576.4	486.7	47.4	43.9	12.9	11.2	0.7
India	100,870[c]	—	—	105.5[b]	—	—	—	—
China	35,250	2450	18.0	30.0	8.8[d]	1.9	—	—
Japan	8,007	2882[b]	125.4	75.1	36.7[b]	—	2.0	0.7
New Zealand	15,200	—	290.0	350	90.0[b]	—	6.1	6.3
Australia	9,373	—	351.8	120.3	117.4	6.8	11.9	3.9

Source: IDF, The world dairy situation 2008. *Bulletin of the International Dairy Federation* 432/2008, 2008.

Note: —, not available.

[a] Including ewe's and goat's milk.

[b] Including ewe's, goat's, and buffalo's milk.

[c] Including buffalo's milk (54,380 tons).

[d] Data from 2005.

TABLE 9.2

Range or Average Composition (%) of Milk Constituents from Different Mammals, and Total World Production in 2007

Source	Total Solids	Fat	Total Protein	Casein	Whey Protein	Lactose	Ash	World Production (Million Tons)
Cow	12.3–14.5	3.4–5.5	3.0–4.0	2.8	0.6	4.6–7.0	0.7	563.7
Buffalo	16.0–17.0	6.0–7.5	4.3–4.7	3.6	0.9	4.3–4.7	0.8	82.5
Goat	11.5–13.5	3.4–4.5	2.8–3.7	2.5	0.4	3.9–4.8	0.8	13.8
Sheep	16.0–20.0	6.0–8.5	5.5–6.5	4.6	0.9	4.0–4.7	1.0	8.7
Camel	13.5–16.0	5.0–5.5	3.5–4.5	2.7	0.9	5.0–6.0	0.7	NA
Mare	10.0–12.0	1.0–2.0	1.6–1.8	1.3	1.2	6.0–7.0	0.5	NA
Yak	17.8–18.0	6.5–9.0	5.5	NA	NA	5.0–6.0	0.9	NA

Sources: Adapted from de Ramesh, C.C. et al., *Manufacturing Yogurt and Fermented Milks*, Blackwell Publishing, Oxford, U.K., 2006; IDF, The world dairy situation 2008. *Bulletin of the International Dairy Federation* 432/2008, 2008.

Note: NA, data not available.

a more reliable way. Besides starters, other microorganisms (adjunct cultures) can be added to milk for the purpose of aspect, formation of flavor, and taste. Probiotics, nonpathogenic microorganisms that when ingested in adequate amounts exert a positive influence on the consumer's health, can also be incorporated into fermented milk products. This chapter reviews some historical, technological, and microbiological aspects of the principal classes of fermented milks, with a special emphasis of those that are spread all over the world.

Milk can be consumed in its fluidic form or transformed into a variety of distinct products, of which fermented milk products are among the most important dairy products from both nutritive and economical points of view (Kosikowski and Mistry 1997, de Ramesh et al. 2006, Robinson and Tamime 2006). Total production amounts to 21 million tons in 2007, as compared to 11 million tons in 1997 (IDF 2008). Initially, fermented milks were probably produced accidentally. Microbial production of lactic acid from lactose causes the coagulation of milk by forming a gel when the isoelectric point of the caseins is reached (around pH 4.6). In the gel, fat and the aqueous phase are entrapped. LAB species are among the best suited microorganisms to grow rapidly in milk with concomitant production of lactic acid. Not surprisingly, the fermentation process is mainly brought about by a series of different LAB members (FAO/WHO 2003). The term LAB embraces a diverse set of bacteria producing lactic acid as the major end product from carbohydrate utilization. They are non-sporulated, anaerobic, aerotolerant microorganisms presenting limited biosynthetic capabilities, thus requiring a rich medium to grow and a series of growth factors, such as amino acids, vitamins, purines, and pyrimidines (Carr et al. 2002). Typical LAB members belong to the genera *Lactococcus*, *Lactobacillus*, *Leuconostoc*, and *Pediococcus*. Nowadays, although phylogenetically unrelated, *Propionibacterium* and *Bifidobacterium* species are also considered among the LAB, because they are frequently found in the same ecological niches and used for similar industrial applications (Parente and Cogan 2004). Development of LAB types to high cell densities during fermentation modifies milk constituents (protein and fats)

through their proteolytic and lipolytic complex systems (Smit et al. 2005). This contributes to the final rheological and sensorial characteristics of fermented products. Moreover, lactic acid and other bacterial metabolites produced during growth (H_2O_2, diacetyl, bacteriocins) further improve stability and safety to fermented products by inhibiting spoiling and pathogenic microorganisms. Thus, fermentation conserves all the critical nutrients of milk and modifies others, enhancing its nutritive and healthy benefits.

Approximately 400 generic names are applied to traditional and industrialized milk fermented products (Robinson and Tamime 2006), although in essence the list of real different varieties is much shorter. Adapting a classification scheme proposed by Robinson and Tamime (1990), which takes into account the kind of microorganisms dominating the fermentation and the majority sensory metabolites of the fermented products, two fundamentally different fermented milk classes can be proposed.

Class I: Lactic fermentations, in which LAB species lead the fermentation changes; products within this group constitute by far the largest number worldwide. They can be subdivided in three subclasses depending on the microbial types driving the fermentation:

- Subclass Ia: mesophilic type; e.g., natural acidified milk, cultured milk, cultured cream, cultured buttermilk, *filmjölk*, and *långfil*
- Subclass Ib: thermophilic type; e.g., yogurt, Bulgarian buttermilk, *zabadi*, *dahi*
- Subclass Ic: probiotic/therapeutic type; e.g., acidophilus milk, *yakult*, bifidus milk

Class II: Fungal lactic fermentations, where LAB and yeasts species cooperate to generate the final product. These fermentations can be further separated in two subclasses:

- Subclass IIa: alcoholic milks; e.g., *kefir*, *koumiss*, acidophilus yeast milk
- Subclass IIb: moldy milks; e.g., *viili*

The diversity of fermented milks can be further increased by the use of milk from different animal species and/or breeds, and the possibility of a large variety of mixtures. More varieties can be obtained by addition of sugar, fruits, condiments, grains, and the application of preservation methods, such as concentration, drying, or freezing. Technologies influence texture and flavor, conditioning the use of the final product (as a staple meal, snack, drink, dessert, condiment, ingredient of cooking, etc.). Consumer trends of fresh dairy fermented products continue to grow in nearly all markets, with the exception of a few where consumption is already high such as in the Netherlands and Sweden (IDF 2008).

9.3 Dairy Starter Cultures

Traditionally, food and feed fermentations were carried out by commensal microorganisms present in the raw materials or present adventitiously, entering the process through contamination from tools, the environment, and manufacturers. However, the natural microbiota of milk is inefficient, uncontrollable, and unreliable, or is

destroyed altogether by the heat treatments applied in some processes. Consequently, modern technology makes use of carefully selected microorganisms that are intentionally added to pasteurized or sterilized milk for controlling the fermentation processes in a more predictable way.

9.3.1 Primary and Secondary Starters

Depending on the principal function, added microorganisms are referred to as starters or primary cultures (if they participate in the acidification), and adjunct, maturing, or secondary cultures (for flavor, aroma, and maturing activities) (Topisirovic et al. 2006). The main species involved as primary cultures in dairy include *Lactococcus lactis*, of *lactis* and *cremoris* subspecies, *Leuconostoc* spp., *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *delbrueckii*, *Lb. delbrueckii* subsp. *lactis*, and *Lactobacillus helveticus* (Parente and Cogan 2004). Not all of them are used in every dairy fermented product; instead, mesophilic (*Lactococcus* and *Leuconostoc* spp.) species are used if the temperature throughout the manufacturing is maintained around 30°C–35°C, while thermophilic species (*S. thermophilus* and *Lactobacillus* species) are used when the technology includes higher manufacturing temperatures.

Secondary cultures are also used in particular dairy commodities, especially in cheese making. These include *Propionibacterium freudenreichii*, *Brevibacterium linens*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Penicillium roqueforti*, and *Penicillium camemberti*. The major role of these microorganisms is to lead the textural, organoleptical, and biochemical changes occurring during ripening. Besides primary and secondary cultures, in some dairy products a series of other LAB species is usually present in high numbers. Among others, *Lactobacillus casei*, *Lactobacillus plantarum*, *Enterococcus faecium*, *Enterococcus durans*, *Lactobacillus salivarius*, and *Staphylococcus* spp. are included. These organisms are referred to as nonstarter lactic acid bacteria (NSLAB) microbiota (Parente and Cogan 2004). The specific role of NSLAB is not well understood; some authors argue that these bacteria participate in both acidification and maturation; because of this, well-characterized NSLAB strains imparting desirable properties and having no pathogenic and virulence determinants are sometimes used as adjunct cultures.

9.3.2 Technological Roles

Primary starters are involved in the production of lactic acid from lactose. Lactic acid production needs to occur early and in a very short time. Besides having some taste, lactic acid causes a lowering of the pH and the redox potential (Eh), which contributes to the microbial safety of dairy products by inhibiting opportunistic and pathogenic microorganisms. Starter strains may also produce antimicrobial compounds (H_2O_2, bacteriocins), which further enhance the competing ability of LAB in milk. Starters are also responsible for the production of a series of volatile compounds, such as acetaldehyde, ethanol, acetoin, and diacetyl, and key flavor components in many dairy products (Smit et al. 2005). Heterofermentative species can also produce CO_2 from both lactose and citrate, which contributes to the typical aspect of some cheeses (eyes), or the refreshing appeal of some fermented milks. Secondary starters are more varied from both taxonomical and functional point of views: NSLAB

organisms, propionibacteria, corynebacteria, staphylococci, yeast, and molds may all contribute to the final organoleptical properties of fermented products. Because activity of secondary starters is only essential during ripening, initial high numbers are not needed. In fact, microorganisms naturally present in milk or contaminants from manufacturing tools and environment are relied upon in much traditional fermentations. However, improvements in milk hygiene and a need for standardization are promoting the generalized use of secondary starters.

9.3.3 Types of Starter Cultures

Starter cultures can be classified on the basis of their function, as stated above, but also according to their optimal temperature of growth or their composition. The most common classification of starters is based on the complexity of the culture and the way it is produced. Species and strains available today as starters are derived from "natural starters" (NS) of undefined composition. These cultures are reproduced daily in the fermentation facilities by some form of back-slopping (Parente and Cogan 2004). Natural starters are still in use throughout the world for artisan and industrial manufacturing of many fermented milks and cheeses. The best NS propagated under controlled conditions resulted in "mixed-strain starters" (MSS), an undefined mixture of LAB species and strains having reduced intrinsic variability as compared to natural starters. As opposed to MSS, "defined-strain starters" (DSS) are composed of one or more strains (up to 13–15); these were first used in New Zealand for Cheddar cheese making in the 1930s (Parente and Cogan 2004). The rationale behind using DSS is for an optimized, highly reproducible performance, combined with exposure to phage attack of a limited number of strains at a time. The phage attack continues to be the principal problem of industrial dairy fermentations; consequently, phage resistance is a key character of starter strains (Sturino and Klaenhammer 2004). Identification of bacteriophage-resistant LAB, selection of phage-insensitive mutants, and transference of natural resistance mechanisms among starter strains are current strategies aimed to minimize this problem. Irrespective of their type, commercial starters are found in a variety of presentations, including liquid, dried, frozen, and freeze-dried cultures. Each culture presentation has advantages and disadvantages; liquid cultures have a very short storage life, and the air-drying process may affect strain composition by selective killing; in contrast, freezing and freeze drying increases viability and activity, but freezing methods are more expensive, and frozen cultures need a continuous cold chain, which is difficult to implement in developing and underdeveloped countries.

9.3.4 Specific Starter Cultures

Specific starters are cultures that are developed specifically for a single product to conserve key organisms and maintain their relative ratio. Specific starters are thought to preserve the sensorial characteristics of traditional products. Furthermore, specific starters would allow reproducing the organoleptical properties of fermented products made of raw milk from pasteurized (or sterilized) milk. In fact, typing of traditional dairy fermentations is usually aimed at the identification and selection of candidate strains for designing specific starters (Beukes et al. 2001, Simova et al. 2002, Patrignani et al. 2006). Moreover, among LAB in traditional fermentation, robust

strains could be found for complementing industrial starters currently in use. In particular, strains having distinctive and new biochemical, aromatic, and bacteriophage profiles are actively sought after (Siezen et al. 2008). Conversely, the microbial typing of fermented milks may help in the choice for suitable commercial starters.

9.3.5 Probiotic Cultures

Besides conventional primary and secondary starters, which are mainly used for technological purposes, there is an increasing market and industrial demand for products containing microorganisms providing health benefits (probiotics). Probiotics have been defined "as nonpathogenic microorganisms that when ingested in adequate amounts exert a positive influence on the host's health" (FAO/WHO 2002). Probiotics are thought to contribute to health maintenance by enhancing metabolic (production of organic acids, vitamins), protective (inhibition or exclusion of harmful bacteria, antitoxin and anticarcinogenic activities), and trophic (immunomodulation) activities (Guarner and Malagelada 2003). A majority of the probiotics currently in use belong to a few species of the *Lactobacillus* and *Bifidobacterium* genera, but many strains of other bacterial (streptococci, propionibacteria, *Bacillus* spp.) and fungal species (*Saccharomyces boulardii*) are also used (Ouwehand et al. 2002). The definition of probiotics does not exclude microorganisms from traditional fermentations from having beneficial health effects. In fact, the probiotic idea was proposed by Élie Metchnikoff after observing healthy and prolonged life in people consuming traditional Bulgarian *yogurt* (Metchnikoff 1907).

9.4 Representative Fermented Milk Types

Variations in the different technologies applied for the manufacturing and ripening of different fermented milks has given rise to a panoply of different fermented products. In addition, these technologies have put a strong selection pressure on the microorganisms able to develop in each particular fermentation. The next section is devoted to reviewing a series of fermented milk types that may be considered representative of the principal classes in terms of economical aspects and worldwide production.

9.4.1 Natural Fermented Milks

This class of fermented milks is undoubtedly the simplest and probably the oldest. Not surprisingly, the traditional manufacture of nonheated natural fermented milk (NFM) from raw milk is spread worldwide. The origin of this practice is difficult to establish, but it can be assumed that was soon after the first human populations settled themselves some 15,000 years ago around the Middle East when the way of life changed from food gathering to agriculture (Robinson and Tamime 2006). From those ancient times up to now, the production of NFM spread across the world, each region using the available type of milk. Evidence of such products can still be found in large areas of Africa, Middle East, Asia, and even in Europe. Products such as *ergo* from Ethiopia; *rayeb*, *lben*, *laban*, *kad*, *zabady*, and *zeer* from Morocco and Northern African and Middle East countries; *roub* from Sudan; *amasi* (*hodzeko*, *mukaka wakakora*) from Zimbabwe; *filmjölk* and *långfil* from Sweden; *chhurpi*, *mohi*, *shoyu*, *philu*,

and *somar* from the Himalayan regions; and many others. A common feature of NFM is the development of mesophilic LAB species, which are responsible for lowering of the pH and the production of the most typical sensorial compounds. Two basically different NFM subclasses can be distinguished: inoculated and non-inoculated NFM. The latter type is made by leaving plain milk at room temperature until enough acidity is formed and the coagulum appears. Depending on the preferences, it can be stored for days of weeks, during which stronger flavors are developed. Inoculated NFM is manufactured by adding a portion of previous NFM to the new batch. In either case, *Lc. lactis* of both *lactis* and *cremoris* subspecies are found among the dominant microbiota. It is also common to find species of mesophilic lactobacilli (*Lb. plantarum* and *Lb. casei/Lactobacillus paracasei*), as well as *Leuconostoc*, *Enterococcus*, and *Pediococcus* species. In warm climates, other lactobacilli species, such as *Lb. helveticus*, *Lactobacillus fermentum*, and/or *Lactobacillus acidophilus*, can also develop (Gonfa et al. 2001, Mathara et al. 2004, Patrignani et al. 2006). As raw milk is used in most traditional NFM, moderate to high (up to 10^8 cfu/g) levels of yeasts species are always present (Gadaga et al. 2000, Gonfa et al. 2001, Benkerroum and Tamime 2004). Yeasts are able to multiply in milk, and may result in spoilage or, conversely, in enhancement of flavor. The major species include *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *Candida lusitaniae*, and others (Gadaga et al. 2000, Benkerroum and Tamime 2004). Micrococci, coliforms, and pathogens (*Staphylococcus aureus*, *Bacillus cereus*) are found occasionally (Gonfa et al. 2001), stressing the need for improving the microbial safety of these natural products.

NFM manufactured from pasteurized and/or sterilized milk (at an either artisanal or industrial scale) are surely safer. Industrial NFM are inoculated with acidifying and aromatic starter cultures, while artisanal products are usually inoculated by backslopping techniques. In the latter case, a strong pressure for strains growing rapidly in milk and supporting high lactic acid levels is applied in every transfer. Analysis in our laboratory of an inoculated NFM of unknown origin made from ultrahigh temperature (UHT) sterilized milk showed a bacterial consortium of three species. One strain each of *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* cohabited with a single *Lb. plantarum* strain. All three strains were found in three batches sampled three times at 2-month intervals, indicating a high degree of stability of the consortium (B. Mayo and A. Alegría, unpublished). NFM is also the basis for more processed commodities, such as butter and its by-product buttermilk, cottage cheese, cheeses, and whey, as the manufacturing process of all these products starts by an initial acidification of milk (Figure 9.1). Some of these products are partially dried (*leben zeer*, *than*), preserved in oil (*labneh anbaris*, *shanklish*), mixed with spices (*shanklish*, *mish*), mixed with wheat products (*kishk*), etc. Butter is made by churning fat-enriched milk or cream, in a process that separates the fat fraction of the milk from the whey. Milk and cream do not normally coagulate for making butter; however, they are frequently acidified either by natural means or by inoculation with mesophilic starters, among which the presence of diacetyl-producing bacteria (*Lc. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc cremoris*) is considered essential. Cheese is the generic name of a group of fermented milk-based food products produced worldwide in a great variety and diversity of forms, textures, and flavors. Acid-induced milk gels are very stable if left undisturbed, but if accidentally or intentionally broken, curd and whey separates. Removing of whey gives rise to a product that can be consumed fresh or stored for long periods if properly salted or dehydrated. In fact, heavily salted cheeses

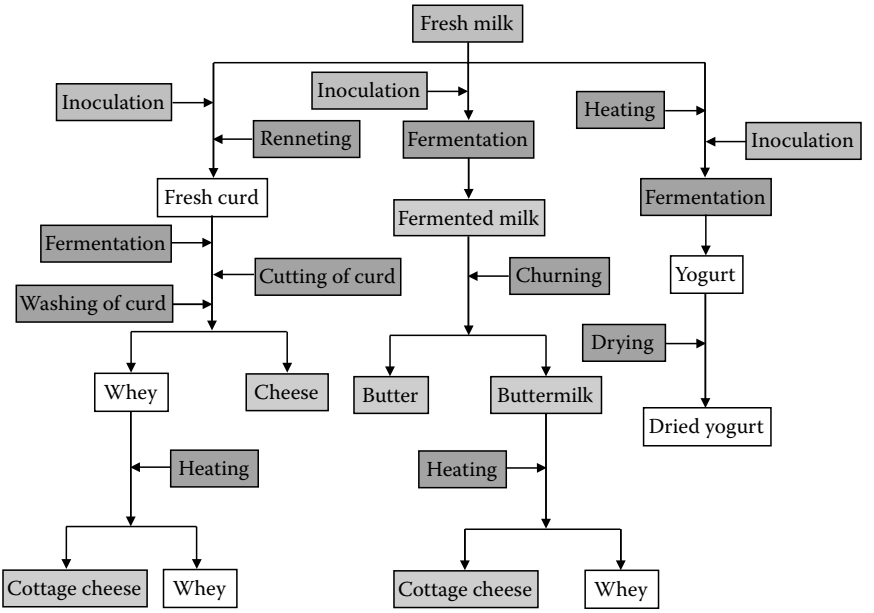

FIGURE 9.1 Simplified flow scheme for manufacturing some fermented milk dairy products. Gray tones in boxes identify similar or equivalent materials, technological processes, and intermediate and final products.

are still widespread throughout the Middle East and the Balkans (e.g., *feta*, *domiati*, *lighvan*) and representative remnants of air- and sun-dried cheeses still survive in areas of North Africa and the Middle East (Kosikowski and Mistry 1997). Today, acid-coagulated cheeses (cottage cheese, cream cheese, *quarg*, *fromage frais*, *queso blanco*) may still represent 25% of the total cheese production, and in some countries are the principal varieties (Kosikowski and Mistry 1997). Cheese will not be further covered in this chapter, and the reader is addressed to several excellent books covering key aspects of ancient and modern issues in its technology and science (Law 1999, Fox et al. 2000).

Examples of NFM from the Himalayan regions prepared by the mountain people from cow's and yak's milk are *dahi*, *mohi*, *gheu*, soft *chhurpi*, *chhu*, *somar*, *maa*, *philu*, and *shyow* (Tamang 2010). Some of the milk products such as *dahi* (curd), *mohi* (butter milk), *gheu* (butter) are familiar in all regions of the Himalayas, while others (*chhurpi*, *chhu*, *philu*, etc.) are confined mostly to the Tibetans. *Somar* is exclusively prepared and consumed by the Sherpas of Nepal and Sikkim living in high altitudes. The practice of using a standard starter culture is uncommon; however, use of the back-slopping technique into freshly boiled milk is frequent (Dewan and Tamang 2006). LAB are the dominant microorganisms in the Himalayan fermented milk products with loads above 10^8 cfu/g (Dewan and Tamang 2006, 2007). Lactic rods are represented by 86%, whereas cocci represent the remaining 14%. It is interesting to note that in *philu*, a fermented product made from yak's milk, only rods have been detected among the majority microorganisms, which suggests that yak's milk drives a selection for particular microbial types and, consequently, products made from yak's

milk may harbor a distinctive microbiota (Dewan and Tamang 2007). The probiotic properties of indigenous microorganisms isolated from the cheese-like product *chhurpi* have been recently reported (Tamang et al. 2000).

9.4.2 *Filmjölk* and *Långfil*

Filmjölk is Swedish mesophilic fermented milk made by fermenting cow's milk with diverse strains belonging to *Lc. lactis* and *Leuconostoc mesenteroides* species (Kosikowski and Mistry 1997). The bacteria metabolize lactose into lactic acid during growth, and produce a limited amount of diacetyl, which gives *filmjölk* its characteristic aroma. *Filmjölk* is similar to other cultured dairy products but compared to *yogurt* tastes less sour. Homemade *filmjölk* is produced by adding a small amount of an active batch of *filmjölk* to pasteurized milk, and then leaving the stuff to ferment for 1 or 2 days at room temperature or in a cool cellar. Nowadays, *filmjölk* is industrially produced by most accredited Swedish dairy companies. Variants of *filmjölk* having different fat content, flavors, sugars, and herbs can readily be found on the market. *Långfil* is a kind of *filmjölk* having an almost elastic texture due to the development of *Lc. lactis* subsp. *lactis* and subsp. *cremoris* variants that produce extracellular polysaccharides (Toba et al. 1991), as in *viili* (see below). *Långfil* only comes unflavored and is eaten sometimes with ground ginger.

9.4.3 Buttermilk and Cultured Buttermilk

The original buttermilk came directly from the butter churn. The product is acidified with microorganisms present naturally in the cream. The buttermilk contains many small butter flakes carried over from the butter-making process, giving the product a characteristic mouth feel and flavor. Today, buttermilk has been mostly replaced by cultured buttermilk. This new commodity is manufactured from skim milk fermented by mesophilic lactic (*Lc. lactis* subsp. *lactis* and subsp. *cremoris*) and aroma cultures (*Leuconostoc* spp. and *Lc. lactis* subps. *lactis* biovar. *diacetylactis*). The milk is heated at 85°C for 30 min to increase the viscosity of the product, preventing, at the same time, wheying-off, and destroying undesirable and pathogenic bacteria. After fermentation, the resulting curd is broken and stirred slowly, cooled, and slightly salted (Kosikowski and Mistry 1997). Fruit condiments, essences, and butter flakes can be added to plain cultured buttermilk. Cultured buttermilk is very popular in the Netherlands and in the United States.

9.4.4 Sour Cream

Originally, this product was the result of natural acidification of milk cream. Today, different formulations with regard to the fat percentage are on the markets. The manufacture of *sour cream* is started by an initial standardization and homogenization of the cream at high temperature, which serves as a pasteurization process. Finally, cream is inoculated with 2%–5% lactococci (*Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*) and aroma-producing bacteria (*Lc. lactis* subsp. *lactis* biovar. *diacetylactis* and/or *Leuconostoc* spp.). The incubation proceeds for 16–18 at 22°C until the pH approaches 4.6. Sour cream with 18% fat and 9% milk solids is consumed in North America, while in France and other European countries a product having

35%–50% fat (called *créme fraiche*) is more popular. The latest research on sour cream involves the manipulation of *Lc. lactis* strains by culturing (García-Quintáns et al. 2009) and genetic engineering techniques (Hugenholtz et al. 2000) to overproduce diacetyl, the key aroma compound of this product.

9.4.5 Yogurt

Yogurt and yogurt-related products represent fermented milks that are acidified at high temperature. In warm climates, these temperatures can probably be attained by direct exposure to sun light. However, fermentation would usually take place in the neighborhood of the cooking area. Traditional *yogurt* production techniques can also include boiling of the milk before acidification. Whatever the processes, this assures the selection of thermophilic and acidophilic microorganisms.

9.4.5.1 History and Technology

Yogurt is a widely consumed highly nutritious fermented milk, defined by the Codex Alimentarius (FAO/WHO 2003) as a coagulated milk product resulting from the fermentation of milk by *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (formerly *Lactobacillus bulgaricus*). Until the 1950s, production and consumption of *yogurt* were confined to communities in Eastern Europe, the Balkans, Middle East, and India (Kosikowski and Mistry 1997). A general perception of the beneficial health effects associated to its consumption increased production in the developed world since the 1960s. Today, yogurt is the major commercial fermented milk around the world. Among the factors contributing to the great success of this fermented milk, the image of a natural product, attractive organoleptic characteristics (fresh, acidulated taste, and pleasant flavor), nutritional value, prophylactic and therapeutic properties, and moderate cost (due to the high productivity of the production lines) can all be mentioned (Tamime and Robinson 2007). Yogurt can be produced from the milk of cow, buffalo, goat, sheep, yak, and other mammals, although cow's milk is predominant in industrial production. Varieties of yogurt available include plain (set), fruit flavored, whipped, drinking type (stirred), smoked, dried, strained, and frozen (Tamime and Robinson 2007). In fact, availability of yogurt choices in the market is thought to have contributed to the rise in consumption. In some countries, the term yogurt is restrained to the fermented milk made by using exclusively these two bacterial types, while in others incorporation of probiotic cultures is allowed. In the latter case, *Lb. acidophilus*, *Bifidobacterium* spp., *Lb. casei*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, and *Lactobacillus johnsonii* are among the commonest adjunct cultures. In restrictive countries, the term "yogurt-like product" has been proposed as an alternative for cultured yogurt (i.e., when *Lb. delbrueckii* is substituted by other *Lactobacillus* species for fermentation) or for yogurt containing probiotic bacteria (Guarner et al. 2005).

Traditional yogurt manufacture involved spontaneous acidification of milk (with or without boiling) at moderately high temperature (between 40°C and 50°C). Either the heating or the high incubation temperature or both select thermophilic microorganisms, among which *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* are dominant. Yogurt cultures are homofermentative and ferment lactose in a similar manner, although *Lb. delbrueckii* can produce up to 4% lactic acid, while *S. thermophilus* produces only between 0.6% and 1.1%. The role of these two LAB species in yogurt

manufacture includes milk acidification and synthesis of aromatic compounds, but they further influence flavor, texture, appearanc,e and overall quality attributes of the finished product. The recent availability of the complete genome sequences of these two species (Bolotin et al. 2004, van de Guchte et al. 2006) has shown a perfect adaptation of these organisms to the milk environment. In this way, simplification of superfluous metabolic routes and acquisition of key characters allowed *S. thermophilus* and *Lb. delbrueckii* to be highly competitive in milk. Furthermore, yogurt-associated bacteria display a kind of symbiotic relationship during growth in milk, a property known as protocooperation, which has been supported by genetic findings. *Lb. delbrueckii* stimulates *S. thermophilus* by releasing essential or stimulatory amino acids through its proteolytic system. Conversely, *S. thermophilus* produces formic acid and CO_2 from urea, compounds that encourage the growth of *Lb. delbrueckii.*

Industrial yogurt manufacture involves a preliminary milk fortification with nonfat powdered milk, milk protein concentrate, or condensed skim milk to increase total solids (up to 12%–13%) and get a thicker consistency. After mixing, ingredients are homogenized at 15–20 MPa and pasteurized at high temperature (85°C–88°C for 30 min). This severe heating step has two main functions. First, heat-resistant bacteria and their spores are killed, making the mixture essentially sterile. Second, the major whey proteins (α-lactalbumin and β-lactoglobulin) are completely denatured. In these conditions, their amino acid residues are exposed having the capability of binding water, thus minimizing whey syneresis and stabilizing firmness of the gel (Tamime and Robinson 2007). Pasteurized milk is promptly cooled to the desired incubation temperature, usually between 40°C and 45°C, and inoculated with *S. thermophilus* and *Lb. delbrueckii* strains. Cultures are added to the mix to give an initial cell concentration of about 10^7 cfu/mL. Inoculated mixes are incubated at 40°C–45°C for 4–6 h or until a titratable acidity (as lactic acid) of 0.8%–0.9% is reached (pH between 4.4 and 4.6). For set varieties, the inoculated mix is pumped to containers, which are sealed and fermented; after cooling the product is ready for marketing. In contrast, manufacture of stirred yogurt requires breaking of the curd by gentle stirring before filling the containers. In either case, post-acidification is avoided by cooling the product below 10°C. In fact, yogurt is maintained at 2°C–4°C throughout the distribution chain, which not only prevents further activity of the starters but also retards spoilage by yeasts and molds.

9.4.5.2 Nutritive Value and Therapeutic Benefits

The desirable typical flavor of *yogurt* comes from a mixture of lactic acid, carbonyl compounds (acetaldehyde, acetone, diacetyl, acetoin), nonvolatile acids (pyruvic, oxalic, succinic), volatile acids (formic, acetic, propionic), and a large series of degradation products from the catabolism of proteins, fats, and lactose. In general, *yogurt* has less lactose and more lactic acid, galactose, peptides, free amino acids, and free fatty acid than milk (Tamime and Robinson 2007). Although milk and yogurt have similar mineral composition, availability of some minerals, such as calcium, may be enhanced in yogurt. In addition to its high nutritional value, yogurt is endowed with therapeutic potential. Beneficial health effects are presumed to depend on the ability of *S. thermophilus* and *Lb. delbrueckii* to reach the gastrointestinal tract alive, where they persist and/or multiply (Guarner et al. 2005). Accordingly, the term yogurt is only applied in some countries if viable cell counts are found in the product, while

a distinctive term refers to products containing nonviable organisms ("pasteurized yogurt") (del Campo et al. 2005). However, there have been conflicting studies concerning the culture recovery of *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* from fecal samples after *yogurt* ingest; while some authors reported recovery of both bacterial types (Mater et al. 2005, Elli et al. 2006), some others have failed to do so (del Campo et al. 2005). Among the health benefits, improvement of lactose intolerance symptoms among lactose maldigestors has been clearly demonstrated (Rizkalla et al. 2000, Labayen et al. 2001, Pelletier et al. 2001). This physiological effect is thought to be due to a lowering of the lactose content in yogurt by 20%–30% as compared to milk, but also to the contribution of bacterial β-galactosidases to increase enzymatic activity in human intestines. Yogurt has also been used in the management of acute diarrhea disorders, as recommended by the FAO/WHO (2003). Yogurt feeding in children with acute watery diarrhea decreased stool frequency and shortened the duration of diarrheal episodes (Boudraa et al. 2001). A further benefit may account by its recognized immunomodulation capacity (Meydani and Ha 2000). Yogurt consumption seems to enhance the immune response, particularly in immunocompromised populations such as the elderly. Yogurt consumption has also been associated with decreased risk of progression and promotion of colon cancer by modulating cell proliferation and increasing cellular apoptosis (Rachid et al. 2002).

9.4.5.3 Yogurt-Related Traditional Products

Fermented milks manufactured with thermophilic starters (subclass I) include not only yogurt but also other fermented milk products related to yogurt, such as Bulgarian buttermilk, *zabady*, and *dahi* (Kosikowski and Mistry 1997, Robinson and Tamime 2006). Bulgarian buttermilk is a type of cultured buttermilk fermented with *Lb. delbrueckii* subsp. *bulgaricus*. As *Lb. delbrueckii* can produce up to 4% lactic acid, growth of this bacterium produces more tartness than the mesophilic starters. Yogurt made domestically in Egypt is called *zabady*. The traditional preparation recipe starts by boiling raw milk for a few minutes, followed by cooling to about 45°C. Then a portion of *zabady* from a previous batch is added as a starter (back-slopping). The inoculated milk is transferred to porcelain pots or plastic cups, incubated at about 42°C until coagulation, and then cooled to avoid further acidity development (El-Baradei et al. 2008). *Dahi* or Indian yogurt is a lesser-known ethnic, fermented milk product consumed in India, Nepal, Pakistan, Bangladesh, Sri Lanka, and Bhutan. Traditional methods of *dahi* preparation involve boiling of fresh yak's or cow's milk in a vessel (Tamang 2010). Then, milk is cooled to room temperature and a small quantity of previously prepared *dahi* is added. The mixtures are left for 1–2 days in summer or for 2–4 days in winter at room temperature for natural fermentation (Dewan and Tamang 2007).

9.4.6 Acidophilus Milk

Acidophilus milk (AM) was one of the first probiotic milks derived from Metchnikoff's observations, which coincidentally occurred around the first isolation of *Lb. acidophilus* (formerly *Bacillus acidophilus*) by the German microbiologist E. Moro in 1900 from the feces of a breast-feeding infant. The bacterium was found lately to be a normal inhabitant in the human intestinal tract, mouth, and vagina. *Lb. acidophilus*

strains are considered to fulfill most of the basic criteria of probiotics: survival in the gastrointestinal transit, bile and acid tolerance, and production of antimicrobials. Strains of this species were reclassified into six species based on DNA–DNA hybridization; *Lb. acidophilus*, *Lactobacillus amylovorus*, *Lactobacillus crispatus*, *Lactobacillus gallinarum*, *Lb. gasseri*, and *Lb. johnsonii* (Berger et al. 2007). In the past, AM was marketed by fermenting sterilized milk at 37°C for 24 h with *Lb. acidophilus* strains from the human gastrointestinal tract (Kosikowski and Mistry 1997). Under these conditions, titratable acidity reached 1%–2% in AM, and its strong acid flavor decreases palatability, making it a poor table beverage. Its popularity declined rapidly when sweetened yogurt began to dominate the market. Today, a nonfermented (sweet) acidophilus milk (SAM) is preferred. Manufacturing of sweet SAM involves the incorporation of a concentrate culture of *Lb. acidophilus* into sterilized low-fat milk (de Ramesh et al. 2006). The bacterium can also be added to plain *yogurt* after fermentation by *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*. Furthermore, additional probiotic microorganisms, e.g., bifidobacteria, can also be included. The exact mechanisms by which dietary cultures of *Lb. acidophilus* affect the heath status of consumers are not yet clear. Positive health effects can be mediated through pathogen colonization competition, production of antimicrobial substances, and/or stimulation of immune functions (reviewed in Sanders and Klaenhammer 2001). Probiotic *Lb. acidophilus* strains have also been shown to inhibit aberrant crypt formation in mutagenized rats; this lower activity is indicative of a decrease in the risk of colon cancer. Additionally, adequate daily intakes led to a significant decrease in the levels of toxic amines in the blood of dialyzed patients with small bowel bacterial overgrowth. Recently, the probiotic strain *Lb. acidophilus* La-5 has been reported to produce conjugated linoleic acid (CLA) (Macouzet et al. 2009), a potent anticarcinogenic agent.

9.4.7 *Yakult*

Yakult is a Japanese probiotic milk product made by fermenting a mixture of skimmed milk with a special strain (Shirota) of the bacterium *Lb. casei*. The Yakult Company (Yakult Honsha Co., Ltd.) was created in 1935 by Dr. Minoru Shirota from the Medical School of Kyoto University. Official claims state that the name is derived from *jahurto*, an older form of *jogurto*, the Esperanto word for yogurt. After its introduction in Japan and Taiwan, *yakult* was first sold in the Western world in Brazil in 1966, due to the large number of Japanese immigrants in this country, before it was marketed elsewhere. The claimed benefits of *yakult* are supported by an array of scientific studies (reviewed recently by Kiwaki and Nomoto 2009), ranging from the maintenance of gut flora, modulation of the immune system, regulation of bowel habits, and alleviation of constipation. It also seems to be effective in curing of various gastrointestinal infections. More recently, *yakult* has introduced a line of milk- and soy-based fermented beverages for the Japanese market containing a probiotic *Bifidobacterium breve* strain (YIT 4065) (Tsuji and Namoto 2009).

9.4.8 Milk Products Fermented with LAB and Yeasts

Fermented milk products that are manufactured using starter cultures containing yeasts include acidophilus yeast milk, *kefir*, *koumiss*, and *viili* (Kosikowski and Mistry 1997, de Ramesh et al. 2006). Of these, *kefir* is widely produced in many areas

in Russia and the Balkans, but its consumption has been extended through the entire world. *Viili* is a single fermented milk-type product that includes the filamentous yeast *Geotrichum candidum* as a starter in its manufacture. Particular attention is being paid at present to the probiotic and health-promoting properties of all these products.

9.4.8.1 Kefir

9.4.8.1.1 History

Although no clear definition of what *kefir* is exists, it is a viscous, acidic, and mildly alcoholic milk beverage produced by fermentation of milk with a kefir grain as the starter culture (FAO/WHO 2003). Kefir grains are cauliflower-like florets of white to yellowish-white color and having a slime but firm texture (Figure 9.2). The kefir grain is an inert polysaccharide matrix in which a relatively stable and specific microbial community composed of different lactic acid bacteria, acetic acid bacteria, and yeast species coexists in a complex symbiotic relationship. After the fermentation, the grain is recovered and can be reused to inoculate a new fermentation, in a way similar to the back-slopping practice. Thought inert, the grain grows and increases in size, which allows the original grain to be easily divided in portions after several transfers in milk. This product is manufactured under a variety of names in different manufacturing areas of the Balkan-Caucasian region, including *kephir*, *kiaphur*, *kefer*, *knapon*, *kepi*, and *kippi*. Kefir grains are supposed to have developed spontaneously after storing milk in animal-based containers, such as pelt-made recipients, and/or intestinal and bladder vessels. Grain formation may have happened several times in the history and at different geographical locations.

At present, there exists a great variety of kefir grains showing different microbial communities and having grain-specific distinctive sensorial properties. *Kefir* has a unique, slightly acidic taste, along with some effervescence caused by the carbon dioxide produced by the yeasts. Further flavor is imparted by a low concentration of ethanol (usually below 2%) and a variety of aromatic substances, such as acetaldehyde, acetoin, and diacetyl (Beshkova et al. 2003, Farnworth 2005). Acetaldehyde

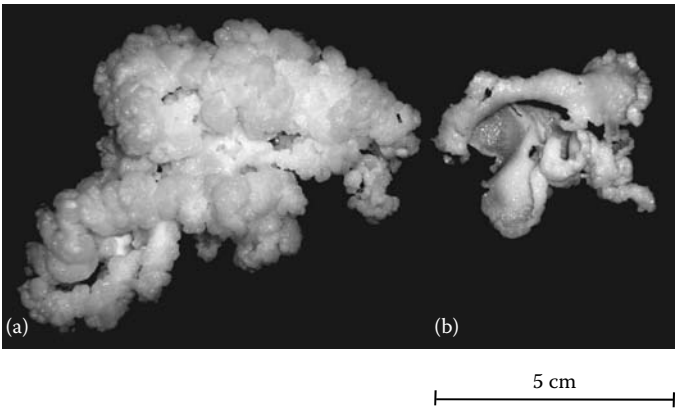

FIGURE 9.2 Photo of two distinct kefir grains: (a) yellowish, cauliflower-like grain; (b) whitish, foil-like grain.

and acetoin increase in concentration during fermentation and storage (Güzel-Seydim et al. 2000). In agreement with this, most LAB species from *kefir* are able to produce acetoin, acetaldehyde, or both. The type of milk (bovine, ovine, or caprine) used for making *kefir* further influences the sensorial characteristics of the beverage (Wszolek et al. 2001). Recently, kefir grains have been conditioned to grow on soy milk (Liu and Lin 2000), although the sensorial properties of this product were not reported.

9.4.8.1.2 Microbiology and Technology

Grains pass their genuine properties in successive transfers in milk or after appropriate drying. The microbiota of kefir grains is remarkably stable, retaining its activity for years if preserved under appropriate conditions. Bacteria and yeast species reported in the literature for *kefir* and kefir grains have been summarized in an excellent recent review (Farnworth 2005). The different groups of microbes present in the grains are active at different stages of the fermentation. *Lc. lactis* subsp. *lactis*, including the biovar. *diacetylactis*, and *Lc. lactis* subsp. *cremoris* strains are loosely associated with the grains and are mostly responsible for acidification during the first hours of fermentation. Yeasts, acetic acid bacteria, and flavor-producing microorganisms of *Lactobacillus* and *Leuconostoc* genera grow much slower, resulting in a slow production of aroma compounds, alcohol, and CO_2. *Lactobacillus kefiri*, *Lactobacillus kefiranofaciens*, *Lactobacillus kefirgranum*, *Lactobacillus parakefir*, and others species such as *Lb. helveticus*, *Lb. delbrueckii*, *Lb. casei*, *Lb. brevis*, *Lb. paracasei*, and *Lb. plantarum* are frequently isolated from the grains (Rea et al. 1996, Kuo and Lin 1999, Garrote et al. 2001). The diversity of LAB species in kefir grains of different geographical origin includes occasional detection of strains of *Lb. acidophilus*, *Leuc. mesenteroides*, and *S. thermophilus*. Acetic acid bacteria have received less attention, although they are supposed to be essential in both the microbial consortium and the product (Rea et al. 1996). In spite of this, levels of acetic acid bacteria (around 10^5 cfu/g) and acetic acid (from 4 to 14 mmol/kg) have been reported to present high intersample differences.

Kefir-specific yeasts play a key role in the sensorial properties of *kefir*. In accordance with this, the standard for *kefir* states 10^4 cfu/g as the minimum number of yeasts in the beverage (FAO/WHO 2003). They are responsible for flavor and aroma formation, but also for the production of alcohol and the fuzzy taste of CO_2. Among the yeasts, *Kluyveromyces marxianus* var. *lactis*, *Torulaspora delbrueckii*, *S. cerevisiae*, *Candida kefir*, *Saccharomyces unisporus*, *Pichia fermentans*, and *Yarrowia lipolytica* have all been detected (Simova et al. 2002, Wang et al. 2008). Of note, the lactose-fermenting species in the kefir grain (such as *K. marxianus*) always occur in association with lactose-negative yeasts (such as *S. cerevisiae* and *Candida* spp.). The presence of a minimum number of lactose-fermenting yeasts (around 10^5 cfu/mL) assures production of alcohol and formation of the typical yeasty flavor. Microbial diversity of kefir grains and *kefir* was traditionally assessed by culturing methods, and the identification of species was based on phenotypic features that proved to be unreliable. At present, there is an active research on the microbial diversity of *kefir* by using modern techniques, including polyphasic approaches combining phenotypic and genotypic methods (Mainville et al. 2006), as well as the use of a panoply of culture-independent microbial techniques, such as denaturing gradient gel electrophoresis (DGGE) (Wang et al. 2006, Chen et al. 2008), Fourier-transformed infrared spectroscopy (FT-IRS) (Bosch et al. 2006), and construction and analysis of

libraries of conserved genes (16S rDNA) (Ninane et al. 2007). By means of these new methods, most cultured species have been identified. In addition, other microorganisms have been detected, including the occasional presence of *Escherichia coli* and *Pseudomonas* spp.

Homemade batches of *kefir* are produced by incubating milk with kefir grains at 20°C–25°C for 18–24 h. At the end of the fermentation, grains are rinsed several times with water and transferred to initiate a new batch. It has been found that the product has a different microbiological profile to that of the grains, and therefore cannot be used to inoculate a new batch of milk (Simova et al. 2002). In spite of this, industrial-scale production of *kefir* involves an initial production of a starter with the kefir grains, which are used to inoculate (1%–2%) a second batch of *kefir* that constitutes the actual commercial product (Simova et al. 2002, Witthuhn et al. 2005). A rationale approach to industrial production would involve the development of defined starter cultures, which would lead to obtaining a uniform and stable product. Attempts have been made to produce *kefir* from mixtures of pure cultures, including LAB and yeasts species isolated from kefir grains (Beshkova et al. 2002). Indeed, multispecies of commercial starter cultures are available from several starter companies and research institutes. The increasing popularity of *kefir*-containing products has prompted the use of *kefir* starters for cheese making. These have proven to be suitable for the production of brine-ripened cheeses with respect to quality and sensory characteristics (Goncu and Alpkent 2005, Kourkoutas et al. 2006).

9.4.8.1.3 Nutritive Value and Therapeutic Benefits

Kefir has had a long history of health benefits in Eastern European and Middle Eastern countries, where it is associated with general well-being. Lactic and acetic acid bacteria produce a wide range of antimicrobial compounds, including organic acids (lactic and acetic), carbon dioxide, hydrogen peroxide, ethanol, diacetyl, and peptides (bacteriocins), exerting inhibitory action against food-borne pathogens and spoilage microorganisms (Leroy and de Vuyst 2004). *Kefir* can reduce symptoms of lactose intolerance by providing an extra source of β-galactosidase. Hertzler and Clancy (2003) have shown that commercial *kefir* produced with a defined mix of bacteria and yeasts was equally as effective as yogurt in reducing the amount of hydrogen in the breath of lactose maldigestors. Although the mechanism of action is not yet clear, *kefir* has been shown to inhibit and suppress several cancer types and metastasis. These effects are thought to be mediated through the exopolysaccharides. Shiomi et al. (1982) were the first to report the antitumor effects of a water-soluble, high molecular weight (about 1,000,000 Da) polysaccharide from kefir grains. Using mice as the animal model, the polysaccharide was able to inhibit Ehrlich carcinoma and sarcoma 180 as compared to controls receiving no treatment. In a similar way, a water-insoluble fraction containing *kefir* polysaccharides proved to inhibit metastasis of highly colonized B16 melanoma (Furukawa et al. 2000). Other authors suggested that the antitumor activity of *kefir* might be due to its antioxidative properties (Güven et al. 2003). They proved that *kefir* was more effective than vitamin E in protecting mice against oxidative damage. Stimulation of the immune system has also been proposed as a mechanism whereby *kefir* bacteria exert a direct beneficial effect (Cross 2002). Beneficial action may also be mediated through different bioactive peptides formed during fermentation (or even during the digestion) (Matar et al. 2003).

9.4.8.2 Koumiss

Koumiss is a natural fermented dairy product from the Caucasian area. It is also produced under other names such as *kumis*, *kumys*, or *kymys*. Furthermore, similar products are produced in Central Asia (Mongolia and China), where they are called *chigee* and *airag* (Kosikowski and Mistry 1997). In fact, it is believed that Mongol tribes developed the product around the thirteenth century. *Koumiss* is defined by the use of mare's milk in its manufacture; mare's milk contains less casein and fatty matter than cow's milk (Table 9.2). *Koumiss* presents a dispersed coagulum giving the product a smoother taste as compared to *kefir*. A higher alcohol content (up to 3%) than in *kefir* with a concomitant production of higher carbon dioxide levels is also characteristic of *koumiss*. The traditional manufacture of *koumiss* involves storing of mare's milk in animal skins bags, where a natural or induced (inoculated) acidification process takes place. Dry *koumiss* is usually maintained from season to season, and the distinctive starters are transferred from one generation to another within families for back-slopping. LAB and yeast species are both responsible for acidification and the final sensorial properties of the product. *Lb. casei*, *Lb. helveticus*, and *Lb. plantarum* have been identified as the dominant lactobacilli species. With a lower frequency, other species such as *Lb. coryniformis*, *Lb. paracasei*, *Lb. kefiranofaciens*, *Lb. curvatus*, and *Lb. fermentum* have also been detected (Ying et al. 2004, Watanabe et al. 2008, Wu et al. 2009). In decreasing percentages, *S. unisporus*, *K. marxianus*, *Pichia membranaefaciens*, and *S. cerevisiae* have all been identified from homemade *koumiss* (Ni et al. 2007, Watanabe et al. 2008). In modern manufacture, cow's milk tends to replace mare's milk as the starting material, for which cow's milk for *koumiss* production has to be adapted for mimicking mare's milk. Traditionally, this was done by diluting cow's milk with water and the addition of some sugar. More recently, efforts have been made to adapt cow's milk by membrane technology (ultrafiltration and nanofiltration) (Kücükcetin et al. 2003). In traditional manufacturing, *koumiss* has been considered not only as a kind of nutritive food, but also as an ancient medical remedy. Research on the health benefits of *koumiss* has yet to be conducted, but the microbial similarity of this product with *kefir* makes it feasible to find similar bioactive compounds of microbial origin (exopolysaccharides, peptides) in both products.

9.4.8.3 Acidophilus Yeast Milk

Acidophilus yeast milk is made by mixing *Lb. acidophilus* with a special yeast species, *S. boulardii*. *S. boulardii* is a tropical strain of yeast first isolated from lychee and mangosteen fruits in 1923 by the French scientist Henri Boulard, after observing natives of Southeast Asia chewing on the skin of these fruits in an attempt to control symptoms of cholera. It is related to, but distinct from, *S. cerevisiae* in several taxonomic, metabolic, and genetic properties (Malgoire et al. 2005). It has been shown to be nonpathogenic and nonsystemic (remaining in the gastrointestinal tract rather than spreading elsewhere in the body), and grows at an unusually high temperature of 37°C (Kotowska et al. 2005). *S. boulardii* is capable of utilizing the milk constituents as a growth substrate, maintaining cell counts exceeding 10^6 cfu/mL (Lourens and Viljoen 2001). There are numerous randomized, double-blind, placebo-controlled studies showing the efficacy of *S. boulardii* in the treatment and prevention of several gastrointestinal disorders.

9.4.8.4 Viili

Viili is a traditional Finnish fermented milk product originally made in the summer as a way to preserve milk excesses. It is also known as *viilia*; and similar and related products are *piima*, *pitkapiima*, and *viilipiima*, from Finland; *långfil* and *tatmjolk* from Sweden; *taette* from Norway; *ymer* from Denmark; and *skyr* from Iceland (Tamime 2005). Most of these products share a thick and sticky consistency, with some degree of stretchiness plus a subtle sweet taste. *Viili* is produced by fermenting milk with special strains of *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* producing extracellular polysaccharides (EPS) (Macura and Townsley 1984). Typical of *viili* is the presence of *G. candidum*, a filamentous fungal species exhibiting properties of both molds and yeasts, which develop on the product surface forming a velvety layer similar to that in Camembert and Brie cheeses. *G. candidum* consumes lactate, lowering the acidity of the product, and produces the characteristic moldy aroma of *viili* (Kahala et al. 2008). EPS production among lactococci has been described as an unstable character, easily being lost upon repeated transfer of cultures or by growing them at abnormal temperature during propagation. Recently, the genes associated with EPS production have been located in plasmids (van Kranenburg et al. 2000). Polysaccharides act as a food stabilizer, preventing syneresis and graininess and providing the product with a natural ropiness (Macura and Townsley 1984). The final thickness of the product depends on the culture and the milk fat content. Beyond their rheological role, EPS have recently been claimed to protect *Lc. lactis* cells from phage attack (Deveau et al. 2002), and to promote development of beneficial populations in the gut after consumption (prebiotics) (Ruas-Madiedo et al. 2006). In traditional home manufacture, inoculation was done by back-slopping. In addition to EPS-producing *Lc. lactis*, mesophilic aroma producers (*Lc. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuc. mesenteroides* subps. *cremoris*) are usually found among the dominant LAB. The industrial production of *viili* began in the late 1950s. It involves standardization of milk, homogenization, and heating at 80°C–82°C for 20–22 min. After cooling at around 20°C, the product is heavily inoculated (up to 4%) with MSS cultures (Kahala et al. 2008) and the *G. candidum* strains selected. Natural starters may also contain other yeasts, such as *K. marxianus* and *P. fermentans* (Wang et al. 2008). The fermentation time is ≈20 h and the final pH of the product around 4.3. Regular fresh *viili* can be found in virtually every grocery store, and a wide variety of *viili*-type products are now available, including low-fat, low-lactose, fruit-flavored variants, and probiotic *viili*. These products are consumed mainly at breakfast and as a snack. However, there is a nonmoldy, high-fat-content variety that is used for cooking (Tamime 2005).

9.5 Conclusions

The great variety and diversity of current fermented dairy products seem to be a reflection of human history from ancient times up to the present. Yogurt, the most successful fermented milk all around the world and manufactured today by scientifically sound technology in modern dairy factories, is basically similar to some traditional products (*dadi*, *zabady*) manufactured thousands of years ago. Many traditional dairy products have social value and generate income in economically

depressed regions of the globe; consequently, continued production will contribute to increasing the standard of living in areas that manufacture these products. However, where possible, fermentation and storage conditions should follow strict hygienic and formulation guidelines to ensure good quality and safety of old and new dairy products. Similar to yogurt, other traditional dairy products (such as *kefir*) are gaining popularity; it is thus possible that in the near future their manufacture could be upgraded into sophisticated industries and produced on a worldwide scale. Besides traditional products, health-oriented fermented milks including those carrying currently-in-use and future probiotic microorganisms could provide consumers with health benefits beyond traditional nutrition, thus contributing to sustaining human health and well-being.

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10

Fermented Fish Products

Junus Salampessy, Kasipathy Kailasapathy, and Namrata Thapa

CONTENTS

10.1 Introduction

Fermentation was one of the first methods used to produce and preserve foods and has been practiced for thousands of years. It provides ways to preserve food products, enhance nutritive value, destroy undesirable factors, improve appearance and taste of some foods, salvage raw materials that otherwise are not usable for human consumption, and reduce energy used for cooking (Peredes-Lopez and Harry 1988). Nowadays, fermentation continues to play an important role in providing varieties of quality foods for human and domestic stock consumption. In fermentation, raw materials are converted into products through activities of endogenous enzymes or microorganisms (bacteria, yeasts, and molds). The process can be a natural process, in which desirable microorganisms grow preferentially, or a controlled process, in which isolated and characterized fermentative microorganisms (starter culture) are added to the raw material under controlled conditions. Fermentation of fish products, in itself, will not do much in

preserving them, as it will degrade fish muscle proteins into smaller peptides and amino acids that are nutrients for microorganisms. Therefore, fermentation is often combined with the addition of salt or drying to reduce water activity and eliminate proteolytic and putrefying microorganisms. The process can be partial and last for several hours to several weeks, such as in fermentation of fish foods in Africa and *balao balao* in the Philippines, or extensive and last for several months such as in fish paste and sauce preparation. As there are many types of fermented fish products in Asia and around the world, this chapter will only cover various fermented products of significance and or specific in nature. Many of the products are quite similar in the way they are prepared and so will not be included. This chapter will also cover the processing techniques, microbial and chemical changes, nutritional and physiological properties, safety issues, and improving the quality of fermented fish products.

10.2 Fermentation of Fish Products

Fermentation of fish products has been practiced for many years in many parts of the world. There has been reports of fermented fish products as far back to ancient Greece, and the trade of this product, *garum*, was extensive in the Roman era (Beddows 1985). There are many varieties of fermented fish products available today; these include liquid products such as fish sauce also knows as *nampla* in Thailand, *kecap ikan* or *bakasang* in Indonesia, *patis* in the Philippines, *nouc-mam* in Vietnam, oyster sauce, *hoi-sin* sauce, and paste products such as fish and shrimp pastes also known as *belacan* or *terasi* in Indonesia and Malaysia, and dry and semidry fermented fish.

10.3 Fish Sauces

10.3.1 Production of Fish and Seafood Sauces

Preparation of fish sauce is very simple. However, since the fermentation will take place for months, the quality of the raw materials is of great importance. Fish, usually small fish like sardines, are mixed with salt and fermented to obtain a clear liquid product. Fish sauces are mainly produced in Southeast Asian regions and variations in the manufacture do exist between countries and will be discussed here. The manufacture of *bakasang* in Indonesia is rather different from the manufacture of *nouc-mam* in Vietnam, *nampla* in Thailand, or *patis* in the Philippines. *Bakasang* is made from small fish like sardines or the guts of skipjack tuna (*Katsuwonus pelamis*). Skipjack tuna is a major fish caught in Indonesian waters in 2006 and account for 32% of the four major fish caught in Indonesia (http://statistik.dkp.go.id/). The guts are usually discharged during cleaning and filleting in fresh fish markets or during the smoking preparation of fish; therefore, the preparation of *bakasang* using the guts is a way of utilizing parts of fish that are otherwise wasted. The fish or fish guts are cut into smaller pieces and mixed with salt in the ratio of 1.5 to 3 parts and 5 parts of fish. The mixture is bottled and kept in the kitchen near the fire place to ferment for 3–6 weeks (Ijong and Ohta 1995a, Ijong and Ohta 1996). Some additives like sugar can be added to stimulate fermentation. The fermentation temperature varies from 30°C to 50°C depending on whether the fire place is being used for cooking.

After 3–6 weeks fermentation, a thick, salty light brown liquid is formed with a characteristic aroma and flavor (Ijong and Ohta 1996). *Bakasang* has been produced traditionally for years in eastern Indonesian regions especially in the North Sulawesi province and in Banda Island of the Spice Islands province of Maluku. *Bakasang* production remains a domestic business and a common household and commercial food ingredient, although it is not very popular due to quality concerns and perception.

Another fish sauce from the Southeast Asian region is *patis* from the Philippines. *Patis* production is associated directly with the production of a partially or fully fermented fish or shrimp product called *bagoong*. *Patis* is a supernatant from the fermentation of fish or shrimp in *bagoong* preparation obtained by either decanting and/or pressing or centrifuging *bagoong* after fermentation. *Patis* is considered a high salt content food ingredient, and the quality is better from the longer periods of fermentation of fish or shrimp (Olympia 1992).

The Vietnamese fish sauce *nouc-mam* is made from fish or sometimes shrimp. Traditionally, the fish is ground, pressed by hand, then placed in earthen jars layer by layer with salt in between the layers. The approximate ratio of fish to salt is 3:1. The jars are then closed tightly and buried almost completely in the ground. In the first stage of fermentation, a bloody liquid (*nouc-boi*) is decanted carefully, and the remaining fish is allowed to ferment further for 6–18 months depending on the size of fish. Some of this *nouc-boi* is added back to the jar, and some is added at a later date. The high quality *nouc-mam* is obtained from the first separation of the supernatant (Beddows 1985). The remaining undigested material is then mixed with hot brine to get low quality *nouc-mam* (Lopetcharat et al. 2001). The low quality of the *nouc-mam* can be improved either by addition of food additives such as caramel, molasses, roasted maize, or roasted barley to the fish before the hot brine extraction. Another common way to improve the quality is by mixing in high quality *nouc-mam* to get a better quality, lower-grade *nouc-mam* (Beddows 1985).

Fish sauce from Thailand, *nampla*, is a popular fish sauce in many countries, including the United States. Just like in other Southeast Asian countries, *nampla* production and consumption was a domestic business. However, industrialization of *nampla* has expanded the product in terms of quality and marketability. *Nouc-mam* preparation is rather different; *nampla* is made by first cleaning the fish to wash out any unwanted materials as well as reducing the number of microorganisms in the raw materials (Lopetcharat et al. 2001). The fish is then mixed with salt in a ratio of 2:1 to 3:1. The mixture is then transferred into fermentation tanks such as wooden barrels or large earthen jars and placed in between two bamboo mats, one at the bottom of the barrels or jars and the other at the top of the mixture loaded with heavy weight to keep the fish in the brine produced from osmotic dehydration of the fish. The liquid level will increase to the top of the mixture within the first week of fermentation. The fish will continue to ferment in the open areas, and after up to 18 months, the first batch of supernatant is transferred into ripening tanks and is allowed to ripen for 2–12 weeks. First-grade *nampla* is obtained (Saisithi et al. 1966, Lopetcharat et al. 2001, Wongkhalaung 2004). Second-grade and low-grade *nampla* is obtained in a manner similar to the production of low-grade *nouc-mam* except that Mikei water, nitrogen-rich by-products from monosodium glutamate (MSG) production, is added to improve the quality of the *nampla* (Beddows and Ardeshir 1979, Lopetcharat et al. 2001). In northeastern Malaysia, a fish sauce called *budu* is made using leftovers from fish drying or whole fish when the weather is not suitable for drying fish. The fish, three parts, is mixed with two parts of salt and placed into concrete fermentation tanks and then covered with plastic sheets.

A heavy weight is loaded on the covered fish to enhance osmotic dehydration of the fish and keep them immersed in the brine produced. The fish is allowed to ferment for 3–12 months, and then the fermented fish is ground coarsely, mixed with tamarind and palm sugar, and then boiled. Once cooled, the mixture is filtered and bottled (Beddows 1985).

Fish sauces are produced in other parts of the Asian continent. In China, especially in Guangdong and Fujian provinces, a fish sauce called *yu-lu* is commonly made from small fish like sardines or anchovies. *Yu-lu* is prepared in the same manner as *nampla* (Jiang et al. 2007). In Japan, *shottsuru* and *ishiru* are two types of fish sauces commonly produced. *Shotturu* is made of *Perciformes trichodontidae* and is mainly related to the Akita prefecture, while *ishiru* is made from a variety of fish and cattlefish (Lopetcharat et al. 2001). In Korea, *jeotkuk* is a fish sauce prepared in a slightly similar way as *patis*, in which the whole fish is mixed with 20% salt and fermented at a rather low temperature of about 20°C. This process is applied to the preparation of whole fermented fish, *jeotkal*, after fermentation for 2–3 months. Extended fermentation for 6–12 months will produce *jeotkuk*. *Jeotkuk* is used mainly as an ingredient in *kimchi* preparation or as condiment (Lee 1993).

The fish sauces mentioned above are notably Asian products, in particular from the Southeast Asian region. However, fish sauce is also produced in other countries like Greece. A product called *garos* is produced by fermenting fish viscera, particularly the liver. Four to nine parts of fish liver are mixed with one part of salt, depending on size, and fermented for 8 days to 8 weeks. The fermentation process proceeds very quickly probably due to the high concentration of enzymes present in the raw material (Beddows 1985). *Garos* preparation seems to be similar to the preparation of *garum*, a fish sauce of the Roman era.

The sauces discussed above are sauces made from fish fermentation. There are, however, other sauces made from seafoods. Oyster sauce, for instance, is a popular sauce in Chinese culinary. Oyster sauce is a thick sauce usually served as a mixing sauce to boiled green vegetables such as Chinese yellow-flowering cabbage, *bakchoi*, or Chinese lettuce. Oyster sauce is prepared by boiling oyster meat for 20 min, and then the meat is separated from the juice. While the meat is used for making dried oysters, the juice is used to prepare oyster paste or sauce. Prior to aging, the juice is concentrated by boiling and then aged for 1 month or longer (Chen 1993). Regardless of regional variations of process, the core process for fish sauce preparation is the same. Fish sauce production is an anaerobic process, therefore lipid oxidation and accumulation of oxidized flavor compounds are minimized. The presence of halophilic and halotolerant bacteria in the medium and low pH environment help prevent the growth of putrefying bacteria (Gopakumar 1997). The hydrolysis of proteins during fermentation is mainly caused by trypsin-like enzymes, although the action of these enzymes are partly minimized by the presence of high salt concentration, thus slowing the autolysis process (Orejana and Liston 1982). High concentrations of salt also control the type of microorganism in favor of halophilic and halotolerant bacteria such as *Pediococcus halophilus* while retarding or killing some pathogenic microorganisms such as *Escherichia* sp. during fermentation (Lopetcharat et al. 2001). At the beginning of the fermentation process, osmotic dehydration of the fish flesh takes place resulting in the fish being immersed in pickle. The fermentation will then proceed as a result of the action of exogenous and endogenous enzymes. The endogenous enzymes are naturally present in the guts and intestines of fish (Gopakumar 1997). Various organic compounds released during fermentation are responsible for the characteristic aroma and odor of fish sauces. Figure 10.1 shows the flowchart of production of some fish sauces.

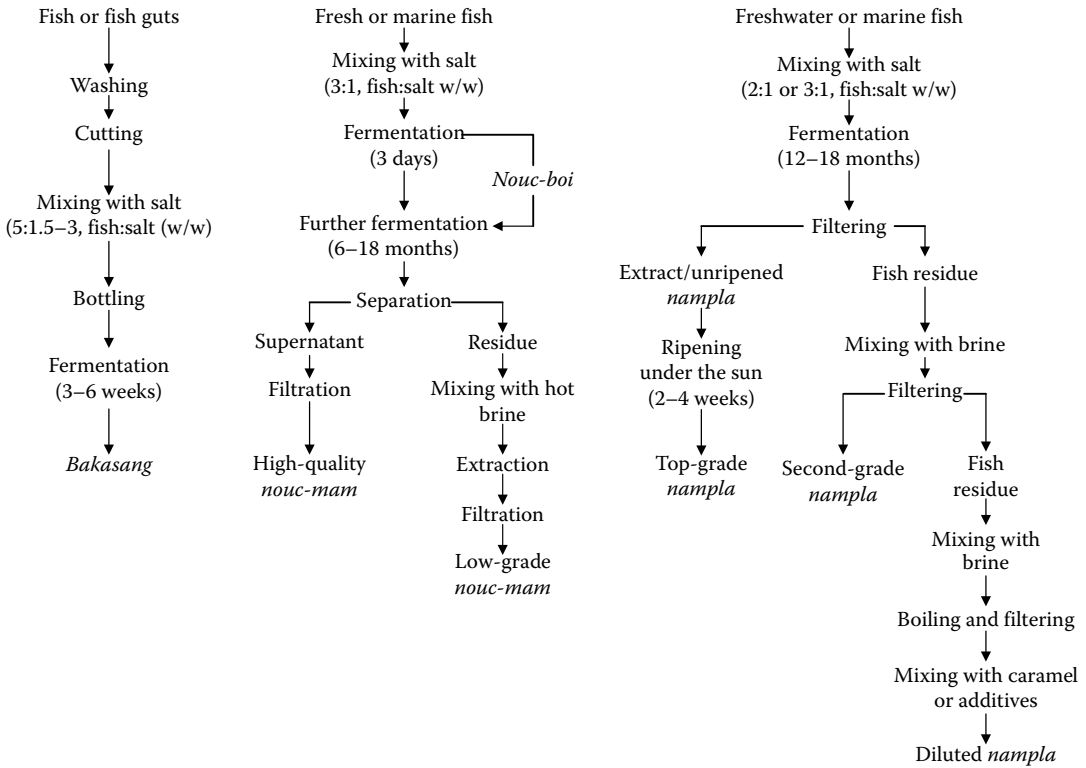

FIGURE 10.1 Flow chart of traditional *bakasang*, *nouc-mam*, and *nampla* production. (Adapted from Ijong, F.G. and Ohta, Y., *J. Sci. Food Agric.*, 71, 69, 1996; Beddows, C.G., *Microbiology of Fermented Foods*, Wood, B.J.B. (ed.), Vol. 2, Elsevier Applied Science, London, U.K., 1985; Lopetcharat, K. et al., *Food Rev. Int.*, 17, 65, 2001.)

10.3.2 Microflora of Fish Sauce

Microflora play an important role in the production of fish sauces. A high salt concentration of fish sauce means that only halophilic and halotolerant bacteria are able to grow. The presence of microflora during fish fermentation enhances the degradation of fish proteins and develops flavor and aroma. Table 10.1 shows the changes of microflora during fermentation of the Indonesian fish sauce *bakasang*. These bacteria can be classified into proteolytic enzyme-producing bacteria and flavor- and aroma-producing bacteria. Bacteria that produce proteolytic enzymes include *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Staphylococcus* sp., *Halococcus* sp., *Halobacterium salinarium*, and *H. cutirubrum* (Thongthai et al. 1992, Lopetcharat et al. 2001). *Staphylococcus* strain 109 and *Bacillus* spp. are bacteria that are involved in the flavor and aroma development of fish sauce (Saisithi et al. 1966, Fukami et al. 2004a,b).

During fermentation, the microflora of the mixture changes preferentially. The number of microorganisms increases rapidly during the first 10 days of *bakasang* fermentation. This increase corresponds to the decrease in the pH of the mixture. The decrease of pH may be attributed to the production of lactic acid by lactic acid bacteria (LAB). After 20 days of fermentation, the number of microorganisms decreases, with *Lactobacillus* sp., *Streptococcus* sp., and *Pediococcus* sp. being the predominant microorganisms (Ijong and Ohta 1996). In *nampla*, the number of bacteria is higher during the first month of fermentation and decreases thereafter. Although there is no data to show the type of microflora changes during *nampla* fermentation, microbial analysis of 9 month old *nampla* reveals that *Bacillus* sp., *Coryneform* sp., *Streptococcus* sp., *Micrococcus* sp., and *Staphylococcus* sp. are present in the product (Saisithi et al. 1966). *Nampla* microflora is, therefore, quite similar to the microflora of traditional *bakasang* or laboratory *bakasang* made from sardine catch in Japanese waters (Ijong and Ohta 1995a, 1996). In addition, an extremely halophilic bacteria, *H. salinarium*, has been also identified in *nampla*. This microorganism produces extracellular proteases that may play an important role in the fermentation process (Thongthai et al. 1992).

The total bacterial count of *patis* decreases rapidly during the first 6 months of fermentation and decreases slightly during the extended fermentation. Most of the microorganisms are facultative anaerobes (Olympia 1992). However, there is no data to indicate the type of microflora present in *patis*. As *patis* is a by-product of *bagoong* fermentation, the microflora may be quite similar to that of *bagoong*. Korean fermented

TABLE 10.1

Microflora Changes during Fermentation of Indonesian Fish Sauce *Bakasang*

Fermentation (Days)	Microflora (Genera)[a]
0	*Pseudomonas*, *Enterobacter*, *Moraxella*, *Micrococcus*, *Streptococcus*
4	*Lactobacillus*, *Pseudomonas*, *Enterobacter*, *Moraxella*, *Micrococcus*, *Staphylococcus*, *Streptococcus*
10	*Streptococcus*, *Pediococcus*, *Micrococcus*, *Pseudomonas*, *Enterobacter*
20	*Streptococcus*, *Pediococcus*, *Micrococcus*
30	*Streptococcus*, *Pediococcus*, *Micrococcus*
40	*Streptococcus*, *Pediococcus*, *Micrococcus*

Source: Ijong, F.G. and Ohta, Y., *J. Sci. Food Agric*., 71, 69, 1996.

[a] These are the predominant microflora identified during fermentation.

fish products made from anchovy have been shown to include of *Bacillus cereus*, *Clostridium setiens*, *Pseudomonas halophillus*, and *Serratia marcescens* in the final stage of fermentation (Sands and Crisan 1974). Although it is not mentioned that this product is a fish sauce, it is quite likely to be a sauce as volume rather than weight is used in characterizing the amount of samples. As can be seen, the microflora of various traditional fish sauces shows little difference. These differences can be associated with different species of fish used (hence their microflora), the environment where the fish is caught, the microflora of the salt used for fermentation (Sands and Crisan 1974), and cross contamination from the worker's hands and/or tools used during preparation. The presence of microflora in fish fermentation also contributes to the flavor and aroma of fish sauce. *Staphylococcus* sp., for instance, has been identified in fish sauces. This group of bacteria has been used as a starter culture to improve flavor and color in meat products (Berdagué et al. 1993, Fukami et al. 2004b), and *S. xylosus* has been found to improve the odor of fish sauce (Fukami et al. 2004a). Microorganisms have also been concluded to be in action to produce volatile fatty acids (VFA) in fish sauce by alteration of amino acids (Beddows et al. 1980).

10.3.3 Chemical Changes during Fish Sauce Fermentation

During fermentation of fish, proteins, fat, and glucose are converted into peptides and amino acids, fatty acids, and lactic acids by the action of enzymes and microorganisms. Proteins are the major constituent of fish, therefore the degradation of proteins represents the major changes taking place during fish sauce fermentation. The chemical compositions of fish sauces are shown in Table 10.2. The pH of fish sauces decrease during fermentation, and this may be due to the production of lactic acid by LAB (Ijong and Ohta 1996), free amino acids, and large polypeptides (Lopetcharat et al. 2001). LAB have been identified in fish sauces (Ijong and Ohta 1996) that will transform glucose

TABLE 10.2

Chemical Compositions of Some Fish Sauces

	***Bakasang* (g/kg) (Ijong and Ohta 1995a,b)**	***Nouc-mam* (%) (Beddows et al. 1979)**	***Nampla* (mmol/100 mL) (Saisithi et al. 1966)**	***Budu* (%) (Beddows et al. 1979)**
pH	5.84–6.30	NA	6.2–6.4	5.6
NaCl	84.2–180.0	NA	27.9–30.3 (%)	26.3
Total nitrogen	140.0–174.4	1.90–2.28	49–140	1.77
Fat	10.0–30.0	NA	NA	0.13
FAN	98.9 (mg/L)	0.2–1.30	NA	1.17
Ammonia nitrogen	NA	NA	8–15	NA
Titratable acid—lactic	NA	NA	5.2–15.8	NA
Volatile acid—lactic	NA	NA	3.3–8.7	NA
Volatile base	NA	0.2–0.7	3.01–14.71[a]	0.12

Sources: Adapted from Ijong, F.G. and Ohta, Y., *J. Fac. Appl. Biol. Sci. Hiroshima Univ*., 34, 95, 1995a; Ijong, F.G. and Ohta, Y., *LWT—Food Sci. Technol*., 28, 236, 1995b; Beddows, C.G. et al., *J. Sci. Food Agric*., 30, 1097, 1979; Saisithi, P. et al., *J. Food Sci*., 31, 105, 1966.

Note: NA, data not available.

[a] In mmol/100 mL.

into lactic acid. In addition, free amino acids and peptides produced during the hydrolysis of fish protein will contribute to a decrease in pH. In general, all fish sauces are slightly acidic having pH values from 5.5 to 6.5. The lipid content of fish sauce is considered low although the lipid content of the raw material is higher. The lipid content of *budu*, for instance, never increases more that 0.13% although the lipid content of the raw material *ikanbilis* is 10 times higher (Beddows et al. 1979). Various acids, including volatile fatty acids, have been identified in fish sauces (Beddows et al. 1980, McIver et al. 1982, Fukami et al. 2002) (Table 10.3). These acids contribute to the flavor of fermented fish sauces. Fish sauces with less volatile fatty acids (VFA) are described as less cheesy and more ammoniacal than those with greater amounts of VFA (McIver et al. 1982). Butanoic acid, for instance, contributes to the cheesy flavor, while 3-methylbutanoic and capronic acids contribute to the rancid flavor (Fukami et al. 2002). The origin of these acids is from lipids, amino acids, or glucose. Caproic and heptanoic acids in the fish sauces may be derived from oxidation of lipids (McIver et al. 1982). Propionic, *n*-butanoic, and *n*-pentanoic acids may be derived from amino acids through bacterial action (Beddows et al. 1980), as well as phenylacetic and 3-phenylpropionic acids that may be derived from phenylalanine (McIver et al. 1982).

TABLE 10.3

Organic Acids Identified in Fish Sauces

	Fish Sauce			
Acids	***Budu*[a] (Beddows et al. 1979)**	***Nampla*[b] (Dougan and Howard 1975)**	***Nouc-mam*[c] (Beddows et al. 1979)**	***Yu-lu*[d] (Peralta et al. 1996)**
Formic	ND	ND	ND	ND
Acetic	2.15	0.25–1.40	0.7–1.4	0.79
Propionic	0.12	0.05–0.67	ND	1.0
N-Butyric	0.23	0.06–0.42	0.35–0.7	3.24
Isobutyric	0	0.06–0.12	ND	8.91
Isopentanoic	0.07	0.03–0.31	ND	0.072
Valeric	ND	NQ	ND	ND
Isovaleric	ND	NQ	ND	ND
Caproic	ND	NQ	ND	ND
Isocaproic	ND	NQ	ND	ND
Heptanoic	ND	NQ	ND	ND
Levulinic	ND	NQ	ND	ND
Benzoic	ND	NQ	ND	ND
Phenylacetic	ND	NQ	ND	ND
3-Phenylpropionic	ND	NQ	ND	ND

Sources: Adapted from Beddows, C.G. et al., *J. Sci. Food Agric*., 30, 1097, 1979; Dougan, J. and Howard, G.E., *J. Sci. Food Agric*., 26, 887, 1975; Peralta, R.R. et al., *J. Agr. Food Chem*., 44, 3606, 1996.

Note: ND, not determined; NQ, not quantified.

[a] Concentration in mg/cm^3.

[b] Concentration in mg/mL.

[c] Concentration in mg/cm^3.

[d] Concentration in mg/L.

Other chemicals are volatile nitrogen compounds present in the fish sauces. During fermentation, proteins as a major source of nitrogen are hydrolyzed to amino acids, peptides, and ammonia. Another compound of note is trimethylamineoxide (TMAO) that will undergo reduction to produce trimethylamine (TMA). TMA has been well identified to cause the fishy odor of fish sauce (Fukami et al. 2002). Other compounds that contribute to the flavor of fish sauces are amides. Together with VFA, some amino acids such as glutamic acid, histidine, and proline are reported to contribute to the meaty flavor of *nampla* (Saisithi et al. 1966).

10.4 Fermented Fish Pastes

10.4.1 Production of Fish Pastes

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. *Hentak* and *tungtap* are fish pastes from Northeast India (Thapa 2002). *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 days. *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). *Tungtap* is a fermented fish paste consumed as a pickle by the Khasia tribes of Meghalaya in Northeast India. *Tungtap* is prepared by mixing salt and dried fish (*Danio* spp.) and fermented in earthen pots for 4–7 days (Thapa et al. 2004).

Bagoong is an undigested residue of partially or fully hydrolyzed fish or shrimp. *Bagoong* is eaten raw or cooked and used mainly as a flavoring agent or in condiment preparation, sautéed with onion and garlic and served as an appetizer with tomatoes or green mangoes, eaten with vegetables, or often as a main source of protein (Olympia 1992). Fish such as anchovy, sardine, and herring, and small shrimp or its roe are used in *bagoong* preparation. The fish are cleaned and mixed with salt in a ratio of 3:1 and placed in vats to ferment (Beddows 1985). The pickle produced during fermentation is drained and constitutes *patis*. The fish is allowed to ferment for several months until it develops the characteristic flavor and aroma of salty and slightly cheese-like odor of *bagoong* (Olympia 1992). Shrimp paste, well known as *terasi* in Indonesia, *belacan* in Malaysia, *kapi* in Thailand, or *ngapi* in Burma, is a popular taste enhancer in many Southeast Asian and Southern Chinese cuisines. Generally, small shrimps are mixed with salt to give a final salt concentration of 10%–15%, or even higher to get a better product. The mixture is then dried on straw mats to reduce the water content to about 50%, then minced or pounded into paste, and allowed to ferment for 7 days. At the end of fermentation, the mixture is broken and sun dried for 5–8 h, then minced again and formed into blocks or balls, and further fermented for about 1 month. This process can be repeated several times if needed.

10.4.2 Microflora of Fermented Fish Paste

As in fish sauce, microflora plays an important role in the preparation of fermented fish pastes. The presence of microorganisms during fermentation contributes to the degradation of proteins and the development of flavor and aroma. The microflora of *hentak* and *tungkap* have been studied (Thapa et al. 2004). The results show that the load of LAB is higher than other microorganisms in the products. The other predominant microorganism present in *hentak* and *tungkap* is *Bacillus* spp. The presence of *Bacillus* spp. in LAB-dominant products is due to their ability to form endospores to survive under prevailing conditions (Crisan and Sands 1975). The microbial contents of *hentak* and *tungtap* show broad diversity ranging from LAB belonging to coccallactics (*Lactococcus*, *Enterococcus*) to species of homofermentative and heterofermentative rods (*Lactobacillus*), endospore-forming rods (*Bacillus*), aerobic coccus (*Micrococcus*), to species of yeast (*Candida*, *Saccharomycopsis*), as well as pathogenic contaminants such as *B. cereus*, *S. aureus*, and *Enterobacteriaceae* (Thapa et al. 2004). The microflora of fish or shrimp paste, *bagoong*, in the Philippines includes *Bacillus* sp., *Micrococcus* sp., and *Moraxella* sp. (Beddows 1985). Shrimp paste *belacan* microflora includes *Bacillus*, *Pediococcus*, *Lactobacillus*, *Micrococcus*, *Sarcina*, *Clostridium*, *Brevibacterium*, *Flavobacterium*, and *Corynebacteria*. The predominant microorganisms are LAB, *Micrococcus*, *Bacillus*, and high-salt-tolerant species (Abdul Karim 1993).

10.4.3 Chemical Changes during Fish Paste Fermentation

During fermentation of fish or shrimp pastes, proteins and fat as well as glucose underdog degradation to produce amino acids and peptides, fatty acids, and organic acids and other nonprotein nitrogen compounds. Shrimp fermentation up to 360 days shows a decrease in amino acid contents. The decrease in amino acids indicates further degradation of amino acids to produce amines, volatile acids, and other nitrogenous substances. This decline also correlates to the Maillard reaction between amino acids and sugar (Peralta et al. 2008).

Fermented fish or shrimp pastes are likely to contain amines as microorganisms involved in fish fermentation, including lactobacilli, and also contain amino acid decarboxylases (Hutkins 2006). Histamine and 2-phenylethylamine have been identified as the most concentrated amines in fish and shrimp pastes, with 2-methylbutylamine being the least concentrated amine. Histamine is the product of the decarboxylation of histidine and is a harmful compound. Fermented prawn paste contains the highest concentration of total amines, while fermented oyster paste contains the lowest concentration of amines (Fardiaz and Markakis 1979).

More than 150 volatile compounds have been identified in fish and shrimp pastes (Cha and Cadwallader 1998). The compounds consist of aldehydes, ketones, alcohols, aromatic compounds, *N*-containing compounds, esters, *S*-containing compounds, and some other compounds. The aldehyde content is high in all fish pastes and low in shrimp paste. Among the aldehydes, benzaldehyde is found in all pastes. Benzaldehyde is reported to possess a pleasant almond, nutty, and fruity aroma (Vejaphan et al. 1988). Ketones are more abundant in all fish pastes, and are much less in shrimp paste. Among the ketones found in fish pastes including shrimp paste is 2,3-butanedione, that has been reported to contribute an intense buttery

and desirable aroma in crustaceans (Tanchotikul and Hsieh 1989). Alcohols are found in abundance in all fish pastes, but are low in shrimp pastes. The low content of alcohol in shrimp pastes correspond to their low aldehyde content. Among the phenolic or aromatic compounds, toluene is more abundant in shrimp pastes, while phenol is more abundant in fish pastes. Toluene and phenol have been reported to give an undesirable aroma in seafoods (Vejaphan et al. 1988). Shrimp pastes contain more *N*-containing compound as compared to fish pastes. Among these *N*-containing compounds is 2-ethyl-3,6-dimethylpyrazine that is found in big-eye herring, hair tail viscera and shrimp pastes. This compound has been reported to contribute to nutty, roasted, and toasted aromas. Pyrazines are formed through the Maillard reaction of amino acids. Various esters have also been identified in fish pastes. Among these is ethylbutanoate that provides a cheesy flavor to anchovy and big-eye herring paste. Four major sulfur-containing compounds found in fish and shrimps pastes are dimethydisulfate, dimethyltrisulfate, isobutylisopropylsulfate, and 3,5-dimethyl-1,2,4-trithiolate. The presence of these *S*-containing compounds may affect the overall flavor because of their low traceholds. Furans are present in fish and shrimp pastes. Furans have been reported to have burnt, sweet, bitter, and coconut-like flavor in some foods (Maga 1979). High content of protein is observed in *ngari*, *hentak*, and *tungtap*, indicating increasing protein intake in the local diet (Thapa and Pal 2007). *Tungtap* has high calcium (5040 mg/100 g) and phosphorus (1930 mg/100 g) contents among the minerals (Agrahar-Murugkar and Subbulaksmi 2006).

10.5 Fermented Fish Foods

10.5.1 Production of Fermented Fish Foods

Most of the fermented fish products mentioned earlier are prepared mainly to be used as flavor enhancers and condiments. There are, however, fermented fish and seafoods that are prepared to be consumed as a staple food. These products can be found in almost every parts of the world including the Scandinavian region in Europe, Africa, Middleast, South Asia, and Southeast Asia. In Iceland, a traditional product called *hákarl* is always available through out the year. *Hákarl* is a fermented food made from shark flesh that has been fermented and sun-dried for months. It has a strong cheese flavor due to high ammonia content and is considered unpalatable by most people. In Scandinavia, whole herring or trout, eviscerated but retaining the roe, are immersed in low concentration brine for 30–40 h, during which vigorous fermentation takes place. These products are known as *surstrømming* for herring and *rakørret* for trout (Beddows 1985). In other European countries such as France and Spain, fermented anchovy products are well known. The fish is beheaded and gutted (or ungutted in Spain), then layered with salt in barrels, weighed down to extract the pickle as it forms, and fermented for 6–7 months. The fish retains its form and no paste is formed.

In Africa, traditional fermented food fish are found in many countries. The preparation method includes fermentation with salting and drying, fermentation and drying without salting, or fermentation and salting without drying. Almost all African countries have fermented fish foods with different names and raw materials used, therefore only selected products will be described here. In Sudan, a fermented fish made from

Alestes sp. or *Hydrocynus* sp. called *fessiekh* is well known. *Fessiekh* is prepared using layered washed fish with salt in a basket or drum, and then weighed down to extract the pickle and fermented for up to 7 days. Then the fish is transferred into larger fermentation tanks and more salt is added and further fermented for 10–15 days. *Fessiekh* is a wet salted product with soft texture and pungent odor and a shiny silvery appearance. The keeping time can be up to 3 months, but is highly susceptible to microbial growth and maggot infestation due to poor quality control (Essuman 1992). In India, various fermented fish products are available. *Ngari* is an ethnic fermented fish product of Manipur, India (Tamang 2010). *Ngari* is made from the fish *Puntius sophore*. The fish is rubbed with salt and dried for 3–4 days, then pressed tightly in an earthen pot, sealed tightly, and fermented for 4–6 months. *Ngari* is eaten as a side dish with cooked rice (Thapa et al. 2004). Another product from the Indian subcontinent is *jaadi* from Sri Lanka. *Jaadi* is a high-salt fermented fish made from a fish like the Indian mackerel (*Rastrelliger kanagurta*), trench sardine (*Amblygaster sirm*), or skipjack tuna (*K. pelamis*). The fish used are usually gutted and cleaned, then layered in a barrel or earthen pot with a mixture of salt and the ripe pods of *goraka* plant in 3:1 ratio (fish to salt/*goraka* mixture). *Goraka* (*Garcinia gamboges*) pods contain several organic acids including tartaric and citric acids. The fish is allowed to ferment for 2–3 months or longer. The pickle formed during fermentation is drained out as fish sauce and used in curry or *sambol* (condiment) preparations (Subasinghe 1993).

Fermented fish foods are also popular in most Southeast Asian countries. Products like *pedah* in Indonesia, *balao balao* in the Philippines, to mention a few, are well-known fermented foods in the respective countries. *Pedah* is a wet fermented fish made from mackerel. The whole fish is first salted in a 3:1 ratio at ambient temperature. The salted fish is then removed from the salt and repacked in basket, then fermented for 2–3 months or longer at about 29°C (Putro 1993). *Balao balao* of the Philippines is fermented cooked rice and shrimp (*Penaeus indicum* or *Macrobranchium* spp.). During fermentation, the mixture becomes acidic and the shrimp shell reddens and soften. *Balao balao* is eaten as an appetizer or as a main dish (Olympia 1992).

10.5.2 Microflora of Fermented Fish Foods

Very little information on the microflora of fermented fish foods is available. However, products like *ngari* have been studied, and are presented here. The load of LAB in *ngari* is higher than other microorganisms present in the product. These include *L. fructosus*, *L. amylophilus*, *L. coryniformis* spp. *torquens*, *L. plantarum*, *Lactococcus plantarum*, *L. lactis* spp. *cremoris*, and *Enterococcus faecium*. Also identified are *B. subtilis* and *B. pumilus*, *Micrococcus*, *Candida*, and *Saccharomycopsis* (Thapa 2002, Thapa et al. 2004). *Bacillus* spp. represent the dominant flora in *ngari* next to LAB due to their ability to form endospores to survive under prevailing conditions (Thapa et al. 2004). The microflora changes during 10 days of *balao balao* fermentation indicate that gram-positive cocci predominate the first 5 days of fermentation, while acid-producing rods predominate the rest of the fermentation period (Mabesa and Babaan 1993).

10.5.3 Chemical Changes during Fish Food Fermentation

As LAB predominate the fermentation of fish food, notable decrease of pH is obvious. During LAB fermentation of *balao balao*, the major chemical change is the

accumulation of lactic acid. This production prevents the decomposition of protein into its basic components such as CO_2 and H_2O (Olympia 1992). In *ngari*, LAB and *Bacillus* sp. show no ability to produce biogenic amines such as tyramine, cadaverine, histidine, and putrcine (Thapa et al. 2004).

10.6 Nutritional and Physiological Properties of Fermented Fish Products

Fish sauces are mainly used as condiments or flavor enhancers in cooking. However, in some Asian countries such as Thailand, Vietnam, and Korea, fish sauce is mixed with rice, and hence can be considered as a staple food. As a product from mainly protein degradation, fish sauce contains high amount of salt, then the nutrient intake from fish sauce is expected to be quite limited. There is no established data to show the amount of fish sauce consumed daily. Since fish sauce is used to give flavor and aroma to rice, some 40–50 mL may be consumed over two meals that will give a salt intake of about 12–13 g/day (Beddows 1985). The proximate and amino acid compositions of some fish sauces and paste are given (Table 10.4). Anchovy is used in the preparation of *nouc-mam*, *nampla*, and *jeotkuk*, while the raw material for *bakasang* is unknown.

Glutamic acid, alanine, isoleucine, serine, and phenylalanine contents are high in *bakasang*. However, glycine, histidine, threonine, and proline contents are low (Ijong and Ohta 1995b). The raw materials used to make *bakasang* are unknown. Usually small fish like sardine and the guts of skipjack tuna (*K. pelamis*) are used, hence could be the reason for the particular amino acid compositions. Also, it has been noted that the concentration of salt used in *bakasang* preparation affected the concentration of amino acids produced. The higher the salt concentration, the lower the amino acid content. This could be due to the slowing of microorganism activities. However, the concentration of amino acids serine, histidine, arginine, and phenylalanine increased as the salt concentration increased. Histidine is the precursor for histamine production through the action of microorganisms, therefore increasing salt concentration may inhibit the transformation of histidine into histamine (Sanceda et al. 1999). The glutamic acid contents of all fish sauces are quite high. This could be due to the use of MSG or the by-product of MSG production to enhance the flavor of fish sauce (Lopetcharat et al. 2001). Fish sauces also contain taurine. Taurine is not naturally present in fish flesh and can be derived from cysteine or cystine (Saisithi et al. 1966). Taurine and taurine analogue have been linked with several beneficial physiological actions including hypoglycemic action, antioxidation, osmoregulation, antihypertension, and neuromodulation (Nara et al. 1978, Kulakowski and Maturo 1984, Huxtable 1992, Ricci et al. 2006). Fish sauces made from salmon, sardine, and anchovy (Okamoto et al. 1995) as well as oyster (Je et al. 2005) have also been identified to contain bioactive peptides against angiotensin-I-converting enzymes (ACE) that can reduce high blood pressure.

Fermented fish and shrimp pastes also contain nutritional properties similar to fish sauces. The amino acid compositions of shrimp paste are quite comparable to fish sauce. In addition, the polyunsaturated fatty acids (PUFAs) present in the

TABLE 10.4

Proximate and Amino Acid Profiles of Some Fish Sauces

	Fish Sauce				
Parameters	***Bakasang* (Ijong and Ohta 1995a)**	***Nouc-mam* (Cho and Choi 1999)**	***Nampla* (Cho and Choi 1999)**	***Jeotkuk* (Cho and Choi 1999)**	**Shrimp Paste (Peralta et al. 2008)**
Protein	14.95	NA	11.6 (2.0)[a] (Wongkhalaung 2004)	NA	NA
Ash	ND	NA	36.4 (30.8)[a] (Wongkhalaung 2004)	NA	NA
Carbohydrate	ND	NA	2.4 (12.7)[a] (Wongkhalaung 2004)	NA	NA
Fat	1.78	NA	Trace	NA	NA
Aspartic acid	0.36	0.43	0.61	0.03	0.49
Glutamic acid	0.73	3.03	1.21	1.80	0.07
Serine	0.47	0.39	0.26	ND	0.19
Glycine	0.07	0.23	0.27	0.59	0.38
Histidine	0.04	0.31	0.27	0.34	0.04
Arginine	0.35	0.15	0.01	0.06	0.37
Threonine	0.06	0.53	0.38	0.09	0.25
Alanine	0.48	0.33	0.67	1.23	0.38
Proline	ND	0.19	0.18	0.32	0.24
Tyrosine	0.19	0.04	0.04	0.03	0.17
Valine	0.48	0.35	0.48	0.68	0.42
Methionine	0.36	0.29	0.17	0.13	0.18
Cysteine	0.28	0.04	ND	0.29	ND
Isoleucine	0.93	0.51	0.30	0.72	0.32
Leucine	0.35	0.90	0.34	1.22	0.67
Phenylalanine	1.04	0.13	0.23	0.07	0.35
Lysine	0.60	0.63	0.97	1.06	0.61
Tryptophan	ND	ND	ND	ND	0.04
Turine	ND	0.17	0.10	0.21	0.92

Sources: Adapted from Ijong, F.G. and Ohta, Y., *J. Fac. Appl. Biol. Sci. Hiroshima Univ*., 34, 95, 1995a; Cho, Y. and Choi, Y.J., Special report for quality estimation of Anchovy sauce, Ministry of Marine and Fisheries, Seoul, 1999 as quoted in Lopetcharat, K. et al., *Food Rev. Int*., 17, 65, 2001; Wongkhalaung, C., Industrialization of Thai fish sauce (nam pla), in Steinkraus, K.H (ed.), *Industrialization of Indigenous Fermented Foods*, 2nd edn., Marcell Dekker, Inc., New York, 2004; Peralta, E.M. et al., *Food Chem*., 111, 72, 2008.

Note: NA, data not available; ND, not determined.

[a] Figure in parentheses are from low-grade fish sauce.

final product, i.e., 20:5n-3 (EPA) and 22:6n-3 (DHA), can survive oxidation during the 360 day fermentation. This is due to the presence of antioxidants in the shrimp paste. Free amino acids (FAA) have been reported to be able to prevent oxidation; however, the Maillard reaction products (MRPs) have been identified as the antioxidants present in shrimp paste (Peralta et al. 2008).

10.7 Safety Issues Concerning Fermented Fish Products

The beneficial aspects of fish sauce consumption, however, can be limited due to the high salt content. Some fish sauce producers used to add a small amount of sugar to cover the explicit salty taste (Wongkhalaung 2004). Various studies have also indicated the presence of pathogenic bacteria in fish sauces. *Staphylococcus aureus* and *Clostridium* sp. have been isolated from *bakasang* (Ijong and Ohta 1995b), suggesting that the production of traditional fish sauce is not very hygienic. Recently, fish sauce consumption has been epidemiologically linked to esophageal cancer in China (Ke et al. 2002). A consumption of around 30 mL/capita/day of fish sauce in Changle county, the highest risk area for stomach cancer in China, is significantly correlated to mortality associated with stomach cancer. Traditional fish sauce, not the commercial fish sauce, had been tested to produce *N*-nitrosomethylurea (NMU) after the sauce is nitrosated at pH 2.0. The production of NMU in the stomach following the administration of fish sauce to experimental pigs and human volunteers has been found to be the cause of the cancer (Deng and Xin 2000). It is believed that fish sauce itself does not contain carcinogenic agents. However, the association between fish sauce consumption and esophageal cancer remains obscure as there is no obvious reason for this. It may be expected that fish sauce may have a profound effect on the metabolism of many hormones, metabolic intermediates, toxic chemicals, and carcinogens that may enhance the effect of environmental carcinogens such as those contained in tobacco tar (Ke et al. 2002). Histamine content of fermented fish products is also a major concern for consumption. Therefore, salt content, the presence of pathogenic microorganisms, the presence of harmful biogenic amines such histamine, and the presence of carcinogenic agents can inhibit the consumption of fermented fish products as a staple food. Other factors such as taste and personal perception can also play a role as determining factors.

10.8 Improving the Quality of Fermented Fish Products

In the light of tackling the safety issues mentioned above, research has been carried out to improve the quality of fermented fish products. This includes the use of five species of LAB, i.e., *L. plantarum* CCRC10069, *L. lactis* spp. *lactis* CCRC 12315, *L. helveticus* CCRC14092, *P. pentosaceus* L, and *P. pentosaceus* S, to produce fish sauce without the addition of salt. The results show that the fish sauce produced in this experiment improved the color and sensory quality of the product, having a buttery and custard-like appearance rather than the normal fish sauce (Yin et al. 2002). Research with *ngari*, *hentak*, and *tungkap* microflora also indicated that the LAB presence in the products indicate the potential of adhesion to gut epithelial cells of the human intestine, advocating their probiotic character (Thapa 2002, Thapa et al. 2004).

10.9 Conclusion

Fermented fish products are common food ingredients used worldwide that possess disadvantages which include unhygienic preparation, limited perception due to high salt content, color, flavor, and aroma, as well as histamine content and the

presence of carcinogenic compounds or their precursors. Some alteration in the production of fermented fish products is needed to enhance their quality and acceptability. Washing raw material before proceeding with fermentation will reduce or eliminate pathogenic microorganisms; hence, more hygienic and safe products can be produced. Salt-reduced products or production of products without the use of salt can be achieved through the use of multiculture LAB. This has been carried out successfully with a combination of five LAB species (Yin et al. 2002). LAB-aided fermentation can also address issues related to color, flavor, and aroma, as their products have better functional properties. The use of washed fish mince, containing less water-soluble materials and compounds such as blood, trimethyl amine oxide (TMAO), urea, and their derivatives as well as free amino acids, in combination with LAB is expected to produce fermented fish products such as fish sauces and pastes with better color, flavor, and aroma, as well as low histamine contents and other undesirable compounds.

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11

Fermented Meat Products

Martin Adams

CONTENTS

11.1 Introduction

Meat is highly nutritious but, in its fresh state, is perishable and can be an agent for the transmission of a range of infections and intoxications. Not surprisingly, therefore, meat was a subject of the earliest conservation methods developed by humankind. These depended largely for their effect on the reduction of water activity (a_w) through removal of available water by drying and salting. This had the effect of controlling growth of normal spoilage microflora associated with the meat but did not necessarily eliminate microbial growth entirely. In the absence of the normal spoilage bacteria, lactic acid bacteria (LAB) in particular were able to grow, especially where there was a moderate reduction in a_w. Their growth enhanced the preservative effect of the reduced a_w and improved the sensory properties of the product in terms of texture and flavor. They also contributed to improved safety compared with the fresh raw materials. Products such as raw hams are sometimes described as fermented meats, although in this case the process is largely enzymatic; any bacterial metabolism involved is not fermentative and its effect can usually be reproduced by replacing nitrate with nitrite in the curing salts. These will not be discussed in detail here.

11.2 History

A number of authors have discussed the origin of fermented meats in antiquity (Pederson 1971, Adams 1986, Zeuthen 2007). It appears that an important point of origin was in the Mediterranean region where the process was particularly favored by the warm, dry climate. Fermented meats are usually presented in the form of a sausage,

FIGURE 11.1 *Nham*: a fermented pork sausage from Thailand.

a name that is derived from the Latin *salsus*, which means salted. Some authors have associated the term salami with Salamis, an ancient city on the Mediterranean island of Cyprus, although it seems more likely that the etymology is related to the Italian *sale* (salt). Historically, several advances in food processing such as canning and irradiation have been closely associated with military needs, and this seems to have been a factor in the development and dissemination of fermented meats. The advantages of high nutritional value, good keeping qualities, and portability made dried sausages an important part of the Roman legionaries' rations as they marched throughout Europe (Shephard 2000). Production of fermented meats seems to have spread from the Mediterranean to Northern Europe from where migration established production in the United States, South America, Australia, and elsewhere (Adams 1986). While this probably represents the main current in the history of fermented meats, it is seldom the case that good ideas occur entirely in a single place and there are products such as the undried, fermented sausage *nham* (Figure 11.1) and other products from Thailand (see later); *urutan*, a Balinese traditional fermented sausage (Antara et al. 2002); and salami-type sausages produced in several regions of China that clearly suggest the independent development of related products elsewhere.

11.3 Classification

Fermented sausages comprise chopped or ground meat that is mixed with other nonmeat ingredients such as curing salts, spices, and flavoring components, and allowed to undergo a lactic fermentation in the course of a drying process. In its essentials, the production of fermented sausage is similar to that of cheese making; both involve salting, drying, and lactic acid fermentation and the products are generally eaten without cooking. Based on these relatively simple processing steps and widespread artisanal production, numerous fermented meats have been developed, in many ways comparable to the plethora of different cheeses available throughout the world. As with cheeses, the most useful system of classification for such a diversity of products is based on their moisture content giving rise to three groups of products: dried, semidry, and undried. Examples of these and some of their key characteristics are given in Table 11.1. Further subdivision is possible based on additional aspects of processing such as whether the product is smoked and/or mold ripened (Lücke 1998).

TABLE 11.1

Classification of Fermented Sausages

Designation	Examples	Moisture Content (%)	Moisture Loss (%)	Moisture: Protein	a_w
Dry (sometimes smoked)	Pepperoni, Milano salami, Genoa salami, *saucisson sec* (France)	25–40	25–50	<2.3:1	0.85–0.86
Semidry (usually smoked)	Summer sausage, *cervelat*, *Thuringer*, *chorizo* (Spain, South America, Philippines), *meguez* (N. Africa), *soudjouk* (Turkey)	40–50	15–30	2.3–3.7:1	0.92–0.94
Undried (spreadable)	*Teewurst*, *Mettwurst, Braunschweiger*, *nham* (Thailand)	50–60	10		0.95–0.96

Source: Adapted from Adams, M.R., *Progr. Ind. Microbiol*., 23, 159, 1986.

An account of the fermented foods of Thailand (Phithakpol et al. 1995) describes a number of different fermented meat products. These usually employ beef or pork, and, as with many European-style products, the slight differences in formulation or processing gives rise to a number of different products (Table 11.2). One notable feature of some of these products is their relatively low fat content compared with European fermented meat products.

TABLE 11.2

Some Fermented Meat Products of Thailand

Local Name	Ingredients	Packaging	Fermentation Period	Storage Life (Ambient Temperature)
Mam-neua (beef) *Mam-moo* (pork)	Ground meat, liver, salt, minced garlic, ground roasted rice, cooked rice	Small intestine or stomach	3–4 days	1 month
Naang-neua (beef) *Nang-moo* (pork)	Pork or lean beef, salt, sugar or honey, fermented bamboo shoots or fresh core of banana stem	Jars	5–10 days	1 month
Nham	Red pork meat, pork rind, garlic, salt, cooked glutinous rice, pepper, whole bird chilli, KNO_3	Banana leaf or plastic casing	3–4 days	1–2 weeks
Sai-krork-prieo	Pork meat, fat and rind, cooked rice, salt, sugar, pepper, spices	Pig intestine	2–3 days	1 week
Som-neua	Ground beef, salt, cooked rice	Banana leaf or bowl	3–4 days	1–2 weeks

Source: Adapted from Phithakpol, B. et al., *The Traditional Fermented Foods of Thailand*, ASEAN Food Handling Bureau, Malaysia, 1995.

FIGURE 11.2 Yak *kargyong* (Himalayan sausage prepared from yak meats). (Photo courtesy of Dr. Jyoti Prakash Tamang.)

The Himalayan people have a variety of traditionally processed smoked, sun dried, air dried, or fermented meat products including ethnic sausages from yak, beef, pork, sheep, and goats (Tamang 2010). Yaks (*Bos grunniens*) are reared in alpine and subalpine regions between 2100 and 4500 m altitude in the Himalayas for milk products and meat (Tamang 2005). Some common as well as some less well-known traditionally processed ethnic meat products of the Himalayas are *kargyong* (Figure 11.2), *satchu*, *suka ko masu*, *kheuri*, *chilu, chartayshya*, *jamma*, and *arjia* (Rai et al. 2009). These are naturally cured without starter cultures or the addition of nitrites/nitrates.

11.4 Preparation

The ingredient mix plays a crucial role in determining the distinctive qualities of a particular product and numerous variations in formulations exist. Typically for a European-style fermented meat, it would comprise

Lean meat	55%–70%
Fat (lard)	25%–40%
Curing salts	3%
Fermentable carbohydrate	0.4%–2%
Spices and flavoring	0.5%
Others (starter culture, acidulant, ascorbic acid)	0.5%

In principle, the meat from any animal could be used to produce a fermented product. There are reports of exotica from sources such as horse, deer, ostrich, and yak (Tamang 2005, Capita et al. 2006, Vural and Özvural, 2007) but pork, beef, mutton, and poultry are the most common. The meat and fat are minced or chopped prior to mixing with the other ingredients in a bowl chopper. These operations are best performed at low temperatures (about −4°C) to avoid smearing of the meat particles

with fat. In most European-style fermented meats, failure to do so would give an aesthetically undesirable appearance since discrete meat and fat particles would not be discernable. Covering meat particles with fat may also impede the drying process and give rise to microbiological problems subsequently. Curing salts are generally a mixture of two or three different salts and play a major role in directing the fermentation process, and ensuring good texture, flavor, and color in the product. The most abundant of the salts is sodium chloride. At least 2% is required to give the product its necessary gel texture by solubilizing the myofibrillar proteins in the meat during initial mixing. Sodium chloride also restricts the growth of the normal meat spoilage flora, which is largely comprised of relatively salt-sensitive gram-negative organisms such as pseudomonades, while allowing growth and acid production by the more halotolerant LAB. In addition, it is an important component of the product flavor.

The beneficial role of nitrate or nitrite in curing appears to have been a fortuitous discovery as a result of using salt (sodium chloride) contaminated with potassium nitrate. It was later found that nitrate acts simply as a reservoir from which nitrite can be produced by bacterial reduction so sodium nitrite or a mixture of sodium nitrite and potassium nitrate are often used now. The nitrite produces the characteristic red color of cured meats following reduction to nitric oxide, which complexes with the red meat pigment myoglobin to produce nitrosomyoglobin. It contributes to flavor by acting as an antioxidant and has a significant effect on the safety of the product by inhibiting the growth of a range of pathogenic organisms, most particularly *Clostridium botulinum*, *Listeria monocytogenes*, and *Salmonella*.

Nitrite itself is toxic and so the levels used are strictly regulated. In the EU, the amounts of nitrite and nitrate that can be used in meat curing are currently restricted to 150 mg/kg potassium nitrite and/or 150 mg/kg sodium nitrate (European Commission 2006). However, the level of nitrite found in products declines markedly during ripening and storage due to a number of reactions competing with those already described such as addition reactions with amino acids and unsaturated fatty acids, and oxidation. The risk that nitric oxide could react with secondary amines leading to the production of carcinogenic *N*-nitrosamines has attracted considerable attention. Under current conditions of practice, this does not appear to be a matter for concern. The increasingly common ingredient, ascorbate, inhibits nitrosamine formation which in any event appears low, as indicated by a number of product surveys (Adams 1986, Silla-Santos 2001, Honikel 2007).

A wide array of different herbs and spices are included at the mixing stage, the precise make up of this mixture depending on the product type. Common ingredients would be mustard, black pepper, garlic, nutmeg, pimento, mace, and coriander. They are added primarily as flavoring ingredients and as such play an important role in conferring the particular character of individual products; for example, the smoked paprika added to the Spanish or Portuguese *chorizo* or the bird peppers added to *nham* in Thailand. However, it is now recognized that they do make other contributions to the keeping quality of the product. They often contain a range of phenolics, many of which will have antioxidant properties. These and other metabolites present in spices and herbs also have antimicrobial effects that can contribute to the inhibition of the normal spoilage flora while allowing the generally less-sensitive LAB to grow. The antimicrobial activity of herbs and spices has been a subject of considerable research effort in recent years, but, in the most part, this has been restricted to descriptive studies on the effect of individual products and their effect on particular

organisms, generally pathogens (see, for example, Nychas and Skandamis 2003). More detailed knowledge will be required before this activity can be used in a predictable and directed way. If herbs or spices are used as the native plant material rather than derived oleoresin preparations then this can further benefit the fermentation. Extracts of clove, cardamom, ginger, celery seeds, cinnamon, and turmeric, all with relatively high manganese content, have been shown to stimulate lactic acid fermentation, an effect which increases with increased manganese content (Zaika and Kissinger 1984). It is thought that manganese helps protect LAB, lacking the enzyme superoxide dismutase, from the damaging effects of reactive oxygen species such as superoxide (Archibald and Fridovich 1981).

Once the ingredients are mixed, the sausage batter, as it is sometimes called, is packed into its casing. Traditionally, natural collagen from the intestinal tract of animals is used, but this has been increasingly replaced by regenerated collagen casings produced from the corium layer of cattle hides. In addition to possessing the desirable properties of being permeable to moisture to allow drying, being able to adhere to the sausage mix so that the casing shrinks with the drying sausage, being permeable to smoke, and being digestible, regenerated collagen casings have the advantage of relative abundance of supply and uniformity of properties compared to intestinal casings. Casings made from cellulose produced from wood pulp may sometimes be used as these possess very similar properties, although they are indigestible and have to be removed before the product is consumed. *Nham* is packed in plastic film and/or banana leaves (Figure 11.1).

European fermented sausages tend to be fermented at lower temperatures and for longer periods than those produced in the United States. This can be as low as around 10°C for some Hungarian and German sausages (Lücke 1998) but is usually below about 26°C. The higher fermentation temperatures in the United States (27°C–38°C) give a faster fermentation, although there is an increased risk of pathogen growth occurring should it be present. The relative humidity during fermentation is generally 85%–90%, 10% lower than the equilibrium relative humidity (ERH) of the sausages. Care is taken to match the rate of moisture loss from the surface of the sausage to migration of water from within the sausage so that case hardening does not occur. After the fermentation or ripening stage, which typically lasts 1–3 days, the sausage is dried at 7°C–15°C for up to 6 weeks at a relative humidity dropping from 85% to as low as 65%, depending on the target moisture content of the product. In a fermented meat such as *nham*, which does not undergo drying, the fermentation takes place at around 30°C for 3–5 days.

11.5 Microbiology—Functional Microorganisms and Safety

In meat fermentations, the selective effect of the environment and processing conditions is vitally important in ensuring a satisfactory fermentation. Many products still rely on this entirely to ensure the growth of the required organisms from within the natural microflora of the ingredient mix. This can be assisted by a procedure known as back-slopping where mix from a previous successful batch is retained and added to the new batch to ensure the introduction of a substantial, healthy inoculum. Even where commercial starter cultures are used, the selectivity of the environment plays a significant role since, unlike in many fermented dairy products, it is not possible

to pasteurize the raw materials to eliminate competitors. In terms of the microflora associated with successful fermentations, LAB are probably preeminent since they are invariably involved in the production of all the fermented meats described here, and increase to levels of around 10^8 cfu/g during the course of fermentation. Other groups of organisms are important in some products as described below but generally achieve levels in the range 10^4–10^6 cfu/g. Numerous studies have been conducted identifying the particular species of LAB associated with different fermented meats. *Lactobacillus* (*Lb*) species are dominant at the end of fermentation with the most common species encountered being *Lb. sake*, *Lb. curvatus*, and *Lb. plantarum* (Leroy et al. 2006). With nitrate cures, which are nowadays more common in Europe than in the United States, gram-positive, catalase-positive cocci such as *Staphylococcus xylosus*, *Staph. carnosus*, *Staph. saprophyticus*, and *Kocuria varians* (formerly known as *Micrococcus varians*) are important for the reduction of nitrate to nitrite. They will also produce a number of significant aliphatic flavor compounds through the degradation of amino acids and fatty acids (Stahnke 1999, Stahnke et al. 2002). These organisms would normally be most active at the start of fermentation and tend to be inhibited as the LAB begin to dominate and the pH declines.

Of the fungi, yeasts are commonly encountered in fermented sausages but their numbers are usually relatively low. They do, however, appear to contribute to the product flavor and decrease its acidity. Molds are particularly suited to aerobic conditions, and reduced pH and water activity at the surface of a fermented sausage, and mold ripening by *Penicillium*, *Aspergillus*, *Mucor*, *Eurotium*, and *Cladosporium* species is a feature of some products. This is especially true for those from Southern Europe that are ripened for long periods and not smoked, where a white covering of mold is a characteristic feature. Studies of naturally mold-ripened sausages have sometimes reported isolation of potentially mycotoxigenic fungi (Leistner 1984, Larson et al. 2001). In one recent study of Italian sausages, about 45% of 160 samples examined were positive for the presence of the mycotoxin ochratoxin A. The toxin was associated with the surface casing; none was detected in the meat itself and the toxin could be removed by brushing or washing the sausages (Iacumin et al. 2009). Concern over the possibility of mycotoxin production has prompted the more widespread use of nontoxigenic mold starter cultures in those products where mold ripening is required. These mostly employ *Penicillium* species (the genus most frequently associated with naturally ripened products) such as *P. nalgiovense* (Leistner 1990). Where surface mold growth is not required it can be prevented by smoking, dipping in sorbate or natamycin (pimaricin), or by vacuum packaging.

Bacterial starter cultures have been used in fermented meats for about 50 years (Adams 1986, Jessen 1995). Freeze-dried *Pediococcus acidilactici*, a strong acid-producing strain, was introduced in the United States as being particularly suited to the quicker, higher temperature fermentations employed there, whereas the first starter culture introduced in Europe was a *Micrococcus* (now *Kocuria*) primarily for nitrate reduction. Since then, more subtle combinations of starters have been introduced and deep-frozen products are available that simplify handling and give a more rapid initiation of fermentation. The benefits of starter use have been demonstrated on numerous occasions and for many product types including the Turkish *soudjouk* and Thai *nham* (Kaban and Kaya 2006, Visessanguan et al. 2006). In addition to *Pediococcus* species, which are generally not commonly found in naturally fermented meats, *Lactobacillus* species such as *Lb. plantarum*, *Lb. sake*, and *Lb. curvatus* are

also available. These are often derived from characteristic isolates of natural fermentations. They are classed as facultatively heterofermentative lactobacilli that breakdown hexoses, such as glucose, homofermentatively to produce mainly lactic acid but are also capable of heterofermentation of pentoses via phosphoketolase when glucose levels are depleted to produce a mixture of lactic and ethanoic acids. Gram-positive, catalase-positive cocci such as *Staph. carnosus*, *Staph. xylosus*, and *Kocuria varians* are also available to reduce nitrate to nitrite, giving rise to the desired color and other effects, but their catalase activity also helps stabilize the color against oxidative destruction by peroxide.

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 effect seen is the aggregate result of a number of antimicrobial factors. In the case of fermented meats, the keeping quality and safety depend 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 have attracted considerable attention since their acceptability as food additives would make any antimicrobial effect they achieve acceptable too.

The antimicrobial activity of LAB has been periodically reviewed often with much attention focused on the relatively minor antimicrobial factors (Adams 2001). However, the central feature common to all LAB is their ability to produce organic acids and decrease the pH as an inevitable consequence of their growth. This is their most important contribution to meat fermentation where, at some stage of the process, they decrease the pH, typically, to a value around 5.2 (although this varies considerably between different products). The rapid fermentation, pH drop, and concurrent decline in the numbers of thermotolerant coliforms in the *nham* fermentation are illustrated in Figure 11.3. One antimicrobial factor other than organic acid that, if not

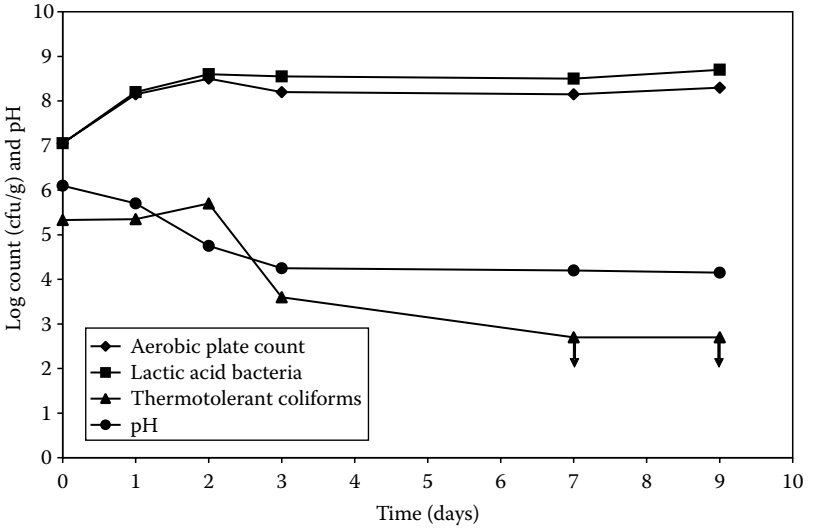

FIGURE 11.3 *Nham* fermentation (arrows indicate < limit of detection). (From Adams, M.R., unpublished.)

generally significant in meat fermentations, may be important in particular cases and may be worth incorporating more widely in starter cultures is the ability to produce bacteriocins. Bacteriocins are proteins or polypeptides produced by bacteria that are inhibitory, usually to closely related species. The precedent for using a bacteriocin in fermented foods comes from the accepted and widespread use of nisin, a bacteriocin produced by strains of *Lactococcus lactis*, in products such as canned foods and processed cheese. Nisin is unusual in that it has a wider range of activity than most bacteriocins since all gram-positive bacteria are sensitive and bacterial endospores are particularly so. Nisin and other bacteriocins with similar properties could be expected to have a protective effect against gram-positive pathogens in fermented meats such as *L. monocytogenes*, *Staph. aureus*, and *Clost. botulinum*, though they would of course inhibit other gram positives such LAB and micrococci that might play some positive role in the fermentation. There have been reports of the relative benefits of adding nisin to fermented sausages (Hampikyan and Ugur 2007) and numerous reports of the production of bacteriocins by LAB isolates from fermented meats (see, for example, Tichaczek et al. 1992, Garriga et al. 1993, Rattanachaikunsopon and Phumkhachorn 2006, Belgacem et al. 2008, Xiraphi et al. 2008). This has also led to the employment of bacteriocin-producing starters in sausage fermentations and the demonstration of useful inhibition of *Listeria* species (Leroy et al. 2006, Ammor and Mayo 2007).

Though some fermented meats are given a final pasteurization treatment to eliminate any pathogens that may be present, many rely entirely on the antimicrobial hurdles described above to assure safety. The epidemiological data on outbreaks caused by fermented meats suggest that three bacterial pathogens are the major concerns: *Salmonella* (Taplin 1982, Van Netten et al. 1986, Cowden et al. 1989), *Staph. aureus* (Barber and Deibel 1972, Metaxopoulos et al. 1981, Warburton et al. 1987), and verotoxin-producing *Escherichia coli* (VTEC) (Anon 1995a,b, Conedera et al. 2007, Sartz et al. 2008). A similar conclusion was drawn from a hazard identification exercise conducted for fermented meats by Beumer (2001). This is further supported by surveillance data from two independent surveys conducted in the United Kingdom in the late 1990s, which showed remarkably similar findings. VTEC was not detected in a total of almost 3500 independent samples, although *Salmonella* was found at similar levels in both surveys; 2 out of 2981 samples in one and 1 out of 455 samples in the other. The levels of *Staph. aureus* were greater than 10^2/g in 1.3% and 1.1% of samples in the two surveys (Adams and Mitchell 2002). Although considerable research effort has been devoted to *L. monocytogenes* in fermented meats, there appear to have been no outbreaks reported. Only one of the two surveys described above looked for *L. monocytogenes*, and it was detected in 3% of 455 samples but only at levels below 10^2/g (Adams and Mitchell 2002). As a result of the outbreaks of VTEC associated with fermented meats, the U.S. Food Safety Inspection Service required producers to validate that their production process could achieve a 5-log reduction in viable numbers of *E. coli* O157. This was based on a worst case scenario, and several products were unable to achieve this goal with their existing process (Beumer 2001). A review of the results of these trials concluded that if *E. coli* O157:H7 is present in sufficient numbers, it is able to survive a range of fermentation and drying processes. Typically the reduction achievable in most processes was 2–3 log cycles and it could be less in some cases. The European fermentation method with its longer fermentation at a lower temperature gave better reductions as did high salt, high nitrite, and low pH (Getty et al. 2000).

TABLE 11.3

Composition of European Salamis Retailed in United Kingdom

Country of Origin	Protein (g/100 g)	Fat (g/100 g)	Carbohydrate (g/100 g)	Sodium (mg/100 g)	Energy (kJ/100 g)
Denmark	13.4	49.7	2.2	1840	2102
France	21.0	37.4	1.9	1700	1771
Germany	20.7	31.5	2.6	1500	1559
Italy	23.4	30.7	0.9	1335	1548

Source: Data from Food Standards Agency, *McCance and Widdowson's The Composition of Foods*, 6th Summary edn., Royal Society of Chemistry, Cambridge, U.K., 2002.

11.6 Nutritional Aspects

The composition and nutritional value of fermented meats has been discussed recently (Demeyer 2007). Though they offer a range of valuable nutrients and vitamins, fermented meats also enjoy the negative aspects of high fat and salt contents, which are associated with cardiovascular diseases, hypertension, and a number of other conditions common in relatively affluent societies (Table 11.3).

In some cases, there are options for reducing the level of these components. Reformulation to achieve lower fat by adopting products such as *nham* as a model is one obvious approach for European and North American fermented meat producers. Improving the quality of the fat used by using fat from animals fed a diet to increase unsaturated fat levels has been tried, although the product was more susceptible to oxidation and the development of rancidity. Other trials using vegetable oils rather than animal fat as an ingredient seem to have enjoyed slightly more success (for a review, see Ansorena and Astiasarán 2007). The critical role that salt plays in the texture, flavor, and microbiological stability of fermented meats appears somewhat more difficult to replace. Decreasing salt will result in losses of these qualities, and the use of alternatives such as potassium chloride and potassium lactate has enjoyed limited success (Ansorena and Astiasarán 2007). On a more positive note, the potential of fermented sausages as a vehicle for probiotic bacteria has been investigated, although their potential health benefits remain to be fully clarified. It may be that established starter organisms used in meat fermentations already possess probiotic potential. Alternatively, it might be possible to incorporate established probiotic strains in fermented meats (Leroy et al. 2006, Ammor and Mayo 2007, Ansorena and Astiasarán 2007). They might then play some useful part in the fermentation process or the product could simply act as a carrier for the probiotic strain and help protect it from stomach acidity in its passage through the gut.

11.7 Conclusion

Fermented meats originated independently in a number of regions around the world, although European-style products now predominate in terms of their scale of production and the degree to which they have been subject to scientific scrutiny. The original

objective of meat fermentation was to preserve an otherwise very perishable product, but with the advent of alternative preservation methods the distinctive sensory properties of the products have assumed greater importance, particularly in the developed world. Studies conducted on fermented meats from other regions frequently reveal their similarity to other, better-known products in terms of their microbial composition and the changes that take place during fermentation/drying. However, in certain key areas such as nutritional and sensory properties, there may be unique aspects which we can learn from and possibly apply more widely in the future.

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12

Ethnic African Fermented Foods

N. A. Olasupo, S. A. Odunfa, and O. S. Obayori

CONTENTS

12.1 Introduction

Traditional fermented foods (TFF) 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 concentrated on the processing technology including the machinery for producing them. Very little

was known about the microbiology or the chemistry of the processes. However, the last 20 years have witnessed an explosion of knowledge in the science of TFF. Many conferences and workshops have been organized on them and important findings have been reported (Halm and Jakobsen 1996). The importance of fermented foods in the African food culture is 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 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 anticholesterolemic effects (Dike et al. 2001), viricidal effects (Gilbert et al. 1983), and antitumor and antileukemic effects (Esser et al. 1983, Shabani et al. 1983). Decreased toxicity has also been reported for some products (Odunfa 1985a,b). Some of these foods have been found to provide useful probiotic effects when they are directly consumed (Mathara et al. 2008a,b).

12.2 History

Fermented foods have been part of the food culture in Africa for many centuries. Evidence for these is from the records of Arab travelers, mostly merchants and geographers, who traveled southward to west and central Africa, and also from archaeological findings. In the period between AD 996 and 1393, records of various kinds of beer made from sorghum were made by Arab travelers in different kingdoms that coincide with present day Ghana, Mali, Senegal, and northern Nigeria (Odunfa and Oyewole 1998). Apart from beer, sour milk production was also widespread particularly in northern Africa and records of these were made for Mali (AD 1392–1393), the people of Walata, an ancient oasis town in present day Mauritania and part of present day Sudan and Morocco (Tantaoui-Elaraki et al. 1983, Dirar 1991). Production of beer (*merissa*) and wine in the kingdom of Meroe, site of present day Sudan, were portrayed by wall drawings from the era 690 BC–AD 323 (Dirar 1993). Special utensils for making the staple *kisra* bread were discovered in archaeological sites, which were traced back to 550 BC. From this era, detailed drawings of wine presses were excavated (Adams 1966).

12.3 Classification

For the purpose of convenience, fermented foods in Africa can be classified on the basis of the substrates used for their preparations or the nature of the finished products into the following major groups (Olasupo 2006):

1. Fermented nonalcoholic cereal substrate
2. Starchy root crops
3. Fermented animal proteins
4. Fermented vegetable proteins
5. Alcoholic drinks

Information on details of the different fermented foods of Africa is shown in Tables 12.1 through 12.5. Many fermented cassava (*Manihot esculenta* Crantz) products are taken as a major part of the meal in Africa. These can be classified into six groups, namely, fermented cassava flour (*lafun*, *kanganga*, *luku*), roasted cassava grits (*gari*, *agbelima*, *kapok*, *popogari*), fermented cassava mash (*fufu*, *akpu*), steamed and fermented cassava chips (*chikwangue*, *ntuka*), fermented steamed cassava grits (*atieke*), and smoked fermented cassava balls (e.g., *pupuru*).

TABLE 12.1

Fermented Nonalcoholic Cereal-Based Foods of Africa

Product	Area of Production	Substrate	Microorganisms
Ogi	Nigeria, Benin	Maize, sorghum, or millet	*Lactobacillus* sp. and yeast
Koko and *kenkey*	Ghana	Maize, sorghum, or millet	*Lactobacillus* sp. and yeast
Mahewu (*magou*)	South Africa	Maize, sorghum, or millet	*Lactobacillus delbrueckii* *Lactobacillus bulgaris*
Uji	East Africa	Maize, sorghum, or millet	*Lactobacillus* sp.
Kisra	Sudan	Sorghum	LAB
Injera	Ethiopia	Sorghum	*Candida guillermondii*
Ting	Botswana	Sorghum	LAB
Obusera	Uganda	Millet	LAB
Mawe	Benin	Maize	*Lactococcus lactis* *Pediococcus pentsaceus* *Lactobacillus plantarum*
Bogobe	Botswana	Sorghum	Unknown
Kunu-Zaki	Nigeria	Millet, sorghum	LAB

TABLE 12.2

Fermented Starchy Root Products of Africa

Product	Area of Production	Substrate	Microorganisms
Gari	West Africa	Cassava	*Streptococcus lactis*, *Geotrichumcandidum*, *Corynebaterium manihot*, LAB
Lafun	Nigeria	Cassava	Yeast, LAB
Fufu	Nigeria	Cassava	LAB
Chikawngue	Zaire	Cassava	Yeast, LAB
Cingwada	East and Central Africa	Cassava	Unknown
Kocho	Ethiopia	Ensette or Abyssinian banana (*Ensette ventricosum*)	LAB, yeast
Kivunde	Tanzania	Cassava	LAB, yeast
Agbelima	Ghana	Cassava	*Lb. plantarum*, *Bacillus* sp. *Candida tropicalis*, *Geotrichum candidum*, *Penicillium* sp.

TABLE 12.3

Fermented Animal Proteins of Africa

Product	Area of Production	Substrate	Microorganisms
Maziwa lala	East Africa	Milk	*Streptococcus lactis*, *S. thermophilus*
Nono (milk curd)	Northern part of West Africa	Milk	LAB
Guedj	Senegal	Fish	*Lactococcus lactis*
Bonome (stink fish)	Ghana	Fish	Unknown
Leban (sour milk)	Morocco	Milk	Lactic streptococci, *Leuconostoc lactis*, *Lc. cremoris*
Wara	West Africa	Milk	*Lactococcus lactis*, *Lactobacillus* sp.
Ergo	Ethiopia	Milk	*Lactobacillus* sp., *Lactococcus* sp.

TABLE 12.4

Some Important Fermented Vegetable Foods of Africa

Food	Area of Production/ Consumption	Raw Material	Microorganisms
Dawadawa or *iru*	Most of West Africa especially northern parts	African locust bean (*Parkia biglobosa*), soybean	*Bacillus subtilis*, *B. licheniformis*
Ogiri	Southwestern Nigeria	Melon (*Citrullus vulgaris*)	*Bacillus* sp. (predominant), *Proteus*, *Pediococcus*
Ogiri-nwan	Southeastern Nigeria	Fluted pumpkin bean (*Telfaria occidentalis*)	*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* spp.
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 (*Gossypium lursutum*)	*Bacillus* sp.
Bukalga	Niger, Mali, Sudan, Burkina Faso	Kartade, red sorrel (*Hibiscus sabradiffa*)	*Bacillus subtilis*

12.4 Methods of Production

In the preparation of most fermented foods, the raw materials used include cereals, root crops (cassava), milk, legume seeds, and oil seeds. However, the use of cereals and cassava accounted for a large number compared to other raw materials in the production of indigenous fermented foods (Oyewole 1997). Processing, which is by traditional village methods, usually involves either soaking of raw materials in water contained in a fermenting vat, usually a clay pot, for a period of time or an initial

TABLE 12.5

Fermented Alcoholic Beverages of Africa

Product	Area of Production	Substrate	Microorganisms
(1) From sugary substrates			
Palm wine	Nigeria (south)	Palm sap	Yeasts
Tej	Ghana	Honey	Yeasts
(2) From starchy substrates			
Pito	Nigeria, Ghana	Guinea corn and maize	Molds, yeasts, and *Lactobacillus* sp.
Kaffir beer	South Africa	Kaffir corn or maize	*Lactobacillus* sp. and yeasts
Busaa (maize beer)	East Africa (Kenya)	Maize	Yeast and *Lactobacillus* sp.
Malawa beer	Uganda	Maize	*Candida krusei*
Zambian opaque maize beer	Zambia	Maize	Yeasts
Merissa	Sudan	Sorghum	LAB, acetic acid bacteria
Shekete	Nigeria (south)	Maize	Unknown
Bouza	Egypt	Wheat	Unknown
Talla	Ethiopia	Sorghum	Unknown
Kishk	Egypt	Wheat and milk	*Lactobacillus* sp., yeasts, and *Bacillus* sp.
(3) From other substrates			
Agadagidi (plantain wine)	Southwestern Nigeria	Plantain	Yeasts and LAB
Cacao wine	Nigeria	Cacao	Yeasts
Alcoholic spirits	Kenya, Nigeria	Molasses or cane sugar	Yeasts
Changaa (Nubian gin)	Nigeria, Ghana	Palm wine	Yeasts
Ogogoro (Akpeteshi)	Nigeria, Ghana	Palm wine	Yeasts

size reduction of the raw material by grating or milling in the wet form before being allowed to ferment (Odunfa 1985a,b, Olasupo 2006). Numerous reports are available in the literature on the methods of preparation of African fermented foods.

12.4.1 Fermented Nonalcoholic Cereal Products

Ogi is a popular traditional infant food and a major staple food in West Africa. It is produced from maize sorghum or millet grains by spontaneous fermentation. The finished product is usually eaten by cooking the sour paste in water as a porridge. *Ogi* shares similar preparation methods with *kenkey*, *banku*, and *mawe* (Figure 12.1). Production usually starts with cleaning, steeping, grinding, and spontaneous fermentation. Variation in steps, sequences, and duration generally determines the product. Among the Yoruba of southwestern Nigeria, *ogi* may be prepared by gelatinizing it into a stiff gel or made into gruel or pap that is popular among infants as a weaning food and as breakfast among adults (FIIRO 2006). *Ogi* in the form of gruel is called

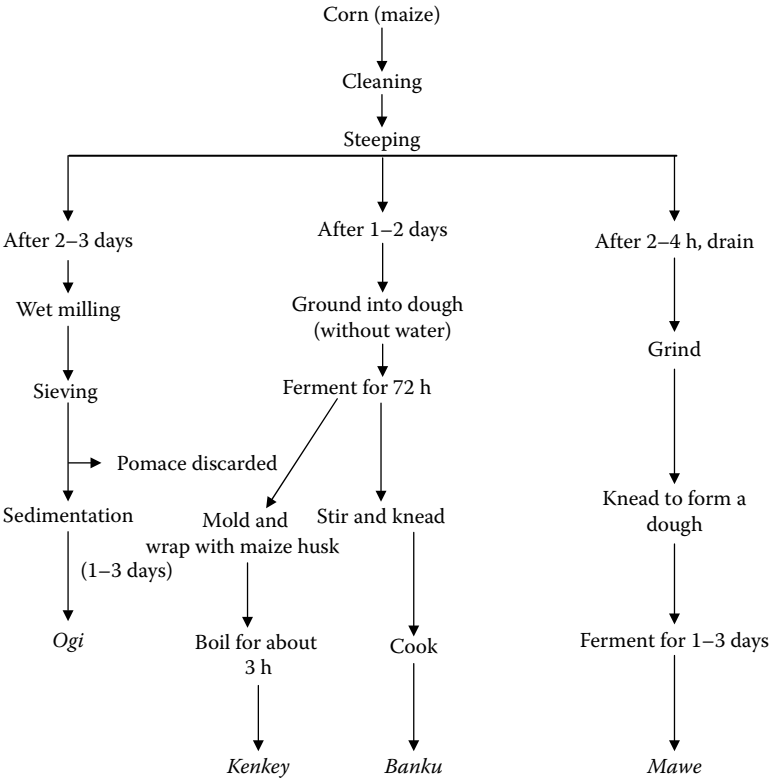

FIGURE 12.1 Flowchart for the preparation of *ogi*, *kenkey*, *banku*, and *mawe*.

akamu by the Hausa people of Northern Nigeria (Akingbala et al. 1987). *Mawe* exemplifies the variety of culinary of African fermented cereals. It is prepared in the form of *akassa* (gelatinized dough), *ablo* (steam-cooked bread), *akpan* (pregelatinized yoghurt-like product), *massa* (fritter), *pate* (fritter), *yeke-yeke* (couscous), or *aklui*, *akluiyonu*, and *koko* (porridges) (Hounhouigan et al. 1994).

12.4.2 Fermented Starchy Root Products

The flow diagrams for the preparation of *gari*, *chiwangue*, *pupuru*, and *atieke* show similarities (Figure 12.2). *Gari* is a gritty product obtained by fermenting fresh cassava roots for 72 h and then frying the dewatered mash (Akinrele 1964, Onabolu et al. 1988, Onyekwere et al. 1989) after fermentation. The processing generally involves several stages including reduction in size, fermentation, dextrinization, partial gelatinization, and tetrogradation (Oyewole et al. 2004, Abimbola 2007). The flow sheet of preparation of *agbelima* and *kivunde* is shown in Figure 12.3. *Agbelima* is a moderately high moisture content off-white fermented cassava meal extensively consumed along entire southern Ghana (Amoa-Awua 1996).

Much of the steps involved in *agbelima* production are similar to those involved in *gari* production, but *kivunde*, which is consumed in East Africa, involves different

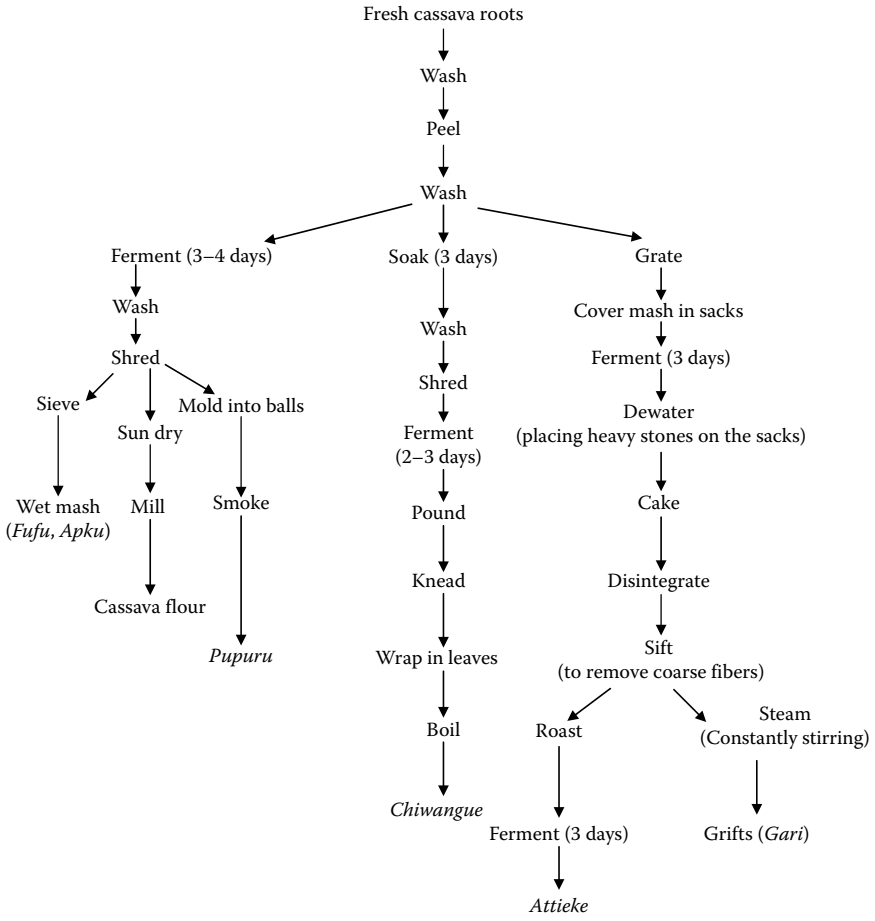

FIGURE 12.2 Flowchart of cassava processing in African fermented products.

steps (Kimaryo et al. 2000). It is not grated but rather cut into small cubes prior to fermentation. Efforts have been made to mechanize *gari* production technology (Onyekwere et al. 1989, Idowu 1990). Development has been mainly in grating, granulating, and frying operations. Another very important starchy root product is *fufu*. It is produced through the submerged fermentation of cassava roots. In an alternative method, the cassava roots are grated before fermentation, and this has been found to give a better product with enhanced consumer acceptability (Achi and Akomas 2006). But whether grated or fermented whole, the product is usually prepared wet and not fried like *gari* or *lafun*. *Lafun* is produced from cassava chunks fermented for a few days submerged in water, sun dried, and milled into flour by the Yoruba people of Western Nigeria (Okafor et al. 1984, Okolie et al. 1992). *Kokonte* in Ghana is also produced the same way (Amoa-Awua 1996, Amoa-Auwa et al. 1996). Cassava-based dishes are usually prepared in the form of a stiff porridge, by stirring the flour, grit, or mash in boiling water, and are usually eaten with vegetable soup (FIIRO 2006).

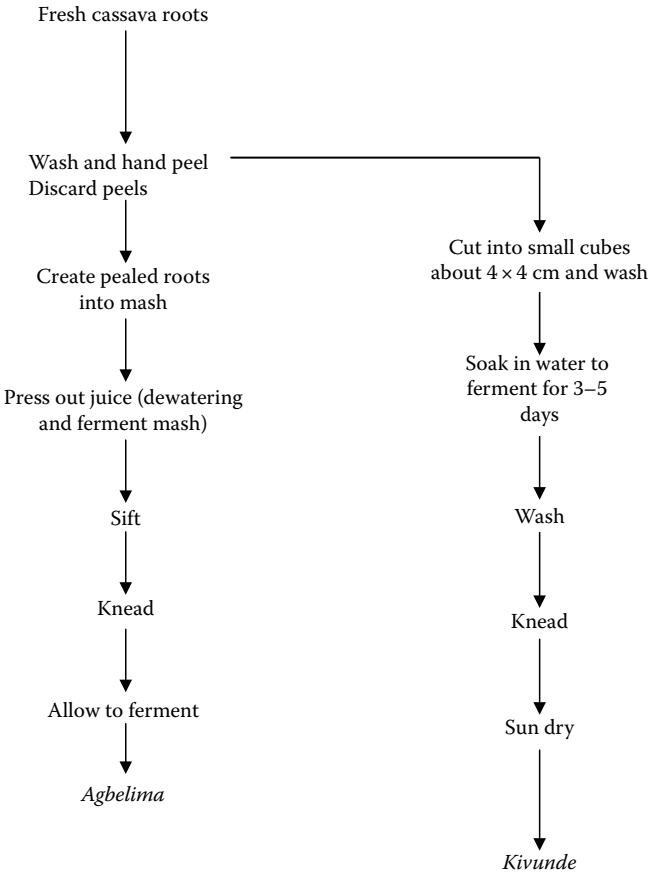

FIGURE 12.3 Flow diagram for the preparation of *agbelima* and *kivunde*.

12.4.3 Fermented Animal Proteins

Traditional fermented dairy products in Africa include different types of cheese, yoghurt, and butter with different flavor and texture and nomenclature depending on the locality. All these are usually fermented spontaneously by lactic acid bacteria present in the environment and vessel or through back-slopping. Various additives such as wood ash, animal, blood, urine, or sometimes leafy vegetables may be added depending on the custom in the area (Odunfa and Oyewole 1998, Olasupo et al. 2004). *Wara* (white cheese), *nono* (yoghurt), and *ergo*, all produced from cow's milk, and occasionally sheep's and goat's milk, show marked variation in their production methods (Figure 12.4). According to Gonfa et al. (2001), *ergo* is one of the several fermented milk products produced in Ethiopia by small holder farmers using traditional methods. Others are *itutu* (fermented sour curd), *kibe* (traditional butter), *neter kibe* (traditional ghee), *ayib* (cottage cheese), *arerra* (sour defatted milk), and *augat* (whey). They share a close relationship in the methods of preparation (Figure 12.5). *Kussa* is a traditional storage utensil which may be a calabash, clay pot, or hollowed wood. Smoking is done using burned leaves of selected plants. Cleaning is done at

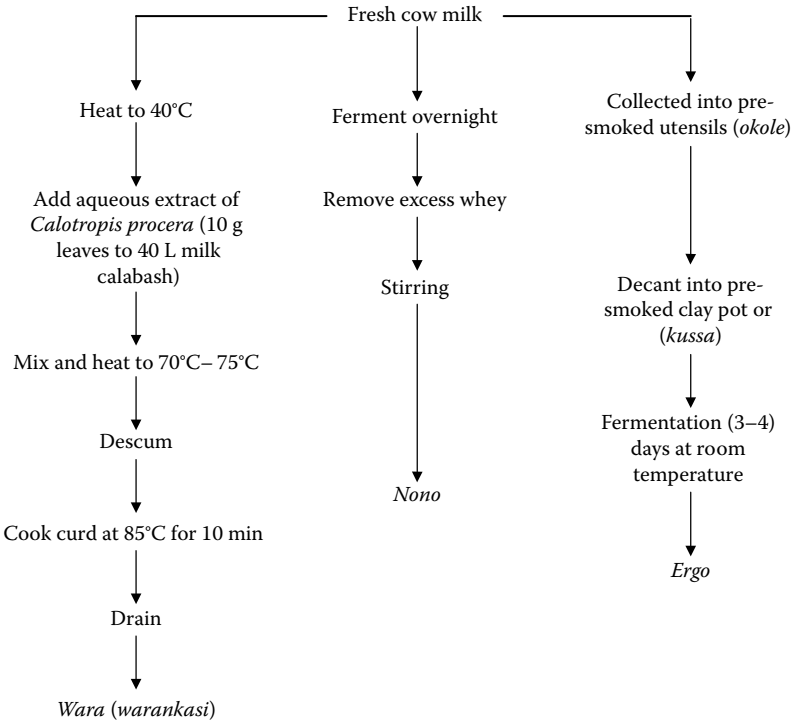

FIGURE 12.4 Flowchart for the preparation of *nono*, *ergo*, and *wara*.

room temperature using plant fiber materials commonly known as *foxso*. *Wesso* is a traditional utensil, which may be either a calabash, a large clay pot, hollowed wood, or animal skin for the purpose of churning. *Ergo* is flavored with fresh leaves of *Rita chalepensis* var. *tenuifolia*, *Coriandrum sativum*, and mixed with mashed *Allium sativum* and green *Capsicum annuum* before serving (Gonfa et al. 2001).

Another fermented dairy product that has received increasing attention in recent times is *kule naoto*, which constitutes the principal nutritional source of the Maasai people of Southern Kenya and Northern Tanzania in the East African Rift valley (Mathara et al. 2004). It is spontaneously fermented from raw milk in custom-made specially treated wooden gourd. The milk and gourd pretreatment includes addition of fresh cow's blood and rubbing the interior of the gourd with a burnt stick from an *Olea africana* tree. The dominant microorganisms are *Lactobacillus* spp. (Mathara et al. 2004, Patrigani et al. 2006, Mathara et al. 2008a). *Guedj* from Senegal (Toury et al. 1970) and *bonome* from Ghana (Nerguaye-Tetteh et al. 1978) are African fish products obtained by the putrefactive fermentation of fish by enzymes in the fish and endogenous bacteria. In both cases, fermentation is followed by drying in the sun for 2–4 days in baskets on straw mats (Odunfa and Oyewole 1998).

12.4.4 Fermented Vegetable Proteins

Fermented vegetable proteins (FVP) are fermented legumes and oilseeds that are generally used as soup-flavoring condiments. The list of major fermented legumes is shown

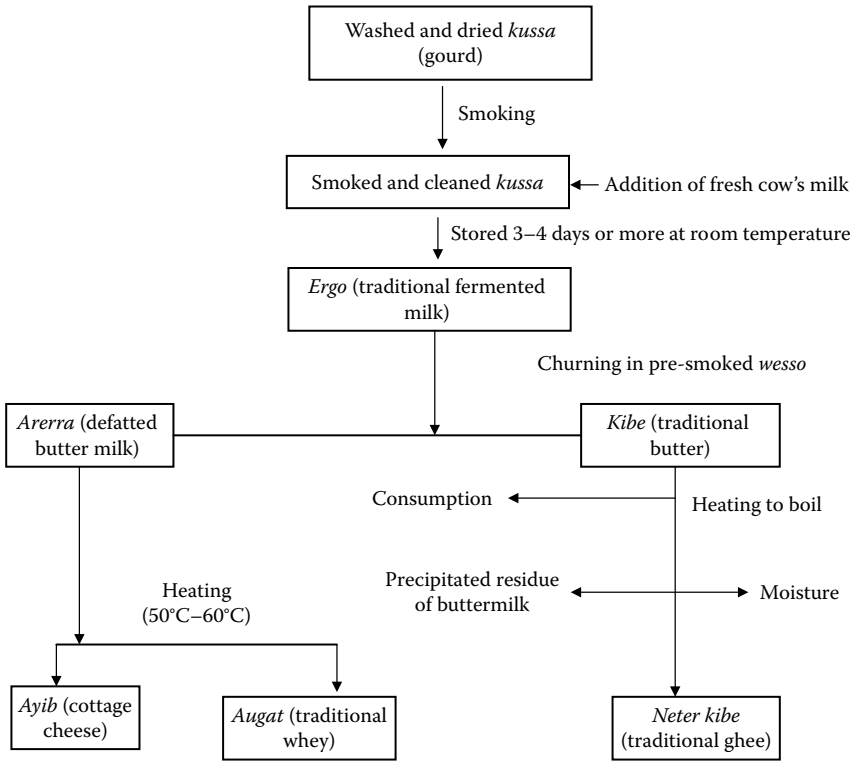

FIGURE 12.5 Flow scheme for the processing of various traditional milk products in Ethiopia.

in Table 12.4. These legumes and oilseeds of Africa are rich in proteins (Eladawy and Khalil 1994, Barminas et al. 1998); they undergo alkaline fermentation brought about by extensive hydrolysis of the protein content to amino acids and ammonia resulting in a high pH (Odunfa 1986). Most of these legumes are inedible in their unfermented state. The seeds of many of them are collected from trees growing in the bush or in places where they are concentrated, which are called *parklands*. The only cultivated seeds are herbaceous and which include soybean, melon, and fluted pumpkin. Apart from the flavoring attributes, they contribute to the protein intake of consumers. The significance of flavoring condiments can be appreciated by the fact that most of the meals in many parts of west central and southern Africa are made from starchy roots and grains and have to be eaten with soups to which these condiments are essential inputs.

The traditional method of preparation is generally laborious and time- and energy-consuming and usually carried out with rudimentary utensils. There are minor differences in different localities. However, the essential steps are similar. The steps involved are shelling/decorticating and dehulling of the seeds. The seeds are washed and then wrapped in several layers of leaves. In some other methods, they are spread in calabashes that are then stacked together and wrapped in several jute bags and left to ferment. These conditions create low oxygen tension during the fermentation. The fermentation time varies with different seeds. Generally, it varies from 48 to 120 h

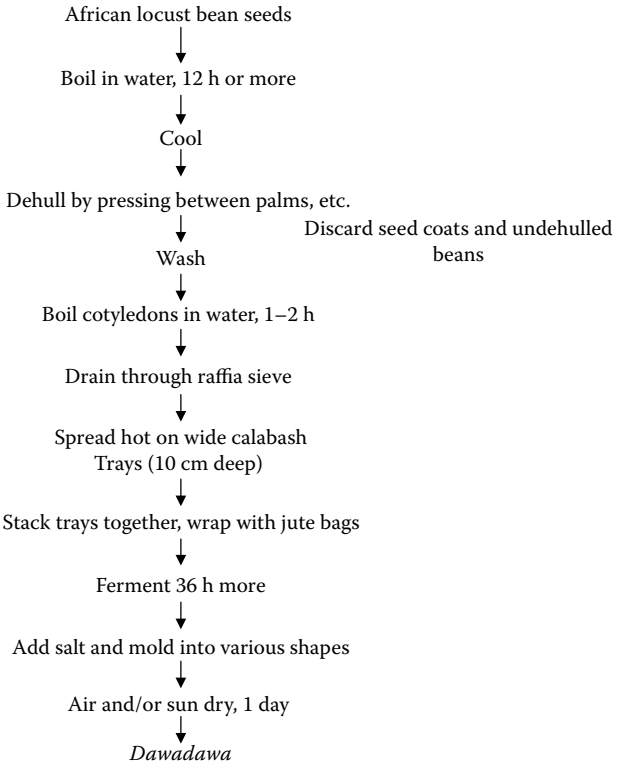

FIGURE 12.6 Flow sheet for the preparation of *dawadawa* (*iru*).

(5 days). Perhaps the most elaborate is the process of *iru* production (Figure 12.6), because the testa is hard and adheres tightly to the cotyledon; the procedure for its dehulling is tedious. It involves cooking the beans in cast iron pots for 12 or more hours to soften the seeds. The boiled seeds are transferred into a mortar and pressed by foot or pounded by the mortar (Odunfa 1986). Sand or other abrasive materials may be added to aid the removal of the testa. The dehulled seeds are washed thoroughly and the testa removed by sifting with baskets or other local sieves. The washed dehulled beans are boiled again for 1–2 h. A softening agent such as native potash rock salt (KCO_3, $KHCO_3$) may be added.

Subsequently, the hot beans are drained though baskets and spread on clean calabash trays in layers of about 4 cm thick. Two or three calabash trays are stacked together and wrapped in jute bags to provide a humid and warm atmosphere. Fermentation takes about 48 h though it may take up to 4 days in some parts of Senegal (Ndir et al. 1994). During the fermentation, the temperature of the fermenting mash rises up to 40°C from an ambient of 30°C (Odunfa 1981a). The bean surface turns from light brown to dark brown with a gray covering, and the beans become sticky. The fermented beans are mixed with salt and shaped into balls ready for sale. Another practice involves drying the beans. Some local additives may be added before drying. The dried cake has a moisture content of between 9% and 19%.

While the fresh *iru* must be sold within 3 days if not refrigerated, the dried *iru* may be stored for up to a year.

Fermentation of African locust bean differs from the other traditional fermented vegetable foods with regard to the strongly exothermic nature of the process. In all other products, there is only a slight (1°C–2°C) increase over the initial ambient temperature. Exothermic fermentation stops soon after the jute coverings are removed. The fermented beans have an ammonical smell. The fermented beans have a grayish sticky mucilage covering and a strong ammoniacal smell, much like the Japanese *natto* and Indian *kinema*. Generally, the fermentation of proteins is the only solid-state fermentation among other African fermented foods, which are in a liquid medium. The flowchart of production of *iru* or *dawadawa* from the seeds of African locust bean (Figure 12.6) is typical of fermentation of most FVP from Africa used as condiments. Characteristically, there is boiling of the cotyledon to soften it, and fermentation is by natural uninoculated solid-substrate fermentation (Campbell-Platt 1980, Eka 1980, Odunfa 1983, Odunfa and Oyewole 1998, Achi 2005).

Ogiri is produced from melon seeds (*Citrullus vulgaris*). The melon seeds are boiled until they are very soft. They are wrapped in leaves in baskets and allowed to ferment for about 5 days (Abaelu et al. 1990, Aniche et al. 1993). The fermenting mash is then smoked for about 2 h and the mash is ground. The product is an oily paste. Other condiments such as *okpehe* from the seeds of mesquite (*Prosopis africana*), *owoh* from cotton seed (*Gossypium*), *ugba* from oilbeans (*Pentaclethrae macrophylla*), *ogiri-igbo* from castor oil seed (*Ricinus communis* Schrad), and *ogiri-nwan* from pumpkin beans (*Teflaria occidentale*) are produced using variations of the above methods (Odunfa 1988, Elfaki et al. 1991, Achi 1992, Sanni et al. 2002, Dike and Odunfa 2003).

12.4.5 Alcoholic Beverages

African fermented alcoholic drinks, unlike their European counterparts, contain a mixture of alcohol and acid. The flowcharts for the production of common types such as *burukutu* and *merissa* are shown in Figure 12.7. The cereal-based types may be from maize, sorghum, or millet. They characteristically have two fermentation stages: lactic acid fermentation and alcoholic fermentation. In the case of *merissa*, produced from sorghum, there are two alcoholic fermentation stages. It is also noteworthy that since the process is spontaneous and uncontrolled, further fermentation of ethanol to acetic acid by *Acetobacter* sp. occurs. *Kaffir* is an alcoholic drink that has been developed to industrial scale production. It is made from malted sorghum and corn grits. The methods of production of other beers such as *pito*, *busaa*, *shekete*, *bauoza*, the Ethiopian *talla*, and the Sudanese *hulu nur kishk* and *agadagidi* have been well elucidated by Odunfa and Oyewole (1998). Palm wine is produced from the sugary sap of raffia palm (*Rafia hookeri*) or oil palm (*Elaeeis guineense*). The sap is collected by cutting the unexpanded flower spathe and tying a gourd to it. The juice trickles into the gourd. Fermentation of the juice takes place spontaneously in the gourd (Odunfa 1985a,b, Iwuoha and Eke 1996). *Tej* is a yellow, sweet alcoholic wine consumed in Ethiopia. It is produced from diluted honey (1:4). Fermentation is carried out in a clay pot previously smoked over an olive hop stem fire. Incubation is carried out in a warm place for 2–3 days after which the wax and scum are removed. A second fermentation lasting 8 days follows the addition of boiled hops. The wine is clarified by filtering through cloth (Steinkraus 1979).

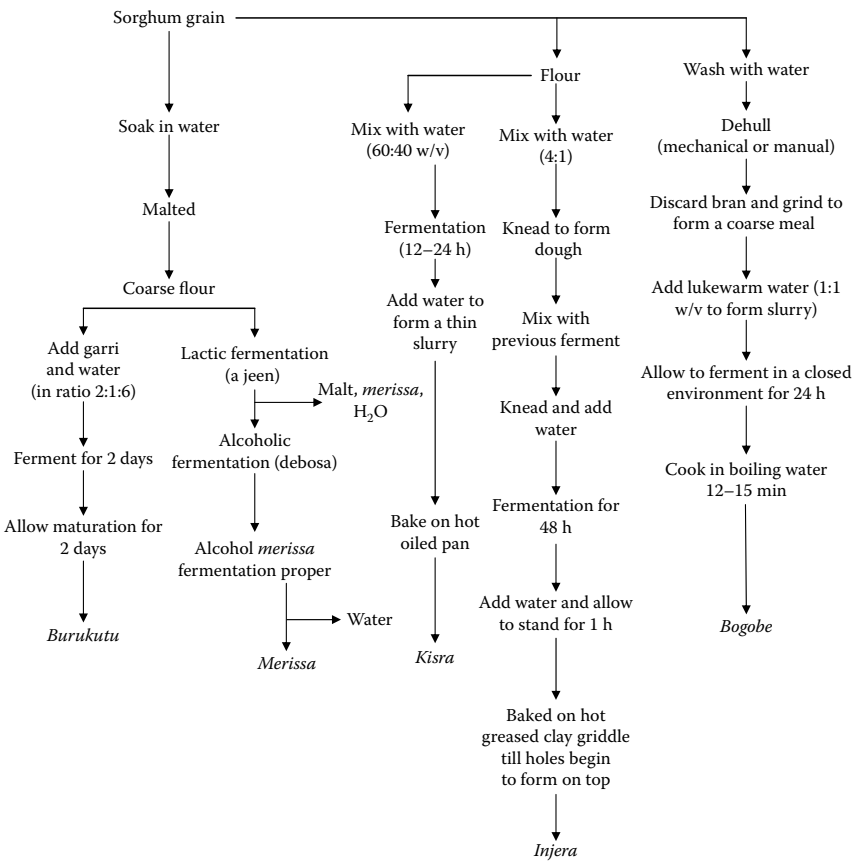

FIGURE 12.7 Flowchart for the preparation of *burukutu*, *merissa*, *kisra*, *injera*, and *bogobe*.

12.5 Microbiology

12.5.1 Functional Microorganisms

12.5.1.1 Fermented Nonalcoholic Cereal Substrates

The organisms mainly involved in the fermentation of *ogi* are *Lactobacillus plantarum*, *Candida krusei*, and *Debaromyces hansenii* (Odunfa and Adeyele 1985). Other organisms isolated from fermenting grains but not yet proven to play a prominent role include *Lactobacillus fermentum*, *Lb. brevis*, *Lactococcus lactis*, *Saccharomyces cerevisiae*, *Enterococcus faecalis*, and *Enterobacter cloacae* (Akinrele 1970, Adegoke and Babalola 1988). Lowering of pH to 4 during steeping for 24 h (Figure 12.1) has been found to favor the activity of key fermenters such as *Lb. plantarum* (Akinrele 1970). *Corynebacterium* hydrolyzes corn starch to form organic acids while *S. cerevisiae* and *C. mycoderma* contribute to flavor development (Odunfa and Oyewole 1998). Apart from *Lactobacillus* sp., the fermented Ghanaian cereal products *koko* and *kenkey* have been found to involve *Pediococcus cerevisiae*, *Leuconostoc mesenteroides*, and *Leuc. fermentum*, which are

thought to play a major role in "doughing." Souring of *uji* is mainly due to the activity of *Lb. plantarum*, although heterofermentative strains of *Lb. fermentum*, *Lb. cellobiosus*, and *Lb. buchneri* have been reported in early stages of fermentation (Mbugua 1987). The fermentation of native-made *mahewu* is mainly by *Streptococcus lactis* (Hesseltine 1979) but in cottage-scale manufacture *Lactobacillus delbrueckii* and *Lb. bulgaricus* are used (Olasupo 2006). The fermentation of *mawe* is mainly by *Lb. fermentum*, *Lb. brevis*, *Candida krusei*, *C. keyr*, and *C. glabrata* (Hounhouigan et al. 1994).

12.5.1.2 Starchy Root Crop Products

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 lactic acid bacteria including *Streptococcus* spp., *Lb. plantarum*, and *Leuconostoc* sp. (Abe and Lindsay 1978, Ngaba and Lee 1979, Ofuya and Nnajiofor 1989, Okafor and Ejiofor 1990, Oyewole and Odunfa 1991) 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* as a result of production of esters and aldehydes (Odunfa and Oyewole 1998).

The special role of LAB in the fermentation of cassava into *fufu* has been well elucidated (Adegoke and Babalola 1988, Oyewole 1990, Oyewole and Odunfa 1990, 1991, Giraud et al. 1993, Westby and Choo 1994, Raimbault 1995). The retting process leading to the softening of the cassava roots during *fufu* production is known to be due to some pectinolytic microorganisms (Oyewole and Odunfa 1992, Ampe et al. 1995). Apart from LAB, unique organisms such as *Bacillus* sp., *C. tropicalis*, and *Penicillium* are important flora in the fermentation of cassava to produce Ghanaian *agbelima* (Amoa-Awua 1996). Other cassava products such as *chikawgue* from Zaire, *kivunde* from Tanzania, and *kocho* from Ethiopia are dominated mainly by LAB and yeasts.

12.5.1.3 Microorganisms in Fermented Animal Proteins

A broad array of lactobacilli and other lactic acid producers have been isolated from fermented African milk products (Elfaki et al. 1991, El-Baradei et al. 2005). *Maziwa lafa*, a homemade fermented sour milk, is characterized by *Streptococcus lactis*, *S. thermophilus*, and *Lactobacillus casei* (Odunfa and Oyewole 1998). *Nono*, a yoghurt common in the Guinea Savannah region of West Africa, has been shown to be fermented mainly by *Lactobacillus acidophilus* and *Lactococcus cremoris* (Akinyanju 1989), although other species of *Lactobacillus* and non-hemolytic *Streptococcus* have also been isolated. Other fermented milk products such as *wara* (cheese), *ergo*, jben (Moroccan white cheese), and *leban* (sour milk from Morocco) also contain similar organisms (Ouadghiri et al. 2005, Olasupo 2006). LAB isolated from *ergo* by Gonfa et al. (1999) include *S. thermophilus*, *E. faecalis* var. *liquefaciens*, *Streptococcus bovis*, *S. mitis*, *S. agalactiae*, *Lb. cremoris*, *Leuconostoc dextranicum*, *Leuc. lactis*, *Lactobacillus xylosus*, and *Lb. lactis*. *Micrococcus* sp. was also isolated (Gonfa et al. 2001). It is of interest that a number of LAB involved in traditional fermented dairy products have been found to produce bacteriocins effective against some very important food-borne pathogens (Olasupo et al. 1994b, 1997, 1999). As indicated in Table 12.3, microorganisms associated with fermented fish products in Africa (*guedj* and *bonome*) are yet to be fully elucidated (Olasupo 2006).

12.5.1.4 Microorganisms Associated with Fermented African Vegetable Proteins

Unlike fermented starchy roots, cereals, and animal proteins earlier discussed, LAB do not play a predominant role in the fermentation of African vegetable proteins. As shown in Table 12.4, the organisms mostly involved are the low G + C content gram-positives such as *Bacillus* and *Micrococcus* species and members of the family enterobacteriaceae. The organisms involved in the fermentation of traditional FVP are mainly the *Bacillus subtilis* group, which includes *B. subtilis*, *B. licheniformis*, and *B. pumilus* (Ouoba et al. 2004). 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 1981b) while *Staphylococcus* sp. and *Micrococcus* sp. play a subsidiary role in *iru* (Odunfa and Komolafe 1989) and *ugba* fermentations (Obeta 1983). Also, *Staphy. saprophyticus* is almost always associated with the fermentation. Others found irregularly are LAB and enterobacteriaceae (Odunfa and Oyewole 1998).

Since the major component of most of the fermented legumes and oilseeds is protein, *Bacillus* species serve to breakdown the protein into amino acids (Isu and Njoku 1997). Proteolytic activity has also been found to increase constantly throughout the course of fermentation. All *Bacillus* species isolated from fermented *iru* were proteolytic and 97.3% were lipolytic, and all were found able to grow at 50°C (Odunfa and Oyewole 1986). Although *Staphylococcus* species is also present in the fermentation, its role has not been well investigated in *iru* fermentation. The species obtained were *S. xylosus* (55%), *S. saprophyticus* (33%), and *S. hominis* (11%) (Odunfa and Komolafe 1989). Their occurrence in the fermenting beans increased from 0.3% at 24 h to 42% at 48 h. They may play a role in proteolysis as 86% of the isolates were proteolytic. In products where salt is added before fermentation, the role of *Staphylococcus* may be enhanced. It has also been shown that the proteolytic activities of *Bacillus* are reduced with increased salt content (Dike and Odunfa 2003).

Apart from proteolysis, other important functions of microorganisms are production of flavor components, production of vitamins and essential fatty acids, and degradation of flatus factors. Significant increases in thiamine and riboflavin have been observed in *ugba* and *ogiri-igbo*; these have been ascribed to riboflavin synthase reported in *B. subtilis* (Odunfa 1986). Also there is reported reduction in the contents of stachyose, raffinose, and melibiose contents in fermented foods (Sarkar et al. 1997). These are ascribed to sucrase activities as reported by Aderibigbe and Odunfa (1990) and possibly α-galactosidase activities of the other microorganisms in the fermenting mash, predominantly *Staphylococcus* spp. and LAB among which α-galactosidase activities are common (Odunfa and Oyewole 1998). An important enzyme in FVP fermentation is proteinase. Proteinases such as serine proteinase and neutral proteinase of *Bacillus* species have been characterized and identified and show both proteolytic and esterolytic activities (Aderibigbe and Odunfa 1988). The serine proteinases have a hydrophobic nature; these characteristics of high pH optima and their ability to act at low water activity make them adaptable to fermentations on solid substrates. Various attempts have been made to develop starter cultures, mostly based on *B. subtilis*, for the fermentation of vegetable proteins (Aderibigbe and Odunfa 1990, Mbugua et al. 1992, Mbajunwa et al. 1998, Isu and Ofuya 2000, Ouoba et al. 2003, Omafuvbe et al. 2004, Amoa-Awua et al. 2006). However, *B. subtilis* isolates from foods have been known to vary widely

in their characteristics, specifically growth rate, production of enzymes such as proteinases and carbohydrases, and the types of proteinases, etc. An approach to developing starter cultures is the use of a mixture of strains with complementary characteristics. Oguntoyinbo et al. (2007) selected starter cultures for the production of *okpehe* based on rapid growth, high amylolytic and proteolytic activities, as well as other functional properties such as high polyglutamic acid production and bacteriocin production.

12.5.1.5 Alcoholic Beverages

Fermentation of palm wine, which is one of the most important alcoholic beverages in West Africa, is mainly by yeasts *S. cerevisiae* and *Schizosaccharomyces pombe*, and *Lb. plantarum* and *Leuc. mesenteroides* (Faparusi et al. 1973, Okafor 1975a,b, 1978). The yeasts are responsible for alcohol production while the acids result mainly from the activity of the bacteria. Also isolated in the fermenting mixture for the production of *burukutu* are organisms like yeasts such as *S. cerevisiae* and *S. chavelieri* and bacteria such as *Leuc. mesenteroides* and *Lactobacillus* sp. However, upon maturation the predominant microorganisms are *Acetobacter* spp. and *Candida* sp. (Faparusi et al. 1973). The fermentation of *pito* on the other hand is by *Candida* sp.; whole souring is brought about by *Geotrichum candidum* and *Lactobacillus* sp. (Odunfa and Oyewole 1998). Souring of the mash in the production of *kaffir* beer is by *Lactobacillus*, while top-fermenting yeast *S. cerevisiae* is used for alcoholic fermentation.

The souring of maize for *busaa* production is by lactic acid bacteria, namely *Lactobacillus helveticus*, *Lb. salivarus*, *Pediococcus damnosus*, and *Pediococcus partulus* as well as yeasts such as *C. krusei* and *S. cerevisiae*. Fermentation is however carried out by a mixture of bacteria and yeasts, namely *C. krusei* and *L. casei* var. *rhamnosus*. As shown in Table 12.5, the production of other alcoholic beverages such as Malawi beer, Zambian opaque maize beer, *merissa*, and *kishk* involves lactic acid bacteria. The fermentation of alcoholic drinks from other substrates such as plantain (*agadagidi*) and cocoa is however mainly dependent on the activity of yeasts.

12.5.2 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 (Odunfa and Adewuyi 1985, Mensah et al. 1988, Oyewole and Sanni 1995, Mensah 1997, 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* and *nono* (Olasupo et al. 2002). 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 (Kingmakono et al. 1994, Nout et al. 1989, Ogbonna et al. 2001, Ozumba et al. 2002, Ogunshe et al. 2006). Cooked *ogi* was found to exhibit antibacterial activity against *E. coli* (Odugbemi et al. 1991). *Ogi* called "dogik" produced using starter cultures *Lb. acidoplilus* DK 77 and *Lb. pentosu*s DK 99 has antimicrobial activity against diarrheagenic bacteria (Olukoya et al. 1994). Although high coliform counts have been reported from locally produced

kunu-zaki (Onuorah et al. 1987), 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 (Adeyemi and Umar 1994, Inyang and Dabot 1997, Olasupo et al. 2000). A number of bacteriocin-producing LAB have been isolated from fermented milk products from Nigeria (Olasupo et al. 1994a,b, 1997). Nisin produced by *Lc. lactis* isolated from *wara* has been found to inhibit pathogenic species including *Listeria monocytogenes*, *L. innocua*, *Clostridium butyricum*, *Cl. perfringens*, and *Bacillus cereus* (Olasupo et al. 1999). Antibacterial activity against bacteria causing diarrhea has been linked with lactic acid bacteria involved in the fermentation of *uji* (Mbugua and Njenga 1991). Also, lactic acid production during fermentation of *tef* to produce *injera* has been found to lower the population of potential pathogenic organisms (Nigatu and Gashe 1994a,b).

The microorganisms involved in the fermentation of FVP-based foods also affect the safety of the fermented products. Most of the raw materials for FVP 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. 2007a) 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 antimicrobials such as hydrogen peroxide, bacteriocins, diacetyls, and secondary reaction products (Amoa-Awua 1996). According to Odunfa and Oyewole (1998), the main significance of cassava fermentation is that it yields a product that can be stored for prolonged periods. It has also been reported that lactic acid fermentation for the production of *mawe* reduced the enterobacteria population below detection levels (<log 1.7 cfu/g) after 24 h of fermentation (Hounhouigen et al. 1994). Similarly, it was shown recently that LAB exhibited a biopreservative 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" (Casalta and Montel 2008, Jacques and Casaregola 2008, Pottier et al. 2008, Ogier et al. 2008). Antimicrobial activities have also been reported in cottage cheese, *maasai* fermented milk, and Ethiopian sour milk (Arocha et al. 1992, Abdullah et al. 1993, Ashenafi 1993, Isono et al. 1994). Also, fermentation helps to reduce bacterial toxinogens and aflatoxins, such as those produced by *Staphylococcus* sp., clostridia, bacilli, and aspergilli (Olasupo 2006).

12.5.3 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% 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 (Mkpong et al. 1990, Vasconcelos et al. 1990, Essers 1995, 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 (Eka 1980, Svanberg and Sandberg 1988, Aderibigbe and Odunfa 1990). Factors responsible for flatulence, indigestion, and diarrhea are also reduced during fermentation. Odunfa (1982) showed that there was significant reduction of oligosaccharides such as sucrose, raffinose, and stachyose, and protease inhibitors in fermented locust beans (*iru*). In the fermentation of castor oil bean for *ogiri* production, a toxic protein, ricin, and other toxic constituents, ricinine and ricinoleic acid, were eliminated during the fermentation (Odunfa 1985a,b). During the fermentation of melon seeds for the preparation of *ogiri*, the aflatoxin content was reduced and after 4 days no aflatoxin was found (Ogunsanwo et al. 1989).

12.5.4 Nutritional Properties

Fermentation results in a lower proportion of dry matter in the food, and the concentration of the vitamins, minerals, and protein appear to increase when measured on a dry weight basis (Adams 1990). One of the reasons for the increasing interest in fermented foods is their ability to promote the functions of the human digestive system in a number of positive ways (Sahlin 1999, Abu Tarboush et al. 1997, Enujiugha 2003). A large percentage of Africa's over 500 million people live below the poverty line, with diets poor in protein and other essential nutrients. Generally, a significant increase in the soluble fraction of a food is observed during fermentation (Sahlin 1999). 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 (Kiers et al. 2000, Oboh 2006). The organisms involved in these fermentation processes, particularly *Bacillus* sp., produce proteolytic enzymes, which hydrolyze proteins to amino acids and peptides (Odunfa 1981b; 1985a,b, 1988; Antai and Ibrahim 1986; Steinkraus 1991; Addy et al. 1995; Leejeerajumnean et al. 2001). *Bacillus* strains from fermenting locust beans have been found to produce glutamic acid and extracellular proteinases (Aderibigbe et al. 1990, Ogbadu et al. 1990).

The proximate composition of some FVP and their raw materials indicated that the major components are protein and fat. 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).

The major component of the carbohydrates of legumes is starch, raffinose, and stachyose (Odunfa 1985a,b). In addition, soybean has verbascose and melibiose (Odunfa 1985a,b). Arabinogalactan and galactomanan have been reported in African locust bean (Aderibigbe and Odunfa 2001).

During fermentation, the oligosaccharides are hydrolyzed to simple digestible sugars (Ouoba et al. 2005, 2007a,b). Assay of the fermenting mash of African locust bean showed activities of α- and β-galactosidases and sucrase (Odunfa 1983) with the former being the highest. Other enzyme present are galactanase, glucosidases and fructofuranosidases, and polygalacturonases. These enzymes are produced by *Bacillus* spp., *Staphylococcus* sp., and lactic acid bacteria, the latter groups producing α-galactosidase. The nutritional significance of hydrolysis of oligosaccharides is the drastic reduction in the level of indigestible carbohydrates which produce flatulence (Odunfa 1983).

Although oil constitutes a major component of the legumes and oilseeds, lipolytic activities are inconsistent. Low lipolytic activities were detected in *dawadawa* (Ikenebomeh 1982, Odunfa and Oyewole 1986). These lipolytic activities are attributed to *Staphylococcus* species in the fermentation. During fermentation, the free fatty acid fraction (FFA) reduced from 0.6% to 0.1% w/w in the fermented seeds. There were no significant differences qualitatively in the FFA of unfermented and fermented seeds, the components being palmitic acid, stearic acid, oleic acid, and linoleic acid (Odunfa and Adesomoju 1985, Ouoba et al. 2003).

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 (Odunfa 1983, Olasupo 2006). Higher levels of folic acid have also been reported. Of 24 foodstuffs analyzed, *iru* (dawadawa) has the second highest folate content (Odunfa 1983).

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 a alcohol content of only 3% (Ekundayo 1969). *Kishk* prepared from wheat and milk is a highly nutritious food, having a protein content of about 25.3%. It is highly digestible and of high biological value (Odunfa 1999). When milk is fermented to *wara*, lactose is reduced from 4.6% to 0.2%, protein from 3.7% to 14.8%, and fat from 4.3% to 13.5% (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). Homemade *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. For example, Olukoya et al. (2000) developed a nutritionally improved *ogi* using starter cultures of *Lb. acidophilus* (DK 52) and *Lb. pentosus* (DK 77). It was found that the reducing sugar content, protein content, and essential amino acids were significantly higher

in the newly developed *ogi* product. Similarly, Teniola and Odunfa (2001) reported significant increase in amino acid (specifically lysine and methionine) content of *ogi* fermented with mutants of lactic and yeast cultures.

12.6 Market Potential

African fermented foods are a group of foods that are produced at homes, villages, and cottage industries (Okafor 1983). Both in rural and urban areas, market for these products abound, but usually 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). *Kunun-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 (Inyang and Dabot 1997). 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 (O'Hair 1990, Hahn 1989). 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 (Oluwole et al. 2004, Onyekwere et al. 2004, FIIRO 2006). With such improvements, there is hope that this energy-rich source and other cassava-based products such as *agbelima* (Amoa-Awua 1996) and *kivunde* (Kimaryo et al. 2000) will leave the realm of localities to become very important on the global market as transnational staple foods.

Fermented African milk products occupy a very important place in the diet of pastoral and nomadic peoples of North Africa, and East and the sub-Saharan Africa. Fermented milk products are consumed as beverages and foods and are of higher market value than raw milk (Motarjeni and Nout 1995). Among the Maasai of East Africa, Rift Valley in Kenya, and Northern Tanzania, fermented products such as *kule naoto* constitute not only the major source of protein but the major part of most diets (Mathara et al. 2008a,b). The fermented vegetable product *iru* (*dawadawa*) is the most important condiment in Nigeria and in many countries of West and Central Africa (Campbell-Platt 1980). It is one of the few fermented African food products that is now available in well-processed packaged forms (Dadawa, a brand name different from the traditional name, *dawadawa*) in stores.

12.7 Conclusion

Fermented African foods have long histories and are highly diverse in their culinary. The organisms involved in the fermentation are restricted to a few groups of yeasts and bacteria. Starchy roots and cereals are fermented mainly by lactic acid bacteria

and yeasts, with yeasts playing more prominent roles in fermenting alcoholic beverages. FVP are produced mainly by the activities of proteolytic bacteria and are used mainly as condiments. Fermented animal proteins are mainly dairy products in the Sahara and in the savannah and hills and valleys of East Africa. Basically, they are produced by traditional methods in village kitchens and cottages. In recent years, there has been fruitful research into the microbiology and technology of production processes. This has been demonstrated for *mahewu* and *kafir* in southern Africa and *gari*, to some extent in Nigeria. The use of starter cultures on a large/industrial scale and application of hazard analysis critical control point (HACCP) offer greater opportunities of tapping into the large market potential of ethnic African fermented foods.

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13

Tea, Coffee, and Cacao

Ulrich Schillinger, Louis Ban-Koffi, and Charles M. A. P. Franz

CONTENTS

13.1 Introduction

Tea, coffee, and cacao are popular beverages consumed all over the world. They are all cultivated exclusively in tropical and subtropical climates. Tea, coffee, and cacao originate from three different continents. The tea plant is an evergreen of the Camellia family that is native to China, Tibet, and northern India; coffee originates from the subtropical forest ecosystems of the Ethiopian highlands, and cacao is native to Central America where it was first domesticated by the Aztecs. Tea, coffee, and cacao have in common relatively high concentrations of alkaloids that are stimulatory for humans. Two structurally related compounds, theobromine and caffeine, are especially important. Theobromine is a bitter alkaloid from the cacao plant and is also present in the leaves of the tea plant. It is a cyclic adenosine monophosphate (cAMP)

phosphodiesterase inhibitor. Caffeine is known to be the main stimulatory compound in coffee (0.9%–2.6%) and tea (black tea leaves contain 3%–3.5% caffeine). In addition to the aroma of these beverages, the high concentration of these psychostimulants has contributed to the popularity of drinking coffee and tea. Coffee, tea, and cacao develop their distinctive color, flavor, and aroma during a fermentation process involving various microorganisms. Most of the teas are produced by a natural oxidation process without microorganisms; however, there are also microbial fermented teas like *kombucha* or *puer* tea. The fermentation process is of crucial importance for the quality of all products. Cacao and coffee require a roasting process before they acquire their full flavors.

13.2 Tea

13.2.1 Green, White, *Oolong*, and Black Teas

Today tea is the second most popular beverage in the world after water. Tea plants belong to the Theaceae family and two main varieties exist: *Camellia sinensis* var. *sinensis* and *Camellia sinensis* var. *assamica*. The *assamica* varieties generally have large leaves, are rich in flavonols and caffeine, contain high polyphenol oxidase activity, and are best suited for processing black tea, whereas the *sinensis* varieties have small leaves, have more nitrogenous compounds than flavanols, have a relatively low polyphenol oxidase activity, and are suited for processing green tea (Hara et al. 1995b). The first apical leaves are harvested manually or with equipment and are processed by different methods resulting in the four basic types of tea: black tea, *Oolong* tea, green tea, and white tea. Green and white teas are unoxidized teas. White tea is the least processed tea. In order to prevent oxidation of the catechins, the leaves are immediately fired or steamed—a process called fixing—and there is no rolling, breaking, or bruising of any kind. Green tea is processed in a similar way: after a period of withering for about 8–24 h, leaves are steamed or pan fried and then rolled up in various ways. The processing of *Oolong* tea requires a special operation unique to this type of tea. After withering, the leaves are rotated using a special machine, and this process (rotating) causes friction between leaves, disrupts cellular organization at the edge of the leaves, and brings about a limited degree of oxidation before the leaves are steamed in order to inactivate the enzymes (Hara et al. 1995a). Black tea, the most widely produced tea accounting for 75% of world production, requires a full oxidation of the leaf polyphenols. For this purpose, after withering, the leaves are rolled by the use of an orthodox roller or by CTC (crushing, tearing, and curling) machines in order to macerate them. As a result of the rolling process, the enzymes and catechins come into close contact and are exposed to air, initiating a multistage enzymatic process resulting in the deep color and unique flavor of black tea. Unfermented teas contain catechins, semifermented black *Oolong* contains both catechins and theaflavins, and fully fermented black tea contains catechins, theaflavins, and polymeric thearubigins (Friedman 2007).

Tea drinking originated in China, and the word tea is derived from *t'e* of the Chinese Fukien dialect. The first authentic reference to tea was made in an ancient Chinese dictionary in AD 350, and the first exclusive book on tea was published by the Chinese tea expert Lu Yu in AD 780 However, with the exception of Japan,

tea drinking did not spread to other parts of the world until the beginning of the seventeenth century. It was the Portuguese and Dutch traders who first imported tea to Europe, with regular shipments by AD 1610.

13.2.1.1 Antibacterial and Antifungal Activities of Tea

Tea leaves produce polyphenolic compounds, the six so-called catechins and the methyl-xanthine alkaloids caffeine, theobromine, and theophylline. The polyphenolic tea compounds are reported to show activity against foodborne pathogens, viruses, and fungi. Numerous pathogenic bacteria including *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, methicillin-resistant *Staphylococcus aureus*, and *Mycoplasma pneumoniae* were inhibited by tea extracts or tea polyphenols (Friedman 2007). *B. cereus*, for instance, was inhibited by seven green tea catechins at nanomolar levels, and some flavonoids were even more active against this pathogen than medicinal antibiotics such as tetracycline or vancomycin at comparable concentrations (Friedman et al. 2006). Moreover, in several studies, anticariogenic/cariostatic effects of tea compounds were observed, and antimicrobial activity against bacteria responsible for tooth decay such as *Streptococcus mutans* and *S. sobrinus* were reported (Sakanaka et al. 1989). Both black and green tea infusions inhibited salivary amylase in human volunteers and thus reduced the cariogenic potential of starch-containing foods by suppressing the release of fermentable carbohydrates in the oral cavity (Zhang and Kashket 1998). *Oolong* tea polyphenols can possibly prevent dental caries by inhibiting adherence of *S. mutans* to tooth surfaces as a result of a reduction in the hydrophobicity of these bacteria. *Oolong* tea extracts may also inhibit the caries-inducing activity of *mutans* streptococci by reducing their rate of acid production (Matsumoto et al. 1999).

13.2.1.2 Functional Properties of Tea

Results of epidemiological and experimental studies indicate that black or green tea provides some protective effect against some types of cancer, particularly of the digestive tract, and may prevent cardiovascular diseases. Tea catechins are the most powerful antioxidants among the known plant phenols, and protect low-density proteins (LDL) and very-low-density proteins (VLDL) against oxidation by aqueous and lipophilic radicals. Tea catechins reduce cholesterol absorption from the intestine, lowering the solubility of cholesterol and enhancing the fecal excretion of cholesterol and total lipids (Dufresne and Farnworth 2000). Quercetin and theanine, the major amino acids in green tea, reduce blood pressure in animals and in man and thus lower the risk for the development of cardiovascular diseases. Quercetin also has an antiallergic effect by inhibiting hyaluronidase activity and histamine release. However, more human investigations are needed to validate these observations.

13.2.2 Microbial Fermented Teas and Tea Products: *Puer* Tea, *Fuzhuan* Brick Tea, *Kombucha*, *Miang*

13.2.2.1 Puer *Tea*

Puer tea is a unique microbial fermented tea traditionally produced mainly in the Yunnan province of China and is consumed in large amounts among Chinese people

since hundreds of years. Like black tea, it is produced by full fermentation. Its processing, however, is different from that of black tea as it undergoes a natural oxidation process through enzymes originally existing in the tea leaves (Mo et al. 2008). During the *puer* tea fermentation process, fresh leaves are heat treated to inactivate polyphenol oxidases (Mo et al. 2008). There are two types of *puer* tea: in case of the raw *puer* tea, the leaves are simply softened by steaming and compressed into different sizes before the natural fermentation starts. The production of cooked *puer* tea is a more complex and relatively recent process developed in the early 1970s. In this case, the fermentation is initiated artificially. The dry black leaves are laid out in thick piles in a well-heated room and are sprayed with water and covered with a canvas or tarpaulin for a few weeks. During this fermentation period, the tea polyphenols are more intensively oxidized by the action of microorganisms and environmental oxygen than in the black tea fermentation process (Abe et al. 2008, Mo et al. 2008). The color turns from green to brown or brownish red, and a particular fragrance is produced. *Puer* tea acquires a characteristic flavor and has numerous health beneficial properties. Aging of *puer* tea is an essential process, allowing the tea's aromatic bouquet to develop while mellowing the tannins and reducing the astringency. It is the only tea whose aromatic profile improves during storage for several years.

Hypolipidemic effects (Sano et al. 1986) and antioxidative properties (Jie et al. 2006) of *Puer* tea have been demonstrated in animals. Statins were identified in *puer* tea extract, and these compounds are known to prevent cardiovascular disease and mortality of patients and reduce the relative risk of major coronary events in the population. Antimutagenic and antimicrobial activities of *puer* tea were also reported. Water extracts of *puer* tea showed some activity against *Staph. aureus* and *B. cereus*, but not against *Escherichia coli* (Wu et al. 2007). The information on the microbial community involved in *puer* tea fermentation is scarce. *Aspergillus niger* was identified as a predominating microorganism, although other fungi such as *A. glaucus*, species of *Penicillium*, and *Rhizopus*, and the yeast *Saccharomyces* were also isolated (Mo et al. 2005, Jeng et al. 2007). A very recent study using DGGE analysis as a culture-independent method to investigate the fungi which play a role in *puer* tea fermentation revealed that *A. niger* and *Blastobotrys adeninivorans* are the major fungi involved (Abe et al. 2008). Bacterial strains belonging to species of *Actinoplanes* and *Streptomyces* were also isolated from *puer* teas by other authors (Jeng et al. 2007).

13.2.2.2 Fuzhuan *Brick Tea*

Fuzhuan brick tea is another microbial fermented tea characterized by a fungal fermentation involving *Aspergillus*, *Penicillium*, and *Eurotium* as the major organisms (Mo et al. 2008). It is uniquely found in China.

13.2.2.3 Miang

In the northern part of Thailand, fermented tea leaves called *miang* are consumed as a snack with salt or with condiments such as roasted coconut or shredded ginger (Figure 13.1).

Young tea leaves are collected, tied into small bundles, and steamed for 2 h or more to inactivate the polyphenol oxidases (Phithakpol et al. 1995). After cooling, the leaf bundles are carefully packed in a cement tank lined with banana leaves, and covered with banana leaves or a plastic sheet. The leaves are pressed tightly and weighted down

FIGURE 13.1 Fermented and pickled tea leaves (*miang*). (Photo courtesy of Dr. Somboon Tanasupawat.)

to exclude oxygen, and thus to discourage the growth of yeasts and aerobic spoilage bacteria (Phithakpol et al. 1995). Fermentation takes about 3 or 4 months. *Miang* is traditionally used during religious ceremonies and funerals in Thailand (Sasaki et al. 2007). The traditional *miang* tea garden forms the agroforestry and community forest system in northern Thailand (Sasaki 2008). Various lactic acid bacteria are involved in the fermentation and contribute to the preservation of the product by acid production. *Lactobacillus plantarum*, *Lb. pentosus*, *Lb. vaccinostercus*, *Lb. pantheris*, *Lb. fermentum*, and *Lb. suebicus* were among the *Lactobacillus* species isolated from fermented tea leaves (Tanasupawat et al. 2007). Enterococci such as *E. casseliflavus* and *E. camelliae* were also isolated from this product (Sukontasing et al. 2007). Three novel species were described as components of the lactic population of *miang*: *Lactobacillus thailandensis*, *Lb. camelliae*, and *Pediococcus siamensis* (Tanasupawat et al. 2007).

13.2.2.4 Kombucha

Kombucha is a slightly carbonated tea beverage consumed worldwide, but historically in China. It is typically prepared by fermenting black tea that has been sweetened with sugar. The recipe may vary slightly. In general, black tea leaves are infused in boiling water sweetened with sucrose (50–150 g/L) for about 10 min. After removing the tea leaves, the tea is poured into a wide-mouth clean vessel and is acidified by the addition of vinegar or the so-called tea fungus, which is actually a floating cellulose mat (the cellulosic pellicle) formed by a symbiotic association of yeasts and acetic acid bacteria from a previous fermentation. The jar is carefully covered with a clean cloth and the preparation is allowed to incubate at room temperature for 10–12 days. The final product comprises organic acids, vitamins, minerals, and tea components, resembling the taste of cider (Greenwalt et al. 2000).

The acetic acid bacteria and the yeasts present in *Kombucha* form a powerful symbiosis able to inhibit the growth of potential contaminating bacteria (Dufresne and Farnworth 2000). *Acetobacter xylinum* has been shown to be the primary

bacterium in the mat able to produce acetic acid and gluconic acid. It also synthesizes the floating cellulose network, which is a prerequisite for close association with the yeasts. The yeast flora of *kombucha* includes the genera *Brettanomyces* (56%), *Zygosaccharomyces* (29%), and *Saccharomyces* (26%) (Mayser et al. 1995), and the spectrum of species may vary considerably due to geographic, climatic, and cultural conditions as well. Also, the species of wild yeasts and bacteria present in the fermentation may vary with local conditions. *Zygosaccharomyces kombuchaensis* was described as a species commonly associated with *kombucha* (Kurtzman et al. 2001). However, other *Zygosaccharomyces* species such as *Z. bailii*, *Z. bisporus*, and *Z. microellipsoides* were also reported to belong to the yeast flora of this tea beverage. In addition, *Schizosaccharomyces pombe*, *Torulaspora delbrueckii*, *Rhodotorula mucilaginosa*, *Candida stellata*, and *Brettanomyces bruxellensis* were isolated from *kombucha* (Teoh et al. 2004). The fermentation appears to be initiated by osmotolerant yeasts and is then succeeded and ultimately dominated by acid-tolerant species (Teoh et al. 2004). The metabolic and ecological interactions occurring during *kombucha* fermentation are not well investigated. The yeasts primarily convert sucrose into glucose and fructose and produce ethanol and carbon dioxide with a preference to fructose as a substrate. The ethanol is then oxidized by the acetic acid bacteria to produce acetic acid which in turn is able to stimulate the yeasts to produce ethanol (Liu et al. 1996). Gluconic acid is produced from glucose by *Acetobacter.*

Kombucha has gained popularity because of the apparent health benefits resulting from regular consumption. Stimulation of the immune system, digestion, liver function improvement, some detoxification activity, and reduction of obesity are examples of reported health claims. However, until now, there is little scientific evidence for the health-promoting properties of the *kombucha* beverage. The detoxifying property of *kombucha* was considered by several researchers to be due to the capacity of glucuronic acid to bind toxin molecules in the liver. However, these days the presence of glucuronic acid in *kombucha* has been doubted (Greenwalt et al. 2000). Gluconic acid derivatives may have been misidentified. In some *in vitro* studies, antibacterial activity of *kombucha* against pathogenic bacteria such as *Helicobacter pylori*, *Staph. aureus*, *E. coli*, and *B. cereus* was reported, and this inhibitory effect probably is related to the acetic acid content of *kombucha* (Steinkraus et al. 1996, Greenwalt et al. 1998). More research is needed to find scientific evidence for health effects of this fermented tea and to understand the mechanisms behind these. There is also a lack of knowledge how the biological activities of tea components such as catechins interact with compounds specifically produced during *kombucha* fermentation, and how the components of the tea are changed by the metabolic activity of the complex microbial association of *kombucha*.

13.3 Coffee

The name of coffee is derived from the southwestern province of Ethiopia called Kaffa, indicating that the origin of coffee lies in Ethiopia. The occurrence of wild coffee plants in various regions of Africa also indicates that coffee is a native of this continent. From Ethiopia, coffee spread to Arabia where coffee beans were first roasted and brewed. By the fifteenth century, it was a very popular drink with the Arabs and the word coffee may also be derived from the ancient Arabic *gahwah*. From there, coffee spread to Italy in the year 1600. In 1615, Venetian merchants imported the first sacks of coffee

FIGURE 13.2 Coffee tree with ripe beans. (Photo courtesy of Dr. Louis Ban-Koffi.)

to Western Europe, and coffee houses were soon springing up throughout Europe. The Dutch became the first to cultivate coffee commercially in plantations during the seventeenth century and to establish the coffee industry. The coffee plant (Figure 13.2) is a woody perennial evergreen plant that belongs to the Rubiaceae family.

This family is composed of about 400 genera and 5000 species (Pursglove 1976). Only the genus *Coffea* is used in the production of coffee. Only two species, *Coffea arabica* Linné and *Coffea canéphora* Pierre, produce edible coffee beans and are cultivated across the world. These are also known as the Arabica and Robusta varieties, respectively. *C. arabica* or Arabica coffee, accounts for 75%–80% of the world's production (Assiedu 1989). It is the oldest known and cultivated coffee variety. *Coffea canéphora* or Robusta coffee is a more resilient plant. It currently meets 30% of the worldwide demand for coffee. Coffee plants are cultivated in more than 50 tropical countries including Brazil, which is the largest producer of coffee, Vietnam, Colombia, Indonesia, Ethiopia, Mexico, and India. *C. arabica* grows best at an altitude ranging from 600 to 2400 m and produces the best quality of coffee, while *C. canephora* is grown at a lower altitude (from sea level to 1000 m) and is more tolerant to warm conditions and less susceptible to disease than *C. arabica*. *C. canephora* grows in certain swamp areas but it also particularly grows in western Africa, coasts of the Congolese basin, and in Angola.

13.3.1 Coffee Processing

Coffee berries and their seeds undergo several processes before they become the familiar roasted coffee. The seeds that are required for the making of coffee are surrounded by various coverings and membranes. The so-called coffee cherries are drupes consisting of an exocarp (skin), an outer mesocarp (pulp), an inner mesocarp (mucilage), and a fibrous endocarp (parchment) surrounding a seed. The ripe cherries are harvested either by picking by hand (Figure 13.3), stripping from the tree of both unripe and overripe beans, or collecting using a harvesting machine. There are basically two methods of coffee processing that differ in complexity and the quality of the resultant coffee: the wet method and the dry method.

FIGURE 13.3 Harvest of coffee cherries. (Photo courtesy of Dr. Louis Ban-Koffi.)

In the wet process, the hand-picked mature cherries are mechanically de-pulped using a pulping machine, which removes all the extraneous material and leaves the beans surrounded by the parchment and a layer of mucilage. The beans covered with the slippery mucilage are sorted by density and then put in cement tanks with water and are allowed to ferment. The objective of the fermentation is to degrade the slimy mucilage adhering firmly to coffee beans by pectinolytic enzymes. The time required for the breakdown of the mucilage may vary and is dependent on temperature, thickness of the mucilage layer, and concentration of pectinolytic enzymes present. The ideal fermentation time is reported to be 24–48 h for best quality parchment production. From the fermentation tanks, the beans are washed before moving to drying patios and dried to 10%–12% moisture content. In the wet processing, the time required for drying is 1–2 weeks. In the final stage, the beans are hulled to remove the parchment skin. The wet method is mainly used for the processing of Arabica coffee in Colombia, Central America, Kenya, East Africa, and Hawaii.

The dry method is simpler than the wet process, and is mainly used for Robusta coffee which has a thin pulp that allows direct drying (Fowler et al. 1998). In Brazil and Ethiopia, however, Arabica coffee is also processed by sun drying. In the dry process, the coffee cherries are spread on drying grounds or mats in layers approximately 3–4 cm thick and are exposed to sun. They are heaped at night in order to avoid rewetting and respreading each day (Silva et al. 2008). The length of time for drying depends on the sun shine and may vary from 3 to 4 weeks.

13.3.2 Microbiology of Coffee Fermentation

During the initial stages of fermentation, gram-negative bacteria, especially Enterobacteriaceae, are the predominating microorganisms. These bacteria occur on the fresh coffee berries and probably belong to the natural epiphytic population. *Enterobacter* seems to be most frequently isolated. During an investigation of the wet coffee fermentation in the Jima province in Ethiopia, most of the strains isolated from

coffee berries were identified as *Enterobacter cloacae* followed by *Klebsiella oxytoca* and *Hafnia alvei* (Holzapfel and Müller 2007).

Silva et al. (2008), when studying natural dry coffee fermentation in Brazil, reported a lower incidence of Enterobacteriaceae (<10^4 cfu/g) as compared to sporulating gram-positive bacteria, which constituted the predominant bacterial population. About 80% of their bacterial isolates belonged to the genus *Bacillus* (*B. subtilis* and *B. cereus* group), and bacilli were found throughout fermentation, drying, and storage (Silva et al. 2008). Among the Enterobacteriaceae, *Enterobacter* and *Serratia* were the genera most frequently isolated although *Pseudomonas*, *Klebsiella*, and *Acinetobacter* were also found. Some of these are reported to show pectinolytic activity, however, to which extent those organisms are involved in mucilage degradation is not clear. No increase in the number of pectinolytic organisms was observed by Avallone et al. (2001) during wet fermentation. Enterobacteriaceae that can be isolated from pectin media such as *Enterobacter dissolvens*, *Erwinia herbicola*, and *K. pneumoniae* produce only pectate lyase, which is unable to depolymerize highly methylated pectins of coffee mucilage. Therefore, their contribution to pectin degradation may be limited.

In the wet process, the viable bacterial counts increase from 10^2 to 10^3 cfu/g to more than 10^8 within 24 h of fermentation (Holzapfel and Müller 2007). Lactic acid bacteria became predominant and cause a reduction in pH, which prevent the development of spoilage bacteria. The role and importance of LAB in coffee fermentation is not well known. Their metabolic activity may affect the quality of the coffee. LAB obviously do not play a role in the dry fermentation of coffee in Brazil. In the early stage of wet fermentation, the genera *Leuconostoc* and *Weissella*, in particular, seem to be present in high numbers and probably initiate the acidification. Homofermentative and heterofermentative lactobacilli, as well as Enterococci, can be typically isolated at later stages of fermentation. Among the 60 *Leuconostoc* strains isolated from coffee fermentation in Jima, Ethiopia, 23 were *Leuc. mesenteroides,* 19 were *Leuc. citreum*, and 17 were identified as *Leuc. pseudomesenteroides* (Schillinger et al. 2008). These are probably the most frequent *Leuconostoc* species involved in coffee fermentation. During the same study (Schillinger et al. 2008), one isolate was classified as a new species, *Leuc. holzapfelii* (De Bruyne et al. 2007). *Weissella cibaria* and *W. soli* were also for the first time described as belonging to the LAB population involved in the fermentation of coffee berries in East Africa. The *Lactobacillus* strains that can be isolated from such fermentations mostly belong to the species *Lb. plantarum* and *Lb. brevis* (Holzapfel and Müller 2007).

Yeasts may also be present at high levels during the coffee fermentation. Total yeast counts of between 4.0×10^4 and 5×10^7 cfu/g were reported in the wet processing of *C. arabica* in Tanzania, with their numbers increasing during fermentation (Masoud et al. 2004). Several strains of the predominating species *Pichia anomala* and *P. kluyveri* produce polygalacturonases and, therefore, may be involved in the degradation of pectin during coffee fermentation (Masoud and Jespersen 2006). Also, in the dry process yeast numbers increase during fermentation. In the investigation by Silva et al. (2008) on the microbial succession of the dry process, bacterial populations predominated until the eighth day of fermentation and then decreased to less than 10% of the total isolates, and an increase in the yeast population was observed. *Debaryomyces hansenii* and *Pichia guilliermondii* were the yeasts most frequently isolated in this study. These and other yeast species with pectin lyase activity such as *P. burtonii*, *D. polymorphus*, and *P. holstii* may contribute to the utilization of

pectin in coffee mucilage. Filamentous fungi were also present throughout the entire process. *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium* were the most common genera (Silva et al. 2008).

The microbial succession of bacteria, yeasts, and filamentous fungi is affected by the coffee berry and bean moisture, temperature, competition for substrates, and the enzymatic capacity of the colonizing species (Silva et al. 2008).

13.3.3 Mycotoxin Production

Many of the fungi naturally occurring on coffee beans are potentially capable of mycotoxin formation. Ochratoxin A is the most important mycotoxin in coffee. It is a toxic metabolite produced by *Aspergillus* and *Penicillium* strains and has carcinogenic, teratogenic, genotoxic, and immunosuppressive properties. Ochratoxin A was detected in green coffee beans in concentrations ranging between 0.2 and 360 µg/kg. It is mainly produced by *Aspergillus ochraceus*, *A. carbonarius*, and *A. niger*. In Thailand, the most important fungi with the potential to produce ochratoxin A were reported to be *A. westerdijkiae* for the northern Arabica coffee and *A. carbonarius* for the southern Robusta coffee (Noonim et al. 2008). A high moisture content of the coffee beans and a high relative humidity during storage and transport of coffee are the main risk factors in mold and OTA formation. OTA may already be present on the cherries at harvest and/or may be formed during sun drying and also during improper storage at high temperature and high relative humidity. The drying process (under humid conditions) is probably the most crucial step as the cherries have a high moisture content (about 60% of water at harvest) enabling mold growth and toxin formation during the initial 3–5 days of drying on the outer part of the cherries (Bucheli and Taniwaki 2002). Wet processing appears to be less susceptible to infection by ochratoxin-producing molds probably because of the removal of the fruit pulp, which was found to be an excellent substrate for OTA-producing *A. carbonarius* strains (Joosten et al. 2001). A water activity below 0.8 will prevent OTA production in green coffee and the conditions of 10%–12% MC and 50%–70% RH are considered appropriate for safe storage of green coffee without quality loss.

13.4 Cacao

Cacao has been used by mankind for more than 2000 years either as a food, beverage, or medicine (Schwan and Wheals 2004). Cacao originally stems from the tropical forests of South America as the cacao tree grows only close to the equator (Figure 13.4).

Cacao was introduced in Europe by the Spanish conquistadores. The importance of the cacao tree for humankind is emphasized by its name, i.e., *Theobroma cacao*, as the genus name literally means “food for the gods” (Schwan and Wheals 2004). The cacao tree belongs to the family Steruliaceae and two subspecies are recognized within *T. cacao*: *criollo* and *forastero*, which are further divided into various varieties. *Trinitario* is a third group often referred to in the literature and is a hybrid between *criollo* and *forastero* varieties (Wood and Lass 1985, Fowler et al. 1998). Following the Spanish conquest of Mexico, cacao cultivation spread to the Caribbean islands, parts of South America, and later across the Pacific to the Philippines, Sulawesi, and

FIGURE 13.4 Cacao tree with mature and immature pods. (Photo courtesy of Dr. Louis Ban-Koffi.)

Java. Toward the end of the nineteenth century, cacao was taken from Brazil across the Atlantic to Ghana where it formed the basis for cacao production in West Africa. Today, cacao is grown in a 20°C belt north and south of the equator. The mean (over 1 month) minimum temperature in most cacao-growing regions is 18°C, and the mean maximum temperature is 32°C. A high rainfall of 1000–4000 mm/year is required and the dry season should preferably be shorter than 3 months and not totally dry. Cacao is obtained by grinding fermented, dried, roasted, and peeled cacao seeds. Cacao, similar to coffee or tea, contains a high concentration of alkaloids that act as stimulatory agents for humans. Despite this, cacao is not considered a natural stimulant but rather as a food, as it contains a high nutritional value. Cacao seeds contain 52% fat, 14% protein, 6% starch, 1.5%–2.1% theobromine, 0.2% caffeine, and 6%–7% catechin tannins (Bickel-Sandkötter 2001).

The fruit of the cacao tree is a pod containing 20–30 (*criollo*) or 30–40 (*forastero* and *trinitario*) beans (often also referred to as seeds when fresh and unfermented) embedded in a mucilaginous pulp (Figure 13.5).

The pods develop from pollinated flowers emerging directly out of the bark on the stem of the cacao tree. Raw *forastero* beans are violet in cross section and produce a strong cacao flavor upon proper processing, whereas *criollo* beans in the raw condition are white, ivory, or very pale colored and have a weaker but very aromatic flavor. The *criollo* beans tend to be bigger and rounder and have a lower fat content compared to *forastero* beans (Wood and Lass 1985). *Criollo* and *trinitario* are considered "fine" cacao, often sold at higher prices than *forastero*. The concentration of anthocyanins in *forastero* beans is approximately 0.5%; these compounds do not occur in the *criollo* beans. *Criollo* cotyledons, on the other hand, contain procyanidin. These differences

FIGURE 13.5 Cacao beans embedded in mucilaginous pulp. (Photo courtesy of Dr. Louis Ban-Koffi.)

are reflected in the fermentation times, where *criollo* beans need to be fermented for a shorter time period of 2–3 days, while *forastero* beans need to be fermented for 5–7 days (Schwan et al. 1995). The *forastero* tree furthermore is less sensitive to growing conditions and to disease, and thus delivers higher yields. Because of this, more than 90% of the produced cacao in the world stems from *forastero* trees or from hybrid *trinitario* trees. The most important cacao-producing countries today include Ivory Coast, Ghana, Brazil, Nigeria, Cameroon, Malaysia, Indonesia, and Ecuador (Kostinek and Franz 2007). Ivory Coast is the largest cacao producer in the world.

13.4.1 Fermentation of Cacao Beans

The pulp of the cacao bean is the actual substrate of the fermentation. The pulp is a rich medium for microbial growth and contains 82%–87% water, 10%–15% sugars (mainly glucose, fructose and sucrose), 2%–3% pentosans, 1%–3% citric acid, and 10%–15% pectin (Roelofson and Giesberger 1947, Thompson et al. 2001, Ardhana and Fleet 2003). The high pectin concentration as well as presence of other polysaccharides (1%–2%) makes the pulp viscous (Roelofson and Giesberger 1947, Pettipher 1986). The glucose/fructose to sucrose ratio changes with the degree of maturity, with unripe pods containing a higher proportion of sucrose, and ripe pods containing mainly glucose and fructose (Packiyasothy et al. 1981). Proteins, amino acids, and vitamins (mainly vitamin C) are also present in the pulp. Each seed consists of two cotyledons and one embryo, contained in a seed coat, and the seeds within the ripe and undamaged cacao fruit are almost germ free. Raw cacao has an astringent unpleasant taste and needs to be fermented, dried, and roasted to obtain the characteristic taste and flavor. The primary processing steps in cacao production include harvesting, pod breaking, fermentation, and drying. The fermentation and drying steps are often simply referred to as "curing" (Wood and Lass 1985, Lopez and Dimik 1995, Nielsen 2006).

During harvesting, the cacao fruits are carefully removed from the stem of the tree and then broke, opened (Figure 13.6) with a wooden hammer, and the beans are removed by hand.

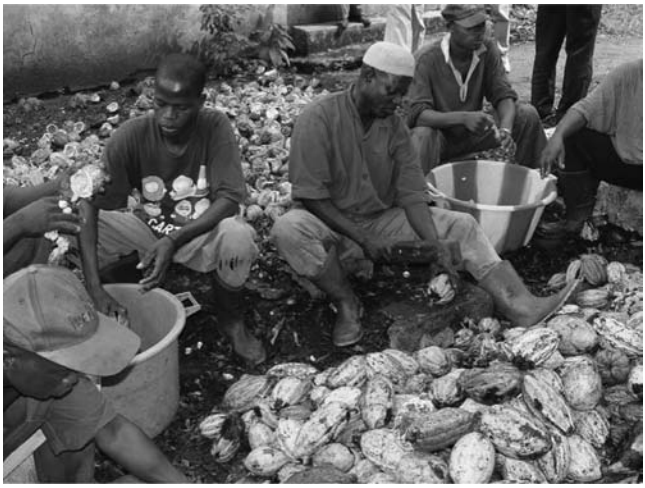

FIGURE 13.6 Cacao pods breaking. (Photo courtesy of Dr. Louis Ban-Koffi.)

The seeds covered with the wet pulp are collected in baskets and brought to the fermentation facility. In many cacao-producing areas, however, it is common practice to harvest the pods over some days before the collected fruits are transported to the fermentation facility. This is considered beneficial for the fermentation, as it results in a more rapid increase in temperature during fermentation and consequently in a faster fermentation. This is probably because sucrose is converted to glucose and fructose during the storage period (Rohan 1963, Tomlins et al. 1993, Nielsen 2006). At the fermentation facility, they are either fermented in heaps on plantain leaves (heap fermentation) or in flat (but relatively deep) trays (tray fermentation).

After opening of the pods, the cacao seeds are spontaneously contaminated, via contact with the pod outer coating, knives, hands of workers, ground, and carrying basket surfaces, with a large variety of microorganisms (Schwan et al. 1995). Several methods are used to ferment the beans. The most currently used is heap fermentation that is carried out on banana leaves or on tarpaulin (Figures 13.7 and 13.8); other methods are box fermentation (Figure 13.9) and tray fermentation. The heap and box fermentations are mainly practiced in Ivory Coast.

The fermentation can be divided into two important events: first, organic acids, ethanol, and heat are generated as a result of microbial activities, and second, complex

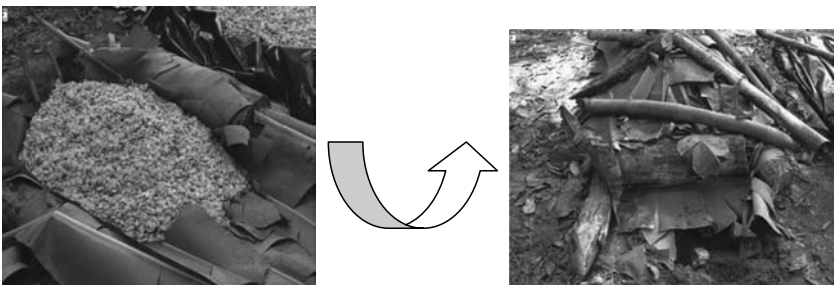

FIGURE 13.7 Heap fermentation on banana leaves. (Photo courtesy of Dr. Louis Ban-Koffi.)

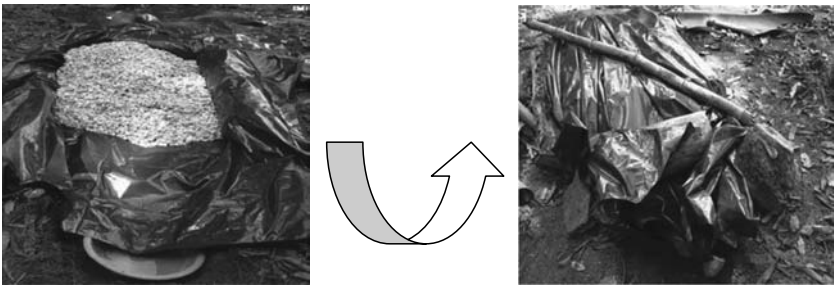

FIGURE 13.8 Heap fermentation on plastic sheet. (Photo courtesy of Dr. Louis Ban-Koffi.)

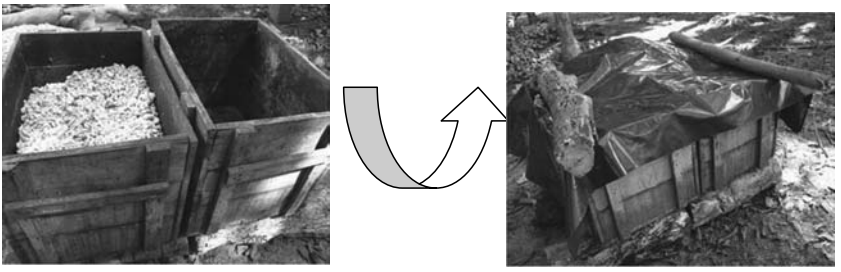

FIGURE 13.9 Box fermentation in wooden boxes. (Photo courtesy of Dr. Louis Ban-Koffi.)

biochemical reactions are initiated by the diffusion of microbial metabolism products within the cotyledons. Thus the fermentation aims to remove residual pulp, kill the embryo, and improve the aroma, flavor, and color of the bean. Removal of the mucilaginous pulp during the fermentation is very important, as remaining pulp would inhibit drying the beans to microbiologically stable water content (Roelofson 1958, Wood and Lass 1985, Schwan et al. 1995, Lopez and Dimik 1996, Nielsen 2006, Kostinek and Franz 2007). During the 3–13 days fermentation, it is necessary to turn (mix) the heaps once or twice daily in order to obtain a uniform fermentation, to provide sufficient aeration, and to keep the temperatures under 45°C–50°C (Baker et al. 1994, Nielsen 2006).

Fermentation times vary widely from country to country and even from farm to farm. No strict definition of when to terminate the fermentation has been formulated but the experienced farmer knows when to stop fermenting and when to start drying. This is based on his observations on the smell of the fermenting mass (i.e., development of an acetic acid odor from the activity of acetic acid bacteria), the internal and external appearance of the beans, and the falling temperature of the fermenting mass (Forsyth and Quesnel 1956, 1963, Nielsen 2006). Upon drying, the moisture content of the beans must be brought from the initial 40%–60% to 6%–7% to avoid growth of molds. Furthermore, biochemical reactions important to flavor and taste and color development must take place during drying. While from the point of avoiding fungal growth drying should be as fast as possible, from the flavor and aroma point of view drying should not be too fast. Thus, it has been established that drying should take at least 48 h to allow proper flavor development (Wood and Lass 1985, Faborode et al. 1995, Thompson et al. 2001, Nganhou et al. 2003, Nielsen 2006).

13.4.2 Microbiology of Cacao Fermentation

The physical and chemical changes in the fermenting mass provide a special ecosystem for a succession of subsequently developing microorganism groups including filamentous fungi, yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB), and *Bacillus* species (Ardhana and Fleet 2003, Kostinek and Franz 2007). In early publications, filamentous fungi were not regarded as very important for the fermentation (Roelofson and Giesberger 1947, Roelofson 1958). More recent investigations have shown, however, that fungi already occur at the beginning of fermentation at 10^2–10^3 cfu/g and that they reach levels of 10^6–10^7 cfu/g within 24–36 h. After this, their numbers are reduced to below the detection limit for the subsequent fermentation but they reappear again at the end of fermentation. As filamentous fungi can possess pectinolytic enzymes, they may play an important role in the degradation of pectin in the pulp in the early stages of fermentation (Ardhana and Fleet 2003). Furthermore, they possess amylases and may be important in the breakdown of starch in the cacao beans during the generation of flavor and aroma. Also, lipolytic enzymes produced by filamentous fungi could play an important part in the generation of flavor and aroma, as the beans contain a high percentage (more than 50%) of fat (Hansen 1975a,b, Lehrian and Patterson 1983). The following molds were isolated from cacao fermentations so far: *Aspergillus fumigatus*, *A. glaucus* (*Eurotium herbariorum*), *Mucor* spp., and *Aspergillus* spp. In an investigation of molds from cacao fermentation in Indonesia, *Penicillium citrinum* was isolated as the predominant mold, followed by *A. versicolor* and *A. wentii*. Furthermore, *P. purpurogenum* and *P. ochrochloron* were also isolated. As the pulp is rich in easily fermentable sugars such as glucose and fructose and has a low pH of about 3.0–3.5 resulting from the presence of citric acid, it is especially yeasts that dominate in the first 12–48 h of the fermentation, reaching 10^7–10^8 cfu/g. The yeasts that occur include *Kloeckera, Hanseniaspora*, *Saccharomyces*, *Candida*, *Pichia*, and *Kluyveromyces* spp., among which *Saccharomyces* species occur most often. In a recent study by Nielsen et al. (2007a), *Hanseniaspora guilliermondii* was the predominant yeast during the initial fermentation phase, and *Pichia membranifaciens* dominated among yeasts during the later phase of the fermentation. Their most important tasks are (1) to remove citric acid from the pulp and thus to increase the pH from approximately 3.5–4.2 so that bacteria can grow, (2) production of ethanol at low oxygen concentrations, (3) production of organic acids (oxalate, succinate, malate, and acetate), (4) production of volatile organic compounds (fusel oils, fatty acids, and fatty acid esters) which are precursors for the later chocolate aroma, and (5) production of pectinolytic enzymes which lower the viscosity of the pulp thus enabling a better aeration of the fermenting mass.

Because of citrate removal and a consequent rise of pH in the fermenting mass, as well as an increasingly aerobic environment, bacteria start to grow in the fermenting cacao mass. LAB grow to levels of 10^6–10^8 cfu/g in the first 36–48 h of the fermentation, after which their numbers start to decrease. The main LAB genera involved include *Lactobacillus*, *Leuconostoc*, and *Lactococcus*, but in some studies the genera *Pediococcus* and *Weissella* were also predominant in the fermentation (Camu et al. 2008, Kostinek et al. 2008). *Lb. fermentum*, *Lb. plantarum*, *Leuc. mesenteroides*, and *Lc. lactis* predominated in cacao fermentations in Trinidad during the first 24 h of fermentation (Ostovar and Keeney 1973). Passos et al. (1984) identified *Lb. casei*, *Lb. plantarum*, *Lb. delbrueckii*, *Lb. acidophilus*, *Lb. brevis*, *Lb. lactis* (=*Lb. delbrueckii* spp. *lactis*),

Ped. dextrinicus (=*Lactobacillus dextrinicus*), and *Ped. acidilactici* as predominant LAB species occurring in cacao fermentations from Brazil. Generally, *Lactobacillus* species occurred during the early phase, while *Lactococcus* species occurred in the later phase of the fermentation. In most of these early studies, *Lb. plantarum* was found to predominate the LAB in the cacao fermentation. Ardhana and Fleet (2003) also showed that *Lb. cellobiosus* (=*Lb. fermentum*) belonged to the predominant *Lactobacillus* species involved in cacao fermentation in Indonesia, and that they could be isolated with a frequency of 60%–85% over a 48 h time period. In more recent investigations based on polyphasic taxonomical approaches, we (Kostinek et al. 2008) and Camu et al. (2008) could also show that the majority of the isolates from Nigerian and Ghanaian cacao fermentations consisted of *Lb. plantarum* strains and *Lb. fermentum* strains, respectively. In addition, Camu et al. (2008) identified *Weissella confusa* and *W. paramesenteroides* to be predominantly associated with the fermentation, and in subsequent studies they could identify a novel *Weissella* spp., i.e., *Weissella ghanensis*, to be associated with the fermentation (De Bruyne et al. 2008). In our studies, we also showed that *P. acidilactici* was predominant in Nigerian cacao fermentations (Kostinek et al. 2008). In the study of Nielsen et al. (2007a), *Lb. fermentum* was the predominant LAB in the Ghanaian cacao fermentation and several other LAB including *Lb. plantarum*, *Leuc. pseudomesenteroides*, *Leuc. pseudoficulneum* (= *Fructobacillus pseudoficulneus*) as well as *P. acidilactici* were also detected during fermentation. A novel *Lactobacillus* species, *Lb. ghanensis*, was discovered and described in a subsequent study (Nielsen et al. 2007b).

Acetic acid bacteria (AAB) (*Acetobacter*, *Gluconoacetobacter*, and *Gluconobacter* spp.) grow in the last aerobic phase of the cacao fermentation to levels of 10^6–10^9 cfu/g, depending on the amount of aeration during the fermentation (Schwan et al. 1995). They oxidize the ethanol produced from yeasts and LAB to acetate and further to CO_2 and H_2O. Some of the AAB also oxidize lactate. Nielsen et al. (2007a) showed that *Acetobacter syzygii*, *A. pasteurianus*, and *A. tropicalis* were the predominant AAB in Ghanaian cacao fermentations. Camu et al. (2008) reported *A. pasteurianus*, *A. ghanensis*, and *A. senegalensis* and a potentially new *A. lovaniensis*-like species to predominate in Ghanaian cacao fermentations. The exothermic reactions of the AAB led to a temperature increase in the fermenting cacao mass (Schwan 1998) and the organic acids produced during the fermentation diffuse into the bean and reduce the pH which inhibits germination of the embryo. This inhibition is essential for aroma production and prevents quality loss by germination (Schwan 1998).

Aerobic spore-forming bacteria dominate during the later phases of the fermentation, reaching approximately 10^7–10^8 cfu/g. At this time, the conditions in the fermenting mass have become less acidic and much more aerobic. Furthermore, *Bacillus* and *Geobacillus* species can tolerate temperatures of 45°C–50°C, which occur in the later fermentation phase (Ardhana and Fleet 2003). Aerobic bacteria isolated from fermenting cacao include *Bacillus subtilis*, *B. cereus*, *B. licheniformis*, *B. coagulans*, *B. pumilus*, and *Geobacillus stearothermophilus* (Schwan et al. 1986, Nielsen et al. 2007a). *Bacillus* spp. produce diverse chemical compounds, e.g., 2, 3 butanediol and pyrazine, as well as lactic and acetic acids, which can contribute to the acidification of the fermentation, but sometimes also to flavor defects in cacao beans (Schwan et al. 1986). Furthermore, these bacteria produce, similar to certain filamentous fungi, proteases, amylases, and lipases (Ardhana and Fleet 2003). Pathogenic and toxinogenic bacteria (other possibly than *B. cereus*) have not been described to occur in the

fermentation, probably as a result of the relatively high acetic acid concentrations present in the fermenting pulp.

13.4.3 Biochemical Changes during Processing

The taste and aroma of the cacao depends on the fermentation, the drying, and the subsequent roasting of the cacao beans. Fresh cacao beans contain in their cotyledons two types of parenchymal cells: protein–lipid cells containing stored proteins, fats, and starch, as well as polyphenol cells. As mentioned above, the combined effect of increasing temperature and acetic acid and ethanol penetrating the testa and entering the beans as the fermentation progresses kills the embryo, and causes breakdown of the protein–lipid and polyphenol-containing storage cells in the cotyledon and diffusion of their contents (Biehl 1973, Lopez et al. 1987, De Brito et al. 2000, Thompson et al. 2001, Nielsen 2006). These then come into contact with plant and microbial enzymes. The biochemical changes in the cacao bean can be divided into two phases. In the first phase, the so-called anaerobic hydrolytic phase, the concentration of free amino acids and peptides increases as a result of proteolytic breakdown of proteins. These are very important for the later roasting step and for the development of cacao aroma. (Ziegler and Biehl 1988). Anthocyanins rapidly break down to anthocyanidins and sugars (galactose and arabinose) with reductions of up to 93% after 4 days having been reported (Forsyth and Quesnel 1957, Lehrian and Patterson 1983, Wollgast and Anklam 2000, Nielsen 2006). Sucrose is converted to glucose and fructose with the latter thus increasing in concentration, while the polyphenol content is decreased. Furthermore, the amounts of threobromine and caffeine are decreased as they diffuse out of the bean. In the second, aerobic and oxidative phase, the polyphenols (including the anthocyanidins) are oxidized and polymerize into insoluble high molecular weight compounds (tannins) during fermentation and drying. Bitterness and astringency are thus reduced. The reactions which began in the oxidative phase continue, until the enzymes are inhibited as a result of too little water being present. After this, the beans are roasted and reactions take place in which the cacao flavor develops. The Maillard reaction, the degradation of proteins, the caramelization of sugars, and the development of volatile compounds such as pyrazine all are involved in cacao aroma development (Schwan and Wheals 2004). The majority of microorganisms are killed during roasting at 120°C–140°C but because of the high fat content a sterile product is not obtained. Mycotoxins are also not destroyed at these temperatures.

Clearly the fermentation of cacao is a complex process that depends on the concerted action of a wide variety of microorganisms. A number of studies have shown which microorganisms are predominantly associated with the fermentation, but clearly we still do not know all of the organisms involved. This becomes clear from the fact that recent studies have shown quite a few novel species of LAB or AAB to be associated with the fermentation (Nielsen et al. 2007b, Cleenwerck et al. 2007, 2008; De Bruyne et al. 2008, 2009). Furthermore, some differences in the microbial compositions are clearly highlighted by the various studies (e.g., the occurrence of pediococci in some fermentations and not in others), and it is unclear whether environmental conditions select for specific species, and if so which species. It is also not yet clear which flavor compounds are created during the roasting stage, and whether the fermentation and flavor can be controlled by using starter cultures. These are exciting areas for future research.

13.5 Conclusion

Beverages such as tea, cacao, and coffee are tremendously important products that have found worldwide acceptance among consumers and thus are immensely important also for global trade and economy. It is, however, not only the plant variety and the quality of the raw material which finally dictates the quality and the price of the end product, but also the types of microorganisms present and their succession within the fermentation have a major impact on quality. Common among all these fermentations is that these are associated with a tremendously complex microbial diversity and furthermore rely on an intricate succession of these microorganisms to convert the raw material to quality end products. The main groups of beneficial microorganisms associated with these fermentations that appear to be necessary for quality include yeasts and lactic acid bacteria, and in the case of cacao fermentation also acetic acid bacteria. The main fermentation activities of fermenting away the pulp of the fruit and killing the embryo of the beans, thus preventing germination, are major determining processing factors in the production of cacao and coffee. The above fermentations, however, are not easily controlled. Thus, unraveling the complex microbiota associated with the fermentation and determining the roles of strains and their interaction will be the first steps in better fermentation control. We have indeed come a long way in the understanding of the fermentations of tea, coffee, and cacao. We even found completely new bacterial species associated with all these products, yet we have not come closer to better fermentation control and need more scientific investigation into these very important beverage fermentations.

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14

Probiotic and Prebiotic Fermented Foods

Kasipathy Kailasapathy

CONTENTS

14.1 Introduction

Consumers are increasingly becoming aware of their personal health, and expect the foods they consume not only to be safe and healthy but also capable of preventing illnesses. In both the developing and developed countries, an aging population, stress-associated busy lifestyle, and a self-medication approach to the prevention of health-related issues rather than curing them are factors that are increasingly contributing to narrow the interphase between food and drugs. Foods are no longer liked by consumers solely for taste and providing nutritional needs, but also because of their ability to provide specific physiological benefits above and beyond their basic nutritional values. A fermented functional food or beverage imparts a physiological benefit that enhances overall health, helps to prevent or treat a disease/condition, or improves

physiological or mental performance via an added functional ingredient, processing modification, or biotechnology (Shah 2001).

A fermented food can be considered as functional if it could be satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way which is relevant to either the state of well-being and health or the reduction of the risk of a disease. A food can be made functional by improving the bioavailability of a particular component. Functional food science will serve to establish claims based either on enhanced function or disease risk reduction (Roberfroid 2002).

Functional foods for gut health currently make up the largest segment of the functional food market in Europe, Japan, and Australia (Heasman and Mellentin 2001). The significant market drivers for growth of functional foods include: increased consumer health awareness, increased popularity of healthy foods, aging population demographics, stress-associated lifestyles, search for food-based drug substitutes, self-medication approach promoted by busy lifestyles, organics and natural remedies, side effects of drugs, and rapidly developing health conscious markets. Development of functional food products in recent years has focused on dietary supplements that may favorably influence gut microbial composition and activities. A common perception is that microbes are dangerous, a misconception fueled by an abundance of information regarding pathogenic bacteria, yeast, and viruses, and their associated morbidities and mortalities. A growing body of evidence, however, shows that the human colon contains not only pathogenic species but also nonpathogenic and health-promoting species called probiotics. The number of microbial cells harbored by every human is approximately 10^{14}, and is estimated to be 10–20 times greater than the number of mammalian cells of the host (Savage 1997, Suau et al. 1999). This microbiota functions in such a manner that the colon is the most metabolically active organ in the body and possibly having a very significant nutritional role. Dietary supplementation is a feasible route by which the large gut microbiota composition and activities can be modulated. In recent times, there has been a growing appreciation for the important role of gut microbes in human and animal health, be it through mediation of intestinal development and innate immunity or digestion of food and protection of the host against diseases (Reid 2008).

Probiotics are live microbial food supplements that have been in use in recent years and are available incorporated into many food products, primarily fermented dairy foods. The positive health effects attributed to lactic acid bacteria and foods fermented with these bacteria have been long recognized. Bacteria that produce lactic acid and are perceived to exert beneficial properties such as improved lactose digestion and resistance to pathogens are the common probiotics.

Prebiotics are non-digestible food components (e.g., oligosaccharides) that are resistant to human digestive enzymes and pass to colonic regions undigested, undergo selective fermentation, and provide nutrients for the colonic commensal bacteria. Fructose oligosaccharides are able to modify the gut flora composition in favor of bifidobacteria. Prebiotics have better survival capabilities than probiotics. Thus the probiotic approach and the prebiotic approach to the development of functional foods are fundamentally different, though both contribute to overall gut health.

A combined strategy that includes both probiotic and prebiotic technologies is known as synbiotics. For example, Cardarelli et al. (2008) reported a synbiotic,

petit-suisse, cheese incorporating probiotic bacteria and prebiotics such as inulin. This chapter discusses the science and technology of probiotics, probiotic strains, prebiotic substances, their efficacy, challenges in incorporating probiotics and prebiotics into foods, viability issues, physiological benefits, packaging considerations, and product examples. This chapter also discusses the efficiency and evaluation of prebiotics and the differences between probiotic, prebiotic, and synbiotic approaches to maintain gut health.

14.2 Probiotic Functional Foods

14.2.1 Probiotic Bacteria

Probiotic bacteria have been shown to possess immunomodulatory activities in human and mouse models, demonstrating the potential to improve human health and to treat diseases. Many health-benefiting effects have been described for probiotic bacteria including reduction of cholesterol, ammonia, and other toxic compounds; the restoration of normal intestinal microflora following antibiotic therapy; and potential treatment of gastrointestinal tract (GIT) disorders among other health effects (Gomes and Malcata 1999, Shah 2007, Prado et al. 2008). Due to such associations with health benefits, probiotic bacteria are incorporated into many foods and beverages, particularly dairy products, which have a large consumer market. *Lactobacillus acidophilus* is a widely used probiotic in yogurts and other dairy products. The Majority of probiotic microorganisms are strains of *Lactobacillus* and *Bifidobacterium* (both are found in the human intestine); however, it is not limited to these genera, with *Bacillus cereus* (animal probiotic), *Clostridium butyricum*, and *Saccharomyces boulardii*, among others, also classified as being probiotic (Prado et al. 2008). Nonetheless, for a microorganism to be considered a probiotic, validation of health benefits, strain identification, and other characteristics are required.

14.2.2 Properties of Probiotic Bacteria

The properties used for probiotic classification reflect the ability of microorganisms to survive transition through the GIT including acid and bile resistance, attachment abilities to intestinal epithelial cells, human intestinal colonization, antimicrobial substance production, and conveyance of beneficial effects on human health (Collins et al. 1998, Holzapfel et al. 1998, Kailasapathy and Chin 2000, Tuomola et al. 2001, Morelli 2007, Pineiro and Stanton 2007, Prado et al. 2008). Emphasis has also been placed on nonpathogenicity of probiotic bacteria and generally regarded as safe (GRAS) characteristics (Collins et al. 1998, Liong 2008, Prado et al. 2008). In addition, Kailasapathy and Chin (2000) stated that enzyme and oxygen stability and demonstrable efficacy and safety are also common criteria for the selection of probiotic bacteria. Food products containing probiotic bacteria are exposed to these elements; therefore, stability of probiotic bacteria in food products is also considered essential for the conveyance of health benefits to consumers as well as the strain being nonpathogenic. Adhesion abilities of probiotic bacteria to intestinal epithelial cells are also considered a classification requirement for probiotic bacteria. Colonization of the intestinal tract by pathogens is inhibited by probiotic bacteria that competes for nutritional metabolites

and inhibits the adhesion of pathogens to the intestinal epithelium (Holzapfel et al. 1998, Blum et al. 2002, Corthesy et al. 2007). In addition, the ability of probiotic bacterial cells to adhere to intestinal epithelial cells potentially stimulates the immune system (Tuomola et al. 2001). Probiotic bacteria also produce lactic and acetic acids subsequently lowering the pH of the intestinal environment, making it uninhabitable for pathogenic microorganisms (Kailasapathy and Chin 2000).

A probiotic must have good stable properties so that it can be cultured and incorporated into food products without loosing viability and functionality or creating unpleasant flavors or textures in the product. Selection criteria for probiotic bacteria include survival in acidic gastric juices, and alkaline bile secretions and in the presence of hydrolytic and proteolytic digestive enzymes during their transit through the gut. Additionally, probiotic bacteria must be capable of colonizing the GIT; they must be safe and have the potential to maintain their efficiency during the shelf life of the product. Other requirements include the growth and survival of the organism under the fermentation conditions required for its commercial manufacture. The organism must grow well in simple media to high cell concentrations, and survive centrifugation, freeze drying, and freezing. The probiotic culture must also withstand incorporation into a range of food matrices, and be able to survive the shelf life of the food product. Careful screening of probiotic bacteria for their technological suitability can also allow the selection of strains with the best manufacturing and food technology characteristics. However, even the most robust probiotic strains are limited in the range of food applications to which they can be applied. In addition, bacteria with exceptional functional health properties are often ruled out due to technological limitations. The viability of probiotics has been both a marketing and technological challenge for many food processing industries. The standard for any food sold with health claims derived from the addition of probiotics is that it must contain at least 10^6–10^7 cfu of viable probiotic bacteria per gram (FAO/WHO 2001). Viability during the shelf life of the product and survival in the GIT to populate the human gut are two important issues in the provision of health benefits by probiotics. Additionally, factors related to the technological and sensorial aspects of probiotic food production are of importance since only by satisfying the demands of consumers can the food industry succeed in promoting the consumption of functional probiotic products in the future. In certain cases, nonviable probiotic bacteria may also have beneficial effects on health (Ouwehand and Salminen 1998). Despite the recognition of the possible physiological benefits of nonviable probiotics, viability of the bacteria is nevertheless considered one of the most important characteristics of probiotics, and the term viable is included in most definitions of probiotic bacteria.

14.2.3 Beneficial Effects of Probiotics

Many health benefits of probiotic bacteria have been reported, including enhancement of immunity against intestinal infections, general immune enhancement, prevention of diarrheal diseases, prevention of colon cancer, prevention of hypercholesterolemia, improvement in lactose utilization, prevention of upper gastrointestinal tract diseases, and stabilization of the gut mucosal barrier (Kailasapathy and Chin 2000). Probiotic microbes have been linked to a range of beneficial effects on host health; some are well documented, and others need further assessment. There is an increasing number of proposed health benefits targeted outside the gastrointestinal tract. An important

reason for the increasing proposed health benefits of probiotic bacteria is the increasing awareness of the role of the gut microbiota in the pathogenesis of a number of clinical conditions, which has provided a rationale for attempts to treat or prevent these conditions by modulating gut microbiota. Additionally, the crucial role of gut microbiota in the development and the modulation of host immune functions have become evident. Hence, administration of probiotics may provide a means for directing the host immune responses, both locally and systemically.

The use of probiotic cultures stimulates the growth of preferred microorganisms, crowds out potentially harmful bacteria, and reinforces the body's natural defense mechanisms (Gismondo et al. 1999, Dunne et al. 2001). The probiotic effect may also operate in a variety of ways to achieve resistance to a wide range of infectious agents. The mechanisms of antipathogenic effects may also be through decreasing the luminal pH by the production of organic acids (acetate, lactate, or propionic acids), rendering vital nutrients unavailable to pathogens, changing the redox potential of the intestinal environment, producing bacteriocins, competing with pathogens for receptor sites on the intestinal wall (competitive exclusion), and producing hydrogen peroxide and antimicrobial or other inhibitory substances (Gilliland and Speck 1977, Hughes and Hoover 1991, Kailasapathy and Chin 2000). The activities of probiotics may include cell-mediated immune responses, including activation of the reticuloendothelial system, augmentation of cytokine pathways, and stimulation of pro-inflammatory pathways such as interleukin regulation (Gill et al. 2001, Paturi 2007).

Currently, the best-documented health effects are the treatment and prevention of rotavirus diarrhea, and the improvement of lactose digestion by consuming yoghurt. The efficiency of the management of antibiotic-associated diarrhea has recently been confirmed in several metaanalyses. The prevention of urinary tract infections is also well documented. However, other promising therapeutic applications requiring further clinical evidence include prevention of eczema, prevention of relapse of ulcerative colitis, treatment of pouchitis, blood pressure lowering effects, prevention of necrotizing enterocolitis, treatment of bacterial vaginosis, and prevention of *Clostridium-difficile*-associated diarrhea. Treatment of allergies, adjuvant effects on antibiotic therapies against *Helicobacter pylori*, treatment of constipation, treatment of irritable bowel syndrome, and treatment of short bowel syndrome also appear promising. It is widely accepted that the health benefits of probiotic bacteria are strain dependent and strain specific and that the documented health effects of one strain are not applicable to others.

14.2.4 Viability Issues of Probiotic Bacteria

Viability of probiotics is often considered a requirement for the beneficial health effects, and it is, by definition, a requirement for microbes to be considered a probiotic. Based on current reports, viable probiotics appear to be superior in mediating a number of health benefits. The viability of probiotic cultures in functional foods is therefore a critical issue of their functionality. The viability and metabolic activity of probiotic bacteria are important considerations because the bacteria must survive in the food during its shelf life and during transit through the acidic conditions of the stomach, and must resist any degradation by hydrolytic enzymes and bile salts in the small intestine. It is essential that products sold with any health claims meet the recommended criterion of a minimum of 10^6–10^7 cfu/g viable probiotic bacteria (FAO/WHO 2001).

14.2.5 Applications of Probiotic Cultures in Functional Foods

The wide range of claimed health benefits of probiotics has stimulated the rapid expansion of the market for probiotic products worldwide. Because of these perceived health benefits, probiotic bacteria have been increasingly included in fermented dairy products, including yoghurts, soft and hard cheeses, dairy spreads, margarine, ice cream, frozen fermented dairy desserts, and fermented milks. Probiotic foods can be made in two ways: (1) fermentation of raw ingredients by probiotics, with or without starter cultures, and (2) addition of suitable concentrations of probiotics to the finished product. Probiotic fermentation of raw ingredients allows the bacteria to multiply and impart distinctive flavors and organoleptic changes to the food. The probiotic strains selected for each different food system will affect the qualities of the final product, depending on the type and amount of acids and other metabolites that are produced. Hetero- and homofermentative lactobacilli will impart different flavors during fermentation. Homofermenters produce an "acidic sour taste" from lactic acid and heterofermenters yield an "sharp acidic taste" from the production of acetic acid. When probiotic bacteria are added to the final product just before packaging (e.g., in some acidophilus milk), bacterial survival rather than growth is the important factor. Bacteria must be added at a suitable concentration to remain greater than 10^6 cfu/g food for the shelf life of the product. Unplanned bacterial growth during this time may produce undesirable changes such as postacidification during storage, resulting in a less acceptable produce.

Whether probiotics are used in the manufacture of the food product or are added postproduction, the most important factor is the ability of the produce to convey the desired health benefits. Strain selection, food composition, the use of prebiotics, and food parameters such as pH will determine the successful survival of the bacterial species. Product taste, appearance, composition, and marketing will determine the success of the produce in the market place. In addition to their application in dairy products, probiotic bacteria are now being applied to more new food products including fermented cereals, infant formulas, beverages, and some therapeutic foods. Over 70 products all over the world including sour cream, butter milk, yoghurt, powdered milk, and frozen desserts contain *Bifidobacterium* and lactobacillus (Shah 2001). In the recent past, a number of new products incorporating probiotics have been researched. These include probiotic orange juice (Luckow and Delahunty 2004), oat-based cereal bars incorporating *Bifidobacterium lactis* Bb-12 (Ouwehand et al. 2004), dry fermented sausages incorporating probiotic bacteria (lactobacillus strains) (Pennacchia et al. 2004), and a nonfermented frozen vegetarian dessert containing *B. lactis*, *Lactobacillus rhamnosus*, and *Lactobacillus paracasei* subsp. *paracasei* (Heenan et al. 2004). Other possible developments include novel applications of probiotic bacteria in the food service industry.

14.2.6 Factors Affecting the Survival of Probiotic Bacteria

Analysis of probiotic products in many different countries has confirmed that probiotic strains exhibit poor survival in foods such as yoghurts and fermented milks, with cell numbers being much lower than the recommended levels at the expiry date (Kailasapathy and Rybka 1997, Shah 2000, Lourens-Hattingh and Viljoen 2001). Probiotic preparations such as tablets, powders, etc., may contain lower viable counts.

A number of factors such as acid, alkali, hydrogen peroxide produced by yoghurt bacteria, the concentration of lactic acid and acetic acids, interaction of the probiotic species with the yoghurt starters, buffering capacity, whey proteins, sugars, incubation temperature and fermentation time, and the fat content of the yoghurt can affect the survival of probiotic bacteria in dairy fermented foods (Shah 2000, Vinderola et al. 2002). Processing of probiotic foods involve heat treatment (pasteurization), pumping, homogenizing, ultrafiltration and stirring (incorporation of air), freezing (frozen dairy products), addition of ingredients which can be antimicrobial (e.g., salt), drying (powdered milk products), packaging (oxygen entry into product during storage), unfavorable storage conditions (e.g., postacidification in yoghurt), heat shock (in cold-chain manufacture and storage), and the possible development of antimicrobial compounds secreted by the starter cultures during fermentation.

14.3 Prebiotic Functional Foods

A prebiotic is defined as a nondigestible food ingredient that affects the host by selectively targeting the growth and/or activity of one or a limited number of bacteria in the colon, and thus has the potential to improve host health (Gibson and Roberfroid 1995). For food ingredients to be classified as prebiotics, they must (1) neither be digested nor absorbed in the stomach or small intestine; (2) act as a selective food source for one or a limited number of potentially beneficial commercial bacteria in the large intestine; (3) change the colonic microbiota ecosystem toward a healthier composition; and (4) induce luminal or systemic changes that improve the health of the host. Through the process of fermentation, colonic bacteria are able to produce a wide range of compounds that have varying potential effects on gut physiology, as well as other systemic influences. Better resistance to pathogens, reduction in blood lipids, antitumor properties, improved nutrition absorption, hormonal regulation, and immune stimulation may all be possible through gut microflora manipulation. One approach advocates the oral intake of live microorganisms (probiotics). However, survival after ingestion is difficult to guarantee and to prove and is very strain dependent. The prebiotic approach dictates that nondigestible food components are specifically fermented in the colon by indigenous bacteria thought to be of beneficial value (e.g., bifidobacteria, lactobacilli) so that these selectively increase in numbers.

14.3.1 Types of Prebiotics

The principal substrates for bacterial growth in the colon are dietary carbohydrates that have escaped digestion in the upper gastrointestinal tract. These include starch, nonstarch polysaccharides, unabsorbed sugars, nondigestible oligosaccharides, and sugar alcohols. While any food ingredient that enters the large intestine is a prebiotic candidate, it is the selectivity of the fermentation in the complex, mixed bacterial gut environment that is critical and required for a prebiotic effect. There are a number of substances that are loosely termed prebiotics. Many of these, however, fail to meet the criteria outlined above. Slippery elm, psyllium husks, guar gum, and pectin could best be considered as colonic foods rather than prebiotics as they appear to lack the selectivity of fermentation that is required of probiotics. Other substances,

such as polydextrose and larch arabinogalactans have been shown to increase the growth of beneficial bacteria in human trials. Certain carbohydrates, oligo- and polysaccharides, occur naturally and meet the criteria of prebiotics (Gibson and Roberfroid 1995).

14.3.2 Health-Related Aspects and Claims

14.3.2.1 Protection against Cancer

Many researchers believe that the colonic microflora has an important role to play in the development of bowel cancer. Prebiotics may protect against development of colon cancer through the selective modulation of microflora (increase of bifidobacteria and decrease of bacteroides, clostridia, and fusobacteria and/or gram-positive cocci) as well as production of protective metabolites. The colonizing cells of bifidobacteria produce lactic acid, thereby lowering the pH and creating a bactericidal environment for putative enteropathogens, thus developing a favorable microenvironment in the gut. This may also involve the modulation of specific bacterial enzymes such as beta-glucoronidase. Prebiotics also increase the production of short-chain fatty acids in the colon; butyrate is a common fermentation product that may inhibit the genotoxic activity of nitrosamines and of hydrogen peroxide in human cells and possibly induce a more differentiated phenotype including colorectal tumor cells (Reddy 1998, 1999). Prebiotic substances may also modify colonic metabolism away from protein and lipid metabolism, thus it is possible to shift the bacterial metabolism in the colon toward less harmful end products. For example, there could be a metabolic shift away from proteolytic fermentation by clostridia to a saccharolytic one.

14.3.2.2 Effect on Pathogens

Prebiotics demonstrate the ability to improve resistance to pathogens by increasing bifidobacteria and lactobacilli which in turn produce acid that lowers the luminal pH to levels below those at which pathogens can effectively compete. Many of the lactobacilli (e.g., *Lb. acidophilus*) are able to secrete natural antibiotics, which could exert a broad spectrum of bacterial activity. Among bifidobacteria, some species are capable of exerting antimicrobial effects on various gram-positive and gram-negative intestinal pathogens (Dave 1998).

14.3.2.3 Improved Mineral Uptake (Calcium)

The stimulation of mineral absorption by prebiotics leading to an increase in calcium and magnesium concentration at the level of the large intestine has been reported. An increase (about 65%) in calcium absorption was observed by Brommage et al. (1993) with 5% oligofructose or other nondigestible carbohydrates. Ingestion of oligofructose also decreased or prevented the loss of bone mass, calcium, and phosphorous from the bones of gastrectomized anemic rats (Ohta et al. 1998). The improved absorption of the minerals induced by prebiotics could be associated with reduction in pH of the ileal, cecal, and colonic contents due to the fermentation of prebiotics, such as inulin, resulting in a significant production of short-chain fatty acids. This is likely to increase calcium solubility and overall levels in the gut. Increased bacterial

breakdown of phytates (that bind divalent cations such as calcium and magnesium) could also release the bound minerals. Human clinical trials have also confirmed that the consumption of prebiotics such as chicory inulin or oligofructose results in increased calcium absorption (Coudray et al. 1997).

14.3.2.4 Lowering of Serum Lipids (and Cholesterol)

A large number of animal studies provide convincing evidence of the beneficial effects of prebiotics such as inulin and oligofructose on blood lipid levels. There is a marked reduction of serum triglycerides that occurs via reduction of fatty acid synthesis in the liver. It has been reported (Roberfroid et al. 1997, Delzenne and Kok 1999) that the decreased *de novo* lipogenesis in the liver is the result of a coordinated reduction of the activity of all lipogenic enzymes as a result of consistent intake of oligofructose. Thus, oligofructose could modulate inulin concentration possibly via effects on the secretion of gut hormones. Hence, oligofructose also decreases serum inulin and glucose.

14.3.2.5 Immunological Effects

There is an increasing awareness that immune functions and health are responsive to the bacteria resident in the GIT. Changes in the populations of GIT bacteria caused by diet, antibiotics, or other means can alter enteric and systemic immune responses. For example, a decrease in cell concentration of bifidobacteria in the colon, especially during aging, could be an important cause of decreased immunity. Lactic acid bacteria are thought to stimulate both nonspecific host defense mechanisms and certain types of cells involved in the specific immune response. The result is often an increased phagocytic activity and/or elevated immunological molecules such as IgA, which may affect pathogens such as *Salmonella* and rotavirus. As prebiotics achieve a similar outcome to lactic acid bacteria (i.e., improved composition of the gut microflora), similar effects may occur through their ingestion.

14.3.2.6 Prevention of Infectious Diseases

Prebiotics can act in a number of ways to help prevent pathogen colonization and translocation: (1) selective stimulation of gut lactic acid bacteria that allow them to effectively compete for luminal nutrients and exclude pathogens competitively in the intestines; (2) the metabolic end products of increased lactic acid fermentation of prebiotics provide additional nutrient source for intestinal mucosal cells, and (3) organic acids and antibacterial proteins (bacteriocins) secreted by lactic acid bacteria could effectively inhibit pathogen proliferation in the GIT. It has been reported that the introduction of inulin and oligofructose enhances the luminal cell concentrations of bifidobacteria at the expense of pathogenic bacteria. When oligofructose is added to pure cultures of *Bifidobacterium infantis* and two potential pathogens (*Escherichia coli* and *Clostridium perfringens*), a rapid decline in pathogen numbers occurred compared with a glucose control (Wang and Gibson 1993). A number of other studies have confirmed the prebiotic effect of inulin and oligofructose inhibiting pathogen growth and subsequent colonization.

14.3.3 Functional Food Applications of Prebiotics

Oligosaccharides are used in beverages, for example, in soybean oligosaccharide drinks in Japan (Suntory Ltd., Osaka Japan). Increasingly, oligosaccharides are being incorporated into probiotic yoghurts and yoghurt drinks to produce synbiotic products. The effect of prebiotics on the characteristics of dairy products has been reported. Ipsen et al. (2001) reported a gradual coarsening of the acid-induced milk protein network, increased syneresis, increased permeability, and a tendency for a lower shear stress with increasing amounts of inulin in yoghurt manufacture. However, Dello et al. (2004) found that yoghurt containing inulin had a stable color and water activity and did not undergo syneresis during storage, similar to that of other fiber-containing yoghurts. Bozanic et al. (2001, 2002) found that the firmness of fermented goat and cow milks and yoghurts improved upon the addition of inulin. Recently, Aryana and McGrew (2007) reported that the chain length of the inulin polymer affected some characteristics of fat-free yoghurts incorporated with probiotic bacteria such as *Lactobacillus casei*. They found that the flavor scores of yoghurts made with inulin of short-chain length were significantly higher than for yoghurt incorporated with longer chain length inulin. However, the yoghurts with longer chain lengths demonstrated better body and texture compared to the control and yoghurts made with short-chain inulin. Seydin et al. (2005) found that yoghurt containing inulin had a good flavor and a smooth texture. Organoleptic scores for set and fermented milks containing inulin and *Bifidobacterium bifidum* were greater than the control (Ibrahim et al. 2004). The viability of lactic acid bacteria in fermented milk was enhanced by inulin (Sadek et al. 2004). Recently, a *petit-suisse* cheese supplemented with both inulin and oligofructose seems to be most promising because of the simultaneous achievement of high fructan contents, good viable counts of *Lb. acidophilus* and *Bifidobacterium animalis* subsp. *lactis*, and sensory acceptance (Cardarelli et al. 2008). Niness (1999) reported that inulin has been successfully used to replace fat in baked goods, table spreads, fillings, dressings, and frozen desserts. Oligosaccharides are also widely used in confectioneries including desserts, jellies, ice creams, bakery products including biscuits, breads and pastries, spreads such as jams and marmalades, and infant milk formulas. It has been long thought that the gut flora of breast-fed infants is dominated by bifidobacteria and that this is not the case for formula-fed infants.

As prebiotics exploit the use of nonviable dietary components to improve gut health, the range of foods into which they can be added is much wider than that for probiotics, where culture viability needs to be maintained. Potential applications for probiotics as food ingredients to improve the gut health of the consumer include beverages, health drinks, sauces, cereal bars, biscuits, snack bars, soups, salad dressings, etc. Some nonfood applications have also been proposed for oligosaccharides including cosmetics and mouth washes.

14.4 Future Trends

Numerous human feeding studies have shown that the human gut microbiota can be modulated with probiotics, prebiotics, and synbiotics to increase the numbers and activity of bifidobacteria and lactobacilli. All three microbiota management tools have also shown some positive health outcomes against specific disease conditions.

As clinical evidence of the beneficial effects of probiotics and prebiotics accumulates, the food, nutraceutical, and pharmaceutical industries will formulate new and innovative probiotic-based therapeutic products. New synbiotic products, containing both probiotic bacteria and prebiotic carbohydrates, will be developed. Though yoghurts containing inulin and bifidobacteria are available, the development of synbiotic products is still in its infancy. There is still only limited evidence of enhanced health efficiency due to synbiotics over that of the probiotic alone or the prebiotic alone. In addition, specific strains of probiotics are not yet being selected for their ability to act synergistically with a specific prebiotic carbohydrate. Thus, future developments will include fermented functional foods where matching of probiotic bacterial strains with specific prebiotic carbohydrates will assume greater importance. Yoghurts and fermented milks have spearheaded the development of synbiotic functional foods. The tendency is for other fermented products such as cheese, dairy–vegetable blended spreads, frozen desserts, and cereal and meat products to follow. Designer synbiotic products delivering specific health benefits will be the next phase of development. This will also include food and pharmaceutical products. Synbiotic technology also has a huge potential in the development of new and innovative products in food service and catering industries. The advantage provided to food manufacturers by the physicochemical properties of oligosaccharides, combined with the health benefits of probiotic bacteria and prebiotic substances, should ensure that the synbiotic functional food market will continue to expand.

14.5 Conclusion

The importance of functional foods and other natural health products has been well recognized in connection with health promotion, disease risk reduction, and reduction in health care costs. Probiotic and prebiotic fermented foods play an important role in the gut health of consumers. Development of innovative health-based fermented products incorporating highly efficient strains of probiotic bacteria and highly bioactive prebiotic substances will increase in the near future. Simultaneously, there is a need for improved and cost-effective fermentation technology for maximizing synbiotic health properties of new and innovative fermented foods.

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15

Health Aspects of Fermented Foods

Mariam Farhad, Kasipathy Kailasapathy, and Jyoti Prakash Tamang

CONTENTS

15.1 Introduction

Campbell-Platt (1994) defined fermented foods as those foods that have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modifications to the food. However, to the microbiologist, the term "fermentation" describes a form of energy-yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is incompletely oxidized, and an organic carbohydrate acts as the electron acceptor (Adams 1990). This definition means that processes involving ethanol production by yeasts or organic acids by lactic acid bacteria are considered as fermentations. They also include the direct consumption of fungal fruit bodies or mushrooms. Fermented foods have immense

functional and therapeutic values possessing antioxidant, antimicrobial, probiotic, low-cholesterol, essential amino acid, bionutrient, and some important bioactive or health-benefit compounds; some of these are considered as potential sources of medical therapy for humans (Tamang 2007). Fermented foods assist in (1) the preservation of milk by the generation of lactic acid and possibly antimicrobial compounds; (2) the production of flavor compounds (e.g., acetaldehyde in yoghurt and cheese) and other metabolites (e.g., extracellular polysaccharides) that will provide a product with the organoleptic properties desired by the consumer; (3) the improvement of the nutritional value of food, as in, for example, the release of free amino acids or the synthesis of vitamins; and (4) the provision of special therapeutic or prophylactic properties against cancer and control of serum cholesterol levels (Parvez et al. 2006). Fermented probiotic foods are aimed to improve gut health, and they currently represent the largest segment of the functional foods market in Europe, Japan, and Australia (Saarela et al. 2002). This chapter outlines the health benefits of fermented foods due to either increased activity of beneficial cultures or increased bioactivity of certain compounds during fermentation and the overall effect to provide physiological benefits (Figure 15.1) over and above their potential to provide basic nutrition.

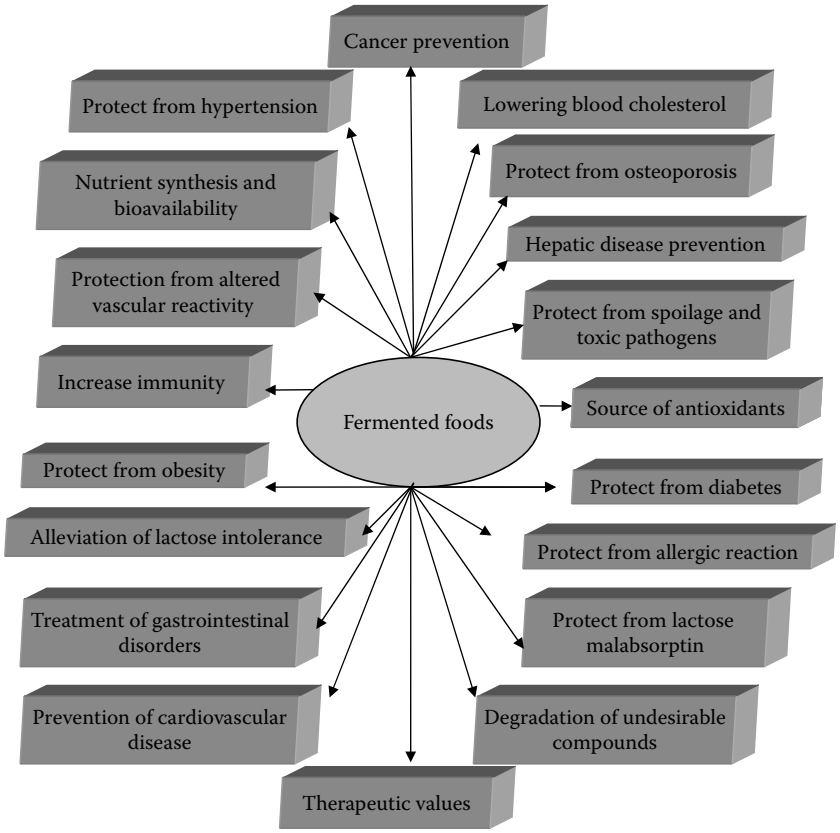

FIGURE 15.1 Various health benefits of fermented foods.

15.2 Protection from Hypertension

Elderly patients with hypertensive disease who consumed fermented milk with a starter containing *Lactobacillus helveticus* and *Saccharomyces cerevisiae* experienced reduced systolic and diastolic blood pressures (Hata et al. 1996). Whole-grain foods are rich in fiber and provide complex carbohydrates, phytochemicals, minerals, and vitamins (Anderson et al. 2000a). Some whole-grain foods deliver omega-3 fatty acids and resistant starch and oligosaccharides. Some specific components of foods (e.g., some fruits) are rich in potassium, which could have major effects on blood pressure (Appel et al. 1997). Early clinical research with diabetic individuals showed that high fiber, high-carbohydrate diets reduced the blood pressure when these individuals were placed on such diets (Anderson 1983). Subsequent studies also suggested that the high fiber intake might reduce the blood pressure level. Probably the increased intake of whole grains would slow the development of hypertension and lower blood pressure in hypertensive individuals (He and Whelton 1999, Anderson 2003).

15.3 Cancer Prevention

Fermented foods may influence intestinal detoxification and immune status, which may be associated with developing colon cancer, and thus it is believed that probiotics play a protective role in colon cancer risk reduction (Saikali et al. 2004, Cabana et al. 2006). An inverse relationship between the consumption of fermented dairy products (containing lactobacilli or bifidobacteria) and the incidence of colon and breast cancers has also been reported in epidemiological and population-based case-control studies (Van't Veer et al. 1991). Fermented food products from lactic acid bacteria may confer a variety of important nutritional and therapeutic benefits to consumers, including antimutagenic and anticarcinogenic activities (Fernandes and Shahani 1990, Danone 2001, Lee et al. 2004).

For example, fermented red beet has a beneficial health effect as it prevents the proliferation of tumor cells. Fermentation of vegetable and vegetable juices produces lactic and acetic acids and make them nutritious, palatable, and wholesome fermented foods. Lactic acid protects the body against various infections and liver diseases. It also improves digestion and increase immunity by protecting body from various physiological infectious agents (Karovicova and Kohajdova 2005). *Kefir* is a traditional popular Middle Eastern beverage. Many researchers have investigated the benefits of the consumption of *kefir.* It cleans effectively the whole body system by which it helps to establish a balanced inner ecosystem for optimum health and longevity. It is easily digested and it provides beneficial bacteria and yeast, vitamins, minerals, and complete proteins and overall it is a nourishing food that contributes to a healthy immune system as well as helps patients suffering from AIDS, chronic fatigue syndrome, herpes, and cancer. It was used for the treatment of tuberculosis and cancer when modern medical treatment was not available (Otes and Cagindi 2003).

Fermented cabbage, cabbage juice, and sauerkraut contain *s*-methylmethionine, which reduces the risk of tumorigenesis in the stomach. Cabbage contains isothiocyananins and indoles that are responsible for anticancer effects in cancers of the colon, breast, lung, forestomach, and liver (Kris-Etherton et al. 2002, Karovicova and

TABLE 15.1

Gross Composition of Some Ethnic, Fermented Soybean Products

	g/kg			
Fermented Soy Products	**Moisture**	**Protein**	**Fat**	**Soluble Carbohydrate**
Sufu (red)	555	146	57	58
Sufu (white)	565	144	112	48
Natto	585	165	100	101
Miso	475	168	69	136
Chianga (chunky)	486	116	52	272
Tempe	640	183	40	110

Source: Adapted from Liu, T.S., *Soybeans: Chemistry, Technology, and Utilization*, Chapman and Hall, New York, 1997; O'Toole, D.K., *Soy-Based Fermented Foods*, City University of Hong Kong, Hong Kong, China, 2004.

Kohajdova 2005). Soybean-fermented sauces and pastes, such as the Korean *chungkokjang*, the Japanese *natto*, and the Indian *kinema*, are involved in the process of enzymatic hydrolysis of proteins and produce peptides and amino acids, which confer health benefits (Tamang and Nikkuni 1998, Lee 2004). Some peptides produced from soybean sauces and pastes exert a number of health benefits such as ACE inhibition, and anti-thrombotic and anticancer effects (Shon et al. 1996). The gross composition of some fermented soybean products is shown in Table 15.1.

15.4 Lowering Blood Cholesterol

Mann and Spoerry (1974) reported that the consumption of fermented milk was associated with reduced serum cholesterol levels in the Maasai people. This primary report stimulated much interest in the cholesterol-lowering effects of fermented milks and lactic acid bacteria. Several animal studies have shown that administration of fermented milks or specific strains of lactic acid bacteria are effective in lowering blood cholesterol levels. A controlled short term meta-analysis (4–8 weeks) study ($n = 6$) with 425 subjects (male and female with different initial cholesterol levels) reported that the consumption of yoghurt containing *Enterococcus faecium* (Gaio) is effective in reducing both total and low-density lipoprotein cholesterol by 4% and 5%, respectively, compared with a control group (Agerholm-Larsen et al. 2000). Whole grains appear to exert some protective effects to reductions in serum LDL cholesterol values (Truswell 2002). Abnormalities of serum LDL cholesterol may be the single greatest risk factor for atherosclerotic disease in western populations (Navab et al. 1996). Whole-grain oat products are effective for decreasing the serum LDL cholesterol level (Ripsin et al. 1992). Whole-grain barley products are also effective for reducing serum LDL cholesterol values (McIntosh et al. 1991). While in wheat products with moderate soluble fiber are less effective, for lowering the LDL-cholesterol-properties (Anderson et al. 1991). Fermented whole-grain foods have a potentially positive role for altering the following risk factors: serum LDL cholesterol values, serum HDL

cholesterol values, hypertriacylglycerolemia, hypertension, diabetes, obesity, coronary heart disease, insulin resistance, antioxidant status, hyperhomocysteinemia, vascular reactivity, and the inflammatory state (Anderson 2003). Fermented soybean products and their functional foods reduce blood cholesterol levels and increase digestibility and the nutritional value (Lee 2001, 2004). It has been reported that in fermented products, acidophilus milk significantly reduced the serum total and LDL cholesterol in pigs, but had no effect on serum HDL cholesterol or triacylglycerols (Danielson et al. 1989), and similarly acidophilus yoghurt reduced serum cholesterol levels (Jones et al. 1985). Another study revealed that in pigs, certain strains of *Lactobacillus acidophilus* may act directly on cholesterol in the alimentary tract and thus may reduce the serum cholesterol level (Gilliland et al. 1985). The fermented beverage *kefir* plays an important role in controlling high cholesterol levels and in this way it protects us from cardiovascular damage (Otes and Cagindi 2003).

15.5 Protection from Osteoporosis

Natto is a popular fermented product in Japan for more than 400 years made from soybeans cultured with *Bacillus subtilis* (synonym, *B. subtilis* subsp. *subtilis*; former name, *Bacillus natto*) (Murooka and Yamshita 2008). *Natto* contains saponin and isoflavones, fibrinolytic enzymes, vitamin K_2, and dipicolinic acid, which are generated by soybeans and *natto* bacteria (Hosoi and Kiuchi 2003). After the start of the fermentation by *natto* bacteria, the concentration of vitamin K_2 increases to 124 times that in the soybeans itself (Yanagisawa and Sumi 2005). Vitamin K_2 stimulates the formation of bone, which might help to prevent osteoporosis in older women in Japan. *Tempe* is a traditional Indonesian granular fermented soybean-based food and due to the superior nutritive qualities and metabolic regulatory functions, *tempe* is now being produced in Japan (Murooka and Yamashita 2008). Newly improved methods for *tempe* production have been developed that raise levels of aminobutyric acid (Aoki et al. 2003) and isoflavones. Nakajima et al. (2005) prepared a new isoflavone-enriched *tempe* by adding soybean germ (hypocotyls) that contains large amounts of isoflavones. The isoflavones have estrogen-like functions, and thus it is expected to alleviate symptoms of osteoporosis after menopause (Brzezinski et al. 1997) and suppress the onset of arteriosclerosis because it improves the metabolism of lipids, such as cholesterol (Crouse et al. 1999, Stein 2000). *Kefir* is a fermented grain beverage made from *kefir* grains, a probiotic fermented milk beverage, an example of symbiosis between yeast and bacteria, shown to be beneficial to health (Lopitz-Otsoa et al. 2006).

15.6 Protection from Spoilage and Toxic Pathogens

Lactic acid bacteria are used for fermentation to produce functional foods and have been added as starter cultures in dairy, meat, vegetable, and beverage products (Campbell-Platt 1987). The fermented food products have an extended shelf life, new aromas, and different consistencies (Nordvi et al. 2007). The preservation of lactic acid bacteria fermented foods is due to the production of lactic acid and other organic acids, which contribute to the reduction of pH and thus inhibit growth of a wide range of pathogenic and spoilage organisms (Vandenbergh 1993, Geisen and

Holzapfel 1996). The major northern European fermented fish products are made from the spontaneous fermentation of fish and products include *gravlaks* from salmon (*Salmo salar*), *rakørret* from trout (*Salmo trutta*), and *tidbits* and *surströmming* from Atlantic herring (*Clupea harengus*) (Nordvi et al. 2007). A number of different types of bacteria, including *Pedicoccus*, *Lactobacillus*, *Leuconostoc*, *Micrococcus*, and *Staphylococcus* species, are used to produce these fermented fish products (Campbell-Platt 1987). The texture of the fermented fish is modified compared to the raw fish fillet and the shelf life of these products is extended due to the high level of salt that reduces water activity (Knöchel 1983). Fermented, dry-cured meat sausages made from raw meat with a high content of pork back fat is a well-established technology. During the drying and ripening process, the fermented meat products develop their characteristics, such as flavor, consistency, and appearance.

15.7 Protection from Altered Vascular Reactivity

Coronary heart disease (CHD) is a multifactorial disease characterized by long-term degenerative changes in the walls of arteries, which result in narrowing of the lumen of blood vessels and limiting the blood supply to vital organs such as the heart. New evidence suggests that the effects of estrogen on vascular reactivity may have greater protective effects on premenopausal women from CHD (Reis et al. 1994, Subbiah 1998). Estrogens promote appropriate vasodilatation of coronary arteries when increased blood supply is required (Mendelsohn and Karas 1999). Preliminary studies suggest that soybean isoflavones have similar effects on peripheral blood vessels in human subjects (Honore et al. 1997, Nestel et al. 1997, Williams and Clarkson 1998). Whole grains also may provide phyto-estrogens that are likely to have similar protective effects on blood vessels (Slavin et al. 1997). It is possible that phyto-estrogens from whole grains have a protective effect on vascular reactivity and reduce risk for vascular events related to arterial spasm. Alterations in HDL levels or function may be the second most important lipoprotein disturbance linked to CHD (Gowri et al. 1999, Rubins et al. 1999, Anderson 2003). Dietary fibers usually have a neutral effect on serum HDL cholesterol values. Psyllium intake may increase apolipoprotein A-I, a protective component of HDL (Anderson et al. 2000b). Intake of soybean protein tends to increase serum HDL cholesterol values slightly but not significantly (Anderson 1995).

15.8 Prevention of Cardiovascular Disease

A series of recent studies has revealed that moderate consumption of wine is healthier than that of other alcoholic beverages and provides more health benefits than those who abstain from alcoholic beverages. When compared to beer drinking, wine drinking is significantly associated with higher IQ, higher parental educational level, and higher socioeconomic status. Alcohol consumption at a moderate level of two drinks or so per day may have protective effects, and cardiovascular benefits have been claimed for wine (Criqui and Ringel 1997, Klatsky et al. 1997). The Mediterranean diet, which includes moderate wine consumption, also has cardiovascular benefits (De Longeril and Salen 1999, Menotti et al. 1999). Only two studies have considered the extent to which the public have come to view moderate drinking as having health

benefits. Hall (1996) reported that in Australia, 39% of the population (among males, younger people, the better educated, and more frequent drinkers) believed that alcohol consumption had cardiovascular benefits compared to 0%, 5 years earlier. In addition, 54% of those surveyed believed that alcohol promoted relaxation. Ogborne and Smart (2001) showed that most adults in Canada (57%) who moderately consumed wine believed that they had cardiovascular health benefits. Prescott et al. (1999) reported that male wine drinkers may have a lower risk of lung cancer than those who drink beer or spirits. The researchers also suggest that the protective effect of wine may be related to the antioxidant properties of wine. Berger et al. (1999) suggest that people who consume as little as one alcoholic drink per day may significantly reduce their risk of stroke, but drinking more does not increase the benefit. In conclusion, the authors also added that "light-to-moderate consumption of alcohol (one to seven drinks per week) may reduce the risks of total stroke and ischemic stroke." It has been reported 40 years ago that fermented whole-grain cereals protect from coronary heart disease and a number of different studies supported the concept that the cereal food is one of the major contributors to western diseases including coronary artery disease (Anderson et al. 2000a). Whole-grain intake appears to protect from the development of CHD and diabetes. Emerging science regarding health benefits and mechanisms strongly supports these nutrition guidelines. Low intakes of antioxidants and vitamins (mainly vitamin E) appear to be associated with high risk for CHD (Gey et al. 1993, Regnstrom et al. 1996). Whole grains are excellent sources of antioxidants, vitamins, and phytochemicals (Anderson 2003).

15.9 Source of Antioxidants

Cao et al. (1996) found that vegetables, such as kale, beet, broccoli, spinach, shallot, potato, carrot, and cabbage, have high levels of antioxidant activities. Their study also indicated that each type of vegetable has different levels of antioxidant activity, contributed by different antioxidant components, such as α-tocopherol, β-carotene, vitamin C, selenium, or phenolic compounds (Rabinkov et al. 1998). The importance of antioxidant constituents of vegetables are maintaining health and protection from coronary disease and cancer. The presence of organosulfur compounds of garlic inhibit the peroxidation of lipids and possess antioxidant and free-radical-scavenging activity (Karovicova and Kohajdova 2005).

Another study found that red beet is the most important source of betalaine, which is a prominent antioxidant compound and free-radical-scavenging agent (Strack et al. 2003). Carrot is a major source of β-carotene; carotenoids are thought to scavenge free radicals and other oxidants involved in disease processes. The antioxidant properties of β-carotene had also been observed *in vitro* and *in vivo* (Karovicova and Kohajdova 2005). Carotenoids may help to reduce risk of developing tumors in various tissues (Desobry et al. 1998) by interfering with the metabolic activation of carcinogens, which damage DNA, proteins, and lipids. Oxidative stress induced DNA damage is significantly reduced during carrot juice intervention due to the presence of carotene (Cao et al. 1996). Increase in total phenol content, which is one of the indicators of antioxidant activity, has also been reported in *chungkokjang*, a Korean fermented soybean food (Shon et al. 2007), in *douchi*, a Chinese fermented soybean food (Wang et al. 2007), and in *kinema* (Tamang et al. 2009a).

15.10 Nutrient Synthesis and Bioavailability

Probiotic activity in fermented foods during the preparation of cultured foods or in the digestive tract has been shown to improve the quantity, availability, and digestibility of some dietary nutrients (Parvez et al. 2006). Lactic acid bacteria are known to release various enzymes and vitamins into the intestinal lumen. Fermentation with lactic acid bacteria increases the levels of folic acid, niacin, and riboflavin in yogurt, and also increase folic acid in bifidus milk and *kefir* (Deeth and Tamime 1981, Alm 1982). The enzymatic hydrolysis of probiotic bacteria may enhance the bioavailability of protein and fat and also may increase the production of free amino acids, short-chain fatty acids (SCFA), lactic acid, propionic acid, and butyric acid (Fernandes et al. 1987). When absorbed, these SCFAs contribute available energy to the host and thus may protect the host against pathological changes in the colonic mucosa (Leavitt et al. 1978, Rombeau et al. 1990, Leopold and Eileler 2000, Rolfe 2000). The SCFA concentration also helps to maintain an appropriate pH in the colonic lumen (Mallett et al. 1989). In general, whole-grain foods are rich in fiber and provide complex carbohydrates, phytochemicals, minerals, and vitamins (Anderson et al. 2000a), while some whole-grain foods deliver omega-3 fatty acids, resistant starch, and oligosaccharides. Some specific foods (e.g., some soy foods) are rich in fiber and also deliver soy protein and isoflavones (Anderson 1995). In some cases, specific components of foods (e.g., some fruits) are rich in potassium, which could have major effects on blood pressure (Appel et al. 1997). Whole grains are among the healthiest food choices that individuals make. These foods are rich in fiber and phytochemicals and have a wide variety of health benefits (Anderson 2003). Some of the minor components of whole grains, including omega-3 fatty acids and certain minerals (e.g., Zn, Cr and Mg), may contribute to their protective effect from CHD (Slavin et al. 1997). Soybean isoflavones exert antithrombotic and antiplatelet aggregating effects (Wilcox and Blumenthal 1995).

Tongnual and Fields (1979) reported that lactic acid fermented rice improves nutritional value and available lysine content in rice. The main bacteria used for bacterial fermentation are *Lb. bulgaricus* and *Streptococcus thermophilus*. *Lactobacillus* and *Bifidobacterium* and their species and subspecies are well known for their probiotic properties (Rebucci et al. 2007). These microorganisms are delivered in freshly fermented dairy products. Recently, probiotics are also being used in fermented meat products to improve the nutritional value of these products as functional foods (Pennacchia et al. 2004, Leroy et al. 2006).

15.11 Protection from Allergic Reactions

Fermented foods may exert a beneficial effect on allergic reactions, in lactose intolerance, decrease of the serum cholesterol concentrations, increase the bioavailability of nutrient bioavailability, and improve urogenital health (Galdeano and Perdigon 2006). Soy sauce is a liquid seasoning traditionally used in Japan and currently used in cooking worldwide (Yokotsuka 1986). Several recent studies suggest that soy sauce contains certain bioactive components and also reported various biological activities, including anticarcinogenic, antimicrobial, antioxidative, and antiplatelet activities,

and the inhibition of an angiotensin-I-converting enzyme (Kataoka et al. 1997, Ando et al. 2003). New immunological functions of soy sauce are also reported with respect to allergy, such as hypoallergenicity and antiallergic activity (Kobayashi 2005). Many researchers have investigated the benefits of the consumption of *kefir* and more than a thousand years of consumption records showed that the microorganisms in *kefir* are not pathogenic. *Kefir* is used for a variety of health conditions including metabolic disorders, atherosclerosis, and allergic disease (Otes and Cagindi 2003). Probiotics modulate allergic reactions and exert beneficial effects by improving mucosal barrier functions and microbial stimulation of the immune system (MacFarlane and Cummings 2002).

15.12 Protection from Diabetes

Recent studies indicated that high fiber intake is associated with a significant reduction in the prevalence of diabetes (Meyer et al. 2000). They also reported that the highest quintile of whole-grain intake was associated with a significant reduction of risk (21%) for developing diabetes. Those earlier data are supported by clinical studies indicating that the increased whole-grain intake increases insulin sensitivity (Pereira et al. 2002) and has a favorable impact on gut-hormone and insulin responses after meals (Juntunen et al. 2000). It is also possible that the increased intake of whole grains would decrease the prevalence of diabetes and improve blood glucose values in diabetic individuals (Anderson 2003). Several studies indicated that the high carbohydrate intake together with high fiber intake decreases insulin requirements in diabetic individuals and increases sensitivity to insulin in nondiabetic individuals (Fukagawa et al. 1990). In another study, Pereira et al. (2002) recently reported that short-term increases in the intake of whole grains increased the insulin sensitivity of overweight hyperinsulinaemic adults. Thus, it is possible that the increased whole-grain intake would improve the insulin sensitivity and decrease risk for CHD for hyperinsulinaemic individuals (Anderson 2003).

15.13 Protection from Obesity

Epidemiological data from the last 30 years, observational studies, human experimental research, and clinical trials supported the role of dietary fiber in the development and management of obesity (Anderson and Bryant 1986). The early study by Mickelsen et al. (1979) demonstrated the weight-reducing benefits of high-fiber bread. Many clinical studies also reported the weight-loss benefits from the increased intake of fiber (Russ and Atkinson 1985). Therefore, it seems possible that the increased intake of whole grains would decrease the risk of developing obesity. An increased dietary carbohydrate intake and a decreased dietary fat intake decrease postprandial hypertriacyl glycerolaemia (Anderson 2003). A recent study evidenced the effects of dietary fiber in decreasing fasting serum triacylglycerol values (Chandalia et al. 2000). Several other studies indicate that the increased intake of whole grains is likely to decrease postprandial serum triacylglycerol values (Anderson 2000, Chandalia et al. 2000).

15.14 Increased Immunity

Many gut-related inflammatory diseases occur due to the altered gut microecology and inflammation and is accompanied by imbalances in the intestinal microbiota (Gill and Guarner 2004). However, an immune response can be induced by resident bacteria (Salminen et al. 1995). Previously, Duchmann et al. (1995) observed that healthy individuals are tolerant to their own microbiota and such tolerance is disturbed in patients with inflammatory bowel diseases. From different studies, it has been reported that patients with rheumatoid arthritis and allergic disease had their gut microbiota either altered or disturbed (Kalliomaki et al. 2001, Kirjavainen et al. 2002). Bifidobacteria are the natural inhabitants of the human large intestinal tract, and it has been reported that large numbers of *Bifidobacterium* form a barrier against pathogens by prohibiting colonization or by controlling the intestinal pH level through the release of acetic and lactic acids (Lauer and Kandler 1976). Another study also reported that these bacteria stimulate the immune response of the host (Sekine et al. 1985). For these reasons, *Bifidobacteria* have been incorporated into various types of commercial fermented products such as yogurts, fermented milk, baby milk powder, pharmaceutical tablets, and animal feed additives (Hughes and Hoover 1991).

Host defense by itself against foreign challenge is elicited by the immune system, which consists of the innate and the acquired immune systems that induce both the systemic and the mucosal immune responses (Galdeano and Perdigon 2006). The innate and adaptive immune systems are two interdependent parts of a single integrated immune system. At the gut mucosal level, the innate immune response not only provides the first line of defense against pathogenic microorganisms but also provides the biological signals that instruct the adaptive immune system to elicit a response. The cells that play a critical role in initiating the innate immune response, such as the macrophages and the dendritic cells, which are specialized phagocytes, participate in the cellular and molecular clearance as well as in the defense against infection. These phagocytic cells may develop a receptor system called pattern recognition receptors, which are able to recognize molecular patterns associated with pathogens present in the surface (Galdeano and Perdigon 2006). These receptors are activated by pathogens (Akira et al. 2001). One of the most intensely studied families of pattern recognition receptors is the Toll-like receptor (or TLR) family. TLRs play a central role in alerting antigen-presenting cells in the presence of pathogenic material (Dunzendorfer et al. 2004). TLRs can activate the innate immune response, mainly during inflammation, before the adaptive immune response gets activated (Ahmad-Nejad et al. 2002, Heil et al. 2003).

15.15 Alleviation of Lactose Intolerance

Lactose malabsorption in adult populations varies from 5% to 15% in northern European and American countries, and 50% to 100% in African, Asian, and South American countries (Gill and Guarner 2004). It is well known that probiotic bacteria incorporated with starter cultures in yogurt improves lactose digestion in many

lactose-intolerant people, and the beneficial effect is due to the presence of microbial β-galactosidase (lactase enzyme) (Oberhelman et al. 1999). In different human studies, consumption of fresh yogurt (with live yogurt cultures) demonstrated better lactose digestion and absorption than with the consumption of a pasteurized product (with heat-killed bacteria) (Pedone et al. 2000, Marteau et al. 2002).

15.16 Hepatic Disease Prevention

Hepatic disease, also known as hepatic encephalopathy (HE), which affects the liver, can be life threatening. The exact pathogenesis of HE still remains unknown. The probiotics (*S. thermophilus*, Bifidobacteria, *Lb. acidophilus*, *Lb. plantarum*, *Lb. casei*, *Lb. delbrueckii bulgaricus*, and *E. faecium*) with therapeutic effects have multiple mechanisms of action that could disrupt the pathogenesis of HE by lowering the portal pressure with a reduction in the risk of bleeding, and thus may make them superior to conventional treatment (Cunningham-Rundles et al. 2000, De Santis et al. 2000, Gorbach 2000, Guslandi et al. 2000, Shanahan 2001, Solga 2003).

15.17 Treatment of Gastrointestinal Disorders

A number of studies involving humans suggest that lactic acid bacteria in fermented foods can decrease the incidence, duration, and severity of some gastric and intestinal illnesses, such as diarrhea (Marteau et al. 2002). Bunte et al. (2000) reported that ingested bacteria (*Lactobacillus paracasei* LTH 2579) in dry-fermented sausage can be recovered from human feces, and it is suggested that the fermented bacteria may contribute to the microbial ecosystem of the gastrointestinal tract (Farnworth 2003). Fermented meat products such as dry sausages are considered to be a suitable carrier of probiotics into the human gastrointestinal tract (Rebucci et al. 2007). The popular beverage *kefir* contains a number of essential elements required for health, such as vitamins, minerals, and essential amino acids. *Kefir* is also a rich source of vitamin B, calcium, amino acids, folic acid, and vitamin K. It was used for the treatment of gastrointestinal disorders when modern medical treatment was not available (Otes and Cagindi 2003). The chemical composition and nutritional values of *kefir* are shown in Table 15.2.

15.18 Prevention against Diarrhea and Inflammatory Bowel Disease

Consumption of probiotic-fermented foods is reported to be beneficial for alleviating many types of diarrhea such as antibiotic-associated, infantile, and travelers diarrhea and diarrheal diseases in young children caused by rota viruses (Marteau et al. 2002). Probiotic therapy is reported to shorten the duration of acute diarrheal illness in children (Gill and Guarner 2004). Studies have shown that the consumption of certain strains of lactobacilli improved the symptoms of inflammatory bowel disease (IBD),

TABLE 15.2

Chemical Composition and Nutritional Values of *Kefir*

Components	**100 g**
Energy	*65 kcal*
Fat (%)	3.5
Protein (%)	3.3
Lactose (%)	4.0
Water (%)	87.5
Milk acid (g)	0.8
Ethyl alcohol (g)	0.9
Lactic acid (mg)	1
Cholesterol (mg)	13
Phosphatateds (mg)	40
Essential amino acids (g)	
Tryptophan	0.05
Phenylalanine + tyrosine	0.35
Leucine	0.34
Isoleucine	0.21
Threonine	0.17
Methionine + cystine	0.12
Lysine	0.27
Valine	0.22
Vitamins (mg)	
A	0.06
Carotene	0.02
B_1	0.04
B_2	0.17
B_6	0.05
B_{12}	0.5
Niacin	0.09
C	1
D	0.08
E	0.11
Mineral content (*g*)	
Calcium	0.12
Phosphor	0.10
Magnesium	12
Potassium	0.15
Sodium	0.05
Chloride	0.10
Trace elements	
Iron (mg)	0.05
Copper (µg)	12

TABLE 15.2 (continued)

Chemical Composition and Nutritional Values of *Kefir*

Components	**100 g**
Molybdenum (μg)	5.5
Manganese (μg)	5
Zinc (mg)	0.36
Aromatic compounds	
Acetaldehyde	
Diacetyl	
Acetoin	

Source: Adapted from Renner, E. and Schaven R., *Nahrwerttabellen für milch und milchprodukte*, Verlag B. Renner. Köhner K. G, Gieben, Germany, 1986; Hallé, C. et al., Les kéfirs: Des associations Bactéries lactiqueslevures, in *Bactéries lactiques: Aspects fondamentaux et technologiques*, Vol. 2, eds. De Roissart, H. and Luquet, F.M. Lorica, Uriage, France, 1994, pp. 169–182; Otes, S. and Cagindi O., *Pak. J. Nutr*., 2, 54, 2003.

paucities, and ulcerative colitis (Ouwehand et al. 2002, Parvez et al. 2006). From several investigations, it has been found that lactic acid bacteria may also improve intestinal mobility and relieve constipation possibly through the reduction of gut pH (Mallett et al. 1989, Leopold and Eileler 2000, Sanders and Klaenhammer 2001). The combination therapy with probiotics may benefit patients with IBD (Campieri and Grionchetti 1999, Schultz and Sartor 2000, Shanahan 2001). Intestinal bacteria may initiate abnormal immune responses due to defects in mucosal barrier function, which is seen in inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. It has also been reported that the modulation of the intestinal flora may be helpful in the treatment of Crohn's disease (Cabana et al. 2006). Probiotics exhibit a direct effect on the gut for the prevention and treatment of inflammatory and functional bowel disorders (Parvez et al. 2006). Theoretically, probiotics might improve the balance and reduce gas accumulation within the bowel. It was also reported that *Saccharomyces boulardii* can decrease diarrhea in irritable bowel syndrome, but was not effective in alleviating other symptoms of the syndrome (Marteau et al. 2001). In a double-blind clinical trial, administration of *Lb. plantarum* strain reduces abdominal pain, bloating, flatulence, and constipation in patients with irritable bowel syndrome (Motta et al. 1991, Nobaek et al. 2000, Steidler et al. 2000, MacFarlane and Cummings 2002). Bacteria in the gut flora can produce intestinal gas but they also consume gas (Gill and Guarner 2004). A recent study suggested a potential role of the intestinal microbiota in the modulation of inflammation in the intestine and joints. Normal gut physiology is molded by the interaction between the intestinal microbiota and the host's GI tissues, including motility, absorption and secretion, and intestinal permeability (Verdu and Collins 2004, Parvez et al. 2006).

15.19 Degradation of Undesirable Compounds

Functional microorganisms present in fermented foods produce desirable amounts of enzymes that may degrade unsatisfactory or antinutritive compounds, and thereby convert the substrates into consumable products with enhanced flavor and aroma. Bitter varieties of cassava (*Manihot esculenta*) tubers, the main staple crop in West Africa, contain the cyanogenic glycoside linamarin, which can be detoxified by natural fermentation into a product called gari by species of *Leuconostoc*, *Lactobacillus*, and *Streptococcus*. Thus the fermented cassava is rendered safe to eat (Westby and Twiddy 1991). Species of *Lactobacillus*, *Lacotococcus*, *Leuconostoc*, and *Pediococcus*, isolated from fermented vegetables and tender bamboo shoots of the Eastern Himalayas, were found to degrade antinutritive factors such as phytic acids and flatulence-causing oligosaccharides (Tamang et al. 2009b). During *tempe* fermentation, a trypsin inhibitor is inactivated by *Rhizopus oligosporus* and eliminates flatulence-causing indigestible oligosaccharides, such as stachyose and verbascose into absorbable monosaccharides and disaccharides (Hesseltine 1983). Microorganisms associated with *idli* batter fermentation reduce the phytic acid content of the substrates (Reddy and Salunkhe 1980).

15.20 Therapeutic Values

Koumiss, an acidic, alcoholic beverage from Russia, has therapeutic uses, particularly in the treatment of pulmonary tuberculosis (Auclair and Accolas 1974). Kosikowski (1977) reported that more than 50 Russian sanatoria offer *koumiss* treatment for tuberculosis. *Kvass*, a rye/wheat-based sour-alcoholic beverage from Ukraine, can provide protection to the digestive tract against cancer (Wood and Hodge 1985). Consumption of *natto* in Japan prevents hemorrhage caused by vitamin K deficiency in infants (Ueda 1989). Ethnic, mild-alcoholic beverages from the Himalayas such as *kodo ko jaanr/chyang*, *bhaati jaanr*, *poko*, etc., have been consumed for therapeutic uses (Tamang 2010). The Chinese fermented soybean food *douchi* reduces high blood pressure (Zhang et al. 2006). Drinking the Chinese fermented tea, *puer*, prevents cardiovascular disease (Mo et al. 2008). In Korea, the popular fermented vegetable *kimchi* has several therapeutic uses (Park et al. 2006, Kim et al. 2008, Lee and Lee 2009).

15.21 Conclusion

The health benefits of fermented functional foods are expressed either directly through the interaction of ingested live microorganisms (bacteria or yeast) or indirectly because of ingestion of microbial metabolites produced during the fermentation process (biogenic effect). As we know more of the role of microorganisms in human nutrition, immune function, and disease resistance, the number of fermented products available will increase in the market. New research supports the effectiveness of fermented food products or probiotics for the treatment and prevention of infectious disease and antibiotic-associated diarrhea. Fermented food therapy also

has been applied to a wide range of health disorders, such as in immune system compromise and treatment of gastrointestinal disorders. Fermented foods with an impact on health will also remain an important functional ingredient in the future. New strains will be identified and foods will be developed to fulfill the needs of specific consumer groups. Increased understanding of the viability of probiotic bacteria, and interactions between gut microbiota, diet, and the host will open up new possibilities of producing new ingredients for nutritionally optimized foods, which promote consumer health through microbial activities in the gut. Gene technology is also playing an important role for the development of new strains, with gene sequencing allowing for an increased understanding of the mechanisms and functionality of probiotics, which is required for the improvement of current fermented foods. In addition, industry-based probiotic research will focus on increasing the shelf life of fermented foods and increasing the survival rate of probiotics through the intestinal tract by introducing new stress-tolerant strains and improving handling and packaging procedures to ensure that the desired health benefits are delivered to the consumer.

Introduction of new fermented food products containing beneficial bacteria will emerge, such as cereals, energy bars, cheese, juices, disease-specific medicinal foods, and infant foods. The establishment of the identity of beneficial bacteria in fermented foods will serve to accelerate the development and availability of a range of new healthy fermented food products. Fermented foods have been a part of the human diet for centuries, and it may become important in the diets of future space travelers.

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16

Packaging Concepts for Enhancing Preservation of Fermented Foods

Kasipathy Kailasapathy

CONTENTS

16.1 Introduction

Fermentation has been a traditional method used to produce and preserve foods and had been practiced in many parts of the world for thousands of years. The origin of fermentation may have begun with the storage of surplus milk, which resulted in a fermented product the next day. Next to drying, fermentation is the oldest food preservation method. Fermented foods are those foods that have been subjected to the action of one or more organisms or enzymes, and biochemical changes cause significant modification to the starting materials (Campbell-Platt 1994). Fermented foods are of great significance because they provide and preserve vast quantities of nutritious foods in a wide variety of flavors, aromas, and textures, which enrich the human diet (Steinkraus 1994). As a process, fermentation consists of the transformation of simple raw materials into a range of value added, nutritive, and therapeutic products by utilizing the phenomena of the growth of microorganisms and their activities on various substrates. This involves knowledge of microorganisms to understand the biochemical and metabolic process of fermentation. Like all other processed foods, fermented foods result from a manufacturing process involving

selection of raw materials, preparatory treatments, the fermentation operations, preservation, packaging, and storage. Hence fermented foods may be considered as safe foods for humans because their manufacture involves a combination of hurdles (low pH, low water activity, presence of competitive microflora, end products of fermentation that inhibit spoilage bacteria, curing salts, etc.) to pathogen survival or growth (Gounadaki et al. 2007). However, improper manufacture, preservation, and, especially, inadequate packaging will lead to a fermented product unsafe for human consumption.

One of the most important functions of packaging is to protect the food from physical damage, physicochemical degradation, and microbial spoilage. Without proper protection, the food may become unappetizing, less nutritious, and unsafe to consume. The required packaging protection depends on the stability and fragility of the food, the desired shelf life of the food package, and the distribution chain. Good package integrity is also required to protect against loss of hermetic condition and microbial penetration (Lee et al. 2008). Fermented foods needs special packaging as in these foods fermentation continues though at a much lower rate; however, to maintain product attributes, such as flavor, texture, and color throughout the shelf life of the product, efficient and intelligent packaging concepts are required. In some cases, such as probiotic fermented products, there is a need to maintain the viable numbers to at least 10^6–10^7 cfu/g of viable cells during the shelf life (FAO/WHO 2001). Careful considerations should be given in packaging such probiotic fermented products to provide suitable environmental conditions to maintain the viable numbers. Fermented foods may become contaminated with components or degraded products due to product–package interactions. Chemical and physical changes taking place during the package–product contact may affect organoleptic characteristics and decrease the quality of the packaged food. Physical changes may occur because of decreased structural, mechanical, and barrier properties of the materials commonly used in the food packaging. As a result, external microbiological and chemical contaminants may migrate through the packaging into the food. In fermented products where lactic acid is produced, it could penetrate into the structure of the plastic polymer packaging and form stubborn bacterial films on polymer surfaces (Steinka et al. 2006). Plastic materials dominate food packaging materials due to many advantages. Use of polyvinyl chloride (PVC) for packaging fermented alcoholic spirits did not succeed because of its poor compatibility with spirits and migration of plasticizers into the product and concern over the carcinogenicity of vinyl chloride monomer. In early 1980s, however, PET came into use as a food-grade substance for use as a container, which proved to have good compatibility with spirits (Flouros et al. 2003). During the middle ages, a number of fermented foods and beverages were developed depending on the availability of raw materials, environmental conditions, and the taste preferences of the local people. Knowledge of some of these products that originated in ancient times has developed, and these products are now being manufactured on a commercial scale. Many products, however, are still poorly understood, and a large number of these products are made in traditional ways as cottage industries and not commercialized, especially the packaging technology for these products need to be developed before they can be commercialized. This chapter includes some of the principles and issues of the packaging technologies that could be useful in enhancing the preservation of many fermented foods and beverages.

16.2 Effect of Oxygen on the Viability of Microorganisms in Fermented Foods

In fermented probiotic foods such as yoghurt, ensuring the viability of probiotic cultures during manufacture (fermentation and storage) is important to deliver a large number of probiotic bacteria to consumers for therapeutic efficiency. Hence, assuring maximum survival rate of probiotic bacteria up to the shelf life of the unopened package becomes important. One of the main causes of postfermentation and in-package reduction in viability of probiotic bacteria is the dissolved oxygen content in the yoghurt (Miller et al. 2002). Miller (2003) showed that dissolved oxygen content (ppm) in stirred yoghurts increased over storage period at 4°C (Table 16.1). He used oxygen probes to measure the dissolved oxygen content in yoghurts. The readings were taken at 3, 33, and 53 mm below the surface, in the center of yoghurt containers. Probiotic bacteria are derived from the human intestine, an anaerobic environment, hence it is logical that their survival in air is severely limited.

A number of by products produced by bacteria during fermentation lower the oxidation–reduction potential. A positive potential favors the growth of aerobic bacteria, while a negative potential favors the growth of anaerobes. Fermented foods often have a low oxidation–reduction potential, helping to inhibit the growth of aerobic spoilage organisms (Miller 2003). Oxygen content and redox potential are important factors for viability of bacteria in fermented foods during storage (Bruner et al. 1993). The sensitivity to oxygen of some probiotic bacteria such as *Bifidobacterium* spp. is due to a lower NAD oxidase and peroxidase activity, and also because they lack catalase and superoxide dismutase activity (Shimamura et al. 1992). The production of yoghurt can involve non-intentional inclusion of oxygen, particularly in stirred-type yoghurts. Stirring during fermentation and subsequent pumping into containers are two steps that allow much oxygen to be incorporated. In addition, the ingress through packaging materials during storage also causes an increase in the in-package oxygen content (Miller et al. 2003b). Indeed, the viability of *Bifidobacterium* spp. can be shown as a function of oxygen permeability through the packaging material

TABLE 16.1

Dissolved Oxygen Content in Stirred Yoghurts (Values Are Mean of Four Replicates) during 4 Weeks Storage at 4°C

Sample	Day 1	Day 13	Day 24
3 mm center	6.916	6.916	6.887
33 mm center	7.750	6.479	7.449
53 mm center	6.479	7.668	9.576
Mean	7.047	7.020	7.971
Std. deviation	0.646	0.602	1.419

Source: Adapted from Miller, C.W., A study of packaging methods to reduce the dissolved oxygen content in probiotic yoghurts, PhD thesis, University of Western Sydney, Sydney, New South Wales, Australia, 2003.

FIGURE 16.1 Packaging used for fermented dairy products incorporating probiotic bacteria.

(Ishibashi and Shimamura 1993). The exclusion of oxygen during the fermented foods such as yoghurts would prove costly. Few current packaging techniques are capable of barring oxygen ingress into the final product container. Glass jars and aluminum-laminated plastics may be neither convenient nor practical packaging materials although they would be rather effective to prevent oxygen entry into product containers. Polyethylene and polystyrene do not have sufficient oxygen barrier properties and, therefore, are unsuitable. Examples of packaging used for fermented dairy foods incorporating probiotic bacteria are shown in Figure 16.1.

16.3 Active Food Packaging

Packaging may be termed active when it performs some desired role other than providing an inert barrier to external conditions (Hotchkiss 1994, Rooney 1995). Active packaging refers to the incorporation of certain additives into the packaging film or within packaging containers with the aim of maintaining and extending product shelf life (Day 1989). An example is when a plastic package has adequate moisture barrier but an inadequate oxygen barrier. Active packaging solutions could be the inclusion of an oxygen scavenger or incorporation of an antimicrobial agent if microbial growth is limiting the quality of the product. Active packaging includes additives or freshness enhancers that are capable of scavenging oxygen; absorbing carbon dioxide, moisture, ethylene and/or flavor/odor taints; releasing ethanol, sorbates, antioxidants and or/other preservatives; and/or maintaining temperature control (Rooney 1995, Day 2001).

16.3.1 Oxygen Scavengers

Oxygen can have considerable detrimental effects on foods. Oxygen scavengers can therefore help maintain food product quality by decreasing food metabolism, reducing

oxidative rancidity, inhibiting undesirable oxidation of labile pigments and vitamins, controlling enzymatic discoloration, and inhibiting the growth of aerobic microorganisms (Rooney 1995, Day 2001). Vermeiren et al. (1999) described techniques in active packaging that are capable of scavenging oxygen from the headspace of packages as well as some methods of removing dissolved oxygen from foodstuffs. Oxygen scavengers are by far the most commercially important category of active packaging. Commercialization of oxygen-scavenging polyethylene terephthalate (PET) bottles, bottle caps, and crowns for beers and other beverages has contributed to increased global market for oxygen scavengers. Many of the well known oxygen scavengers are contained in small sachets. The active oxygen scavenger is separated from the food by keeping it in a small, highly oxygen permeable sachet that is labeled "do not eat." The main advantage of using such oxygen scavengers is that they are capable of reducing oxygen levels to less than 0.01%, which is lower than the typical 0.3%–3.0% residual oxygen levels achievable by modified atmospheric packaging (MAP). Oxygen scavenger technology is particularly useful in hot and humid climate, which is conducive to mold spoilage of products.

It should be noted that there is a potential risk of accidental ingestion of the scavenging material, which could jeopardize commercial success and could prove detrimental to a product's reputation. However, development of oxygen-scavenging adhesive labels that can be applied to the inside of the packages and the incorporation of oxygen-scavenging materials into laminated trays and plastic films have enhanced this technology and eliminated the risk of accidental ingestion. This system will also allow contact with the product and, therefore, is capable of directly removing dissolved oxygen in the product. In the United Kingdom, oxygen-scavenging adhesive labels are used for a range of sliced, cooked and cured meat and poultry products that are particularly sensitive to deleterious light- and oxygen-induced color changes (Day 2001). These labels are also used in bakery goods and dried food ingredients, cured fish, powdered milk, confectionery, and snack food (Day 2001).

Maloba et al. (1996) reported an active packaging system to protect sunflower oil from oxidation during storage. The active component was incorporated into the package. It uses a photosensitized dye to excite oxygen diffusing through the plastic to its singlet stage, which was then absorbed into the polymer by a singlet oxygen acceptor. This method proved successful in its application, extending the shelf life of the oil by reducing the effect of oxidative rancidity. The limitation of this system is that in order for it to work, a constant source of light was needed that was essential to keep the dye active in its role of exciting oxygen. Such a requirement would not be a problem in products such as cooking oils, as they do not need refrigeration. However, this system will fail with refrigerated products such as dairy products. Indeed, when milk is exposed to light, it suffers a marked reduction in vitamin B content. Hence milk, which was originally packed in clear glass bottles, is now packaged in paper board cartons, which includes a thin layer of material that does not allow UV light penetration into the milk. Oxygen scavengers for fermented beverages such as beer and wine, where various nonmetallic reagents and organometallic compounds have an affinity for oxygen, have been incorporated into bottle closures, crowns, and caps or blended into polymer materials so that oxygen is scavenged from the bottle headspace and any entering oxygen is also scavenged.

16.3.2 ZERO2™ Oxygen-Scavenging Materials and Applications

Packaging issues involving the need for scavenging include headspace and dissolved oxygen present at the time of container closure of most food and beverages as well as diffusion of oxygen into the product through the packaging material during product storage. ZERO2 scavenging polymer composition meet these requirements, and these materials have been synthesized from food-grade polymers and extruded into films. ZERO2 is the trade name for a range of oxygen-scavenging plastic packaging materials developed by Food Science Australia, North Ryde, New South Wales, Australia and partly owned by Visy Pak Industries, Melbourne, Victoria, Australia. This system has been named as ZERO2 by Rooney (1999). It involves an organic compound that has been incorporated into a polymer for use as a layer in a laminated packaging film. Importantly, it can be added to any plastic packaging material and hence can be formed into any required shape (Figure 16.2). In contrast to Maloba et al.'s (1996)

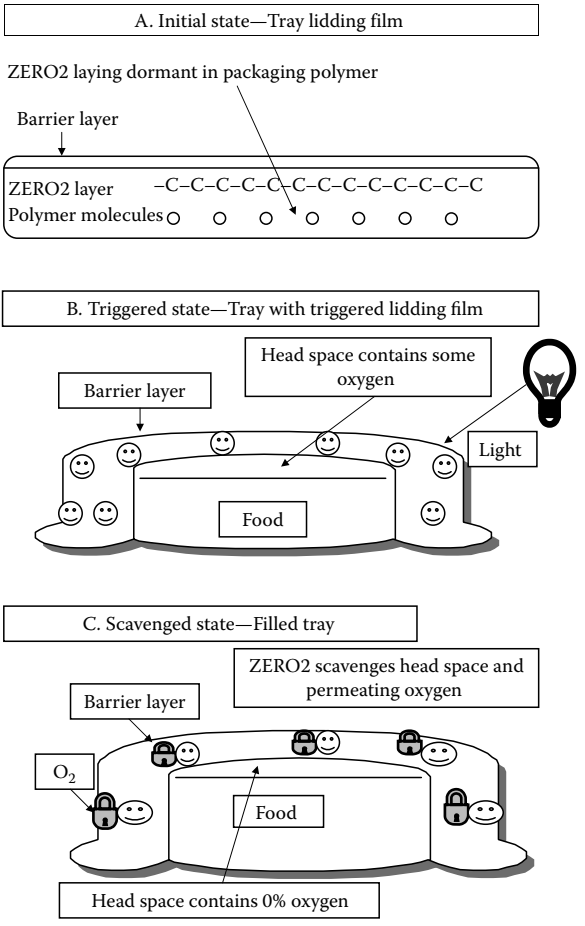

FIGURE 16.2 How ZERO2 works. (Adapted from Miller, C.W., A study of packaging methods to reduce the dissolved oxygen content in probiotic yoghurts, PhD thesis, University of Western Sydney, Sydney, New South Wales, Australia, 2003.)

active packaging system, ZERO2 does not require a constant source of light. It does, however, rely on light to begin the scavenging process. It can be stored inactive until required, which represents a great advantage over the other active materials. The light needed to activate the packaging is of a specific wavelength, meaning normal light conditions do not affect the organic compounds (Miller 2003). The ZERO2 package would be exposed to the correct wavelength of light prior to filling. The subsequent oxygen-scavenging action proceeds in a chain reaction. This packaging system may be suitable for fermented and refrigerated products such as yoghurt but would require an outer layer of high gas barrier material to prevent the scavenger being overwhelmed by oxygen migrating through the package (Miller 2003).

Alcoholic beverages such as beer and white wine are susceptible to rapid oxidative deterioration. Using ZERO2 materials, shelf life extensions of at least 33% have been achieved for bag-in-box wine and beer in multilayer PET bottles. Cheese and processed meats are examples of refrigerated foods that are normally packaged under modified atmospheres (Rooney 2000). The shelf life of these products is significantly affected by headspace oxygen concentration. Cheese requires a gas composition of carbon dioxide and oxygen levels below 1%. Results of packaging in laminates with or without a ZERO2 layer suggests that common spoilage molds can be inhibited completely with little or no carbon dioxide. Sliced smoked ham can be prevented from discoloration under refrigerated cabinet lighting conditions when the packaging laminates scavenge the initial oxygen concentration of 4% to very low levels. Development of ZERO2 and other similar technologies are aimed at inhibiting the widest range of oxygen-mediated food degradation processes (Rooney 2000).

16.3.3 Reduction of Dissolved Oxygen in Fermented Foods (Yoghurt)

Probiotic bacteria are incorporated into commercial yoghurts to deliver health benefits to consumers. To gain maximum therapeutic benefits, the probiotic bacteria must remain viable over the shelf life of yoghurt. However, a number of studies have reported that viability of these bacteria decreases significantly during the shelf life of probiotic yoghurt (Iwana et al. 1993, Shah et al. 1995, 2000, Rybka and Fleet 1997). Yoghurt contains a significant concentration of dissolved oxygen. Dissolved oxygen may be a significant cause for the reduction of probiotic viability. The current packaging materials used for yoghurt packaging may play a negative role, promoting oxygen uptake in the product.

When commercial stirred-type probiotic yoghurt was evaluated for dissolved oxygen (using a method developed using Clarke-type oxygen microelectrodes, Miller et al. 2003a), over a 6 week storage period from manufacture to expiry date, the results indicated a rising level of dissolved oxygen in the yoghurts over time. The dissolved oxygen was found to be not distributed homogenously within the yoghurt, and this indicated different rates of oxygen diffusion into the yoghurt at various points in the packaging. At some locations (near packaging weak spots), the concentration of dissolved oxygen reached saturation, which is completely an unfavorable environment for the survival of anaerobic probiotic bacteria incorporated into the yoghurt (Miller et al. 2002). When yoghurts were stored in packaging materials with improved gas barrier properties, decreased levels of dissolved oxygen during storage was reported. Nupak™ (Visy Pak, Melbourne, Victoria, Australia), a polyethylene-based packaging container with an added gas barrier layer, was used to pack stirred yoghurts.

The composition of the laminate is HIPS/tie/EVOH/tie/PE, with a transmission rate measured to be 0.005–0.01 mL/packaging/day at 23°C and 50%–60% RH. The HIPS and PE layers provide moisture barriers and the EVOH layer acts as the gas barrier (Miller et al. 2002). In stirred-type yoghurt manufacture, the coagulum is stirred briefly during fermentation to impart a smooth texture and the yoghurt packed in HIPS. This process allowed for the inclusion of oxygen in a number of unit operations such as online injection of fruit puree into the yoghurt before packaging. In contrast, in set-yoghurt manufacture, the yoghurt mix fermented in the final container, hence less or no agitation compared to stirred yoghurt; therefore, there is less chance to have oxygen inclusion during manufacture. Miller et al. (2003b) reported studies where dissolved oxygen levels in set and stirred yoghurts (stored for 42 days at 4°C) packed in HIPS and Nupak materials with or without an oxygen-scavenging polymer. The aim of these studies was to produce yoghurt with minimal oxygen content using different packaging systems. The results showed that the optimum reduction in dissolved oxygen was achieved by fermenting set-type yoghurt in an oxygen-barrier container, supplemented with an oxygen scavenger and sealed with an anaerobic headspace. Miller et al. (2003b) suggested that this method would produce yoghurts most conducive to the survival of probiotic bacteria, providing consumers with a product of increased health benefits.

16.3.4 Other Examples of Oxygen Scavengers

In Japanese fish processing industry, oxygen scavengers are used in packaging dried products (e.g., sea weed, salmon jerky, sardines, shark fin, cod squid) and smoked products (e.g., salmon) (Ashie et al. 1996). These products are stored at ambient temperatures and have low a_w (<0.85) and hence reduced microbial deterioration; however, the effect of oxygen scavengers is to prevent oxidative reactions such as discoloration and mold growth. When fresh fish and fish products such as yellow tail and salmon roe, and sea urchin are stored at frozen conditions and packaged with oxygen scavengers, the primary role of this scavenging activity is to prevent oxidation and discoloration in addition to inhibiting bacterial growth (Ashie et al. 1996). Different oxygen scavengers are chosen depending on the amount of oxygen to scavenge (pack size and nature of material) and product a_w. Oxygen scavengers for high a_w foods react faster compared to scavengers for dry foods but in general the absorption is slow and exothermic. Removal of oxygen from package interiors improves shelf life by suboptimizing the environment for aerobic microbiological growth and for adverse oxidative reactions such as rancidity. These principles may be applied for packaging fresh, frozen, and dried fermented products. Preservative packaging for fresh meats should maintain acceptable color, odor, and flavor for the product, while allowing the development of desirable characteristics associated with aging and retarding the onset of microbial spoilage (Taylor 1985). Such effects can be achieved by packaging meats under various atmospheres of oxygen, carbon dioxide, carbon monoxide, and/or nitrogen. The atmosphere within a container may change during extended storage, because of interaction between components of the atmosphere and the product, and/or because of transmission of gases into or out of the package through the packaging film (Stiles 1991). The appearance of raw meat has major effects on the purchasing decisions of consumers (Cornforth 1994). For red meats, consumers prefer bright red muscle tissue and white rather than yellow fat. The color of muscle tissue in red meat is determined

by the quantity and chemical state of the muscle pigment myoglobin. The deoxy form is a dull, purple color that consumers consider unattractive. The function of myoglobin is to transfer oxygen from blood to the muscle tissue cells. Myoglobin, hence, reacts rapidly with oxygen to give the bright red form oxymyoglobin. The fraction of pigment in the oxymyoglobin form is dependent on the partial pressure of oxygen to which the pigment is exposed (Livingston and Brown 1981). Myoglobin can also react with oxygen to give the stable, oxidized form metmyoglobin (Faustman and Cassens 1990). Meat with the dull, brown color of metmyoglobin is considered undesirable by most consumers (Renerre 1990).

When meat is packaged using modified atmosphere, it must provide a barrier to the exchange of gases between the package and the ambient temperature. However, the gas barrier properties of the packaging materials differ for different types of packaging and differing commercial functions of the packs. Materials such as nylon or polyethylene have nominal oxygen transmission rates of greater than 100 cc/m^2/24 h/atm under stated conditions of humidity and temperature. These are used for bulk packaging that is expected to contain the product for only a couple of days. However, packaging films used for modified atmospheric packages usually have oxygen transmission rates between 10 and 100 cc oxygen/m^2/24 h/atm, while packaging designed to contain products for the longer possible times is likely to be composed of materials with oxygen transmission rates less than 10 cc/m^2/24 h/atm (Jenkins and Harrington 1991). Carbon dioxide, the essential component of any effective modified atmosphere for meat, is highly soluble in both muscle and fat tissues (Gill 1988). Unlike carbon dioxide, the solubility of oxygen in muscle and fat tissues is low. However, oxygen is converted to carbon dioxide by the respiratory activities of both muscle tissue and bacteria. Although both gases are lost through packaging films when both are at concentrations above those of air, the carbon dioxide dissolved in tissue buffers decreases in carbon dioxide concentrations. Hence with modified atmospheres rich in oxygen, it is usually found that oxygen concentration declines with time of storage, but that carbon dioxide concentration alters little after the initial dissolution of the gas in the tissues (Nortje and Shaw 1989). If containers with oxygen-rich atmospheres are to be stored for relatively long times, the volume of the pack atmosphere should be about three times the volume of the product, to avoid excessive decreases of oxygen concentrations (Holland 1980). A number of studies have attempted to evaluate if oxygen scavengers can be used to prevent permanent discoloration of red meats in atmospheres with initial concentration about 1% or transient discoloration of meats in atmospheres with very low concentrations of residual oxygen. Although some success with the atmosphere of the former type has been reported (Doherty and Allen 1998), the general utility of such an approach may be not successful because the muscle tissue itself acts as a very efficient oxygen scavenger (Gill and McGinnis 1995).

16.3.5 Other Examples of Gaseous Forms of Scavengers, Emitters, Releasers, and Absorbers

Commercial sachet and label materials can be used to scavenge or emit carbon dioxide especially for fresh roasted or ground coffee that produces significant volumes of carbon dioxide. Fresh roasted or ground coffees cannot be left unpackaged since they will absorb moisture and oxygen and less desirable volatile aromas and flavors. However, if coffee is hermetically sealed in packs directly after roasting, the carbon

dioxide released will build up within the packs and eventually cause them to burst (Subramanyam 1998). To prevent this, a carbon dioxide scavenger is used. Carbon dioxide scavenger sachets and labels are most common and used for canned and foil pouched coffees in Japan and the United States (Day 1989, Rooney 1995). Pack collapse or partial vacuum can also be a problem for foods packed with an oxygen scavenger. To avoid this problem, dual action oxygen scavenger/carbon dioxide emitter sachets and labels have been developed, which absorb oxygen and generate an equal volume of carbon dioxide. The main food applications for these dual action oxygen scavengers/carbon dioxide emitter products are packaging nuts and sponge cakes (Naito et al. 1991, Rooney 1995).

In horticultural produce to retard respiration and subsequent senescence, ethylene scavengers are used (Abeles et al. 1992, Rooney 1995). Activated carbon-based scavengers with various metal catalysts can effectively remove ethylene. Dual-action ethylene scavenger and moisture absorber sachets have been marketed in Japan that contain activated carbon, a metal catalyst, and silica gel, and are capable of scavenging as well as acting as a moisture absorber (Abeles et al. 1992, Rooney 1995). Ethanol-emitting films and sachets are used as antimicrobial agents. They are effective against mold but also inhibit growth of yeasts and bacteria. For example, ethanol emitters are particularly useful in extending mold-free shelf life of bakery products. Preservative releasers such as antimicrobial and antioxidants packaging films are used in packaging meat, fish, bread, cheese, fruit, and vegetables. Due to increasing consumer demand for reduced antioxidants and other additives in foods, there has been an increased use of antioxidant packaging films. Plastic manufacturers show increasing interest in the use of naturally approved food ingredients, for example, vitamin E for polymer stabilization instead of synthetic antioxidants developed specifically for plastics (Rooney 1995). Excess moisture is a major cause of food spoilage. Soaking up moisture by using desiccants would extend shelf life by inhibiting microbial growth and associated degradation of texture and flavor. Several companies manufacture moisture absorbers in the form of sachets, pads, sheets, and pellets. For packaged dried-food applications, desiccants such as silica gel, calcium oxide, and activated clays and minerals are used within permeable plastic sachets. For dual-action purposes, these sachets may also contain activated carbon for odor absorption or iron powder for oxygen scavenging (Rice 1994, Rooney 1995). In addition to moisture-absorber sachets for humidity control in packaged dried foods, moisture-drip absorbent pads, sheets, and blankets are used to control liquid exudates in high a_w foods such as meats, fish, poultry, fruit, and vegetables. These moisture-drip devices contain polymers such as carboxymethyl cellulose (CMC), starch copolymers, and polyacrylate salts, which have a strong affinity for water. Moisture-drip absorber pads are commonly placed under packaged fresh meats, fish, and poultry to absorb unsightly tissue drip exudate (Rooney 1995).

16.4 Antimicrobial Food Packaging

The principle of antimicrobial packaging is to inhibit or kill spoilage and pathogenic organisms contaminating foods. The antimicrobial function can be achieved by incorporating antimicrobial agents into the packaging polymers, and/or employing antimicrobial polymers that satisfy conventional packaging requirements. When the

packaging system acquires antimicrobial activity, the packaging system (or material) limits or prevents microbial growth by extending the lag period and reducing the growth rate or decrease live counts of microorganisms (Han 2000). Primary objectives of any antimicrobial packaging are safety assurance, quality maintenance, and extension of shelf life of the product. Antimicrobial packaging could play a role in food security assurance. The mechanisms of antimicrobial action by antimicrobial agents are different depending on the target microbes. Some antimicrobial agents inhibit essential metabolic pathways of target microorganisms while others affect the structural integrity of cell wall membranes. For example, lysozyme destroys cell walls without the inhibition of metabolic pathways and results in physical cleavages of the cell wall, while lactoferrin and EDTA act as coupling agents of essential cationic ions and charged polymers. Two major functions of microbial inhibition are microbial-cidal and microbial-static effects. In the case of microbial-static effects, the packaging system has to possess the active function of maintaining the concentration above the minimal inhibitory concentration during the entire storage periodin order to prevent the regrowth of target microorganisms.

Traditional preservation methods sometimes consist of antimicrobial concepts, which include sausage casings of cured/salted/smoked meats, smoked/pottery/oak barrels for fermentation, and salt pickle jars. The basic principle of these traditional preservation methods and antimicrobial packaging is hurdle technology. The additional antimicrobial function of the packaging is another hurdle to prevent the degradation of total quality of packaged foods while satisfying the conventional functions of moisture and oxygen barriers as well as physical protection. The microbial hurdles may not contribute to protection from physical damage. However, it provides significant protection against spoilage microorganisms and pathogens, which cannot be achieved by conventional moisture- and oxygen-barrier packaging materials. Various antimicrobial agents may be incorporated in the packaging system, which may include chemical antimicrobials, antioxidants, biotechnology products, antimicrobial polymers, natural antimicrobials, and gas. Chemical antimicrobial agents are the most common substances used in the industry. They include organic acids, fungicides, alcohols, and antibiotics. The use of antibiotics as package additives is not approved for the purpose of antimicrobial functions and is also controversial because of the possibility of the development of resistant microorganisms. Antioxidants are effective antifungal agents due to the restrictive oxygen requirement of molds. Food-grade chemical antioxidants could be incorporated into packaging materials to create an anaerobic atmosphere inside packages, and eventually protect the food against aerobic spoilage (Smith et al. 1990). Since the package did not contain oxygen, the partial pressure difference of oxygen is formed between the outside and inside of packaging materials. Hence to maintain the low concentration of oxygen inside the package, the packaging system requires high oxygen-barrier materials such as ethylene vinyl alcohol (EVOH), polyvinylidene chloride (PVDC), or aluminum foils that prevent the permeation of oxygen. In addition to antioxidants, a multi-ingredient oxygen-scavenging system, such as commercial oxygen-absorbing sachets, can be used to reduce oxygen concentration inside the package. Various bacteriocins that are produced by microorganisms also inhibit the growth of spoilage and pathogenic microorganisms. These fermentation products include nisin, lacticins, pediocin, diolococcin, and propionicins (Daeschul 1989, Han, 2002). These biologically active peptides possess strong antimicrobial properties against various bacteria. Other

nonpeptide fermentation products such as reuterin also demonstrate antimicrobial activity. Natural polymers such as chitosan exhibit antimicrobial activity. Short- or medium-size chitosan possesses good antimicrobial activity, while long-chained chitosan is not effective. The use of natural plant extracts is desirable for the development of new food products and new active packaging systems. Some plant extracts such as grapefruit seed, cinnamon, horse radish, and clove have been added to packaging systems to demonstrate effective antimicrobial activity against spoilage and pathogenic bacteria. Gaseous antimicrobials may have advantages compared to solid/solute types of chemical antimicrobial agents. They can be vaporized and penetrated into any air space inside packages that cannot be reached by nongaseous antimicrobial agents. An ethanol sachet is one example of a gaseous antimicrobial system. Headspace ethanol vapor can inhibit the growth of molds and bacteria.

Antimicrobial agents can be incorporated into a packaging system through sample blending with packaging polymers, immobilization, or coating differently depending on the characteristics of packaging system, antimicrobial agent, and food. The blended antimicrobial agents can migrate from packaging materials to foods, while the immobilized agent cannot migrate. Antimicrobial agents are impregnated into packaging materials before final extrusion (Han and Floros, 1997, Nam et al., 2002), dissolved into coating solvents (Rodrigues and Han 2000, Rodrigues et al. 2002), or mixed into sizing/filling materials of paper and paperboards (Nadarajah et al. 2002). The coating process can also produce an antimicrobial packaging system. Coating over prepackaged products or edible coating on the food itself can produce an extra physical barrier layer that also contains antimicrobial agents. An antimicrobial agent has its own specific inhibition activity against each microorganism. The selection of the antimicrobial agent should be dependent on its activity against a target microorganism. Growth of potential spoilage microorganisms in a food is predictable when characteristics of food products such as pH, water activity, composition, and storage temperature are considered. The design of an antimicrobial packaging system requires controlled release technology and microbial growth kinetics. When the migration rate of an antimicrobial agent is faster than the growth rate of the target microorganism, the antimicrobial agent will be depleted before the expected storage period, and the packaging system will lose its antimicrobial activity because the packed food has an almost infinite volume compared to the volume of packaging material and the amount of antimicrobial agent. Consequently, the microorganism will start to grow after the depletion of the antimicrobial agent. However, when the release rate is too slow to control the growth of the microorganism, it can grow instantly before the antimicrobial agent is released. Hence the release rate of the antimicrobial agent from the packaging material to food is specifically controlled to match the release rate with the growth kinetics of the target microorganism. Physical and mechanical integrity of packaging materials is affected by the incorporated antimicrobial agents. If the antimicrobial agent is compatible with the packaging materials and does not interfere with the polymer–polymer interactions, adequate amount of the antimicrobial agent may be impregnated into the packaging material without any physical and mechanical integrity deterioration (Han 1996). However, an excess amount of antimicrobial agent that is not capable of being blended with packaging materials will decrease physical strength and mechanical integrity (Cooksey et al. 2000). Since the antimicrobial agent is contacting the food or migrating into the food, the organoleptic property and toxicity of the antimicrobial agent should be satisfied to avoid deterioration in quality

and to maintain the safety of the packaged foods. The antimicrobial agents may also possess strong taste or flavor, such as bitter or sour taste and possibly an undesirable aroma that can influence the sensory quality adversely. In the case of antimicrobial edible protein film-coating applications, the allergenicity or chronic disease caused by edible protein materials, such as peanut protein, soy protein, and wheat gluten, should be considered before use (Han 2001).

16.5 Modified Atmospheric Packaging

In modified atmospheric packaging (MAP), the air in the package is replaced with a different gas or gas mixture. The packages containing food products are flushed and filled with nitrogen gas or gas mixtures. As a consequence of change in the gas atmosphere, the shelf life of the product is increased significantly because the modified atmosphere slows down the degradation process, particularly microbial spoilage reactions. During storage, the gas composition may change as a consequence of permeability of packaging material and chemical and biological activities of the packaged food. A decrease in carbon dioxide level in packages has been reported when lobster was stored under modified conditions due to dissolution of the gas in the lobster tissue (Ruiz-Capillas and Moral 2004). Another method of changing the package atmosphere is through vacuum packaging, which prevents oxidative rancidity; however, it favors proliferation of anaerobic pathogenic microorganisms, especially under temperature abuse conditions. At chilled storage temperatures, the shelf life of vacuum packaged fish may be compatible with that of MAP products such as salmon and trout and is doubled compared with air-stored fish (Sivertsvik et al. 2002).

The three main gases used in MAP are oxygen, carbon dioxide, and nitrogen, the choice of gas totally dependent upon the food product being packed. Used singly or in combination, these gases are commonly used to balance safe shelf life extension with optimal organoleptic properties of the food. Carbon dioxide is colorless gas that dissolves readily in water to produce carbonic acid, which increases the acidity of the solution and reduces pH. The solubility of carbon dioxide increases with decreasing temperature, hence the antimicrobial activity of carbon dioxide is greater at temperatures below 10°C. The high solubility of carbon dioxide can result in the package collapsing due to reduction of headspace volume. However, in some MAP applications, pack collapse is preferred, for example, in wrapped cheese for the purpose of retail sale. Oxygen is a colorless and odorless gas and promotes several types of deteriorative reactions in foods including fat oxidation, browning reactions, and pigment oxidation. Most of the consumer spoilage bacteria and fungi require oxygen for growth. Therefore, to increase the shelf life of foods the pack atmosphere should contain a low concentration of residual oxygen. However, in certain foods, a low concentration of oxygen can result in quality and safety problems (e.g., unfavorable color changes in red meat pigments, senescence in fruits and vegetables, and growth of food poisoning bacteria), and this must be taken into account when selecting the gaseous composition for a packaged food. Nitrogen does not support the growth of aerobic microbes and therefore inhibits the growth of aerobic spoilage bacteria but does not prevent the growth of anaerobic bacteria. The low solubility of nitrogen in foods can be used to prevent pack collapse by including sufficient nitrogen in the gas mix to balance the volume decrease due to carbon dioxide going into solution. Selection of the most

appropriate packaging materials is essential to maintain the quality and safety of MAP foods. Flexible and semirigid plastics, and plastic laminates are the most common materials used for MAP foods. Most MAP films are multilayer structures formed from several layers of different plastics. Using co-extrusion, laminates, or coating technologies, it is possible to combine different types of plastics to form films, sheets, or rigid packs. Plastic packaging for MAP applications is most commonly found in the form of flexible films for bags, pouches, pillow packs and top webs, or as rigid or semirigid structures for base trays, dishes, cups, and tubs. Commonly used plastic flexible laminates are produced from polyethylene (PE), polypropylene (PP), polyamide (nylons), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyvinylidene chloride (PVDC), and ethylene vinyl alcohol (EVOH). Rigid and semirigid structures are commonly produced from PP, PET, PVC, and expanded polystyrene.

The main food groups that are packaged using modified atmospheric packaging includes raw meat and poultry; cooked, cured, and processed meat products; fish and fish products; fruits and vegetables; and dairy products (Table 16.2). Microbial growth and oxidation of the red oxymyoglobin pigments are the main spoilage mechanisms that limit the shelf life of raw red meats. In raw meat packaging, appropriate oxygen concentrations in the pack atmosphere are necessary to maintain the desirable red color of the oxymyoglobin pigment. In highly pigmented red meats such as venison and wild boar, higher concentrations of oxygen may be required. To inhibit growth of aerobic spoilage bacteria in red meats, a gas mixture containing 20%–30% carbon dioxide (which inhibits bacterial growth) and 70%–80% oxygen is used. A gas/product ratio of 2:1 is usually recommended. However, maintenance of recommended chilled temperatures and good hygienic practices throughout the slaughter house, MAP, and retail chain are critically essential in ensuring safety and extended shelf life of red meat products. This is particularly important in the case of poultry meat as it provides a good medium for the growth of pathogenic microorganisms, including some that are not inhibited by carbon dioxide. In packaging poultry meat with MAP, it has been reported that carbon dioxide concentrations greater than 15% caused discoloration of meat (Ogilvy and Ayres 1951); however,

TABLE 16.2

Recommended MAP Conditions for Various Foods

Food Type	Temperature (°C)	Oxygen (%)	Carbon Dioxide (%)	Reference
Bread	Ambient	0	100	Smith et al. (1990)
Hard cheese	1–4	0	100	Subramanyam (1998)
Soft cheese	1–4	0	20–40	Subramanyam (1998)
Lean fish	0–2	30	40	Garthwaite (1992)
Oily/smoked				
Fish	0–2	0	60	Garthwaite (1992)
Beef	−1 to 2	60–80	20–40	Blakistone (1998)
Pork	−1 to 2	30	30	Floros (1990)
Poultry	−1 to 2	0	25–35	Blakistone (1998)

Source: Adapted Devlieghere, F. et al., Modified atmosphere packaging (MAP), in *The Nutrition Handbook for Food Processors*, eds. C.J.K. Henry and C. Chapman, CRC Press, Boca Raton, FL, 2002, pp. 342–370.

later it was confirmed that development of gray tinges on poultry meat may be due to high residual oxygen levels (Gill 1990).

The principal spoilage mechanisms that limit the shelf life of cooked, cured, and processed meat products are microbial growth, color change, and oxidative rancidity. The color of cooked meats is susceptible to oxidation, and it is important to have only low levels of residual oxygen in packs. MAP using carbon dioxide/nitrogen mixes (gas compositions of 25%–50% carbon dioxide and 50%–75% of nitrogen) along with a gas/product ratio of 2:1 is widely used to maximize the shelf life and inhibit the development of oxidative off-flavors and rancidity. Processed meat products (e.g., sausages, frankfurters) generally contain sodium metabisulfite, which is an effective preservative against a wide range of spoilage microorganisms and pathogens. Cooked, cured, and processed meat products containing high levels of unsaturated fat are liable to be spoiled by oxidative rancidity; however, MAP with carbon dioxide/nitrogen mixtures is effective at inhibiting this undesirable reaction. Potential food-poisoning hazards are primarily due to microbial contamination or growth resulting from postcoking, curing, or processing contamination. These can be minimized by using recommended chilled temperatures, good hygiene, and handling practices.

MAP is used to control spoilage of fish due to the breakdown of tissue by the fish's own enzymes (autolysis of cells), growth of microorganisms, as well as oxidative reactions. However, MAP has no direct effect on autolysis of fish tissue. Oxidative reactions are more important as shelf life limiters in fish compared with other fresh meat, because sea food has a higher content of polyunsaturated lipids. Storage temperature has a major effect on fat oxidation that occurs even at frozen temperatures. There are several microorganisms that are of particular importance when dealing with MAP of fish products; these include *Clostridium botulinum*. Use of carbon dioxide effectively inhibits the growth of some of these species. The aerobic spoilage organisms tend to be replaced by slower-growing and less odor-producing bacteria, particularly lactic acid bacteria such as lactobacilli during storage. Because fish and shell fish contain much lower concentrations of myoglobin, the oxidation status of this pigment is less important than that in other meats. Consequently, there is potential to use higher levels of carbon dioxide, for example, 40%. However, because of the high moisture content and the lipid content of some species, nitrogen is used to prevent pack collapse. In case of MAP of raw fish products, risk can be effectively eliminated if storage temperature is held at 3°C or below and if the shelf life is limited to no more than 10 days.

MAP has the potential to extend the shelf life of many fruits and vegetables. Respiration is affected by the intrinsic properties of fresh produce as well as various extrinsic factors, including ambient temperature. It is accepted that the potential shelf life of packed produce is inversely proportional to respiration rate. Respiration rate increases by a factor of 3–4 for every 10°C increase in temperature. In MAP, the respiration of fruits and vegetables is reduced to extend shelf life while maintaining quality. Respiration can be reduced by lowering the temperature, lowering the oxygen concentration, increasing the carbon dioxide concentration, and by the combined use of oxygen depletion and carbon dioxide enhancement of the pack atmosphere. If the oxygen concentration is reduced beyond a critical concentration, which is dependent on the species and cultivar, then anaerobic respiration will be initiated (Kader et al. 1989). Anaerobic respiration or anaerobiosis is usually associated with undesirable odors and flavors and a marked deterioration in product quality. While increasing the carbon dioxide concentration will also inhibit respiration, high concentrations may

cause damage in some species and cultivars. The use of MAP atmospheres containing low concentrations of oxygen and elevated carbon dioxide concentrations may permit the growth of psychotropic protease-negative strains of *C. botulinum*. This problem can be avoided if the packs are stored at below 3°C for not more than 10 days. Increasingly, gas packing fresh produce along with carbon dioxide/oxygen/nitrogen gas mixtures is being used. This approach may have benefits is reducing enzymatic browning reactions before a passively generated equilibrium-modified atmosphere has been established. MAP also has the potential to extend shelf life of a number of dairy products. These include fat-filled milk powders, cheeses, and fat spreads. In general, these products spoil due to the development of oxidative rancidity in the case of dried milk powders and/or the growth of microorganisms, particularly yeasts and molds in the case of cheese.

16.6 Conclusion

As fermentation technology evolves, suitable packaging technology needs to keep pace with the development. The future of any innovation in packaging depends upon the extent to which it can satisfy the requirement of the product packaged. Commercial development will be driven by needs as perceived in the food fermentation industry or in other related industries. New developments in packaging should address viability issues of live microorganisms (e.g., probiotics incorporated dairy fermented foods). Processing and packaging of dairy fermented foods such as yogurts incorporate substantial quantities of oxygen and in addition it has been shown that oxygen gains entry into fermented foods through the packaging material of the container over the shelf life. In this respect, the use of oxygen-scavenging plastics as chemical barriers to permeation should provide product shelf lives closer to those foods using metal cans. Active packaging is an exciting area of food technology that can confer many preservative benefits on a wide range of fermented food products. The objectives of this technology are to maintain sensory quality and extend the shelf life of foods while at the same time maintaining nutritional quality and ensuring microbial safety. Oxygen scavengers are by far the most commercially important subcategory of active packaging in recent times. However, other active packaging technologies such as carbon dioxide scavengers and emitters, moisture absorbers, and temperature control packaging will be increasingly used in the future.

Antimicrobial packaging systems are particularly important in fermented foods to inhibit growth of spoilage and pathogenic microorganisms, and contribute to the improvement of food safety and the extension of shelf life of the packaged food. Many factors need to be considered in designing an antimicrobial packaging system; however, most factors are closely related to the characteristics of antimicrobial agents, packaged foods, and target microorganisms. Packing food in a modified atmosphere can offer extended shelf life and improved product presentation in a convenient container, making the product more attractive to the retail consumer. However, MAP cannot improve the quality of a poor quality food product. It is therefore essential that the food is of highest quality prior to packaging in order to optimize the benefits of modifying the pack atmosphere. Good hygienic practices and efficient temperature control throughout the cold chain for perishable products are required to maintain the quality benefits and extended shelf life of MAP foods.

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