Jyoti Prakash Tamang *Editor*

Ethnic Fermented Foods and Alcoholic Beverages of Asia

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Editor
Jyoti Prakash Tamang
Department of Microbiology
School of Life Sciences
Sikkim University
Gangtok, Sikkim, India

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Preface

Asia has a long history of preparation and consumption of various types of ethnic fermented foods and alcoholic beverages based on available raw substrates of plant or animal sources and also depending on agroclimatic conditions of the regions. About 90 % of the Asian ethnic fermented foods are naturally fermented by both culturable and unculturable microorganisms. Diversity of functional microorganisms in Asian ethnic fermented foods and alcoholic beverages consists of bacteria (lactic acid bacteria and *Bacillus* species, micrococci, etc.), amylolytic and alcohol-producing yeasts, and filamentous molds. Microorganisms establish on relevant substrates for survival and produce bioactive compounds that enrich the human diet, thereby promoting health benefits to consumers.

This book has 15 chapters covering different types of ethnic fermented foods and alcoholic beverages of Asia. I tried to cover all Asian countries for this book, but could not get contributors for book chapters from many countries. However, I am grateful to all contributing authors who accepted our invitation to write this book. Some of them are well-known scientists and researchers with vast experiences in the field of fermented foods and beverages. We are happy to bring all of them in the same platform, bringing out this book, and thanks to Prof. Tek Chand Bhalla, Dr. Namrata Thapa, and Dr. Savitri (India); Prof. Yearul Kabir and Dr. Mahmud Hossain (Bangladesh); Prof. Tika Karki, Dr. Pravin Ojha, and Dr. Om Prakash Panta (Nepal); Dr. Saeed Akhtar, Dr. Majid Hussain, Dr. Tariq Ismail, and Dr. Muhammad Riaz (Pakistan); Prof. Sagarika Ekanayake (Sri Lanka); Dr. Werasit Sanpamongkolchai (Thailand); Prof. Sh.Demberel, Dr. D. Narmandakh, and Dr. N. Davaatseren (Mongolia); Dr. Yoshiaki Kitamura, Dr. Ken-Ichi Kusumoto, Dr. Yukio Magariyama, Dr. Tetsuya Oguma, Dr. Toshiro Nagai, Dr. Soichi Furukawa, Dr. Chise Suzuki, Dr. Masataka Satomi, Dr. Kazunori Takamine, Dr. Hisanori Tamaki, and Dr. Sota Yamamoto (Japan); Prof. Dong-Hwa Shin, Prof. Cherl-Ho Lee, Dr. Young-Myoung Kim, Dr. Wan-Soo Park, Dr. Jae-Ho Kim, and Dr. Moonsil Lee Kim (South Korea); Dr. Maryam Tajabadi Ebrahimi, Dr. Neda Mollakhalili Meybodi, and Dr. Amir Mohammad Mortazavian (Iran); Dr. Francisco B. Elegado, Dr. Maria Teresa M. Perez, Dr. Shara Mae T. Colegio, Dr. Charina Grace B. Banaay, Dr. Bernadette C. Mendoza, Dr. Vanessa Marie T. Lim, Dr. Andrea Therese R. Gervasio, and Dr. Marilen P. Balolong (Philippines); Prof. Ingrid Suryanti Surono (Indonesia); and Dr. Vu Nguyen Thanh and Dr. Nguyen Thi Viet Anh (Vietnam). We are also grateful to Springer for bringing out this comprehensive book on important topics. We hope this book will be read by researchers, students, teachers, nutritionists, dieticians, food entrepreneurs, agriculturalist, government policy makers, ethnologists, sociologists, and electronic media persons who keep interest on the health benefits of fermented foods and beverages. Though there are hundreds of research articles, review papers, and limited books on fermented foods and beverages, this book *Ethnic Fermented Foods and Alcoholic Beverages of Asia* is the first of this kind on compilation of various ethnic fermented foods and alcoholic beverages of Asia.

I dedicate this book to Asian ethnic people who invented their indigenous knowledge of traditional food fermentation technology and have created a platform for research to study in depth molecular microbiology, nutrition, and bioactive compounds in ethnic fermented foods and beverages.

Gangtok, India Jyoti Prakash Tamang

Contents

Contributors

Saeed Akhtar Institute of Food Science and Nutrition, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan

Nguyen Thi Viet Anh Food Industries Research Institute, Thanh Xuan, Hanoi, Vietnam

Marilen P. Balolong Department of Biology, College of Science, University of the Philippines Manila, Manila, Philippines

Charina Grace B. Banaay Institute of Biological Science, University of the Philippines Los Baños, College, Laguna, Philippines

Tek Chand Bhalla Department of Biotechnology, Himachal Pradesh University, Shimla, Himachal Pradesh, India

Shara Mae T. Colegio Institute of Biological Science, University of the Philippines Los Baños, College, Laguna, Philippines

N. Davaatseren Department of Strategic Policy and Planning, Ministry of Food and Agriculture, Ulaanbaatar, Mongolia

Sh. Demberel Mongolian University of Life Sciences, Ulaanbaatar, Mongolia

Maryam Tajabadi Ebrahimi Department of Biology, Faculty Member of Science, Islamic Azad University, Central Tehran Branch, Tehran, Iran

Sagarika Ekanayake Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

Francisco B. Elegado National Institutes of Molecular Biology and Biotechnology, University of the Philippines Los Baños, College, Laguna, Philippines

Soichi Furukawa College of Bioresource Science, Nihon University, Fujisawa, Kanagawa, Japan

Andrea Therese R. Gervasio Department of Biology, College of Science, University of the Philippines Manila, Manila, Philippines

Mahmud Hossain Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh

Majid Hussain Institute of Food Science and Nutrition, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan

Tariq Ismail Institute of Food Science and Nutrition, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan

Yearul Kabir Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh

Tika Karki Department of Biotechnology, Kathmandu University, Kathmandu, Nepal

Jae-Ho Kim Korea Food Research Institute, Seongnam-si, Gyeonggi-do, Republic of Korea

Young-Myoung Kim Gijang Mulsan CO.LTD, Suwon-si, Kyunggi-do, Republic of Korea

Moonsil Lee Kim Rhode Island College, Providence, RI, USA

Yoshiaki Kitamura Food Research Institute, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan

Ken-Ichi Kusumoto Food Research Institute, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan

Cherl-Ho Lee Korea University, Seoul, South Korea

Vanessa Marie T. Lim Department of Biology, College of Science, University of the Philippines Manila, Manila, Philippines

Yukio Magariyama Food Research Institute, National Agriculture and Food Research Organization, Tsukuba, Ibaraki Japan

Bernadette C. Mendoza Institute of Biological Science, University of the Philippines Los Baños, College, Laguna, Philippines

Neda Mollakhalili Meybodi Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Research Center for Food Hygiene and Safety, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Amir Mohammad Mortazavian Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Toshiro Nagai Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan

D. Narmandakh Mongolian University of Life Sciences, Ulaanbaatar, Mongolia

Tetsuya Oguma Japan Soy Sauce Technology Center, Chuoku, Tokyo, Japan

Pravin Ojha Food Research Division, Nepal Agricultural Research Council, Lalitpur, Nepal

Om Prakash Panta Department of Microbiology, National College, Kathmandu, Nepal

Wan-Soo Park World Institute of Kimchi, Gwangju Metropolitan City, Republic of Korea

Maria Teresa M. Perez National Institutes of Molecular Biology and Biotechnology, University of the Philippines Los Baños, College, Laguna, Philippines

Muhammad Riaz Institute of Food Science and Nutrition, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan

Werasit Sanpamongkolchai Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Chatuchak, Bangkok, Thailand

Masataka Satomi National Research Institute of Fisheries Science, Japan Fisheries Research and Education Agency, Yokohama, Japan

Savitri Department of Biotechnology, Himachal Pradesh University, Shimla, Himachal Pradesh, India

Dong-Hwa Shin Shindonghwa Food Research Institute, Kangnam-ku, Seoul, Republic of Korea

Ingrid Suryanti Surono Food Technology Department, Faculty of Engineering, Bina Nusantara University, Serpong-Tangerang, Indonesia

Chise Suzuki Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan

Kazunori Takamine Education and Research Center for Fermentation Studies, Faculty of Agriculture, Kagoshima University, Kagoshima, Japan

Hisanori Tamaki Education and Research Center for Fermentation Studies, Faculty of Agriculture, Kagoshima University, Kagoshima, Japan

Jyoti Prakash Tamang Department of Microbiology, School of Life Sciences, Sikkim University, Gangtok, Sikkim, India

Vu Nguyen Thanh Food Industries Research Institute, Thanh Xuan, Hanoi, Vietnam

Namrata Thapa Department of Zoology, School of Life Sciences, Sikkim University, Gangtok, Sikkim, India

Sota Yamamoto Research Center for the Pacific Islands, Kagoshima University, Kagoshima, Japan

Author Biography of Professor Jyoti Prakash Tamang

Professor Dr. **Jyoti Prakash Tamang** is one of the authorities of global fermented foods and beverages for the last 28 years. He did his Ph.D. in microbiology at North Bengal University, India, in 1992, and postdoc research works at the National Food Research Institute, Tsukuba, Japan, in 1995, and Institute of Hygiene and Toxicology, Germany, in 2002. He was given the prestigious National Bioscience Award of the Department of Biotechnology by the Government of India in 2005 and Gourmand Best Cookbook Award of Paris in 2010. He is a Fellow of National Academy of Agricultural Sciences (2012), Fellow of Indian Academy of Microbiological Sciences (2010), and Fellow of Biotech Research Society of India (2006). He has published more than 135 research papers and authored several books including (1) *Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values* (2010), (2) *Fermented Foods and Beverages of the World* (2011), and (3) *Health Benefits of Fermented Foods and Beverages* (2015) all published by CRC Press, Taylor & Francis Group, USA. He has one patent and has produced several Ph.D. students. He is a member of several prestigious national and international academics including the International Yeast Commission, Asian Federation of Lactic Acid Bacteria, etc. Prof. Tamang is a professor in the Department of Microbiology and also dean of the School of Life Sciences at Sikkim University, a national university at Gangtok.

1 History of Fermented Foods in Northeast Asia

Cherl-Ho Lee and Moonsil Lee Kim

1.1 Introduction: Where and Who?

Northeast Asian culture has been generally considered as part of Chinese civilization because currently a large portion of this region is occupied by the People's Republic of China (PRC), and the relatively small nations around the PRC have been simply understood as strongly influenced by Chinese civilization in terms of politics, economy, and culture. However, Northeast Asian culture is composed of many different ethnic groups that have developed their own identities and distinctive cultures throughout history. Therefore, before starting a discussion on the fermented food in Northeast Asia, it should be clarified where and about whom we are talking in this chapter.

The definition of Northeast Asia varies according to the context in which it is discussed. From an economic view, it indicates China, South Korea, and Japan, as these nations are dominating current economic growth in the region. In terms of the regional security of Northeast Asia, North Korea is at the center of this issue in relation to the surrounding countries of South Korea, China, Japan, and Russia. Sometimes people use the term "Northeast Asia" to indicate much broader territories including Mongolia, Taiwan, and eastern regions of the Russian Federation and Siberia. In this chapter on the fermented foods of Northeast Asia, we define Northeast Asia as the northeastern subregion of Asia that includes the Korean Peninsula, the Japanese archipelago, Manchuria, the Russian Far East, and the Chinese northeast coast, along with the Bohai Sea and the Yellow Sea (Map 1.1). In particular, the fermented food culture and technologies developed in the Korean Peninsula and the surrounding area of the Korea Strait will be the focus of discussion in this chapter.

The people who occupied Northeast Asia were first introduced in the Chinese history book, *Hou Hanshu* (*History of the Later Han Dynasty*) in the fifth century AD, as "eastern archers (*dongyi* 東夷)," collectively referring to several ethnic groups and tribes in Northeast Asia (Ban, fifth century). This Chinese word "*dongyi*" has been traditionally translated as "eastern barbarians" from the Sino-centric perspective, but here we suggest interpreting this word as "eastern archers" since the character "yi 夷" was originally composed of a combination of two words, "big (大)" and "bow (弓)." The mural painting of the Koguryŏ (ca. 37 BC–668 AD) tomb of Muyongchong in Manchuria that depicted horse

C.-H. Lee (✉)
Korea University, Seoul 136-701, South Korea
e-mail: chlee@korea.ac.kr

M.L. Kim
Rhode Island College, Providence, RI, USA
e-mail: mkim@ric.edu

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Map 1.1 Northeast Asia

riders with big bows supports the possibility that the Dongyi were people who had good archery skills, as the name indicates. The *History of the Later Han Dynasty* describes Dongyi people who were good at horse riding and archery and who established several proto-states in Manchuria, the Korean Peninsula, and the Japanese archipelago, such as Puyŏ, Dongye, Okchŏ, Koguryŏ, Samhan, and Wa. This indicates that as early as the third century BC, the Dongyi had already formed a unique culture that differed from the well-known Chinese civilization that had thrived in Central and Southern China. However, it was much earlier that Chinese people recognized these archers (*yi*) as non-Chinese, as we can see from the oracle bone inscription of the Shang period (1600–1046 BC) in China, dating to around 1200–1046 BC, that reads "…the king orders to campaign against the Yi" (Ebrey 1993).

This historical evidence implies that several ethnic groups called Dongyi who had occupied Northeast Asia and competed with Chinese people since around 1000 BC or earlier must have had stable food resources that sustained the people and their communities during famines. Historical and archaeological remains tell us that the fermented foods they enjoyed, such as fermented fish, fermented vegetables, and fermented soybean products, were the most reliable and stable sources for obtaining protein throughout the year. The dietary culture of the Dongyi people, based on various fermented foods, was the result of the early use of fermentation technology, which originated in this region. In this chapter, before an in-depth scientific analysis of traditional fermented foods in Northeast Asia, the birth and the development of the fermentation technology of the Dongyi will be discussed. In particular, the geographical and environmental background and the primitive pottery culture will be examined as prerequisites of the birth of fermentation technology in Northeast Asia.

1.2 Pottery and Dietary Culture of Northeast Asia

The trace of earlier people in this region can be found in archaeological discoveries. Archaeological remains suggest that this region was occupied by Early Paleolithic people who immigrated through Mainland China, Siberia, or South Asia as early as the Early Pleistocene period (~1,600,000 BP, Bae 2010). The early existence of human beings in this region is evidenced by some Early Paleolithic remains (1,800,000–300,000 BP) of the Early/Middle Pleistocene period on the northeast Chinese mainland and Korean Peninsula. Several Early Paleolithic sites, such as the Zhoukoudian (of Peking man, dated 680,000–780,000 years old) and Jinniushan sites, have been excavated in Northeast China and Manchuria. The earliest hominin fossils in the Korean Peninsula excavated from central South Korea (Chŏngokni) and in Pyŏngyang (Daehyŏndong) have been dated to the early Middle Paleolithic sites (350,000–40,000 BP), but lithic assemblage was discovered in Komunmoru, Jangsanni, and Jangdongni that seem older than Chŏngokni (Bae 2010). In addition, Middle Paleolithic remains were found on the Korean Peninsula and in South Manchuria, and numerous Late Paleolithic (40,000–10,000 BP) sites were also found on the Korean Peninsula, South Manchuria, and Japanese archipelago. These sites indicate an increase in population and a dispersal of people in this region of Northeast Asia during the Paleolithic period.

Paleolithic hominin survived by mobile hunting and mountain foraging. They preyed on deer, wild pig, bison, and roe. These animals provided people with meat, gut, and blood. Animal meat, intestine, and blood were probably the main foodstuff for these people, with the use of vegetable supplements, such as grass seeds, tree nuts, and wild fruits and roots. Those who reached the Korean Peninsula gradually adapted themselves to their new environment as they changed dwelling site and diet habits. The meat-centered diet culture of the early hominin in Northeast Asia gradually changed to an omnivorous culture by the end of the Paleolithic Age. According to archaeological remains from the Korean Peninsula, peoples there increasingly consumed tree nuts and acorns, wild fruits, berries and grapes, grass seeds, roots, and young buds of trees and ferns. The appearance of the pollen of grass, rice (Gramineae), and beans (Leguminosae, Papilionoideae) also increased in Late Paleolithic remains (Lee 1998).

Paleolithic hominin of Northeast Asia who lived in mountain caves at the beginning gradually moved to the lower plains and riverbanks by the Late Paleolithic Age (Lee 1998). As they could now obtain abundant food around their dwelling sites, they could stay longer in these areas. Beginning to inhabit one single place, they reduced their mobile hunting practices and instead obtained more food by collecting seeds of grass and barnyard grass, millet, and wild beans. Gradually they developed skills in storing food resources by drying. Step by step, they became accustomed to collecting mollusks like frogs and snails in damp ground and clams and shellfish in rivers and beaches.

As they transferred dwelling site from the hill and cave to the coastline and riverside, ancient Northeast Asians experienced yet another significant change in their dietary culture. They began increasingly to consume marine products while also still enjoying tree nuts, acorns, wild grains, fruits, vegetables, and roots. Archaeological evidence such as numerous shell mounds along the coastal line of the Korean Peninsula and the Japanese archipelago clearly proves that the dietary habit of the people changed at the beginning of the Holocene period (ca. 10,000 BC) as their dwelling sites moved to the coastline (Lee 2001).

Interestingly, the increased consumption of marine products coincided with the invention of primitive pottery. Around the Late Paleolithic Age, by 6000 BC, the use of *Jeulmun* (Korean) or

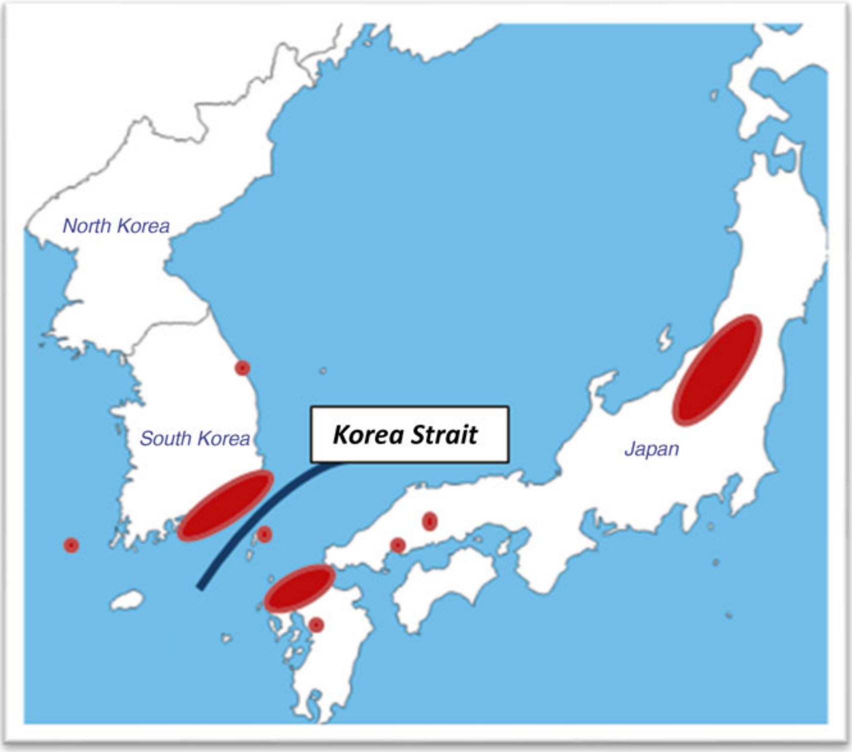
Jomon (Japanese) pottery had spread over the region of the Korean Peninsula and the Japanese archipelago. Archaeologists call this period the *Jeulmun* period in the Korean Peninsula and the *Jomon* period in the Japanese archipelago (from ca. 8000 BC). This period is comparable to the western Neolithic period, although people in Northeast Asia had not started agriculture yet at this point. Western archaeology distinguishes the Neolithic Age from the Paleolithic by the use of polished stone tools and the start of agriculture, since these two events appear to have taken place at the same period around 8000 BC in Europe. However, this chronicle is unsuitable to that of Northeast Asia, where there are indications of the use of ground stone tools some 30,000 years old, and primitive earthenware of 12,000 years old has been discovered, while the oldest evidence of agriculture is only ca. 5000 years old (Barnes 1993). Therefore, we suggest referring to this period from the birth of earthenware to the beginning of agriculture in Northeast Asia as the "Primitive Pottery Age" in order to distinguish it from the European Mesolithic culture (Lee 1999).

The Korean Peninsula functioned as a land bridge connecting the seasonal movements of Paleolithic hunters from the north of Manchuria to the south of the Japanese archipelago, with the Korea Strait as an obstacle obstructing their journey. Consequently, people gathered around the coastal region of the Korea Strait, making it the cultural center for the creation of the Primitive Pottery Age (Lee 1999). It is supported by the fact that the early Primitive Pottery Age remains are located in the Korean Peninsula (Dongsamdong, Dadaepo, Chilgok, Sangnodaedo, Yokjido, Sohuksando, Osanri) and the Japanese archipelago (Kosijima, Nishikaratsu, Iwasita, Senpukuji, Todoroki, Kamikuroiwa, Mawatari, Yagimata, Isigoya, Hasitate, Tazawa, Ozawa) (Han 1983). In particular, many remains have been found around Korea Strait coastal areas including the southeast portion of the Korean Peninsula and northwest of Kyushu Island (Map 1.2).

The use of earthenware itself may have been enough to create a revolutionary impact that dramatically changed the production and processing of food in the Korea Strait region between 6000 and 3000 BC, prior to the period of Neolithic agricultural settlements. The marine foods obtained from the coastal regions and riversides were difficult to dry, easily decomposed by autolysis, and rapidly spoiled by microbial growth, so people needed to consume them instantly, and therefore they were not likely to rely much on marine food. However, the invention of earthenware enabled them to cook perishable foods easily and store them longer for eating. This cultural trend of seafood consumption preceding farm products still remains today: Koreans and Japanese are the only people in the world who consume seaweed and laver as daily food and eat more fish and shellfish than meat.

Moreover, earthenware was used for cooking fish and vegetables in seawater (Lee 1999). Before man knew the salty taste from marine foods, people used to take minerals (salt) from either animal blood or intestine. People alive during the *Jeulmun* and *Jomon* periods possibly enjoyed cooking seafood with vegetables, roots, and grains with seawater in an earthen vessel as they came to understand that the salty taste enabled them to eat more vegetables and plants, which enabled them to survive on plants when game was scant. Using pottery and seawater to cook marine food resources and vegetables can be related to the origin of "hot pot culture," the so-called *chigae* culture in Korea and *nabe* culture in Japan, which are the most characteristic Northeast Asian foods today. This culture is similar to that of the primitive people living in the coastal region of Papua New Guinea today who still use seawater as a salty ingredient for cooking (Ishige 1976).

In about 3000–2000 BC, which is referred to as the Late Neolithic Age by Korean archaeologists, polished stone tools were replaced by chopped stone tools, and in using these new tools, farming agriculture began. This period is actually a Neolithic Age according to the European standard since it marks the start of agricultural farming. Tribes based around agriculture and fishery settlements emerged in Northeast Asia during this period. This Neolithic period was followed by the Bronze Age in the Korean Peninsula



Map. 1.2 Locations of the early Primitive Pottery Age remain in the coastal region of Korea Strait

around 1200 BC when the megalithic culture represented by dolmens and menhirs developed.

Earthenware played another significant role in the birth of a new dietary culture in Northeast Asia during the Bronze Age: that of using soybeans in this region. When the horse-riding people of the north, the *Yemaek* tribe of Northeastern Dongyi, came to the south of the Korean Peninsula and became agricultural farming settlers, they began to use wild soybeans as a stable protein source to replace meat from their animal herds. It has been proposed that the *Maek* tribes, who settled broadly in Manchuria and the Korean Peninsula, are the first users of soybeans for food in history (Lee 1984). Chinese history books and literature also indicate that soybean cultivation was concentrated in the region of Northeast Asia, in particular, Manchuria and the Korean Peninsula, since the Bronze Age (Choi 2009). As they began relying on soybeans, the people of Northeast Asia invented and developed the process of making soybeans into food by soaking them in water and cooking them in earthen vessels to eliminate the anti-nutritional factors in the bean. An earthen vessel excavated from Paldang in Korea, which has traces of soybeans on the surface, supports the theory that these peoples used pottery for cooking soybeans in Central Korea beginning in the Bronze Age.

Most of all, the invention of pottery brought with it the birth of fermentation technology since storing wet cereals, meats, fish, and vegetables in earthen pots naturally produces fermented foods. The invention of fermentation technology could provide relatively abundant nutrients to people compared to previous periods, so it must be related to the sudden increase in population during this period (Lee 2001). For example, cooked soybeans, saltwater, and pottery could have resulted in the birth of fermented soybean products which played an important role not only as a supplementary source of protein but also as palatable sauces enjoyed with cereals, vegetables, and meat. It seems that the *Maek* tribe of the Dongyi, who were originally meat-eating nomads, enjoyed or invented the soy sauce that has been used for *Maek-chŏk* or *Bulgoki*, roasted meat marinated with soybean sauce, which is one of the favorite foods of Korean people to this day.

The use of soybean product and fermentation technology had continued and developed during the Iron Age that began in Northeast Asia around 500 BC (Nahm 1988). During this period, several proto-states and early states such as Puyŏ, Dongye, Okchŏ, Koguryŏ, and Samhan were established in Manchuria and the Korean Peninsula by the Dongyi people. According to ancient Chinese historical records, such as the *History of the Later Han* (*Hou Hanshu*) and *Records of the Three Kingdoms* (*Sanguo Zhi*, 3rd c. AD), people in these states, the Dongyi, enjoyed drinking wine made of grain. Also, it is written that Chinese people acquired a new kind of soybeans (*rongshu*) from the region of the Dongyi and spread it into China. In *Qiminyaoshu*, the term of *Gaolidou*, literally meaning "soybeans of Koguryŏ," was used for indicating a good quality of soybeans (Choi 2009). This evidence indicates that the Dongyi largely cultivated high-quality soybeans as their staple food. With fermentation technology, they must have been able to acquire stable and nutritious food sources, which contributed to the creation of proto-states and fully developed state-level society during the Iron Age in Northeast Asia.

Fermented food products such as rice wine, soy sauce, soybean paste, and fish sauce seem to have become the most important food in the dietary culture in and around the Korean Peninsula by the seventh century AD. According to the earliest Korean history, *Samguk Saki* (1145 AD), rice, rice wine, oil, honey, soy sauce, soybean paste, dried meat, and fish sauce were the most important food items prepared for a wedding in the royal family in Silla in the year 683 AD. It seems that the introduction of Buddhism to the Northeast Asian region from the third to fourth centuries AD contributed to the development of fermented food, thus accelerating the decrease of meat consumption and encouraging the spread of vegetarian food habits. During the 1000-year period following the introduction of Buddhism, nomadic meat-eating culture gradually disappeared. In its place, the extensive use of salted vegetables and soybean products thrived as the major food source of the Northeast Asian diet. This process also coincided with the invention and development of *Onggi*, glazed earthenware which have been used as containers for fermented products for well over a 1000 years and continue to be used to this day.

People reaching all the way to the northeast end of the continent adapted themselves to their new geographical, ecological, and cultural environments by developing their own dietary cultures and technologies. The dietary culture of Northeast Asia and the birth of fermentation technology in this region in particular have been examined in the context of the use of earthenware beginning in the prehistoric period. The invention of pottery was a significant prerequisite for the long history of fermented food, which dominates the dietary culture of Northeast Asia today. Considering the early appearance of earthenware for food cooking and storing in Northeast Asia, cereal alcoholic beverages, vegetable pickles, and fermented fish and meats were possibly made prior to the beginning of agriculture in this region. The next section of this chapter will be dedicated to a detailed analysis of the history and development of the technology of cereal alcoholic fermentation, vegetable fermentation, fish and meat fermentation, and soybean fermentation in Northeast Asia.

1.3 Origin and Development of Fermentation Technology in Northeast Asia

1.3.1 Cereal Alcoholic Fermentation

Alcohol fermentation is considered one of the oldest food-processing technologies human beings have ever had. The production of an alcoholic drink from a cereal is a much more sophisticated affair than making fruit wine, but the process of invention of fermented beer in Northeast Asian culture is nevertheless a mystery. Since the oldest archaeological evidence of the rice-wine crock was found in the remains of the Shang period around 2000 BC in Central China, it is believed that Northeast Asian people have also possibly prepared an alcoholic beverage from grains using fermentation technology since prehistoric times (Lee 1984).

The process of making an alcoholic drink from a cereal requires two separate biochemical steps: saccharification and fermentation. Saccharification is to hydrolyze the starch in the cereal to fermentable sugars, and fermentation is the process of converting sugars to ethyl alcohol and carbon dioxide by yeast (Lee 2001). In areas with high temperature and high humidity, mold growth is a natural process in a container storing wet starchy materials, for example, plant seeds, millet, barnyard millet, nuts, beans, and tubers. Some molds like the *Rhizopus* species produce enzymes which can hydrolyze raw starch and convert it into sugars. When a sufficient amount of moisture is provided, the sugar is transformed into alcohol by the yeast existing in nature. The resulting alcoholic food or beverage with an attractive aroma is produced in several days in summer after adding a small amount of water to starchy material in a crock. This is a natural process which can be observed even among primitive people. It is likely that the history of cereal alcoholic fermentation began with uncooked starchy ingredients; thus, the use of pottery may mean the start of cereal alcoholic fermentation (Lee 2001).

According to the book of *The Generational Origins* (*Shi Ben* 世本), Yi Di first prepared alcoholic drinks at the command of the daughter of Emperor Yu, the legendary Yu the Great who established the Xia Dynasty of China (ca.2070–ca.1600 BC). It is likely that the people of this period invented a new method, probably using ferment, to make beer with grains as the same book proclaims that a man named Shao Kang later prepared an alcoholic beverage from millet (Huang 2001).

The earliest written record of alcohol drinking culture in Northeast Asia appears in one of the Chinese histories, *Records of the Three Kingdoms* (*Sanguo Zhi*). According to this book, Dongyi people enjoyed alcoholic beverages during their statewide festivals called *muchŏn* (in Dongye, ca. third century BC to third century AD), *yŏnggo* (in Puyŏ, ca. second century BC–494 AD), and *dongmaeng* (in Koguryŏ, 37 BC–668 AD) (Nahm 1988).

As people recognized the process of making alcoholic beverages by fermentation, people also invented two different methods for saccharification in ancient civilizations: using the amylases in sprouted grains, and making ferments, or useful microorganisms, grown dominantly on wet seeds and grains. In Northeast Asia, ferments of various kinds were invented for the process of the saccharification of grain alcoholic beverage, so as in Central and Southeast Asia (Table 1.1). For example, in Korea, when ferment, called *nuruk*, is mixed with cooked rice and water in about a 1:1:4 ratio, alcoholic fermentation takes place and is normally completed within 1 week in summer days. When it is strained with a sieve into turbid liquid, it is called rice beer, *makkolli* or *takju*, and when filtered with fine cloth into clear liquid, it becomes fine rice wine, *chŏngju* (Lee 2001, 2009).

1.3.2 Origin of *Kimchi* Fermentation

Kimchi is a unique fermented vegetable product with a long tradition in Korea. It remains today a main side dish served alongside cooked rice and other dishes. The kinds of *kimchi* add up to more than 50 depending on the use of raw materials and processing methods and also on the season and locality of preparation. As there still remains a local tradition of using seawater for making salted cabbage on the southern coast and islands of Korea today, it can be assumed that the origin of *kimchi* is related to the prehistoric dietary culture of storing vegetables using seawater and pottery.

Vegetable pickles and fermented vegetables are a widespread dietary tradition not only in Asia but also in Europe. These incorporate a heterofermentative bacterium, *Leuconostoc mesenteroides*, that produces both lactic acid and acetic acid from sugars in vegetables and grows actively until the pH goes down to 4.8. When *Leuconostoc*

Table 1.1 Names of fermentation starters in different countries and their major ingredients

Country	Name	Ingredients commonly used	Shape	Microorganism
China	*Qu*	Wheat, barley, millet, rice (whole grain, grits, or flour)	Granular or cake	*Rhizopus*, *Amylomyces*
Korea	*Nuruk*	Wheat, rice, barley (whole grain, grits, or flour)	Large cake	*Aspergillus*, *Rhizopus*
	Meju	Soybean (whole seed)	Large ball	*Aspergillus*, *Bacillus*
Japan	*Koji*	Wheat, rice	Granular	*Aspergillus*
Indonesia	*Ragi*	Rice (flour)	Small cake	*Amylomyces*
Malaysia	*Ragi*	Rice (flour)	Small cake	*Amylomyces*
Philippines	*Budbod*	Rice, glutinous rice (flour)	Small cake	*Muor*, *Rhizopus*
Thailand	*Loogpang*	Bran	Powder	*Amylomyces*
India	*Marchaa*	Rice	Flat cake	*Hansenular*, *Mucor*

Lee (2001, 2009)

mesenteroides stop growing at the lower pH, other homofermentative bacteria like *Lactobacillus plantarum*, which produce mainly lactic acid only, start to grow, and the vegetable becomes very sour like the sauerkraut of Germany (Lee 2001). Many of the lactic-acid-fermented vegetables are made in anaerobic conditions by packing vegetables in sealed containers like ensilage, making the resulting products very sour. The vegetable pickles described as *zhe* in Chinese classical literature appear to be this type of product (Nout et al. 2014).

On the other hand, the vegetable pickles traditionally made in Northeast Asia including the Korean Peninsula are made by salting and subsequent lactic-acid fermentation and are not so strong in a sour taste. In the process of *kimchi* fermentation, the lactic-acid fermentation of vegetables yields a sour taste by keeping cabbage or turnip slices immersed in 3 % brine for 3–4 days. In such conditions, *Leuconostoc mesenteroides* are the suitable candidates dominating the system at the initial stage of fermentation (Lee 1994). This condition resembles that of primitive men putting foraged vegetables into a jar holding seawater, and with almost no exception, the result would be lactic-acid fermentation. It indicates that the Korean-style pickle, *kimchi*, originates from the natural fermentation of withered vegetables stored in seawater made in the Primitive Pottery Age (Lee 2001).

The traditional salted-vegetable dish of Korea was transformed into today's form of *kimchi* with the introduction of red pepper. The route of the propagation of red pepper into the Korean Peninsula is still debated, but it is known that red pepper had already been utilized in Korea in the fifteenth century or earlier (Kwon 2011).

1.3.3 Origin of Fish Fermentation

If one mixes seafood, which easily putrefies, with lactic-acid-fermented vegetables and lowers the pH below 4.5, one can prevent the proliferation of harmful microorganisms, and therefore the seafood can be stored over a long period of time and later consumed (Lee 2001). In these conditions, because of the low salt concentration, fish decomposes rapidly by autolysis with its own intestinal enzymes, and a strong flavor or putrid stench is formed. The smell and taste created in this process would be unacceptable to modern men, but to the people of primitive eras, who relied on rough plant materials like acorns, plant roots, grass seeds, etc., it likely reminded them of the savory taste of stored animal meats and intestines. In fact, some fermented fish products made in different regions of the world have too strong a flavor to be consumed by other people outside that region. Therefore, under conditions where harmful microorganisms do not prevail, the putrefaction and fermentation are distinguished only according to the subjective judgment of consumers.

Seen from such a perspective, the mixture of low-salt cured seafood with lactic-acid-fermented vegetables would be an essential condiment for people at the transitory stage between a meat diet and a vegetarian diet and can be seen as an archetype of the lactic-acid-fermented fish products, like *sikhae* in Korea, which are widely consumed in East Asia today. High-salt-fermented fish products, like *jŏtkal* in Korea, must have been developed at later stages, when abundant salt production was possible. At an even later stage after having raised the salt concentration, people came to add *nuruk* in order to achieve the rapid decomposition of fish as well as to reduce the strong putrid stench by the action of the enzymes in *nuruk*. This is the origin of *jang* (醬 in Korean or *jiang* in Chinese) which has been used widely in Northeast Asia and China as the major preserved food and condiment (Lee 2001).

The first description of *jang* appears in *Zhou Li* (*Rites of Zhou*) of the second century BC in China. It notes that *jiang* has two types, *hai* (醢) and *xi* (醯), and records the methods of its preparation (Lee 2001). *Hai* is made from sun-dried meats of fowl, beast, and fish which are ground into powder; mixed with rice wine, salt, and ferments made from millet; packed into a jar; and sealed and aged for 100 days. *Xi* is made from the same materials as *hai*, but acidic plum juice is added to give a sour taste. It is apparent that *jiang* was originally made from meat and is a kind of meat sauce, unlike the fermented soybean products which are commonly called *jiang* or *jang* today (Lee 1984). It can be said that *jiang* is a high-class condiment developed by 1000 years of experience, which applies the same principle of fish fermentation which might have been developed by the people in the Korea Strait region during the Primitive Pottery Age (Lee 2001).

1.3.4 Origin of Soybean Fermentation

Since major Western encyclopedias, such as the *Encyclopedia Americana* and *Great Soviet Encyclopedia*, claim that the origin of soybeans is China, it is generally understood that soybeans were first cultivated and consumed by the Chinese in Mainland China. However, according to botanical and historical research, it was the region covering South Manchuria and the Korean Peninsula where soybeans originated with the most abundant wild varieties (Kwon et al. 2001). Archaeologists generally agree that the history of soybean cultivation is about 4000 years long, as the traces of soybean use are found in the Bronze Age (ca. 1500 BC) in remains in the Korean Peninsula (Lee 1984). Soybeans appear to have been first introduced to China in the Eastern Zhou Period of the seventh century BC and further transferred to Southern China, Southeast Asia, and Japan from the third century BC to the seventh century AD (Choi 2004). Soybeans were further introduced to the Eastern Himalayas in India by the Mongolian-origin races (Tamang 2015). Accordingly, people in the Korean Peninsula and South Manchuria, the so-called Dongyi, were the first to consume soybeans as a food using earthen vessels as cooking facilities. By using earthen vessels, they were able to boil seeds in water and could thus solve the problem of trypsin inhibitor in soybeans.

During the early stages of soybean utilization, the Northeastern Dongyi had probably first invented *shi* (豉), the ancient Chinese term for Korean *meju*, by keeping cooked soybeans packed in straw mats or cloth during hot summer months. The need for condiments to replace meaty flavors for the bland cereal diet would be the most probable motivation for the northern nomads settled in the Korean Peninsula to produce fermented soybean products (Lee 2001). According to Huang (2000), there are no references to *shi* in the pre-Qin (221–209 BC) literature, but there is no doubt that by late Western Han, *shi* had become a major commodity in China. Books written from the Eastern Han to the Tang periods (100–800 AD), for instance, *Bowuzhi* and *Xin Tangshu*, describe the letter *shi* (豉) as a dialect coming from the northern region and a special product of Bohai, a nation founded by the refugees from defeated Koguryŏ (37 BC–668 AD) (Lee 1984, 2001). Therefore, we can conclude that the origin of fermented soybean products is the Bronze Age in the Korean Peninsula and South Manchuria by Northeastern Dongyi. The written record of soybean sauce and paste utilization in Korean literature appears during much later years, such as in the records on a princess marriage in King Sinmun's reign (683 AD) of Silla. According to S.W. Lee (1990), fermented soybean products were first introduced to China during the first century BC and to Japan in the sixth century AD (Fig. 1.1). Fermented soybean products were developed in China, Korea, and Japan according to local ingredients and dietary traditions, much as the typical hot soybean paste of Korea, *kochujang*, had developed with the introduction of red pepper into the Korean Peninsula (Nout et al. 2014).

1.4 Fermented Soybean Products in Northeast Asia

1.4.1 Korean *Kanjang* and *Doenjang*

Meju is prepared from cooked soybeans. Soybeans are soaked in water overnight, cooked for 2–3 h, and mashed by pounding. It is then shaped like a brick or a ball and dried in the sun and kept in a stack covered during the night for several days. During this period, mold typically grows on the surface, especially *Aspergillus oryzae*, and the inside becomes laden with bacteria, typically *Bacillus subtilis*. Enzymes from the mold and bacteria hydrolyze the soybean proteins into amino acids and turn the carbohydrates into sugars and organic acids (Lee 2001). The amino acids and sugars interact with each other to create a browning reaction, resulting in the characteristic dark brown color and meaty flavor of *meju*. Well-fermented *meju* is immersed in brine in an earthen jar and ripened for several months. The brown

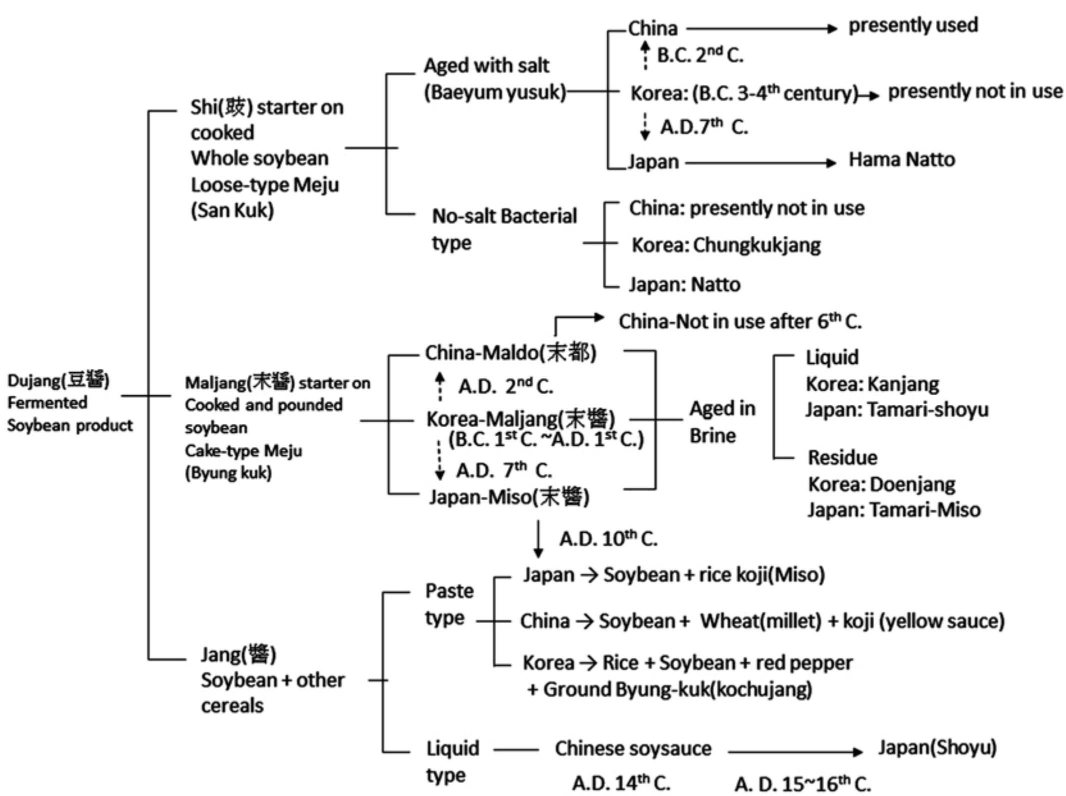


Fig. 1.1 The origin and interchange of Dujang (fermented soybean products) in East Asia (Lee 1990, 2009)

color and meaty flavor leach out into the brine. During this period, salt-tolerant yeasts grow in the mash, especially *Saccharomyces rouxii*, which produces the aroma of soybean sauce. The liquid part is soy sauce (*kanjang*) and the precipitates are soybean paste (*doenjang*). Soy sauce produced by this method is boiled once and can be stored in an earthen jar for years. The flavor of soy sauce gets richer as the storage time grows, just as the flavor of wine becomes smoother as it ages. It has been said in Korea that the taste of food in a household is dictated by the taste of their fermented soybean products.

1.4.2 Japanese *Shoyu* and *Miso*

Japanese people modified the *meju* preparation method in early 1900s by using a controlled fermentation technology incorporating a pure culture of mold isolated from the traditional starter (Shurtleff and Aoyaki 1976; Nout et al. 2014). The mold, normally *Aspergillus oryzae*, is grown on cooked rice or cooked wheat grits to make *koji*. It is mixed with cooked soybeans for further fermentation and then ripened in the brine. Soybean paste (*miso*) and soy sauce (*shoyu*) are made separately; for *shoyu*, *koji* is made with cooked defatted soybean flake and wheat grits and then mixed with brine for aging. After 4–6 months of aging, it is filtered to obtain *shoyu*, the liquid part, and the solid part is discarded. *Miso* is prepared by using *koji* made with cooked rice or other cereals which is mixed with cooked soybeans and salt and then mashed into a paste and ripened. These processes make industrialization of the products easy. The flavor of Japanese *shoyu* and *miso* is mild and sweet compared to their Korean counterparts. Korean people prefer the strong flavor of traditional soy sauce and soybean paste, just as European people distinguish Roquefort from processed Cheddar cheese.

1.4.3 Korean *Chŏngkukjang*

Soybeans are cooked and covered with a straw mat or cloth and then placed on the warm stone floor, *ondol*, for 3–4 days until the mucous string is formed. This is then mixed with chopped ginger, chopped garlic, and salt and pounded slightly until the bean kernels are separated into halves and is then stored in an earthen jar (Fig. 1.2). The strong smell of fermented soybeans is partially masked by the ginger and garlic smell, which also creates the characteristic *chŏngkukjang* flavor. The spicy seasoning of *chŏngkukjang* is thus prepared in 3–4 days, while ordinary soybean paste, *doenjang*, which uses *meju* as a fermentation starter, takes over 6 months for complete ripening. The mucous substance in *chŏngkukjang* is peptido-polysaccharides produced by *Bacillus subtilis* (Lee 2001; Nout et al. 2014).

Japanese *natto* is a modified form of *chŏngkukjang*. *Natto* is fermented soybeans grown with *Bacillus subtilis* on cooked soybeans. The fermented soybeans with mucous string are consumed directly without further processing, so it is a non-salt-fermented product. However, *natto* is not generally accepted by Korean people. It is always mixed with spices and used for the cooking of vegetable stew as a meaty-flavored condiment. The amount of *chŏngkukjang* added to the stew is large enough to supplement protein to the diet significantly. The fermented bean halves floating and mixing in the vegetable stew are seen as a healthy sign.

In the urbanized lifestyle of today—the so-called apartment culture—what Korean elders miss most is the stimulating savory smell of *chŏngkukjang* emanating from their kitchen. Since Korean young people are known for disliking the smell, neighbor-conscious apartment life does not allow for the preparation of *chŏngkukjang* stew at home. Consequently, *chŏngkukjang* stew is an important menu item for traditional Korean food restaurants, where elderly people prefer to go. *Chŏngkukjang* was also called *Jeonkukjang* in the old days. "Chŏngkuk" means the Chinese empire "Qing," while "Jeonkuk" means "Warring States." What all these names imply is that this product was made for extraordinary situations, for example, wartime or famine conditions, to meet the urgent need for nutritious savory food ingredients.

Fig. 1.2 Flowchart for *chŏngkukjang* making

Soybeans

↓

Soaking in water overnight

↓

Cooking for 2-3h

↓

Cover with rice straw and put on warm floor (ca. 40°c) for 2 days

↓

Mucous string formed

↓

Crushed by mixing salt

↓

Pack in an earthen jar

↓

Chŏngkukjang

1.4.4 Korean *Kochujang*

The basic tastes for European cultures are sweet, sour, bitter, and salty, and Japanese people add here *umami*, the meaty taste. Korean people add another one: hot or pungent taste. The most remarkable difference between Korean food to that of neighboring Japan and China is the strong pungent taste of red pepper in most Korean dishes. *Kochujang* is a unique hot bean paste seasoning popular in Korea. It is made from fermented soybean starter, *meju*, and malt made from barley (Fig. 1.3). As seen in Fig. 1.3, malt powder is mixed with cereal porridge made from rice, glutinous rice, or barley. Enzymes in the malt hydrolyze the starch into sugars and dilute the consistency of the mixture (Lee 2001; Nout et.al. 2014). *Meju* powder, red pepper powder, and salt are added to the partly saccharified porridge with thorough mixing to make a paste which is then put in an earthen jar. The top is covered by salt in order to prevent mold growth. The jar is then placed in a sunny place for further fermentation. The proteins in the soybeans and cereals degrade into amino acids, producing a meaty flavor. During the fermentation, a wonderful harmony of the meaty flavor from hydrolyzed proteins and the sweet taste of hydrolyzed starches with the pungent taste of red pepper and salty taste is achieved, and a new characteristic flavor stimulating the appetite of Koreans is formed (Shin et.al. 2012).

Kochujang is the queen of fermented soybean products in Korea. The shiny red color, rich and smooth consistency, and stimulating hot-sweet-meaty aroma are enough to trigger your central nerve to cry for a bowl of warm rice. One can empty a bowl of rice with *kochujang* alone as a side dish. It is a good source of protein, and it contains essential fatty acids and vitamins, espe-

Fig. 1.3 Flowchart of *kochujang* making

Cereal flour (rice, wheat or barley)

↓

Make into porridge by heating and then cool to 50°C

↓

Add malt powder and stirring in warm state

↓

Add *meju* powder, red pepper powder and salt to make paste

↓

Put the paste in earthen crock

↓

Spread salt on the top and seal with net and cover to keep from fly

↓

Put the crock in sunny place for 3-4months

↓

Kochujang

cially vitamin A. A bowl of cooked rice and a fresh cucumber with enough amount of *kochujang* for dressing is an excellent lunch menu in the summer for Korean people, just as a hotdog covered with tomato ketchup might be for European people.

1.5 Role of Fermented Food in Northeast Asia

Fermented food has long been at the center of the dietary culture of Northeast Asia. For example, the traditional Korean diet is composed of rice as a staple food, supplemented with soybeans, *kimchi* as a side dish, and fermented soybean products (*jang*) and fish products (*jŏtkal*) as the main condiments. Thus, fermented foods are the major condiments providing the taste and palatability to Korean meals. Historically, *kimchi* was an important vitamin and fiber source for Koreans during winter when green vegetables were not available. Korean rice wine was an essential item for ancestor-memorial services, wedding ceremonies, and religious and communal rituals as a medium for accessing the spiritual realm. Many of the founding myths of ancient nations in Northeast Asia note the abundance of affairs incorporating grain-wine drinking, with communal and religious rituals often ending with drinking and dancing overnight.

Since the taste of foods in a household was heavily dependent on the taste of soybean sauce and paste, *jang* fermentation was considered one of the most important annual events for families of Northeast Asian culture. People believed that the taste of *jang* was related to the family's well-being. Since the grain wine for ancestral rituals and religious ceremonies was brewed at home, each district and household had their own unique method of wine brewing, and numerous kinds of grain wine were produced. For example, a number of books describing rice-wine brewing were published and sold in the seventeenth to nineteenth centuries of the Chosun Kingdom in Korea. The advanced fermentation technology developed in and around the Korean Peninsula seems to have been influenced by the dietary culture of surrounding countries and people. For example, soybean fermentation tradition and rice-wine brewing technologies were transferred to China and Japan. This can be supported by the fact that the Matsuya shrine of Kyoto, Japan, keeps the tablet of Master Jin from Silla as the god of rice wine.

In East Asia, a crude starter culture, *nuruk*, has been used for cereal fermentation. It was made by solid fermentation mold grown on a cereal substrate. Although the names of fermentation starters used in East Asia are different in each country (see Table **1.1**), the microorganisms involved and the processing methods are similar. The microflora and substrate vary with the climate and geographic conditions. Figure 1.4 compares the processing methods of Korean *nuruk*, Japanese *koji*, Indonesian *ragi*, and Filipino *budbod* (Lee 2001) (Fig. 1.4).

Japanese *koji* is made with the pure culture of *Aspergillus oryzae* and the enzyme activity is relatively high. It produces the light and bland taste of rice wine, which is preferred by women and young people. On the other hand, Korean *nuruk* is made by natural mixed culture fermentation, and the resulting rice wine has a deep and complex flavor. In the case of soybean sauce, the pure-cultured Japanese *koji* produces a mild- and sweet-tasting sauce suitable for table dipping sauces, while naturally fermented Korean *meju* produces a strong and sharp flavor which is useful for soup and in cooking other dishes.

Whole wheat flour or grits	Polished rice
↓	↓
Add water to 30-40% moisture	Soak in water 17 hr. at 25°C.
↓	↓
Wrap in a cloth and press in a molder to make cakes (5 cm thick, 10-30 cm diameter)	Drain excess water
↓	↓
Incubate 10 days at 30-45°C	Steam for 70 min and cool to 35°C
↓	↓
Incubate 7 days at 35-40°C	Inoculate with *A. oryzae*
↓	↓
Dry 2 weeks at 30°C	Incubate at 27-28°C for 50 hrs
↓	↓
Age 1-2 months at room temp.	Dry
↓	↓
Nuruk **(Korea)**	***Koji*** **(Japan)**

Fig. 1.4 Flowcharts for the preparation of solid-fermented starters made in different countries in the Asia-Pacific region

Rice flour	Glutinous rice
↓	↓
Moisten with water or sugar cane juice to form a thick paste	Wet ground
↓	↓
Inoculate with *Ragi* powder from a former batch	Mix with water, *onwad* roots and ginger
↓	↓
Flatten into cakes or mold into hemispheres	Mold into flattened cakes or balls
↓	↓
Place on a bamboo tray and cover with muslin	Sprinkle with powdered *bubod*
↓	↓
Incubate at 25-30°C for 2-5 days	Incubate 3 days
↓	↓
Dry	Dry
↓	↓
Ragi **(Indonesia)**	***Bubod*** **(Philippines)**

Fig. 1.4 (continued)

1.6 Conclusion

The Korea Strait region is considered one of the original sites of fermentation technology in East Asia. Archaeological findings and geographical considerations on the movement of the ancient people in the Late Paleolithic and Early Neolithic era in this region support the contention that fermentation technology began as early as 6000 BC during the Primitive Pottery Age in the Korea Strait region, comprising the southeast coast of the Korean Peninsula and the northwest coast of Kyushu in the Japanese archipelago. By using earthen vessels/jars, which are able to hold wet organic materials for the first time in human history, microbial growth was able to occur as a spontaneous phenomenon. Alcoholic fermentation (rice wine), fish fermentation (*sikhae* and *joetkal*), vegetable fermentation (*kimchi*), and then soybean fermentation (*doenjang*) in later periods (ca. 1000 BC) have all been developed in this region. These fermented foods, which have a long storage life and abundant nutrients formed by the microbial conversion process, have contributed greatly to the food availability and nutritional status of the people in this region through history.

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Ethnic Fermented Foods and Beverages of India

2

Jyoti Prakash Tamang, Namrata Thapa, Tek Chand Bhalla, and Savitri

2.1 Introduction

India covers an area of 3,287,263 km^2, extending from the snow-covered Himalayan heights to the tropical rain forests of the south. The mainland of India extends between latitudes 8°4′ and 37°6′ north, longitudes 68° 7′ and 97° 25′ east, and measures about 3214 km from north to south between the extreme latitudes and about 2933 km from east to west between the extreme longitudes. The climate of India can broadly classify as a tropical monsoon. In spite of much of the northern part of India beyond the tropical zone, the entire country has a tropical climate marked by relative high temperatures and dry winters.

India has 29 states and 7 Union territories including 640 districts, 497 cities, 5767 tehsils, and over 6 lakh villages. The population of India is 1,210,854, 977 with 623,724,248 males and 586,469,174 females (Census 2011). India has more than 2000 ethnic groups, and in every major religion represented, ethnic group of India comprises of Indo-Aryan (72 %), Dravidian (25 %), and Mongoloid and others (3 %) (Census 2011). India is a home to various religions, Hinduism (80.5 %), Muslim (13.4 %t), Christians (2.3 %), Sikhism (1.9 %), Buddhism (0.8 %), Jainism (0.4 %), and others (0.7 %t). Diverse people in India speak more than 1652 languages and 216 dialects. The Indian constitution recognizes 23 official languages (Census 2011) (Map. 2.1).

2.2 Indian Food Culture

India has one of the oldest civilizations in the world with a kaleidoscopic variety and rich cultural heritage. In the sacred book of Hindu called *Bhagavad Gita*, foods may be classified into three different types based on the property, quality, and sanctity; these are sattvika, rajasika, and tamasika (Tamang and Samuel 2010). "Sattvika" food denotes food for prosperity, longevity, intelligence, strength, health, and happiness, which

J.P. Tamang (✉)
Department of Microbiology, School of Life Sciences, Sikkim University, 6th Mile, Gangtok, Tadong 737102, Sikkim, India
e-mail: jyoti_tamang@hotmail.com

N. Thapa
Department of Zoology, School of Life Sciences, Sikkim University, 6th Mile, Gangtok, Tadong 737102, Sikkim, India
e-mail: namumani@hotmail.com

T.C. Bhalla • Savitri
Department of Biotechnology, Himachal Pradesh University, Summer Hill, Shimla 171005, Himachal Pradesh, India

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Map. 2.1 Map of India

includes fruits, vegetables, legumes, cereals, and sweets. "Rajasika" food signifies activity, passion, and restlessness, which include hot, sour, spicy, and salty foods. "Tamasika" food is intoxicating and unhealthy, which generally causes dullness and inertia. Hindu foods follow the concept of purity and pollution, which determines interpersonal and intercaste relationships (Kilara and Iya 1992). Kitchens of the Brahmin Hindu produce two types of meals, *kaccha*, meaning uncooked and unripe, and pakka, meaning ripe and cooked. "Kaccha" foods are highly vulnerable to contamination, and, therefore, there are strict codes of cooking, serving, and eating this food at kitchen. "Pakka" food is fried, so it is not vulnerable (Misra 1986). Hindus are traditionally vegetarians, but many non-Brahmins are nonvegetarians. Since the cow is considered to be sacred, Hindus do not eat beef. Brahmin Hindus do not eat garlic, onion, and intoxicants. Foods are offered to shrines/temples for worshipping gods, spirit possession, and feeding domestic and some wild animals including birds on religious occasions. Ethnic foods have social importance for celebrations particularly during festivals and social occasions.

The Indian food is spicy, and salt is added directly while cooking; seasonings such as soy sauce and monosodium glutamate (MSG) are never used. People have developed the different methods of eating foods in history of dietary culture. The dietary culture of India is a fusion of the Hindu-Aryan culture and the Tibetan-Mongolian culture influenced by the ancient Chinese cuisines with modifications based on ethnical preference and sensory likings over a period of time (Tamang 2010a). Countries boarding with other countries have closed cultural affinity, which has influenced the dietary cultures of both regions.

The use of fingers of hands to grasp food items for feeding remains a tradition of Hindu, Buddhist, and Muslim dietary cultures. Practice of washing hands and mouth before taking meals and after meals was common during Vedic period (1500–800 BC) (Prakash 1987), which is a part and parcel of dietary rules and etiquette of the Hindus. There is no mention of use of table cutlery or chopstick in the ancient dietary culture of the Hindus and Muslims.

2.3 History of Indian Fermented Foods

Dietary culture carries the cultural history of ethnic communities (Tamang and Samule 2010). Traditional fermentation, smoking, drying, and salting processes were developed by ancient people to preserve the foods for consumption and to improve their nutritional value, a remarkable step in the food cultural history of human being. Fermented foods and beverages are socially and culturally widely acceptable food items in local cuisines (Tamang 2010b, c). 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; Tamang 2010a; Tamang and Samule 2010). The native skills of food fermentation have been passed from mothers to daughters and fathers to sons through the traditional knowledge of the elders which include grandmothers/grandfathers, mothers/fathers and village elders, self-practice, family tradition, community knowledge, and neighbors.

Jalebi, fermented cereal-based pretzel-like product, has been known in northern Indian areas since 1450 AD and is probably of Arabic or Persian origin (Gode 1943). Records of Indian *dosa* and *idli* go back to 1100 AD (Gode 1943). Tamil poet Chavundaraya has historically described preparation of *idli* in 1025 AD (Iyengar 1950). In a book *Manasollasa* written in Sanskrit in 1130 AD, *idli* preparation from fine wheat flour and spices such as pepper powder, cumin, and asafetida has been described (Shrigondekar 1939). *Dosa*, a traditional fermented pancake food made from rice and black gram, was first noted in the Tamil Sangam literature in India about sixth century AD (Srinivasa 1930). *Dhokla*, a fermented mixture of wheat and Bengal gram of western India, was first mentioned in 1066 AD (Prajapati and Nair 2003). *Idli* and *dosa* are the cultural foods of Tamil, Telugu, Malayalam, and Kannada people of Dravidian origins in India. Besides being used as breakfast, south Indians eat these cultural foods in every religious and social occasion. *Dhokla* and *khaman* are socially attached to the food culture of every Gujarati in India elsewhere. *Siddu*, a fermented wheat food of Himachal Pradesh in India, is served hot with *ghee* (butter) or *chutney* (pickle) in rural areas as a special dish in the customary occasions (Tamang 2010a). *Chilra* or *lwar* is ethnic fermented buckwheat or barley, which is traditionally prepared during marriage ceremonies and festivals in Himachal Pradesh. *Marchu*, a traditional fermented wheat flour product in the form of flat bread, is eaten during local festivals (*phagli*, *halda*) and religious and marriage ceremonies in Lahaul in Himachal Pradesh. It is customary for a daughter to take *marchu* whenever she visits her maternal home from in-laws or vice versa in Himachal Pradesh (Thakur et al. 2004).

In the Himalayas, fermented vegetables such as *gundruk*, *sinki*, and *goyang* have cultural significance among the Nepali. There is an oral history on invention of *gundruk* and *sinki* which was documented in details by Tamang (2010a). The word *gundruk* might have originated from the Newar (one of the major ethnic groups of the Nepali) word "gunnu" meaning dried taro stalk (Tamang 2010a). Invention of pit fermentation method for acidification and preservation of perishable vegetable in the Himalayas is unique to the Himalayas (Tamang 2010a). *Kinema*, a fermented sticky soybean of Kirat Nepali, might have originated in east Nepal boarding Darjeeling hills and Sikkim in India around 600 BC to 100 AD during the Kirat dynasty (Tamang 2001). The word *kinema* was derived and originated from the word *kinamba* of the Limboo language of Kirat race; *ki* means fermented and *namba* means flavor (Tamang 2010a). Celebration of festivals with *selroti*, an ethnic fermented rice food, is a cultural aspect of the Nepali in Himalayas (Yonzan and Tamang 2009). *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 a customary to hand over a basket full of freshly fried *selroti* to bride's parents by the groom during marriage among the Nepali. Traditionally, 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), which contains freshly fried *selroti*, as gift.

In Hindu cow is regarded as a sacred animal, and its milk and milk products are used in every religious and cultural function. Development of dairy system in ancient India has been mentioned in some of the historical records. Mention of cow and importance of milk products was referred in *Rig Veda*, the oldest sacred book of the Hindus (Prajapati and Nair 2003). *Veda* and *Upanishad* mentioned the origin of *dahi* and fermented milk products during 6000–4000 BC, one of the oldest fermented milk products of the Hindus (Yegna Narayan Aiyar 1953). Preparation and consumption of *dahi* have been recorded since 2000 BC in India (Prakash 1961). It is well known in ancient Indian history that *dahi*, buttermilk, and *ghee* (butter) widely consumed milk products during Lord Krishna's time about 3000 BC (Prajapati and Nair 2003). *Dahi* plays an important part in the socioreligious habits in Indian subcontinent and is considered as a sacred item in many of their festivals and religious ceremony both by Hindu and Buddhists. Many Indian ethnic fermented milk products have cultural aspects in dietary system and have been consumed for more than 3000 years. *Lassi*, buttermilk, is a by-product obtained in the preparation of butter (*ghee*) from *dahi* by traditional methods and is the most common nonalcoholic refreshing beverage in the hot climate of India. *Misti dahi* (sweetened *dahi*, *mishti doi*, *lal dahi*, or *payodhi*) is a sweetened fermented milk product of Bengali in India. *Shrikhand* is an ethnic concentrated sweetened fermented milk product of Gujarati and Rajasthani of 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 north and western part of India. For both Hindu and Buddhists, *gheu* or butter is a sacred item in all their religious ceremonies and is used in the birth, marriage, death, as well as in other prayers as sacred offerings. *Ghee* (butter) is given to a newborn baby along with honey by the father to protect from any disease; this is a tradition among the Hindus. *Ghee* is also used for lighting the lamps for Gods and Goddesses in Hindu temples and Buddhist monasteries. *Somar* an ethnic fermented milk product from cow or yak is generally consumed to increase the appetite and to cure digestive problems by the Sherpa of highlanders in the Himalayas (Tamang 2005). Historical evidences prove that the present-day fermented milk products were originally developed by the west Asian nomads who use to rear and breed cattle for milk and milk products. The Aryan-Hindu pastoral system has influenced the preparation and consumption of milk and dairy products in the early settlement in the Indian Himalayas (Tamang 2012).

Unlike Chinese-type fermented fish, the Himalayan fish products are slightly different and mostly dominated by dried and smoking process. Fermentation of fish is restricted to *ngari* and *hentak* in Manipur and *tungtap* in Meghalaya in India, while the rest of the fish products are dried or smoked which include *sidra*, *sukuti*, and *gnuchi* of Nepal, Darjeeling hills, and Sikkim and *karati*, *lashim*, and *bordia* of Assam in India (Thapa et al. 2004, 2006, 2007). Fermented fish foods are deeply associated with food culture of the Meitei in Manipur, which are prepared and eaten in every festival and religious occasions. Consumption of fish products in the Himalayas, though it is an important diet, is comparatively less than other fermented products such as vegetable and dairy products. This may be due adoption of pastoral system of agriculture and the consumption of dairy products in these regions.

A sizable number of populace 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 family feast, and the remaining meat is smoked above the earthen oven to make *suka ko masu* for future consumption. Tibetans, Bhutia, Drukpa, and Lepcha people of the Himalayas slaughter yaks occasionally and consume the fresh meat, and the remaining flesh of the meat is smoked or preserved in open air called *satchu*. Making of *kargyong*, ethnic fermented sausages made from yak-minced meats using intestine as natural casing, might have started long time before the Tibetans embraced Buddhism probably 3000 years ago (Tamang 2010a). The ethnic people of the Western Himalayas in Uttarakhand, Himachal Pradesh, and Ladakh prepare *chartayshya*, fermented meat product especially during festival, and offer to ancestors before eating (Rai et al. 2009).

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 *Vedas*, *Soma* was credited with great medicinal power (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). There are brief descriptions of ethnic alcoholic drinks in historical documents during British rule in Indian hills of Darjeeling and the then Sikkim kingdom (Hooker 1854; Risley 1928; Gorer 1938). The mixed starter cultures of the Himalayas *marcha* or *phab* might have originated from south China during migration of Mongoloid races to the Himalayas (Tamang 2010a). Since the method of preparation of amylolytic starters for alcohol production such as of *nu-chu* of China and *marcha* of India is very identical, mycelia molds consisting of *Mucorales* are dominant in *marcha*, along with alcohol-producing yeasts (Tamang 2010c).

In Indian Himalayas, 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 she carries the indigenous knowledge to her in-laws after marriage. Traditionally women exclusively do the preparation of ethnic mixed starters, and *marcha* is prepared by the Limboo and Rai castes of the Nepali. Marital status is a strong determinant in the preparation of *marcha* by the Rai castes of the Nepali who allow only widows or spinsters to make *marcha* (Tamang et al. 1996). Drinking of alcohol is a part of the social provision to the majority of ethnic people of the Himalayas except for Brahmin Hindu and Muslims where alcohol is taboo (Tamang 2010a). *Jaanr* and *raksi* are essential to solemnize the marriage ceremony of non-Brahmin Hindu Nepali and the Buddhist tribes. Eloping is a common practice in the Himalayas. Traditionally relatives of the boy usually after 3 days pay a visit to the girl's parents with bottles of locally prepared ethnic distilled liquor *raksi* to respect the verdict of her parents and pay the penalty for elopement. Once the girl's parents grant the consent, freshly prepared *raksi* is served to signify the union of two families and the marriage is thus solemnized (Tamang 2005). Such practice of bridging between two families by a bottle of alcoholic drink is common only among the Himalayan people, mostly the non-Brahmin Nepali. Ethnic alcoholic beverages have strong ritual importance. In the Himalayas and Northeast India, people offer distilled alcoholic liquors to family of Gods and ancestors and also alcoholic liquors are used in spirit possession. Those who come to offer condolences gathered at a funeral or a memorial service for the deceased are served with alcoholic beverages. Distilled liquor is offered during the construction of a new house preferably in the first foundation pillar by the non-Brahmin Nepali and the Tibetans (Tamang 2010a).

2.4 Diversity of Ethnic Fermented Foods in India

Very few have realized that India is the center of the diverse food culture comprising fermented and non-fermented ethnic foods and alcoholic beverages. Ethnic fermented foods are defined as foods produced by the 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, which modify the substrates biochemically and organoleptically into edible products that are culturally and socially acceptable to the consumers (Tamang 2010b). Indian people eat plants, both domesticated and wild with varieties of recipes. It is interesting to note that unlike many countries, India singly has more than 1000 different types of familiar and less-familiar ethnic fermented foods and alcoholic beverages prepared and consumed by the diverse ethnic people of India, which include milk, vegetable, bamboo, soybean, lentils, meat, fish, cereal, and alcoholic beverages. Diverse microorganisms ranging from filamentous fungi to enzyme and alcohol-producing yeasts, lactic acid bacteria, bacilli, and micrococci are associated with fermentation and production of ethnic foods and alcoholic drinks (Tamang et al. 2015), and some of them have health benefits (Tamang 2015a). Ethnic foods are fermented naturally, except the alcoholic beverages, which are produced by using consortia of microorganisms in the form of dry, cereal-based starter (Tamang 2010c). Daily per capita consumption of ethnic fermented foods and alcoholic beverages in Sikkim was 163.8 g representing 12.6 % of total daily diet (Tamang et al. 2007b). However, no such data is available for other states of India. Some of the ethnic fermented foods and beverages prepared and consumed in various parts of India are listed in Tables 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, and 2.9. Ethnic fermented foods and beverages prepared

Table 2.1 Ethnic fermented cereal foods of India

Fermented product	Substrate	Nature and use	Area	References
Ambali	Millet flour and rice	Thick fermented batter; staple	South India	Ramakrishnan (1979)
Appam/*kallappam* or *vellayppam*	Rice	Like *idli*; staple	Kerala	Ramakrishnan (1979)
Babru	Wheat or rice flour	Pancake; breakfast, snack	Himachal Pradesh	Kanwar et al. (2007)
Bagpinni	Roasted barley flour "sattu" with *chhyang* (fermented beverage)	Ball-like, snack	Himachal Pradesh	Savitri and Bhalla (2007)
Bhatooru/*bhaturu*	Wheat or barley flour	Baked breads; staple	Himachal Pradesh	Savitri and Bhalla (2012)
Bedvin roti	Wheat flour, black gram, and walnut	Baked; staple	Himachal Pradesh	Thakur et al. (2004)
Chilra	Wheat and buckwheat flour	Like *dosa*; staple	Himachal Pradesh	Thakur et al. (2004)
Gulgule	Wheat flour	Cooked as flat pancake, snack	Himachal Pradesh	Thakur et al. (2004)
Hakua	Rice	Strong off-flavor; therapeutic uses	Darjeeling hills, Sikkim	Tamang (2010a)

(continued)

Table 2.1 (continued)

Fermented product	Substrate	Nature and use	Area	References
Jalebi	Wheat flour	Crispy sweet, deep-fried pretzels; snacks	All	Chitale (2000)
Koozh/koozhu	Sorghum, pearl millet, little millet, and foxtail millet flour	Slightly sour, cooked; breakfast	Tamil Nadu	Thirumangaimannan and Gurumurthy (2013)
Marchu	Wheat flour	Baked breads; staple	Himachal Pradesh	Thakur et al. (2004)
Rabadi	Wheat, barley, pearl millet, or maize	Mild acidic, thick slurry-like product; drink	North and West India	Gupta et al. (2002a, b)
Nan	Wheat flour	Leaved bread; baked; staple	North India	Batra (1986)
Pazhaya sadham	Rice	Cooked, breakfast	Tamil Nadu	Kumar et al. (2012)
Puda/pudla	Maize, Bengal gram	Solid food, pancake; snack food	Tamil Nadu	Kumar et al. (2012)
Selroti	Rice-wheat flour	Pretzel-like, deep fried; bread; staple	Darjeeling hills, Sikkim, Northeast India, Uttarakhand, Himachal Pradesh	Yonzon and Tamang (2009, 2010)
Seera	Wheat grains	Dried, sweet dish	Himachal Pradesh	Thakur et al. (2004), Savitri et al. (2012)
Siddu	Wheat flour, walnut/opium seeds/black gram	Steamed bread, oval shaped; staple	Himachal Pradesh	Thakur et al. (2004)
Tchog	Roasted barley flour, *chhang*	Solid ball-like dough; staple	Himachal Pradesh	Savitri and Bhalla (2007)

Table 2.2 Ethnic fermented cereal-legume mixture foods of India

Fermented product	Substrate	Nature and use	Area	References
Adai dosa	Rice, Bengal gram, red gram, black gram, green gram	Like *dosa*, breakfast	Tamil Nadu	Chavan and Kadam (1989)
Chakuli	Rice and black gram	Fermented batter, fried; breakfast	Orissa	Roy et al. (2007)
Chhuchipatra pitha	Rice and black gram	Pancake, snack	Orissa	Roy et al. (2007)
Chitou	Rice, black gram, sugar, and grated coconut	Fermented batter, baked, snack	Orissa	Roy et al. (2007)
Dosa	Rice and black gram	Thin, crisp pancake; shallow fried, staple	South India	Soni et al. (1985, 1986)
Enduri pitha	Rice and black gram	Fermented batter; steamed; snack	Orissa	Roy et al. (2007)
Handwa	Rice, red gram, and Bengal gram flour	Fermented, baked, snack	Gujarat	Sheth and Chakraborthy (2003)
Idli	Rice and black gram	Mild acidic, soft, moist, spongy; breakfast food	South India	Soni and Sandhu (1989b, 1991)
Munha pitha/podo pitha	Rice and black gram	Spongy-like *idli*; staple	Orissa	Roy et al. (2007)

Table 2.3 Ethnic fermented legume foods of India

Fermented product	Substrate	Nature and use	Area	References
Aagya	Soybean	Alkaline, sticky	Arunachal Pradesh	Sharma and Pegu (2011)
Amriti	Black gram	Rings, deep fried; snack	West Bengal	Steinkraus (1996)
Aakhone	Soybean	Alkaline, sticky, Paste; side dish	Nagaland	Singh et al. (2014)
Bari	Soybean	Alkaline, sticky, soup	Sikkim	Tamang (2010a)
Bekang	Soybean	Alkaline, sticky, paste, curry	Mizoram	Chettri and Tamang (2015)
Dhokla	Bengal gram and rice	Mild acidic, spongy; snack	Gujarat	Joshi et al. (1989)
Hakhu mata	Soybeans	Alkaline, sticky, paste, curry	Manipur	Singh et al. (2007b)
Hawaijar	Soybean	Alkaline, sticky, curry	Manipur	Jeyaram et al. (2008b, 2009)
Khaman	Bengal gram	Mild acidic, spongy; breakfast food	Gujarat	Joshi et al. (1989)
Kinema	Soybean	Alkaline, sticky; curry	Sikkim, Darjeeling hills, Assam	Sarkar et al. (1994) and Tamang (2003)
Masauyra	Black gram	Cone-shaped hollow, brittle, and friable, similar to North Indian *war*i or *dhal bodi* and South Indian *sandige*; side dish	Sikkim and Darjeeling	Chettri and Tamang (2008)
Papad	Black gram	Circular wafers; snack	All	Tamang (2010b)
Peron naming	Soybeans	Alkaline, paste, ball	Arunachal Pradesh	Singh et al. (2007b)
Peruyaan	Soybean	Alkaline, sticky, side dish	Arunachal Pradesh	Tamang et al. (2012)
Sepubari	Black gram	Fermented paste, side dish	Himachal Pradesh	Thakur et al. (2004)
Iibi chhurpi	Soybean	Alkaline, after fermentation, crushed, dried, side dish	Arunachal Pradesh	Singh et al. (2007b)
Tungrymbai	Soybean	Alkaline, sticky, curry, soup	Meghalaya	Chettri and Tamang (2015)
Vadai	Black gram	Paste, fried patties; snack	South India	Soni and Sandhu (1990b)
Wadi	Black gram	Ball-like, brittle; condiment	North India	Batra and Millner (1974)
Yanni perung	Soybean	Alkaline, paste, ball; side dish	Arunachal Pradesh	Singh et al. (2007b)

Table 2.4 Ethnic fermented milk foods of India

Fermented product	Substrate	Nature and use	Area	References
Chhu/sheden	Cow or yak milk	Soft, strong flavored; curry	Darjeeling hills, Sikkim	Dewan and Tamang (2006)
Chhur chirpen	Yak milk and crab apple	Pressed, light yellowish brown, side dish	Arunachal Pradesh	Singh et al. (2007a)
Chhur singba/chhur mingba	Yak milk	Pressed, light yellowish brown, side dish	Arunachal Pradesh	Singh et al. (2007a)
Chhura	Dzomo (crossbreed of cow and yak), yak milk	Hard mass, masticator	Ladakh	Raj and Sharma (2015)
Chhurpi (soft variety)	Cow or yak milk	Soft, cheese-like; curry, pickle	Sikkim, Darjeeling hills, Arunachal Pradesh	Singh et al. (2007a), Tamang et al. (2000), and Savitri and Bhalla (2007)
Chhurpi (hard variety)	Cow or yak milk	Hard mass, masticator	Sikkim, Darjeeling hills, Arunachal Pradesh, Ladakh, Himachal Pradesh, Uttarakhand	Katiyar et al. (1991) and Pal et al. (1996)
Chhurpupu	Yak/cow milk	4–5 years old *chhurpi*	Arunachal Pradesh	Singh et al. (2007a)
Churkham	Fresh and old *chhurpi*	Soft cheese packed in yak skin and sun dried, eaten as masticator, mouth freshener	Arunachal Pradesh	Tiwari and Mahanta (2007)
Dahi	Cow/buffalo/yak milk	Curd; savory	All	Sarkar (2008) and Patil et al. (2010)
Dudh chhurpi	Cow milk	Hard mass, masticator	Darjeeling hills, Sikkim	Tamang (2010a)
Ghee/gheu	Cow/buffalo milk	Butter	All	Tamang (2010a)
Khalari	Cow/buffalo/goat milk	Semisoft, cottage cheese, side dish	Kashmir	Dubey (2010)
Lassi	Cow/buffalo milk	Buttermilk; refreshing beverage	All	Padghan et al. (2015)
Maa/maar	Yak milk	Butter	Sikkim, Ladakh	Tamang (2001) and Raj and Sharma (2015)
Marchang	Yak ghee and barley flour "kongpu"	Side dish	Arunachal Pradesh	Singh et al. (2007a)
Mohi	Yak milk	Buttermilk; refreshment	Himalayan regions	Dewan and Tamang (2007)
Misti dahi	Cow/buffalo milk and sugar	Mild acidic, thick gel, sweet	West Bengal, Orissa	Ghosh and Rajorhua (1990)
Paneer	Whey of cow milk	Soft, cheese-like product; curry	All	Tamang (2010a)

(continued)

Table 2.4 (continued)

Fermented product	Substrate	Nature and use	Area	References
Pheuja or suja	Tea-yak butter	Fermented butter tea	Ladakh	Tamang (2010a)
Philu	Yak milk	Cream; fried curry with butter	Sikkim	Dewan and Tamang (2007)
Phrung	Yak milk	Hard mass, masticator	Arunachal Pradesh	Tamang (2010a)
Shrikhand	Cow, buffalo milk	Acidic, sweet, viscous	Western and Southern India	Ghosh and Rajorhua (1990)
Shyow	Yak milk	Curd; savory	Sikkim, Ladakh	Tamang (2010a)
Somar	Milk	Paste, flavored; condiment	Darjeeling hills, Sikkim	Dewan and Tamang (2007)
Tara	Dzomo (crossbreed of cow and yak), yak milk	Buttermilk; refreshment	Ladakh	Raj and Sharma (2015)

Table 2.5 Ethnic fermented vegetable and bamboo shoot products of India

Fermented product	Substrate	Nature and use	Area	References
Fermented vegetable products:				
Anishi	Leaves of *Colocasia* sp.	Fermented; sour; curry	Nagaland	Tamang and Tamang (2009b)
Antramthu	Mustard leaves	Dried/semiliquid; pickle with rice	Assam	Chakrabarty et al. (2014)
Goyang	Leafy vegetables	Freshly fermented; juice as condiment, soup	Sikkim, Darjeeling hills	Tamang and Tamang (2007)
Gundruk	Fresh leaves of local vegetables	Dried, sour-acidic; soup, pickle	Sikkim, Darjeeling hills, Northeast India, Uttarakhand, Himachal Pradesh	Tamang (2010a) and Deka (2012)
Hungrii	Leaves of *Brassica* sp.	Sour, acidic; by pit fermentation; side dish	Nagaland	Jamir and Deb (2014)
Inziangsang	Leafy vegetables	Dried, sour; soup, curry	Nagaland and Manipur	Tamang et al. (2005)
Inziang-dui	Mustard leaves	Liquid, sour; condiment	Nagaland	Tamang (2010a) Manipur
Khalpi	Cucumber	Sour; pickle	Sikkim	Tamang et al. (2005)
Sinki	Radish tap root	Dried, sour-acidic; soup, pickle	Sikkim	Tamang and Tamang (2009b)
Tsutocie	Cucumber	Thick sluggish green paste; condiment	Nagaland	Jamir and Deb (2014)
Fermented bamboo shoots products:				
Bastanga	Shoots of *Dendrocalamus hamiltonii*, *Bambusa tulda*	Sour-acidic; curry	Nagaland	Jamir and Deb (2014)
Ekung	Young bamboo tender shoots	Sour-acidic; curry, soup	Arunachal Pradesh	Tamang and Tamang (2009b)
Eup	Young bamboo tender shoots	Dry, acidic; curry, soup	Arunachal Pradesh	Tamang and Tamang (2009b)
Hirring	Topmost part of tender bamboo shoots	Sour-acidic; curry, soup	Arunachal Pradesh	Tamang and Tamang (2009b)

(continued)

Table 2.5 (continued)

Fermented product	Substrate	Nature and use	Area	References
Lung-siej	Young bamboo shoots	Sour-acidic; curry	Meghalaya	Mao and Odyuo (2007)
Mesu	Young bamboo tender shoots	Sour; pickle	Sikkim, Darjeeling	Tamang and Tamang (2009b)
Miya mikhri	Young bamboo shoots	Sour, acidic, solid; curry with vegetables	Assam	Chakrabarty et al. (2014)
Soibum	Succulent bamboo shoots	Sour-acidic; curry	Manipur	Tamang et al. (2008) and Romi et al. (2015)
Soidon	Bamboo shoot tips	Sour-acidic; curry	Manipur	Tamang et al. (2008)
Soijim	Bamboo shoot	Liquid, sour; condiment	Manipur	Tamang et al. (2005)
Thunkhiang	Bamboo shoot	Solid, sour; curry	Assam	Chakrabarty et al. (2014)
Tuaithur	Bamboo shoot	Solid, wet, sour; curry, pickle	Assam	Chakrabarty et al. (2014)
Tuaihroi	Bamboo shoot	Solid, dry, sour; curry	Assam	Chakrabarty et al. (2014)

Table 2.6 Ethnic preserved and fermented fish products of India

Products	Substrate	Nature and use	Area	References
Ayaiba	Fish	Smoked fish; pickle, curry	Manipur, Mizoram	Tamang et al. (2012)
Bordia	*Pseudeutropius atherinoides*	Dried, salted	Assam, Manipur, Mizoram, Arunachal Pradesh	Thapa et al. (2007)
Chucha	Small fish species (*Gudusia chapra*, *Amblypharyngodon mola*, *Aspidoparia morar*, *Chela laubuca*, *Puntius ticto*, etc.)	Sun-dried fish product	Assam	Sarmah et al. (2014)
Gnuchi	*Schizothorax* spp., *Labeo* sp.	Smoked	Darjeeling hills, Sikkim	Tamang et al. (2012)
Godak	*Puntius* spp.	Semi-fermented, non-salted product; 4–6 months fermentation; curry/pickle	Tripura	Dhar et al. (2012)
Hentak	*Esomus danricus*, petioles of *Alocasia macrorrhiza*	Fermented paste	Manipur	Thapa et al. (2004)
Hidal	Small fish	Semi-fermented	Assam	Muzaddadi (2015)
Hukoti	Small	Dried; side dish	Assam	Sharma et al. (2013)
Japangangnagtsu	Crab (*Scylla* sp.) with sesame	Fermented; side dish	Nagaland	Jamir and Deb (2014)
Karati	*Gudusia chapra*	Dried, salted	Assam, Meghalaya	Thapa et al. (2007)
Lashim	*Cirrhinus reba*	Dried, salted	Assam, Meghalaya	Thapa et al. (2007)
Lona Illis	Indian shad (*Tenualosa ilisha*)	Salted, fermented	Tripura	Mazumdar and Basu (2010)
Mio	Fish	Dried; curry	Arunachal Pradesh	Tamang et al. (2012)

(continued)

Table 2.6 (continued)

Products	Substrate	Nature and use	Area	References
Naakangba	Fish	Sun dried; pickle, curry	Manipur, Nagaland	Tamang et al. (2012)
Naduba siyan	Fish	Sun-dried fish product	Assam	Sarmah et al. (2014)
Nah-grain	Small fish	Dry, solid; pickle, curry	Assam	Chakrabarty et al. (2014)
Namsing	*Puntius* spp., *Amblypharyngodon mola*, *Channa* spp., along with some plant materials like *Colocasia* (*Alocasia macrorrhiza*) or Shizu leaves (*Euphorbia nerifolia*)	Sun-dried fish products; curry	Assam	Kakati and Goswami (2013a, b)
Ngari	*Puntius sophore*	Fermented	Manipur	Thapa et al. (2004) and Devi et al. (2015)
Sepaa	Small fish	Dried	Tripura, Nagaland, Arunachal Pradesh	Muzaddadi (2015)
Shidal	*Puntis*	Semi-fermented, non-salted product; 4–6 months fermentation; curry/pickle	Tripura, Assam	Muzaddadi and Basu (2003) and Muzaddadi (2015)
Sidra	*Puntius sarana*	Dried; curry	Eastern Nepal, Darjeeling hills, Sikkim, Bhutan	Thapa et al. (2006)
Sukako maacha	*Schizothorax* spp.	Dried/smoked; curry	Eastern Nepal, Darjeeling hills, Sikkim, Bhutan	Thapa et al. (2006)
Sukuti	*Harpadon nehereus*	Dried, salted; curry	Eastern Nepal, the Darjeeling hills, Sikkim, Bhutan	Thapa et al. (2006)
Tungtap	*Danio* sp.	Fermented; side dish/pickle	Meghalaya	Thapa et al. (2004), Rapsang et al. (2011) and Rapsang and Joshi (2012)

Table 2.7 Ethnic preserved and fermented meat products of India

Products	Substrate	Nature and use	Area	References
Bagjinam	Pork	Fermented pork; curry	Nagaland	Tamang (2010a)
Honoheingrain	Pig/boar meat	Hard, dried meat; curry	Assam	Chakrabarty et al. (2014)
Arjia	Large intestine of chevon	Sausage; curry	Uttarakhand, Himachal Pradesh	Tamang (2010a) and Oki et al. (2011)
Chartayshya	Chevon	Dried, smoked meat; curry	Uttarakhand, Himachal Pradesh	Tamang (2010a) abd Oki et al. (2011)
Faak kargyong	Pork	Sausage, soft or hard, brownish; curry	Darjeeling hills, Sikkim	Rai et al. (2009, 2010a)
Jamma	Intestine of chevon, finger millet	Sausage, soft; curry	Uttarakhand, Himachal Pradesh	Oki et al. (2011)
Jang kap	Buffalo skin	Fermented; cooked; side dish	Nagaland	Jamir and Deb (2014)
Lang kargyong	Beef	Sausage, soft; curry	Darjeeling hills, Sikkim, Ladakh	Rai et al. (2009, 2010a)
Lang satchu	Beef	Dried, smoked meat; curry	Darjeeling hills, Sikkim	Rai et al. (2009)
Lang chilu	Beef fat	Hard, used as an edible oil	Darjeeling hills, Sikkim	Rai et al. (2009)
Luk chilu	Sheep fat	Hard, solid, used as edible oil	Darjeeling hills, Sikkim	Rai et al. (2009)
Lang kheuri	Beef	Chopped intestine of beef; curry	Darjeeling hills, Sikkim	Rai et al. (2009)
Suka ko masu	Buffalo meat	Dried, smoked; curry	Darjeeling hills, Sikkim	Rai et al. (2010a)
Yak chilu	Yak fat	Hard, edible oil	Darjeeling hills, Sikkim	Rai et al. (2009)
Yak kargyong	Yak	Sausage, soft; curry	Darjeeling hills, Sikkim, Arunachal Pradesh	Rai et al. (2010a)
Yak kheuri	Yak	Chopped intestine of yak; curry	Darjeeling hills, Sikkim	Rai et al. (2009)
Yak satchu	Yak meat	Dried, smoked meat; curry	Darjeeling hills, Sikkim	Rai et al. (2009)

Table 2.8 Ethnic dry amylolytic starters for alcohol production of India

Fermented product	Substrate	Nature and use	Area	References
Bakhar	Rice flour, ginger	Starter to ferment alcoholic beverage – *pachwai*	North India	Hutchinson and Ram Ayyar (1925)
Balam	Roasted wheat flour and spices	Starter to ferment alcoholic beverage – *jaan*	Uttarakhand	Roy et al. (2004)
Dhehli	Herbal mixture of 36 herbs and roasted barley flour	Starter to ferment alcoholic beverage – *sura*	Himachal Pradesh	Thakur et al. (2004) and Savitri and Bhalla (2007)
Emao	Rice, herbs	Starter to ferment alcoholic beverages	Assam	Tamang (2010a)
Hamei	Rice, wild herbs	Dry, mixed starter to ferment alcoholic beverages	Manipur	Jeyaram et al. (2008a, 2009)
Humao	Rice, barks of wild plants	Dry, flat, cake-like starter for *judima* production	Assam	Chakrabarty et al. (2014)
Ipoh/siye	Rice and powder of seeds and bark of locally available plants	Starter to ferment alcoholic beverages – *apong* and *ennog*	Arunachal Pradesh	Tiwari and Mahanta (2007)
Keem	Wheat; plants	Starter to ferment alcoholic beverages – *soor*	Himachal Pradesh	Rana et al. (2004)
Khekhrii	Germinated rice	Starter to ferment alcoholic beverages – *zutho/zhuchu*	Nagaland	Jamir and Rao (1990) and Jamir and Deb (2014)
Malera/treh	Wheat flour	Starter to ferment alcoholic beverages – *bhatooru/chilra*	Himachal Pradesh	Savitri and Bhalla (2007)
Mod pitha	Rice and 31 plant materials	Starter to ferment alcoholic beverages – *sujen*	Assam and Arunachal Pradesh	Deori et al. (2007)
Marcha	Rice, wild herbs, spices	Dry, mixed starter to ferment alcoholic beverages	Darjeeling hills, Sikkim, Northeast India	Tamang et al. (1988) and Tamang and Sarkar (1995)
Phab	Wheat, wild herbs	Dry, mixed starter to ferment alcoholic beverages	Sikkim, Ladakh, Arunachal Pradesh	Tamang (2010a)
Pham/phab	Rice and leaves of *Solanum khasianum*	Starter to ferment alcoholic beverages – *themsing*, *chhang*, *arrak*, *kinnauri*	Arunachal Pradesh, Jammu and Kashmir, Himachal Pradesh	Singh et al. (2007a, b) and Angmo and Bhalla (2014)
Ranu dabai	Rice, herbs	Starter to ferment alcoholic beverages – *jhara* or *harhia*	West Bengal	Ghosh and Das (2004)
Ranu goti	Rice, herbs	Starter to ferment alcoholic beverages – *handia* and *mahua*	Central India	Kumar and Rao (2007)
Thiat	Rice powder, powder of *Amomum aromaticum* Roxb. leaves	Starter to ferment alcoholic beverage – *kiad*	Meghalaya	Samati and Begum (2007)
Vekur pitha	Rice, leaves of some local plants	Starter to ferment alcoholic beverages – *ahom*	Assam	Saikia et al. 2007

Table 2.9 Ethnic fermented beverages and alcoholic drinks of India

Fermented product	Substrate/starter	Nature and use	Area	References
Ahom	Rice and *vekur pitha*	Aromatic, alcoholic and sweet; refreshing drink	Assam	Saikia et al. (2007)
Angoori/kinnauri	Grapes	Distillate; drinks	Himachal Pradesh	Thakur et al. (2004)
Apong/ennog	Rice	Mild alcoholic beverage	Assam, Arunachal Pradesh	Pegu et al. (2013)
Ark/ara	Barley, apple, wild apricot	Light brown drinks	Himachal Pradesh	Kanwar et al. (2007)
Aara	Cereals	Clear distilled liquor; alcoholic drink	Arunachal Pradesh	Tamang (2010a)
Atingba	Rice	Mild alcoholic, sweet-sour, food beverage	Manipur	Jeyaram et al. (2009)
Bhaati jaanr	Rice and *marcha*	Mild alcoholic, sweet-sour, food beverage	Darjeeling hills, Sikkim	Tamang and Thapa (2006)
Bhang-chyang	Maize rice/barley and *pham*	Extract of *mingri*; alcoholic beverages	Arunachal Pradesh	Singh et al. (2007b)
Buza	Barley	Thick liquor	Ladakh	Tamang (2010a)
Chakti	Jaggery	Filtrate; drinks	Himachal Pradesh	Thakur et al. (2004)
Chhang/chyang/chee	Finger millet/barley and *phab*	Mild alcoholic, slightly sweet-acidic; alcoholic beverage	Sikkim, Ladakh, Himachal Pradesh	Savitri and Bhalla (2007), Tamang (2010a), and Targais et al. (2012)
Chulli	Apricot	Alcoholic beverages; filtrate; alcoholic drink	Himachal Pradesh	Thakur et al. (2004)
Daru	Cereal	Alcoholic beverages; filtrate; jiggery	Himachal Pradesh	Sekar and Mariappan (2007)
Dekuijao	Rice	Alcoholic beverage, drunk directly	Nagaland	Chakrabarty et al. (2014)
Duizou	Red rice	Alcoholic drink	Nagaland	Jamir and Deb (2014)
Ennog	Rice, paddy husk	Black rice beer	Arunachal Pradesh	Tamang (2010a)
Faapar ko jaanr	Buck wheat and *marcha*	Mild alcoholic, slightly acidic; alcoholic beverage	Darjeeling hills, Sikkim	Tamang (2010a)
Gahoon ko jaanr	Wheat and *marcha*	Mild alcoholic, slightly acidic; alcoholic beverage	Darjeeling hills, Sikkim	Tamang (2010a)
Ghanti	Apple and apricot	Distillate; drinks	Himachal Pradesh	Roy et al. (2004)
Handia	Rice and *ranu*	Alcoholic beverage	Central India	Kumar and Rao (2007)
Haria	Rice and *bhakar/dabai*	Alcoholic beverage	Bihar, Jharkhand, West Bengal	Sha et al. (2012a, b, c) and Ghosh et al. (2014)
Jann/jaan	Rice, wheat, and other cereals and *balam*	Mild alcoholic, slightly acidic; alcoholic beverage	Uttarakhand	Das and Pandey (2007)

(continued)

Table 2.9 (continued)

Fermented product	Substrate/starter	Nature and use	Area	References
Jao ko jaanr	Barley and *marcha*	Mild alcoholic, slightly acidic; alcoholic beverage	Darjeeling hills, Sikkim	Tamang (2010a)
Jhara	Rice and *ranu dabai*	Sweetness, bitter taste, alcoholic beverage	West Bengal	Sekar and Mariappan (2007)
Jou	Rice	Alcoholic beverage	Nagaland	Tamang (2010a)
Judima	Rice	Alcoholic beverage, drunk directly	Assam	Chakrabarty et al. (2014)
Juharo	Rice	Distilled liquor, drunk directly/with water	Assam	Chakrabarty et al. (2014)
Juhning	Rice	Alcoholic beverage; drunk directly	Assam	Chakrabarty et al. (2014)
Kanji	Beet root and carrot and *torami*	Thick filtrate, drunk directly	South India	Sura et al. (2001) and Kingston et al. (2010)
Kiad	Rice and *thiat*	Distillate; drunk directly	Meghalaya	Samathi and Begum (2007)
Kodo ko jaanr	Finger millet and *marcha*	Mild alcoholic, slightly sweet-acidic; alcoholic beverage	Eastern Himalayas	Thapa and Tamang (2004)
Lugri	Barley	Alcoholic beverages	Himachal Pradesh, Ladakh	Thakur (2013)
Madhu	Rice and *kekhrii*	Distilled liquor	Nagaland	Dahiya and Prabhu (1977)
Mahua	Dried corollas of *Madhuca longifolia* and *ranu*	Distilled liquor	Central India	Kumar and Rao (2007)
Mingari/lohpani	Maize, rice, or barley and *pham*	Alcoholic beverages	Arunachal Pradesh	Shrivastava et al. (2012)
Makai ko jaanr	Maize and *marcha*	Mild alcoholic, sweet-sour, food beverage	Darjeeling hills, Sikkim	Tamang (2010a)
Nchiangne	Red rice	Distilled liquor	Nagaland	Tamang (2010a)
Nduijao	Rice	Alcoholic beverage, drunk directly	Nagaland	Chakrabarty et al. (2014)
Opo	Rice millet	Soft, alcoholic beverage	Arunachal Pradesh	Shrivastava et al. (2012)
Pachwai	Rice and bhakar	Alcoholic beverage	West Bengal and Northern parts of India	Thakur et al. (2004)
Pona	Rice	Mild alcoholic, sweet-sour, food beverage; paste	Arunachal Pradesh	Tamang (2010a)
Rak	Apple, wild almond, apricot, and jiggery and *phab*	Filtrate; drunk directly	Himachal Pradesh	Thakur et al. (2004)
Raksi	Cereals and *marcha*	Clear distilled liquor; alcoholic drink	Eastern Himalayas	Kozaki et al. (2000)

(continued)

Table 2.9 (continued)

Fermented product	Substrate/starter	Nature and use	Area	References
Ruhi	Rice	Distilled liquor	Nagaland	Dahiya and prabhu (1977)
Sez	Rice	Fermented cooked rice; mild alcoholic; snack	Uttaranchal	Roy et al. (2004) and Sekar and Mariappan (2007)
Soor	Fruits and *keem*	Distillate; drinks	Himalayan region	Rana et al. (2004)
Sujen	Rice and *mod pitha*	Sweet taste	West Bengal and Assam	Deori et al. (2007)
Sura	Finger millet	Food beverage; staple	Himachal Pradesh	Thakur et al. (2004)
Simal tarul ko jaanr	Cassava tuber and *marcha*	Mild alcoholic, sweet-sour; food beverage	Darjeeling hills, Sikkim	Tamang (2010a)
Themsing	Finger millet/barley	Alcoholic beverages	Arunachal Pradesh	Singh et al. (2007)
Toddy or *tari*	Palm juice	Thick alcoholic drinks	South India	Batra and Millner (1974) and Shamala and Sreekantiah (1988)
Yu	Rice	Distilled liquor	Manipur	Singh and Singh (2006)
Zu	Rice	Alcoholic beverages	Assam	Chakrabarty et al. (2014)
Zutho/*zhuchu*	Rice	Milky white, alcoholic beverage	Nagaland	Teramoto et al. (2002)

and consumed in India are categorized as follows: (i) ethnic fermented cereal foods, (ii) ethnic fermented cereal-legume mixture foods, (iii) ethnic fermented legume/soybean foods, (iv) ethnic fermented milk foods, (v) ethnic fermented vegetable foods, (vi) ethnic fermented and traditionally preserved fish products, (vii) ethnic fermented meat products, (viii) ethnic amylolytic starters, and (ix) ethnic alcoholic beverages and drinks. Some of the ethnic Indian fermented foods and beverages have been studied by several researchers for the last 30 years which have been documented in this chapter.

2.5 Indian Fermented Cereal Foods

2.5.1 Ambali

Ambali, prepared by fermentation of millet flour and rice, is an easily digestible food of South India. Millet flour is combined with water to make a thick batter and the batter is fermented for 14–16 h. This fermented batter is then added to partially cooked rice with continuous stirring and is further cooked. After cooling to ambient, buttermilk is added and served. Microbiological studies of *ambali* revealed the presence of *Leuconostoc mesenteroides*, *Lactobacillus fermentum*, and *Streptococcus faecalis* during fermentation (Ramakrishnan 1979).

2.5.2 Bhatooru

Bhatooru (*sumkeshi roti*, *tungi roti*) is an ethnic leavened bread or *roti* and constitutes the staple diet of rural population of Himachal. It is traditionally prepared by using wheat flour or sometimes barley flour. Inoculum, *malera* (which mainly consists of lactic acid bacteria and yeasts), along with water is added and kneaded as dough and left for 3–5 h for fermentation. The fermented dough is then made into *roti*, spread on cotton/woolen sheet (*pattu*, etc.) and covered to allow further fermentation, and then baked (Savitri and Bhalla 2007). The microflora of the fermented dough was mainly dominated by yeast (*Saccharomyces cerevi-*

siae), lactic acid bacteria (*Lactobacillus plantarum*), and *Bacillus* sp. The gas-producing *Leuconostoc* sp. also appeared at 4 h of fermentation causing leavening of dough (Savitri and Bhalla 2012).

2.5.3 Jalebi

Jalebi is one of the most popular sweet dishes of India, which is a crispy sweet, deep-fried pretzel made from wheat flour and eaten as snack food (Chitale 2000). *Jalebi* is prepared by mixing wheat flour with *dahi* (curd), adding water in it and leaving overnight at room temperature. The thick leavened batter is squeezed through an embroidered hole (about 4 mm in diameter) in thick and durable cotton cloth and deposited as continuous spirals into hot edible oil, frying both sides until gold and crisp. After about a minute, these are removed from fat and with a sieved spatula are submerged for several seconds in hot sugar saffron-scented syrup, which saturates their hollow insides. Skill is required to master the uniform shapes of *jalebi*. Often rose or *kewda* (*Pandanus tectorius*) water and orange food color are added to the syrup (Ramakrishnan 1979; Batra 1981). The rheological parameters of *jalebi* batter can be controlled by adjusting the moisture content of the batter system (Chakkaravarthi et al. 2009). It is eaten as snack when hot. *Lb. fermentum*, *Lb. buchneri*, *Lb. bulgaricus*, *Streptococcus lactis*, *E. faecalis*, *S. thermophilus*, and yeasts *Saccharomyces bayanus*, *S. cerevisiae*, and *Hansenula anomala* were present in jalebi (Batra and Millner 1974, 1976; Soni and Sandhu 1990b).

2.5.4 Nan

Nan is an ethnic leavened bread of India, made from wheat flour. Wheat flour is thoroughly mixed with butter, baking powder, *dahi* (curd), milk, salt, sugar, and water to make thick dough. The dough is fermented for 3–5 h at room temperature. Fermented dough is sheeted between the palms of the hand to about 2–3 mm thickness, wetted a little and pasted on inner wall of the *tandoori* oven. The baked product called *nan* has typical soft texture and flavor. *Nan* is baked in a specially designed oven known as *tandoori* with a temperature range of 300–350 °C. It is also baked on live coal or flame for a short time. *Nan* is eaten as a staple food with vegetable or *dhal* (legume soup) and meats. *Saccharomyces kluyveri* is the dominant yeast in *nan* (Batra 1986).

2.5.5 Rabadi

Rabadi is an ethnic fermented cereal-based food of India. It is prepared by mixing flour of wheat, barley, pearl millet or maize with buttermilk in an earthen or metallic vessel and then allowing the mixture to ferment in the open sun for 5–6 h in the hot summer, and after fermentation, it is boiled, salted to taste, and consumed (Gupta et al. 1992a). *Ped. acidilactici*, *Bacillus* sp., and *Micrococcus* sp. were reported from *rabadi* (Ramakrishnan 1979). Phytic acid content decreased during *rabadi* fermentation (Mahajan and Chauhan 1987; Gupta et al. 1992b). Single and mixed culture fermentation of pearl millet with yeast (*S. cerevisiae* or *S. diastaticus*) and LAB (*Lb. brevis* or *Lb. fermentum*) was developed to prepare *rabadi* using pearl millet (Khetarpaul and Chauhan 1990a, b). Pearl millet-based *rabadi* prepared using developed starters has been found to increase bioavailability of minerals (Khetarpaul and Chauhan 1989), improve starch and protein digestibility (Khetarpaul and Chauhan 1990a), increase total soluble sugar with decrease in starch (Khetarpaul and Chauhan 1990b), and degrade antinutrients (Khetarpaul and Chauhan 1991).

2.5.6 Selroti

Selroti is a unique fermented cereal food of the Nepali people residing in the Himalayan regions of India. It is a popular fermented ring-shaped, spongy, pretzel-like, and deep-fried food consumed during religious festivals and special occasions (Yonzan and Tamang 2009). During preparation, rice is soaked in cold water for 6–8 h. Soaked rice is pounded into rice flour and mixed thoroughly with about 25 % refined wheat flour, 25 % sugar, 10 % butter or fresh cream, and 2.5 % spices/condiments containing large cardamom, cloves, coconut, fennel, nutmeg, and cinnamon. Milk is added, kneaded into soft dough, and finally made into batter with easy flow. Batter is left to ferment naturally at ambient temperature (20–28 °C) for 2–4 h during the summer and at 10–18 °C for 6–8 h during winter. The fermented batter is squeezed as continuous ring onto hot edible oil, fried until golden brown, and drained out from hot oil by a poker or spatula. *Selroti* is served as confectionary bread. Lactic acid bacteria mainly *Leuconostoc mesenteroides*, *Enterococcus faecium*, *Pediococcus pentosaceus*, and *Lactobacillus curvatus* and yeasts belonging to *Saccharomyces cerevisiae*, *Saccharomyces kluyveri*, *Debaryomyces hansenii*, *Pichia burtonii*, and *Zygosaccharomyces rouxii* were identified from *selroti* (Yonzan and Tamang 2010). The most prevalent LAB and yeasts in *selroti* batters were *Leuc. mesenteroides* (42.9 %) and *S. cerevisiae* (35.6 %). Molds and pathogenic bacteria were not detected (Yonzan and Tamang 2010). *Selroti* has the following nutritive value: moisture (42.5 %), pH (5.8), acidity (0.08 %), ash (0.8 % DM), protein (5.7 % DM), fat (2.7 % DM), carbohydrate (91.3 % DM), food value (410.3 kcal/100 g DM), Na (8.9 mg/100 g), P (29.7 mg/100 g), and Ca (23.8 mg/100 g) (Yonzan and Tamang 2010).

Selroti batters produced using a mixture of pure culture strains of *Leuconostoc mesenteroides* BS1:B1 and *Saccharomyces cerevisiae* BA1:Y2 at 28 °C for 4 h had organoleptically scored the highest acceptability (Yonzan and Tamang 2013). This was also correlated by decrease and increase in pH and acidity of the fermenting batters, respectively, from 0 to 4 h. The consumers' preference trial showed that *selroti* batter prepared by a mixture of *Leuc. mesenteroides* BS1:B1 and *S. cerevisiae* BA1:Y2 was more acceptable than *selroti* batters prepared by conventional method (Yonzan and Tamang 2013). *Selroti* prepared by using a consortium of starter cultures had advantages over the traditional method.

2.5.7 Seera

It is a nutritious, easily digestible snack food made in Bilaspur, Kangra, Hamirpur, Mandi, and Kullu districts of Himachal Pradesh. In the preparation of *seera*, wheat grains are soaked in water for 2–3 days to allow natural fermentation. After fermentation, grains are ground and steeping is done to allow the starch grains and some proteins to settle down, and then bran is removed. The settled solids are then sun dried and the dried material is called *seera*. This dried material is made into slurry by soaking in water, which is then poured into hot *ghee*, and sugar is added, cooked, and served as sweet dish/snack. It holds special significance to the village people in Bilaspur where during drought *seera* it is offered to "*Jal Devta*" (god of water) for rain. People suffering from jaundice/hepatitis are also given *seera*. It is also used during fast. The microflora isolated from *seera* comprised mainly yeasts (*Saccharomyces cerevisiae*, *Cryptococcus laurentii*, and *Torulaspora delbrueckii*) and bacteria (*Lactobacillus amylovorus*, *Cellulomonas* sp., *Staphylococcus sciuri*, *Weissella cibaria*, *Bacillus* sp., *Leuconostoc* sp., and *Enterobacter sakazakii*) (Savitri et al. 2012).

2.6 Indian Fermented Cereal-Legume Mixture Foods

2.6.1 Adai Dosa

This is the traditional delicacy prepared from rice and a variety of legumes (Bengal gram, red gram, black gram, green gram) in South India especially in Tamil Nadu. Legume seeds and rice are soaked in water for 2–3 h and then ground into a coarse batter and left to ferment for 2–3 h during summer and overnight during winters after the addition of some salt. After the completion of fermentation, the coriander leaves, chopped onions, curry leaves, asafoetida, and grated coconut are added to the batter. A small amount of batter is spread onto an oily plate and toasted in low flame and served hot with coconut chutney and jaggery. *Pediococcus* sp., *Streptococcus* sp., and *Leuconostoc* sp. have been found to be associated with *adai dosa* fermentation (Chavan and Kadam 1989).

2.6.2 Dosa

Dosa is also an ethnic fermented rice-black gram food of South India. It is a light, shallow-fried, thin crisp pancake, made from finely grounded rice and dehulled black gram (Steinkraus 1983). *Dosa* batter is very similar to *idli* batter, except that both the rice and black gram are finely ground. Bacteria alone or in combination with yeasts were found to be responsible for the fermentation of *dosa*, and ordinarily the microorganisms developed during the initial soak, and fermentation is sufficient to bring about the fermentation (Soni et al. 1986). Overall increase in batter volume, microbial load, total nitrogen, soluble proteins, reducing sugar, and decrease of pH has been noted after 30 h fermentation of *dosa* (Soni et al. 1985). The combination of *Saccharomyces cerevisiae* and natural bacterial flora was found to be the best microbial factor for standardizing the dosa fermentation (Soni and Sandhu 1989a). The millet-based *Dosa* contained high proportions of protein (15–18 %), fat (8.5–9.8 %) and carbohydrate (69–72 %) compared to the rice-based *dosa* (Krishnamoorthy et al. 2013).

2.6.3 Idli

Idli is an ethnic fermented rice-black gram food of South India. It is an acid-leavened and steamed cake made by natural fermentation of a thick batter made from coarsely ground rice and dehulled black gram. *Idli* cakes are soft, moist and spongy, and sour in flavor and are eaten as breakfast in South India. The predominant bacteria responsible for souring as well as production of gas are *Leuconostoc mesenteroides* in *idli* fermentation (Mukherjee et al. 1965). Yeasts have also been reported in *idli* mostly *Saccharomyces cerevisiae*, *Debaryomyces hansenii*, *Hansenula anomala*, *Torulopsis candida*, and *Trichosporon beigelii* from Soni and Sandhu (1989b, 1991) and Thyagaraja et al. (1992). Yeasts in *idli* fermentation contribute to leavening and flavor development and result in enhanced contents of thiamine and riboflavin (Soni and Sandhu 1989b). *Leuc. mesenteroides*, *Lb. delbrueckii*, *Lb. fermenti*, *Lb. coryniformis*, *Ped. acidilactici*, *Ped. cerevisiae*, *Streptococcus* sp., *Ent. faecalis*, *Lact. lactis*, *B. amyloliquefaciens*, *Cand. cacaoi*, *Cand. fragicola*, *Cand. glabrata*, *Cand. kefyr*, *Cand. pseudotropicalis*, *Cand. sake*, *Cand. tropicalis*, *Deb. hansenii*, *Deb. tamarii*, *Issatchenkia terricola*, *Rhiz. graminis*, *Sacch. cerevisiae*, *Tor. candida*, and *Tor. holmii* are present in idli (Steinkraus et al. 1967; Venkatasubbaiah et al. 1984; Sridevi et al. 2010). *Idli* makes an important contribution to the diet as a source of protein, calories, and vitamin B complex, compared to the raw unfermented ingredients (Steinkraus et al. 1967; Reddy et al. 1981). Exopolysaccharide from *Leuconostoc lactis* KC117496 isolated from *idli* batter has been isolated and characterized (Kalki and Shetty 2015). The millet-based *Idli* contained high proportions of protein (15–18 %), fat (5.0–6.2 %) and carbohydrate (72–74 %) compared to the rice-based *idli* (Krishnamoorthy et al. 2013).

2.7 Indian Fermented Soybean Foods

2.7.1 Aakhone

Aakhone or also called *axone* is an ethnic fermented sticky soybean food of Sema Naga in Nagaland, similar to *kinema* (Tamang 2015b). Aakhone contains *Bacillus subtilis* and *Proteus mirabilis* (Singh et al. 2014). During preparation, soybean seeds are soaked and cooked, and beans are wrapped in fresh leaves of banana or *Phrynium pubinerve* Blume (Family, Marantaceae) or *Macaranga indica* Wight (Family, Euphorbiaceae) and kept above the fireplace to ferment for 5–7 days (Mao and Odyuo 2007). The maximum shelf life of freshly fermented *aakhone* is 1 week. Fresh *aakhone* is molded and made into cakes and dried above the earthen oven. Sometimes, each fermented bean is separated by hand and dried in the sun for 2–3 days. Dried *aakhone* is stored in containers for future consumption. Pickle is made from freshly fermented *aakhone* mixed with green chili, tomato, and salt. The dried *aakhone* cakes are cooked with pork and are eaten as side dish with steamed rice.

2.7.2 Bekang

Bekang is an ethnic fermented soybean food commonly consumed by Mizo in Mizoram (Tamang 2015b). It is also similar to *kinema*. During traditional method of preparation of *bekang*, small-sized dry seeds of soybean are collected, cleaned, and soaked in water for 10–12 h. Excess water is dewatered and beans are boiled for 2–3 h in an open cooker until the beans become soft. Excess water is drained off and wrapped in fresh leaves of *Callicarpa arborea* (Family, Verbenaceae) locally called "nuhlhan" or in leaves of *Phrynium* sp. (Family, Marantaceae), locally known as "hnahthial." The wrapped beans are kept inside the small bamboo basket. The basket is then placed near the earthen oven or warm place and is allowed to ferment naturally for 3–4 days. Sticky soybean with emission of ammonia odor is produced which is liked by the local consumers. The product is called *bekang* in Mizoram. *Bekang* is consumed as it is or made into curry with addition of salt, green chilies, and tomatoes. It is consumed as side dish with steamed rice. *Bekang* is sold in the local markets by Mizo women, who earn their livelihood (Tamang et al. 2009a). On the basis of a combination of phenotypic and molecular characterization using ARDRA, ITS-PCR, and RAPD-PCR techniques, species of *Bacillus* isolated from *bekang* were identified as *Bacillus brevis* (2 %), *B. circulans* (7.5 %), *B. coagulans* (6.5 %), *B. licheniformis* (16.5 %), *B. pumilus* (9.1 %), *B. sphaericus* (4.6 %), *B. subtilis* (51.8 %), and *Lysinibacillus fusiformis* (2 %) (Chettri and Tamang 2015). *B. subtilis* BT:B9 from *bekang* accounted for the highest production of PGA (2.8 mg/ml each) among the other strains tested (Chettri and Tamang 2014). *Enterococcus faecium* BAV:E2 isolated from *bekang* showed high degree of hydrophobicity of 71.6 % indicating probiotic property (Chettri and Tamang 2014). *Bekang* possesses antioxidant and free radical (DPPH and ABTS) scavenging activity (Chettri and Tamang 2014).

2.7.3 Hawaijar

Hawaijar is a traditional fermented soybean alkaline food of Manipur. It is prepared from local variety of small-seeded soybean grown in hilly terraces of Manipur (Jeyaram et al. 2009). It is similar to *kinema*. Small-sized soybean seeds are selected, washed, and boiled in an open cooker for 2–3 h. Excess water is drained off, cooled to ~40 °C, and then packed the whole soybean seeds in a small bamboo basket having a lid. The basket is lined with fresh leaves of fig plant (*Ficus hispida*) locally called "assee heibong" in Meitei language or banana leaves. After placing cooled soybean seeds inside the basket, the lid is closed loosely, and the basket is kept nearby the kitchen or warm place for natural fermentation for 3–5 days. Emission of typical ammonia odor and appearance of sticky texture on the cooked soybean seeds are determined as good quality *hawaijar* by the Meitei. The maximum shelf life of *hawaijar* is 7 days without refrigeration. Sometimes, it is sun dried for 2–3 days and stored for several weeks for future consumption. Unlike *kinema*, the practice of cracking and addition of ash is not adopted by the Meitei women in *hawaijar* production. *Hawaijar* is produced by the Meitei women, and men support in the process (Tamang 2015b).

A special curry called "chagempomba" is commonly prepared by the Meitei in Manipur and is eaten with steamed rice. *Hawaijar* is eaten directly or used as a condiment or mixed with vegetables to make curry in the Manipuri cuisine (Singh et al. 2007b). The Meitei women commonly sell *hawaijar* in local markets throughout Manipur. Despite of its popularity, there is no organized food sector for mass scale production of *hawaijar* in Manipur. The product is still prepared at home and many women are dependent upon the product for livelihood. Bacteria isolated from *hawaijar* were identified as *Bacillus subtilis* (dominant functional bacterium), *B. licheniformis*, *B. cereus*, and other non-bacilli bacteria *Staphylococcus aureus*, *S. sciuri*, and *Alcaligenes* spp. (Jeyaram et al. 2008b; Keishing and Banu 2013). *Hawaijar* showed higher specificity toward fibrin produced by *Bacillus* spp. (Singh et al. 2014).

2.7.4 Kinema

Kinema is an ethnic fermented soybean food of India, Nepal, and Bhutan and is one of the oldest cultural foods of Asia (Tamang 2010a). Daily per capita consumption of *kinema* in Sikkim in India is 2.3 g (Tamang et al. 2007b). Soybeans are washed and soaked overnight, and soaked soybeans are taken out and put into the container with freshwater and boiled for 2–3 h until they are soft. Excess water is drained off, and the cooked soybean seeds are filled into the wooden mortar and are cracked lightly by a wooden pestle to split the cotyledons. This practice of cracking cooked seeds of soybeans is observed only during *kinema* production unlike *natto* and *chungkokjang*, probably to increase the surface areas for speed fermentation by aerobic spore-forming bacteria (Tamang 2001). 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 locally grown fresh fern (*Glaphylopteriolopsis erubescens*) or *Ficus* (fig plant) and banana leaves. The basket is covered in a jute bag and left to ferment naturally at ambient temperatures (25–40 °C) for 1–3 days above an earthen oven in the kitchen. During summer, the fermentation time may require 1–2 days, while in winter it may require 2–3 days. The maximum shelf life of freshly prepared *kinema* is 2–3 days during summer and 1 week during winter without refrigeration. Drying in the sun for 2–3 days may prolong it. Dried *kinema* is stored for several months at room temperature.

A number of species of *Bacillus* have been isolated from *kinema*, which include *Bacillus subtilis*, *B. licheniformis*, *B. cereus*, *B. circulans*, *B. thuringiensis*, and *B. sphaericus* (Sarkar et al. 1994, 2002). However, *B. subtilis* is the dominant functional bacterium in *kinema* (Sarkar and Tamang 1994; Tamang and Nikkuni 1996). Besides bacilli, *Enterococcus faecium*, two types of yeasts, *Candida parapsilosis* and *Geotrichum candidum*, were also isolated from *kinema* samples (Sarkar et al. 1994). It is observed that rich microbial diversity in various sources particularly soybean, equipment, and leaves as wrapping materials harness microbiota for spontaneous fermentation of *kinema* (Tamang 2003). With the decline in protein nitrogen content, the nonprotein and soluble nitrogen contents increase during *kinema* fermentation (Sarkar and Tamang 1995). Significant increase in relative viscosity of *kinema* during maturation at 5 °C and 10 °C was observed (Tamang and Nikkuni 1998). Keeping freshly prepared *kinema* below 10 °C for 1 day stabilizes the quality of the product by preventing the further biological activity of microorganisms and shows better stickiness which is a very important sensory property of *kinema* (Tamang and Nikkuni 1998). Organoleptically, the monoculture fermentation of soybean by *B. subtilis* MTCC 2756 produces the best *kinema* because of a pleasant nutty flavor and highly sticky texture and also minimizes the conventional fermentation time, maintains better hygienic conditions and consistency, and increases levels of soluble protein (Tamang 1999).

Inexpensive soybean extract broth after adjusting pH to 7 as medium is prepared for enrichment of *B. subtilis* spores instead of discarding the soybean extract after autoclaving soybeans (Tamang 1999). Moreover, nutrient broth, conventionally used for enrichment of *B. subtilis* spores, is composed of expensive beef extract, which is not acceptable to the majority of the Hindu population in the Himalayas. *Kinema* prepared by using *B. subtilis* KK2:B10 strain which is harvested in soybean extract broth is dried in an oven at 70 °C for 10 h and ground aseptically to make pulverized starter. The 1 % of pulverized starter instead of *B. subtilis* is added aseptically to autoclaved soybeans and fermented to get *kinema*. The consumers' preference trials show that *kinema* prepared by using pulverized starter under optimized conditions is more acceptable than market *kinema* (Tamang 1999). Water-soluble nitrogen and formol nitrogen contents are higher in *kinema* prepared by using pulverized starter than market *kinema* (Tamang 1999). Increased water-soluble nitrogen in *kinema* helps in digestibility and has high amount of formol nitrogen which contains free amino acid supplements that impart better taste to *kinema* (Nikkuni et al. 1995). Application of ready-to-use pulverized starter may appear appropriate in *kinema* production for marginal *kinema* producers in the Himalayas since it is cost-effective and easy to handle (Tamang 2010a).

Kinema has several health benefits including antioxidant, digested protein, essential amino acids, vitamin B complex, low cholesterol content, etc. (Tamang 2010a), which can be considered as a functional food. *Kinema* is the cheapest source of plant protein as compared to milk and animal products on the basis of protein cost per kg. It contains all essential amino acids (Sarkar et al. 1997) and is rich in linoleic acid, an essential fatty acid (Sarkar et al. 1996). Total amino acids, free amino acids, and mineral contents are increased during *kinema* fermentation (Sarkar and Tamang 1995; Nikkuni et al. 1995; Tamang and Nikkuni 1998). Phytosterols (cholesterol-lowering effect) is increased during *kinema* fermentation (Sarkar et al. 1996). Riboflavin and niacin increases in *kinema* during fermentation (Sarkar et al. 1998). *Kinema* has antioxidant activities (Tamang et al. 2009a, b). Due to large amount of Group B saponin contents, *kinema* claims to have health-promoting benefits (Omizu et al. 2011). Safety of *kinema* production was conducted using HACCP model (Rai et al. 2014).

In the phylogenetic relationships among bacilli isolated from *kinema* (India), *chungkokjang* (Korea), and *natto* (Japan), similar fermented sticky soybean foods of Asia reveal that all bacilli strains belonged to *B. subtilis* (Tamang et al. 2002). This suggests that *B. subtilis* strains responsible for fermentation of sticky soybean food in Asia might have originated from the same stock. Another theory was proposed that non-salted fermented soybean foods were originated in Yunnan province of China, which was the center of the hypothetical triangle (Nagai and Tamang 2010).

2.7.5 Peruyaan

Peruyaan is an ethnic fermented soybean food of Apatani tribes in Arunachal Pradesh state of India. The word *peruyaan* has been derived from the Apatani dialect; *perun* means beans and *yannii* means packing in leaves (t al. 2009a, b). During the traditional preparation of *peruyaan*, soybean seeds are collected, washed, and cooked for 2–3 h till the beans become soft. The excess water is drained off and is cooled for sometime. The cooked soybeans are kept in a bamboo basket (vessel) lined with fresh ginger leaves locally called as “taki yannii.” The basket is loosely covered with ginger leaves and is kept on the wooden rack above the fireplace 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 with steamed rice. It is mixed with hot water, chilies locally called as “tero,” and salt and directly consumed without frying or cooking unlike *kinema* curry preparation. *Bacillus subtilis*, *B. amyloliquefaciens*, *Vagococcus lutrae*, *Ped. acidilactici*, and *Ent. Faecalis* are bacteria isolated from *peruyaan* (Singh et al. 2014).

2.7.6 Tungrymbai

Tungrymbai is an ethnic fermented soybean food of Khasi and Garo in Meghalaya state of India. During production, soybean seeds are collected, cleaned, washed, and soaked in water for about 4–6 h (Agrahar-Murungkar and Subbulakshmi 2006; Jeyaram et al. 2009; Sohliya et al. 2009). The soaked soybeans are cooked for about 1–2 h till all the water is absorbed. Cooked beans are allowed to cool, are packed with fresh leaves of *Clinogyne dichotoma* locally called "lamet," and are placed inside the bamboo basket and covered by thick cloth. The covered basket is kept over the fireplace and cooked beans are fermented naturally for 3–5 days to get *tungrymbai*. *Tungrymbai* is mashed and put into a container with water, boiled till water evaporates, and stirred continuously. It is mixed with fried onion, garlic, ginger, chili, grinded black sesame locally called "til," and salt. A thick curry is made and is served as side dish with steamed rice by Khasi in Meghalaya. Pickle is also made from *tungrymbai*. Khasi women are commonly seen selling *tungrymbai* packed in fresh leaves of "lamet" or banana at the vegetable markets of Shillong. On the basis of a combination of phenotypic and molecular characterization, species of *Bacillus* isolated from *tungrymbai* were identified as *Bacillus licheniformis* (25.5 %), *B. pumilus* (19.5 %), and *B. subtilis* (55 %) (Chettri and Tamang 2015). *Bacillus subtilis* TS1:B25 isolated from *tungrymbai* showed high production of polyglutamic acid (Chettri and Tamang 2014). *Enterococcus faecium* TM2:L6 isolated from *tungrymbai* showed probiotic property (Chettri and Tamang 2014). *Tungrymbai* possesses antioxidant and free radical (DPPH and ABTS) scavenging activity (Chettri and Tamang 2014).

2.8 Indian Ethnic Non-soybean Legume Foods

2.8.1 *Dhokla* and *Khaman*

Dhokla is an ethnic fermented spongy-textured product of India prepared from Bengal gram and rice product 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 of Gujarat in India and is made solely from seeds of Bengal gram. Dry seeds of Bengal gram and white polished rice are washed, soaked, and ground, and salt and water are added to make a thick paste. The slurries are left for natural fermentation in a warm place (30–32 °C) for 8–10 h. The spongy-textured product *dhokla* is ready. It is steamed for 10–15 min and is eaten as snack. *Leuc. mesenteroides* and *Enterococcus faecalis* are essential and responsible for leavening of batter and acid-producing microorganisms during *dhokla* fermentation (Joshi et al. 1989). Acetoin and volatile fatty acids at their optimum concentration imparts characteristic flavor to *dhokla* (Joshi et al. 1989).

2.8.2 Masyaura

Maseura or *masyaura* is an ethnic fermented non-soybean product prepared from black gram by Gorkha of Northeast India. It is a cone-shaped hollow, brittle, and friable product. *Maseura* is similar to North Indian *wari* or *dhal* *bodi* and South Indian *sandige*. Black gram seeds are cleaned, washed, soaked, dehulled, and ground into thick paste using mortar and pestle. Water is carefully added while grinding until paste becomes sticky, which is then made hand molded into small balls or cones. The mixture is placed on a bamboo mat, fermented in open kitchen for 2–3 days, and then sun dried for 3–5 days (Chettri and Tamang 2008). *Maseura* can be stored in a dry container at room temperature for a year or more. It is used as condiment or adjunct to vegetable. Bacteria isolated from *masyaura* were identified as *Bacillus subtilis*, *B. mycoides*, *B. pumilus*, *B. laterosporus*, *Pediococcus acidilactici*, *P. pentosaceus*, *Enterococcus durans*, *Lb. fermentum*, and *Lb. salivarius* and yeasts *Saccharomyces cerevisiae*, *Pichia burtonii*, and *Candida castellii* Chettri and Tamang 2008). The nutritional values of *Masyaura* are moisture (8–10 %), pH (5.6–6.3), protein (8–10 %), and carbohydrate (67–70 %) (Dahal et al. 2003; Chettri and Tamang 2008). Increase in soluble protein, amino nitrogen, nonprotein nitrogen, thiamine, and riboflavin has been observed in *maseura* of Nepali (Dahal et al. 2003).

2.8.3 Wari

Wari is an ethnic Indian fermented black gram product, similar to *maseura*. It is a dry, hollow, brittle, spicy, and friable ball with 3–8 cm in diameter and 15–40 g in weight (Batra 1986). Seeds of black gram are soaked in water for 6–12 h, dewatered, dehulled, and ground into a smooth, mucilaginous paste. The dough is mixed with inoculum from a previous batch, salt, and spices including asafetida, caraway, cardamom, clove, fenugreek, ginger, and red pepper. The mixture is allowed to ferment at room temperature for 1–3 days, hand molded into balls, and dried for 2–8 days on bamboo or palm mats (Batra and Millner 1976). *Wari* is used as condiment and is mixed with vegetables as side dish. Yeast species of *Candida krusei*, *C. vartiovaara*, *Kluyveromyces marxianus*, *Trichosporon beigelii*, *Hansenula anomala*, *Saccharomyces cerevisiae*, *Leuc. mesenteroides*, and *Lb. fermentum* have been isolated from *wari* (Batra and Millner 1974, 1976; Batra 1981, 1986; Sandhu and Soni 1989). Increase in total acids, soluble nitrogen, free amino acids, thiamine, riboflavin, and cyanocobalamin has been observed during *wari* fermentation (Soni and Sandhu 1990a).

2.9 Indian Ethnic Fermented Dairy Products

2.9.1 Chhurpi

Two types of *chhurpi*, hard and soft, are popular among the ethnic people of Sikkim and Arunachal Pradesh in India (Tamang 2010a). A hard variety of *chhurpi* is prepared from yak milk in high-altitude mountains (2100–4500 m) and has the characteristic of gumminess and chewiness. Cream is separated from milk and the skimmed milk is boiled and curdled by adding whey. After straining, the coagulum is cooked until the remaining water dries up. The highly stringy mass is wrapped in a cloth and fermented under pressure at room temperature for about 2 days. After pressing, the mass is sliced and allowed to dry by keeping above earthen oven for about a month. Soft *chhurpi* is a cheese-like fermented milk product with slightly sour taste (Tamang et al. 2000). Buttermilk is cooked for about 15 min till a soft, whitish mass is formed. This mass is sieved out and put inside a muslin cloth, which is hung by a string to drain out the remaining whey. It is eaten as curry as well as pickle, mixed with wild edible ferns or prepared as soup.

A soft variety of *chhurpi* is sold in all local markets. According to its age, *chhurpi* is of three types, i.e., *chhursingba*, *chhur chirpen*, and *chhurpupu* (Singh et al. 2007a).

Lb. farciminis, *Lb. paracasei* subsp. *paracasei*, *Lb. confuses*, *Lb. bifermentans*, *Lb. plantarum*, *Lb. curvatus*, *Lb. fermentum*, *Lb. paracasei* subsp. *pseudoplantarum*, *Lb. alimentarius*, *Lb. kefir*, *Lb. hilgardii*, *Enterococcus faecium*, and *Leuconostoc mesenteroides* were isolated from soft chhurpi (Tamang et al. 2000; Dewan and Tamang 2007). The proximate composition of soft *chhurpi* consists of moisture 73.8 %, pH 4.2, acidity 0.61 %, ash 6.6 % DM, protein 65.3 % DM, fat 11.8 % DM, carbohydrate 16.3 % DM, Ca 44.1 mg/100 g, Fe 1.2 mg/100 g, Mg 16.7 mg/100 g, Mn 0.6 mg/100 g, and Zn 25.1 mg/100 g (Tamang 2010a; Tamang et al. 2012). The proximate composition of hard *chhurpi* consists of moisture 3.9–13 %, pH 5.3, acidity 0.3 %, ash 6.6–7.7 %, protein 53.4–68.5 %, fat 7.7–12.3 %, and carbohydrate 20.4–23.2 % (Katiyar et al. 1991; Pal et al. 1996).

2.9.2 Chhu

Chhu or *sheden*, an ethnic fermented milk product of Bhutia, Lepcha, Mongpa, and Tibetan living in Northeast India, is a strong-flavored traditional cheese-like product prepared from yak milk. It has a rubbery texture with a slightly sour taste and strong flavor (Dewan and Tamang 2006). *Shyow* is churned in a bamboo or wooden vessel, with addition of water to produce *maa* and *kachhu*. The latter is cooked for 15 min till a soft, whitish mass is formed, sieved out and put inside a muslin cloth, which is hung by a string to drain out the remaining whey. *Chhu* is placed in closed vessel to ferment the product further after which it is consumed (Dewan and Tamang 2006). It is prepared into a curry and soup. *Lb. farciminis*, *Lb. brevis*, *Lb. alimentarius*, *Lb. salivarius*, *Lc. lactis* subsp. *cremoris*, and yeasts *Saccharomycopsis* and *Candida* were isolated from chhu (Dewan and Tamang 2006). Chhu contains moisture of 75.5 %, pH 6.3, acidity 0.15 %, ash 1.9 % DM, protein 58.4 % DM, fat 5.8 % DM, carbohydrate 33.9 % DM, Ca 111 mg/100 g, Fe 4.5 mg/100 g, Mg 64.3 mg/100 g, Mn 3.1 mg/100 g, and Zn 87.6 mg/100 g (Tamang 2010a).

2.9.3 Dahi

Indian fermented dairy products are mostly natural fermented milk products produced spontaneously or by using back-sloping method. In back-slopping, a part of a precious batch of a fermented product is used to inoculate the new batch (Josephsen and Jespersen 2004). Examples of naturally fermented milks of India are *dahi*, *lassi*, *misti dahi*, *shrikhand*, *chhu*, *chhurpi*, *mohi*, *philu*, *shoyu*, and *somar* (cow/buffalo/yak milk) (Sarkar 2008; Patil et al. 2010; Tamang 2010a; Thapa and Tamang 2015).

Dahi (curd) is a popular ethnic fermented milk product consumed by a large section of the population throughout India either as a part of daily diet or as a refreshing beverage. The word *dahi* comes from Sanskrit word "dadhi" (Yegna Narayan Aiyar 1953). *Dahi* is obtained by lactic acid fermentation of cow or buffalo milk. *Dahi* is well known for its palatability and nutritive value (Rathi et al. 1990). It resembles plain yogurt in appearance and consistency and differs in having less acidity. During traditional method of preparation of *dahi*, fresh milk of cow or buffalo is boiled in a vessel, cooled to room temperature, and transferred to a hollow wooden vessel or container. A small quantity of previously prepared *dahi* (serves as source of inoculums; back-sloping technique) is added to boiled and cooled milk. This is left for natural fermentation for 1–2 days during summer and for 2–4 days during winter at room temperature. The duration of fermentation depends on the season as well as on the geographical location of the place. *Dahi* is consumed directly as a refreshing nonalcoholic beverage and savory. *Dahi* is also considered as sacred food item in many of their festivals and religious ceremony both by Hindu and Buddhists. It is also used as adhesive to make "tika" with rice and colored powder during the Hindu festival of Nepali called "dashain" and is applied to foreheads by the family elders (Tamang 2005). It is used to prepare many ethnic milk by-products such as *lassi* (buttermilk) in India and *mohi* in Nepal as nonalcoholic refreshing beverage and butter (*ghee*). *Lb. bifermentans*, *Lb. alimentarius*, *Lb. paracasei*, *Lact. lactis*, *Strep. cremoris*, *Strep. lactis*, *Strep. thermophilus*, *Lb. bulgaricus*, *Lb. acidophilus*, *Lb. helveticus*, *Lb. cremoris*, *Ped. pentosaceus*, *P. acidilactici*, *W. cibaria*, *W. paramesenteroides*, *Lb. fermentum*, *Lb. delbrueckii* subsp. *indicus*, *Saccharomycopsis* sp., and *Candida* sp. were isolated from *dahi* (Ramakrishnan 1979; Mohanan et al. 1984; Dewan and Tamang 2007; Patil et al. 2010).

2.9.4 Lassi

Lassi is a refreshing traditional summer beverage of North India, originally from Punjab, prepared by blending *dahi* with water, salt, and spices until frothy (Padghan et al. 2015). A traditional *lassi* is sometime flavored with sugar, rose water/lemon, mango, and strawberry of other fruit juices. *Lassi* is considered as digestive, nutritive, and useful in gastrointestinal ailments. The health benefits of *lassi* are the result of biologically active components that are present in native milk and also due to their suitably modulated activities produced through the action of lactic acid bacteria, recognition of the immense therapeutic and nutritional value, and use for the treatment of diarrhea, dysentery, chronic specific and nonspecific colitis, piles, and jaundice. *Lassi* is very low in fat but it has large amount of beneficial bacteria or their breakup products in the form of amino acids, peptides, vitamins, minerals, etc., which are useful in human and animal health (Padghan et al. 2015). The composition of *lassi* consists of water 96.2 %, fat 0.8 %, protein 1.29 %, lactose 1.2 %, lactic acid 0.44 %, ash 0.4 %, calcium 0.6 %, and phosphorous 0.04 % (Sukumar 2004). *Lassi* is a probiotic product due to the presence of *Lb. acidophilus* and *Strep. thermophilus* as active cultures (Patidar and Prajapati 1988).

2.9.5 Misti dahi

Misti dahi (sweetened dahi, *misti doi*, *lal dahi*, or *payodhi*), analogous to caramel-colored set-style sweetened yogurt, is a sweetened fermented milk product prepared particularly in West Bengal (India) and Bangladesh from cow or buffalo's milk (Tamang 2010b). *Misti dahi* is mildly acidic, thick gel, and sweet in nature which is actually a sweetened curd and has a savory-like taste with a light brown color and firm body with a cooked or caramelized flavor. Traditionally, it is prepared by heating milk and sugar cane (sweetener; palm jiggery is also used in some varieties) in an open pan at about 60–70 °C for 6–7 h, to evaporate part of the moisture and to develop a marked cooked flavor, slightly brown color, viscosity, and other physicochemical changes. After cooling to 30 °C, the mixture is inoculated with previously kept commercial starter culture and is transferred to earthen pots; curdling takes place at room temperature overnight (Ghosh and Rajorhua 1990). Presently, it is now produced industrially and marketed in different parts of the country. The dominant microorganisms present in *misti dahi* are *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lactococcus lactis* subsp. *lactis*, and *Saccharomyces cerevisiae* (Ghosh and Rajorhua 1990; Gupta et al. 2000).

2.9.6 Philu

Philu is an ethnic fermented, cream-like dairy product, with an inconsistent semisolid texture, and is consumed by the Tibetans, Bhutia, Sherpa, and Mongpa of Northeast India (Tamang 2010a). Fresh cow/yak milk collected in cylindrical bamboo vessels (called *dzydung* by the Bhutia) or in wooden vessels (called *yadung*) is slowly swirled around the walls of these vessels by rotating them for a few minutes. Sometimes a thick mesh of dried creeper is kept inside the vessel to increase the surface area for the *philu* to stick. A creamy mass sticks to the walls of the vessels and around the creeper. Milk is then poured off and utilized elsewhere. The vessel is kept upside down to drain out the remaining liquid, and the process is repeated daily for about 6–7 days until a thick, white creamy layer is formed on the vessel walls and the creeper surface. The soft mass, *philu*, is scraped off and stored in a dry place for consumption. Rich gravy from *philu* is prepared as a side dish along with boiled rice. *Lb. paracasei* subsp. *paracasei*, *Lb. bifermentans*, and *Enterococcus faecium* were isolated from *philu* (Dewan and Tamang 2007). The nutritional values of *philu* are moisture (38.2 %), pH (4.3), acidity (0.61 %), ash (3.6 % DM), protein (52 % DM), fat (32 % DM), carbohydrate (12.5 % DM), (Ca) 34.9 mg/100 g, Fe (0.8 mg/100 g), Mg (16.9 mg/100 g), Mn (0.9 mg/100 g), and Zn (27.1 mg/100 g) (Tamang 2010a).

2.9.7 Shrikhand

Shrikhand is a semisolid sweetish sour traditional fermented milk product of Indian origin and is very popular in Gujarat, Maharashtra, Northern parts of Karnataka, and Madhya Pradesh (Ghosh and Rajorhua 1990). The name *shrikhand* is derived from the Sanskrit word "Shikharini" (Patel and Chakraborty 1988). *Shrikhand* is obtained from curd by partial draining of whey, to which sugar, fruits and nuts are added. It is prepared by whole milk of cattle, buffalo or any diary milk, and heating will be done for 15 min at 95 °C and cool it down to 30 °C. After cooling, previous curd culture is added and incubated it at 30 °C for 8 h till the acidity reaches 1 % (curd formation). Curd is separate by using muslin cloth and tied it for 8 h and *chakka* is formed. In last stage of *shrikhand* preparation, *chakka* is mixed with sugar and cardamom for color and flavor development (Swapna and Chavannavar 2013). It is extensively used as a sweet dish after meals. *Shrikhand* constitutes 39.0 % moisture and 61.0 % of total solids, of which 10.0 % is fat, 11.5 % proteins, 78.0 % carbohydrates, and 0.5 % ash, on a dry matter basis, and pH of about 4.2–4.4 (Boghra and Mathur 2000). *Shrikhand* is known for its high nutritive, characteristic flavor, taste, palatable nature, and possible therapeutic value (Swapna and Chavannavar 2013). *Lactococcus lactis* subsp. *lactis*, *Lc. lactis* subsp. *diacetylactis*, *Lc. lactis* subsp. *cremoris*, *Strep. thermophilus*, and *Lb. delbrueckii* subsp. *bulgaricus* were reported from *shrikhand* (Swapna Chavannavar 2013; Singh and Singh 2014).

2.9.8 Somar

Somar, a soft paste, brownish with strong flavor, is an ethnic fermented milk (yak/cow) product of Sikkim traditionally consumed by Sherpa (Tamang 2010a). Buttermilk (*tara* in Sherpa dialect) is cooked till a soft, whitish mass is formed, and the mass is sieved out with a cloth or plastic sieve. The product is called *sherkam* (same as fresh soft *chhurpi*) and is kept in a closed vessel for 10–15 days to ferment the product further. The final fermented product is called *somar*. In another traditional way, *somar* is cooked with milk, *mar* (butter) and turmeric to produce a soft-brown paste *somar*. This type of *somar* is stored for 4–7 months. *Somar* soup is consumed with rice. *Lb. paracasei* subsp. *pseudoplantarum* and *Lactococcus lactis* subsp. *cremoris* were isolated from *somar* (Dewan and Tamang 2007). The nutritional values of *somar* are moisture (36.5 %), pH (6), acidity (0.1 %), ash (2.7 % DM), protein (35 % DM), fat (15.4 % DM), carbohydrate (46.9 % DM), Ca (31.2 mg/100 g), Fe (0.4 mg/100 g), Mg (13.7 mg/100 g), Mn (0.5 mg/100 g), and Zn (17.2 mg/100 g) (Tamang 2010a).

2.10 Indian Ethnic Fermented Vegetables

2.10.1 Goyang

Goyang is an ethnic fermented wild plant food, generally prepared by the Sherpa women of high mountains in the Himalayan regions of India (Tamang and Tamang 2009). Leaves of wild edible plant (*Cardamine macrophylla* Willd) are collected, washed and cut into pieces, squeezed to drain off excess water, and tightly pressed into the bamboo baskets lined with two to three layers of leaves of fig plants. The top of the baskets is then covered with fig plant leaves, and they fermented naturally at room temperature 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 drying for 2–3 days and it is stored for several months. *Goyang* is eaten as soup. *Lb. plantarum*, *Lb. brevis*, *Lc. lactis*, *Enterococcus faecium*, *P. pentosaceus*, and yeasts *Candida* spp. were isolated from *goyang* (Tamang and Tamang 2007).

2.10.2 Gundruk

Gundruk is an ethnic fermented vegetable of the Gorkha in India. Daily per capita consumption of *gundruk* is 1.4 g with annual production of 3.2 kg/house in Indian state of Sikkim (Tamang et al. 2007b). *Gundruk* is prepared from fresh leaves of local vegetable called *rayo-sag* (*Brasicca rapa* subspecies *campestris* variety *cuneifolia*), mustard, and cauliflower which are wilted and shredded, crushed mildly, and pressed into an earthen jar or container, which is made airtight. 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 at room temperature. *Gundruk* is eaten as soup or pickle. *Lb. fermentum*, *Lb. plantarum*, *Lb. casei*, *Lb. casei* subsp. *pseudoplantarum*, and *P. pentosaceus* have been isolated from *gundruk* (Tamang et al. 2005). *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* (Tamang and Tamang 2010). *Lb. plantarum* MTCC 9483 and *P. pentosaceus* MTCC 9484 are selected as a starter for production of *gundruk* (Tamang and Tamang 2010). Some LAB isolated from *gundruk* showed strong acidification, antimicrobial properties, abilities to degrade anti-nutritive factors, and probiotic character (Tamang et al. 2009a, b). *Gundruk* is considered as good appetizer (Tamang 2010a).

2.10.3 Inziangsang

Inziangsang or *ziangsang* is an ethnic fermented leafy vegetable product of Naga living in Nagaland and Manipur. It is very similar to *gundruk*. Leaves of mustard locally called *hangam* are crushed and soaked in warm water. Leaves are then squeezed to remove excess water and put into airtight container and fermented at room temperature for 7–10 days. Like *gundruk*, freshly prepared *inziangsang* is sun dried for 4–5 days and stored in a closed container. Freshly fermented *inziangsang* juice is also extracted, instead of sun drying, by squeezing with hand and concentrated by boiling. The liquid form of fermented extract is called *ziang dui* and the concentrated paste is *ziang sang* (Tamang and Tamang 2009). It is consumed as soup. Fermented extract *ziang dui* is used as condiment. *Lb. plantarum*, *Lb. brevis*, and *Pediococcus acidilactici* were isolated from *inziangsang* (Tamang et al. 2005).

2.10.4 Khalpi

Khalpi is an ethnic fermented cucumber product of Sikkim and Darjeeling hills. During its preparation, ripened cucumber is cut into pieces and sun dried for 2 days and then put into a closed bamboo vessel and fermented naturally at room temperature for 3–5 days. *Khalpi* is consumed as pickle. *Leuc. fallax*, *P. pentosaceus*, *Lb. brevis*, and *Lb. plantarum* are the dominant LAB in *khalpi* fermentation (Tamang et al. 2005). *Lb. plantarum* MTCC 9485, *Lb. brevis* MTCC 9486, and *Leuc. fallax* MTCC 9487 were selected as a mixed starter for commercial production of *khalpi* (Tamang and Tamang 2010).

2.10.5 Sinki

Sinki is an ethnic fermented radish tap root commonly prepared in the Himalayan regions of India by pit fermentation. Pit fermentation of *sinki* preparation is a unique type of biopreservation of perishable radish by lactic acid fermentation in the Himalayas (Tamang 2010a). 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 taproots are wilted for 2–3 days, crushed, 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 several years in an airtight container. It is eaten as soup or pickle. *Lb. plantarum*, *Lb. brevis*, *Lb. casei*, and *Leuc. fallax* were isolated from *sinki* (Tamang and Sarkar 1993; Tamang et al. 2005).

2.11 Indian Ethnic Bamboo Fermented Foods

2.11.1 Ekung

Ekung is an ethnic fermented bamboo shoot product of India, prepared in Arunachal Pradesh. A pit of about 3–4 ft is dug in the forest, bamboo baskets are laid into the pit and lined with leaves, and 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. *Lb. plantarum*, *Lb. brevis*, *Lb. casei*, and *Tetragenococcus halophilus* were isolated from *ekung* (Tamang and Tamang 2009).

2.11.2 Eup

Eup is a dry fermented bamboo tender shoot food commonly prepared and consumed by different tribes of Arunachal Pradesh (Tamang 2010a). Bamboo shoots are chopped into small pieces and fermented in similar manner as in *ekung*. Fermentation is completed within 1–3 months. After fermentation, the fermented product, now, *eup*, is again cut into smaller pieces and then sun dried 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. *Lb. plantarum* and *Lb. fermentum* were isolated from *eup* (Tamang and Tamang 2009).

2.11.3 Hirring

Hirring is a fermented topmost whole bamboo shoot product, commonly prepared in Arunachal Pradesh of India. Outer leaf sheaths of tender bamboo shoots are removed. The topmost tender edible portions are either cut longitudinally into two to three 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 its contents are fermented for 1–3 months. Baskets are taken out from pits after the fermentation, and *hirring* is ready for consumption. *Hirring* is kept for 2–3 months at room temperature. It is consumed as side dish mixed with vegetables, meat, and fish, along with steamed rice. *Lb. plantarum* and *Lc. lactis* are the functional LAB in *hirring* (Tamang and Tamang 2009).

2.11.4 Mesu

Mesu is an ethnic fermented bamboo shoot product of Darjeeling hills and Sikkim. Young 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. Opening of the vessel is covered tightly with leaves of bamboo and left to ferment under natural anaerobic conditions for 7–15 days. *Mesu* is eaten as a pickle. *Lb. plantarum*, *Lb. brevis*, *Lb. curvatus*, *Leuc. citreum*, and *P. pentosaceus* have been isolated from *mesu* (Tamang and Sarkar 1996; Tamang et al. 2008).

2.11.5 Soibum

Soibum is an ethnic fermented bamboo shoot food of Manipur. Thin slices of young bamboo shoots are packed compactly into this chamber, 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 and is left for 6–12 months for fermentation. After fermentation, *soibum* is stored for 10–12 months. Different dishes are prepared from *soibum* in Manipur of India such as *ironba*, *athongba*, *kangou*, and *chagempomba* (Tamang 2010a). *Lb. plantarum*, *Lb. brevis*, *Lb. coryniformis*, *Lb. delbrueckii*, *Leuc. fallax*, *Leuc. Lact. lactis*, *Leuc. mesenteroides*, *Ent. durans*, *Strep. lactis*, *B. subtilis*, *B. licheniformis*, *B. coagulans*, *B. cereus*, *B. pumilus*, *Pseudomonas fluorescens*, *Saccharomyces* sp., and *Torulopsis* sp. were isolated from *soibum* (Sarangthem and Singh 2003; Tamang et al. 2008; Jeyaram et al. 2010). Recently Romi et al. (2014) differentiated two yeasts *Meyerozyma guilliermondii* and *Meyerozyma caribbica* isolated from *soibum* using internal transcribed spacer (ITS) restriction fingerprinting.

Some lactic acid bacteria isolated from *soibum* such as *Lb. brevis* showed maximum phytic acid degradation ability (19.33 U/ml), and *Lb. xylosus* had highest protease activity (64.2 nmol/ml) and also exhibited lipolytic activity and *Lb. plantarum* (SM2) showed highest degree of hydrophobicity (Sonar and Halami 2014). Increase in free amino acids has been observed during fermentation of *soibum* (Giri and Janmejay 1994). *Soibum* exhibited significant radical scavenging activity and α-glucosidase inhibitory activity (Sonar et al. 2015).

2.11.6 Soidon

Soidon is a fermented tip of matured bamboo shoot product commonly consumed in Manipur of India. Outer casings and lower portions of tips of matured bamboo shoot are removed; entire tips are submerged in water in an earthen pot. Sour liquid of previous batch is added as 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 fermentation to enhance flavor of *soidon*. After fermentation, *soidon* is removed from the pot and is stored in closed container at room temperature for a year. It is consumed as a curry as well as pickle with steamed rice. *Lb. brevis*, *Leuc. fallax*, and *Leuc. lactis* have been isolated from *soidon* (Tamang et al. 2008; Jeyaram et al. 2010). Microbial community and population dynamics during natural bamboo shoot fermentation for production of *soidon* were studied using cultivation-dependent and cultivation-independent molecular approaches and revealed three-phase succession of autochthonous lactic acid bacteria to attain a stable ecosystem within 7 days natural fermentation of bamboo shoots (Romi et al. 2015). *Weissella* spp. (*Weissella cibaria*, uncultured *Weissella ghanensis*) and *Lactococcus la*ctis subsp. *cremori*s predominated the early phase (1–2 days), which was joined by *Leuconostoc citreum* during the mid-phase (3 days), while *Lactobacillus brevis* and *Lb. plantarum* emerged and became dominant in the late phase (5–7 days) with concurrent disappearance of *W. cibaria* and *Lc. lactis* subsp. *cremoris*. *Lc. lactis* subsp. *lactis* and uncultured *Lb. acetotolerans* were predominantly present throughout the fermentation (Romi et al. 2015).

2.11.7 Tuaithur

Tuaithur is an ethnic fermented bamboo shoot product with sour-acidic taste prepared and consumed by the *Hrangkhol*, *Baite*, and *Hmar* tribes of North Cachar Hills district of Assam (Chakrabarty et al. 2014). *Dimasa* calls it as *miyamikhri*. It is similar to other ethnic fermented bamboo shoot products of Northeast India and the Himalayas such as *mesu* of Nepal, Darjeeling hills, Sikkim, and Bhutan; *soidon* and *soibum* of Manipur; *ekung* and *hiring* of Arunachal Pradesh; and *lung-siej* of Meghalaya (Tamang and Tamang 2009). During the traditional method of preparation of *Tuaithur*, young tender shoots of bamboo (*Dendrocalamus hamiltonii* Nees. et Arn. ex Munro, *Melocanna bambusoides* Trin., and *Bambusa tulda* Roxb.) are collected, their outer hard casings are removed, and inner portion is then chopped into small pieces with a knife. The chopped pieces are washed thoroughly with clean water, drained well, and pressed tightly in cylindrical vessels made of bamboo or in bottles. The vessel is made airtight with a lid and it is fermented under natural anaerobic condition for 6–7 days. It is kept in an airtight container or in sealed plastic bags for few months at ambient temperature. *Tuaithur* is prepared as curry with dry fish or meat product mixing with salt. It is also made as pickle and can be preserved for several months. *Tuaithur* is sold in the local markets during the months of June to September. *Tuairoi* is a similar dry fermented bamboo tender shoot product consumed by the *Hrangkhol* and *Baite* tribe of Assam. *Lb. plantarum*, *Lb. brevis*, *P. pentosaceus*, *Lactococcus lactis*, *Bacillus circulans*, *B. firmus*, *B. sphaericus*, and *B. subtilis* were isolated from *tuaithur* (Chakrabarty et al. 2014).

2.12 Indian Ethnic Preserved and Fermented Fish Products

2.12.1 Gnuchi

Gnuchi is a traditional smoked fish product of Lepcha of Sikkim. Fishes (*Schizothorax richardsonii* Gray, *Labeo dero* Hamilton, *Acrossocheilus* spp., *Channa* sp.) are collected from river, kept on a big bamboo tray to drain off water, degutted, and mixed with salt and turmeric powder. Fishes are separated according to their size. The bigger-sized fishes are selected and spread in an upside-down manner on "sarhang" and are kept above the earthen oven in the kitchen. The small-sized fishes are hung one after the other in a bamboo stripe above the earthen oven and kept for 10–14 days (Thapa et al. 2006). *Gnuchi* is kept at room temperature for 2–3 months and is eaten as curry. Microorganisms of *gnuchi* are bacteria *Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Lc. plantarum*, *Leuconostoc mesenteroides*, *Enterococcus faecium*, *E. faecalis*, and *Pediococcus pentosaceus* and yeasts *Candida chiropterorum*, *C. bombicola*, and *Saccharomycopsis* spp. (Thapa et al. 2006).

2.12.2 Hentak

Hentak is also a fermented fish paste prepared from a mixture of sun-dried fish powder and petioles of aroid plants in Manipur. Finger-sized fish (*Esomus danricus* Hamilton) is washed thoroughly, sun dried, and crushed to powder. Petioles of *Alocasia macrorrhiza* are cut into pieces, washed, and then exposed to sunlight for 1 day. An equal amount of the cut pieces of the petioles of *Alocasia macrorrhiza* is mixed with powdered fish, and a ball-like thick paste is made, kept in an earthen pot, tightly sealed, and fermented for 7–9 days (Thapa et al. 2004). *Hentak* is consumed as curry and is also used as condiment. Sometimes it is given to women in the final stages of their pregnancy or patients recovering from sickness or injury (Sarojnalini and Singh 1988).

Bacteria *Lc. lactis* subsp. *cremoris*, *Lc. plantarum*, *Enterococcus faecium*, *Lb. fructosus*, *Lb. amylophilus*, *Lb. corynifomis* subsp. *torquens*, *Lb. plantarum*, *Bacillus subtilis*, *B. pumilus*, and *Micrococcus* and yeast species of *Candida* and *Saccharomycopsis* were isolated from *hentak* (Thapa et al. 2004). The nutritional values of *henta*k are moisture (40.0 %), pH (6.5), ash (15.0 %), protein (32.7 %), fat (13.6 %), carbohydrate (38.7 %), food value (408.0 kcal/100 g), Ca (38.2 mg/100 g), Fe (1.0 mg/100 g), Mg (1.1 mg/100 g), Mn (1.4 mg/100 g), and Zn (3.1 mg/100 g) (Thapa and Pal 2007).

2.12.3 *Karati*, *Bordia*, and *Lashim*

Karati, *bordia*, and *lashim* are sun-dried and salted fish products of Assam. Fish is washed and rubbed with salt and dried in the sun for 4–7 days. The sun-dried fish products are stored at room temperature for 3–4 months for consumption. *Karati* is prepared from *Gudusia chapra* Hamilton, *bordia* is prepared from *Pseudeutropius atherinoides* Bloch, and *lashim* is prepared from *Cirrhinus reba* Hamilton. These fish products are eaten as side dish. Bacteria *Lc. lactis* subsp. *cremoris*, *Leuc. mesenteroides*, *Lb. plantarum*, *Bacillus subtilis*, and *B. pumilus* and yeast *Candida* were isolated from *karati*, *bordia*, and *lashim* (Thapa et al. 2007).

2.12.4 Ngari

Ngari is a fermented fish product of Manipur consumed by Meitei (Thapa et al. 2004). During its production, fish (*Puntius sophore* Hamilton) is rubbed with salt, sun dried for 3–4 days, washed briefly and spread on a bamboo mats, and filled and pressed tightly in an earthen pot by leg. To the inner wall of the pot, a layer of mustard oil is applied before filling up the fishes. The pot is sealed airtight and then stored at room temperature for 4–6 months. It is kept for more than a year at room temperature. *Ngari* is eaten daily as a side dish with cooked rice. It is sold in local markets.

The microbial composition of *ngari* consists of bacteria *Lactococcus lactis subsp. cremoris*, *Lc. plantarum*, *Enterococcus faecium*, *Lb. fructosus*, *Lb. amylophilus*, *Lb. corynifomis subsp. Torquens*, *Lb. plantarum*, *Bacillus subtilis* and *B. pumilus*, and *Micrococcus* and yeast species of *Candida* and *Saccharomycopsis* (Thapa et al. 2004). Devi et al. (2015) reported several bacteria from *ngari* using PCR-denaturing gradient gel electrophoresis (DGGE) and amplified ribosomal DNA restriction analysis (ARDRA)-based grouping and 16S rRNA gene sequence similarity analysis such as the dominant bacteria *Staphy. cohnii* subsp. *cohnii* (38.0 %), *Tetragenococcus halophilus* subsp. *flandriensis* (16.8 %), a novel phylotype related to *Lactobacillus pobuzihii* (7.2 %), *Enterococcus faecium* (7.2 %), *Bacillus indicus* (6.3 %), and *Staphylococcus carnosus* (3.8 %). The study showed the presence of additional species, in which *Kocuria halotolerans* and *Macrococcus caseolyticus* disappeared during fermentation while *Clostridium irregulare* and *Azorhizobium caulinodans* were detected throughout the fermentation (Devi et al. 2015). The nutritional values of *ngari* are moisture (33.5 %), pH (6.2), ash (21.1 %), protein (34.1 %), fat (13.2 %), carbohydrate (31.6 %), food value (381.6 kcal/100 g), Ca (41.7 mg/100 g), Fe (0.9 mg/100 g), Mg (0.8 mg/100 g), Mn (0.6 mg/100 g), and Zn (1.7 mg/100 g) (Thapa and Pal 2007).

2.12.5 Shidal

Shidal is non-salted and semi-fermented ethnic fish product of Tripura prepared using minor carps (*Puntius* spp.) in specially designed earthen pots (Muzaddadi and Basu 2003). It has several local names like *seedal*, *seepa*, *hidal*, and *shidol* in Assam, Tripura, Arunachal Pradesh, and Nagaland (Kakati and Goswami 2013a, b; Ahmed et al. 2013). During preparation of *shidal*, *Puntius* sp. are sun dried, filled them in vats/earthen pots, and fermented naturally for 4–6 months under anaerobic condition till the product gains a characteristic odor, texture, and appearance (Ahmed et al. 2013). *Shidal* is a delicacy for most of the tribal, Bengali, and other people of Tripura for its characteristic taste and flavor. *Staphy. aureus*, *Micrococcus* spp., *Bacillus* spp., and *E. coli*. were isolated from *shidal* (Muzaddadi and Basu 2003, 2012; Muzaddadi 2015).

2.12.6 Sidra

Sidra is a sun-dried fish product commonly consumed by the Gorkha. Fish (*Puntius sarana* Hamilton) is collected, washed, sun dried for 4–7 days, and stored at room temperature for 3–4 months. *Sidra* pickle is a popular cuisine. The microbial composition of *sidra* consists of bacteria *Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Lc. plantarum*, *Leuconostoc mesenteroides*, *Enterococcus faecium*, *E. faecalis*, *Pediococcus pentosaceus*, and *Weissella confuse* and yeasts *Candida chiropterorum*, *C. bombicola*, and *Saccharomycopsis* spp. (Thapa et al. 2006).

2.12.7 Suka ko maacha

Traditionally smoked fish product in Sikkim is called *suka ko maacha*. The hill river fishes "dothay asala" (*Schizothorax richardsonii* Gray) and "chuchay asala" (*Schizothorax progastus* McClelland) are collected in a bamboo basket from the river or streams and are degutted, washed, and mixed with salt and turmeric powder. Degutted fishes are hooked in a bamboo-made string and are hung above the earthen oven in the kitchen for 7–10 days. It can be preserved for 4–6 months and is eaten as curry. Microorganisms of *suka ko maacha* are bacteria *Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Lc. plantarum*, *Leuconostoc mesenteroides*, *Enterococcus faecium*, *E. faecalis*, and *Pediococcus pentosaceus* and yeasts *Candida chiropterorum*, *C. bombicola*, and *Saccharomycopsis* spp. (Thapa et al. 2006).

2.12.8 Sukuti

Sukuti is also very popular sun-dried fish product cuisine of the Gorkha. Fish (*Harpadon nehereus* Hamilton) is collected, washed, rubbed with salt, sun dried for 4–7 days, and stored for 3–4 months. *Sukuti* is consumed as pickle, soup, and curry. It is also commonly sold at local markets. Bacteria *Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Lc. plantarum*, *Leuc. mesenteroides*, *Enterococcus faecium*, *E. faecalis*, and *Pediococcus pentosaceus* and yeasts *Candida chiropterorum*, *C. bombicola*, and *Saccharomycopsis* spp. were isolated from *sukuti* (Thapa et al. 2006).

2.12.9 Tungtap

Tungtap is a traditional fermented fish paste of Khasi in Meghalaya. Sun-dried fish (*Danio* spp.) is washed briefly and is mixed with salt; sun-dried fish is kept in the earthen pot, made airtight, and fermented for 4–7 days (Thapa et al. 2004). It is consumed as pickle and curry. Bacteria *Lc. lactis* subsp. *cremoris*, *Lc. plantarum*, *Enterococcus faecium*, *Lb. fructosus*, *Lb. amylophilus*, *Lb. corynifomis* subsp. *Torquens*, *Lb. plantarum*, *Lb. puhozihii*, *Bacillus subtilis*, *B. pumilus*, and *Micrococcus* and yeast species of *Candida* and *Saccharomycopsis* are present in *tungtap* (Thapa et al. 2004; Rapsang et al. 2011; Rapsang and Joshi 2012). The nutritional values of *tungtap* are moisture (35.4 %), pH (6.2), ash (18.9 %), protein (32.0 %), fat (12.0 %), carbohydrate (37.1 %), food value (384.4 kcal/100 g), Ca (25.8 mg/100 g), Fe (0.9 mg/100 g), Mg (1.6 mg/100 g), Mn (0.8 mg/100 g), and Zn (2.4 mg/100 g) (Thapa and Pal 2007).

2.13 Indian Ethnic Preserved and Fermented Meat Products

The native skills of the Indian Himalayan people in preservation methods of locally available raw meat can be justified by making sausage-like products using unappealing animal parts such as scraps, organ meats, fat, blood, etc. Traditional sausage-like meat product is also made with the leftover parts of the animal. Hence, leftover meats are used for *kargyong* (ethnic sausage-like products) making that may be the first concern of the high-altitude dwellers (Rai et al. 2010a) and that has high nutritional value (Rai et al. 2010b). The ethnic people of Uttarakhand and Himachal Pradesh prepare many ethnic meat products (Rai et al. 2009).

2.13.1 *Arjia*

Arjia is boiled and smoked as *jamma* and used to prepare curry dishes or deep-fried sausage (Oki et al. 2011). The preparation of *arjia* is similar to that of *jamma*. However, a mixture of chopped lungs of goat, salt, chili powder, *Zanthoxylum* sp., and fresh animal blood is stuffed into the goat's large intestine instead of small intestine.

By 16S rRNA and phenylalanyl-tRNA synthetase (*pheS*) gene sequencing, lactic acid bacteria from *arjia* were identified as *Enterococcus durans*, *E. faecalis*, *E. faecium*, *E. hirae*, *Leuconostoc citreum*, *Leu. mesenteroides*, *Pediococcus pentosaceus*, and *Weissella cibaria* (Oki et al. 2011).

2.13.2 Chartayshya

Chartayshya is a chevon (goat) meat product consumed by the ethnic people of Uttarakhand state of India and West Nepal. During the preparation of *chartayshya*, red meat is cut into pieces and strung together on a long thread which is hung from bamboo stripes or wooden sticks and then exposed in the corridors of the houses for 15–20 days (Rai et al. 2009). The prepared *chartayshya* is usually kept at room temperature for several weeks before it is eaten as a component of curry dishes. Similar product is named as *karki sha* or *karchisha*/*nyasha* in Lahaul Spiti district of Himachal Pradesh where ethnic people use it for preparation of soups during winters. *Ent. faecalis*, *Ent. faecium*, *Ent. hirae*, *Leuc. citreum*, *Leuc. mesenteroides*, *Ped. pentosaceus*, and *Weissella cibaria* were isolated from *chartayshya* (Oki et al. 2011).

2.13.3 Honoheingrain

Honoheingrain is an ethnic fermented pork or boar (wild pig) product consumed by *Dimasa* tribe of North Cachar Hills of Assam (Chakrabarty et al. 2014). Freshly killed wild boar/hog (*Sus scrofa* L.) is collected for its preparation. Upper skin along with its hair is removed with a knife and washed thoroughly and dipped into boiled water for a few minutes to remove excess hair. It is then cut into several small pieces, kept in a bamboo mat above the kitchen oven which is about 2–3 ft above the fireplace, and fermented naturally for 4–5 days till it completely dries. *Honoheingrain* is made into curry. During "*hirimdi puja*" of *Dimasa*, the pig is sacrificed to Goddesses. *Honoheingrain* curry is prepared to solemnize the marriage ceremony.

Microbial diversity in *honoheingrain* includes *Lb. brevis*, *Lb. plantarum*, *Leuc. mesenteroides*, *Ent. faecium*, *Bacillus cereus*, *B. pumilus*, *B. firmus*, *B. circulans*, *B. stearothermophilus*, *Micrococcus*, and *Staphylococcus* and yeasts *Debaryomyces hansenii* and *Saccharomyces cerevisiae* (Chakrabarty et al. 2014.

2.13.4 Jamma or Geema/Juma

Jamma or *geema*/*juma* is an ethnic fermented sausage of the Western Himalayas prepared from chevon meat. Red meat is chopped into fine pieces and mixed with 1 % ground finger millet (*Eleusine coracana*), 0.5 % wild pepper locally called "*timbur*" (*Zanthoxylum* sp.), 0.5 % chili powder, and salt (Rai et al. 2009). A little fresh animal blood is also added. The meat mixture is made semiliquid by the addition of water and is poured into the small intestine of the goat with the help of funnel. Both ends of the filled intestine are tied, and then it is pricked randomly to prevent bursting while boiling. After boiling for 15–20 min, the stuffed intestine is smoked above earthen oven in the kitchen for 15–20 days if they are not eaten immediately after boiling. The method of preparation of *jamma* is similar to *kargyong* of the Eastern Himalayas (Rai et al. 2009). It is eaten as cooked sausage or as a curry component. Lactic acid bacteria from *jamma* were identified as *Enterococcus durans*, *E. faecalis*, *E. faecium*, *E. hirae*, *Leuconostoc citreum*, *Leu. mesenteroides*, *Pediococcus pentosaceus*, and *Weissella cibaria* (Oki et al. 2011).

2.13.5 Kargyong

Kargyong is a sausage-like meat product of Sikkim and Arunachal Pradesh prepared from meat. Meat (yak/beef/pork) with its fat is chopped finely; combined with crushed garlic, ginger, and salt; and mixed with water. The mixture is stuffed into the segment of gastrointestinal tract locally called *gyuma*, used as natural casings with 3–4 cm in diameter and 40–60 cm length. One end of the casing is tied up with rope, and other end is sealed after stuffing and boiled for

20–30 min. Cooked sausages are taken out and hung in the bamboo stripes above the kitchen oven for smoking and drying for 10–15 days to make *kargyong* (Rai et al. 2009). *Kargyong* is eaten after boiling for 10–15 min, sliced and made into curry or fried sausage. Bacteria from *Kargyong* were identified as *Lb. sake*, *Lb. divergens*, *Lb. carnis*, *Lb. sanfransisco*, *Lb. curvatus*, *Leuc. mesenteroides*, *E. faecium*, *Bacillus subtilis*, *B. mycoides*, *B. thuringiensis*, *Staphylococcus aureus*, and *Micrococcus* and yeasts *Debaryomyces hansenii* and *Pichia anomala* (Rai et al. 2010a). The nutritional values of yak *kargyong* are moisture (21.9 %), pH (6.9), ash (2.8 % DM), protein (16.0 % DM), fat (49.1 % DM), carbohydrate (32.0 % DM), and food value (634.5 kcal/100 g) (Rai et al. 2010b).

2.13.6 Satchu

Satchu is a dried meat (beef/yak/pork) and is consumed by the Tibetans, Bhutia, Lepcha, Drukpa, and Sherpa in the EN. Red meat of beef or yak and also pork is sliced into several strands of about 60–90 cm and is mixed thoroughly with turmeric powder, edible oil or butter, and salt. The meat strands are hung in the bamboo stripes or wooden stick and are kept in an open air in corridor of the house or are smoked above the kitchen oven for 10–15 days as per the convenience of the consumers (Rai et al. 2009). *Satchu* can be kept at room temperature for several weeks. Deep-fried *satchu* is eaten as side dish. Bacteria from s*atchu* were identified as *Pediococcus pentosaceus*, *Lb. casei*, *Lb. carnis*, *E. faecium*, *B. subtilis*, *B. mycoides*, *B. lentus*, *S. aureus*, and *Micrococcus* and yeasts *D. hansenii* and *Pichia anomala* (Rai et al. 2010a).

2.13.7 Suka ko masu

Suka ko masu is a dried or smoked meat product prepared from buffalo meat or chevon (goat meat). The nonvegetarian Gorkha consumes it. It is prepared by cutting the red meat of buffalo or chevon (goat meat) into stripes up to 25–30 cm and mixing it with turmeric powder, mustard oil, and salt. Mixed meat stripes are hung on bamboo and kept above the earthen oven in the kitchen and smoked for 7–10 days and stored for several weeks (Rai et al. 2009). It is eaten as curry with cooked rice. Bacteria isolated from s*uka ko masu* were identified as *Lb. carnis*, *E. faecium*, *Lb. plantarum*, *B. subtilis*, *B. mycoides*, *B. thuringiensis*, *S. aureus*, and *Micrococcus* and yeasts *Debaryomyces hansenii* and *Pichia burtonii* (Rai et al. 2010a).

2.14 Indian Amylolytic Starters

Ethnic people of the Indian Himalayan prepare nonfood starter cultures for long centuries. A consortium of mycelial or filamentous molds, amylolytic and alcohol-producing yeasts, and LAB with rice or wheat as the base in the form of dry, flattened, or round balls of various sizes (Tamang 2010c). The starter is inoculated with 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 the Philippines, *chiu*/*chu* in China and Taiwan, *loogpang* in Thailand, *nuruk* in Korea, and *men* in Vietnam (Tamang 2010c), which are used as starters for a number of fermentations based on rice and cassava or other cereals in Asia.

Ethnic fermented foods and beverages have importance in the context of the Himalayan food ecosystems and in terms of culture, tradition, cost-effective production, and nutrition. Due to cultural adaptation for consumption, and the preserved nature of the products, the majority of the Himalayan fermented foods can be considered essential for food and nutritional security of the region (Tamang 2010a). The native skills of alcohol production by "starter culture" technique and using traditional distillation apparatus are well recognized. The consortium of microorganisms (starter culture) is preserved in rice or wheat base, as a source of starch, together with the use of glucose-rich wild herbs to supplement the carbon sources for growing microorganisms (Thapa and Tamang 2004).

Unlike mixed culture starters of the other Asian countries, *marcha* is usually prepared by wrapping kneaded dough cakes in fern fronds with the fertile side touching them. This may be due to abundance of ferns locally called "pire uneu" {*Glaphylopteriolopsis erubescens* (Wall ex Hook.) Ching} in the Himalayas. Probably, germination of spores in sori helps to maintain the warmth of the fermenting mass in cold climates. Preparation of *marcha* is similar to those of other starter cultures of Asia. *Marcha* makers believe that addition of wild herbs give more sweetness to the product. Addition of chilies and ginger during *marcha* preparation is to prevent growth of undesirable microorganisms that may inhibit the growth of native functional microorganisms in *marcha*. Studies of Soedarsono (1972) in *ragi*, an Indonesian rice-based starter culture, reveal that certain spices inhibit many undesirable microorganisms at the time of fermentation. Hesseltine (1983) speculated that the spices, which are known to be inhibitory to many bacteria and molds, are the agents that select the right population of microorganisms for fermentation. Ethnic amylolytic starter culture-making technology reflects the traditional method of subculturing desirable inocula from previous batch to new culture using rice as base substrates in India (Tamang 2010a). This technique preserves the microbial diversity essential for beverages production.

2.14.1 Bakhar

Bakhar is the traditional starter used in the preparation of *Pachwai* (Indian rice beer) in Northern regions of India. For the preparation of starter, ginger and other plant materials are dried, ground, and added to rice flour. Water is added to make a thick paste, and small round cakes of 1.0–1.5 cm in diameter are made and inoculated with powdered cakes from previous batches. The cakes are then wrapped in leaves, allowed to ferment for 3 days and then sun dried. *Rhizopus* sp., *Mucor* sp. and at least one species of yeast have been reported from *bakhar* (Hutchinson and Ram Ayyar 1925).

2.14.2 Dhehli

Dhehli is fermentation starter used in preparation of *sura*, a millet-based alcoholic beverage in Lug valley of Kullu district in Himachal Pradesh. *Dhehli* preparation is an annual community effort, in which elderly people go to forests on the 20th day of *Bhadrapada* month (usually 5th or 6th of September) and collect approximately 36 fresh herbs. Some of the important herbs used in *dhehli* preparation are *Pistacia integerrima* (*kakar shinga*), *Solanum xanthocarpum* (*katari*), *Clitoria ternatea* (*kkayal*), *Aegle marmelos* (*bhel*), *Viola cinerea* (*banaksa*), *Cannabis sativa* (*bhang*), *Trachyspermum copticum* (*ajwain*), *Micromeria biflora* (*chharbara*), *Spiranthes australis* (*bakarshingha*), *Saussurea* sp. (*bacha*), *Bupleurum lanceolatum* (*nimla*), *Drosera lunata* (*oshtori*), *Salvia* sp. (*kotugha*), *Arisaema helleborifolium* (*chidi ri chun*), and *Fragaria* sp. (*dudlukori*). The collected herbs are crushed with a large conical cavity (*ukhal*) using a wooden bar (*mussal*), and the extract and the plant biomass are added in to the flour of roasted barley and are roughly kneaded. This is put into a wooden mold to give the shape of a brick and dried (Savitri and Bhalla 2007; Thakur et al. 2004). Main lactic acid bacteria isolated and identified from *dhehli* are *Enterococcus faecium* and *Lactobacillus plantarum*. Among the yeasts, *Saccharomyces fibuligera* and *Saccharomyces cerevisiae* were identified (Thakur 2013).

2.14.3 Hamei

Hamei is a dry, round to flattened, solid ball-like mixed dough inocula used as starter cultures to prepare *atingba*, an alcoholic beverages in Manipur. Local varieties of rice, not soaked or soaked and then dried, are crushed and mixed with powdered bark of "yangli" (*Albizia myriophylla* Benth.) and a pinch of previously prepared powdered *hamei*. The dough is pressed into flat cakes and kept over paddy husk in a bamboo basket, covered by sackcloths for 2–3 days at room temperature, and then sun dried for 2–3 days (Jeyaram et al. 2009). Women sell *hamei* in local markets in Manipur. Filamentous molds are *Mucor* spp. and *Rhizopus* spp.; yeasts *Saccharomyces cerevisiae*, *Pichia anomala*, *P. guilliermondii*, *P. fabianii*, *Trichosporon* sp., *Candida tropicalis*, *C. parapsilosis*, *C. montana*, and *Torulaspora delbrueckii*; and lactic acid bacteria *Pediococcus pentosaceus* and *Lb. brevis* (Tamang et al. 2007a; Jeyaram et al. 2008a, 2011).

2.14.4 Humao

Humao is the traditionally prepared mixed amylolytic dough inocula used as a starter for the production of various indigenous alcoholic beverages and drinks in Assam (Chakrabarty et al. 2014). *Humao* is a dry, flat-rounded and oval, creamy white to dusty white, solid starter ranging from 1.5 to 10.8 cm in diameter with the weight ranging from 20 to 25 g. Dimasa calls it *humao*, Hrangkhol calls it *Chol*, and Jeme Naga calls it *Nduhi*. During the traditional method of preparation, bark of *Albizia myriophylla* Benth. (Family Mimosaceae) is used. Sticky rice was soaked for 10–12 h at room temperature, and there after it was crushed with bark of *Albizia myriophylla* and 1–2 % of previously prepared starter in the form of powder. The mixture is then made into paste by adding water and kneaded into flat-rounded and oval cakes of varying sizes and kept it for 1–3 days at 25–30 °C and sun dried. The traditional method of preparation of *humao* is protected as hereditary trade and passes from mothers to daughters. *Ped. pentosaceus*, *Bacillus polymyxa*, *B. Licheniformis*, *B. Stearothermophilus*, *Debaryomyces hansenii*, *Saccharomyces cerevisiae*, and species of *Mucor* and *Rhizopus*, have been recovered from *humao* (Chakrabarty et al. 2014).

2.14.5 Malera/Khameer and Treh

Malera/*khameer* and *treh* are the traditional inoculum used for preparation of some fermented foods. It is a portion of a previous leftover fermented dough or fermented slurry. Every time fermentation is carried out, some portion of dough/slurry is kept so that it can be used next time. The microbiological analysis of *malera* revealed that it was a consortium of microorganisms which mainly consisted of lactic acid bacteria and yeast. *Lactobacillus plantarum* (MTCC 8296), *Leuconostoc* sp. and *Saccharomyces cerevisiae* (MTCC 7840) were isolated from different samples of *malera* (Savitri and Bhalla 2012).

2.14.6 Marcha

Marcha is an ethnic amylolytic starter to produce fermented beverages and alcoholic drinks in the Himalayan regions of India (Tamang 2010a). *Marcha* is a dry, round to flattened, creamy white to dusty white, solid ball-like. Glutinous rice is soaked, crushed, ground, and mixed with roots of *Plumbago zeylanica*, leaves of *Buddleja asiatica*, flowers of *Vernonia cinerea*, ginger, red dry chili, and 1–2 % of previously prepared *marcha*. The mixture is made into a paste by adding water and kneaded into flat cakes of varying sizes and shapes. This is then placed individually on the ceiling floor, 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 *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. *Marcha* is the Nepali word. Different ethnic communities of the Himalayas call it by their vernacular names such as *phab* (Tibetan), *poo* (Drukpa), *khesung* (Limboo), *bharama* (Tamang), *bopkha* or *khabed* (Rai), *buth* or *thanbum* (Lepcha), *manapu* (Newar), *hamei* (Meitei), *thiat* (Khasi), *humao* (Dimasa), *pham* and *ipoh* (Apatani), *bakhar* (people of Himachal Pradesh in India), and *balan* (people of Uttarakhand in India). *Marcha*-making technology reflects a 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 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 (Tamang 2010a).

Species of mycelial molds present in *marcha* are *Mucor circinelloides* forma *circinelloides*, *Mucor* sp. close to *M. hiemalis*, *R. chinensis*, and *R. stolonifer* variety *lyococcus* (Tamang et al. 1988). Amylolytic and alcohol-producing yeasts isolated from *marcha* are *Saccharomycopsis fibuligera*, *Sm. capsularis*, *Saccharomyces cerevisiae*, *S. bayanus*, *Pichia anomala*, *P. burtonii*, and *Candida glabrata* (Tsuyoshi et al. 2005). *Sm. fibuligera* is the most dominant yeasts in *marcha* (Tamang and Sarkar 1995). 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 2004). Among LAB *P. pentosaceus*, *Lb. bifermentans*, and *Lb. brevis* are present in *marcha* (Tamang and Sarkar 1995; Tamang et al. 2007a). LAB impart flavor, antagonism, and acidification of the substrates in *marcha* (Tamang et al. 2007a). Hesseltine et al. (1988) isolated *Mucor* and *Rhizopus* spp. from *marcha* samples of Nepal. Uchimura et al. (1990) reported yeast *Saccharomycopsis* and molds *Penicillium* sp. and *Aspergillus* sp. in *poo* or *phab* (*marcha* of Bhutan).

2.15 Indian Alcoholic Beverages and Drinks

A number of cereals and fruit-based fermented alcoholic beverages are prepared and consumed by ethnic people in rural and tribal areas of different states in India. In contrast to barley malt used in preparation of beer, *ragi*, rice, and barley are used as starting materials in the preparation of Indian traditional beverages. Some of the ethnic fermented alcoholic beverages are discussed below:

2.15.1 Bhaati Jaanr

Bhaati jaanr is the Indian Himalayan sweet-sour, mild alcoholic food beverage paste prepared from rice and consumed as a staple food (Tamang 2010a). Rice is cooked and spread on a bamboo mat for cooling; 2–4 % of powdered *marcha* is sprinkled over 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 during summer and 7–8 days during 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 beverage. The similar product is called *poko* in Nepal. Occasionally *bhaati jaanr* is stored in an earthenware crock for 6–9 days, and thick yellowish white supernatant liquor locally called *nigaar* is collected at the bottom of the earthenware crock. *Nigaar* is drunk directly with or without addition of water. Alcohol content of *bhaati jaanr* is 5.9 % (Tamang 2010a). *Bhaati jaanr* is an inexpensive high-calorie staple food beverage for the postnatal women and ailing old persons in the villages who believe that it helps to regain their strength. Maximum activities of saccharification and liquefaction of rice are observed on third day of fermentation (Tamang and Thapa 2006). *Saccharomycopsis fibuligera* and *Rhizopus* spp. and *Mucor* spp. contribute 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. Increase in mineral contents mostly calcium, iron, sodium, potassium, and phosphorus is also observed in *bhaati jaanr* due to fermentation (Tamang and Thapa 2006).

2.15.2 Chhang/Lugri

This is an indigenous rice beer made in the tribal belt of Lahaul and Spiti district of Himachal Pradesh. The preparation of *chhang* involves solid-state fermentation as no additional water is added to the ingredients, i.e., cooked rice and *phab* (the traditional inoculum). *Chhang* is also prepared from barley; however, it takes longer time (1 week) to ferment and beverage is called *lugri* in Lahaul. The traditional vessel made of metal or stone used to store *chhang* is called "*uthi*" in Lahaul, and it is served in traditional jugs called "*chapkiayan*."

Chhang/*lugri* is a very popular fermented beverage served during *phagli* (traditional New Year of *Lahulis*) and marriage ceremonies. This beverage is also of religious significance as it is sprinkled on guests as *shagun* (tribal custom). Microorganisms include *Lactobacillus plantarum*, *Lb. casei*, *Enterococcus faecium*, and *Pediococcus pentosaceus*. *Saccharomyces cerevisiae*, *Saccharomyces fibuligera*, *Pichia kudriavzevii*, and *Candida tropicalis* have been isolated and identified from *chhang* (Thakur 2013).

2.15.3 Haria/Harhia

Haria/*harhia* is a rice-based fermented beverage that is popular among tribal people in West Bengal and east-central India (Ghosh and Das 2004; Ghosh et al. 2014). The main ingredient of this beverage is boiled rice (*Oryza sativa* L.), which is mixed with a traditional starter called *bakhar*, and fermented within an earthen pot for 3–4 days. For generations, *haria* has been consumed by the tribal people of India as a staple as well as for its ethnopharmacological benefits (Ghosh et al. 2014). Microbiological studies revealed the presence of *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Saccharomycopsis* sp., *Zygosaccharomyces cidri*, *Pichia*, *Candida tropicalis*, *Candida musae*, *Candida nitratophila*, and *Issatchenkia* in *haria* along with some of the species of lactic acid bacteria (Sha et al. 2012a, b, c). The quantities of molds and yeasts were highest at second day of *haria* fermentation and then declined; however, lactic acid bacteria and *Bifidobacterium* sp. increased concurrently during the fermentation (Ghosh et al. 2015). Accumulation of starch hydrolytic enzymes along with different types of malto-oligosaccharides like maltotetraose, maltotriose, and maltose and increase in antioxidant during *haria* fermentation were also noted (Ghosh et al. 2015).

2.15.4 Judima

Judima or *zu* is a mild alcoholic beverage, with distinct sweet aroma, prepared from steamed glutinous rice in Assam (Chakrabarty et al. 2014). Different ethnic groups of people in Assam call it by their own dialect such as *judima* (by *Dimasa*), *juhning* (*Hrangkhol*), *jeme naga* (*Deuijao* and *Nduijao*), and *remalu baitui* and *bumong baitui* (*Baite*). *Dimasa* prefers sticky rice (*Oryza sativa* L., local cultivar "Bairong"/"Maiju-walao"/"Maiju-walao-gedeba"/"Maiju-hadi") for better quality of*judima* preparation, or sometimes if sticky rice is not available, they mix 80 % ordinary rice with 20 % of sticky rice. During the traditional method of preparation of *judima*, the sticky rice is cleaned, washed, and cooked, and excess water is drained out. Cooked rice is spread over in bamboo mats or banana leaves for cooling; nowadays, polythene sheet is also used, and then ~1 % of *humao* is mixed thoroughly and added a little amount of water. The fermenting mass is placed in fresh banana leaves and again externally covered by another leaves of banana or polythene sheet and fermented for 1–2 days (during summer) or 3–4 days (during winter) at room temperature. When sweet flour is emitted (saccharification), the fermented mass is transferred in a *khulu* (triangle-shape bamboo-made cone) covered by banana leaves or hollow earthen pot. Now, an empty vessel or *hundy* is kept below *khulu*, and the juices are collected in the vessel (it requires 1–2 days) which is called as *judima*. After collection of *judima*, left out rice, called *jugap*, is distilled to make high alcohol content liquor, locally called *juharo*. *Judima* is similar to *bhaati jaanr* of Sikkim (Tamang and Thapa 2006).

Judima/*zu* is drunk directly with or without water. After celebration of the traditional rituals and festivals, *judima* is served with meats. *Juharo*, the distilled liquor, is more alcoholic and acidic in taste. It is a traditional diet for women in villages who believe that it helps them to regain their strength. Ethnic people believe that the quality of *zu* depends upon the richness of the family, i.e., better quality of *judima* is prepared by rich family. Best quality of *judima* is pineapple juice in color or yellowish red in color. The color of the products depends on the quality of rice. Besides home consumption, *judima* is sold in the local market of NC Hills by some people who are economically dependent upon *judima*.

Besides food culture, *judima* is an integral part of ritual for *Dimasa*. During religious festivals, freshly prepared *judima* is offered to the family of God and Goddesses. During birth, a drop of *judima* is administered in the lips of a newborn baby to protect from any devil forces. *Judima* and *juharo* are essential to solemnize their marriage ceremony. Traditionally, a newly wedded bride visits her parents' house once in a year; when she returns back to her husband's house, she should carry the *Judima*. The traditional festival "*busu-dima*" of *Dimasa* is celebrated with freshly prepared *judima*. In death ceremony, freshly prepared *judima* is offered to death persons and also to ancestors. Guests are also served with *judima*. Without *judima*, no celebration or religious ceremony is completed.

Lactic acid bacteria *Ped. pentosaceous*, *Bacillus circulans*, *B. laterosporus*, *B. pumilus*, and *B. Firmus* and yeasts *Debaryomyces hansenii* and *Saccharomyces cerevisiae* were isolated from *judima* (Chakrabarty et al. 2014).

2.15.5 Kanji

Kanji is an ethnic Indian strong-flavored mild alcoholic beverage prepared from beet root and carrot by natural fermentation (Batra and Millner 1974). It is drunk as mild alcoholic refreshing drink in India. Alcohol content in *kanji* is 2.5 % and pH is 4.0 showing the product as mild alcoholic and with acidic taste (Sura et al. 2001). During its preparation, carrots or beets are washed, shredded, and mixed with salt and mustard seeds and placed in earthen pot and allowed to ferment naturally at 26–34 °C for 4–7 days. Sometimes, the mixture is inoculated with a portion of a previous batch of *kanji*. After fermentation, 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*, yeast-containing fermented rice gruel, is used as starters for *kanji* production. *Hansenlu anomala*, *Candida guilliermondii*, *C. tropicalis*, and *Geotrichium candidum* are involved in *kanji* fermentation (Batra and Millner 1974). *Leuc. mesenteroides*, *Pediococcus* spp., and *Lactobacillus dextranicum* were isolated from *kanji* fermentation (Sura et al. 2001). Kingston et al. (2010) reported *Lb. paraplantarum* and *Lb. pentosus* from *kanji* based on rep-PCR identification method.

2.15.6 Kodo ko jaanr

Kodo ko jaanr or *chyang* is one of the most popular ethnic fermented finger millet (*Eleusine coracana*) beverages of the Himalayan regions of India with mild alcohol (4.8 %) and sweet taste (Tamang 2010c). *Kodo ko jaanr* has several synonyms as used by different ethnic groups of the Himalayan people such as *chyang* (Tibetan, Ladakh, Drukpa) (Bhatia et al. 1977), *mandokpenaa thee* (Limboo), and *mong chee* (Lepcha) (Tamang 2005). During its production, finger millet seeds are cleaned, washed, and cooked for about 30 min, excess water is drained off, and 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 the mixture is fermented at room temperature for 2–4 days. The saccharified mass is transferred into an earthen pot or bamboo basket which is made airtight and fermented for 3–4 days during summer and 5–7 days during 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 edge 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 in a side near the bottom to avoid passing of grits. Water is added twice or thrice after sipping of the extract. Consumption of fermented finger millet beverages in exclusively decorated bamboo or wood-made vessel called *toongbaa* is unique in the Himalayas (Tamang 2010a). *Kodo ko jaanr* liquor is believed to be good tonic for ailing persons and postnatal women. After consumption, residual or grits of *kodo ko jaanr* are used as fodder for pigs and cattle. This is a good example of total utilization of substrate as food and fodder, and also the discarded grits contain nutrient used as animal feed.

Marcha used as amylolytic starter supplements all functional microorganisms in *kodo ko jaanr* fermentation (Thapa and Tamang 2004). Mycelial molds have roles only in the initial phase of fermentation mostly in saccharification of the substrates. Yeasts *Pichia anomala*, *Saccharomyces cerevisiae*, *Candida glabrata*, *Saccharomycopsis fibuligera*, and LAB *Pediococcus pentosaceus* and *Lactobacillus bifermentans* have been recovered in *kodo ko jaanr* samples. The population of filamentous molds, which were originated from *marcha*, declines daily during in situ fermentation of *kodo ko jaanr* and finally disappears after the fifth day (Thapa and Tamang 2006). *Sm. fibuligera* and *R. chinensis saccharify* and liquefy millets starch into glucose and produce alcohol in situ fermentation of *kodo ko jaanr*. Fermentation of finger millet enhances bioenrichment of minerals such as Ca, Mg, Mn, Fe, K, and P, contributing to mineral intake in daily diet of the Himalayan rural people (Thapa and Tamang 2004). Because of high calorie, ailing persons and postnatal women consume the extract of *kodo ko jaanr* to regain the strength. *Chyang* contains more riboflavin, niacin and pantothenic acid and folic acid than the substrate (Basappa 2002). The essential amino acids also increase in *chyang* (Bassapa et al. 1997).

2.15.7 Raksi

Raksi is an ethnic alcoholic drink of the Himalayas with characteristic aroma and distilled from the traditional fermented cereal beverages (Kozaki et al. 2000). *Raksi* is a common term 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 40×30×25 cm for 2–3 h continuously over firewood in an earthen oven (Tamang 2005). A perforated container locally called *phunga* is placed above the main cylindrical vessel inside which a small metallic collector called *poini* is kept on an iron tripod called *odhan* to collect the distillate called *raksi*. Another a metallic vessel with cold water is placed above the *phunga* as condenser. The bottom of the condenser vessel is plastered by mud with the tip of the *phunga* 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 for 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 distil 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 *Rhododendron*-grown regions of the Himalayas (Tamang et al. 1996). The alcohol content of *raksi* is 22–27 % (v/v) (Tamang 2005). *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.

2.15.8 Toddy or Tari

Toddy or *tari* is an ethnic alcoholic drink of India prepared from palm juice. There are three types of *toddy* (Batra and Millner 1974): (1) *sendi*, from the palm; (2) *tari*, from the *palmyra* and date palms; and (3) *nareli*, from the coconut palm. *Geotrichum*, *Saccharomyces*, and *Schizosacchoramyces* spp. of yeast 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).

2.15.9 Tchang/Jhar and Rokshi

The tribal people of Sikkim consume beverages called *tchang/jhar* and *rokshi*, in which *tchang* is prepared using millet (*Paspalum* sp.) and *rokshi* is prepared using certain plants and plant parts. The fermented millet is transferred into bamboo or wooden cylindrical cups. Lukewarm water is poured inside the cups and drunk by suction through a small pipe of bamboo. It can be repeated until the flavor is available. *Canna edulis* Ker Gawl. and *Zea mays* L. are the important plants used in the preparation of *rokshi* by the ethnic people of Sikkim, namely, Lepcha, Bhutia, and Nepali. *Jhar* and *rokshi* samples contain filamentous molds and yeasts (*Mucor cicinelloides*, *Rhizopus chinensis*, *R. stolonifer* var. *lyococcus*, *Saccharomycopsis fibuligera*, *Saccharomyces cerevisiae*, *Hansenula anomala*, *Pediococcus pentosaceus*, and *Lactobacillus* sp.) (Sekar and Mariappan 2007).

2.15.10 Yu

Yu also known as *atingba yu* or *puk-yu* is the ethnic fermented rice-based beverage of Manipur. For *yu* preparation, the cooked rice is spread and kept in dim light for cooling. It is then mixed up with starter material, *hamei*, and mixture is then filled into a pot, which is previously cleaned and dried over fire. The mouth of the pot is covered with a clean cloth and kept in sunlight for 3–4 days during summer. During winter, the mixture is fitted in a bamboo basket internally wrapped with the leaves of *teak* (*Tectona grandis*), *Ficus hispida*, banana (*Musa paradisiaca* Linn.), giant *taro* (*Alocasia indica*), etc., and baked in direct sunlight after covering the mouth of the basket by coarse cloth for 5–6 days for fermentation (Singh and Singh 2006).

2.15.11 Zutho

Zutho or *zhuchu* is an ethnic alcoholic beverage of Mao Naga prepared from rice. Rice is soaked overnight, drained off, pounded into flour, put in a big bamboo bucket and mixed with boiling water, stirred, and left for cooling; amylolytic starter locally called *khekhrii* powder is added and left about 6–8 h for brewing after which the whole mixture is poured into a big earthen jar (Mao and Odyuo 2007). More water is added to make the volume up to the neck and kept for fermentation for 3–4 days during which it forms a profuse whitish froth to get *zutho* which has sweet taste with acidic flavor (Mao 1998). The similar alcoholic beverage called *nchiangne* is prepared from red rice in of Nagaland. *Saccharomyces* cerevisiae was isolated from *zutho* (Teramoto et al. 2002). It contains 5 % (v/v) alcohol, pH 3.6, and acidity 5.1 % (Teramoto et al. 2002).

2.16 Conclusion

In India, diversity of ethnic fermented foods is related to diversity of ethnicity with unparallel food culture of each community. Microbial diversity ranges from filamentous molds to enzyme-producing to alcohol-producing yeasts and Gram-positive and few Gram-negative bacteria with several functional properties. There is a relationship between the human life and microorganisms. Indian ethnic fermented foods and beverages have biological functions enhancing several health-promoting benefits to the consumers due to functional microorganisms associated with them. Calculation of per capital consumption ethnic fermented foods and alcoholic beverage in every state in India is an urgent need to be addressed by the food policy makers.

Most of these ethnic fermented foods are prepared on household level by the women, thus exploiting the capabilities of microorganisms unknowingly leading to increase in shelf life of these foods. However, rapid urbanization and modernization have affected the time-tested traditional technologies for preparation of fermented foods. If we continue to ignore this important issue, the time is not far when our indigenous traditional knowledge will disappear before it could be validated and improved on the scientific criteria. Thus, the documentation of the ethnic fermented food products is much needed at the present time, and there is a need to characterize the traditional fermented products by studying their biochemical, microbiological, and the nutritive aspects, to provide scientific base for their present status and improvement by quality addition.

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Ethnic Fermented Foods and Beverages of Bangladesh

3

Mahmud Hossain and Yearul Kabir

3.1 Introduction

3.1.1 Bangladesh Geography

Bangladesh lies between latitude 20° and 27°N and longitude 88° and 93°E. The Bay of Bengal is on the southern coast of Bangladesh; most of it is surrounded by India with a small common border with Myanmar in the southeast (Fig. 3.1).

3.1.2 Agriculture in Bangladesh

Although Bangladesh achieved lower middle income country status in 2015 (WB 2015), agriculture remains the largest employer in the country by far; 47.5 % of the population is directly employed in agriculture and around 70 % depends on agriculture in one form or another for their livelihood (Hamid 2015). Agriculture is the source of food for people through crops, livestock, and fisheries; the source of raw materials for industry and of timber for construction; and a generator of foreign exchange for the country through the export of agricultural commodities. It is the motor of the development of the agro-industrial sector, including food processing, input production and marketing, and related services. As the main source of economic linkages in rural areas, agriculture is fundamental in reducing poverty, which remains a predominantly rural phenomenon. The role of agriculture is also fundamental in promoting nutritious diets, especially in the countryside where production and consumption patterns are closely linked. According to the Household Income and Expenditure Survey (HIES) of 2010, 35.2 % and 21.1 % of the population in rural areas lives below the upper and lower poverty line, respectively (BBS 2011). It also has a fundamental role in the sustainable valorization and preservation of natural resources and in preserving and promoting the resilience to natural calamities and climate change of rural communities and agro-ecological systems. However, as Bangladesh develops, and other economic sectors grow (such as ready-made garments), the share of agriculture in gross domestic product (GDP) has naturally declined (Hamid 2015). During the fiscal year 2012–2013, the broad agriculture sector contributed 16.77 % to the total GDP (Hamid 2015). The contributions of crop, fishery, livestock, and forestry subsectors to the GDP were 9.49 %, 3.68 %, 1.84 %, and 1.76 %, respectively. Provisional estimates show that the contribution of the broad agriculture sector to GDP in 2013–2014 would be 16.33 % (Hamid 2015; BER 2014). Nearly three fifths of the agricultural GDP comes from the crop subsector; the other contributors in order of magnitude are fishery, livestock, and forestry.

M. Hossain • Y. Kabir (✉)
Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka 1000, Bangladesh
e-mail: ykabir@yahoo.com

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Fig. 3.1 Map of Bangladesh

Rice and jute are the primary crops, but maize and vegetables are assuming greater importance. In response to the expansion of irrigation networks, some wheat producers have switched to the cultivation of maize, which is used mostly as poultry feed. Tea is grown in the northeast area. Because of Bangladesh's fertile soil and normally ample water supply, rice can be grown and harvested three times a year in many areas. For a number of reasons, Bangladesh's labor-intensive agriculture has achieved steady increases in food grain production despite the often-unfavorable weather conditions: these factors include better flood control and irrigation, a generally more efficient use of fertilizers, and the establishment of better distribution and rural credit networks. Following independence in 1971, agricultural production in Bangladesh increased at around the rate of 2 % per year, and the growth rate accelerated during the 1990s and early 2000s to around 4 % per year (Hamid 2015). According to Food and Agriculture Organization Statistics (FAOSTAT) for 2012, Bangladesh is the sixth largest rice-producing country in the world.

3.1.3 Ethnic People

Bangladesh is largely ethnically homogeneous, and its name derives from the Bengali ethno-linguistic group that comprises 98 % of the population (IBP 2012). The Chittagong Hill Tracts, Sylhet, Mymensingh, and North Bengal divisions are home to diverse indigenous peoples. Many dialects of Bengali are spoken throughout the region. The dialect spoken by those in Chittagong and Sylhet is particularly distinctive. According to the census of the Bangladesh Bureau of Statistics (BBS), in 2011 the population was estimated at around 160 million (BBS 2012). About 89 % of Bangladeshis are Muslims, followed by Hindus (8 %), Buddhists (1 %), and Christians (0.5 %).

The Bengali ethno-linguistic group also spans the neighboring Indian province of West Bengal.

Minority ethnic groups include Meitei, Khasia, Rakhain, Santhals, Chakma, Garo (tribe), Biharis, Oraons, Mundas, and Rohingyas. Biharis are Urdu-speaking, non-Bengalis who migrated from the State of Bihar and other parts of northern India during the 1947 partition and are concentrated in the Dhaka and Rangpur areas (Nabi 2006). In the 1971 independence war many of them sided with Pakistan, as they stood to lose their positions in the upper levels of society (Heitzman and Worden 1989). Hundreds of thousands went to Pakistan and those who remained were interned in refugee camps. Refugees International has called them a "neglected and stateless" people because they are denied citizenship by the governments of Bangladesh and Pakistan (Refugees of Nowhere 2006). As more than 40 years have passed, two generations of Biharis have been born in these camps. Biharis were granted Bangladeshi citizenship and voting rights in 2008 (Refugees of Nowhere 2006).

Bangladesh's tribal populations are of Sino-Tibetan descent and differ markedly in their social customs, religion, language, and level of development (Heitzman and Worden 1989). They speak Tibeto-Burman languages and most are Buddhist or Hindu (Heitzman and Worden 1989). The four largest tribes are Chakmas, Marmas, Tipperas, and Mros. Smaller groups include the Santals in Rajshahi and Dinajpur, and Khasis, Garos, and Khajons in the Mymensingh and Sylhet regions (Heitzman and Worden 1989).

3.1.4 Food and Culture

People can connect to their cultural or ethnic group through similar food patterns. Immigrants often use food as a means of retaining their cultural identity. People from different cultural backgrounds eat different foods. The ingredients, methods of preparation, preservation techniques, and types of food eaten at different meals vary among cultures. The areas in which families live—and where their ancestors originated—influence food likes and dislikes. These food preferences result in patterns of food choices within a cultural or regional group.

Generally, the Bangladeshi take meals three times a day. Also, in the evening they eat snacks. Lunch is the main dish for Bangladeshis. Almost all Bangladeshis take plain rice at lunch. Most of them take different kinds of vorta and bhaji (sautéed or fried vegetable with green or roasted dried chilies and other spices), which are very popular. They also take various kinds of spicy fishes with it. So, it is called Mas-a-Vaat-a-Bangali (i.e., the land of rice and fishes). The staple food of the people in Bangladesh is rice and fish. One of the most relished items in this country is a platter of *panta ilish*, which is a dish made with fermented rice and fried hilsa fish accompanied by *dal* (pulse), onions, fresh green chilies, and pickles. It is a special dish, which is cooked to celebrate the first day of Bengali New Year. Bangladesh also enjoys a variety of rather delightful sweets that include *roshgulla*, *sandesh*, *jilapi*, and a huge variety of milk-based sweets.

Bangladesh is a primarily Muslim country, which affects all aspects of its food culture. Food beliefs and practices result from religious beliefs (Kwon and Tamang 2015). For instance, Bengali cuisine will never feature any pork dishes, nor will one ever see wine as an ingredient in any dish. Religious beliefs and myths have immense influence on the traditional food culture of the country. During the Ramadan, Muslims fast during daylight hours, eating and drinking only before dawn and after sunset.

3.1.5 Ethnic Fermented Foods

Ethnic fermented foods and drinks are produced using native knowledge of locally available raw materials from plant or animal sources, either naturally or by adding starter cultures that contain microorganisms (Tamang 2010). Microorganisms are primarily functional bacteria, mostly lactic acid bacteria, yeasts, and filamentous molds, that cause biotransformation of the raw or cooked materials of plant or animal origin during fermentation, enhancing nutritional value; prolonging the shelf life; improving flavor, texture, and aroma; and also exerting several health-promoting benefits (Steinkraus 1996;

Table 3.1 Ethnic fermented foods and beverages in Bangladesh

Food category and name	Substrate	Nature of product	Microbes	Uses
1. **Fermented cereals**				
Jilapi	Wheat flour	Crispy sweet, deep-fried pretzels; snacks	Yeasts, LAB	Snacks
Amitri	Black lentils	Crispy sweet, deep-fried pretzels; snacks	Yeasts, LAB	Snacks
Pauruti	Wheat flour	Leavened bread	Yeasts	Breakfast
Pantavaat	Water-soaked rice	Rice	LAB	Breakfast
2. **Fermented/dry fish products**				
Chepa sukuti	Fish; dried fish	Curry	LAB, yeasts	Curry
Lonailish	Fish	Curry	LAB	Curry
Shidal	Fishes	Solid, bilaterally compressed paste	LAB *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp., yeast	Curry
3. **Fermented legumes**				
Papad	Daal	Thin ruti	*Candida krusei* and yeast	Cracker-like snacks
Kumrabori	Chaal Kumra and daal	Bori		Curry
4. **Fermented milk products**				
Dodhi/doi	Cow milk	Curd; savory	LAB, yeasts	Dessert
Ghee	Cow milk	Butter	LAB, yeasts	Cooking
Lassi	Cow milk	Milk; refreshing beverage	LAB, yeasts	Drinks
Matha	Liquid left behind after churning butter out of cream from cow milk	Buttermilk; refreshing beverage	LAB	Drink
5. **Beverage**				
Bangla maad	Rice	Liquid	Yeast	Drinks
Tari	Palm	Liquid	Yeast	Drinks
Borhani	Milk	Savory	LAB, yeast	Drinks
6. **Pickles**				
Aachaar	Different kinds of fruits and vegetables	Curry type product	*Leuconostoc mesenteroides*, LAB	Side dish
Chutney	Vegetables, fruit, and tamarind	Paste	*Leuconostoc mesenteroides*, LAB, yeast	Side dish/sauce for snacks

LAB lactic acid bacteria

Hansen 2002; Tamang et al. 2007). Fermented foods are generally palatable, safe, and nutritious (Campbell-Platt 1994; Geisen and Holzapfel 1996; Kwon 1994).

Without having any scientific knowledge about microorganisms, Bangladeshi local people developed many foods of their choice and know what needs to be done to get the desired food products. The microbiology, biochemistry, nutrition, functionality, toxicology, food safety, and biotechnology of their fermented foods are unknown to the producers, who are simply practicing an age-old food fermentation technology. Several different types of familiar ethnic fermented foods and beverages of the Bangladesh are listed in Table 3.1.

Fig. 3.2a *Jilapi*

3.1.6 Ethnic Fermented Foods and Beverages in Bangladesh

3.1.6.1 *Jilapi*

Jilapi is a traditional sweet dessert of Bangladesh that is formed in crunchy, sweet, deep-fried multiple circular shapes that are soaked in sugar syrup just after frying. The popularity of *jilapi* can be attributed to its attractive shape, crisp texture, and juicy mouth feel (Chakkaravarthi et al. 2009; Balaswamy et al. 2012). *Jilapi* is prepared by mixing wheat flour (*moyda*) with *doi* (curd), adding lukewarm water to make dough, and leaving it 8–10 h room temperature for fermentation. The thick leavened batter is squeezed through an embroidered hole (about 4 mm in diameter) in thick and durable cotton cloth, deposited as continuous spirals into hot edible oil, and fried on both sides until golden and crisp. After about few minutes, these are removed from oil with a sieved spatula and then submerged for several minutes in hot sugar rose-scented syrup, which saturates their hollow insides (Nivedita et al. 2013; Chakkaravarthi et al. 2009; Balaswamy et al. 2012; Rao et al. 2006) (Fig. 3.2a, 3.2b). People eat *Jilapi* all year round but consumption increases during the month of Ramadan (Muslim people fast 1 month in a year). The lactic acid bacteria (LAB) *Lactobacillus (Lb.) fermentum*, *Lb. buchneri*, *Lb. bulgaricus*, *Streptococcus (Strep.) lactis*, *Strep. thermophilus*, and *Enterococcus faecalis*, and the yeasts *Saccharomyces (S.) bayanus*, *S. cerevisiae*, and *Hansenula anomala*, are the microorganisms mainly responsible for fermentation (Batra and Millner 1976; Soni and Sandhu 1990).

Fig. 3.2b *Jilapi*

3.1.6.2 *Amriti*

Amriti is a dessert popular in Bangladesh that is made by deep-frying black lentils or mung dal flour batter in a kind of circular flower shape which then soaked in essence sugar syrup. This dish is not to be confused with jilapi, which is made of comparatively thinner material and is sweeter than *amriti* (Fig. 3.3a, 3.3b). Black lentils or *mung dal* are soaked in water for a few hours, stone-ground into a fine batter, and put aside for a few hours. The batter is then poured into oil or ghee in geometric patterns. Before frying the batter, sugar syrup is prepared, usually flavored with cardamom and saffron. The fried material is then dipped in sugar syrup until it expands in size and soaks up a significant amount of the syrup (Keshavrao 2013).

Fig. 3.3a *Amriti*

Fig. 3.3b *Amriti*

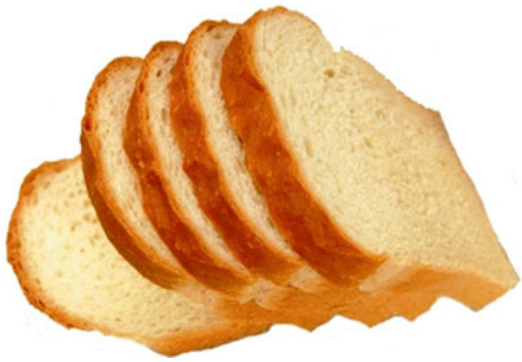

Fig. 3.4a Bread (sliced)

Fig. 3.4b Bread (*bonruti*)

3.1.6.3 *Pauruti*

Pauruti or bread (sliced form) is also used as a staple food in Bangladesh but it is comparatively expensive and it is essentially limited to the higher class, although there is another type of cheaper bread called *bonruti* (round soft bread) (Fig. 3.4a, 3.4b) that is more popular among people with less income such as rickshaw pullers. There are five simple ingredients: whole wheat flour, lukewarm water, fresh yeast, salt, and a small amount of sugar. All the ingredients are mixed and kept for 6–8 h, until the dough becomes double in size, then is poured into a box and baked in an oven. In case of *bonruti*, the dough is just made into a round shape and baked. The microorganism is yeast.

Fig. 3.5a *Pantavaat*

Fig. 3.5b *Pantavaat*

3.1.6.4 *Pantavaat*

Among all the fermented cereals, *pantavaat* (water-soaked rice) is the most popular food in Bangladesh in the mass of the people, especially the people living in rural areas. It is prepared by soaking boiled rice, generally leftover, in water overnight. Traditionally, it is served in the morning with salt, onion, and chili. It is also a very popular dish on the day of *Pohela Boishak* (Bengali New Year) (Fig. 3.5a, 3.5b) with hilsa fish, pickles, and dried processed fish (*shutki vorta*). There are many variations of the dish, but a common one is made by soaking cooked rice in water overnight. Rice is boiled the usual way, then the liquid starch is drained. The rice is cooled in air temperature for 3–4 h, then cool water is added so that about an inch of water rises above the rice. The rice is generally covered with a light piece of fabric. Twelve to 18 h later, *pantavaat* is ready to serve. Care must be taken to cover the dish during the long soaking to avoid contamination. The soaked rice is usually eaten in the morning with salt, lime, chili (either raw or roasted), and onions (sliced or whole), mostly for flavor. The water is discarded before consumption. During the soaking process, a small amount of alcohol is produced in *pantavaat* (Nasrullah 2003). The taste of *pantavaat* is a bit sour, which makes it more popular.

3.1.6.5 *Chepa Shutki*

Chepa shutki is a home-processed indigenous semi-fermented food, prepared from a small-sized fish. It is prepared by the traditional method of fermentation and it does not cost much or require any technical knowledge. The people prefer *chepa shutki* because of its characteristic taste and flavor, and also for its low cost compared with larger commercially important fish as table fish (Khanum et al. 1999; Mansur 2007). *Chepa shutki*, the most common processed food made from traditional small-sized fish commonly called *punti*, is very popular in Bangladesh (Khanum et al. 1999). Although *chepa shutki* serves as a cheap source of protein, knowledge about its nutritional quality is very limited.

Such foods are important for a number of reasons; for example, (i) they are important in nutrition for poor and economically deprived people, and (ii) they generally involve a low-cost processing method. For these reasons, semi-fermented foods are very important for a developing country such as Bangladesh. A large number of people of our country, especially those in the Mymensingh, Netrokona, Kishorgonj, Bhrammonbaria, Jamalpur, and Tangail regions, are engaged in the production and marketing of this semi-fermented product; this has a vital role in increasing their socioeconomic condition (Mansur 2007). Semi-fermented foods are mainly produced during winter season because of the availability of raw material, favorable weather conditions, and lower price (Nayeem et al. 2010).

Fig. 3.6a *Chepa shutki*

Fig. 3.6b *Chepa shutki*

On the basis of this product, a large marketing chain has developed in our country. *Chepa shutki* is a rich source of protein, and its protein contains a well-balanced amino acid composition (Kakati and Goswami 2013) (Fig. 3.6a, 3.6b).

3.1.6.6 *Lona ilish*

Lona ilish is a salt-fermented product from *hilsa* that is very popular in Bangladesh. The technology of *lona ilish* originated in Bangladesh about 100 years ago (Majumdar and Basu 2010). It is assumed that the technology evolved during the glut period when no preservation techniques except sun-drying and salting were available (Majumdar and Basu 2010). Sun-drying was not suitable for high-fat fishes such as *hilsa* because the fish rapidly becomes rancid on being exposed to the sun (Majumdar and Basu 2010). In addition, sun-drying was difficult during the continuous spell of rain in July–August that corresponds to the main glut period. It is very popular because of its typical flavor, aroma, and texture (Fig. 3.7a, 3.7b). *Lona ilish* is traditionally prepared by dry salting the diagonally cut *hilsa* chunks followed by fermentation in saturated brine in a metal container until the appearance of the characteristic flavor and texture. It is kept immersed in the fermenting medium until consumption. The fermentation period is around 3–4 months. Usually, *lona ilish* is washed several times with fresh plain water to reduce the salt concentration and then cooked to eat as curry with plain boiled rice (Majumdar and Basu 2010). The microorganisms involved during the fermentation of *lona ilish* are *Bacillus* spp. and *Micrococcus* spp. (Majumdar and Basu 2010).

Fig. 3.7a *Lona ilish*

Fig. 3.7b *Lona ilish*

3.1.6.7 *Shidal*

Shidal is a salt-free fermented fish product indigenous to Bangladesh. It is very popular because of its strong flavor. The appearance of the product

Fig. 3.8a *Shidal*

Fig. 3.8b *Shidal*

is solid, bilaterally pasty, and the shape of the fish remains almost unchanged except a little disintegration near the belly and caudal portion. The color of the best quality product is a dull white that gradually becomes slightly brownish to deep brownish on continuous exposure to air (Fig. 3.8a, 3.8b). The strong odor permeates the air in and around the storage area and gives the area a condensed and characteristic smell of *shidal*. Traditional *shidal* is prepared by raw small fish dried in the sun and then soaked in water (washing preferably in running water), followed by overnight drying at room temperature by spreading on a bamboo mat. Then the partially dried fish are filled in oil-smeared fermenting containers (*matk*a) up to the neck portion with strong compaction. The mouth portion is then sealed with a cover paste made from dry fish, and the cover paste is wrapped with paper or banana leaves and left undisturbed for 3–4 days before replacement of the cover leaf/paper with a thick layer of clay. Last, fermentation is allowed for 3–5 months at ambient temperature. The final product (*shidal*) is taken out after careful removal of the clay seal and cover paste (Majumdar et al. 2016) *Shidal* has a very short storage life once it is removed from the *mutka*. In the preparation of *shidal*, neither food additives/preservatives nor starter culture is added during the processing steps (Muzaddadi and Basu 2003). Several microorganisms such as LAB, *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp., and yeast are responsible for the fermentation of *shidal* (Ahmed et al. 2013).

3.1.6.8 *Papad*

Papad is a tortilla-like savory food in Bangladesh. This thin, wafer-like product is eaten as a crackly snack or appetizer with meals after roasting or deep-frying in oil. Black gram flour or a blend of black gram with Bengal gram, lentil (*Lens culinaris*), and red gram or green gram (*Vigna radiata*) flour is hand kneaded with a small quantity of peanut oil, common salt (about 8 %, w/w), 'papad khar' (salt warts produced by burning a variety of plant species, or from very alkaline deposits in the soil) and water, and then pounded into a stiff paste. The dough (sometimes spices are added) is left to ferment for 1–6 h. The fermented dough is shaped into small balls that are rolled into thin, circular flat sheets (10–24 cm diameter, 0.2–2 mm thick) and generally dried to 12–17 % (w/w) moisture content (Math et al. 2004; Aidoo et al. 2006; Kumar et al. 2011) (Fig. 3.9a, 3.9b). Open sun-drying is one of the most primitive methods of papad drying, and it is still practiced in Bangladesh despite the many

Fig. 3.9a *Papad* (dried)

Fig. 3.9b *Papad* (fried)

Fig. 3.10a *Kumrabori* (raw)

Fig. 3.10b *Kumrabori* (dried)

disadvantages associated with it. *Candida krusei* and *S. cerevisiae* are involved in the preparation of papad (Shurpalekar 1986).

3.1.6.9 *Kumra bori*

To prepare the *bori*, the necessary ingredients are pulse/grain legumes (*Mash Kolai dal*), white pumpkin (*Chaal Kumra*), water, and salt. *Bori*-making is a laborious process (Haider 2014). Pulses are soaked in water the night before so their skin loosens easily. The soaked pulses are pestled to a dough and mixed with grated, smashed white pumpkin, water, and salt proportionately to achieve the sticky texture required. After completing this process, *boris* are prepared and dried on clean, rough cloth or bamboo sheets in the sun. It takes 3–4 days to make these usable (Fig. 3.10a, 3.10b).

3.1.6.10 *Dodhi/Doi*

Dodhi/Doi (curd) is a popular fermented milk product of Bangladesh and is also used for the preparation of a number of other ethnic milk products such as *ghee*, *lassi*, and *matha*. Cow milk usually is boiled to evaporate the water, sugar is added according to desired sweetness; waiting to cool to lukewarm temperature is followed by adding some old dodhi/doi as a source of LAB and fermenting for 15–25 h to obtain dodhi/doi (Fig. 3.11a, 3.11b) (Tamang 2010). Along with taste, dahi has many health benefits (Lin and Yen 1999; Agarwal and Bhasin 2002) and, in the tropical climate, it has greater storability as compared to milk.

Fig 3.11a *Dodhi/doi*

Fig 3.12a *Ghee*

Fig 3.11b *Dodhi/doi*

Fig 3.12b *Ghee*

3.1.6.11 *Ghee*

Ghee is a butter locally prepared from cow's milk, with a typical flavor and aroma. *Dadhi* is churned to produce *ghee* (butter) and *matha* (buttermilk). The churning can be done in a number of ways. The most common and easiest method is by using a long bamboo vessel. *Dahi* is poured into the bamboo vessel and is churned by lifting and lowering of the *madani* inside the bamboo vessel [the *madani* is made of a long stick (*shar*) with a circular or star-shaped flat wooden disc (*pangra*) at one end]. The churning is done for 15–30 min, with the addition of either cold or warm water, as the weather demands, to facilitate better separation of the *ghee* from the liquid *matha/buttermilk*. In the process of this churning, a big lump of soft *ghee* is formed and seen floating on the *matha*. It is carefully lifted out with the hand and transferred to another vessel. Freshly prepared *ghee* is purified further by boiling until the oily liquid separates from the unwanted dark-brown precipitate (Fig. 3.12a, 3.12b). The purified *ghee* is used to prepare various foods including *pulao* (rice boiled with ghee and other spices), *shemai* (rice vermicelli), *jorda* (sweet colored rice), with mashed potato, etc. The LAB *Lactococcus lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* are the microorganisms used (Dewan 2002; Tamang 2010).

3.1.6.12 *Lassi*

In Bangladesh *lassi* is prepared by *dodhi/doi* blended with chill water, sugar, and a pinch of salt. Sometimes ice cubes are mixed with it (Fig. 3.13a, 3.13b). *Lassi* is very popular during the summer season as people become very thirsty as well as it is at Iftar time during holy Ramadan.

Fig 3.13a *Lassi*

Fig 3.14a *Matha*/buttermilk

Fig 3.13b *Lassi*

Fig 3.14b *Matha*/buttermilk

3.1.6.13 *Matha* (Buttermilk)

Buttermilk is named *matha* in Bangladesh. During *ghee* production the remaining liquid part is *matha* (Fig. 3.14a, 3.14b). *Matha* is consumed as a cooling beverage during hot days and also to overcome tiredness. Microorganisms such as the LAB *Lactobacillus alimentarius*, *Lactococcus lactis* subsp. *lactis*, and *Lc. lactis* subsp. *cremoris*, and the yeasts *Saccharomycopsis* spp. and *Candida* spp., are responsible for the production of buttermilk (Dewan and Tamang 2007).

3.1.6.14 Bangla *Maad*

Bangla *maad* is an alcoholic drink prepared from rice that is consumed in Bangladesh although at low frequency. Making Bangle *maad* at home takes several days: the necessary ingredients are glutinous rice, all-purpose flour, a yeast ball, and hot water (Chang 1995) (Fig. 3.15a, 3.15b). First, glutinous rice is put into a saucepan and the rice is covered with hot water and allowed to soak for 1 h. Then, drain the rice through a strainer and put the drained rice into a double-boiler with a lid or a steamer pan. Steam the rice for 25 min. Drain again and rinse the steamed rice with warm water. Return the rice to the saucepan. Put the ball of yeast into a bowl and mix the crumbled yeast with all-purpose flour. Add the yeast mixture to the warm rice and stir. Push the rice evenly against the sides of the pan, leaving a well in the middle. Cover the pan with plastic wrap. Place the pan lid over the plastic. Wrap the pan in a

Fig 3.15a *Bangla maad*

Fig 3.15b *Bangla maad*

Fig 3.16a *Tari*/palm wine

Fig 3.16b *Tari*/palm wine

heavy towel and place it in a warm place such as a water-heater closet. Allow to stand for 4–5 days. Spoon the rice into cheesecloth and squeeze the liquid into a jar. Pour the rest of the clear liquid to the jar, put the lid on, and refrigerate.

3.1.6.15 *Tari*

Palms are fermented to produce an alcoholic beverage called palm wine, or toddy, named *tari* in Bangladesh (Aidoo et al. 2006) There are three types of toddy: 'sendi,' obtained from wild date palm (*Phoenix sylvestris*); 'tari,' from palmyra palm (*Borassus flabellifer*), and date palm (*Phoenix dactylifera*); and 'nareli (nira),' from coconut palm (*Cocos nucifera*) (Batra and Millner 1974). There is an art in binding the flower spathes, pounding them to cause the sap to flow properly by cutting the spathe tip, and collecting the sap into the ear then into pitchers that contain yeasts and bacteria from left-over toddy from previous lots. The fermentation starts as soon as the sap flows into the pitcher. Palm wine is either consumed fresh as it is brought down from the tree or fermented for up to 24 h. The freshly cut sap is generally a dirty-brown sweet liquid having 10–18 % w/w sugar, which after fermentation results in the formation of a product containing as much as 9 % (volume in volume, v/v) ethanol (Steinkraus 1996). The palm wine fermentation is always an alcoholic–lactic–acetic acid fermentation, involving mainly yeasts and lactic acid bacteria. In the fermenting sap, *Saccharomyces (S.) cerevisiae* is invariably present, but lactic acid bacteria such as *Lactobacillus plantarum*, *Lactobacillus mesenteroides*, or other species of bacteria such as *Zymomonas mobilis* and *Acetobacte*r spp. can vary (Fig. 3.16a, 3.16b). The other yeast types include *Schizosaccharomyces pombe*, *Saccharomyces chevalieri*, *S. exiguus*, *Candida* spp., and *Saccharomycodes ludwigii* in the samples of coconut palm wine (toddy). *S. cerevisiae*, *S. pombe*, *Kodamaea ohmeri*, and *Hanseniaspora occidentalis* are characterized as the maximum ethanol producers in toddy (Joshi et al. 1999). The yeasts, especially *Saccharomyces* spp., are largely responsible for the characteristic aroma of palm wine (Uzochukwu et al. 1999).

3.1.6.16 *Borhani*

Borhani is a yogurt-like beverage that is very popular in parties where very rich food is served, especially at a wedding party in Bangladesh. It is mixed with several types of spices and herbs such as ground pepper, ground mustard, mint leaves paste, coriander leaves paste, cumin seeds, green chilies, rock salt, sugar, and water (Fig. 3.17a, 3.17b), followed by blending all the ingredients until they become smooth; it is then kept in the refrigerator to chill.

Fig 3.17a *Borhani*

Fig 3.17b *Borhani*

3.1.6.17 *Aachaar*

Aachaar are made from certain varieties of vegetables and fruits that are finely chopped and marinated in brine or edible oils along with various spices (Fig. 3.18a, 3.18b). Some varieties of fruits and vegetables are small enough to be used whole.

The most common pickles are made from mango and olive. Others include garlic and green or red chili peppers. A wide variety of spices may be used during the pickling process, such as asafoetida, red chili powder, turmeric, or fenugreek. Salt is generally used both for taste and for its preservative properties. Homemade pickles are prepared in the summer and kept in the sun while stored in porcelain or glass jars with airtight lids. The high concentrations of salt, mustard oil, and spices act as preservatives. Many commercially produced pickles use preservatives such as citric acid and sodium benzoate. Pickles made by traditional methods contain Lactobacillus, produced by fermentation in brine (salt and water) (Tamang 2010).

3.1.6.18 *Chutney*

Chutneys can be made from almost any combination of vegetables, fruits, herbs, and spices in Bangladesh (Fig. 3.19a, 3.19b). Chutneys are usually grouped into sweet or hot forms; both forms usually contain spices, including chilis, but differ by their main flavors. Vinegar, citrus, tamarind, or lemon juice may be added as natural preservatives, or fermentation in the presence of salt may be used to create acid. Chutneys may be ground with a mortar and pestle. Spices are added and ground, usually in a particular order; the wet paste thus made is sautéed in vegetable oil, usually sesame, or peanut oil. Electric blenders or food processors can be used as labor-saving alternatives to stone grinding. Spices commonly used in chutneys include fenugreek, coriander, cumin,

Fig 3.18a *Aachaar*

Fig 3.19a Coriandar *chutney*

Fig 3.18b *Aachaar*

Fig 3.19b Tomato *chutney*

and asafoetida. Other prominent ingredients and combinations include cilantro, capsicum, mint, tamarind, coconut, onion, tomato, red chili, green chili, garlic, peanut, dhaniya (coriander), and pudina (mint).

3.2 Conclusion

The fermentation technologies practiced by the people of Bangladesh reveal the strong coexistence of these people with nature and its utilization for survival advantages. Fermented food of both animal and plant origins is a daily component of the diet of the people of Bangladesh. Climatic conditions, in addition to the availability of raw materials, have a determinative major role in the type of fermented foods produced and consumed by the people. There is enormous scope for development of value-added ethnic fermented foods and beverages in Bangladesh by adopting appropriate methodologies directed toward (a) selection, propagation, and preservation of microbial strains, (b) genetic improvement of the strains and use of genetically modified organisms for enhanced productivity, (c) fermentation process improvement using enzymes and immobilized systems, (d) production and improvement of raw materials used for fermentation, and (e) the study of probiotic activity of the organisms.

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Ethnic Fermented Foods of Nepal

4

Tika Karki, Pravin Ojha, and Om Prakash Panta

4.1 Introduction

Nepal is located between latitude 26°22′ and 30°27′ north and longitude 80°04′ and 88°12′ east (Dahal et al. 2005a, b). Nepal has three broad ecological regions running from east to west having a total area of 147,181 km^2. The three belts are classified as Mountain, Hill, and Terai compromising 41 %, 36 %, and 23 %, respectively, of the total area (Kayastha 2012). Within the average 192 km width of the country, climatic condition ranges from subtropical in the south to arctic in the north (Kandel 2011). The population of Nepal is 26,494,504 with a population growth rate of 1.35 per annum. Terai constitutes 50.27 % (13,318,705) of the total population, while Hill and Mountain constitute 43 % (11,394,007) and 6.73 % (1,781,792), respectively. There are 126 caste/ethnic groups. Chhetri is the largest caste/ethnic group having 16.6 % of the total population followed by *Brahman*-Hill (12.2 %), *Magar* (7.1 %), *Tharu* (6.6 %), *Tamang* (5.8 %), *Newar* (5 %), *Kami* (44.8 %), *Musalman* (4.4 %), *Yadav* (4 %), and *Rai* (2.3 %). There are 123 languages spoken as mother tongue in Nepal. Nepali is spoken as mother tongue by 44.6 % of the total population followed by *Maithili* (11.7 %), *Bhojpuri* (5.98 %), *Tharu* (5.77 %), *Tamang* (5.11 %), *Newar* (3.2 %), *Bajjika* (2.99 %), *Magar* (2.98 %), *Doteli* (2.97 %), and *Urdu* (2.61 %). There are ten types of religion in Nepal. Hindu is followed by 81.3 % (21,551,492) of the population followed by Buddhism (9 %; 2,396,099), Islam (4.4 %; 1,162,370), Kirat (3.1 %; 807,169), Christianity (1.4 %; 375,699), Prakriti (0.5 %; 121,982), Bon (13,006), Jainism (3214), Bahai (1283), and Sikhism (609) (CBS 2012). Nepal is divided into four main physiographic zones, High Himal (above 5000 m), High Mountains (3000–5000 m) with alpine or subalpine climate, Mid-Hills (1000–3000) with temperate or subtropical climate, and Lowlands (below 1000 m) with tropical climate (MFSC/GEF/UNDP 2002). Nepal is a sovereign country which is rich in biodiversity and natural resources due to its diverse geography, ecosystem, and cultures. Nepal is in the top 25th and 11th position on biodiversity in the world and Asia, respectively (NINPA 2005). On the geographical scale, Nepal occupies only 0.03 % of the world while occupying 0.3 % of

T. Karki (✉)
Department of Biotechnology, Kathmandu University, Kathmandu, Nepal
e-mail: ktika@ku.edu.np

P. Ojha
Food Research Division, Nepal Agricultural Research Council, Lalitpur, Nepal
e-mail: projha84@gmail.com

O.P. Panta
Department of Microbiology, National College, Kathmandu, Nepal
e-mail: pantaop@gmail.com

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Asia, yet it harbors about 2 % of flowering plants, 3 % of pteridophytes, 6 % of bryophytes, 600 indigenous plant species, and 319 species of exotic orchids of world flora. It is the home to 8 % of the world's population of birds (more than 848 species), 4 % of all the mammals of the earth, and 11 % of the world's 15 families of butterflies (more than 500 species) (Weaver 2001). The various ethnic groups of Nepal consumed a variety of traditional fermented foods such as *masyura*, *kinema*, *gundruk*, *sinki*, *khalpi*, *dahi*, *jand*, etc., and non-fermented foods such as *khir*, *dhakani*, *khoa*, *chaku*, etc. (Dahal et al. 2005a, b). Some fermented foods and beverages of Nepal are shown in Table 4.1.

4.1.1 *Gundruk*

Gundruk is a non-salted fermented food product prepared by spontaneous lactic acid fermentation of leaves or seedlings of the Brassicaceae family, such as radish, cauliflower, rape, mustard, etc. *Gundruk* making seems to have been evolved as a means of preserving vegetables when they are plentiful in peak harvest season (Karki et al. 1983a; Kharel et al. 2010). *Gundruk* is dry in nature and a prized typical indigenous vegetable product and believed to have existed in the Nepalese culture since time immemorial (Karki et al. 1986; Kharel et al. 2010; Tamang 2010). It occupies an eminent place in the Nepalese diet

Table 4.1 Ethnic fermented foods and beverages of Nepal

Foods	Substrates	Nature and uses	Microorganisms	Regions of consumption in Nepal	References
Gundruk	Leaves or seedlings of the Brassicaceae family	Dry solid, curry and pickle	Lactic acid bacteria	All over Nepal	Karki et al. (1983a)
Sinki	Radish	Dry solid, curry and pickle	Lactic acid bacteria	All over Nepal	Ojha and Katuwal (2009)
Tama/*mesu*	Bamboo shoot	Sticky solid, curry	Lactic acid bacteria	Hill region and inner Terai of Nepal	Dahal (2014)
Khalpi	Cucumber	Wet oily solid, pickle	Lactic acid bacteria	Mid- and eastern region of Nepal	Maharjan (2014)
Murcha	Rice, wheat	Round white cakes, green chunks	Molds, lactic acid bacteria, ethanol-fermenting yeast		Rai and Subba (2003)
Jaand	Millet, rice, buck wheat	Turbid liquid, alcoholic beverage	*Aspergillus oryzae*, lactic acid bacteria, ethanol-fermenting yeast	All over Nepal	Kharel et al. (2010)
Hyaun thon	Rice	Red turbid liquid, undistilled alcoholic beverage	*Aspergillus oryzae*, *Rhizopus* spp., lactic acid bacteria, ethanol-fermenting yeast	Mainly Kathmandu valley	Dangol (2006)
Rakshi	Millet, buck wheat	Clear liquid, distilled alcoholic beverage	Saccharifying molds, lactic acid bacteria, ethanol-fermenting yeast	All over in Nepal	Rai (1991a, b)
Kinema	Soybean	Solid and sticky curry, soup	*Bacillus* spp.	Eastern Nepal	Karki (1986a); Tamang (2010)

and is eaten with great relish. *Gundruk* preparation is generally confined to household level and purpose. A crude estimate reveals that only about 2% of *gundruk* is produced in commercial amounts. *Gundruk* made from the mustard leaves is believed to be the best and also supposed to have medicinal value to many people (Kharel et al. 2010).

4.1.1.1 Methodology of *Gundruk* Preparation

The typical process of *gundruk* making is shown in Fig. 4.1. For the production of *gundruk*, leaves of *rayo sag* (*Brasicca rapa* subspecies *campestris* variety *cuneifolia*), mustard (*Brasicca juncea*), cauliflower (*Brasicca oleracea* variety *botrytis*), radish leaves, and cabbage (*Brasicca oleracea* variety *capitata*) are wilted, shredded, crushed mildly, and pressed into an earthen jar or container. It is then pressed by a wooden ladle and made airtight. Then these leaves are fermented naturally for about 7–10 days till acidic flavor is developed. Freshly fermented *gundruk* is removed from the jar and sun dried for 3–4 days and is stored for 2 years or more (Karki et al. 1983a, b, c, d; Shrestha 2010; Tamang 2010).

Gundruk is eaten as a soup or pickle (Fig. 4.2). Soup is made by soaking *gundruk* in water for 10 min, and then water is squeezed out and fried with chopped tomatoes, onions, chilies, turmeric powders, and local spices along with soybean. Now the boiled mass is served hot with steamed

Green mustard leaves or any other leafy vegetables kneaded by hand

↓

Placed in earthen pot (narrow mouthed)

↓

Pressed tightly by wooden ladle or handle up to the very neck of the pot. Straw is put a top of the cover

↓

Placed in the pot for sun-drying during day-time

↓

Placed nearby over (mud) fired by wood during night, warm water frequently added to the pot so as to keep the mass warm

↓

The process repeated until the fermentation is complete (5-7 days)

↓

Dried in sun by spreading

↓

Gundruk (dry)

Fig. 4.1 Traditional process of *gundruk* making (Karki 1986a)

Fig. 4.2 Dried *gundruk*

rice. *Gundruk* soup is a good appetizer in a bland and starchy diet. Similarly, lemon juice, salt, and oil along with chopped potatoes and fried soybean are mixed to make pickle (Karki 1986a, b; Upadhyaya 2002; Tamang 2010).

4.1.1.2 Nutritional Aspects of *Gundruk*

Karki et al. (1983b) reported 0.8 % and 1 % acidity as lactic acid in mustard *gundruk* and cauliflower *gundruk*, respectively, after fermentation for 7 days at 20–22 °C. Palmitic acid (26.8 %) was found to be the dominant fatty acid followed by linoleic (13.9 %), linolenic (12.8 %), and oleic (10.3 %) in mustard *gundruk*. However, cauliflower *gundruk* consists of linolenic acid (28.9 %) as the dominant fatty acid followed by palmitic acid (20.6 %). Karki et al. (1983c) reported the total nitrogen of mustard *gundruk* and cauliflower *gundruk* to be 3.38 % and 1.56 %, respectively. Glutamic acid (13.6 %) was found to be dominant in mustard *gundruk*, whereas proline (15.2 %) was dominant in cauliflower *gundruk*. Karki et al. (1983d) reported that the dominant flavor compounds in mustard *gundruk* are cyanides (15.7 %) followed by alcohols (12.3 %), isothiocyanates (8.5 %), and esters (4.1 %). However, cauliflower *gundruk* consists of alcohols (50 %) as the major flavor compounds followed by cyanides (6.5 %), isothiocyanates (6.1 %), and esters (3.2 %). Tamang (2006) reported moisture, pH, acidity, ash, protein, fat, carbohydrate, calcium, sodium, and potassium of gundruk to be 15 %, 5.0, 0.49 % as lactic acid, 22.2 % DM (dry matter), 38.7 % DM, 2.1 % DM, 38.3 % DM, 234.6 mg/100 g, 142.2 mg/100 g, and 677.6 mg/100 g, respectively. Shrestha (2010) reported moisture, crude fiber, ash, calcium, and iron content of *gundruk* made from cabbage to be 7.92 %, 14.65 g/100 g, 0.68 g/100 g, 2253 mg/100 g, and 86.4 mg/100 g, respectively. The acidity was found to be 1 % as lactic acid.

4.1.1.3 Biochemical Changes During *Gundruk* Fermentation

Karki et al. (1983a) reported that pH changed from initial value of 5.0 to 4.0, 4.3 and 4.1 in mustard, rape and radish leaves *gundruk* respectively, after 6 days of fermentation. However acidity increased from 0.3 % to 1 % as lactic acid for mustard *gundruk*, 0.5–0.8 % as lactic acid for rape leaves *gundruk*, and 0.45–0.9 % as lactic acid for radish leaves *gundruk*. Karki et al. (1986) found that *L. plantarum*, *P. pentosaceus*, and *L. cellobiosus* change pH from 6.2 to about 4.5 in 6 days of fermentation of komatsuna vegetable, reducing sugar to less than 0.1 % from 0.4 %, and acidity from less than 0.1 % as lactic acid to 0.8 %, 0.55 %, and 0.48 %, respectively, in the same vegetable.

Karki et al. (1983b) found that palmitic acid increased from 23.1 % to 26.8 % in mustard leaves *gundruk* fermentation, but there is slight change in oleic acid, linoleic acid, and linolenic acid. Karki et al. (1983c) found that percentage of nitrogen decreased from 3.56 to 3.38 % during mustard leaves fermentation for 6 days. Glutamic acid increased from 3.4 to 13.6 %, whereas asparagine decreased from 17.8 % to 1.2 % in mustard leaves fermentation for 6 days. Similarly, valine, cystine, methionine, glycine, alanine, and lysine increased during fermentation of mustard leaves, but serine, glutamine, proline, and tyrosine were found to decrease during the same fermentation. Karki et al. (1983d) reported an increase in cyanides and esters from 1.9 % to 15.7 % and 1 to 4.1 %, respectively, with a decrease in alcohol from 28.8 to 12.3 % in mustard *gundruk* fermented for 6 days.

4.1.1.4 Microbiology of *Gundruk*

Karki et al. (1983a) found *Pediococcus* and *Lactobacillus* as main two genera in mustard leaves *gundruk* fermentation. Homofermentative

Lactobacilli were identified as *L. plantarum*, *L. casei* subsp. *casei*, and *L. casei* subsp. *pseudoplantarum*. Authors identified heterofermentative lactobacilli which were found to be *L. cellobiosus* and also identified heterofermentative tetrads as *P. pentosaceus*. Authors found that heterofermentative strains carry out fermentation for initial 3 days; however, after that, the homofermentative strains dominate. They also found that *L. plantarum* and *P. pentosaceus* mainly carried out *gundruk* fermentation and to lesser extent by *L. cellobiosus*. Tamang et al. (2005) found the lactic acid bacteria (LAB) count log to be 7.3 cfu/g. Authors identified 95 strains from *gundruk*, among which 22 strains were homofermentative rods and 73 were tetrad-forming cocci.

4.1.1.5 Improvements in *Gundruk* Processing

Karki et al. (1986) found that *gundruk* prepared with *L. plantarum* gives the highest amount of lactate compared to *gundruk* fermented with *P. pentosaceus* and *L. cellobiosus*, whereas *gundruk* fermented with *P. pentosaceus* yields the highest amount of acetate. The authors found that *P. pentosaceus* among the above three strains in a single fermentation was smaller in the second day and reached the peak on the fourth day and thereafter slowly decreased, whereas the remaining two strains were maximum within 2 days and decreased thereafter. The synergistic action of all three species is required for desirable flavor, aroma, and stability of *gundruk*.

4.1.2 *Mesu/Tama*

Bamboo shoots are tender bamboo plants which are 20–30 cm long and narrow to a point (wt >1 kg), and their size and weight depend mainly upon the location, depth, and nutrition of soil watering and drainage conditions, rainfall, temperature, and soil fertility (Farrelly 1984). The emerging fresh young bamboo shoots are harvested and used as vegetables. They are used in numerous Asian dishes and are available in markets in various sliced forms, fresh, fermented, and canned version (Midmore 1998). The fermented form of bamboo shoots was used as a highly prized vegetable by the Asian countries (Yamaguchi 1983). *Mesu/tama* (Fig. 4.4) is a traditional fermented bamboo shoot used for curry and pickle in different parts of Nepal and India. *Tama* is a non-salted naturally fermented food product from tender bamboo shoots (DFTQC 2001). The word *mesu* is directly derived from the *Limbu* culture where "me" means the young bamboo shoot and the "su" means sour taste (Tamang 1998).

4.1.2.1 Nutritional Composition of Bamboo Shoot

Bhatt et al. (2003) reported that moisture content, protein content, and carbohydrate content of four edible bamboo shoots range from 75.5 % to 87 %, 3.56 to 3.9 %, and 5.23 to 6.12 %, respectively. Similarly, NMBA (2009) published the composition of seven edible bamboo shoots and reported that moisture, protein, and carbohydrate contents range from 85.98 % to 92.37 %, 1.98 to 3.29 %, and 3.83 to 9.94 %, respectively. NMBA (2009) reported that hydrocyanic acid was found to be from 0.032 to 0.13 %, and Choudhary et al. (2010) reported that maximum amount is at the tip and lowest amount at the base for almost all edible varieties.

4.1.2.2 Methodology of *Mesu* Preparation

People living in Nepal, India, and Bhutan consume varieties of domesticated and wild bamboo shoot fermented products (Sharma 1989). The products prepared by fermentation of bamboo shoot are named differently in different regions. The methodology of preparation of *mesu/tama* is shown in Fig. 4.3.

During preparation, tender shoots of bamboo (*Dendrocalamus sikkimensis*, *D. hamiltonii*, *Bambusa tulda*, etc.) are collected, their outer hard casings are removed, and the inner portion is then chopped into small pieces with a knife. The chopped pieces are washed thoroughly with clean water, drained off, and pressed tightly into a bamboo-made cylindrical vessel. This vessel is

Fig. 4.3 Traditional process of *mesu*/*tama* making (DFTQC 2001; Tamang et al. 2009)

Young bamboo shoot

↓

Peel outer edible coating

↓

Chopped the inner part, wash with water, cut in pieces

↓

Press tightly into vessel, make air tight

↓

Fermentation for one month to two years(depend on locality)

↓

Mesu/Tama

made airtight with a lid, placed in an upside-down position to drain out any liquid, and allowed to ferment under natural anaerobic conditions for 7–15 days. Fermented product is eaten as a curry, pickle, or soup. It is sold in the local markets during the months of July and September, when young bamboo tender shoots are plenty, and they are preserved by the method of fermentation by some rural women who are dependent upon this product for their livelihood (Sarngthem and Singh 2003; Kharel et al. 2010).

4.1.2.3 Nutritional Composition of Fermented Bamboo Shoot

DFTQC (2001) reported that acidity of traditionally fermented *mesu* and LAB-fermented *mesu* was 0.94 % and 1.26 %, respectively, fermented for 15 days. Singh et al. (2011) reported the acidity of samples (*soibum*, fermented bamboo shoot similar to *mesu*) collected from Manipur, India, to be 1.34 %. Dahal (2014) reported that the acidity ranges from 1.03 % to 1.26 % as lactic acid of six different samples of *tama* collected from different locations. Singh et al. (2011) reported the crude protein, crude fiber, ash, crude fat, and sugar contents of two samples collected from Manipur, India, to be 1.35–2.76 %, 0.58–0.67 %, 0.11–0.12 %, 0.09–0.1 %, and 1.14–1.72 %, respectively. Similarly, vitamin C and riboflavin were found to be 170.17–174.7 mg/g and 11.23–13.55 mg/g, respectively. Further, calcium and iron were found to be 1283.5–1463.8 ppm and 189.4–219.3 ppm, respectively.

Nirmala et al. (2008) found protein, carbohydrate, and vitamin C to be 2.17 g/100 g, 1.504 g/100 g, and 1.09 g/100 g, respectively, in fermented bamboo shoot. However Dahal (2014) found that total sugar, crude fiber, and total ash range from 15.61 % to 19.66 %, 20.71 to 31.11 %, and 7.24 to 9.1 %, respectively, for six samples of *mesu* collected from six different locations of Nepal. Similarly, Dahal (2014) found that calcium and iron for six samples of *mesu* range from 203.08 to 340.81 mg/100 g and 154.59 to 210.5 mg/100 g, respectively.

Sarangthen and Singh (2013) found phytate, saponin, tannin, alkaloid, and hydrogen cyanide of traditional fermented samples (undefined condition for 60 days) to be 22.46 ± 1.19, 76.47 ± 0.31, 68.21 ± 0.55, 0.34 ± 0.01, and 33 ± 2.65 mg/100 g, respectively, and for LAB-fermented samples (30 °C ± 2 °C for 60 days) to be 24.12 ± 0.12, 84.21 ± 1.53, 52.00 ± 1.58, 0.63 ± 0.03, and 30.23 ± 0.05, respectively. Dahal (2014) reported that phytate and tannin of fermented bamboo shoot were 23.11 ± 0.05 mg/100 g and 52.76 ± 0.05 mg/100 g, respectively.

4.1.2.4 Biochemical Changes During Fermentation

DFTQC (2001) reported change in acidity from 0.25 % to 1.29 % as lactic acid during 10 days of fermentation. Singh et al. (2011) reported that acidity changes from 0.835 % to 1.34 % as lactic acid in 55 days of bamboo shoot fermentation. Dahal (2014) reported that acidity increases from

Fig. 4.4 Fermented bamboo shoot (*tama*/*mesu*)

0.143 % to 0.963 % as lactic acid in 25 days of fermentation. Similarly he found that pH decreases from 6.1 to 3.8 and reducing sugar decreases from 6.53 to 1.36 mg/100 g, respectively. The phytate content of 34.81 ± 0.01 mg/100 g in fresh raw bamboo shoot of *D. hamiltonii* was found to reduce to 23.11 ± 0.05 mg/100 g fresh weight, whereas tannin content of 44.45 ± 0.05 mg/100 g fresh weight in fresh bamboo shoot increases to 52.76±0.05 mg/100 g fresh weight. Sarangthen and Singh (2013) reported that phytate, saponin, tannin, alkaloid, and hydrogen cyanide in traditional fermented sample (undefined condition for 60 days) change from 35.95 ± 1.17, 95.32 ± 0.55, 45.49 ± 0.67, 0.87 ± 0.07, and 224 ± 6.51 mg/100 g to 22.46 ± 1.19, 76.47 ± 0.31, 68.21 ± 0.55, 0.34 ± 0.01, and 33 ± 2.65 mg/100 g, respectively, and in LAB-fermented sample (30 °C ± 2 °C for 60 days) from 30.67 ± 0.69, 103.32 ± 1.58, 31.49 ± 1.50, 0.98 ± 0.06, and 317 ± 6.03 mg/100 g to 24.12 ± 0.12, 84.21 ± 1.53, 52.00 ± 1.58, 0.63 ± 0.03, and 30.23 ± 0.05 mg/100 g, respectively.

4.1.2.5 Microbiology of *Mesu* Fermentation

DFTQC (2001) reported that LAB count increased from 4×10^2 to 32×10^2 in 10 days during fermentation of bamboo shoot. Dahal (2014) reported a maximum LAB count of 7.66 ± 0.777 log cfu/g in 7 days of fermentation and this decreases to 1.98 ± 0.67 log cfu/g in 25 days of fermentation of bamboo shoot. Similarly, LAB count was found in the range from 0.66 to 1.33 log cfu/g for six different samples from different locations of Nepal. Tamang and Sarkar (1993) reported that *Lactobacilli* species such as *L. plantarum*, *L. pentosus*, *L. brevis*, and *L. fermentum* were found in fermented bamboo shoot products. They isolated a total of 327 strains of lactic acid bacteria, representing *L. plantarum*, *L. brevis*, and *Pediococcus pentosaceus* from *mesu*. *Mesu* was dominated by *L. plantarum* followed by *L. brevis*, whereas *P. pentosaceus* was found only in 40–50 % of *mesu* samples. The fermentation was initiated by *P. pentosaceus*, followed by *L. brevis*, and finally succeeded by *L. plantarum* species. Tamang et al. (2008) determined the microbial load on various types of fermented bamboo shoots of the Indian region and found that the total viable count is within the range of 1.66–2.66 log cfu/ml with no significant difference among samples.

4.1.3 *Sinki*

Sinki is a traditional, Nepalese non-salted vegetable product eaten as a good appetizer. According to Nepal Brihat Sabdakosh (1983), the word *sinki* is a curry product developed by souring the radish in the pit and earthen pot. *Sinki* as being strictly a product of Nepal differs significantly from Japanese blanched "*sunki*." In fact, *sinki* has its own traditional charm in both product and making process. *Sinki* (Fig. 4.6) is believed to have existed in Nepalese culture since time immemorial. It is valued for its uniquely appetizing flavor (Steinkraus 1996). The quality of *Sinki* was judged on the basis of acidic taste and typical *sinki* flavor. These two characteristics have been embodied as key indicators of quality. However, the final acidity ultimately depends on the indigenous flora present in vegetables (Shrestha 2002). *Sinki* is more popular among the fermented foods of Nepal. One of the reasons of *sinki* being so popular is because of its raw material "radish" which can be easily cultivated in all seasons in Hilly and Terai region and does not require any specific fertilizer and soil to grow. *Sinki* is a dried food product with pH much below 4 so it is an appetizing product (Rai 1991a, b). The quality of *sinki* has been primarily judged based on the acidic taste and typical *sinki* flavor. However, the quality of *sinki* mainly depends upon the balanced proportion of lactic acid and acetic acid, which is highly desirable to maintain stability in the product (Karki 1986a, b).

4.1.3.1 Nutritional Aspects of Radish

The usually cultivated variety is white radish with a conical shape and which is very pungent (Manay and Shadaksharaswamy 2001). The sharp taste of radish is due to 4-methylthio-trans-3-butenyl-isothiocyanate, which is released from the corresponding glucosinolate after radish is sliced (Burghagen et al. 1999). Shrivastav and Kumar (2002) reported that radish is a high-moisture food with a moisture content of 94.9 %, carbohydrate content of 3.2 %, fiber content of 0.6 % fiber, and mineral content of 0.7 %. Joshi and Sharma (2009) reported titratable acidity, total sugar, and ascorbic acid of radish to be 0.009 % (as lactic acid), 2.04 %, and 12.8 %, respectively. According to Ojha (2007), the acidity of radish was found to be 0.625 % as lactic acid in dry basis.

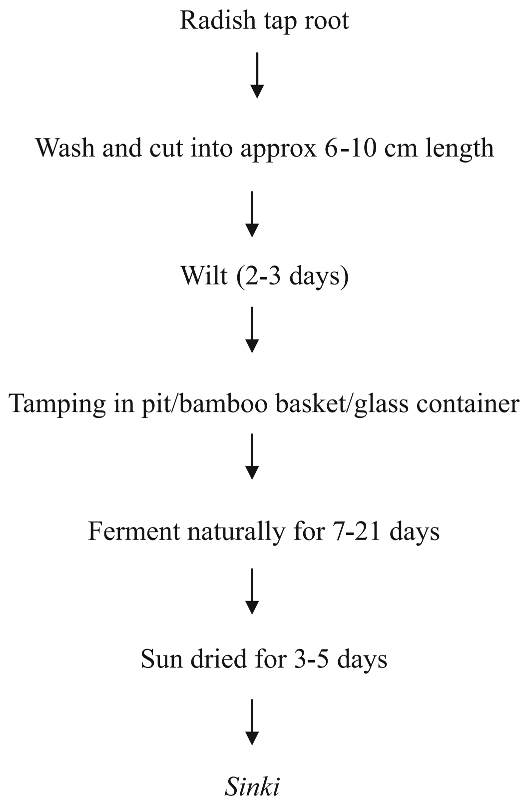

Fig. 4.5 Traditional process of *sinki* making (Ojha 2007; Kharel et al. 2010; Tamang 2010)

4.1.3.2 Methodology of *Sinki* Preparation

Radish is the principal raw material for *sinki* making. In strict sense, *sinki* is prepared only from radish and not from its leaves and other vegetable leaves. It is a sour pickle prepared from radish (Regmi 1982). This is a form of fermented radish (*Raphanus sativus*) tap root and is consumed in the Nepal and in Darjeeling and Sikkim in India. It is prepared during the months of winter when weather is least humid, and there is ample supply of this vegetable (Tamang and Sarkar 1993) (Fig. 4.5).

Sinki making tradition has been equally popular in both the Terai and Hill regions of Nepal. As

Fig. 4.6 Dried *sinki*

far as the type of raw material is concerned, it has been found that *sinki* made from radish in hilly areas is more acceptable. The reason might be due to the compact body and richness in juice as compared to radishes in the Terai region. *Sinki* making process is quite simple and easily conducted by Nepalese villagers. At first, moderately matured radishes with leaves are manually cleaned by removing mud and other foreign matters. Radishes are then withered in the sun for 1 day. Sun drying helps to tender radish, which in turn facilitates filling in the container. Leaves are detached from withered radishes and cleaned again in potable water to remove the remaining mud and foreign matters. Radishes are then crushed under *dhiki* or in between stones. Crushing results in mashing and tearing of compact body of radish into thin flat irregular pieces. It has been found that, usually, radishes of poor quality are used by the villagers for *sinki* making, while good-quality radishes are sold in the market (Shrestha 2002; Ojha 2007; Tamang and Sarkar 1993).

It is consumed as soup and pickle. *Sinki*, with a highly acceptable and attractive flavor, is typically used as a base for soup and as pickle. The soup is made by soaking *sinki* in water for about 2 min, squeezing out the liquid, and frying along with salt, tomato, onion, and green chili. The fried mixture is boiled in rice water. The soup is served hot along with main meals. It is said to be a good appetizer, and people use it for remedies for indigestion. Pickle is prepared by soaking *sinki* in water, squeezing, and mixing with salt, mustard oil, onion, and green chili (Tamang and Sarkar 1993; Kharel et al. 2010; Tamang 2010).

4.1.3.3 Nutritional Aspects of *Sinki*

Tamang and Sarkar (1993) reported 14.6 % protein, 2.5 % fat, 11.5 % ash, acidity 0.72 % as lactic acid, calcium 120.5 g/100 g, potassium 443.1 mg/100 g, and iron 18 mg/100 g in *sinki* on dry basis. Shrestha (2002) reported moisture, ash, crude fiber, acidity (dry matter), protein, fat, calcium, and iron of fresh *sinki* to be 81 %, 14.1 % DM, 51 % DM, 11.1 % as lactic acid (dry matter), 2.8 % DM, 1.1 % DM, 865 mg/100 g, and 105 mg/100 g. Tamang (2006) reported moisture, acidity, ash, protein, fat, carbohydrate, calcium, sodium, and potassium of *sinki* to be 22.8 %, 0.65 % as lactic acid, 14.9 % DM, 1.4 % DM, 68 % DM, 223.9 mg/100 g, 737.3 mg/100 g, and 2329.4 mg/100 g, respectively. Ojha and Katuwal (2009) reported moisture, ash, crude fiber, acidity, protein, fat, calcium, and iron of *sinki* to be 50 %, 13.6 % DM, 52.8 % DM, 13 % as lactic acid, 1.8 % DM, 1.8 % DM, 785 mg/100 g, and 96 mg/100 g. Similarly, the water activity of prepared *sinki* was 0.85.

4.1.3.4 Microbiology of *Sinki*

Natural fermentation of *sinki was* initiated by heterofermentative *L. fermentum*, followed by another heterofermentative *L. brevis*, and finally succeeded by homofermentative *L. plantarum* (Tamang and Sarkar 1993). *Leuconostoc fallax* was also reported with other lactics from *sinki* (Tamang et al. 2005). Ojha and Katuwal (2009) reported that LAB count reduced from 5.2 to 3.2 log cfu/g after exposure of *sinki* to steam for 20 min. Joshi and Sharma (2009) reported bacterial count and fungi count to be 36×10^3 cfu/g and 66 cfu/g in fermented radish with 2.5 % salt added.

4.1.3.5 Biochemical Changes During *Sinki* Fermentation

Tamang and Sarkar (1993) reported that pH of *sinki* drops from 6.72 to 3.3 in 10 days, whereas acidity increases from 0.04 % to 1.28 % as lactic acid after the same days of fermentation. Similarly Joshi and Sharma (2009) reported an increase in TSS from 6 to 8° bx in 9 days and finally drop to 6.5° bx on further fermentation of radish initiated with addition of 2.5 % salt. Similarly pH decreases from 7 to 4 after 18 days of fermentation and titratable acidity as lactic acid from 0.01 % to 0.6 % after the same period of fermentation.

4.1.4 *Khalpi*

Khalpi is one of the important indigenous fermented cucumber (*Cucumis sativus*) products commonly consumed in Nepal and Sikkim, India. *Khalpi* (Fig. 4.8) is a non-salted fermented cucumber (*Cucumis sativus* L.) product, consumed by the Brahmin Nepali in Nepal, Darjeeling hills, and Sikkim. It is the only reported fermented cucumber product in the entire Himalayan region (Kharel et al. 2010; Tamang 2010). For preparation of *khalpi*, matured and ripened cucumbers are used (Tamang 2001). *Khalpi* is a pickle which is mostly prepared from local varieties of cucumber, which are jumbo large in size (50 cm × 20 cm, length × diameter). Salt and mustard powder are also added which selectively control undesirable microorganisms but allow the growth of lactic acid bacteria (LAB). The predominant LAB in *khalpi* is *L. fallax*, *P. pentosaceus*, *L. brevis*, and *L. plantarum* (Tamang et al. 2005). The prepared *khalpi* is sour in taste. It is a good appetizer, adds palatability, and complements the Nepali meal (*dal-bhat-tarkari*) (Kharel et al. 2010).

4.1.4.1 Nutritional Aspects of Cucumber

Fully ripened cucumbers are used for preparing the *khalpi* (Maharjan 2014). The estimated figures for cultivated area, yield, and production of cucumber in Nepal during 2011/2012 were 8970 ha, 13.8 t/ha, and 127,918 metric tons, respectively (MOAD 2013). Cucumbers are composed of 91–97 g of water, 0.3–0.5 g of fiber, 1.8–2.6 g of sugar, 0.3–0.5 g of ash, 0.2 g of fat, 0.6–1.4 g of protein, 0–0.04 mg of carotene, 0.02–0.1 mg of thiamine, 8–19 mg of ascorbic acid, 0.02–0.1 mg of riboflavin, 0.1–0.6 mg of niacin, 6 mg of folic acid, 15.23 mg of calcium, and 0.3–0.8 mg of iron (Pierce 1987). Maharjan (2014) reported moisture, acidity, reducing sugar, and total sugar of cucumber as 95.35 %, 0.12 % as lactic acid, 2.61 %, and 2.89 %, respectively.

4.1.4.2 Methodology of *Khalpi* Preparation

During *khalpi* preparation, ripened cucumber is collected from the field and cut into pieces and is sun dried for 2 days. Now the cut piece of cucumber is seasoned with salt, mustard powder, turmeric, and chili. It is then tamped into a bamboo vessel and made airtight by covering with dried leaves or oil. It is fermented naturally at room temperature for 3–7 days. The completion of fermentation is confirmed by the typical sour flavor and characteristic color. It is prepared during September/October. It is served with steamed rice as pickle (Tamang 2010; Kharel et al. 2010; Maharjan 2014) (Fig. 4.7).

4.1.4.3 Nutritional Aspects of *Khalpi*

Tamang (2006) reported moisture, acidity, ash, fat, protein, carbohydrate, calcium, sodium, and potassium of *khalpi* to be 91.4 %, 0.95 %, 14.2 %

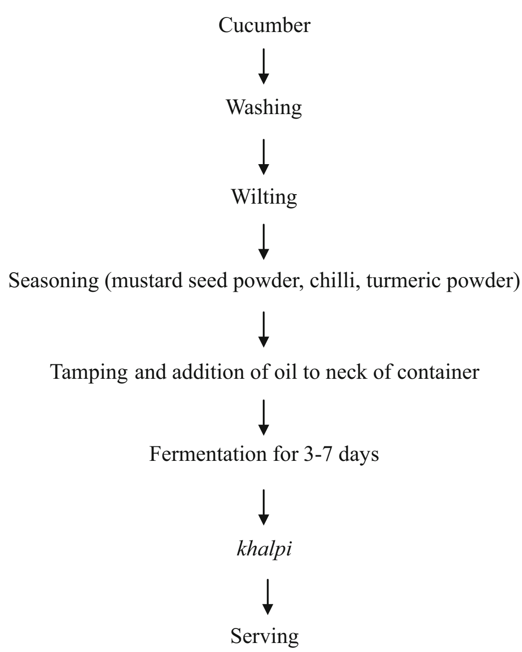

Fig. 4.7 Traditional process of *khalpi* making (Kharel et al. 2010; Maharjan 2014)

Fig. 4.8 *Khalpi* and cucumber (Maharjan 2014)

DM, 2.6 % DM, 12.3 % DM, 70.9 % DM, 6.4 mg/100 g, 2.2 mg/100 g, and 125.1 mg/100 g, respectively. Maharjan (2014) reported that titratable acidity as lactic acid of *khalpi* fermented spontaneously and pure culture was found to be 0.97 % and 1.13 %, respectively, for sample fermented in sunlight for 20 days; however, for sample fermented in the room, the acidity was 0.93 % and 1.02 %, respectively. Reducing sugar and total sugar of spontaneous fermented *khalpi* and pure culture fermented *khalpi* were 0.46 %, 0.63 %, 0.44 %, and 0.46 %, respectively, for fermentation carried out in the sun (Maharjan 2014) (Fig. 4.8).

4.1.4.4 Microbiology of *Khalpi*

Heterofermentative LAB such as *L. fallax*, *L. brevis*, and *P. pentosaceus* are isolated from the initial fermentation stage of *khalpi*. As the fermentation progresses, more acid-producing homofermentative lactobacilli mainly *L. 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 (McDonald et al. 1991). Tamang et al. (2005) reported LAB count of 7.4 log cfu/g in *kahlpi*. The predominant LAB in *khalpi* were *L. fallax*, *P. pentosaceus*, *L. brevis*, and *L. plantarum* (Tamang et al. 2005). *L. plantarum*, *L. brevis, and Leuconostoc fallax* are the major microbes in *khalpi* (Tamang and Tamang 2010). The population of LAB in raw cucumber was very small (10^3 cfu/g) during in situ fermentation that increases significantly to 10^8 cfu/g within 36 h and then remains at the level of 10^7 cfu/g in the final product. Maharjan (2014) reported yeast count of 0.2 log cfu/g in fresh sample of *khalpi* after 14 days of fermentation but did not detect mold. Similarly, LAB count was 10^7 cfu/g after 6 days of fermentation.

4.1.4.5 Biochemical Changes During *Khalpi* Fermentation

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 (McDonald et al. 1991). Tamang and Tamang (2010) reported an increase in titratable acidity from 0.28 % as lactic acid to 1.24 %, whereas there was a drop in pH from 5.6 to 3.2. Maharjan (2014) reported that the titratable acidity of cucumber increases from 0.12 % as lactic acid to 0.4 % after 2 days and finally to 1.1 % after 20 days; however the pH of *khalpi* decreases from 5.73 to 3.5 after 20 days. Reducing sugar of *khalpi* decreases from 2.61 % to 0.4 % after 20 days, whereas the total sugar drops from 2.89 % to 0.6 % after 20 days of fermentation.

4.1.5 *Marcha*

The indigenous starters used for the production of alcoholic beverages in Nepal and certain territories of India are known as *marcha* or *murcha. Marcha* is the Nepali word; the *Lepchas* and the *Limbus* (ethnic group of Nepal) use the word *thamik* and *kesung*, respectively, to the same. *Marcha* is prepared mostly by *Rai*, *Limbu*, *Tamang*, *Gurung*, *Newar*, and *Tharu* communities in Nepal (Rai 1991a, b). It is used in preparation of alcoholic beverages like *jaand*, *rakshi*, *tongba*, etc., from various substrates. Microbiologically *marcha* is a mixed culture and consists of saccharifying molds, fermentative yeast, and acidifying lactic acid (Karki 1986a, b; Tamang and Sarkar 1988). Mold population in *marcha* has been detected at the level of 10^6 cfu/g, whereas the load of yeasts and LAB was 10^8 cfu/g and 10^7 cfu/g, respectively (Tamang 1992). Kobayashi et al. (1961) reported the presence of *Rhizopus oryzae*, *Mucor praini*, and *Absidia lichtheimia* in *murcha*.

The exact period of origin of these starters is not known. The existence and use of these starters have a long history. The indigenous and traditional technology of *marcha* is kept so secretly by some people of the Lubhu area in the Kathmandu valley that is not even taught to daughters but only to daughters-in-law. The normal trend to prepare *murcha* remains between mid-September. *Marcha* is mainly classified into two types based on substrates utilized for the preparation. These are named as *mana* and *manapu. Mana* is prepared from wheat flakes and appears green in color due to the presence of molds, and *manapu* is prepared from rice flour and millet grains. *Manapu* is marketed as round cakes of varying sizes, shapes, and weights (Gajurel and Baidya 1979).

4.1.5.1 Method of Preparation

Marcha preparation varies with ethnic communities. The general method for preparation of *marcha* involves soaking, grinding, and dough preparation along with incorporation of various plants and herbs. For the preparation of *marcha*, glutinous rice (*Oryza sativa*) is soaked in water for 8–10 h at ambient temperature. Soaked rice is crushed in a foot-driven, heavy wooden mortar and pestle. In 1 kg of ground rice, ingredients added include roots of *guliyo jara* or *chitu* (*Plumbago zeylanica*), 2.5 g; leaves of *bhimsen paate* (*Buddleja asiatica*), 1.2 g; flowers of *sengreknna* (*Vernonia cinerea*), 1.2 g; ginger, 5.0 g; red dry chili, 1.2 g; and previously prepared *murcha* as mother culture, 10.0 g. The mixture is then made into a paste by adding water and kneaded into flat cakes of varying sizes and shapes. This is then placed on a straw mat and covered with straw. These are left to ferment for 1–3 days, depending upon the temperature. Completion of fermentation is indicated by a distinct alcoholic aroma and swollen appearance of the *murcha*. Finally, cakes of *murcha* are sun dried for 2–3 days. In north Sikkim, root barks and flowers of wild herbs, locally called *murcha jhar* (*Polygala arillata*), are mixed and ground with water during *murcha* preparation. In eastern Nepal, the *murcha* producer uses more than 42 plants (two ferns, five monocots, and 35 dicots) and their roots and leaves for making *marcha*. The more widely used plants (called *murcha* plants) are *Vernonia cinerea*, *Clematis grewiaeflora*, *Polygala arillata*, *Piper chaba*, *P. longum*, *Plumbago zeylanica*, *Buddleja asiatica*, *Christella appendiculata*, *Polygala* sp., *Elephantopus scaber*, *Inula* sp., and *Scoparia dulcis* (KC et al. 2001).

4.1.6 *Mana*

Mana (Fig. 4.14) is a granular, greenish-type starter prepared from wheat flakes in Nepal. It is prepared by soaking wheat grains in water overnight and steaming for 30 min. Then it is washed and transferred to a bamboo basket for draining water. Flakes are made or ground in a traditional mortar and pestle. The wheat grains are ruptured until they become lumpy. Fermentation is carried out in the floor covered with paddy straw. 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.

In the *Newar* community of Nepal, *mana* is further classified into three of its forms, which are based on the key substrates used. They are *Cho mana*, *Ki mana*, and *Pa mana*. Here *Cho* means wheat, and *Ki* means rice. *Pa mana* refers to *murcha* as stated above.

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 lactic acid bacteria (Nikkuni et al. 1996). *Aspergillus oryzae* and *Rhizopus* spp. are present in *mana* (Nikkuni et al. 1996; Shrestha et al. 2002) (Fig. 4.9).

It is the only amylolytic starters in this region which have *Aspergillus* significant in cereal-based alcoholic fermentation. Among the yeast, *Saccharomyces cerevisiae* are predominant followed by *Candida versatilis* (Karki and Shrestha 1999).

Rice or Wheat
↓
Soaking in Water (Rice: Water =1: 1.25)
↓
Steaming I (Boiling water, 5 min.)
↓
Washing
↓
Steaming II (Boiling water, 5 min.)
↓
Crush to make flakes
↓
Cooling by spreading over floor
↓
Covering by straw and straw mat
↓
Fermenting (30-31°C for 1-10 days)
↓
Appearance of green molds
↓
Drying in Sun
↓
Mana

Fig. 4.9 Process for *mana* preparation in Nepal (Karki and Shrestha 1999)

4.1.7 *Manapu*

Manapu (Fig. 4.13) is an ethnic amylolytic starter from Nepal similar to *marcha* that is prepared from rice flour and millet. *Manapu* preparation is also an example of solid-state fermentation. It serves as a source of various groups of microorganisms that allow selective growth of desirable microflora. It is white to creamy white in color and can be of varying size. For the production of *manapu*, rice or millet is milled to get flour and is mixed with 20 % old *murcha* (seed), 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 30–33 °C for 5–7 days. Freshly fermented dough is sun dried to get *manapu*. The microorganisms present in *manapu* are *S. cerevisiae*, *S. fibuligera*, *C. versatilis*, *Rhizopus* spp., and *P. pentosaceus* (Shrestha et al. 2002). The load of yeast and LAB in *manapu* is 10^5–10^9 cfu/g, and the mold population is 10^7 cfu/g (Shrestha et al. 2002).

4.1.7.1 Microorganisms

The major microorganisms present in *murcha* are filamentous molds such as *Mucor circinelloides*, *M. hiemalis*, *Rhizopus chinensis*, and *R. stolonifer* var. *lyococcus*; yeasts such as *S. fibuligera*, *S. capsularis*, *Pichia anomala*, *P. burtonii*, *S. cerevisiae*, *S. bayanus*, and *C. glabrata*; and LAB such as *P. pentosaceus*, *Lactobacillus bifermentans*, and *L. brevis* (Tamang and Sarkar 1995; Tsuyoshi et al. 2005).

4.1.7.2 Microbial Dynamics During *Marcha* Fermentation

The changes in microbial dynamics for 200 h during wheat and rice *mana* fermentation were analyzed. In wheat *mana*, viable loads of molds, yeasts, and lactic acid bacteria were found to be log 8 cfu/g, log 5.8 cfu/g, and log 3.2 cfu/g, respectively (Karki and Shrestha 1999) (Fig. 4.10).

For rice *mana*, all these counts were within the range of log 8 cfu/g, log 5.8 cfu/g, and log 5 cfu/g, respectively. Similarly changes in the load of various groups of microorganisms were done

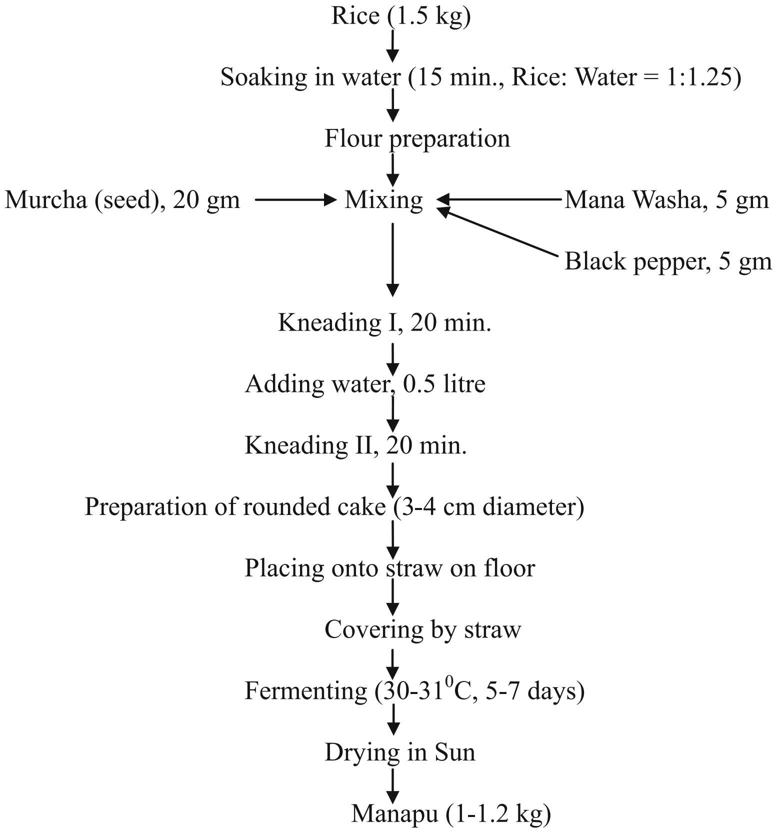

Fig. 4.10 Process for preparation of *manapu* (Karki and Shrestha 1999)

in *manapu* sample for 90 h. The microbial groups analyzed were gram-negative bacteria, coliforms, molds, yeasts, and lactics. Gram-negative bacteria and coliforms decreased to log 3.5 cfu/g and log1.8 cfu/g during that period, whereas viable loads of yeasts, lactics, and molds were within the range of log 8.6 cfu/g, log 7.4 cfu/g, and log 6.9 cfu/g, respectively (Karki and Shrestha 1999) (Figs. 4.11, 4.12, and 4.13).

Fig. 4.11 Kneading various compositions of *murcha*

4.1.8 *Jaand*

Jaand or *jaanr* is a generic term used to represent undistilled alcoholic beverage made from a wide range of carbohydrate-containing substrates. The basic raw materials for the production of *jaand* are rice, millet, maize, wheat, or other substrates containing starch. Rice wine and millet wine are the Nepalese indigenous fermented alcoholic beverages made by the action of *murcha*. They are the alcoholic beverages produced by utilizing starchy material as substrate and ethanol and CO_2 with volatile aromatic compounds as product. The fermentation of cooked rice and gelatinized millet takes place in two steps. The first step is the saccharification and liquefaction of the starch,

Fig. 4.12 Spreading *manapu* on straw mat for fermentation

and the second is the utilization of simple sugars to produce alcohol and CO_2 (Pudasaini 2012). Finger millet (*Eleusine coracana*) is a traditionally important grain used in brewing millet beer in some cultures. It is also the base ingredient for the distilled liquor *rakshi* in Nepal and the indigenous alcoholic drink of the *Sherpa*, *Tamang*, and *Limbu* people. *Jaand* refers to the sweet-sour cereal beer made from grains like finger millet, rice, wheat (*Triticum* spp.), and maize (*Zea mays*) by using *murcha. Jaand* finds a very prominent place in *Limbu* and *Rai* cultures in particular and among the ethnic groups in general. The tradition of offering *jaand* to guests is a unique way of

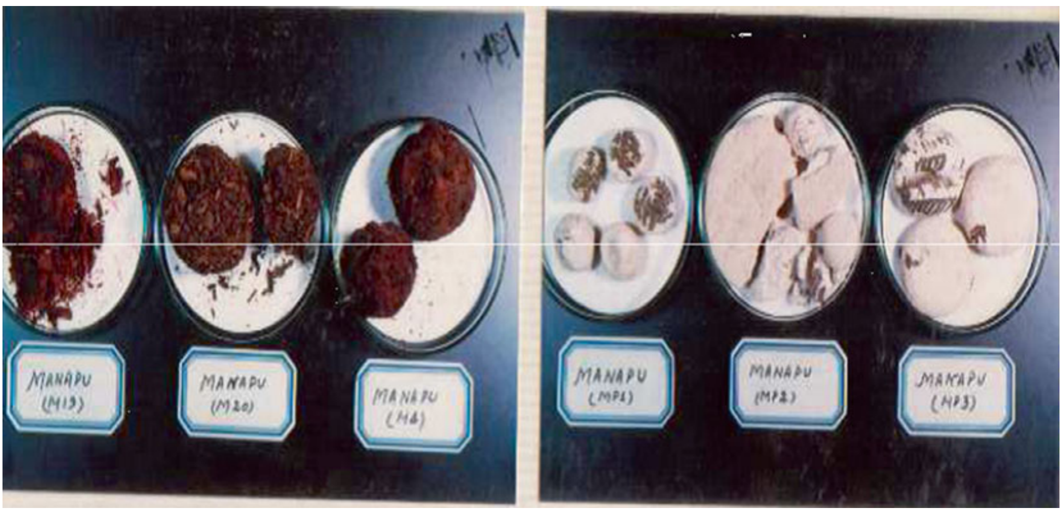

Fig. 4.13 Various types of *manapu* prepared in Nepal

Fig. 4.14 *Mana* prepared from rice and wheat

showing hospitality. *Jaand* is also used in several festive occasions, rituals, and rites, settling disputes, and appeasing deities (Rai 1991a, b).

Jaand is served in different forms. Strained *jaand* is prepared by leaching out the readily extractable contents from the mash with water (usually lukewarm). A strainer made of thin bamboo strips or perforated aluminum strainer is normally used for straining the liquor. This form of beverage is drunk in *lumbha* (small brass bowl) or deep aluminum mugs until satiated (Rai 1991a, b). The beverage is cloudy in appearance and has a very short shelf life of about few hours. The shelf life of strained *jaand* can be extended by pasteurization (Rai et al. 2006).

Marcha is an amylolytic fermentation starter culture cake, made by mixing selected wild plants (called *murcha* plant here after) in the starchy substrate (Tsuyoshi et al. 2005). KC et al. (2001) have listed the use of some 38 *murcha* plants in the east zones (Mechi and Koshi) of Nepal. *Murcha* serves as a source of microorganism (a mixture of saccharifying molds, fermentative yeast, and acidifying lactic acid bacteria derived from *murcha* plant for the fermentation) (Rai and Subba 2003) and has been reported to have a shelf life of about 1 year (Karki 1986a, b). The starter cultures used for rice wine and millet wine production in Nepal are *mana* and *manapu*. There are three major groups of microorganism, namely, yeasts, molds, and bacteria, present in the traditional amylolytic starter where molds produce amylase to degrade starch into fermentable sugar. The substrate of *mana* is wheat which is rich in *A. oryzae*, *Rhizopus* spp., and *Mucor* spp., while the substrate for *manapu* is rice, wheat, or millet flour. *S. fibuligera*, *S. cerevisiae*, and *P. pentosaceus* are the major microorganisms found in *manapu* (Karki 1986a, b).

4.1.8.1 Method for *Jaand* Preparation

Jaand is an alcoholic beverage (undistilled) indigenous to Nepal. It is prepared by solid-substrate fermentation of starchy cereals like corn, rice, wheat, and millet. *Marcha*, a starter culture, is used as the inoculum in traditional fermentation. *Marcha* contains saccharifying molds, lactic acid bacteria, and fermenting yeasts (Kharel et al. 2010). *Jaand* is therefore the result of concerted action of these microorganisms on the cooked cereal.

The basic steps followed in the traditional *jaand* making are cooking of cereal, cooling to room temperature, mixing with *murcha* powder (about 0.5–1 %), leaving it for a day or two for biomass buildup under aerobic condition, and fermenting in tightly plugged containers for 10–15 days. The duration of fermentation may range from a week to several months. During serving, the mash is taken out, mixed with a requisite amount of water, squeezed, and strained, and the cloudy extract is drunk. When *jaand* is pot distilled, it becomes *rakshi*, which is an unaged traditional spirit of varying alcohol content. The flowchart of preparation of *jaand* is shown in Fig. 4.15.

4.1.8.2 Microbiology

Detailed studies on the microbiology of *jaand* are lacking. The total mesophilic aerobic count ranged from 1.3×10^7 to 7.9×10^7 cfu/g during 5 days of fermentation. Mold counts were lower, between 6.3×10^5 and 1×10^6 cfu/g, and even less after 4 days of fermentation. Lactics were enumerated in the order of 6×10^6–5×10^7 cfu/g. Yeast counts showed a steady increase from 1.8×10^6 to 1.3×10^8 cfu/g (Shrestha et al. 2002). Yeasts such as *P. anomala*, *S. cerevisiae*, *C. glabrata*, and *S. fibuligera* and LAB such as *P. pentosaceus and L. bifermentans* have been recovered in millet *jaand* samples. *Mucor* and *Rhizopus* spp. are the molds responsible for saccharification of rice or millet (Thapa and Tamang 2006) (Figs. 4.16 and 4.17).

4.1.8.3 Physicochemical Parameter

The pH of the rice wine and millet wine was in the range of 4.29–4.51 and 4.0–5.0, respectively, while the acidity ranges from 0.63 % to 0.88 % and 0.63 to 1.8 % (as lactic acid) (Pudasaini 2012). The ethanol concentration (v/v) ranges between 4 % and 11 % in most of the *jaand* samples. Typically, *jaand* contains 5–9 % alcohol, 0.8–1.1 % acidity (as lactic acid), 1.6–2.5 % reducing sugar as glucose, 1.6–2.8 % total sugar (as sucrose), 12–14 % starch, 76–80 % water, and

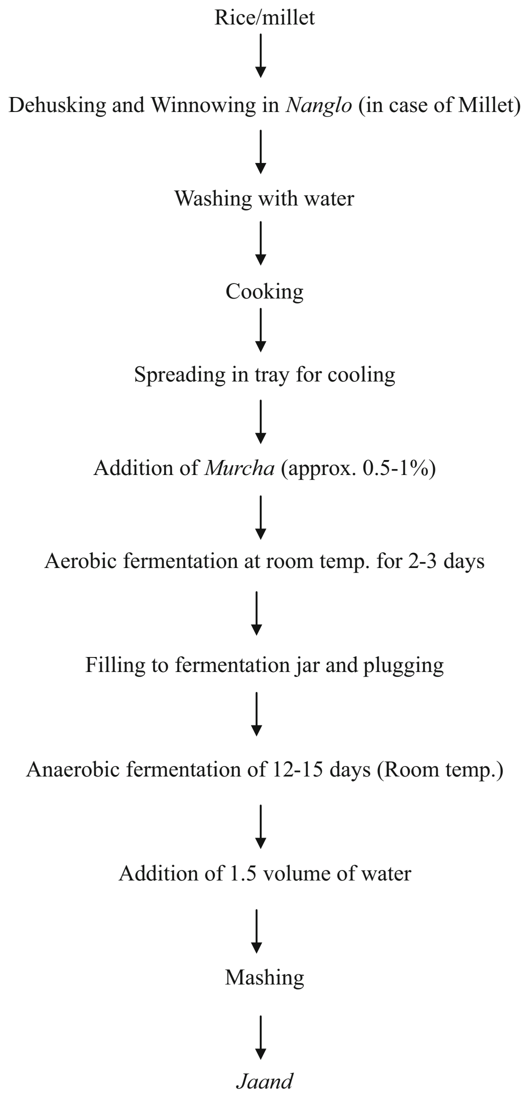

Fig. 4.15 Traditional process of *jaand* making (Kharel et al. 2010)

trace amounts of methanol, ester, aldehydes, and other flavor components (Upadhyaya 2005). Rice brew typically has higher ethanol content (18–25 %) than wine (10–20 %) and beer (3–8 %). The pH of millet *jaand* is 4.1, moisture content is 69.7 %, acidity is 0.3 %, and alcohol content is 5.0 % (Thapa and Tamang 2006).

4.1.8.4 Nutritional Value

Protein content increased up to 17.6–38.8 %; carbohydrate decreased from 86.41 % (in control sterilized rice) to about 77.29–77.71 %. Calorie was around 4 kcal/g for *jaand*. Riboflavin was not detected in the fermented product, and thiamine was found to increase up to 16–32 %. Similarly pyridoxine increased by 50–59 % and the increase in folic acid was nearly 76 %. A significant increase of up to 117–173 % in niacin content was recorded (Shrestha and Rati 2003).

4.1.9 *Rakshi*

Rakshi is an ethnic alcoholic drink with a characteristic aroma, distilled from the traditional fermented cereal beverage *jaand*. Fermented masses of buckwheat, potato, canna, and cassava roots are also distilled to get *rakshi*. *Rakshi* is a common term in Nepali meaning alcoholic drink. *Rakshi* has several other names in different ethnic communities such *asarak* (*Tibetans*, *Bhutia*, *Drukpa*, *Sherpa*), *aarok* (*Lepcha*), *aaerak* (*Tamang*), *aayala* (*Newar*), *sijongwaa aara* (*Limbu*), *aarakha*/*hemma* (*Rai*), *paa* (*Gurung*), *rindho* (*Sunwar*), *dhise* (*Magar*), etc.

Rakshi (also spelt *rakshi*, *rukshi*) is an unaged congeneric spirit obtained by pot distillation of the slurry of *jaand*. The product likens whiskey and has highly varying alcohol content. Several researches have been done on *rakshi* production from different cereals using *murcha* as well as pure culture isolated therefore, but there seems a lack of attention toward process development such as preparation of good starter culture, increasing efficiency of traditional distillation apparatus, and separation of feints and foreshots for improving the quality of *rakshi* (Rai et al. 2006). Traditionally *rakshi* is made by using *murcha* as starter culture as that in preparation of *jaand*.

4.1.9.1 Distillation of Fermented Mash

Traditional fermented beverages prepared from cereals are distilled in a large cylindrical metallic vessel measuring 40 × 30 × 25 cm for 2–3 h continuously over firewood in an earthen oven. Above the main cylindrical vessel, a perforated container called a *phunga* is placed, inside of which a small metallic collector, locally called

Fig. 4.16 Aerobic fermentation of rice by *Rhizopus* spp. and *Aspergillus* spp.

Fig. 4.17 Anaerobic fermentation of rice

paini, is kept on an iron tripod, locally called *odhan*, to collect the distillate (*rakshi*). Another metallic vessel with cold water is placed above the *phunga* as condenser.

The bottom of the condenser vessel is plastered by mud with the tip of the *phunga* to prevent excess ventilation during distillation. Water is replaced three to five times after it is heated. Condensed *rakshi* is collected in a small metallic vessel (*poini*). *Rakshi* prepared after replacing the condensing water three times is known as *teen pani rakshi*; this contains a high amount of alcohol and is traditionally prepared for religious purposes. *Rakshi* 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 *jaand* to get 1–2 l of *rakshi* after replacing condensing water thrice.

Rakshi 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 *rakshi*. This type of *rakshi* is commonly prepared in *Rhododendron*-growing regions of the Himalayas (Tamang et al. 1996; Kharel et al. 2010). The most common fermented beverages used for traditional distillation are prepared from rice and maize.

4.1.9.2 Consumption Pattern

Rakshi is drunk directly without addition of water along with snacks like fried meat, soybeans, or any other curries. It is traditionally drunk by the *matwali* (alcohol drinkers), including non-Brahmin Nepali, *Bhutia*, *Tibetans*, *Lepcha*, *Drukpa*, etc. *Rakshi* is drunk in mountains and high hilly region by people to protect themselves from the cold.

4.1.9.3 Economy

Some people are economically dependent on *rakshi*, which is commonly available in liquor shops, restaurants, and hotels. The trade of *rakshi* is increasing in marginalized people. However the process for *rakshi* has not been mechanized and industrialized. *Rakshi* costs cheaper as compared to other distilled alcoholic beverages.

4.1.10 *Hyaun Thon*

Hyaun thon (Fig. 4.18) is an indigenous undistilled fermented beverage prepared and consumed by the *Newar* community, especially of Kathmandu, since time immemorial (Dangol 2006). The term *hyaun thon* originates from a pair of *Newari* words *hyaun* and *thon* meaning "red" and "beer," respectively. The term *hakuwa* (rice) used by the community in the present context doesn't refer to any particular variety of rice. It actually refers to the "reddened" rice. Among the community, *hyaun thon* is one of the socially and culturally accepted alcoholic beverages. To date, the technology of preparing *hyaun thon* is limited to household purpose following the same traditional method from *hakuwa* and *mana*. *Mana* has a dual purpose in *hyaun thon* preparation: it serves as starter for amylolytic fermentation as well as substrate.

4.1.10.1 Method of Preparation of *Hyaun Thon* and *Mana*

The traditional method of *hyaun thon* preparation uses two basic raw materials (1) *hakuwa* (as the main substrate) and (2) *mana* (as the starter). *Hakuwa* doesn't refer to the wild variety of red rice (*Oryza rufipogon*); it refers to the "reddened rice." In Nepal, the purpose of reddening of rice is especially for the preparation of *hyaun thon*. The reddening is carried out by heaping the freshly threshed paddy in the same threshed paddy plant and covered with the same for about 8–10 days. As a result of fermentation, there is a change in color.

Clean polished *hakuwa* of good quality at least 1 year old is selected for the preparation of *hyaun thon*. The rice is soaked in cold water for over a night. The water is drained and rice is steam cooked in especially designed clay pot called *chappani*. The soft consistency of grain while pressing with finger indicates the completion of cooking. The cooked rice is spread on the clean objects and let to cool to room temperature. The fermentation is carried out in a clay pot which should be sun dried for 5 h before fermentation is carried out. Then the cooked *hakuwa* is mixed with *mana* of the same mass. The mixture is kept in a fermenting vessel, and cold clean water is added to the mixture in the ratio of (1:1:3,

Fig. 4.18 *Hyaun thon* fermentation

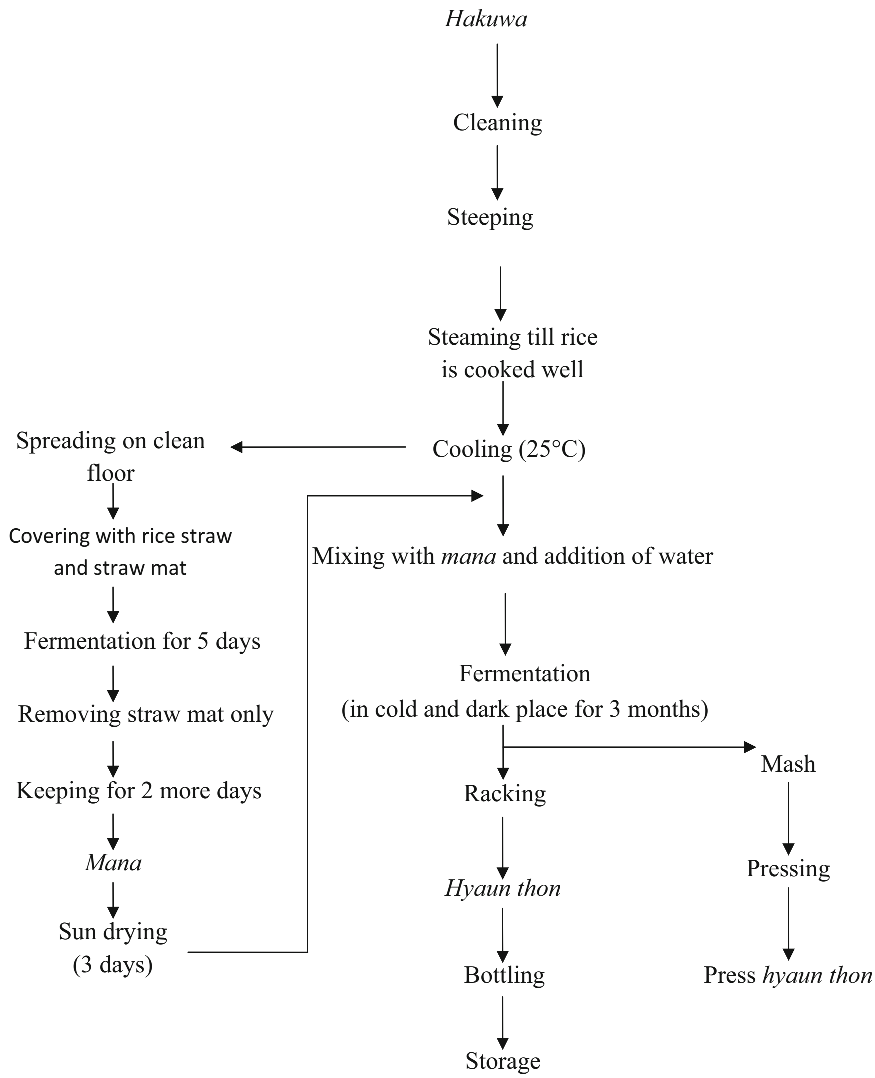

Fig. 4.19 Traditional method of preparation of *mana* and *hyaun thon* (Dangol 2006)

i.e., *hakuwa*/*mana*/water). Then the mass is stirred with a clean stick until it is mixed well, and the gruel mass formed is called "cut." The fermenting vessel is closed tight with a lid and covered with muslin cloth. The vessel is placed in a cold and dark place for 3 months for fermentation. The winter season is considered as the best season for the preparation of *hyaun thon*.

For the preparation of *mana*, the polished reddened rice is taken. The reddened rice is cleaned, washed, and steeped for the whole night at room temperature. The steeped water is drained, and the rice is steam cooked in *hanshi* (especially designed clay pot) and cooled to ambient temperature. The floor is cleaned and cooked rice is spread over the floor maintaining about 1 cm of thickness and is covered with the straw, and then with a straw mat, it is left to ferment for 5 days at ambient temperature. After 5 days, the straw mat is taken out, and fermentation is carried out with straw covered only for 2 days more. Green mold is seen and it is collected and sun dried for 3 days. Thus prepared *mana* is packed in a polythene bag and placed in a dry place at room temperature (Dangol 2006). The flowchart of preparation of *hyaun thon* is shown in Fig. 4.19.

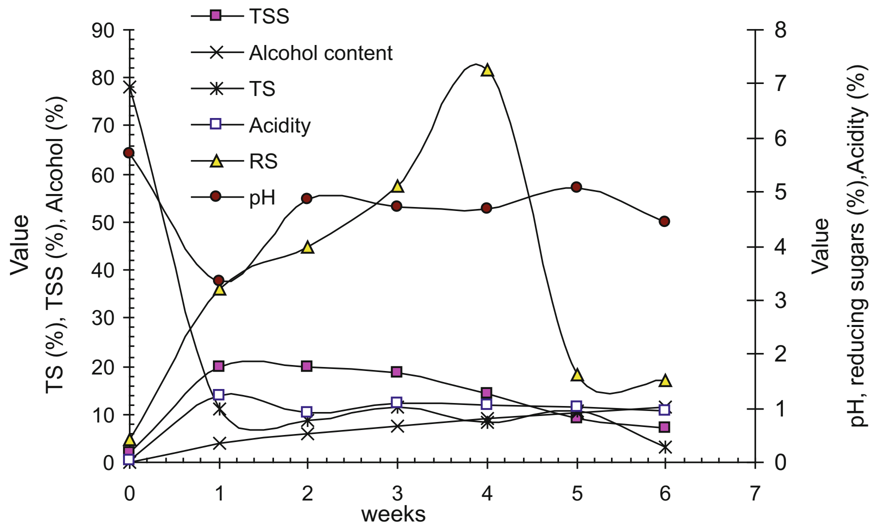

Fig. 4.20 Physicochemical parameters of *hyaun thon* (Dangol 2006)

4.1.10.2 Biochemical Changes

After fermentation for 4 weeks, the pH of the *hyaun thon* dropped from initial 5.7 to 4.3. The acidity (% as lactic acid) of the brew increased to 0.953 % from the initial of 0.025 %. The ethanol content of the product is around 11.5 % after 5 weeks of fermentation. Fermentation of *hyaun thon* using *mana* with initial microbial load of 1.8×10^4 cfu/g at 25 °C for 45 days yielded a product with pH 2.72, acidity 1.62 % (as lactic acid), and ethanol 13.29 % (v/v) (Prajapati 2012) (Fig. 4.20).

4.1.11 *Kinema*

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. 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 restricted to some of the communities only because of their ammoniacal odor (Tamang 2010).

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; and *chungkokjang* of Korea, and all have a characteristic stickiness. *Kinema* is an ethnic, bacilli-fermented, sticky soybean food with a slight ammonia flavor produced by natural fermentation. *Kinema* (Fig. 4.23) is a traditional non-salted soybean fermented food product widely and popularly consumed by *Kirat* ethnic population of eastern hills of Nepal, India, and Bhutan. This product greatly resembles *natto* of Japan and *thua nao* of Thailand and is produced usually in the winter season. *Kinema* is a high-protein food based on soybeans. *Bacillus subtilis* strain was found to be the dominant microflora of *kinema* fermentation (Karki 1986a, b) (Fig. 4.21).

4.1.11.1 Method of Preparation

For the production of *kinema* in Darjeeling, Sikkim (India), and South Bhutan, the small (≈6 mm) yellow-cultivar soybean dry seeds are selected. In eastern Nepal, local consumers prefer dark brown local varieties of soybean seeds rather than yellow-colored seeds for making *kinema* (Tamang and Nikkuni 1998).

The flowchart of preparation of *kinema* is shown in Fig. 4.22. Indigenous method for

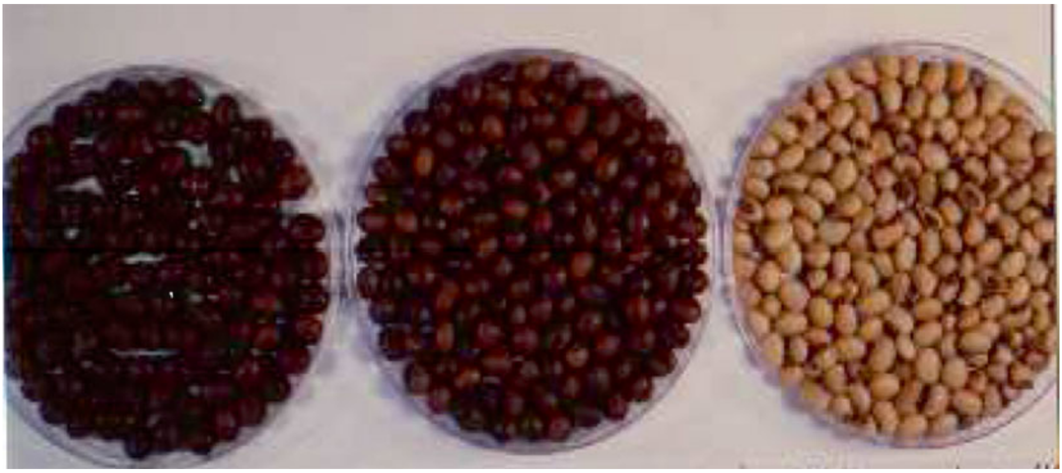

Fig. 4.21 Different varieties of soybeans

Fig. 4.22 Traditional method for preparation of *kinema* in Nepal (Karki 1986)

Raw soybeans washed

↓

Soaked in water for 10-12 hour

↓

Drain off excess water

↓

Cooking in boiling water (3-4 hr)

↓

Crushed by *okhal* as mixing little ash (approx. 1-2%)

↓

Placed in bamboo basket (*doko*) coated with banana leaves

↓

Covered with banana leaves and gunny bags

↓

Kept at warm place for 1-3 days

↓

Kinema

↓

Dried for the purpose of preservation

(Spreaded on *nanglo*, then kept under sunlight for 2-3 days)

Fig. 4.23 *Kinema* made at Terhathum, Nepal

Table 4.2 Chemical composition of *kinema*

Constituent	% Dry weight
Moisture	8.9
Protein	46.2
Fat	18.1
Total sugar	21.2
Reducing sugar	2.1
Crude fiber	7.1
Ash	5.2
Acidity (as lactic)	1.52

Karki (1986a, b)

kinema production starts with washing and cleaning of soybeans. *Kinema* soybeans were washed and soaked overnight at ambient temperature (10–25 °C). Cooking is done at boiling water in an open cooker for about 4 h (until they can be easily crushed between the fingertips) and cracked lightly using a wooden mortar and a wooden pestle. They were then placed in a bamboo basket (*doko*) lined with banana leaves (Karki 1986a, b) or fresh fern leaves (Tamang et al. 1988) which had been dusted with a small amount of firewood ash (about 0.5 %) followed by occasional dusting with a small amount of ash and covered with banana leaves. The bamboo basket was covered with a jute bag and left beside an earthen fire oven to keep it warm (25–40 °C). During summer, the fermentation time may require 1–2 days, while in winter it may require 2–3 days (Tamang 1994). After 3 days of fermentation, the fermented soybeans were spread on a bamboo tray (locally called *nanglo*) lined with newspapers and sun dried for 2 days (Karki 1986a, b). Completion of fermentation is indicated by the appearance of a white viscous mass on the soybean seeds and the typical *kinema* flavor, with a slight odor of ammonia.

The shelf life of freshly prepared *kinema* remains 2–3 days in summer and a maximum of a week in winter without refrigeration. It can be prolonged by drying in the sun for 2–3 days. Dried *kinema* is stored for several months at room temperature. *Kinema* is consumed in the form of soup along with green vegetables or it can be consumed after frying in oil.

4.1.11.2 Microorganisms

B. subtilis is the principal microorganism in *kinema* fermentation (Karki 1986a, b; 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). Hara et al. isolated gamma polyglutamate-producing *Bacillus* strains from *kinema* which is responsible for the sticky nature of *kinema*. Besides bacilli, lactic acid bacterium *Enterococcus faecium* and two types of yeasts, *Candida parapsilosis* and *Geotrichum candidum*, were also isolated from *kinema* samples (Sarkar et al. 1994). *Streptococcus faecalis* were also isolated in large numbers together with heat-resistant, gas-producing, and very less gram-negative microorganisms (Karki 1986a, b). 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*. 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 2000). The microbial load changes during fermentation and was higher (log 9 cfu/g) after 12 h of fermentation. At initial stage of fermentation (0 day), the total viable count of bacteria was 3.7×10^6 (cfu/g) and that of lactic acid bacteria was 3.6×10^5 (cfu/g) (Karki 1986a, b).

4.1.11.3 Biochemistry or Nutritional Composition

Compositional and nutritional analysis of *kinema* has been carried out on the various aspects including proximate composition and influence of process variables on sensory quality of *kinema* (Sarkar and Tamang 1994). The average composition of *kinema* was shown in Table 4.2.

The total amino acid and free amino acid composition of the *kinema* ranged about 46,218 mg/100 g and 5129 mg/100 g (on dry basis) which are higher than soybean. The average value of the ash content of the *kinema* samples was also higher than the similar product which may be due to the firewood ash addition process in *kinema* preparation. Potassium comprised about 30 % of the ash prepared from the *kinema* (Nikkuni et al. 1995). 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).

A remarkable increase in water-soluble nitrogen and trichloroacetic acid (TCA)-soluble nitrogen content is observed during *kinema* fermentation (Sarkar and Tamang 1995). The total amino acid, free amino acid, and mineral contents increase during *kinema* fermentation and subsequently enrich the nutritional value of the product (Nikkuni et al. 1995; Sarkar and Tamang 1995; Sarkar et al. 1997; 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. 1997). Degradation of oligosaccharides has been reported in *kinema* which increases digestibility. *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 comparative study of soybean, *kinema*, *thua nao*, and *natto* was conducted in terms of the total amino acid, free amino acid, and mineral contents which revealed *kinema* as superior among others. *Kinema* is very much similar to Japanese *natto* in terms of nutrient and organoleptic properties (Nikkuni et al. 1995). 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 found to have many functional properties as well including antioxidants, digested proteins, essential amino acids, vitamin B complex, low cholesterol content, etc. (Tamang 2010).

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5 Ethnic Fermented Foods of Pakistan

Saeed Akhtar, Majid Hussain, Tariq Ismail, and Muhammad Riaz

5.1 Introduction

Pakistan, situated in South Asia, has coordinates between 30°N and 70°E and covers a total land and sea area of approximately 796,095 km^2. The country borders India in the east, Iran in the west, China in the north, Afghanistan in the northwest, and the Arabian Sea in the south. Pakistan with majority of its people belonging to the Muslim community (96.4 %) also has various ethnic groups including Punjabi (44.7 %), Pashtun (15.4 %), Sindhi (14.1 %), Saraiki (8.4 %), Muhajir (7.6 %), Balochi (3.6 %), and others (6.3 %). Dietary habits of the peoples of this region reveal typical Pakistani meals to be comprised of cereal-based cuisines, while around 51 % of the population of the country irrespective of their financial capability are reported to have a likeness factor toward the meat group. Besides their staple diet, peoples of different cultures of Pakistan have wide acceptability toward some ethnic fermented foods like "*lassi*" or buttermilk, "*desi ghee*" or butter oil, "*achar*" or pickles of fruit and vegetable origin, preserves, sauces, fermented cereals, and an array of fermented nonalcoholic beverages (Rengel 2004; CIA 2015; SAI 2015) (Table 5.1).

Dating back toward historic periods of primitive fermentation concepts and its application in food industry, mankind has found fermented foods as an essential part of its civilization (Prajapati and Nair 2008). Starting from dairy-based preparations, a vast variety of fermented foods from the cereal, meat, fruit, and vegetable group has emerged as an integral part of almost all cultures of the modern age. Asian civilizations have a tremendous history of fermentation, and hence the process is deployed traditionally to design a variety of healthful and nourishing food commodities (Stanton 1985; Battcock 1998). Fermentation processes are adopted in fermented foods and beverages to achieve the desired biochemical changes for specific taste and flavor of product. Traditional fermentation is accompanied with indigenous microflora that might be utilized as starter culture or may be present on food surface as substrate to onset the fermentation process (Harlander 1992).

Fermentation has been selected as a sustainable and economical strategy to curtail food losses and ensure food security as has been adopted in primitive eras to kill hunger (Chavan et al. 1989; Billings 1998). Fermentation process improves the nutritional status, physical appearance, and sensory features and enhances the shelf stability of the product for a longer period of time (Simango 1997). As has been mentioned above,

S. Akhtar (✉) • M. Hussain • T. Ismail • M. Riaz
Institute of Food Science and Nutrition,
Faculty of Agricultural Sciences and Technology,
Bahauddin Zakariya University, Bosan Road,
Multan 60800, Pakistan
e-mail: saeedbzu@yahoo.com

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Table 5.1 Various forms of fermented foods and their utilization in Pakistan

Foods	Substrates	Nature and uses	Microorganisms	Regions of consumption in Pakistan	Reference
Dahi	Cow/buffalo/goat milk	Dessert, beverage, appetizer	Lactic acid bacteria (LAB)	Southern, central areas of Punjab	Soomro and Masud (2012)
Paneer	Fresh buffalo/cow milk	Raw form, sweets,	LAB	KPK, northern areas	Masud et al. (1992)
Naan	Wheat flour	Meal purpose	Yeast	In all areas of Pakistan	Aslam et al. (1982)
Doli ki roti	Fine flour	Meal purpose	Indigenous microflora	Southern areas	Bhatia and Khetarpaul (2012)
Mango pickle	Green mango	Appetizer	Indigenous microflora	Punjab, Sindh	SMEDA (2007)

besides modifying the taste and aroma profile of consumable goods, fermented foods contribute to improved digestibility and micronutrient availability as well as significantly lower down the concentration of anti-nutritional attributes (Nout 2009; Swain et al. 2014). Being a potential source of probiotics, fermented foods have gained a wide popularity as nutritional therapy to treat gastrointestinal ailments, strengthen the immune system, and reduce the risks of inflammatory disorders and various types of cancer (Parvez et al. 2006). A wide variety of probiotic cultures have been isolated from indigenous fermented foods of Pakistan including *Lactobacillus delbrueckii* subsp. *bulgaricus*, *L. helveticus*, *L. acidophilus*, *L. casei*, and *Streptococcus thermophilus* (Soomro and Masud 2007, 2012; Mahmood et al. 2013).

The application of fermentation techniques in Pakistan and other developing countries of the region is highly desirable as the low-cost technology has introduced a safer and nutritionally reliable class of fermented foods (Cooke et al. 1987; Soomro et al. 2002). Presently, the application of multiple fermentation processes is in practice for manufacturing cereal, milk, meat, fruit, and vegetable-based fermented food products in developing and developed economies of the world (Soni and Sandhu 1990; Hirahara 1998; Pagni 1998). Fermentation practices in foods at household levels have been adopted as cottage industry, and they are among the reasons that more than 70 % of the fermented foods in Pakistan are produced from the small and medium enterprises and from the cottage sector (Bol and de Vos 1997; SMEDA 2007). The chapter in hand discusses a brief description of the indigenous fermented foods of Pakistan and conventional techniques especially with reference to Pakistan's cottage industry.

5.2 Cereal-Based Fermented Foods

Cereal grains being the staple diets of peoples of Pakistan are considered as the energy-dense dietary source of carbohydrates, protein, fiber, and a range of essential micronutrients. Like global food crop production rationales, wheat and rice account for more than 50 % share of cereal production in Pakistan and are used as whole grain, flour, fine starch, farina, and semolina. The Pakistan Bureau of Statistics reported a production of 25,286,000 tons of wheat followed by 6,798,000 tons of rice and 4,527,000 tons of maize in the fiscal year 2013–2014 (PBS 2015). Contrarily to their apparent compositional features, nutritional and sensorial properties of cereal-based products are sometimes compromised and reported inferior in quality as compared to dairy and meat products. Lack of some essential amino acids, presence of anti-nutritional compounds, impaired starch availability, and reduced digestibility are a few highlights of cereals' nutritional concerns that need to be ameliorated while being consumed as staple diet for millions of mouths (Chavan et al. 1989).

A range of techniques and processes have been adopted to counter nutritional inadequacies and organoleptic properties of cereal-based food products, namely, fortification, supplementation, cooking, sprouting, baking, and fermentation (Mattila-Sandholm 1998). Fermented cereal-based products are valued for their unique taste and sensory-appealing aromatic compounds (Steinkraus 1988; Parveen and Hafiz 2003). Fermentation is reportedly linked with improved amino acid composition, digestibility, better total sugar contents, B vitamins, and remarkable reduction in anti-nutritional compounds particularly phytates that account for 0.14–2.05 % of grain composition (Reddy et al. 1982; Chavan et al. 1989). Cereals in Pakistan like other countries of South Asia have a range of applications in indigenous fermented foods. Bread, leavened bread (*naan* also known as *kulcha* in some regions), doughnuts (*balushahi*), pancake preserved in syrup (*jalebi*), molten lava cake (*doli ki roti*), and *andrassay* are among the widely consumed cereal-based fermented food preparations. Supplementation of cereal flours with legumes and lentils is the preferred intervention aimed at overcoming nutritional inadequacies and poor sensorial preferences associated with cereal-based formulations.

5.2.1 Nutritional Aspects of Fermented Cereal-Based Products

Cereals are nourishing mankind since prehistoric times and well known as cradle-to-grave foods (Jonnes 2000). Cereals as whole grain, bread, leavened flat bread, *chapati*, porridges, and ready-to-eat breakfast products have been anticipating more than 50 % of calorie needs of populations and around 50 % of protein requirements. Dietary guidelines being followed globally emphasize on consumption of whole grain cereals to improve complex carbohydrate and fiber intake for the sake of preventing noncommunicable chronic diseases (Alwan 1997).

Cereals being complementary food for growing children provide dietary iron; however, the presence of phytochemicals like phytates strongly compromises iron absorption by generating mineral–phytate complexes and precipitation of minerals. De-phytinization or removal of phytates from cereals particularly wheat bran sheds the beneficial effect on the absorption of iron and zinc, thereby improving the bioavailability of minerals. Degradation of phytates by fermentation is a sustainable strategy to avoid mineral–phytic acid complex formation and improve mineral bioavailability. Lactic acid bacteria have been proven to be the dominating microorganisms for degradation of phytic acids and phenols enabling the end product to be richer in bioavailable mineral elements. Fermented cereals are reportedly low in starch bioavailability making food more palatable for peoples with high glycemic levels (Katina et al. 2005). Techniques for partial or complete replacement of wheat flour with other cereals or legumes are also in practice to design specialty products, e.g., low in gluten, high in quality protein, and rich in complex polysaccharides. Majority of ethnic fermented foods of Pakistan are a balanced mixture of cereals, lentils, and other seeds from Leguminosae. Although advantageous in increasing the rate of fermentation (Zhou et al. 2014), added sugars are still a major health concern for populations with higher obesity index, type 2 diabetes, and certain other chronic ailments. To a greater extent, a variety of ethnic fermented cereal-based products of Pakistan are prepared with high-calorie sweeteners, i.e., table sugar and jaggery. Intended use of sweeteners is primarily to add sweeter taste, attribute caramelized color, and improve flavor and textural properties of the finished good.

Re-modulating dietary plans of communities with certain nutritional inadequacies is a least acceptable strategy particularly in regions with rigorous dietary habits and beliefs. From the house of ethnic and cultural foods, high-glycemic-index cereal-based fermented foods, however, require modification in generic recipes to counter health hazards particularly those associated with high-calorie sweeteners.

5.2.2 Cereal-Based Fermented Products of Pakistan

5.2.2.1 *Doli Ki Roti*

Doli ki roti like fried *puri* is native to Western Pakistan from where the production technology of this indigenous fermented food was hand to hand disseminated in Indian cuisine (Bhatia and Khetarpaul 2012). The name "*doli ki roti*" is primarily linked with the fermentation process of white flour being carried out in earthen pots "*doli*." The product is derived from wheat flour partially substituted with legumes and seasoned with poppy seeds, cinnamon, jaggery (formally known as *gur*), and *jaifal*. Seasoning elements being incorporated in the recipe are supposed to add flavor and add up antimicrobial characteristics against a wide range of spoilage and pathogenic microbial strains. *Doli ki roti* being manufactured by blending cereals with legumes imparts significantly higher levels of protein, i.e., 14.5–17.1 %, whereas it has 3.8–4.7 % of ash, 8.7–10.6 mg/100 g of iron, and 52.7–62.6 mg/100 g of Ca content, respectively (Bhatia and Khetarpaul 2001). The fried bread is often stuffed with legumes, fried vegetables, or minced meat that further add nutrition and taste to the finished good (Fig. 5.1).

5.2.2.2 *Jalebi*

Jalebi is also known as funnel cake being fried and immersed in sugar syrup and is prepared from refined white wheat flour (*maida*) with less than 75 % extraction rate. The product indigenously prepared is unique in taste and is generally served as an Asian dessert on the sacred days or events of Muslim, Sikh, and Hindu communities. Tremendous production and consumption trends of *Jalebi* are observed on cultural festivals and celebrations and in cold winters when the fried product is served with milk as a food of pleasure. Production technology of *jalebi* reveals application of yogurt (*dahi*) as indigenous source of lactic acid bacteria being deployed in fermentation of refined wheat flour (*maida*). Fermentation increases volume of the *jalebi* batter by 9 % (Das et al. 2012). Despite of its nutritional attributes, *jalebi* is a high-calorie food and hence not recommended for patients reported with hyperglycemia (Fig. 5.2).

5.2.2.3 *Andrassay*

Fig. 5.1 Flow diagram of *doli ki roti* production

Inoculum Preparation

Mix 1/2kg sugar in water and heat until sugar is dissolved

↓

Add rice flour in syrup and homogenously mix

↓

Give a stay time to the homogenate for 2-3hrs

↓

Fermentation at 35°C (24h)

↓

Addition of whole wheat flour

↓

Fermentation at 35°C (2h)

↓

Preparation of Dough

Inoculum + wheat flour partially substituted with gram flour

↓

Mixing and kneeding

↓

Fermentation of dough in doli (1hr)

↓

Dividing dough balls

↓

Filling with sprouted, steamed chick pea, closing and flattening with rolling pin

↓

Deep Frying

Fig. 5.2 Flow diagram of *jalebi* production

Batter Preparation

Straight grade flour (Maida), corn flour, sweet yogurt, baking powder, vegetable oil

↓

Homogenous mixing avoiding lumps formation

↓

Gradual mixing with small quantity of water

↓

Add 1 Tbsp vegetable oil and keep mixing

↓

Give a stay time of 10min to smooth batter

↓

Preparation of Sugar Syrup

Add sugar and 1/2tbsp of cardamom powder in water

↓

Cook at low flame until thick string consistency

↓

Squeezing Jalebi batter from muslin cloth in frying oil

↓

Frying until brown

↓

Submerge brown textured jalebi in syrup for 2-3 minutes

↓

Remove from syrup pan and serve hot

Fig. 5.3 Flow diagram of *andrassay* production

Soak white rice in water for 3-4hrs
↓
Dry and grind the grains
↓
Add baking powder, vegetable ghee, sugar and finely beaten egg
↓
Prepare the dough
↓
Give stay time to the dough for 15-20min
↓
Prepare dough balls and shape alike patty
↓
Fry in vegetable oil until brown
↓
Garnish if required with sesame seeds

Andrassay is a product native to the district Kasur of Punjab Province and has exceptional popularity on account of its unique quality attributes. *Kasuri andrassay* either manufactured with wheat flour, sugar, and butter oil; wheat flour, jaggery, and butter oil; or otherwise *khoya*, sugar, and butter oil have wide acceptability among peoples of all ages and races (Fig. 5.3).

5.2.2.4 *Naan* (Leavened Flatbread)

Naan or flatbread is a cheap source of protein and carbohydrates for the peoples of South Asia (Pakistan, Afghanistan, Iran, and India) where the leavened bread is consumed as staple diet (Anjum and Walker 1991). *Naan* is prepared with refined wheat flour, salt, and sugar, while product fermentation is carried out either with yeasts or heterofermentative cultures of *Lactobacilli* spp. to ease fermentation, to increase product digestibility, and to improve nutritional and other qualitative features of the consumer good. *Naan* being prepared with fermented dough is relatively more nutritious than conventional unleavened *chapati* or *roti*. Logically, it is apparent that about 80 % of annual wheat production in Pakistan is consumed either in the form of *chapati* or *naan* and constitutes ~75 % of entire food consumption for a day (Aslam et al. 1982; Anjum et al. 1991). In addition to their pleasant sour taste and appealing flavor, sourdough fermentation process further adds up typical textural properties to the conventional fermented baked products (Fig. 5.4).

5.3 Dairy-Based Fermented Food Products

Mankind has been well renowned to ferment dairy products from ancient times and utilize the cultured milk products since the early 1800s. Milk is a complete diet having essential nutrients in it and can be spoiled easily in hot weather conditions if it is not properly refrigerated. But the people learnt from the fermentation process over the time that spoiled milk (sour milk) having pleasant aroma and taste could be stored for a longer period of time if storage conditions are properly controlled (Medina and Jordano 1994).

Fig. 5.4 Flow diagram of sourdough flatbread (*naan*) production

Preparation of Mother Sponge
Mix straight grade flour, water and freeze dried culture of Yeast (10^6 cfu/g) and *Lactobacilli spp*.(10^3cfu/g)
↓
Manual mixing and incubation for 20h at 30°C
↓
Dough mixing
Straight grade flour, salt, water and sponge mixing
↓
Cover the dough with wet muslin cloth and incubate for 20h at 30°C
↓
Prepare dough balls and shape in round sheet of 5mm thickness
↓
Baking on hot iron plate or earthen oven
↓
Mist water on the baked surface and partially grease with butter oil
↓
Serve hot or store in polyethylene bags

Nowadays, beneficial bacteria are used for the process of fermentation to produce the cultured milk products. Yogurt, sour cream, butter, buttermilk, and various types of cheeses are fermented dairy products.

5.3.1 Dairy-Based Fermented Foods of Pakistan

5.3.1.1 *Paneer*

Paneer is native coagulated dairy product and can be processed when organic acid is added to milk at high temperatures. In Khyber Pakhtunkhwa, Pakistan, *paneer* is prepared from buffalo milk and extensively utilized in cooked vegetables and meat dishes (Masud et al. 1992). It was also declared that the *paneer* prepared from cow milk had lower yield when compared to buffalo milk. But the sensory features of *paneer* prepared from buffalo milk significantly decreased at 4 °C after 1 week of storage.

Paneer is a type of soft cheese from the South Asian region and prepared by coagulation of milk with acid and heat treatment. It is an unripened, non-fermentative, and non-rennet type of cheese and popular in the South Asian region as its utilization can be made possible in many cooked food dishes. Now, *paneer* production is widely spread throughout the world. The most attractive feature of *paneer* is deep frying that could lead to its acceptance for utilization in many food items like fried *paneer* chunks, pakoras, and snacks (Aneja 2007).

Chemical Composition

Buffalo milk is preferred for *paneer* making over cow milk due to its marvelous features as it contains greater amount and size of fat globules and casein protein. Buffalo milk also contains higher concentrations of phosphorus and calcium. All of the ingredients contribute toward giving the spongy characteristics to *paneer* (Sindhu 1996; Ramasamya et al. 1999; Masud 2002) (Table 5.2).

Table 5.2 Nutritional comparison of milk, *paneer*, and whey derived from buffalo and cow milk

	Milk		*Paneer*		Whey	
Constituent (%)	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo
Total solid	13.32b	16.24c	48.74b	49.93c	7.25b	7.83b
Fat	4.2b	7.1c	22.46b	28.86c	0.8b	1.30c
Protein	4.0b	3.7b	21.23b	16.07c	0.45c	0.86b
Lactose	4.4b	4.7b	2.52b	2.33b	5.37b	4.82c
Ash	0.72b	0.74b	2.53b	2.67b	0.63b	0.65b

Source: Masud et al. (1992)

Preparation of *Paneer*

The direct acidification process is used for the preparation of *paneer* from fresh buffalo or cow milk as has been described in the flow diagram in Fig. 5.5 (Kosikowski 1982).

Milk (4 Liters)

↓

Heating at 85°C for 4 minutes

↓

Cooling to 70° C

↓

Addition of Lactic acid (10%)

↓

Constant stirring till complete coagulation

↓

Settling of curd for 5 min

↓

Drainage of whey through muslin cloth

↓

Salting of curd (1.5%)

↓

Moulding and Pressing (2-3 hrs, room temperature)

↓

Weighing and storage of paneer (4° C)

Fig. 5.5 Flow diagram for *paneer* production

5.3.1.2 *Dahi*

Fermented milk appears to be a popular beverage in Central Europe, Russia, Yugoslavia, the Middle East, and Indo-Pakistan under the names of yogurt, *leben*, *goidu*, and *dahi*. A standard *dahi* should possess the qualities of a smooth texture, a semisolid but firm body without any lumps, a velvety appearance, and a pleasant flavor (Wilson 1945; Tiwari and Singh 1964; Mihajlovic et al. 1980; Naeem and Rizvi 1986).

Dahi is a popular dairy product in the Indo-Pak subcontinent, having comparable features with yogurt. In Pakistan, its consumption is next to a beverage called "*lassi*" made from *dahi* which is relished by the most of the people for its refreshing taste and flavor. *Dahi* has been reported to contain a mixed culture of lactic streptococci and lactobacilli in addition to the yogurt organisms *S. thermophilus* and *L. bulgaricus* (Isani et al. 1986; Masud et al. 1991).

In Pakistan, *dahi* share is about 70 % of total fermented dairy products, and it also contributes in the preparation of other products like *makhan* (butter), *desi ghee*, and *lassi* (buttermilk). Processing of these products is inherited down from father to son and so on within the whole community. *Dahi* is fermented by a local microflora of lactic acid bacteria predominantly consisting of *S. thermophilus* and *L. bulgaricus*. They ferment milk lactose to lactic acid that further coagulate milk protein and form a yogurt-like product known as *dahi*. The most common bacteria used for yogurt production are *S. thermophilus* and *L. bulgaricus* (named so because yogurt was first discovered by Europeans among the people of Bulgaria).

Composition, Production, and Consumption of *Dahi*

Dahi is produced in India and its neighboring countries, Pakistan, Bangladesh, and Sri Lanka. *Dahi* can be utilized directly either spiced, salted, or sweetened. It is also consumed with various other food products like *chapati* and rice.

Approximately 9.1 % of total annual milk production (23.0 billion tons) or 100 g per person per day in India is utilized for making *dahi* intended for direct consumption. Consumption of *dahi* is more in summer rather than winter and taken once or twice a day at morning or evening meal. *Dahi* is consumed by the rich and poor alike. The consumption depends upon the nutritional composition, availability of the product, individual food habits, and locality (Steinkraus 1995) (Table 5.3).

Dahi analog to yogurt is a prevalent cultured dairy product in the Indo-Pak subcontinent. Its consumption stands next to whole milk particularly in the summer season. *Dahi* has been reported to contain a mixture of lactic acid bacteria (LAB) in addition to *L. bulgaricus* and *S. thermophilus* which are the mostly used cultures for yogurt making (Masud et al. 1991).

Table 5.3 Nutritional composition of *dahi*

Contents	% value
Water	85–88 %
Fat	5.00–8.00 %
Protein	3.20–2.40 %
Lactose	4.60–5.20 %
Ash	0.70–0.75 %
Lactic acid	0.50–1.10 %
Calcium	0.12–0.14 %
Phosphorus	0.09–0.11 %

Source: Steinkraus (1995)

Raw milk (buffalo/cow)

↓

Heating (90°C for 1min)

↓

Cooling (37°C)

↓

Inoculation (5%, 10%, 15%, 20% inoculums)

↓

Sealing (aluminum foil)

↓

Incubation (37°C for 15 hrs)

↓

Refrigeration (4°C)

Fig. 5.6 Flow diagram for manufacturing of *dahi*

Manufacturing Process of *Dahi*

Dahi can be processed conventionally by raw milk standardization followed by pasteurization, cooling, inoculation, and incubation as portrayed in the flow diagram presented in Fig. 5.6 (Chowdhury and Bhattacharyya 2014).

5.3.1.3 *Lassi*

Lassi is the by-product obtained through the churning process of *dahi* with native impellers when *desi* butter is going to be produced (De 2004). The process for the preparation of *lassi* varies with the change in place. In traditional

Fig. 5.7 Flow diagram for preparation of *lassi*

Standardization of Buffalo milk (4% fat and 8.5% solids-nonfat (SNF))

↓

Heating (85°C for 30 min)

↓

Cooling (approximately 37°C)

↓

Inoculation with (2% active *dahi* culture)

Incubation (16 hrs at 37°C)

↓

Addition of sugar (8%, 10% and 12%)

↓

Mixing with blender (sugar, coagulum)

↓

Cooling of lassi

↓

Storage at refrigerated (4°C)

↓

Physico-chemical and sensory evaluation

method, *lassi* preparation is a time-consuming process as lactic culture takes 12–16 h of fermentation process to acquire the desired level of sourness. The processing line of *lassi* production has been presented in Fig. 5.7 as adopted by Hingmire et al. (2009).

5.3.1.4 *Butter* (*Makhan*)

There are two basic types of butter, i.e., sweet cream and sour cream butter. Sweet cream butter can be prepared from fresh, pasteurized cream, while, in sour cream butter, pasteurized cream is ripened through fermentation process and churning is done to separate the fat from whey. The standardized butter contains 82 % fat in its composition. According to the desire of human beings, various food additives can be used to enhance the utilization of butter, e.g., addition of flavoring, coloring agents, and salts (Fig. 5.8).

5.3.1.5 *Desi Ghee*

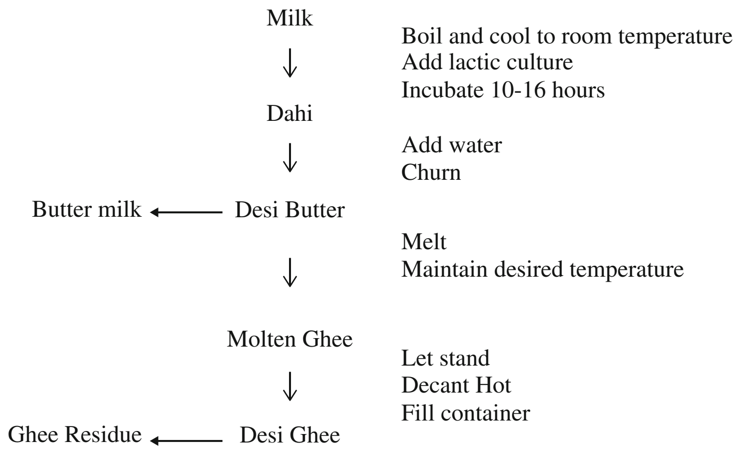

Fig. 5.8 Flow diagram for *butter* and *desi ghee* processing

The word *ghee* is derived from Sanskrit "*Ghrita.*" A Sanskrit verse says "*Ayurghritam*" meaning *ghee* is life. In South Asian countries (Pakistan, India, Bangladesh, Sri Lanka), *ghee* is extensively utilized in food products in various forms.

Why Is *Desi Ghee* Good for the Health?

- "*Desi ghee*" or the butter fat could be a good source of essential vitamins, e.g., vitamins A, D, and E, whereas it may also serve as a good carrier of fat-soluble vitamins vide supplementation to fulfill the nutritional needs of undernourished populations (Narayanan et al. 1954; Fox et al. 2015).
- *Desi ghee* may serve as a good source of MUFA (monounsaturated fatty acid) and hence can perform better than PUFA (polyunsaturated fatty acid) in reducing low-density lipoprotein cholesterol and less frequently negatively affect high-density lipoprotein concentration (Mattson and Grundy 1985; Grummer 1991).
- *Desi ghee* is made up of short-chain fatty acids that have better hydrolysis and absorption properties in monogastric mammals (Carey et al. 1983; Grummer 1991).
- *Desi ghee* is naturally low in *trans* fats as compared to hydrogenated plant fats.
- *Desi ghee* is free of salt unlike butter available in market.
- Ayurveda recommends *desi ghee* because it is good for the whole body.
- Ghee can be used for cooking at high temperatures unlike olive oil. However, a few people believe that *desi* ghee is best consumed when poured on cooked food before consuming. That's the reason why our grandparents added ghee in *daals* before eating or poured on *roti* (Preet 2013).

Method of Production

Ghee is attained by making clear milk fat from various solids at higher-temperature treatment. *Ghee* can be kept for a long period of time at normal temperature as compared to butter that needs a refrigeration system to be stored for longer shelf life (Jana 2012).

- In villages, *ghee* is usually prepared by the method referred above (Fig. 5.8), and this process contributes toward more than 97 % of total ghee processed.
- In this process, whole milk is inoculated to form *dahi*, and curdled milk is churned with a wooden corrugated beater and butter is separated from whey. Then butter is heated in an open pan to convert it into *ghee*.

5.4 Fruit- and Vegetable-Based Fermented Products

Fermented fruits and vegetables have good records of benefiting populations on ground of ensuring food security, improving nutritional inadequacies, social well-being, and livelihood standards. A very opportunistic situation exists for exploiting cost-effective fruit and vegetable fermentation technologies in countries with higher proportion of vulnerable and marginalized populations. A higher rate of deterioration in perishable nutritious food commodities including fruits and vegetables calls for expert's attention to cut down postharvest food losses for the sake of social and economic welfare. Drying, dehydration, freezing, pickling, and canning are a few known options to overcome fruit and vegetable losses. Among the adoptable strategies, fermentation is known as the best suitable technique to preserve fruits and vegetables. Owing to the low financial burden, simplicity in processing, and subsequent handling, fruit and vegetable fermentation has been adopted as a profitable cottage industry in middle- to low-income populations of the developing countries.

Consumption of fruits and vegetables in a variety of ways has been reportedly associated with health promotion and prevention of certain chronic ailments. Epidemiological findings conclude that diet may have a positive role in disease prevention, whereas fruits and vegetables have strong association with reduction in chronic ailments including cardiovascular diseases, certain types of cancers, Alzheimer's disease, diabetes, cataracts, and other age-related degenerative disorders (Hung et al. 2004). Very convincing research-based evidences further suggest for a drift in nutritional habits converting from unhealthy dietary approaches to fruit- and vegetable-based healthier recipes (Halliwell 2007). In global food production, estimates further present encouraging information on consumption of fruits and vegetables in the recent years (Manach et al. 2004).

In line with certain dairy-based fermented foods, fermented cereals, fermented white and red meat recipes, and fermented soybean and by-products, broader prospects exist for utilization and consumption of fruit- and vegetable-based fermented foods in Pakistan. Pickles are one of the delightful cuisines of Pakistan and are referred to be the product of lactic acid fermentation. Pickle formation from various fruits and vegetables involves lactic acid fermentation that work for optimum acid production ensuring adverse condition for the growth of aerobic food spoilage and pathogenic factors and to improve nutritional status of the finished good vide improving nutrient availability and absorbability.

5.4.1 Fruit- and Vegetable-Based Fermented Foods of Pakistan

5.4.1.1 Lime Pickle

Native to Asia, lemon belongs to family Rutaceae and is a flavorful culinary ingredient in preparing certain ready-to-eat meat-based recipes and for garnishing meals and adding flavor to certain drinks, sherbets, puddings, cakes, and confectionaries (Mohanapriya et al. 2013). The nutritional profile of the fruit comprising of alkaloids, flavonoids, and essential vitamins proposes viable biological properties like antimicrobial, antiviral, antipyretic, antidiabetic, antiulcer, and anticancer (Kawaii et al. 2000; Burt 2004; Ortuno et al. 2006). Being rich in vitamin C (ascorbic acid), lime can be used as a potential ethnopharmacological approach to treat and cure scurvy – a vitamin C deficiency disorder (Aronson 2001). A unique phenolic profile of lime rind and juice shows that both are also helpful in wound healing and eye damages. Ascorbic acid from the fruit juice improves dental health, taking care of healthy gums by preventing gum inflammation and bleeding. Folk uses of lime juice as weight loss strategy are in practice in various cultures to offset obesity (Steven 1992). Lemon pickles are homemade cuisine generally consumed to attribute a sweet, sour, and tangy flavor to the recipe in question. Lemon pickles are well known for their properties to ease out stomach disorders and act as appetizer as well as digestive ingredient.

Preparation of Lemon Pickle

Fully ripened limes free from cuts and bruises are selected for pickle manufacturing. Washing with boiled cold water is performed to sanitize the fruit surface and avoid microbiological contaminants to survive in the brine solution. Chlorination on this stage is avoided because the decontaminant (chlorine and chlorine compounds) application on this stage may kill desirable microbial growth and hence may hinder the fermentation process. Lemons with their skin intact are cut in quarters and layering is performed in a fermentation container that could be of glass or plastic origin. Cover the lemon layer with salt free from contaminants and add up another layer of limes over it and continue layering until the pickling container is three-quarter filled. Tightly place a muslin cloth over the container and leave out at a very sunny place to initiate brine formation as the salt application on fresh limes helps to ooze out water from the fruit. It takes around 24 h for brine formation and fermentation to initiate that is usually indicated in the form of bubbles of CO_2 on the surface of brine. Container is further incubated in the same conditions for a period of 1 week to continue the fermentation process. Incubation for lactic acid fermentation as desired in pickle formation is recommended at 21 °C, and the process is continued until no further bubbles appear in the container. Spices of premier quality free from microbiological contaminants particularly molds are added up in the pickle container. General composition of the ingredients depends on the local taste and is comprised of cumin seeds, fenugreek, mustard seeds, dried red chilies, and turmeric powder. Red chilies, fenugreek, and cumin seeds are roasted partially and ground with turmeric powder. A few spoons of mustard oil are heated in a pan; add mustard grains and cook until the grains pop up. Add all the spicy ingredients to the fermented lime and homogeneously mix the ingredients in a container (Fig. 5.9).

Selection of lime
↓ Fully ripened lemon should be used
Washing
↓ Clean, boiled water is used preferably
Cutting
↓ Fruits are cut in quarters
Mixing Salt for Brining
↓ Add 1kg salt in 4kg lime
Fermentation
↓ Leave lime container in sun for 1 week
Addition of Spices
↓
Packaging

Fig. 5.9 Flow sheet for lemon pickle processing

5.4.1.2 *Lasoora* Pickle

Lasoora or *Callicarpa dichotoma* is well known in ethnic cultures for its unique nutritional and medicinal attributes. The seeds or fruiting body of *C. dichotoma* are reported to carry anti-inflammatory features and are also in application as an expectorant, diuretic, purgative, and anti-helmintic ingredient (Kuppast and Nayak 2005; Sharma et al. 2010; Mishra and Garg 2011). Methanolic extracts of the fruit are referred as a good source of antioxidants, predominantly the flavonoids and α-amyrin, octacosanol, botulin, 3, 5, dirhamnoside, taxifolin, and certain fatty acids that bear anti-inflammatory, antiepileptic, and cognition-improving properties (Singh et al. 2010).

Ingredients

- *Lasoora* unripened fruit (500 g)
- Edible oil (preferably mustard oil)
- Refined salt (four tbsps)

- Turmeric powder (three tbsps)
- Fenugreek, coarsely ground seeds (three tbsps)
- Mustard seeds, (three tbsps)
- Asafoetida (one-half tbsps)
- Red chili powder (four tbsps)

Preparation

Prepare a mixture of salt and turmeric powder and rub the slit berries of *C. dichotoma* with the mixture. Heat ~2 cups of mustard oil in a pan to remove moisture traces and cool to lukewarm. Pour the oil over spices and homogeneously mix the ingredients. Add *C. dichotoma* berries in the spice mixture and mix to ensure adequate coating of berries with spices. Transfer all ingredients in a clean dry glass jar and cover the jar opening with muslin cloth. Leave the jar material to settle for 4/5 days and then merge the berries with preheated and cooled mustard oil. Cover the jar tightly and leave the ingredient for aging for a week time or longer. *Lasoora* pickle is also prepared by blanching washed green and gummy berries in boiling water for 2–3 min along with salt and turmeric powder. Drain the berries and dry them to remove adhering moisture from the fruit surface. Partially fry fenugreek, mustard, fennel, and nigella seeds in mustard oil and cool the ingredients. Add salt, crushed red chilies, turmeric powder, and boiled berries to the fried seeds and oil and fill in the glass jar. Homogeneously mix all ingredients and submerge the pickling material in preheated and cooled mustard oil. Tighten the jar lid and keep it in sunlight for a period of 1 week to ease out the fermentation process.

5.4.1.3 *Green Mango* Pickle

Homemade pickles are a traditional business of Pakistani populations; however, urbanization and consumer sensitization have critically affected the traditional food habits as greater urge was recorded among peoples for instantly available ready-to-consume food products. A report published by the Small and Medium Enterprise Development Authority of Pakistan (2007) indicates that 70–75 % of national pickle production comes from small and medium enterprises and household manufacturers, while the ~25 % share is managed by national brands like National Foods, Ahmed Foods, Shan Foods, Mitchell's Food, Young's Food, and other fruit and vegetable processing units (SMEDA 2007). It's worth mentioning that mango pickles to a greater extent from Sindh and Punjab provinces contribute a greater part to national pickle production. In addition to providing a good concentration of probiotics, mango pickles also attribute a range of phenolics compounded in green mangoes including gallic acid, ellagic acid, mangiferin, syringic acid, gentisyl-protocatechuic acid, and quercetin (Akarapach 2012; Tunchaiyaphum et al. 2013). Mango pickles hence could be referred as a good source of phenolic compounds that further attribute functional characteristics to the finished good.

Ingredients

- Green mangoes (10 kg)
- Salt (4 kg)
- Turmeric powder (100 g)
- Mustard seed (0.2–0.4 kg)
- Fenugreek seeds (0.2–0.4 kg)
- Chili powder (0.2–0.5 kg)
- Edible oil (1.0–1.25 kg)
- Asafoetida

Procedure

Fresh, firm, and fully matured green mangoes are selected for pickle manufacturing. Soft and ripened mangoes are not included in the recipe due to their inability to not withstand the fermentation process and loss in product texture and other sensory characteristics. Mango fruits harvested at their early maturity stage and after attaining peak size are the desired raw material for premium quality pickle manufacturing. Green and firm

Fig. 5.10 Flow sheet for mango pickle processing

Green, mature and firm fruit	Mangoes need to be ripened
↓ Sorting	Remove damaged fruit
↓ Washing	Clean and potable water
↓ Draining	
↓ Cutting/Slicing	Use stainless steel and sharp knives
↓ Brining	Add salt (4kg per 10 kg) or 20% salt solution
↓ Draining	Remove the brine in case of 20% brine solution
↓ Spices addition	
↓ Packaging	Add oil and pack in container

mangoes are sorted from the damaged fruit and adequately washed with potable water to remove adhering particles and surface contaminants. After de-stoning, the fruit is cut into quarters or halves. Fruit cuts are held with brine solution (2–3 %) until further processing to avoid a browning reaction. Sharp and stainless steel knives are used to ensure minimum damage to the fruit and avoid mushiness of the finished good. Dry salting and otherwise ready-to-use brine methods are used for mango pickle formation. Turmeric powder is mixed with mango cuts and the fruit–salt layering is performed in such a fashion that salt layer covers the top of fruit cuts. Glass jars covered with muslin cloth are placed under the sun to extract the fruit juice and convert salt into brine to onset fermentation. Complete submergence of mango cuts in brine solution is ensured to avoid microbiological contamination during fermentation. The process is continued for a period of 45 days until complete removal of fruit juice that is indicated by shrinkage and yellowness of the mango cuts. Homogeneously ground spices (mentioned earlier) are mixed with the fermented pickle to add a unique taste and flavor to the finished good. Preheated and cooled edible oil (mustard or other good-quality vegetable oils) is poured on the top of the pickle to aerobically seal the container. Finely ground chili powder can also be used in edible oil to add flavor and color to the pickle. Finally, the jar is tightly closed with a lid and stored in a cool and dry place away from direct sunlight (Fig. 5.10).

5.4.1.4 *Kanji*

Daucus carota L. sub sp. *sativus* (Hoffm.) Arcang (black carrot) has six varieties: yellow (var. *scharrovii Mazk*.), violet (var. *boissieri Schweinf*), pink (var. *rosseus Mazk*.), orange (var. *zhukovskii*

Fig. 5.11 Flow sheet for *kanji* beverage production

Kali Gajar/ purple carrots (250 g)
↓
Peeling
↓
Cutting
↓
Filling of jar/earthen pot with water (1 L) and prepared carrots
↓
Add spices, salts, ground mustard seeds
↓
Stirring
↓
Put in sunny sill for 5-6 days
↓
Stirring and pouring
↓
Separation of juice and pickled carrot pieces
↓
Packaging

Setch.), white (var. *albus Alef.*), and black (var. *vavilovii Mazk.*). The roots of the variety *vavilovii Mazk.* are used to prepare a fermented beverage in some Asian countries, particularly, Pakistan and India, whereby it is known as *kanji*. It is a quite popular remedy for the treatment of indigestion, loss of appetite, and liver disorders. It is prepared and used extensively as an appetizer in early summer season (Baloch 1994). Since long, it is being prepared both in homes as a household remedy and on a small industrial scale by roadside vendors. Despite such long usage history, it is neither characterized analytically nor evaluated biologically, which necessitates the investigation of this remedy to make it an evidence-based product. The tuberous roots of black carrots, the main ingredient of *kanji*, possess diuretic, digestive tract-soothing, hepatoprotective, and uterine stimulating properties (Hung et al. 2004; Halliwell 2007). These are reported to have menstruation-delaying and uterine contraction-inducing effects (Manach et al. 2004). The decoction of roots is efficacious in removing urinary tract stones. The use of infusion of roots for treating edema, flatulence, indigestion, and menstrual problems is another traditional claim (Hung et al. 2004).

The literature review indicated a number of pharmacological studies on roots of different cultivars of the plant. Two studies described the antioxidant activity of carrots using in vitro models (Foster and Duke 1990; Gazzani et al. 1998). The seven different colored varieties of carrots were biofortified to enhance their antioxidant potential, which was then evaluated using DPPH and (2, 2-azino-di-[3-ethylbenzthiazoline-6- sulfonate] ABTS models (Cao et al. 1996). Moreover, the juice of fresh roots had shown hepatoprotective activity in CCl_4- and lindane-induced intoxicated mice and rats (Bishayee et al. 1995; Sun et al. 2009) (Fig. 5.11).

5.5 Conclusion

Fermentation as a traditional food processing technique is in practice in the highlands and plains of *Khyber Pakhtunkhwa*, *Balochistan*, *Gilgit-Baltistan*, *Punjab*, and *Sindh* provinces of Pakistan for generations and potentially anticipating as a potential segment of *Eastern* cuisines. Objectively, fermented foods owing to their higher index of acceptability are not only meeting the dietary needs of a significant size of population but also contributing a characteristic role in managing health issues of undernourished populations. During the last couple of years, massive private and public sector advocacy campaigning has been witnessed in the underdeveloped regions of the country to opt food fermentation as a small and medium food enterprise. Evidently, the exploitation of underutilized food resources from their own farms as delicious and nourishing ready-to-use consumer goods, e.g., pickles, fermented dairy products, baked cereals, confectionary goods, and nonalcoholic beverages, has been emerged as a profitable business to curtail the vulnerability factor of the farming communities. Additional health prospects explored from indigenous fermented foods further enlightened the significance of fermentation on essential biomolecule recovery and added endurance factors to decrease the level of glucose and nondigestible polysaccharides in fermented cereals and enhance the synthesis of certain amino acids. Although the ethnicity in application of fermentation technology in the food industry of Pakistan has a very active and enduring role to satisfy the dietary needs of the population, still there exists a need for application of innovative approaches in conventional to rather more sophisticated pure culturing, process optimization, and product safety ensuring ideal and consistent utilization of locally acceptable and adoptable nourishing food commodities in the country.

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Ethnic Fermented Foods and Beverages of Sri Lanka

6

Sagarika Ekanayake

6.1 Introduction

Sri Lanka is a teardrop or pear-shaped, elongated island, with a wider area in the south that narrows toward the north. The south central area is mountainous. It is located within the tropics, between the northern latitudes of 50 55' and 90 51' and the longitudes of 790 41' and 810 53' (Fig. 6.1). It has a land extent of 65,610 km^2 and receives on average 2000 mm of rain annually (Statistical Abstract 2014). Major agricultural produce is rice which is cultivated during 2 seasons (*Maha* and *Yala*) of a year. Other crops include tea, rubber, and coconut, while other major food crops include millet (*kurakkan*), maize, cowpea, ginger, manioc, fruits, vegetables, etc. In addition, livestock, fish, milk, and eggs contribute to the dietary pattern (Statistical Pocket Book 2015).

Population comprise of people belonging to many different ethnicities, majority being Sinhalese followed by Tamils, Moors, Burghers, and Malays (Statistical Abstract 2014). According to the Department of Census and Statistics report (2001) on the number and percentage of population by district and ethnic group, the Sinhalese are the majority group and account for 82 % of the total population. The Tamil community is 9.4 % of the total population and is subdivided into two groups: Sri Lankan Tamils and Indian Tamils (migrants from India). Muslims (8.2 %) are scattered around the country and comprised of Indian and Malay Moors. Burghers (0.2 %) are the descendants of the Dutch, Portuguese, and English who once colonized Sri Lanka and, by marrying Sri Lankans, settled in Sri Lanka (Department of Census and Statistics report 2001). Hence, the Sri Lankan cuisine is influenced by the Portuguese, Dutch, Malays, Arabs, and South Indians who have left their culinary characteristics and legacies.

6.2 Food Habits and Food Culture

Fermentation is defined as a desirable process of biochemical modification of primary food products (carbohydrates) brought about by microorganisms and their enzymes (Karovicova and Kohajdova 2005). This is a process that occurs naturally and part of the decay process, especially in fruits and vegetables. The health benefits and the enhancement of texture, nutritional value, taste, aroma, and longer shelf life are among the advantages of fermentation (Fellows 2000).

In Sri Lanka, even though not many fermented foods are available, some such foods have been used as a meal or part of a meal for a long time.

S. Ekanayake (✉)
Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
e-mail: sagarikae@hotmail.com

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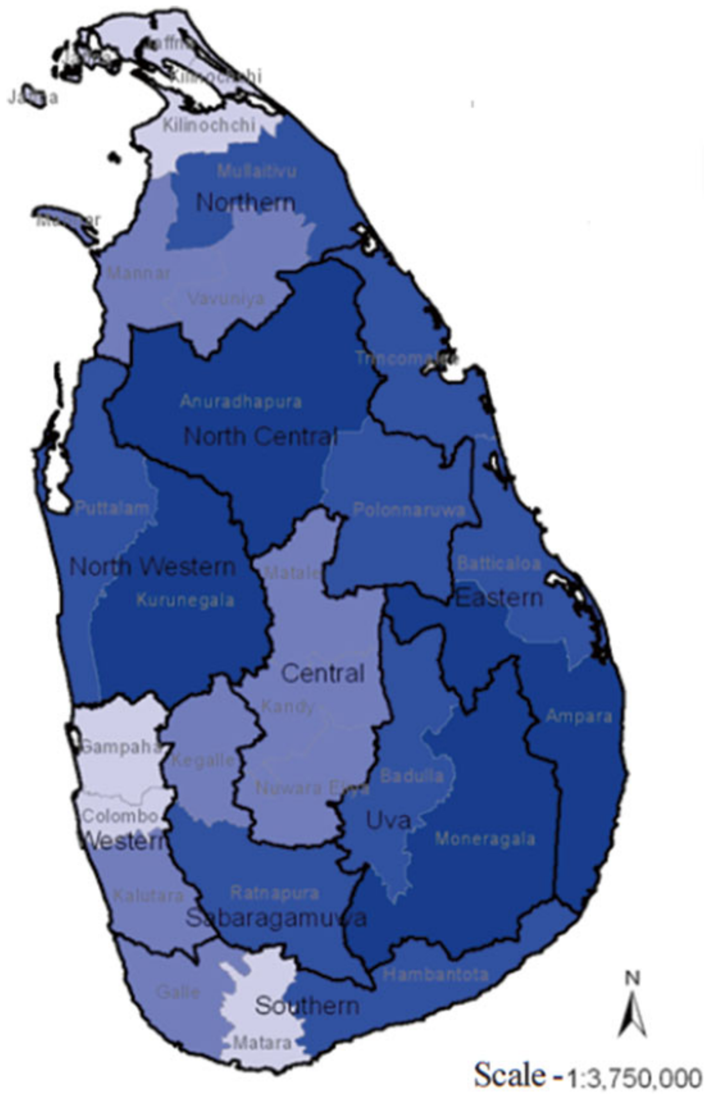

Fig. 6.1 Sri Lanka – land area by district (Source: http://www.statistics.gov.lk/Pocket%20Book/chap01.pdf – Statistical Pocket Book 2015)

The most well known is curd, made from buffalo milk, which has been a delicacy from ancient days. Fermented foods are fitted into the meal pattern in a variety of ways. Of the three main meals, lunch is invariably rice and curry, which comprises of a major portion of rice, one or two vegetables (green leafy vegetables, legume or potatoes, yams or tubers), and fish or meat curry made with condiments. However, most Buddhists avoid eating meat and Hindus avoid beef. Breakfast and dinner include other foods made with rice or wheat flour or bread. String hoppers, hoppers, *roti*, *thosai*, or *idly*, together with one or two side dishes, make the breakfast and dinner, of which some are fermented products. Especially *thosai* and idly together with *wadai* are common foods in the Tamil community. However, the foods that have been restricted to different ethnic groups have now become popular among all communities and available throughout the country.

Hoppers, *thosai*, *idly*, *wadai*, curd, toddy, *jaadi* (cured fish), lime pickle, dried fish, and bread (legacy from the European colonization era) are common fermented foods that have been utilized for a long time. However, yogurt, arrack, flavoring agents like vinegar, Maldive fish, newly developed vegetable pickles, salami, pepperoni, tempeh, and cheese are also commonly available.

Today fermented fish sausages, fish sauces, and fish paste are entering the commercial market.

6.3 Milk-Based Products

The most common and popular lactic acid-fermented preserved foods among children and adults in Sri Lanka are yogurt and curd (Fig. 6.2), normally consumed as a dessert after the main meal. Both are characterized by a significant acidity caused by the production of lactic acid by lactic acid bacteria (LAB).

In curd (*mee kiri* or *kiri*) preparation, fermentation with lactic acid bacteria (*Lactobacillus acidophilus*, *L. bifidus*, *L. bulgaricus*) usually takes place with boiled cow or buffalo milk (Table 6.1). The milk is boiled, allowed to cool,

Fig. 6.2 Curd (*mee kiri*)

Table 6.1 Some common ethnic fermented products of Sri Lanka

Name of product	Substrate	Nature and use	Microorganisms	References
Curd	Cow or buffalo milk	Acidic, sour, gel: dessert	*Lactobacillus acidophilus*, *Lactobacillus bifidus*, *Lactobacillus bulgaricus*	Wikramanayake (1996) and Pathirana et al. (1992)
			Lactobacillus fermentum, *Lactobacillus cellobiosus*, *Streptococcus lactis*, *Streptococcus lactis* subsp. *diacetylactis*	
Toddy	Coconut, palmyra, or *Caryota urens* (kitul) flower sap	Slightly sweet: alcoholic beverage	*Saccharomyces exiguus*, *S. cerevisiae*, *Pichia fermentans*, *S. rosei*, *S. fructuum*, *Torulopsis holmii*, *Torulopsis versatilis*, *Candida robusta*, *Candida lambica*, *Saccharomyces*, *Schizosaccharomyces*, *Brettanomyces*	Samarajeewa (1986), Jayatissa et al. (1978), Liyanage et al. (1981), Theivendirarajah et al. (1977), Vidanapathirana et al. (1983)
Jaadi	Seawater or freshwater fish (fatty)	Pickle, curry, condiment	–	Jayasinghe (2002), and Lakshmi et al. (2010)
Vinegar	Coconut toddy	Acidic: preservative, flavoring	*Acetobacter acetic*, *A. xylinum*, *A. ascendens*	Wikramanayake (1996) and Vidanapathirana et al. (1983)
Maldive fish	Smoked tuna	Hard: flavoring	*Aspergillus flavus*, *A. tamaraii*, *A. niger*, *A. ochraceus*, *Penicillium citrinum*	Mohamed (2013)

and inoculated with a small amount of previous days' culture as a starter and fermented for 24–72 h. LAB produce lactic acid and lower the pH, which, once reaching the isoelectric pH, causes casein precipitation forming curd (Wikramanayake 1996). Fermentation also results in a change in texture and increases the nutrient density. Curd made from buffalo milk, which is consumed with treacle, made from *kitul* (*Caryota urens*) palm, is the most popular form of fermented milk in Sri Lanka (Wikramanayake 1996). Buffalo milk gives a firmer curd than cow milk due to the warm dry conditions of the areas where buffalo milk is used in curd production. The minimum inoculum required for satisfactory curdling was 0.5 g of previously made curd or 34×10^6 colony-forming units (Pathirana et al. 1992).

Enzymatic methods of curdling also cause rapid clotting, but advantages of fermented milk are not found with these forms of curdled milk. The maximum recommended pH is 4.5 and lactic starter culture organisms isolated from curd are reported as *L. fermentum*, *L. cellobiosus*, *Streptococcus lactis*, and *S. lactis* subsp. *diacetylactis* (Pathirana et al. 1992).

6.4 Cereal and Legume Products

Fermentation due to lactic acid bacteria is a natural process brought about by LAB present in the raw food or those derived from a starter culture. Such bacteria in food fermentation of cereals are used in households of Sri Lanka from earlier days. Hoppers (*appa*) are based on a fermented batter, made of rice flour and coconut milk and pan fried. In hopper making, rice grains are soaked overnight, during which naturally occurring microorganisms will result in a population dominated by lactic acid bacteria. The grains are then ground and made to a smooth batter with coconut water and water. Fermenting agent could be toddy, a piece of bread, or yeast which is added to the mixture. The mixture is allowed to rise under normal aerobic conditions (8 h). Endogenous amylase will accelerate the fermentation. The pH produced will be 4.0 or less since cereals are weakly buffered. Hence, growth of bacterial pathogens will be inhibited. Just prior to preparation of hoppers, coconut milk is added and a batter made and cooked in a hot griddle. Hoppers are much like sourdough pancakes or muffins. If palm toddy is the fermenting agent (traditional way), this gives the hoppers a delicious liquor tang. Back sloping is a common practice in this type of food production at small scale or in households (Wikramanayake 1996). Hoppers can be either savory (egg hoppers, milk hoppers) which are eaten accompanied by *lunu miris*, a mix of red onions and spices or sweet (*pani appa*) made by adding jaggery. However, the instant flour mixtures are becoming more popular, but may not have the beneficial effects of fermented products.

Thosai and *idly* are common fermented foods eaten in Sri Lanka, a legacy from the South Indians who inhabit Sri Lanka. Black gram and rice are soaked separately for 4–6 h; mixed in proportion of 1:2 and mashed to a smooth batter and left overnight to rise (lactic acid production); flavored with fried shallots, curry leaves, fenugreek, and cumin; and cooked on a hot griddle greased with sesame oil for *thosai* or without condiments or steamed to make *idly* (Wikramanayake 1996).

6.5 Alcoholic Beverages

Toddy (palm wine; *raa*) is the traditional common fermented alcoholic beverage popular in the villages, made by the fermentation of the sap from coconut (*Cocos nucifera*) (*Pol*, Sinhala; *Tennai*, Tamil); the commonest, palmyra (*Borassus flabillifer*) (*Tal*, Sinhala; *Panai*, Tamil) (in northern parts); and toddy palm (*Caryota urens*) (fishtail; *Kitul*, Sinhala; *Tippilipana*, Tamil) (wet zone) (Samarajeewa 1986). The sap is collected by slicing off the tip of an unopened flower. The sap oozes out from the cut spadix and collected twice daily in a small earthenware pot tied underneath the flower. The fermentation starts as soon as the sap is collected in the pots on the palms, and after straining, the extract is sold on the same day in taverns and is referred to as sweet toddy. It is white and sweet with a characteristic flavor (4–6 % alcohol) and has a shelf life of about 24 h (Fig. 6.3). Fermented coconut toddy contains about 1.8–7.9 g alcohol, 0.29 g sucrose, 0.9–3.0 g invert sugar, and 3.72 g/dL total solids. Fermented sap also contains nitrogen, phosphorus, potassium, calcium, and magnesium <0.5 g/100 mL (Samarajeewa 1986).

The flavor is due to higher alcohols, aldehydes, ketones, and amino acids. More than 75 kinds of wild yeasts and bacteria in toddy have been identified. In fermenting coconut sap, 17 isolates of yeasts belonging to five genera were observed. They included isolates of *Saccharomyces exiguus*, *S. cerevisiae*, *Pichia fermentans*, *S. rosei*, *S. fructuum*, *Torulopsis holmii*, *Torulopsis versatilis*, *Candida robusta*, and *Candida lambica* (Jayatissa et al. 1978) and yeasts of *Saccharomyces*, *Schizosaccharomyces*, and *Candida* from coconut and palmyra palm wines (Liyanage et al. 1981). According to Vidanapathirana et al. (1983), coconut sap fermentation has three phases, in which the first phase (0–20 h) is dominated by bacterial fermentation (*Leuconostoc*, *Lactobacilli*, *Streptococci*, *Bacilli*, and *Enterobacter*) with a drop in pH from 7 to 4 with no alcohol formation. The second phase (30 h–5 days) is dominated by yeasts (*Saccharomyces chevalieri*) with *Pichia* (two strains) and *Candida* (two strains) with a subsequent increase in alcohol with no change in pH (4). Acetic acid bacteria dominate the third phase (Vidanapathirana et al. 1983). In the coconut sap, *Saccharomyces chevalieri* is the main alcohol-producing organism in contrast to *Saccharomyces cerevisiae* observed in many other alcoholic fermentation industries. Of the microorganisms isolated from the kitul palm, yeasts belonging to the genera *Brettanomyces* and *Saccharomyces* had been identified (Theivendirarajah et al. 1977).

Toddy, when fermented, becomes *arrack* (spirit), which comes in varying degrees of strength (Samarajeewa 1986). *Toddy* with an alcohol content of 6.7 % is distilled in copper or stainless steel plants to obtain *arrack* and stored in *halmilla* (*Berrya cordifolia*) vats for maturation (wood also adds a flavor component and color). The matured spirit is blended with water to obtain a final alcohol strength of 33 % by volume, and the product is bottled under various names depending upon maturation (Wikramanayake 1996). The quantity of ethanol produced had been shown to increase by adding the antifermenting agent sodium metabisulfite (up to 200 mg/l) during the collection of sap on the tree. This suppresses the non-ethanol-producing microorganisms and permits ethanol production by pure yeast cultures to different extents as coconut sap is the major raw material

Fig. 6.3 Toddy (*raa*)

for the preparation of the toddy and *arrack* in Sri Lanka (Samarajeewa et al. 1985).

The production of beers is one of the well-established alcoholic fermentation industries in Sri Lanka with a history starting from 1860. The technology for the production is the same as in the Western countries. However, rice (dry milling) is also used in the beer production in addition to malt (wet milling). Milled starting material is mashed and conducted over a period of time at various temperatures in order to activate the enzymes responsible for acidulation of the mash and reduction of proteins and carbohydrates. The optimum pH range for mashing is maintained from 5.1 to 5.6 although values toward lower end (5.1–5.3) are considered optimum. After mashing, the liquid extract (wort) is separated (lautering) from the residual undissolved solid material of the mash. This will produce clear wort which is conditioned by boiling in the kettle. This will stabilize the wort and extract desirable components from the hops. This will allow sterilization (remove microorganisms which can result in off flavors), enzyme inactivation, protein precipitation, color development, production of melanoidins, removal of volatiles, and isomerization of alpha acid in hops into iso-alpha acid, a major contributor to bitterness in beer. Following boiling, the hop debris is separated and wort is cooled (to a temperature of 5–15 °C for bottom-fermented beers or 15–18 °C for top-fermented beers). After cooling, the wort is aerated to increase yeast activity and start the fermentation process. The yeast strain is a major contributor to flavor and character of the beer. Maturation involves secondary fermentation of remaining ferments at a lower rate. Finally beer clarification and stabilization are achieved by filtration (personal communication). The ethyl alcohol content (v/v) in different beers varies from 4 to 10 and pH is maintained at 3–4.8 (Samarajeewa 1986).

Illegally produced alcoholic beverage known as *kassippu* is produced with sugar as the starting material with yeast added for fermentation to proceed. People brew this for domestic consumption and for commercial purposes. Depending on the availability, fruits and other substances are added and the distillation is not properly controlled. It was reported that the brewers hang the herbicide, paraquat bottle with the lid pierced over the distilling cocktail, as they believe that condensed particles act as a catalyst, increasing the concentration and quality of the distillate (Dias 2010).

6.6 Vinegar

Vinegar, translated as sour wine, is one of the oldest products used by man. Vinegar is important as a highly effective food preservative and a flavoring agent. Coconut *toddy* vinegar is produced throughout South Asia particularly in Sri Lanka and has been used as a preservative of perishable food. Natural vinegar produced from coconut toddy is a household industry in Sri Lanka from ancient times using a traditional vat system (Food Processing Technologies 1995).

It is a clear liquid with a strong acetic acid flavor and a hint of coconut flavor (Fig. 6.4). Fresh toddy is strained and yeast fermentation is allowed to occur naturally for 48–72 h. The yeast (*Saccharomyces ellipsoideus* and *S. cerevisiae*) converts sugars to alcohol, which is the substrate required by the *Acetobacter* to produce acetic acid. After 2–4 weeks of settling, the fermented toddy is placed in barrels. Alcohol concentration of 10–13 % is ideal for vinegar production. High alcohol concentration will result in incomplete oxidation of alcohol to acetic acid, whereas lower alcohol concentration will cause loss of acetic acid due to oxidation after formation. The alcohol is oxidized into acetic acid, by acetic acid bacteria (*Acetobacter acetic*, *A. xylinum*, and *A. ascendens*), which are naturally present (Vidanapathirana et al. 1983). During maturing of vinegar, reactions between residual ethanol and acetic acid form ethyl acetate, which imparts the characteristic flavor to the product. The fermented toddy is converted into vinegar in about 3 months. Aging for 6 months results in a pleasantly flavored final product (Wikramanayake 1996; Battcock and Azam-Ali 1998). In addition to the above, vinegar is also produced by coconut

Fig. 6.4 Natural vinegar from coconut

water alcoholic fermentation and acetous fermentation with addition of sugar. The standards of composition of vinegar as specified should contain acetic acid not less than 45 m/v, with no mineral acids, and should be free of *Turbatrix aceti* (Food Act 2007).

6.7 Vegetable and Fruit Pickles

Fermenting fruits and vegetables can bring many benefits to people in developing countries. Other than providing food security and improving the nutrition, such foods can be a source of good income when the waste is considered. Vegetables are classified as "low-acid" foods due to their low levels of acidity and are more prone to deterioration by microorganisms.

Lime pickle (*lunu dehi*) is an integral part of Sri Lankan cuisine. It is a method to preserve the excess of mature lime harvested during the seasons. The limes used are those that have turned yellow from ripening. The other main ingredient, an excess of salt crystals that the limes are stuffed with, acts as the preservative. Lime pickle is prepared by treating limes with dry salt. Limes are cut into quarters and placed in a layer, approximately 2.5 cm deep, into the fermenting container and salt added (4:1 ratio). The raw lime taste is avoided by prolonged drying in the sun ideally till its moisture content is removed and the core of the limes acquires a white coloration. The repeated drying in the sun allows the limes to marinade in salt. Salted dried lime is layered tightly until the container is three quarters full and covered with a cloth. The formation of brine takes about 24 h. As soon as the brine is formed, fermentation starts. Fermentation takes between 1 and 4 weeks depending on the ambient temperature. The final product is a sour lime pickle which can last for about 5 years. The methods of preparation vary, with the ingredients used and their quantities. Addition of vinegar, which also preserves the limes, gives the pickle the desired moisture as well. In addition, instead of chili powder, chili flakes and other spices such as cloves and cardamom as well for the preferred and desired final taste can be added (http://exploresrilanka.lk/2013/03/lunu-dehi-a-friendly-zest/). Spices are added depending on local preference. It is usually eaten as a condiment to enhance the overall flavor of the meal and is favored by many Sri Lankans (Wikramanayake 1996). A small amount of pickle makes a bland diet much more appealing.

Production of fermented capsicum using wet and dry salt method has indicated a salt concentration of 5 % and 5.5 % giving the lowest pH (3–4) following 12 days of fermentation. The microorganisms involved have been identified as *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *L. fermentum*, *Pediococcus pentosaceus*, *Saccharomyces* species, heterotrophic species *Enterobacter cloacae*, *Citrobacter freundii*, *Micrococcus* species, and *Pseudomonas* species (Mahinda 2005). However, following 6 months of fermentation, no microbes have been detected (Mahinda 2005).

6.8 Fish and Meat Products

All fermented fishery products in Sri Lanka are salt based. Of this, nearly 75 % is Maldive fish (*umbalakada*). This product is used to be imported from the Maldive Islands, but now most of it comes mainly from India and local production (Wikramanayake 1996). This is the lightly salted, smoked, dried loin of skipjack tuna. This is widely used as a flavoring agent in most local products/curries and is also said to have been used by Kandyan kings for flavoring their food (1700–1800 era; Edirisuriya 2003).

In the preparation of Maldive fish, skipjack tuna or other tuna varieties are deheaded and gutted and washed. Salt is added to the surface of fish and inside the horizontally cut areas and kept for 30 min. Fish is cooked as well as smoked for about 10–12 h or overnight. The cut pieces are separated from the central bone and all small bone scales are removed. The pieces are subjected to smoking for further 2–4 h. The smoked pieces are then rolled in ash and sun-dried for 7–10 days. Insufficient drying leads to extensive mold growth (State-of-the-art report on selected rural technologies 1995). According to a study with Maldive fish produced in Maldives, a higher percentage of (96 %) Maldive fish has been found to be contaminated with *Aspergillus flavus* (92 %), *A. tamaraii* (96 %), *A. niger* (40 %), *A. ochraceus* (12 %), and *Penicillium citrinum* (60 %). Quantification of aflatoxins from these samples had shown only 2 samples among 25 to be contaminated with above the legal limits (Mohamed 2013). Inhibition of fungal growth especially of *Aspergillus flavus* was achieved at a_w 0.75, and this could be achieved by rapid drying. During the production of Maldive fish, toxigenic fungal growth needs to be controlled to avoid food safety risk (Mohamed 2013).

Dry fish marketed in Sri Lanka generally cannot be categorized as a fermented fishery product due to the process of salting involved and the fact that the product is in various degrees of fermentation. Fermentation will continue until water activity is decreased due to drying. Drying method varies with size of fish, and for small fish, sun-drying is preferred and slat-drying (common during rainy season) is preferred for large and moderately fatty fish. Ventral side is split in smaller fish, whereas dorsoventral splitting is used for large fish, where gills and intestines are removed, washed, cleaned, and spread out at the time of drying (Subasinghe 1993).

Jaadi or pickled fish (Fig. 6.5) is the other important fermented fishery product and is mainly produced locally on a cottage scale. *Jaadi* is a wet-cured traditional fermented fishery product (Weerasinghe 1991). Indian mackerel is the commonly used fatty fish in the preparation of *jaadi* in Sri Lanka especially during the glut. *Jaadi* is a highly salted fermented fishery product consisting of partially hydrolyzed fish flesh where the organs are immersed in the liquid exudates from fish. A low pH (below 4) is maintained by the addition of ripe pods of *goraka* (*Garcinia cambogia*) (Jayasinghe et al. 2000). The curing mixture is made using dried and ground *goraka* (*G. cambogia*) and solar salt (ratio varies). Indian mackerel treated with solar salt in the ratio of 3:1 and by keeping the ratio of fish/*goraka* to 10:1 is reported (Jayasinghe 2002).

Deskined tilapia (*Oreochromis niloticus*) fish without head has also been used effectively to produce *jaadi*. A ratio of 500 g of goraka (*G. gambodiea*) and 100 g of salt with 1 kg of tilapia has produced *jaadi* with acceptable sensory properties (Lakshmi et al. 2010). Production of *jaadi* from tilapia is considered beneficial to overcome the postharvest losses and increase consumption due to lack of taste leading to less popularity among consumers. Presently this is done as a cottage industry along the coastal area, but the product is not produced under standard conditions; hence, the popularity is less.

Salami and pepperoni produced by using starter cultures (*Pediococcus pentosaceus*) on meat are also gaining popularity though they are not traditional products. Meat is cultured with starter cultures and frozen and kept at room temperatures for 3 days and put in a steam chamber (35–40 °C, 3–4 h) and in a controlled atmo-

Fig. 6.5 Pickled fish (*jaadi*)

spheric room (3 weeks) where the temperature and relative humidity are gradually decreased. Depending on the temperature and the relative humidity and the time, salami (5 weeks) or pepperoni is produced. In addition to flavor acquired by bacterial fermentation, artificial flavors are added (personal communication).

6.9 Biochemistry or Nutritional Composition

Very limited data are available on the nutritional quality and biochemical activities of Sri Lankan fermented food products. Most work has been conducted on curd and yogurt, the most popular forms of fermented food products.

6.10 Milk Products

Nutrition-wise, curd and yogurt are both rich in B vitamins and have high protein content that enhances the bioavailability of calcium and are a food for lactose intolerants. Curd from buffalo milk is rich in fat and calcium and sets harder than cow's milk. Set yogurt and fruit yogurt manufactured by different manufacturers on an average had protein contents of 4.0 % and 3.0 %, respectively. Ash and fat contents varied from 0.77–1.3 % to 0.40–3.94 % with fruit-containing samples having a low fat percent (De Silva and Rathnayaka 2014).

The ACE inhibitory percentages of the bovine full-fat milk samples fermented with *L. lactis* subsp. *lactis* NBRC 12007 and *S. cerevisiae* K7 monocultures and the coculture were 33 %, 27 %, and 25 %, respectively (Rasika et al. 2015). The results conclude that the two strains tested were able to hydrolyze milk proteins into ACE inhibitory peptides. It is suggested that these strains be successfully utilized in the dairy industry in manufacturing fermented milk products with ACE inhibitory activity as a dietary supplement and/or as an alternative approach for antihypertensive medication (Rasika et al. 2015).

6.11 *Jaadi*

A total of 40 samples of different varieties of fishes collected from the market and *jaadi* curing yards when evaluated for quality and fungal and insect infestation showed that *jaadi* had a high level of protein. However, the defects of curing process such as inadequate cleaning and salting resulted in low-quality (chemical and microbiological) products (Jayasinghe et al. 2000). The total bacterial count (TBC), oil content, free fatty acid (FFA), peroxide value, and pH of *jaadi* made with Indian mackerel cured for 1 day were 1.42×10^3, 2.2 %, 29.9 %, 0 %, and 2.42 %, respectively (Jayasinghe et al. 2003). However, the overall acceptability was unsatisfactory (2.5). After 2 months of curing, the FFA, peroxide value, oil, TBC, pH, and overall acceptability of *jaadi* were 19.71 %, 0 %, 2.24 %, 1.82×10^3, 3.29, and 5 (best quality), respectively. The changes in oil, peroxide, and FFA indicated that oxidation has not occurred during the first 2 months (Jayasinghe et al. 2003). Further studies on nutritional profile of *jaadi* made with Indian mackerel indicated little change in fat content during a period of 4 months. Among lipids, triacylglycerols dominated the other lipids and lipid-soluble compounds. However, the content of fat can be influenced by the species, season, geographical region, size, gender, age, and maturity. The saturated fatty acids in *jaadi* decline during storage with no change in monounsaturated fatty acids and n-6 PUFA, but a gradual increase in n-3 PUFA is observed. A distinct decline in cholesterol (35.2–11.2 mg/g lipids) has been observed with pickling time with an increase (Ubhayasekera et al. 2005) in the cholesterol oxidation products (COPs) up to the sixth week and a gradual decline during further storage. Oxycholesterol metabolites are considered to be potentially involved in the initiation and progression of major chronic diseases including atherosclerosis, neurodegenerative processes, diabetes, kidney failure, and ethanol intoxication (Sottero et al. 2009). Therefore, regular consumption of pickled fish that has been cured for over 2 months can be a source of considerable amounts of COPs in the diet of the local population in Sri Lanka (Ubhayasekera et al. 2012).

Salted dried fish is also consumed in small quantities after deep frying or as a curry with rice. The composition varies widely with moisture varying from 10 % to 55 % and salt from 1.5 % to 5 %. Drying causes loss of vitamins but the protein content is higher than fresh fish ranging from 25 % to 30 % (Wikramanayake 1996).

6.12 Ethnical Value and Socioeconomy

Curd (*mee kiri* or *kiri*) production in some parts of the country is conducted as a cottage or small-scale industry. The traditional method of production is followed and curd is available in clay pots (Fig. 6.2). In the south and southeast of Sri Lanka, coverage of 84 % of 2 districts has indicated that 1968 producers produce 20,000 pots of curd per day (Pathirana et al. 1992). This has now increased considerably. Especially in the south and east of the country, this is a form of fund generation. It is indicated that by introduction of low-cost, simple, scientific production methods, the quality and hygienic standards of the curd could be improved. Currently large dairy production companies have also entered the market to meet the consumer demand. The largest alcoholic beverage industry in Sri Lanka today is the coconut sap fermentation industry which produces toddy and the distilled beverage "arrack." *Toddy* is bottled and pasteurized to improve the shelf life. These beverages are popular due to the characteristic flavor and the low cost. The other popular beverage in Sri Lanka is beer. Of the three, the coconut is tapped industrially in large scale in the areas of the western coastal belt. The inflorescence of the palmyra palm is tapped in the Jaffna Peninsula and to a small extent in the east coast of Sri Lanka. Fishtail palm is not grown in large scale but is tapped wherever it is available. It exists as a cottage industry for the production of toddy and as a source of sugars, treacle, and jaggery (Chrystopher 1988). The brewery industry has captured the export market with a variety of beers (lager, stout, beer) and caters to local demand as well. The largest one is equipped with state-of-the-art manufacturing facilities that enable it to fulfill the increasing demand and export beer to new and current export markets, such as England, Japan, and Australia.

Manufacture of different cheeses is another industry that has gained value during the last 25 years with few major and small manufacturers producing to cater to the consumer demand. Over 90 % of the cheese in Sri Lanka is imported; however, the cheese produced by these manufacturers is gaining recognition and some are able to provide rural women with an income. Some small-scale manufacturers adjust the textures, flavors, and also packaging size and modes according to the demand and thus are able to sell these domestic cheeses at highly competitive prices. Through this process, women are empowered by giving them access to the market to learn valuable farming and monetary skills and providing them with the reliable income they need to gain financial stability. Vinegar production has also captured the export market and is a source of foreign revenue in Sri Lanka. Fermented foods as functional foods are gaining importance all over the world, and increasing the awareness of such products among the general population is important. Major benefits include enhancement of nutritive value by increasing digestibility, inhibition of growth of most pathogenic bacteria, inhibition of formation of bacterial toxins, and degradation of plant toxins (cyanogenic glycosides). The health benefits (reduction in gastrointestinal disorders such as diarrhea, gastritis, and peptic ulcers, inhibition of cholera bacteria) that can be obtained by consuming such products are not well publicized and hence not known by many people in Sri Lanka. However, in the modern world, people tend to buy convenient foods off the shelf, which are easy to prepare. Therefore, it will be relatively easy to popularize some of these products if they are produced under standard hygienic conditions. More research is needed to identify the lactic acid bacteria in Asian fermented foods and their physiological functions in the human diet.

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7 Ethnic Fermented Foods and Beverages of Thailand

Werasit Sanpamongkolchai

7.1 Introduction

The Kingdom of Thailand is located in the Southeast Asian region and became a member of the ASEAN Economic Community (AEC) in 2015. Thailand consists of five big regions: north, northeast, central, east, and south regions. Every region has its own culture, food, and dialect but the formal language is Thai language. In 2015, Thailand has a population of about 67 million people and 90 % are Buddhist which is a majority, whereas 10 % are Christian, Muslim, etc. (Ministry of Foreign Affairs of the kingdom of Thailand 2015). Since Thailand is located near the equator zone, the climate is hot and humid with three seasons (summer, rainy, and dry), and average temperature for the whole year is 18–34 °C (Tourism Authority of Thailand 2015a). Agriculture is the main occupation of the people, and 80 % of the population are farmers with important crops such as rice, sugarcane, rubber, cassava, etc. (Office of Agriculture Economics 2015) (Fig. 7.1).

Thailand has many varieties of food due to various kinds of herbs, spices, and raw materials. Therefore, Thai people could apply such splendid sources of herbs, spices, and materials for preparation of delicious dishes with delicate taste and aroma. Thai foods are also claimed as healthy food because they contain less fat and oil but plenty of vegetables, spices, and herbs such as lemongrass, etc. (Phetyai 2011). At present, Thai dishes are not popular only in Thailand but also well known in foreign countries such as Europe, the USA, and Japan. Thai restaurants are increasing outside Thailand, too. Thailand has a culture of consuming food in different ways and types depending on the region or areas. Some foods got influence from neighbor countries such as Vietnam, Laos, and Indonesia. Thailand also has many varieties of traditional fermented foods and beverages with different purposes. Some are popular in all areas such as *Nampla,* and soy sauce, but some are consumed in regional areas such as *Thuanao* in the northern area, whereas *Sato* (rice wine) is popular in the northeastern region (Thaniyavarn et al. 2005).

The brief details of traditional fermented foods and beverages in Thailand are shown in Table 7.1. More details of some food and beverage are described in the next pages.

7.2 Thai Traditional Alcoholic Beverage "*Sato*"

As far as you know, Thailand is an agricultural country. Rice is the main crop and is harvested one or two times a year depending on planting

W. Sanpamongkolchai (✉)
Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, 50 Ngam Wong Wan Rd., Chatuchak, Bangkok 10900, Thailand
e-mail: fagiwrk@ku.ac.th

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Fig. 7.1 Map of Thailand (Source: Tourism Authority of Thailand 2015b)

Table 7.1 Some common and uncommon ethnic fermented foods and beverages of Thailand

Name of product	Substrate	Nature and use	Microorganisms	Region of consumption	References
Nampla	Fish, salt	Brown liquid, sauce, seasoning, dipping condiment	*Pseudomonas* sp.	All areas	Kanlayakrit and Boonpan (2008) and Kanlayakrit et al. (2002, 2004, 2013)
			Virgibacillus halodenitrificans		
			Halobacterium salinarum, *Staphylococcus warneri*		
Nham	Minced pork, pork skins, cooked rice, fresh garlic	Fermented semidry sausage, sour, food	*Lactobacillus johnsonii*, *L. plantarum*, *Pediococcus pentosaceus*, *P. cerevisiae*, *Micrococcus varians*	All areas	Twichatwitayakul (1996) and Kwanmuang (2003)
Pla-ra	Fish, salt, rice bran	Brown liquid, sauce, seasoning	*P. halophilus*, *P.* sp., *S. epidermidis*, *S.* sp., *M.* sp., *Bacillus subtilis*, *B. licheniformis*	All areas (except southern Thailand)	Hongthongdaeng (1979)
Kapi	Planktonic crustaceans or small shrimp, salt	Dark paste, chili paste, seasoning	*M. morrhuae*, *S. aureus*, *Staph. epidermidis*, *B. subtilis*, *B. pumilus*, *B. polymyxa*, *B. laterosporus*	All areas	Chotwanawirach (1980)
Sato (*rice wine*)	Glutinous rice, Loog-paeng	Turbid white liquid, alcohol, drink	*Amylomyces* sp., *Aspergillus* sp., *Rhizopus* sp., *Saccharomyces cerevisiae*	Northeastern Thailand	Kanlayakrit et al. (2011)
Soy sauce	Soybean, wheat flour, salt	Brownish liquid, seasoning	*A. oryzae*	All areas	Kanlayakrit and Laohawattanawanit (2006)
Sufu	Soybean curd, *Sato*, red rice	Tofu in red dressing mixture, seasoning	*R. microsporus* var. *oligosporus*, *Monascus purpureus*	All areas	Kanlayakrit and Phromsak (2014)
Thuanao	Soybean	Cooked paste or chip, seasoning	*B.* sp., *B. amyloliquefaciens*, *Klebsiella pneumonia*, *Candida* sp.	Northern Thailand	Tangjitjaroenkun (2002)

area with or without irrigated system. Besides this, alcoholic beverages in Thailand are controlled by the Thai government or monopoly, and the price of alcoholic beverages is quite expensive compared with daily expense. Therefore, farmers or people in the countryside will prepare "*Sato*" themselves before paddy rice harvesting. During harvesting season, farmers will help each other and act as hosts serving and providing food and drink especially *Sato* to the guests who helped them. However, *Sato* was previously illegal due to the regulation of excise tax. Recently, the Thai government has allowed communities to produce *Sato* and sell it in the market.

Thailand has many varieties of alcoholic beverages being sold in the market. Some alcoholic beverages are produced by local manufacturers such as beer, wine, rum, and white liquor. Besides these, Thailand also imports wine, whisky, brandy, and cognac from foreign countries such as from Scotland, France, Spain, etc.

Sato is a traditional alcoholic beverage of Thailand containing about 7–10 % (v/v) alcohol (Kanlayakrit et al. 2011). Main raw materials of *Sato* are glutinous rice, *Loogpaeng* (Thai traditional fermentation starter), and water (Fig. 7.2).

Loogpaeng, *loog-pang*, or *look-pang* is a dry form of starter cake which is used for traditional fermented products from starchy raw materials (Krusong 2014). There are many kinds of *Loogpaeng* such as *Loogpaeng Kaomag* for making sweet rice, *Loogpaeng Lao* for *Sato*, etc.

Fig. 7.2 *Loogpaeng* (Thai traditional fermentation starter)

Loogpaeng consists of rice flour and selected herbs depending on the recipe of producers which generally are household or cottage manufacturers in non-aseptic conditions. The recipes of *Loogpaeng* are a secret and transferred from the previous generation. Herbs in *Loogpaeng* play an important role on the inhibition of pathogenic bacteria or undesirable bacteria, due to the essential oil in herbs. Wannissorn (1984) and Kanlayakrit and Changpha (2010) reported that Thai herbs such as clove, Ceylon leadwort, and mace inhibited the growth of *Acetobacter* sp. and *Bacillus* sp., whereas liquorice promoted fungal growth. Long pepper, black pepper, and Ceylon leadwort also inhibited fungal and yeast growth. Therefore, herbs as mentioned before are not recommended to be use in *Loogpaeng* preparation (Fig. 7.3).

Kanlayakrit et al. (1989), Kanlayakrit and Booranasawettatham (2004, 2005) and Chaownsungket (1978) reported that *Aspergillus*, *Mucor*, *Rhizopus*, *Amylomyces*, and *Penicillium* were fungi found in *Loogpaeng*, whereas *Endomycopsis* and *Saccharomyces* were the existing yeast in *Loogpaeng*. Besides these, *Bacillus*, acetic acid bacteria, and lactic acid bacteria were also detected.

Loogpaeng plays an important role for *Sato* production because the starter contains various kinds of microorganisms such as fungi, yeast, and bacteria, leading to alcohol fermentation and aroma. *Loogpaeng* consists of many kinds of spices, such as garlic, pepper, etc., which will inhibit the growth of undesirable microorganisms (Table 7.2). Moreover, these spices also provide a unique aroma in *Sato*. *Loogpaeng* from each area has different types of microorganism which will affect the quality of *Sato* (Fig. 7.4).

7.3 Production Process of Traditional *Sato*

Sato preparation is quite simple and easy and can be produced in the household especially; it is very popular in the countryside area. Glutinous

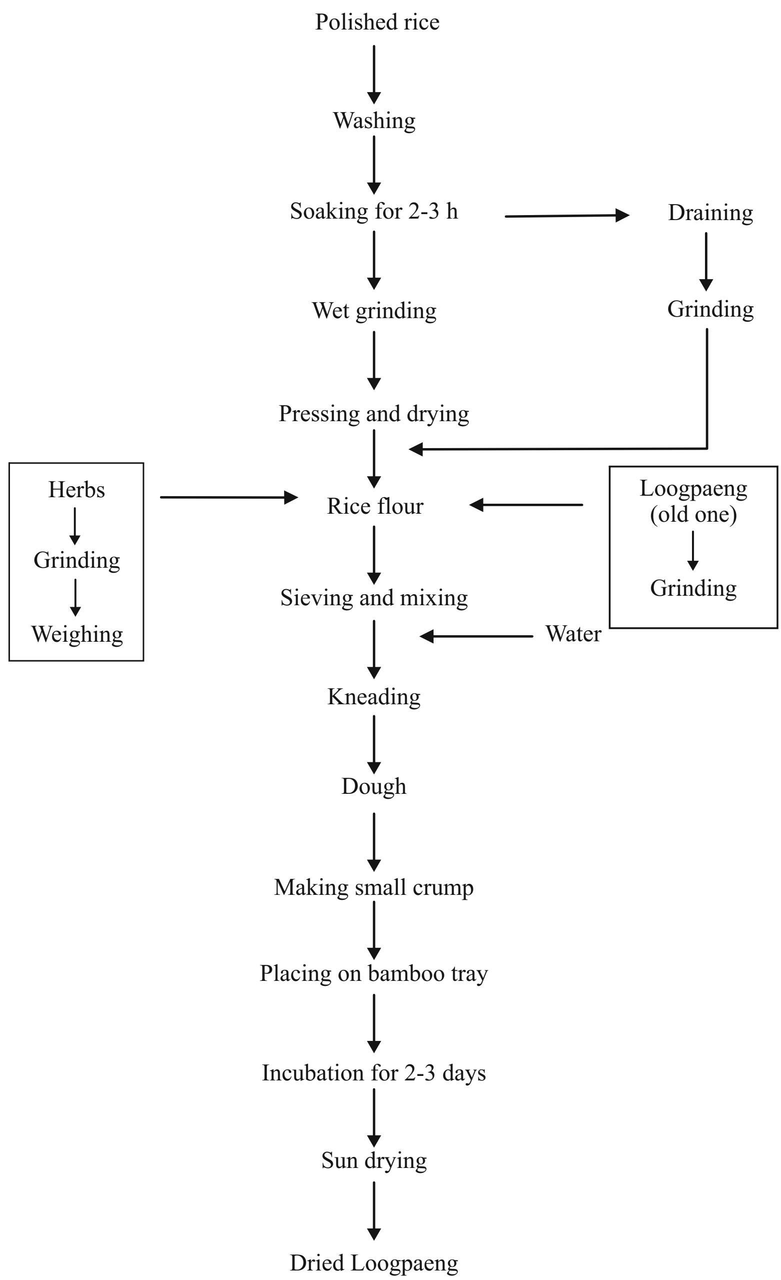

Fig. 7.3 Production process of *Loogpaeng* (Source: Lotong 1992)

Table 7.2 Composition of *Loogpaeng*

Composition	Quantity (g)
Garlic (*Allium sativa*)	40
Ginger (*Zingiber officinale*)	40
Galingale (*Alpinia siamensis*)	20
Liquorice (*Myriopteron extensum*)	40
Pepper (*Piper nigrum*)	6
Long pepper (*Piper chaba*)	6
Shallot (*Allium ascalonicum*)	20
Rice (*Oryza sativa*)	2500

Source: Chatisatienr (1977)

Fig. 7.4 *Sato* preparation in laboratory scale (**a**). Non-pasteurized and non-filtered *Sato* made from glutinous and black glutinous rice (**b**)

rice is first soaked in water overnight and drained to remove the water. After steaming for 30–60 min, cooling and washing with water are performed to remove sticky polysaccharide from rice. Then *Loogpaeng* powder, about 0.1–0.2 %, is mixed with rice, put in jars, and incubated at room temperature for 24–48 h. At this step, steamed rice will be more turbid and sweet due to the saccharification process caused by the amylase enzyme from the fungus. After that, water is added and left for fermentation for 2 weeks (Fig. 7.5). *Sato* after filtration with cheesecloth becomes more clear but slightly turbid. Generally, it contains alcohol, about 7–10 % (v/v), depending on the quality of *Loogpaeng* (Kanlayakrit et al. 2011).

7.4 Microbiology and Biochemistry During Fermentation of *Sato*

After mixing steamed glutinous rice with *Loogpaeng*, the fungus will first grow rapidly because water activity at the beginning is quite low which is not suitable for the growth of yeast and bacteria. Fungi such as *Amylomyces* and *Rhizopus* can produce the amylase enzyme that will hydrolyze starch in glutinous rice into sugar (glucose) (Jantra 2006). At this step, rice will become sweet, and we can use this sweet rice as dessert. When the water is added into sweet rice, the water activity (a_w) is increased; at this condition, yeast including bacteria will grow and alcohol fermentation including aroma occurs. *Sato* contains alcohol at about 7–10 % (v/v). Some aroma of *Sato* comes from the spices used in *Loogpaeng* which give the unique aroma in *Sato* (Kanlayakrit et al. 2011).

7.5 Ethnical Value and Socioeconomy

As described before, traditional fermented alcoholic beverage *Sato* is popular in the northeastern area of Thailand because the people in this region are farmers, and they prepare *Sato* themselves to use for harvesting season. Ten years ago, the Thai government had a policy to promote a community product from each village to activate the economics of Thailand. The Thai Community Product Standard of *Sato* was established by the Thai Industrial Standards Institute (TISI) as shown in Table 7.3. Many communities registered for *Sato* production and sale in the market, but only a few products survived in the market because the quality of *Sato* is not stable due to the quality control system of households or communities not being performed. Moreover, the quality of *Sato* also depends on the quality of *Loogpaeng* which is generally prepared in a small cottage or

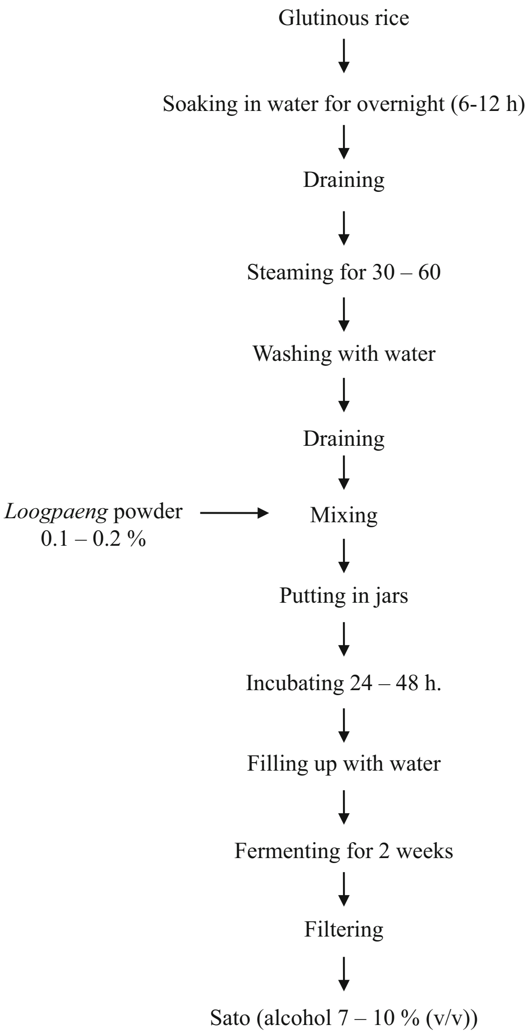

Fig. 7.5 Production process of traditional *Sato* (Source: Changpha 2011)

by a family, and they use old *Loogpaeng* as starter for the next preparation in non-aseptic condition resulting in the unstable *Loogpaeng* quality. Besides these, *Loogpaeng* from various places also has different recipes including herbs and spices, resulting in different microbiologies existing in *Loogpaeng*. This important factor causes the popularity of *Sato* to decrease. The way of life of Thai people is becoming better than the previous time, especially the economic system which increased even in the farmer village; many convenience stores are available with service for 24 h. Alcoholic beverage such as beer and white liquor is easy to buy, and the price is quite cheap compared with the old period. Young generation of Thai people know *Sato* very little because of culture transferred from abroad especially imported alcoholic beverages or foreign alcoholic beverages manufactured in Thailand.

Table 7.3 Thai Community Product Standard of *Sato*

Composition	Quantities
1. Chemical properties	
Alcohol (% v/v)	15≤
Methyl alcohol (mg/L)	420≤
Total SO_2 (my/L)	300≤
Sorbic acid or sorbate salt (mg/L) (calculate as sorbic acid)	200≤
Benzoic acid or benzoate salt (mg/L) (calculate as benzoic acid)	250≤
Cu (mg/L)	5≤
Fe (mg/L)	15≤
Pb (mg/L)	0.2≤
As (mg/L)	0.1≤
Ferrocyanide	Not detected
2. Physical properties	
Foreign matter	Must not detect foreign matter which are not from raw material
Stability	Must not have gas from repeat fermentation

Source: Thai Industrial Standards Institute (2013)

7.5.1 Nampla

Fish sauce is the most popular traditional seasoning used for cooking in most households in Thailand. Besides this, it also is popular in Southeast Asian countries such as Vietnam and the Philippines. Fish sauce is a clear liquid with a reddish-brown color, is salty, and has a unique flavor with 23 % salt content (Figs. 7.6 and 7.7).

7.5.1.1 Method of Preparation

In Thailand, small and large factories of fish sauce are still producing the product using the traditional process that takes about 12–14 months including aging period. Usually, *Nampla* is made from small pelagic fishes from the genus *Stolephorus*, but species of *Scomber*, *Cirrhinus*,

Fig. 7.6 *Nampla* after fermented for 12 months

and *Clupeoides* are also used (Saisithi 1994). Small eviscerated fish are mixed with solar salt in various ratios, but generally the ratio of fish/salt from 1:1 to 1:4 is operated. The mixture is then put into vessels, such as the jars and earthenware containers for home or concrete tank for the industry, and allowed to naturally ferment at room temperature under sunshine for 8–12 months or more. After fermentation is completed, the liquid is drained off to become first-grade *Nampla*, and saturated brine is added to the residue to extract the leftover soluble matter to make lower-quality fish sauce (Figs. 7.8 and 7.9).

7.5.1.2 Nutritional Composition

The physical and chemical properties of *Nampla* are shown in Table 7.4.

According to the standard for local fish sauce (1983) established by the Thai Industrial Standards Institute (TISI), *Nampla* contains sodium chloride not less than 23.0 % (w/v), but total nitrogen is different between the first and second grades.

7.5.1.3 Microorganism

Microflora isolated from fish sauce was investigated and reported in many publications. *Bacillus* sp. was reported by Saisithi et al. (1966), Saisithi (1994), Saono et al. (1974), Choorit and Prasertsan (1992), Phithakpol et al. (1995), Wongkhalaung (2004), and Padongkeittiwong (2001), *Candida claussenii* by Crisan and Sands (1975), *Coryneform* sp. by Liptasiri (1975), *Corynebacterium* sp. by Aroonpiroj (1997), *Halobacterium* sp. by Kanlayakrit and Bovornreungroj (2002); *Lactobacillus* by Saono et al. (1974), *Micrococcus* sp. by Wongkhalaung (2004), *Pediococcus* sp. by Itoh et al. (1985), *P. halophilus* by Saisithi et al. (1966), *Pseudomonas* sp. by Kanlayakrit et al. (2001), *Sarcina* sp. by Saono et al. (1974), and *Staphylococcus* sp. by Kanlayakrit and Boonpan (2008).

There are many varieties of microorganisms found during fish sauce preparation such as *Bacillus* spp., *Coryneform*, *Micrococcus*, *Pseudomonas*, *Achromobacter*, *Flavobacterium* spp., *Vibrio* sp., and *Clostridium* spp. (Davis 1995; Shewan 1961; Hobbs 1991). Some bacteria cause spoilage, but some are useful for solubilization and flavor development (Kanlayakrit and Boonpan 2008). Halophilic bacteria such as *Halobacterium salinarum* are also concerned with aroma formation of fish sauce during aging (Bovornreungroj and Kanlayakrit 2005).

7.6 Nutrition Composition

Fish sauce contains organic nitrogen that could provide a substantial amount of daily nitrogen intake (Amano 1962). Apart from organic nitrogen, there are also vitamins and minerals in fish sauce, for example, vitamin B_{12} is found in large quantities (Amano 1962). Vitamin B_{12} is not available in food from plant, and it is necessary to protect against megaloblastic anemia. The Thai government also enforces in the fish sauce indus-

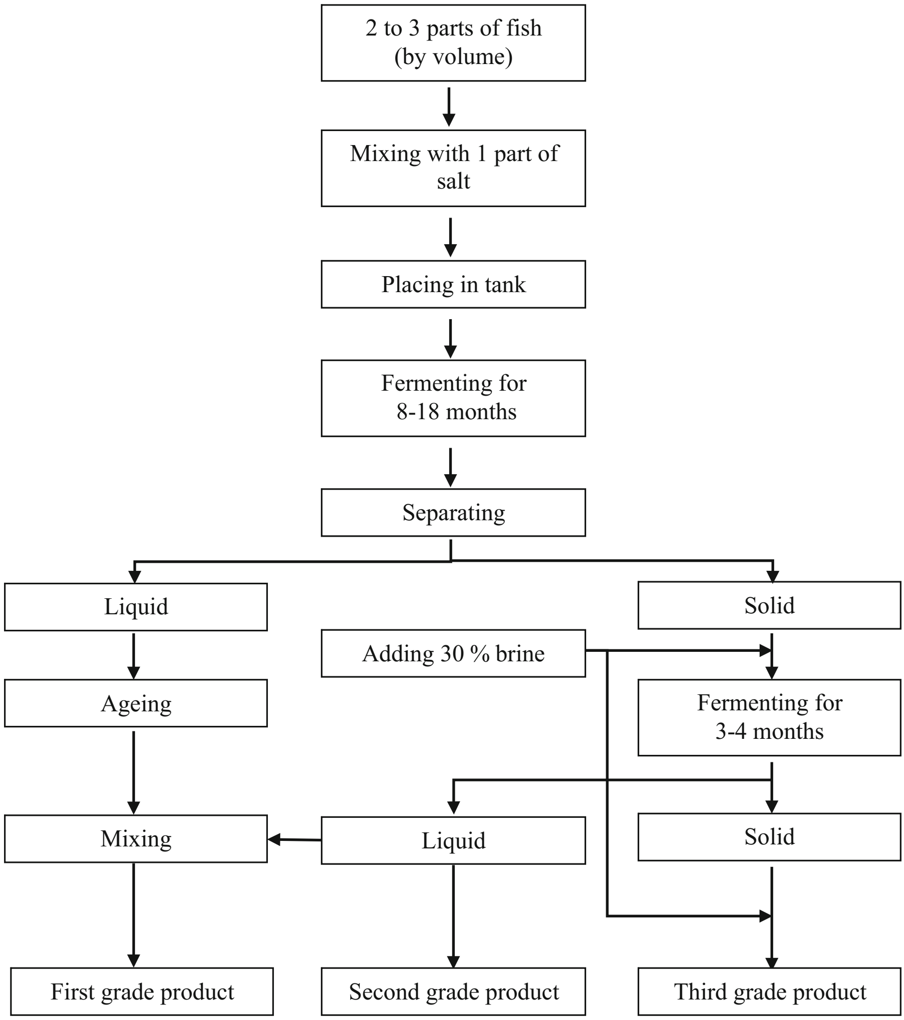

Fig. 7.7 Fish sauce production (Source: Maneepun 1993)

Fig. 7.8 Raw material for fish sauce production

try the addition of iodine supplements such as potassium iodate or the use of iodate table salt for production because iodine deficiency causes cretinism in Thailand especially in the north part that is located far from the sea.

7.7 Ethnical Value and Socioeconomy

Fish sauce is one of the seasonings used for cooking in most households in Thailand. It has the biggest market value share in the seasoning sector. Thailand also can export fish sauce to foreign countries such

Fig. 7.9 Fermentation concrete tank for fish sauce production in industry

Table 7.4 Physical and chemical characteristics of Thai fish sauce

No.	Characteristics	Grade	
		I	II
1.	Relative density NLT*	1.2	1.2
2.	pH	5.0–6.0	5.0–6.0
3.	NaCl (g/l) NLT	230	230
4.	Total nitrogen (g/l) NLT	20	15
5.	Ratio of glutamic acid of total nitrogen NLT	0.4–0.6	0.4–0.6
6.	Amino acid nitrogen (g/l) NLT	10	7.5

Source: Thai Industrial Standards Institute (1983)
*NLT** not less than

as the USA, Japan, Myanmar, etc., with a total amount of about 1600 million baht in 2014 which increased about 47 % compared to five years ago and 75 % compared to 10 years ago. Recently it became more popular in foreign countries because of the increase of Thai restaurants, and Thai foods also are well known as healthy food. Fish sauce could be harmonized with Thai food because it has a unique taste and flavor that fit Thai food.

7.8 Biochemistry

The process of fish sauce production consists of three major factors: (1) hydrolysis, (2) color formation, and (3) aroma formation (Saisithi 1967). At the beginning of the fermentation period under anaerobic condition with salt, the protease enzyme from muscle tissue of fish plays an important role on digesting fish muscle protein to amino acid (Lebez et al. 1971; Montfort and Perez-Tamayo 1975) that resulted in the taste of fish sauce. Besides these, enzymes produced by existing microorganisms in fish such as tryptic enzymes also play a minor important role for the solubilization process (Orejana and Liston 1981; Voskresensky 1965; Kanlayakrit and Bovornreungroj (2003)). The color of fish sauce is developed according to the Maillard reaction between amino groups of protein with reducing sugar from glycogen in fish muscle. Aroma formation is very important in fish sauce. There are three types of aroma in fish sauce: (1) ammonia aroma, (2) cheesy aroma, and (3) meaty aroma. Mclver et al. (1982) found that flavor and taste in fish sauce contain acetic acid, propionic acid, isobutyric acid, isovaleric acid, etc., and major organic acids such as pyroglutamic acid, lactic acid, and acetic acid (Park et al. 2001). Bacteria concerned with aroma in fish sauce are *Halobacterium*, *Pediococcus*, *Staphylococcus*, *Micrococcus*, and *Bacillus* (Saisithi et al. 1966; Kanlayakrit et al. 2002; Suntinanalert 1979).

7.9 Conclusion

Nampla is the most popular seasoning in Thailand used in almost every household for cooking.

Recently *Nampla* consumption is still increasing gradually every year due to economic and population increase. Another reason is that Thailand can export *Nampla* to foreign countries since Thai food is becoming more popular. Besides this, the soy sauce industry in Thailand is also becoming more popular than before. Traditional and new technologies are both being used for soy sauce production. In the case of traditional fermented beverage *Sato*, the popularity is decreasing because the way of life of Thai people is becoming better than in the former times. The economic system has increased even in the farmer village. Many convenience stores are available with 24-h service, and alcoholic beverages such as beer and white liquor are easy to buy and the price is cheaper than before. Young generation of Thai people also know *Sato* very little compared with the previous generation. The reason is that *Sato* is not recognized as a legal beverage because it contains alcohol which is controlled by the government. Recently, the government had the policy which allows communities to produce an alcoholic beverage and sell it in the market. After that, many brand names of *Sato* were produced by household or small communities with lack of quality control especially the quality of *Loogpaeng*. Due to the nonuniformity of *Sato* in the market, this caused the decreased popularity until now.

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Ethnic Fermented Foods and Beverages of Mongolia

8

Sh. Demberel, D. Narmandakh, and N. Davaatseren

8.1 Introduction

Mongolia is located geographically in Northeast Asia, between China and Russia. Although they do not share a border, Mongolia is separated from Kazakhstan by only 36.76 km (22.84 mi). At 1,564,116 km^2 (603,909 square miles), it has a population of about 3 million people. It lies mostly between latitudes 41° and 52°N (a small area is north of 52°), and longitudes 87° and 120°E. Ulaanbaatar, the capital and largest city, is home to about 45 % of the population. The country contains very little arable land, as much of its area is covered by grassy steppe, with mountains to the north and west and the Gobi Desert to the south.

Climate is continental and dry, hot in the summer and extremely cold in the winter, with January averages dropping as low as −30 °C (−22 °F) [39]. A vast front of cold, heavy, shallow air comes in from Siberia in winter and collects in the river valleys and low basins, causing very cold temperatures, whereas the slopes of the mountains are much warmer because of the effects of temperature inversion (temperature increases with altitude). Mongolia is high, cold, and windy. It has an extreme continental climate with long, cold winters and short summers, during which most of its annual precipitation falls. The country averages 257 cloudless days a year, and it is usually at the center of a region of high atmospheric pressure. Precipitation is highest in the north [average of 200–350 mm (7.9–13.8 in.) per year] and lowest in the south, which receives 100–200 mm (3.9–7.9 in.) annually.

Ethnic Mongols account for about 97 % of the population, consisting of Khalkh and other groups, all distinguished primarily by dialects of the Mongolian language. The Khalkhs make up 86 % of the ethnic Mongol population. The remaining 14 % include Oirats, Buryats, and others. Significant ethnic Turkic-speaking Kazakhs constitute 3.9 % of Mongolia's population, and the other Tuvan, Khoton, Chantuu, or Tsaatan are mongolized people with a Turkic origin and speak in Mongolian.

Mongolia is traditionally an agricultural country. In agriculture, about 80 % of the production comes from the animal husbandry sector. The number of livestock at the end of 2014 reached 51.9 million, of which 99 % is private property. In the Mongolian Gobi region, Bactrian camels, and in the mountainous north and west

Sh. Demberel (✉) • D. Narmandakh
Mongolian University of Life Sciences,
IVM, Zaisan- 53, P.O. Box 01,
Ulaanbaatar 17024, Mongolia
e-mail: demberel2002us@yahoo.com

N. Davaatseren
Department of Strategic Policy and Planning,
Ministry of Food and Agriculture,
Government Bldg 9/A Enkhtaivan Avenue 16A,
Ulaanbaatar 13381, Mongolia
e-mail: davaatseren@gmail.com

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regions yak and cattle, are herded. The meat, milk, wool, and hides of the livestock are used. For example, 6.5 thousand tons of raw goat cashmere and 1.7 thousand tons of camel wool are prepared, and 450–500 million liters (l) of milk yield per year is produced of which 70–80 % is processed through traditional methods. In the crop sector, Mongolia is self-sufficient in cereals and potatoes. Cereals, potatoes, and vegetables acclimated to the dry conditions and fast-maturing varieties are selected for cropping. Mongolia is currently focused on developing local varieties of crop cultivation, improving soil fertility, and increasing irrigated crop areas. Currently, about 35 % of the total Mongolian workforce is working in the field of agricultural production and is dependent on this sector for the main sources of their living.

Fermented foods and beverages containing lactic acid bacteria (LAB) have been found to aid in the prevention or treatment of bacterial infections and viral and fungal infections (Tarakanov 1998; Indra 2000). Probiotics bacteria have a cleansing effect on the body, which in turn helps to prevent diseases and excess toxicity including autoimmune disease, allergies, and cancer. The Greek term "pro" means "for" and "bios" means "life" (Demberel and Dugersuren 2014). Probiotics are microbes that protect people and animals from infectious and noninfectious diseases (Tarakanov 1998). There are hundreds of strains of probiotic bacteria. The most common types are *Lactobacillus (Lb.) acidophilus* and *Bifidobacterium. Lactobacillus acidophilus* exists in the small intestine and *Bifidobacterium* exists in the large intestine (Mindell 2004). In Mongolia a very popular probiotic is *Lb. acidophilus*, which is found in yogurt, acidophilus milk, and supplements. Another source of probiotics is a microbial dietary supplement, which can give better effects for human and animal bodies by modulating mucosal and systemic immunity and improving nutritional and microbial balance in the intestinal tract. Lower dosages of probiotics help restore harmony to the internal environment, which is often in disarray in young animals and children. However, overdosage of probiotics will make people unhealthy. For the best results, people and animals have to start at a low dosage in the first weeks and later on increase the dosage (Table 8.1).

Daily intake of foods containing these bacteria (yogurt, acidophilus milk, tablets, and supplements)

Table 8.1 Ethnic fermented foods and beverages of Mongolia

Foods	Substrates	Nature and uses	Microorganisms	Regions of consumption in Mongolia	References
Tarag	Milk and starter culture	Food products and raw materials for milk products	*Enterococcus*	All regions of Mongolia	Watanabe et al. (2008)
			Lactobacillus		
			Pediococcus		
			Streptococcus and yeast (*Candida*, *Issatchenko, Ka, Kl*)		
Airag	Mare's milk and starter culture	Food and diet products for raw materials for distillation alcohol drink/*shimiin arkhi*/	*Enterococcus*	Mainly in the steppe and forest steppe and Gobi region	Watanabe et al. (2008)
			Lactobacillus		
			Lactococcus, *Leuconostoc*, *Streptococcus*, and yeast (*Candida, I, Ka, Kl*)		
Hoormog	Bactrian camel milk	Food and diet products; raw materials for milk products	*Lactobacillus*	Altai and Gobi region	Damdinsuren (2010)
			Streptococcus		
			Saccharomyces		
			Torula		

can improve digestion and decrease constipation, increase absorption of dietary minerals, reduce blood cholesterol levels, and give some protection from intestinal cancer (Damdinsuren 2014; Demberel and Dugersuren 2014).

Probiotics bacteria, also known as intestinal flora, have been added to milk, soy, and cabbage for centuries. Probiotics also have been found to aid in the prevention or treatment of bacterial infections and viral and fungal infection. They support digestive health in several ways. Many strains of probiotics [*Lactobacillus* (*Lb.*) *acidophilus, Lb. casei, Bifidobacterium (Bif.) bifidum*, *Bif. longum,* and the yeast *Saccharomyces (St). boulardii*] produce enzymes within the gastrointestinal tract (Watanabe et al. 2008). These enzymes help to break down foods; the food's nutrition can thus be absorbed, and bloating and gas are reduced. Probiotics are important for processing, digesting, absorbing, and detoxifying foods; using antibiotics can often upset the digestive system because they kill off good bacteria along with bad. According to the professor George Weber, the intestines alone have about 100 trillion bacteria, which outnumber all the cells in human body 10 to 1 (Bondarenko and Matsulevich 2007). Hundreds of different species of bacteria with many different variations are evolving, colonizing, and moving throughout the digestive system. All good bacteria help organisms inside the environment to adapt to outside environmental factors, including diet, stress, climate, and toxins in air and water. Most of the bacteria do not remain alone, because changes in diet in young animals and children cause various changes to physical function. The growth rate of youth is improved greatly by improvement of the diet and changes in the life environment. Bacteria are excreted from an organism through feces, blood, saliva, urine, breath, skin, sweat, etc.

In Mongolia, LAB have been used for different purposes for a long time, such as to make the silage from different plants into animal feeds. LAB can go through the stomach, because it contains acid- and bile-resistant bacteria. LAB have been used to prepare bacterial probiotics, to treat some intestinal disorders in young animals, to improve the quality and taste of vegetable foods and to prolong their life by preserving them, and in processing various fermented milk products.

The fermented milk products that have been mentioned are used in Mongolia. The Mongolian technology of milk processing has specific characteristics as a result of the living habits of

Central Asian nomads. Because of nomadic animal husbandry, milk-processing techniques were developed so that raw milk could be used completely and would not produce waste; the milk thus would also not require further processing and storage. This dairy processing technique was continuously improved from generation to generation. Historically, Mongolians have the greatest respect for milk's importance, because raw milk was the only Mongolian food resource in nomadic agricultural conditions along with wild vegetable tubers and fruits (Indra 2000; Ongoodoi 1991; Gombodash 1996). Mongolian lactic acid bacteria can prevent and treat diarrhea and constipation in young animals and children. Mongolian traditional dairy products resulted from the following specific conditions:

1. Central Asian nomadic livestock husbandry system
2. Climatic conditions
3. Nutritional needs for Mongolians
4. Wastefree technology

In the current situation, as Mongolia is shifting into a market economy, with curtailment of import of various medicines destined for curing and preventing young animal diseases, selective cultivation of useful bacteria among those existing in young animals' digestive tracts, followed by their use in the production of medicines, bacterial probiotics are of great significance. The frequent use of oral antibiotics and sulphanilamide drugs during various ailments of young animals can cause loss of function and disrupt the correct ratio of microbes. These drugs begin to localize and remain in the neonate's digestive tract and cause advanced severity of the disease pathogenesis, as well as negatively affecting digestion. The demand to create probiotics from beneficial bacteria, based on the latest trend of avoiding use of chemical preparations for treatment of animals and using pharmaceuticals with less residue, has been proposed.

Nowadays, in the practice of curing diseases in young animals, the lactic acid bacterial preparations are the most important among the probiotics prepared from various biological active strains in many countries. Probiotics have multiple roles in the maintenance of a healthy large and small intestine and have more significance for the prevention and treatment of diarrhea, constipation, irritable bowel syndrome, inflammatory bowel disease, and colon cancer.

Milk and milk products are especially necessary in two ways: one is the feeding of the young animals and the other is its nutritional value for their diet. It may be stated that healthy animals produce high-quality milk. The Mongolians have an adverb about the kindness of the human heart: "[A pure heart like milk… skillful like a key" (this means if you have a good heart, it is like milk. You will always get more in your life. Milk is an important thing for Mongolian people, as in life.)].

Milk composition and the technology of processing milk products are both based on the quality of milk from pastoral livestock. Throughout history, Mongolians have consumed milk and milk products from five kinds of livestock: cows, mares, camels, ewes, and goats. Nowadays, Mongolian livestock are mostly native breeds and their productivity is relatively low compared with Western countries, but milk from native breeds has a high content of fat, protein, and lactose. This high content of fat, protein, and lactose is the result of the animal's breed and also their nutrition. These milks also have more variability in their uses to make dairy products. Mongolians produce the following dairy products using traditional methods: *urum* or skim milk, white butter, ghee, butter, cream, soft curd, dried curds, yogurt, koumiss, and a fermented milk-alcoholic drink (see Photos 8.8, 8.9, 8.10, 8.20, 8.21 in Appendix). For example, sheep's milk is suitable for making cheese, skim milk (*urum*), dried curd (*aaruul*), and yogurt.

Camel milk has a high content of protein and lactose; it is mostly used for fermented milk products and drinks *(undaa)*. Mare's milk has an abundant amount of lactose, but it is low in protein and fat content. It is used as a raw material for making koumiss, and although yak's milk is rich in fat it is suitable for making skim milk (*urum*), ghee, and butter (Table 8.1).

Table 8.1 Final products from different types of milk in Mongolia

Types of milk	Final product
Sheep's and ewe's milk	Skim milk (urum)
Cow's milk (high protein, lactose)	Dried curd (aaruul)
	Yogurt
	Alcohol drink or distilled vodka (shimiin arkhi)
	Eezgii
	Cheese
	Clabber
Camel's milk (high protein)	Drink (undaa)
Mare's milk (high lactose)	Clabber
Yak's milk (high fat)	Koumiss
	Skim milk (urum)
	Ghee
	Butter

Indra (1983, 2000) and Narmandakh (2005)

Fermented milk products include the following: yogurt (from milk of cows, yaks, ewes, goats), koumiss (beverage of fermented mares' milk), clabber from camel and cattle, cheese, cow's milk drink, cow's and sheep's butter from each clabber, butter of whole milk, etc.

Many Mongolian scientists, including Indra (1983, 2000), Gombo (1985), Baldorji (1980, 1988), Tsendsuren (1989), Batshukh (1995), and Nansalmaa (1998) have studied the physics and chemical characteristics of milk and the composition of milk from Mongolian livestock species. They studied the technology of milk product processing and the traditional techniques for Mongolian milk products. They also reported the microbiological quality of milk and milk products and their methods of manufacturing. Also, Japanese scientists have performed studies, for example, Nakae (1987, 1988), about the chemical and biological properties of milk and milk products from different Mongolian livestock (Table 8.2; Photo 8.4).

The traditional methods for processing milk and milk food products have their own peculiarities, which are deeply related to consumption habits, geographic location, ecological conditions, climate, and methods of rearing livestock. The Mongolian traditional technologies of producing dairy products are rather simple and easy to learn. Their production methods are quick, and it is possible to carry them out in the same amount of time as with other kinds of livestock. Not many days or months are required for processing milk, and it is possible to carry out the processing at the same time as another livestock activity. The technology for processing milk and milk products, especially fermented milk products, has a scientific basis, and the processing activity is based on physical, chemical, and biological procedures.

8.2 Present Situation of Dairy Industry in Mongolia

Mongolia is a country where the pastoral nomadic economy depends on its tradition of herding six kinds of livestock: camels, horses, cattle, yak, sheep, and goats. Six species are commonly raised (see Photos 8.8 and 8.9 in the Appendix), their distribution and frequency depending on ecological conditions and pasture. Agriculture is very important for the country economy and accounts for more than one quarter of the gross national product of Mongolia. Livestock breeding is very important and contributes around 70 % of the gross agricultural output.

Mongolia (see Photo 8.1 in Appendix) has 1,565,000 km^2 of territory and a population of 3.0 million people. Currently, 33 % of the population lives in urban areas (Ulaanbaatar, Darhan, and Erdenet, major cities of Mongolia). There are 51.9 million livestock animals (as of 2014) in Mongolia, including 2.9 million horses, 0.35 million camels, 3.4 million cattle, 23.2 million sheep, and 22.0 million goats. Of all the livestock numbers, about 50 % of the animals are dams; only 60 % of them undergo milking. Mongolia is a country of herders and vast open spaces with low population density (1.9 person/m^2). By the end of 2014 there were 1497 thousand herder families in Mongolia, which has low soil productivity and extremes of climatic conditions, making only a single crop possible per year.

Mongolian herders have traditionally been more dependent on livestock than on crops.

Table 8.2 Chemical composition of milk from Mongolian livestock species

Number	Physics and chemical index of milk	Kind of animal						
		Cow						
		Native	Yak	Hainak[a]	Mare	Camel	Ewe	Goat
1	Density (g/cm)	1027.2–1032.1	1030.5–1034.9	1030–1034.9	1028–1030.0	1037.8–1039,8	1037.8–1039.8	1031–1031.3
2	Acidity (%)	0.18–0.19	0.21–0.22	0.19–0.22	0.081–0.087	0.16–0.17	0.27–0.29	0.18–0.19
3	Fat (%)	3.1–5.4	4.2–8.0	7.1–6.8	2.1–2.4	5.17–5.65	5.52–5.82	5.15–5.81
4	Protein (%)	3.15–3.75	3.7–6.1	3.6–5.15	2.2–3.1	3.91–4.39	5.26–6.48	3.64–3.87
5	Lactose (%)	4.54–4.8	4.84–5.5	4.13–5.15	7.0–7.8	3.97–4.76	3.78–5.0	4.75–4.8
6	Ash (%)	0.72–0.84	0.76–0.93	0.7–1.21	0.3–0.38	0.67–0.87	0.91–1.2	0.87–0.89
7	Total solids (%)	12.51–18.2	13.6–20.0	13.8–19.6	11.6–13.68	13.72–15.67	15.45–18.5	14.41–15.44
8	pH	6.54	6.52	6.45	6.0	6.6	6.7	6.7

Indra (2000) and Tsendsuren (1989)

[a]Hainak is a crossbreed between Mongolia native cow and yak

Mongolian grazing animals are largely dependent on the weather and pasture conditions. In particular, Mongolia's livestock population was decreased by 10 million in three consecutive harsh winters, 2000–2002. Livestock production accounts for 80 % of the agricultural production in Mongolia, serving as the main resource of food for the Mongolian people. The livestock population of Mongolia has witnessed a sharp fall in the past 10 years. With the closure of the fodder farms, factories, and workshops of socialist vintage, this sector has become disorganized (Dept. of Animal Husbandry, Ministry of Food and Agriculture of Mongolia, 2004.12.25.).

The government of Mongolia encouraged the 105th implementation of the national program called the "White Revolution" in 1999. The program was already implemented in 21 provinces of the country. Inhabitants of the capital, all urban centers, and more than 300 district population and administrative units joined in this movement. Since 1992, dairy production in Mongolia has decreased continually, and the annual production has been reduced at least to 1.7 million Mongolian 'Tugrug in 1999 (Ministry of Agriculture of Mongolia 1995–1999, 1999). So far, the amount is continually being curtailed from year to year.

Domestic butter production has almost stopped since 1995. Until 1990, the Mongolian Government was modeled on the Soviet system and in 1990 democrats changed the Mongolian political system from socialism to democracy. The dairy industry changes began in 1990, when Mongolia ceased to be under Soviet control and stopped receiving developmental aid as one of the Eastern Bloc satellites. Since that time, its economy has been changing from a centrally planned socialist system to a free market economy with healthcare delivery reflecting that transition. Financial difficulties remain a major challenge as the country seeks to develop economic self-sufficiency and deliver dairy products to its people. In 1995, legislation was geared to energize a free market system. It was passed and widely supported by merchants. One important step that is still in process is the privatization of the country's assets. In 2000, 61 % of businesses were privately owned, and although a notable improvement has occurred in a small amount of time, the goal of 66 % was not met (Ministry of Agriculture of Mongolia 2000). Yet to be privatized are the large state-owned entities that are more difficult to dissolve and sell. Nonetheless, in 1998 inflation dropped to 6 %, its lowest point since the inception of a capitalist system, with credit attributable to the encouragement of foreign investment by the government (from the www.countystudies.us/mongolia).

It can be thought that all these failures were related to the following points:

1. Very limited feasibility of capital investment or the technological innovation of the dairy industry
2. The collapse of the old investment
3. No available credit system
4. Lack of a management system as well as increase of importing products and reduction of domestic substitution products
5. Selling while transiting into a market economy system from a social economy system that was launched in 1990

Being a country of cattle, cows, and camels, Mongolia is self-sufficient in milk and dairy products. This sector suffered decline in production during the 1990s because of the shortage of raw material and equipment and also the closure of large state-owned enterprises of the centrally planned economy period. The production of milk and dairy products has declined almost 60 fold during 10 years as compared to 1990. However, the sector has picked up in the past 2 years, and production increased 2.8 fold in 2002 over the previous year, reaching 3.2 thousand tons despite three consecutive harsh winters, which resulted in a decline in the cattle population. Almost every aspect of Mongolian society has been shaped by pastoral nomadism, an ecological adaptation that makes it possible to support more people in the Mongolian environment than would be true under any other mode of subsistence. Pastoralism is a complex and sophisticated adaptation to environments marked by extreme variability in temperature and precipitation, on time scales ranging from days to decades. Mongolia's precipitation is not only low on average; it also varies widely and unpredictably from year to year and from place to place. The dates of first and last frosts, and hence the length of the growing season, also vary widely. Such general conditions favor grasses rather than trees and produce prairies rather than forests.

The main species for milk production in Mongolia are cattle and sheep. The productivity of the different species of the livestock there is relatively low compared with developed countries (Table 8.3).

Although herdsmen produce their own dairy products for daily consumption from the herds/flocks and are capable of selling some products in the markets of urban areas, the demand for dairy products for the population in Mongolia cannot be met with only products manufactured by traditional methods. Such a trend is attributed to the urbanization of Mongolia (Table 8.4).

If the dams' portion occupied in the total number of the livestock could be increased from 60 % to 65–70 %, the annual milk consumption per capita of Mongolia would be more than 300 kg. Mongolians are traditionally a nation of nomadic civilizations. Nowadays some herdsmen are living in other remote rural areas 1,400–1,500 km from the capital city and hence the markets of major cities as well, whereas numerous private dairy companies are established in urban areas that are surrounded by major cities and milk products are supplied for their consumption. The largest dairy company in Mongolia is "Suu" Co. Ltd. (see attached Photo 8.5 in Appendix). Today, the Milk Company processes 200 tons of milk daily and produces more than 100 types of dairy products. Most small-scale dairy factories have the capacity to process from 400 to 1000 l of raw milk per day. All factories have their own dairy farms. The main products are pasteurized milk, yogurt, cream, butter, and ice cream in more than

Table 8.3 Milk productive performance of different livestock species

	Duration of lactation (months)		Milk production (kg)	
Species	Exotic	Native	Exotic	Native
Cattle	10	7	3500	650
Horse	–	5	–	250
Camel	–	17.5	–	600
Sheep	5	4	65	45
Goat	5	4	150	65

(Narmandakh 2006, from report science – technological project/1995–1999/Ulaanbaatar)

Table 8.4 Number of livestock and milk production in Mongolia (2012)

Index	Species					
	Camel	Horse	Cattle	Sheep	Goat	Overall
Overall number of livestock (thousand)	305.8	2330.4	2584.6	18,141.4	17,558.7	**40,920.9**
Over all dam animals (thousand)	105.1	700.9	1046.0	8257.1	7639.7	**17,748.9**
The total amount of milk production (mln/kg)	5.8	56.7	338.6	35.9	73.9	**511.0**

Source: Department of Animal husbandry, Ministry of Food and Agriculture of Mongolia 2012

100 kinds of wet and dried curd, cheese, and casein, etc., in Mongolia (see attached Photos 8.6 and 8.7 in Appendix). More than 30 kinds of milk products are also produced. In general, the herdsmen of rural areas make various milk products by themselves. They use the milk from different livestock species and use these products widely for their daily meals. Mongolian dairy product processing techniques were originated and developed under the conditions of nomadic civilization. So, Mongolians do not use a large-scale processing system.

8.3 Effect of Warm Seasons on Dairy Products

There is an increase in dairy production during the warm seasons (summer and autumn), attributable to weather conditions, the reproductive season, and dams beginning extensive production systems for rearing their young. Milk yield is closely related to pasture growth and quality and, in general terms, the amount of milk produced by the yak, cow, goat, ewe, camel, or mare is considered to be no more than the amount needed for the normal growth and development of their newborn. The production of milked animals increases in the warm season because the milked animals eat more fresh grass in the pastures and, after calving, their milk production increases. The milked animals are not milked for the first month after calving, although perhaps for only the first 2 weeks in some areas. During that time, the calf takes all the available milk, including the colostrums, on the day of calving or 2 days after calving. As in other cattle, the quality of the colostrums of milked animals is much better than the milk produced thereafter. Even though Mongolians use colostrums, the people make some dairy products and keep them for wintertime. The milk that comes after calving has so much protein and needs friendly bacteria for the body's intestinal system. The first milk after calving contains many antibodies, which helps young animals and supports their immune systems. After the initial period when the calves or newborn obtain all the milk provided by the dam, it is estimated that the newborn take about one third of the available milk if the milked animals are milked twice daily and about half the milk with once-a-day milking. Sometimes the milked animals such as cow, ewe, or goat produce about a third more milk, in total, if stimulated by milking twice daily compared with once a day. In fact, the milk yield is higher in the months of high pasture growth than either at the beginning or end of the grass-growing season. As referred to earlier, one of the important factors influencing milk yield is pasture production: the quantity, growth status, and nutritive value of the herbage.

All lactating female animals, irrespective of age, parity, or breed type and even location, tend to peak in yield in July and August when grass is at its best in terms of quality and quantity. Before July, although the grass has started to turn green and to grow, the amount of grass available is not high. After August, as the air temperature falls, the nutritive value declines, as the grass produces seeds and then wilts, and the content of crude fiber of the grass is high. Milk composition varies with seasonal grass growth and climate change as does milk yield. Milk solids, lactose, protein, and amino acids in yak milk are at their highest in mid-lactation, and fat percentage increases continuously into late lactation. When the milk yield increases, the dairy product production increases in the warm season. The increase milk in yield comes from many factors: reproduction, meteorology, environment (plants, temperature change), etc. The meteorological factors include temperature and photoperiod and involve physiological mechanisms; the most important nonmeteorological factors are quantity and quality of feedstuffs and disease factors.

Environmental temperature (thermal factors) and possibly emotional factors signal the hypothalamus and central nervous system to increase feed intake, hormonal functions, and heat production and/or loss with resultant declines in milk yield and fertility.

In a thermal environment in which the animal's heat production exceeds heat loss, an increasing amount of heat is stored in the animal's body, resulting in increased body temperature. When the body temperature is significantly elevated, these events include increases in evaporative heat loss by respiration and skin. However, when high temperatures and radiation lessen the ability of the animal to radiate heat from the body, feed intake, metabolism, body weight, and milk yield decrease to help alleviate the heat imbalance. Even though tissue substrates are mobilized, energy metabolism, growth, and lactation decline.

Looking at all the experience of herders and scientists, dairy production increases in the warm season in Mongolia.

8.4 Technological Features of Fermented Foods and Beverages in Mongolia

Mongolian dairy products are utilized not only for human food but also for medicinal and dietetic purposes. It is necessary to increase their manufacture and distribution and improve their economic status. The dairy products are a special food for Mongolian people, but some people have milk allergies because they are lactose intolerant. Lactose intolerance may cause bloating, abdominal pain, and diarrhea after milk is consumed. Mongolian dairy products contain much protein, which can give better effects in liver function. There are several specific characteristics in Mongolian dairy products.

The Mongolian milk technology is based on the cautious recycling of the raw milk and dairy products that do not have any possible economic value. In the milk-processing technology of recycling, the fat, protein, and lactose of the milk are completely utilized through various steps of processing that do not produce any wastes, for example, skim milk (*"urum"*), which is the final product of boiled fresh milk: it is a way of separating fat from milk. Fermentation of the remaining milk for making yogurt and koumiss (fermented mare's milk) causes conversion of lactose into lactic acid and alcohol. The final product of lactose utilization becomes an alcoholic drink (*"shimiin arkhi"*). The boiled curd remains are used to produce *"aarz"* or dried curd *"aaruul"* (see attached Photos 8.8 and 8.9 in Appendix).

Mongolia is a vast territory that is characterized by various ecological regions. There are five major steppe zones with different livestock production capacities. An aimag is an administrative unit. There are 21 administrative units (aimags) in Mongolia: Tuv, Arkhangai, Bayan-Ulgii, Bayankhongor, Bulgan, Darkhan-Uul, Dornod, Dornogobi, Dundgobi, Govi-Altai, Gobisumber, Khentii, Khovd, Khuvsgul, Orkhon, Umnugibi, Uvurkhangai, Selenge, Sukhbaatar, and Uvs Zavkhan. Aimags are divided into soums, and soums are divided into bags (see attached Photo 8.1 in the Appendix). The Khangai-Khosvol region in the northwest is mountainous (see attached Photo 8.2 in the Appendix) with scattered larch forest. It includes Arkhangai, Khovsgol, and part of Bulgan and Zhagvan *aimags*; this is mixed grazing with yaks replacing cattle at the higher altitudes. Selenge-Onon in North Central (Tuv, Selenge, and parts of Bulgan) is the main area of agricultural production. These two regions drain to Lake Baikal. Altai (covering Uvs, Bayangoli, Khovd, and parts of Zhavakan and Gobi-Altai *aimags*) is a high, mountainous area (see attached Photo 8.2 in Appendix) with internal drainage and contains large lakes. In the north of the region the main types of livestock are yaks; there is some localized fodder and horticultural production under irrigation in the lower parts.

The Central and Eastern steppes (comprising Dornod, Hentii, Sukhbaatar, and parts of Dorongobi and Dungovi) are characterized by broad, treeless plains, of which the Herlen River traverses part; the primary activity is herding of horses, cattle, sheep, goats, and camels. The Gobi (mainly Bayankhongor, Omnogobi, much of Ovorkhangai, parts of Dungobi, and Gobi-Altai) is desert steppe and desert (see attached Photo 8.3 in Appendix); used for grazing camels, horses, cattle, and goats with very limited hay harvesting. Drainage is internal, and oases produce vegetables and fruit. For example, the western and southern parts of the country are dry and warm climates and it is a desert zone (Gobi). The northern and central parts are humid, fresh with moderate climate, and it is a forest region (Hangai). The eastern parts are dry and hot and include the relatively low and steppe region (Tal-heer).

Every region has its own tradition for processing dairy products. For example, in some *soums* (the unit of administration of a district) of *Dornot* and *Khentii aimags* (provincial administrative division of Mongolia), provinces of the eastern part including *Bayan-Olgii aimag*, and the provinces of the Western part of the country cannot produce vodka from milk (*shimin arkhi*). Instead of such products, they prefer to make and consume wet or dried curds. Making yogurt is difficult in some *aimags* of Gobi and western regions because of the climatic features, and instead of that the *"shimin arkhi"* (see Photo 8.12 in Appendix) is made from clabber after separation (see Photo 8.19) from its fat. Utilization of milk from some livestock also differs depending on specificity of livestock species and rearing methods; in some provinces of the northern region (called *Khangais*), a few camels are kept. However, there are no milking camels and their milk is not used for making dairy products. Also, mares are not used for milking in *Dornot*, *Khentii*, and *Sukhbaatar aimags*, and ewes are usually not used for dairy food consumption in northern *soums* of *Tov aimag* but are used in some *aimags*. There are seasonal influences, lactation periods, and management systems on the processing of Mongolian milk products after calving. During the early spring the colostrums are used for making various dairy products. Because the colostrums have much protein and minerals, these are processed into making white butter, cottage cheese, and yogurt. At the end of the lactation period in autumn, the milk from different species becomes more concentrated. It is then very suitable for making ghee, *urum,* dried curds, and cheese.

Various kinds of curds and fresh cheeses are widely used products for protein sources in the dry climatic conditions of Central Asia and Mongolia. Because these products are capable of being stored for longer periods, they are said to be specific food products well suited to the nomadic lifestyle (Tsevel 1936, Nyamaa 1980). Although some products obtained through the processing procedures of Mongolian dairy products are resources for making other dairy products, they are also products available for use at any time. In Mongolia are produced the following dairy products using traditional methods: *urum* or skim milk, white butter, ghee, butter cream, soft curd, dried curds, yogurt, koumiss, and a fermented milk-derived alcoholic drink.

There is no processing or intermediate products, because if a product is impossible to consume, it may be interconnected with some specific utilization of nomadic animal husbandry. For example, the *urum* both becomes end product and can be a source for making ghee, white butter, etc. Yogurt, clabber, and boiled curd are final dairy products on the one hand and on the other hand raw materials for wet and dried curds. Those products have specific utilizations, which might appear that intermediate products are used for daily consumption during long-distance migration. They are further processed when the nomads are stationary to distill vodka or *"shimiin arkhi."* Therefore, if some would like to make *"shimiin arkhi,"* then it is necessary to use boiled yogurt, clabber, and koumiss and the remaining part is used for dried curd.

Mongolia has a unique continental climate; therefore, there it has great influence on the processing of milk products. For example, a condition of 10–12 °C dry air is preferable to dry the *urum*. Such processing procedures are impossible to carry out in warm and wet climate conditions. So, in Mongolia *urum* cannot be made in the hot weather of the Gobi desert or will be unsuccessful during rainy days. Drying of curd and freezing of wet curd, butter, and milk are also specific methods that rely on the features of the winter season of Mongolia for their storage.

For processing most of the traditional dairy products of Mongolia, it is necessary to use lactic starter in the first step of the production. The next steps of product processing make it unnecessary to use lactic acid bacteria because there are no continuous microbiological processes. The next steps of product processing create high temperatures that kill all lactic acid bacteria, for example, Mongolian cheese and cheese-like products *(eezgii, aaruul, aarz*, etc.) and other products (milk vodka).

Mongolian dairy product processing techniques have originated and developed under the conditions of nomadic husbandry. So, Mongolians do not use large-scale processing. The simple equipment that Mongolians use for milk processing includes Mongolian-style cauldrons, scoops, and other tools. These cauldrons (see Photos 8.18 and 8.12 in Appendix) are very important in their nomadic life. Mongolians use them not only for boiling tea and preparing the daily meal but also to process the dairy products. As well, they also produce a surplus during summer season and store it to use during winter and spring seasons.

8.5 Characterization of Mongolian Ethnic Fermented Foods and Beverages

Fermented milk products have a major position among Mongolian dairy products. Generally, fermented products include such as yogurt (*tarag* from the milk of cows, yaks, ewes, goats), koumiss (fermented mare's milk), clabber from camel and cattle cheese, cow's milk drink, etc. These products are all made by souring milk and are also daily food for herdsmen; these products serve as intermediate raw materials and ingredients for processing several other food products. The Mongolian milk-processing technology has a specific characteristic as a result of the living habits of Central Asian nomads. There are many kinds of milk products in Mongolia because of their processing activity using milk from various dairy animal species. Mongolia has more than 30 kinds of dairy products and fermented milk products that include more than 36 % of the Mongolian dairy fermented food products [butter-like (urum, ghee, cream, butter, white cream, butter of clabber); cheese-like (cheese, aarz, aaruul, eezgii, huruud, shirgeemel, eerem); yogurt-like (yogurt, koumiss); and beverage and milk vodka (clabber, kefir, tsagaa, undaa, milk vodka)].

Herdsmen who engage in extensive livestock production have specific methods to conserve and prepare the starter culture of fermented milk products in the nomadic condition. The majorities of the fermented milk products are widely used in the diet of Mongolian consumers because they have therapeutic and nutritional effects on the digestive organs and their function (Demberel 2015). From time immemorial Mongolians have been consuming fermented food products and have obtained some sources of starter (see Photos 8.14 and 8.15). Central Asian nomadic inhabitants have domesticated some wild animals and also they could "domesticate" some microorganisms from plants, grass, and fruit that are capable of causing fermentation.

Mongolian people use several plants for the lactic starter source from generation to generation. There are many kinds of lactic acid bacteria and yeasts on the surface of certain plants of Mongolia. Specifically, Mongolian lactic starter cultures were prepared from keeping milk products and specific wild plants. It is a specific characteristic that the Mongolian starter is composed of three microorganisms (lactobacilli, streptococci, and yeast) all together in the living state. Nowadays there is still the custom of using some plants (see Photos 8.16a, b in the Appendix), such as *Rheum undulate* (rhubarb, geshuune), *Artemisa sibersena* (wormwood, sharilj), *Artemisa vulgare* (mugwort, agi), *Rheum ribes* (rhubarb, geshuune), and *Rumex acetosa* (rumex acetosa, isgelen khurgan chikh) to make some dairy products, but such products are disappearing. Those plants are used for lactic starter preparation (Indra 2000). The traditional processing methods for Mongolian fermented milk products have a scientific basis, and these methods are easy to learn and operate.

Starters of yogurt (Mongolian *tarag*), koumiss, and clabber (see Photo 8.14 in Appendix) were found many centuries ago and kept a in pure state from generation to generation, which was not easy in the conditions of the nomadic lifestyle. Nowadays, the world produces more than 80 kinds of fermented dairy products and their origins are different. It is peculiar that although many fermented milk products such as mares' milk, clabber from camels and cows, ewes, goats, cows, yak's yogurt, and the cheeses, curd, and butter have different tastes from one another although they are soured by the same starter. Starter is obtained from yogurt aged for 1–2 days or yogurt filtrated and dried. The yogurt starter is obtained by filtration through a clean white cotton cloth and can be stored for 6–10 months without loss of activity. Before using the filtrate and dried yogurt as starter, it is scaled up in warm milk and mixed thoroughly. Also in making fermented dairy products Mongolians have used extract from a specially prepared starter powder of dried curds, and the remaining clabber and koumiss is put in the skin bag. In conditions of nomadic livestock husbandry, various starters are unavailable for preservation a in pure state, because of the product quality and also the keeping process.

Central Asian nomads resolved intelligently the problem of starter for dairy products because lactic cocci, lactic bacilli, and yeasts as starters are able to grow together and the desired products are made by activating each other and some microorganisms, whereas others are deactivated in compliance with a certain technological regimen for processing fermented dairy products. For example, to activate LAB for making yogurt, the milk is warmed at 45 °C and put under anaerobic conditions. When the temperature reaches 30 °C after gradual reduction below 40 °C, lactic cocci grow and aromatic substances are produced. Yeasts are not suitable to use in yogurt as a starter culture, because yeasts are not capable of growing at high temperature and in anaerobic conditions; thus, they are inactivated during yogurt fermentation. The yeast for koumiss starter is activated from 20 ° to 25 °C in aerobic conditions (Baldorji and Namsrai 1980). In case of the activation of starter, residues of yogurt can be used for further fermentation of the same product. For normal fermentation of the koumiss, only fresh mare's milk is added and stirred or beaten with a wooden stick.

8.6 Mongolian Yogurt (*Tarag*)

One of the most distributed and consumable traditional dairy products for Mongolian households is yogurt. Yogurt is made of milk from the native cow, yak, sheep, and goat. Mostly, boiled milk is used to make yogurt. Mongolian *tarag* has been used for the treatment and control of several intestinal diseases since ancient times. Yogurt contains minerals, amino acids, many vitamins, lactic acid bacteria, alcohol, and aromatic compounds, etc. Mongolian *tarag* is a natural probiotic with live bacteria that are beneficial for young animals and children.

Tsoodol and Munkhtuya (1974) reported the composition and properties of the microorganisms of Mongolian yogurt. They found thermophilic lactic acid bacilli of the Bulgarian bacilli type and local strains of thermopile lactic acid streptococci in the composition of yogurt, which had had a major role in the starter system and were isolated from the local strains. In the past several years, research has provided evidence that among lactic bacilli included in the composition of the microflora of Mongolian yogurt, the local strains, which produce antibiotics against causative agents of dysentery, salmonella, colibacteriosis, and putrefactive bacteria, were frequently observed. Mongolians regularly drink much traditional fermented milk, such as called in Mongolian "*Airag-koumiss*" and "*Tarag-yogurt*," which are made in the traditional way developed by the nomadic people of Mongolia. These fermented milks are not only precious nutritional sources but are also probiotics, which can be used for treatment and as dietary supplement. The following Fig. 8.1 shows the traditional technology of Mongolian yogurt in a farmstead.

8.7 Koumiss (Fermented Horse Milk Beverage: *Airag*)

The traditional method of making koumiss from mare's milk was inherited from remote antiquity by the modern generation of Mongolians. The koumiss is an important product used daily for human consumption during the summer at the same time that mare's raw milk is broadly employed for therapeutic and dietetic purposes. Fermented mare's milk is a product of combined fermentation by lactic acid, alcohol, and CO_2 (the chemical composition of koumiss is presented in Table 8.5, and Fig. 8.2; Photos 8.17 and 8.18 show the technology and equipment to make the traditional Mongolian koumiss/*airag*).

If affected by thermal treatment, koumiss cannot be finished. The native characteristics of mare's milk remain untouched. It is abundant in free amino acids, vitamin C, and a high concentration of lactic acids, ethanol, and CO_2. Because of these characteristics, the koumiss that is produced during fermentation will be changed to a product more digestible and of higher nutrient quality and taste compared with other fermented dairy products. The content of essential amino acids of mare's milk protein are almost equal to the amino acids from meat. The lysine content of mare's milk is higher than cow and ewe's milk and the methionine content is higher than human milk. Sukhbaatar investigated the useful microflora of koumiss, and the techniques of preparation and preservation of

Mention Make a flow sheet

1. Boiling milk (removing skim)

⇩

2. Heating (40–45 °C)

⇩

3. Followed by adding 1–3% of starter and pour back for 5–10 min for proper mixture

⇩

4. Pouring into enameled pot and closed with a lid and put in warm place to reach the titratable acidity at 0.60–0.65 % for 4–8 h

Fig. 8.1 Traditional technology: the manufacture of *Tarag*

Table 8.5 Climate conditions in Mongolia

Seasons	Period		Temperature		Rainfall average
	From (month)	To (month)	Min. (°C)	Max. (°C)	(mm)
Winter	12 (Dec)	2 (Feb)	−40	−10	100–150
Spring	3 (Mar)	5 (Jun)	−18	+20	120–150
Summer	6 (Jun)	8 (Jul)	+5	+30	200–250
Autumn	9 (Sept)	1 (Nov)	−10	+25	125–180

Jambaajamts (1989)

Mention flow sheet

1. Pouring the mare's milk into skin bag or wooden barrel
2. Initiation fermentation with the starter at 25–26 °C
3. Beating 2000–3000 times in the 1.5–2.0 h interval for acidification of milk
4. Maturation period (12–24 h)
5. Koumiss (acidity 1,20 %)
6. Consumption

Fig. 8.2 Technique for making Mongolian koumiss has six steps

starter culture were determined. Also, this scientist noted the lactic streptococci and bacilli and fermentation agents of the koumiss. For making koumiss, lactic bacilli, lactic cocci, and yeasts are added to the fermentation process. The streptococci have a lower resistance to an extreme amount of acidity, and therefore the number of streptococci could be increased in the initial stages of fermentation although reduced in number in the middle and last stage. However, yeasts grow well in an acidic environment and produce biological substances that have a positive effect on bacterial multiplication. The bacterium and yeast are mutually beneficial organisms capable of growing in combination.

Watanabe et al. (2008) reported that the Mongolian traditional fermented milk products *Airag* (koumiss) and *Tarag* (yogurt) had unique microbial compositions as compared with the traditional fermented milk products in other countries, that is, *Lactobacillus (Lb.) helveticus*, *Lb. kefiranofaciens*, and the lactose-fermenting yeast *Kluyveromyces (Kl.) marxianus* were isolated from *Airag* as the predominant isolates, whereas *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. helveticus*, and *Streptococcus (St.) thermophilus* were the predominant isolates from *Tarag*. We also observed the close relationship between the lactose content in milk used to prepare the fermented milk products and the concentration of the lactose-fermenting yeast strains in these fermented milk products. The information obtained here will be useful for screening the beneficial strains from traditional fermented milk products in Mongolia. It will also help to clarify the reasons why these fermented milks have important roles as probiotics

During koumiss making, the beating treatment provides an air supply inside the mass and activates the alcoholic fermentation. Koumiss is received from mare's milk as a result of lactic acid and alcoholic fermentation. Earlier Mongolian people kept koumiss in leather vessels; now they keep koumiss in gouged lime tubs and with special sticks with a fungus-like foot (see attached

Photos 8.13 and 8.17 in Appendix). The mechanism of the treatment effect of koumiss is conditioned by the healthy substances that form it: enzymes, microelements, antibiotics, vitamins A, B_1, B_2, B_{12}, D, E, C (especially much), spirits of wine, lactic acid, carbon dioxide, etc.

Consumption of koumiss is good for alimentary canal activity, metabolism, the circulatory and nervous systems, blood-forming organs, functions of kidneys and the endocrine glands; it also improves immunity. Koumiss is an effective remedy in treatment of pulmonary tuberculosis and tuberculosis, and in treatment of exhaustion, anemia, and disorders of liver function, etc.

8.8 Cows Milk Drink: Beverages (*Undaa*)

The milk drink (*Undaa*) is one of the common beverages for peoples who live in southern Mongolia (Gobi region). For such drinks, boiled milk is chilled to 30–35 ° C or fresh whole milk is poured into a skin bag followed by adding matured koumiss prepared drink as a starter, and the product is beaten for further processing. The agent is used for initial fermentation, but after maturing some part remains and added into other fresh milk to adjust the fermentation level. Fully matured koumiss has gross granules of protein, 2.0–2.4 % acidity, less than 2.5 % of alcoholic content, only a small amount of CO_2 (Indra 2000). Before drinking it has to be diluted with milk to make the strong acidity more mild, or it can be boiled. During the fermentation of koumiss, the beating work may separate the milk fat. Then the separated remaining liquid is utilized for distillation of vodka and the other part; curd is filtered for making *"aaruul" (*dried curd). The following Fig. 8.3 gives the processing procedure of cow's milk drink.

Mention flow sheet

1. Boiled or whole milk of cows
⇩
2. Pouring into skin bag and wooden barrel
⇩
3. Fermentation with matured koumiss or yogurt
⇩
4. Beating by wooden stick
⇩
5. Heating up to 30–35°C
⇩
6. Maturation
⇩
7. Consumption
⇩
8. Add fresh milk into remainder

Fig. 8.3 Processing procedure of cow's milk drink and beverages (*undaa)*

8.9 Camel Clabber (Fermented Camel Milk: Hoormog)

Indra et al. (1988) and Indra (2000) studied the composition of camels' milk and found some changes among total solids, lactose, and fat content. The granulated and ungranulated lactic bacilli, lactic cocci, and yeast in the microbial agent that are related to the fermentation process were studied as a result of camel clabber (Fig. 8.4 and Photo 8.8).

Lactic acid bacteria and yeasts sour camel milk (Batsukh 1995). The fermentation procedure of camel clabber making is principally similar as mare's koumiss and cow's milk drink.

Camel milk soured by lactic acid bacteria and yeasts. The fermentation procedure of camel clabber making is principally similar to mare's koumiss and cow's milk drink (Table 8.6 shows the physicochemical composition, microflora, and bacterial agents of fermentation of the camel clabber) (Table 8.7).

8.10 Butter and Sheep's Butter from Clabber

Butter and sheep's butter from clabber are traditional Mongolia fermented dairy products; these are very delicious, and one takes pleasure in eating them. They also have a rich nutrient con-

Fig. 8.4 Processing procedure of Mongolian camel clabber

Mention flow sheet

1. Camel whole milk (5 L)
2. Pouring into small wooden barrel
3. Adding starter (5 L), pour back 3–5 min; put in the warm place 24–28 h
4. Pouring into skin bag and wooden barrel
5. Adding camel milk, beating 20–30 min at 20–25°C and ferment 10–20 h

Table 8.6 Chemical composition of Koumiss (*airag*)

	Titratable acidity (%)	Chemical substance (%)		
Classification of koumiss		Protein	Fat	Alcohol
Underdone fermentation	0.70–0.80	2.0–1.8	2.2–2.0	0.8–1.2
Fermented	0.90–1.0	1.8–1.7	2.0–1.8	1.2–1.8
Stiffened	1.1–1.2	1.7–1.2	1.3–1.4	1.8–2.0

Baldorji and Namsrai (1980)

Table 8.7 Chemical composition and microorganisms of fermented of camel milk and clabber

	Chemical composition (%)				
Products	Acidity	Protein	Fat	Alcohol	Microflora
Milk	0.17	4.15	5.4	–	*Lactococcus (Lc.) lactis*
Clabber	0.9–1.1	4.0	5.2	1.5	*Lc. lactis, Streptococcus (Str.) thermophilus*
					Lactobacillus (Lb.) bulgaricus, Saccharomyces (Sm.) cerevisiae
					Sm. cartilaginosus

Batsukh (1995)

tent and are suitable for long-term preservation. Butter of clabber is produced from cow and sheep fermented milk by beating. During the fermentation, a small amount of *hiram* (mixture of boiled milk and water) is added. The ratio of *hiram* and fermented clabber is 0.5:10.0 for sheep and 1.0:10.0 for cow. Also, the churning temperature is different for the two species (Fig. 8.5).

8.11 Conclusions

Mongolian fermented foods and beverages (dairy products) can provide the complete nutritional needs for Mongolians. Traditional dairy products are concurrent in the conditions of nomadic animal husbandry and the technology for processing such is used regularly. The ways

Fig. 8.5 Processing procedure of butter from cow and sheep's clabber

Mention flow sheet

1. Cow and sheep's fermented clabber
⇩
2. Adding boiled milk diluted with water and adjust the temperature
⇩
3. Beating 45–50 time per minute
⇩
4. When the beating until to 2500 strokes (It means more than 2500 times)
⇩
5. Add 3–5 L warm milk and continue beating
⇩
6. When the beating until 4500,
⇩
7. Decrease number of beating, continue very slowly
⇩
8. The beating process continue until 5000,
⇩
9. Stop the beating and closed by lid
⇩
10. Keep until 24 h (with out movement)
⇩
11. Take a separate from the clabber and rinse by cold water again to again
⇩
12. Store the paunch and large intestine of animal

of processing of Mongolian ethnic fermented foods and beverages (dairy products) are based on wastefree technology. It is a technique well adapted to the Central Asian nomadic environment and climate conditions.

Mongolia has more than 30 types of dairy products, including yogurt, urum, ghee, aaruul, eezgii, cream, cheese, wet curd, dried curd, butter, ice cream made by various types of animal's milk, koumiss, clabber from cow's and sheep milk, cow's milk huruud, camel's milk huruud, cow's butter, sheep's milk beverage, vodka of koumiss and clabber, cow's butter, and sheep's butter. Each clabber is a major product of unfermented processing.

The fermented milk products occupied more than 36 % of dairy products, such as cow, yak, sheep, and goat yogurt; koumiss from clabber of cow and sheep; cow's butter and sheep's butter from each clabber; butter of whole milk and beverages from clabber, etc. It is a specific characteristic that the Mongolian starter is composed of three microorganisms (lactobacilli, streptococci, and yeast) all together in the living state.

Mongolia has a traditional specific method to obtain starter from wild plants and store the starter by using cotton filtrate for drying in the nomadic condition. Most Mongolia dairy products can be used as final products, and also these are suitable for use as raw materials for making other dairy products.

Appendix

Photo 8.1 Map of Mongolia (Aimag, provincial administrative division of Mongolia; Soum, unit of administration as a district)

Photo 8.2 Mountain area of Mongolia

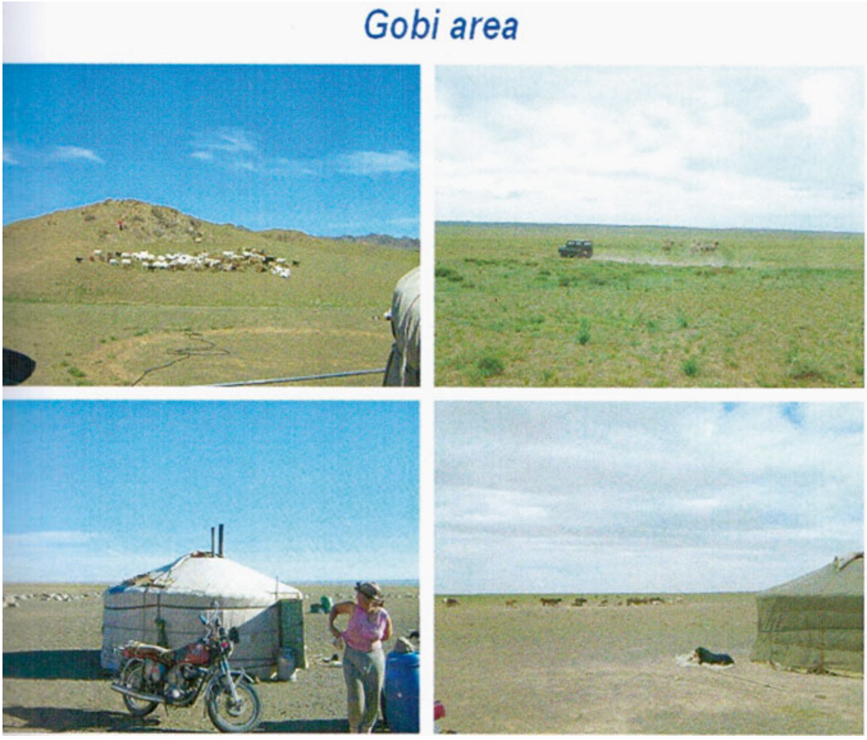

Photo 8.3 Gobi area of Mongolia

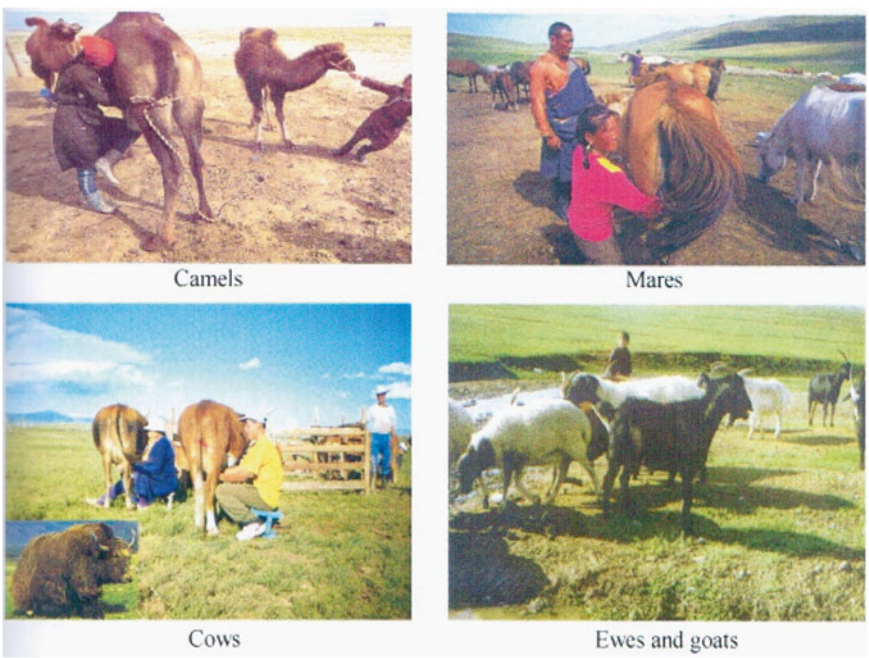

Photo 8.4 Mongolian native breeds of milking animals

Photo 8.5 Largest company of dairy products, ‘Suu’ Company

Photo 8.6 Dairy products produced by “Suu” Company

Photo 8.7 Various Mongolian milk products for therapeutic and dietetic use

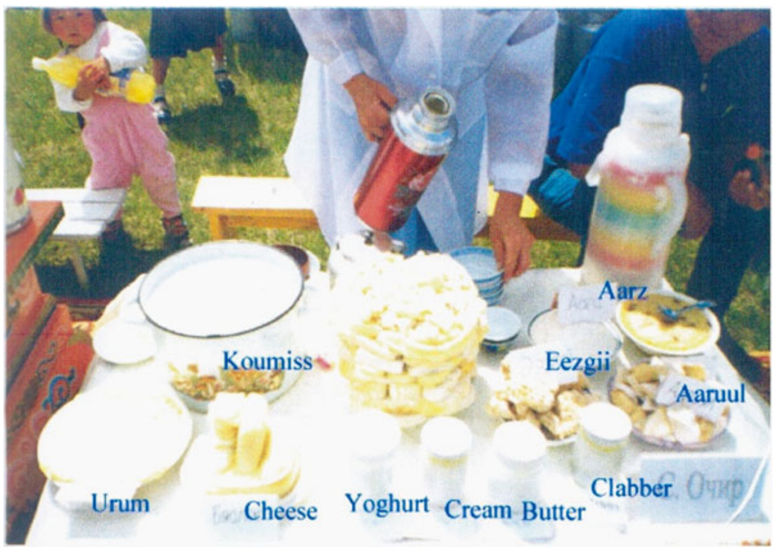

Photo 8.8 Dairy products prepared by traditional methods

Photo 8.9 Technology of butter products

Photo 8.10 Dairy products prepared by traditional methods

Photo 8.11 Technology of Mongolian cheese

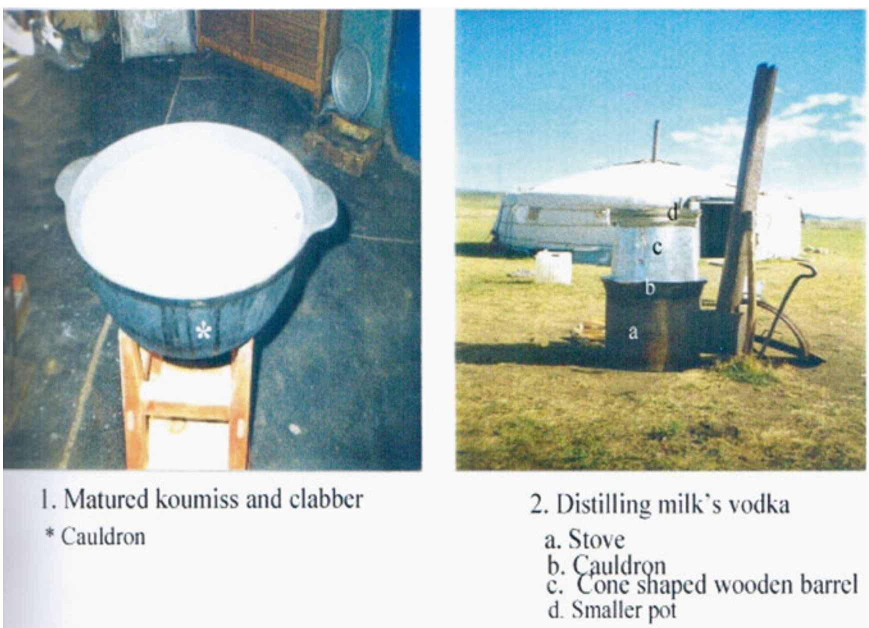

Photo 8.12 Technology of milk's vodka

Photo 8.13 Tools for making fermented milk products in Mongolia

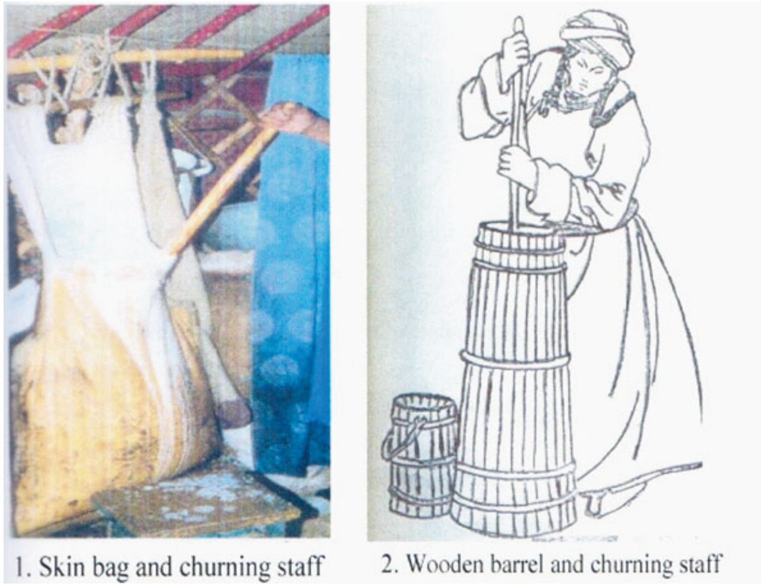

Photo 8.14 Traditional "starter" system (kept from milk products)

A.1. Filtration and storage the wet fermented products (yogurt, clabber, koumiss…etc)

A.2. Filtrate by cotton

A.3. Drying

A.4. Storage

A.5. Prepare the starter

A.6. Use the starter fermented milk products

B. Grinding the dried cheese like products and add the fresh milk

Photo 8.15 Traditional "starter" system (prepared from specific plants, powdered)

1. Collect plants

2. Catch the leaves

3. Washing and drying

4. Making powder

5. Storage

6. Prepare the starter mix with plant powder and fresh milk

7. Use the starter for fermented milk products

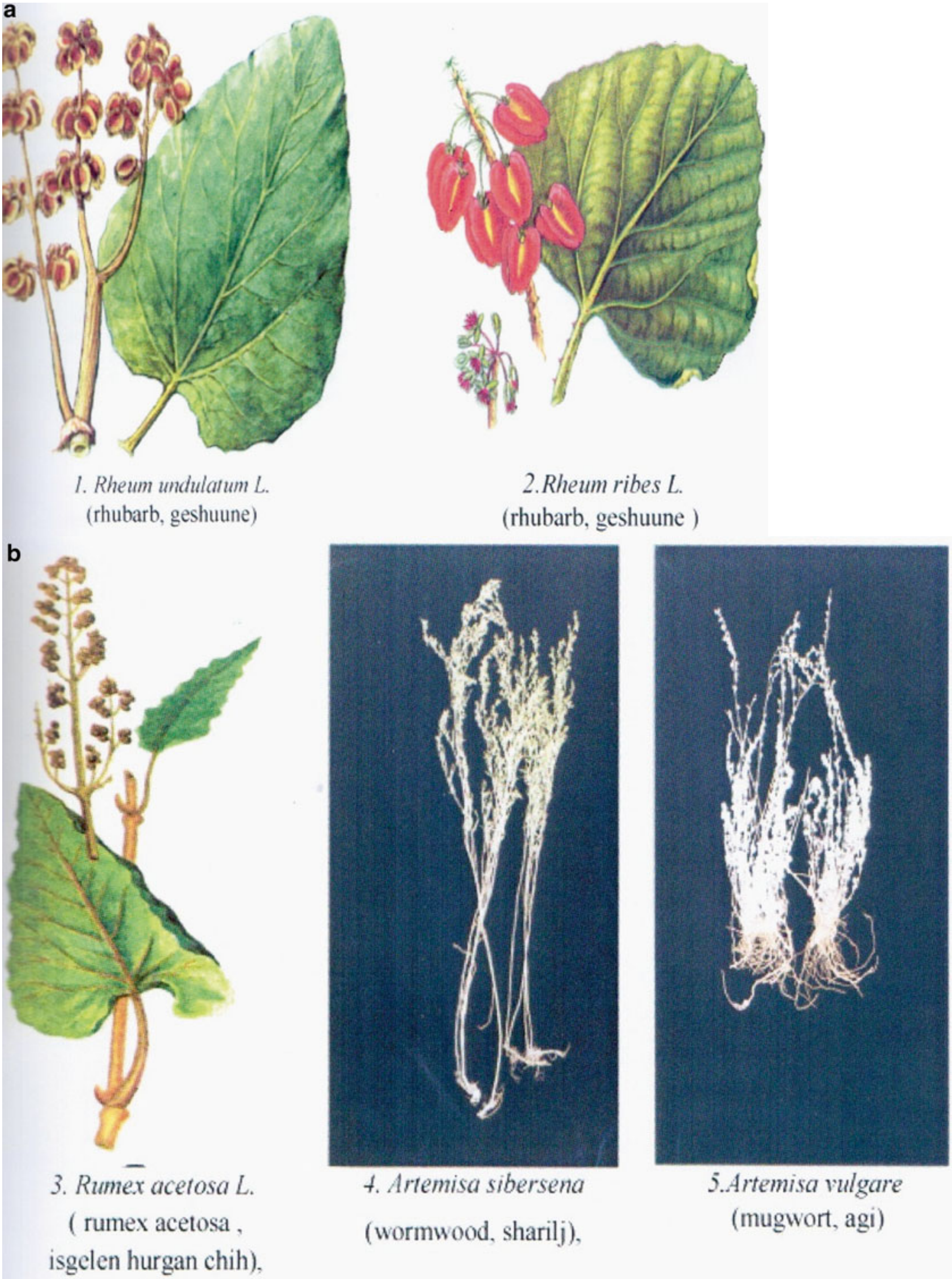

Photo 8.16 (**a**, **b**) Specific plants used as starter in Mongolia

Photo 8.17 Equipment for manufacturing traditional fermented milk

Photo 8.18 Equipment for manufacturing traditional fermented milk

Photo 8.19 Cream separator of milk

Photo 8.20 Mongolian cheese

Photo 8.21 Mongolian yogurt

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9 Ethnic Fermented Foods and Alcoholic Beverages of Japan

Yoshiaki Kitamura, Ken-Ichi Kusumoto, Tetsuya Oguma, Toshiro Nagai, Soichi Furukawa, Chise Suzuki, Masataka Satomi, Yukio Magariyama, Kazunori Takamine, and Hisanori Tamaki

9.1 Introduction

Japan is an island nation surrounded by the sea, which is made up of 4 main big and more than 6800 small islands, located in the east of the Sea of Japan and in the west of the Pacific Ocean. The climate is predominantly temperate but varies greatly because the area lies about 3300 km north to south, from latitudes 24° and 46°N (Fig. 9.1). According to the Köppen climate classification, it is classified as *Dfa* (cold, without dry season, warm summer) in *Hokkaido*, *Dfb* (cold, without dry season, hot summer) in northeast of *Honshu*, *Cfa* (temperate, without dry season, hot summer) in other regions except in several southern islands, and *Am* (tropical, monsoon) (Peel et al. 2007). The main ethnicity group is Japanese (98.5 %) including very small number of minority groups known as the indigenous *Ainu*, 0.5 % Koreans, 0.4 % Chinese, and 0.6 % others (The World Factbook 2015). Almost 70 % of the land is mountain area or covered with forest and agricultural land is only 12.5 %. The most important agricultural and fishery product is Japanese cultivar of paddy rice (8.6 million ton (mt)), and other important ones are vegetables (12 mt), potato (3.3 mt), fruits (3.1 mt), milk and milk products (7.3 mt), meats (3.3 mt), egg (2.5 mt), and fish and shellfish (4.3 mt) (Food Balance Sheet, MAFF 2014).

Until the end of the nineteenth century, Japanese people had not usually eaten mammal

Dr. Soichi Furukawa had suddenly passed away just after preparing this manuscript.

Y. Kitamura (✉) • K.-I. Kusumoto • Y. Magariyama
Food Research Institute, National Agriculture and Food Research Organization, 2-1-12, Kan-nondai, Tsukuba, Ibaraki 305-8642, Japan
e-mail: kitamr@affrc.go.jp

T. Oguma
Japan Soy Sauce Technology Center, 3-11, Koami-cho, Nihonbashi, Chuo-ku, Tokyo 103-0016, Japan

T. Nagai
Genetic Resources Center, National Agriculture and Food Research Organization, 2-1-2 Kan-nondai, Tsukuba, Ibaraki 305-8602, Japan

S. Furukawa
College of Bioresource Science, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan

C. Suzuki
Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization, 2 Ikenodai, Tsukuba, Ibaraki 305-0901, Japan

M. Satomi
National Research Institute of Fisheries Science, Japan Fisheries Research and Education Agency, 2-12-4, Fukuura, Kanazawa-ku, Yokohama 2368648, Japan

K. Takamine • H. Tamaki
Education and Research Center for Fermentation Studies, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan

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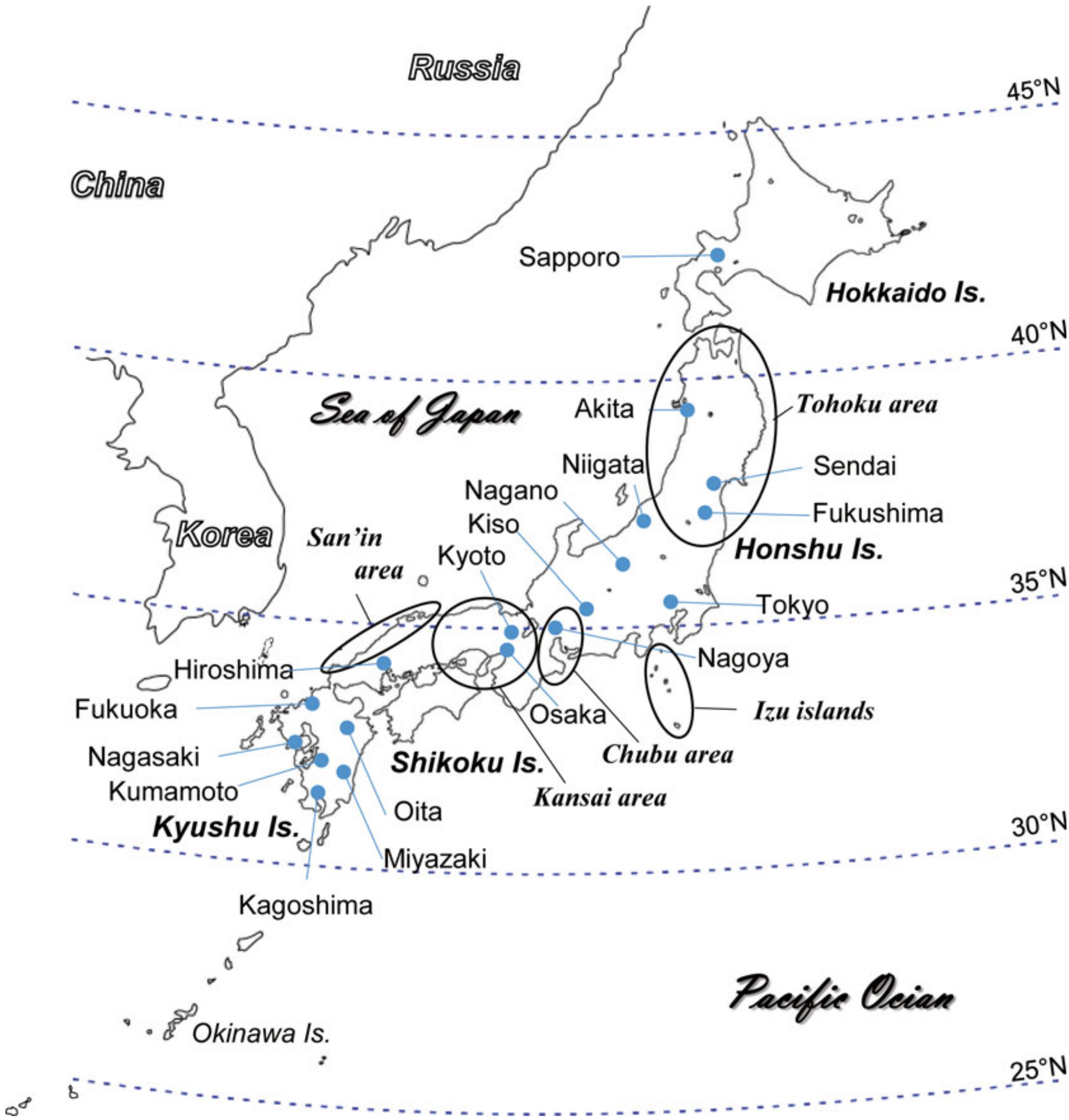

Fig. 9.1 Map of Japan

meats because of a religious practice, and then a special food culture called *Washoku* has been developed. The *Washoku* cuisine is based on a large variety of vegetables, edible wild plants, fish, and shellfish products which are the gifts from the natural feature of various climates in Japan as shown above. The *Washoku* was added to UNESCO's roster of intangible cultural heritages in December 2013 with the backgrounds of traditional cultures (UNESCO 2013). The centerpieces of taste and cooking for *Washoku* cuisine are fermented condiments such as *miso* and *shoyu*, and fermented foods such as *tsukemono*, *natto*, and/or *shiokara* are additionally garnished to enhance the taste of cooked rice.

Fermentation can be considered as one of the beneficial results of long-standing efforts to preserve food materials and utilize them with minimal loss. Various fermented foods were created by taking advantage of domesticated microorganisms, which had been selected and bred for safe and suitable fermentation through ancestral wisdom and trial and error. For example, it was shown by genome analysis that *Aspergillus oryzae*, one of the main *koji* mold strains which has been used in the brewing of *miso*, *shoyu*, *sake*, etc., is completely lacking the ability of aflatoxin production because of multiple mutations and deletions in the ortholog of aflatoxin biosynthesis genes (Kusumoto et al. 1998, 2000; Matsushima et al. 2001), although it is a closely related species to *A. flavus*, an aflatoxin-producing one. On the other hand, *A. oryzae* is assumed to have 1.3–2 times more copies of genes for proteolytic and saccharolytic enzymes which help to bring out the taste and flavor of fermented foods than closely related *Aspergillus* species (Machida et al. 2005). These facts show that strains with phenotype useful for brewing had been selected in the long history of domestication. *Koji* molds (*A. oryzae*, *A. sojae*, *A. luchuensis*, and *A. luchuensis* mut. *kawachii*) were authorized as national microbes of Japan in 2006 by the Brewing Society of Japan, because they are especially important for the living and culture of the Japanese, the industries of foods and alcoholic beverages, and also the field of biotechnology in Japan (Brewing Society of Japan 2006).

Miso and *shoyu* are thought to derive from the cereal *jiang* of ancient China, which was mentioned in *Zhou-li* compiled around two thousand years ago and instructed in *Qi-min Yao-shu*, the oldest agricultural text written around 540 AD, and then have developed independently in Japan (Yoshizawa 2002). Besides *sake*, *nare-zushi*, *shiokara*, and *gyosho* were also described in the text, so recipes for archetypes of these foods and beverages are also assumed to be introduced from ancient China. But present Japanese ethnic fermented foods and beverages have been localized and evolved independently by adapting to Japanese natural features, regional food materials, and customs, and then this unique food culture has been formed (Table 9.1). This chapter introduces comprehensive Japanese traditional fermented foods and beverages that have been supporting Japanese dietary customs as a pivotal role of *Washoku* taste.

Table 9.1 Some common and uncommon ethnic fermented foods and beverage of Japan

Product	Substrate	Nature and use	Microorganisms	References
Miso (soybean paste)	Soybeans, rice or barley, salt	Soft or hard, semisolid, soup, seasonings	*Aspergillus oryzae*, *Zygosaccharomyces rouxii*, *Tetragenococcus halophilus*, *Candida versatilis, and C. etchellsii*	Imai (2009) and Watanabe (2009a)
Koikuchi shoyu	Soybeans and wheat	Common soy sauce The basic seasoning of Japanese cuisine	*A. oryzae*/*A. sojae*, *Z. rouxii*, *C. versatilis*, *T. halophilus*	Soy sauce's Japanese Agricultural Standard[a]
Usukuchi shoyu	Soybeans and wheat	Lighter in color Contains about 10 % more salt than *koikuchi shoyu* Mainly *nimono* (boiled dish)	*A. oryzae*, *A. sojae*, *Z. rouxii*, *C. versatilis*, *T. halophilus*	Soy sauce's Japanese Agricultural Standard[a]
Saishikomi shoyu	Soybeans and wheat	Rich in color, flavor, and aroma Table condiment	*A. oryzae*, *A. sojae*, *Z. rouxii*, *C. versatilis*, *T. halophilus*	Soy sauce's Japanese Agricultural Standard[a]
Tamari shoyu	Soybeans and small amount of wheat	Rich in color and *umami* flavor	*A. oryzae*, *A. sojae*, *Z. rouxii*, *C. versatilis*, *T. halophilus*	Soy sauce's Japanese Agricultural Standard[a]

(continued)

Table 9.1 (continued)

Product	Substrate	Nature and use	Microorganisms	References
Shiro shoyu	Wheat and small amount of soybeans	The lightest in color *Chawanmushi and nimono* Instead of salt	*A. oryzae*, *Z. rouxii*, *T. halophilus*	Soy sauce's Japanese Agricultural Standard[a]
Natto	Boiled soybeans	Alkaline, non-salted, viscous ingredient (mainly with cooked rice)	*Bacillus subtilis*	Nagai and Tamang (2010)
Fukuyama pot vinegar	Rice	Acidic, sour, condiment, drink	*Acetobacter* sp., *Saccharomyces* sp., *Lactobacillus* sp.	Furukawa et al. (2013) and Haruta et al. (2006)
Nuka-zuke	Raw vegetables, *nukadoko* (fermented rice bran, salt, and seasonings)	Pickling vegetables flavored with lactic acid fermentation in *nukadoko*	*Lactobacillus* (*L.*). *plantarum*, *L. namurensis*, *L. sakei*, *L. coryniformis*, *Pediococcus pentosaceus*, *L. lactis*, *L. furfuricola*, *Hansenula* sp., *Debaryomyces* sp., *Pichia* sp.	Ono et al. (2014) and Sakamoto et al. (2011)
Takuan-zuke	Sun-dried *daikon*, salt, rice bran and sugar	Sweet, crunchy taste, brownish white to fluorescent yellow in color	Lactic acid bacteria (LAB), *Debaryomyces* sp., *Torulopsis* sp.	Koizumi (2000) and Matsuoka et al. (2008)
Iburi-gakko	Smoked *daikon*, salt, rice bran and sugar	Takuan with smoky flavor. *Daikon* is smoked instead of drying process. Then pickled as *takuan* (specific in Akita Pref.)	LAB, halotolerant yeasts	Miura and Nakano (1985) and Kasahara and Nishibori (1986)
Yamagawa-zuke/ Tsubo-zuke	Dried *daikon*, salt	Dried *daikon* sprinkled with salt but not in brine. Matured in pots for more than a half year	LAB, halotolerant yeasts	Setoguchi et al. (2005)
Bettara-zuke	Salted *daikon*, *koji*, sugar, rice	Very sweet taste from *koji*, freshly prepared (Tokyo)	LAB, yeasts, *A. oryzae* (enzymes)	Koizumi (2000)
Sagohachi-zuke	Vegetables, salt, *koji*, cooked rice	Sweet taste from *koji*, freshly prepared (Fukushima Pref.)	Yeasts, *A. oryzae* (enzymes)	Miyao (2002)
Nara-zuke	Salted *daikon*, Japanese melon, cucumber, *kasu* (*sake* lee)	Deep brown pickles originated in Nara Pref., soaked in *sake* lee for a long period, resulting a strong, pungent flavor	*L. pentosus*, *L. paracasei*, *Torulopsis* sp., *Saccharomyces* sp., *Pichia* sp., *Hansenula* sp.	Miyao (2002) and Yamagata and Fujita (1974)

(continued)

Table 9.1 (continued)

Product	Substrate	Nature and use	Microorganisms	References
Miso-zuke	Salted vegetables, burdock, cranshaw, *miso*/supernatant of *miso* (*tamari*)	Vegetables with *miso* taste, brown colored	LAB	Miyoshi (1982) and Miyao (2002)
Tamari-zuke				
Kinkon-zuke				
Yamagobo-zuke				
Shio-zuke	Vegetables, salt, seasonings	Pickled with salt, seasonings	*L. mesenteroides*, *Lactococcus lactis*, *L. curvatus*, *L. plantarum*, *L. casei*	Nakagawa et al. (2001)
Hakusai-zuke	Partially dried *hakusai* (*Brassica rapa* var. *pekinensis*)	Pickled with salt, red pepper powder, and other seasonings	*Leuconostoc mesenteroides*, *L. carnosum*, *L. sakei*, *L. curvatus*, *L. lactis*, *L. citreum*	Oyaizu and Ogihara (2009)
Nozawana-zuke	*Nozawana* (*Brassica rapa* L. var. *hakabura*), salt	Pickled with salt, red pepper powder, and other seasonings (Nagano Pref.)	*L. brevis*, *L. fermentum*, *L. curvatus*, *L. plantarum*, *L. coprophilus*, *L. delbrueckii*, *L. mesenteroides*, *L. lactis*	Suzuki et al. (2006) and Kawahara and Otani (2006)
Takana-zuke	*Takana* (*Brassica juncea* var. *integrifolia*), salt	*Asotakana* (Aso in Kumamoto Pref.), *miiketakana* (Chikugo in Fukuoka Pref.), and *kobutakana* (Unzen area in Nagasaki Pref.) pickled with salt. Large leaves are used for rapping or served as thin slices	LAB, halotolerant yeasts	Miyao (2002) Precidia, Ark of Taste
Suguki-zuke	*Sugukina* (*Brassica rapa* var. *neosuguki*) *turnip*, *salt*	Leaves and turnip roots are pickled with salt and matured to add taste and color in a warming chamber called *muro* (Kyoto Pref.)	*L. brevis*, *L. sakei*, *L. plantarum*	Ogihara et al. (2009)
Shiba-zuke	Eggplant, cucumber, red perilla (Japanese basil)	Vegetables and leaves of perilla are fermented, resulting in sour taste, magenta color, and typical flavor (Kyoto Pref.)	*L. plantarum*, *L. casei* subsp. *peudoplantarum*	Shinagawa et al. (1996)
Sunki-zuke	*Sunkina* or *kabuna* (*Brassica rapa*)	A traditional unsalted, fermented vegetable (Kiso area in Nagano Pref.). It is used for soups, topping of buckwheat noodle, and stir-fry	*L. fermentum*, *L. plantarum*, *L. brevis*, *L. kisonensis*, *L. otakiensis*, *L. rapi*, *L. sunkii*	Endo et al. (2008) and Watanabe et al. (2009)

(continued)

Table 9.1 (continued)

Product	Substrate	Nature and use	Microorganisms	References
Gyosho (fish sauce)	Fish, shellfish, salt (*koji*: mold starter, applicable)	Salty, fish odor, *umami*, condiment	*T. halophilus*, *T. muriaticus*, *Staphylococcus* spp., *Chromohalobacter* spp.	Fujii (1992), Fukui et al. (2012), and Satomi et al. (2011)
Fugunoko-nuka-zuke (fermented pufferfish ovaries in rice bran)	Pufferfish ovaries, salt, rice bran	Salty, fish and rice bran odor, sour, side dish, snack for drinking	*T. halophilus*, *Haloanaerobium* spp.	Kobayashi et al. (1995)
Katsuobushi (boiled, smoke-dried, and molded skipjack tuna)	Boiled, smoke-dried, and molded skipjack tuna (mackerel and its relatives are applicable)	*Umami*, aromatic (phenolic compounds), sliced before using soup stock (*dashi*)	*Aspergillus* spp., *A. glaucus*	Fujii (1992)
Kusaya (special salt-dried fish)	Flying fish, amberstripe scad, mackerel scad, etc.	Dried fish, brine has a unique, offensive smell, side dish and snack for drinking	*Pseudomonas* spp., *Marinobacter* spp., *Peptostreptococcus* spp., *Enterococcus* spp., *Marinospirillum* spp., *putative Corynebacterium*	Fujii (1992), Satomi et al. (1997), and Takahashi et al. (2002)
Nare-zushi (fermented sushi)	Crucian carp, mackerel, salmon, sand fish, etc.	Picked fish, sour, sometimes cheese-like flavor, side dish, and snack for drinking	*L. plantarum*, *L. alimentarius*, *L. coryniformis*, *L. sakei*, *L. sanfrancisco*, *L. kefir*, *L. fermentum*, *Lactococcus lactis*, *Pediococcus* sp., *Leuconostoc* spp.	Fujii (1992)
Nuka-zuke (fermented fish in rice bran)	Mackerel, sardine, herring, etc.	Picked fish, salty, fish odor, side dish, and snack for drinking	*T. halophilus*, *T. muriaticus*, *Haloanaerobium* spp.	Kosaka and Ooizumi (2012) and Kosaka et al. (2012)
Shiokara (salted fish)	Squid, organs of skipjack tuna, intestine of sea cucumber, kidney of salmon, etc.	Salty, fish odor, soft texture, side dish, and snack for drinking	*Staphylococcus* spp.	Fujii (1992)
Sake	Rice	Alcoholic beverage	*A. oryzae*, *S. cerevisiae*, *L. plantarum*, *L. leichmanii*	Ishikawa (2002)
Shochu	Sweet potatoes, barley, rice, buckwheat, brown sugar, chestnut, sesame seeds	Distilled alcoholic beverages	*A. luchuensis*, *A. kawachii*, *A. oryzae*, *S. cerevisiae*	Brewing Society of Japan (1991) and Hong et al. (2013)
Awamori	Rice	Distilled alcoholic beverages	*A. luchuensis*, *S. cerevisiae*	Brewing Society of Japan (1991) and Hong et al. (2013)

[a]Ministry of Agriculture, Forestry and Fisheries (2014) Soy Sauce's Japanese Agricultural Standard

9.2 *Miso: Soybean Paste*

9.2.1 Outline

Japanese soybean paste, *miso* in Japanese, is one of the traditional, salted fermented foods mainly made from soybeans, rice, or barley. Japanese people had been eating this food for several hundred years, as *miso* soup or seasonings. There are varieties of *miso* in Japan, according to each district. The main varieties of *miso* are rice *miso*, barley *miso*, and soybean *miso*. The key for variation is type of *koji* (solid fermentation culture of *koji* mold, *A. oryzae*) (Imai 2009). In case of using steamed rice for the solid-state fermentation of *koji* mold in manufacturing of *miso* (this type of *koji* is called rice *koji*), the product is called rice *miso*. In case of using barley *koji*, it is called barley *miso*. In addition, soybean *miso* is unique as it is made of *koji* by using soybean itself as solid fermentation substrate of *A. oryzae*. There are several mixed types of *miso*, i.e., mixture of rice *miso* and the barley one and mixture of the rice one and soybean *miso*. Sometimes the manufacturers produce *miso* using *koji* fermented with wheat, buckwheat, Japanese millet, and coix seed. *Miso* is also classified by characteristic of its physical property. *Miso* where the grain shapes of steamed soybean remain is called "*tsubu-miso*" (granular soybean paste). *Miso* after the fermented materials are ground finely is called "*koshi-miso*" (ground soybean paste). *Miso* where the grain shape of rice *koji* remains is called "*koji-miso*" (*koji* soybean paste). Several types of *miso* are shown in Fig. 9.2.

9.2.2 Methods of Preparation, Mode of Consumption, Culinary, and Microorganisms

The method of preparation for rice *miso* is described as an example. The raw materials for manufacturing *miso* are soybeans cooked by steaming or boiling, rice or barley cooked by steaming, and salt. The amount of soybean used for the fermentation of *miso* is 120 k ton (during 2009, speculated) (Japan Federation of *Miso* Manufacturers Corporation 2014a). Ninety percent of them are imported from the USA, Canada, and China (ibid.). Recently, the use of soybean made in Japan increases slightly. The properties of the soybean used in *miso*, necessary for manufacturing *miso*, are large grain, thin skin, light color in the navel, high absorption of water, easy softening of the grain after steaming, light color (bright yellow) of the grain after steaming, and good fragrance after steaming.

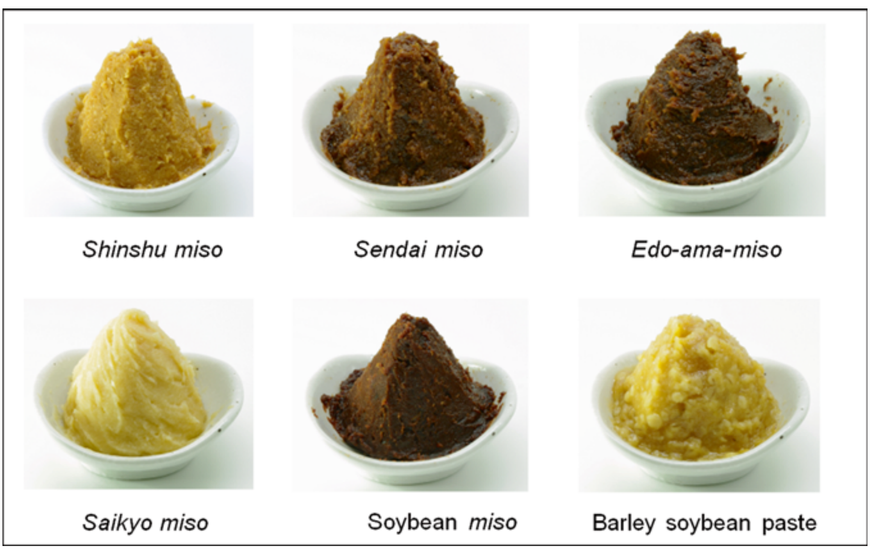

Fig. 9.2 Several kind of soybean paste (All photo files were kindly provided by Dr. Yoshiaki Kitamura (NFRI))

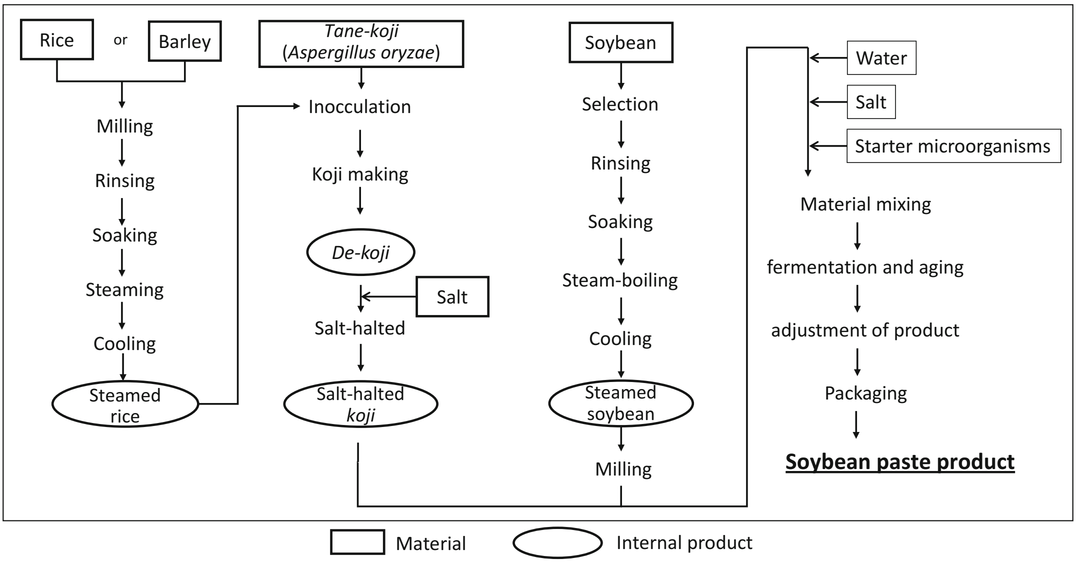

Fig. 9.3 Process of soybean paste manufacturing. Materials for soybean paste are marked by quadrangles. Internal products are marked by ovals

The other points are high contents of total sugar and low lipid content.

The total amount of rice used in the manufacture of *miso* is subject to making *koji*. It is not necessary for the rice to have a specific property like rice wine. The degree of polishing of rice is the same as the one for eating. The salt containing low content of iron and copper is used. The grade of the water for *miso* should be drinkable, and it should contain low content of iron and copper, manganese, and calcium ions. The *koji* starter microorganism is *A. oryzae*. The conidia of this fungus are delivered from companies having special technique for preparing and maintaining *koji* mold and its conidia. For sweet *miso*, the *A. oryzae* strain producing strong amylase activity should be selected, and the strain producing strong protease is for salty paste.

The whole strategy of manufacture for rice *miso* is described in Fig. 9.3. The main process contains *koji* making, treatment of soybeans, material mixing, fermentation and aging, and adjustment of product. Traditionally, in the manufacturer's saying, the most important step for production of *miso* is *koji* manufacture. The second one is boiling soybeans. The third one is material mixture. Especially the quality of *koji* directly concerns the quality of the final product in the process of *koji* making (Imai 2009). The *koji* making contains rice treatment including polishing, rinsing, soaking, steaming, and cooling, inoculating the *koji* mold, fermenting the *koji* mold, and stopping the fermentation by adding salt which is one third of the total amount. The degree of polishing is approximately 10 %, i.e., the weight of the removed bran is 10 % of the unpolished rice. The polished rice is subject to soaking in water to let them absorb water completely. After steaming and cooling, conidia of the *koji* mold are inoculated and mixed with the soaked rice. Then they are incubated at around 30 °C, which is dependent on the engineer of each company. The temperature increases up to 40 °C after some hours of inoculation due to breathing. At this point, the mycelia of the *koji* mold prolong and invade the inside of rice grain. If the degree of invasion is appropriate, enzyme activities necessary for *miso* are supposed to be enough. For the sweet and white to bright yellow type of *miso*, the maximum temperature should be above 35 °C to increase amylase activity. For the salty and deep-colored one, the temperature should be below 30 °C to increase protease activity. The difference of these temperature

Fig. 9.4 Treatment of rice koji during fermentation directly by hand. The photo file and next one were kindly provided by Mr. Takahiro Ogawa (Asahi Breweries Co., Ltd.)

Fig. 9.5 Automatically controlled koji making machine

controls is partly dependent on the gene expression profile of the enzymes. Moreover, manufacturing of sweet *miso* needs *koji* mold of low condition in order to avoid deep coloration. In the history of manufacturing *miso*, *koji* making was performed with multiple sets of wooden tray called "*koji buta*" (*koji*-making tray) (Fig. 9.4). Nowadays the manufacturers introduce the automatic *koji*-making machine (Fig. 9.5).

Treatment of soybeans begins with removing any dust, soil, and hull. Then sometimes soybean grains may be polished and husks are removed to display the beautiful color of *miso*. After rinsing, the grains are soaked in water for a certain number of hours. During soaking, pigments or its precursors (pentose polymer, etc.) are eluted from the grain. Soaked soybean grains are then steamed to denature the protein derived from soybeans, including trypsin inhibitor. After cooling down, the steamed grains are milled with a chopper machine. Then the milled soybeans, rice *koji* mixed with salt, additional water, and the remaining salt are mixed together. This mixture is called *moromi*. In many cases, the starter microorganisms such as salt-tolerant yeast (*Zygosaccharomyces rouxii*) and/or lactic acid bacteria (*Tetragenococcus halophilus*) are simultaneously added in the *moromi* (Imai 2009). They are packed into barrels and kept at atmospheric temperature or sometimes at the warmer condition. At the packing process, it is necessary to remove air from the *moromi* in order to keep an anaerobic condition. Then they are covered with a sheet such as polyethylene and pressed with stone weight on the lid. The *moromi* is fermented for several months to sometimes 1 year or more. This period depends on the type of each product. During late stage of fermentation, *Candida versatilis* and *C. etchellsii* are grown, and they are related to produce fragrance specific to well-fermented *miso* (Suezawa and Suzuki 2007).

The rice *miso* shares ca. 80 % among total *miso* in Japan and is consumed around the whole area of Japan. It is mostly used in *miso* soup with *dashi* (Japanese soup stock made from fish and/or kelp). It is also used as seasoning for cooking of fish or meat. However, interestingly, the taste is different among each district. People living in the northern and eastern part of Japan including Hokkaido, Akita, Sendai, Tokyo, and Nagano prefer dark yellow- to orange- and sometimes brown-colored

Fig. 9.6 Five types of soy sauce (Soy Sauce Information Center 2014)

miso, which tends to be salty according to the low ratio of rice *koji* against soybean and salt. People in the Kansai area (including Kyoto and Osaka) prefer white- to light yellow-colored *miso*. It is sweet according to the high ratio of rice *koji*. In the Tokyo area, there is a sweet type of rice *miso* called "*edo-ama-miso*." "*Edo*" is the ancient name of Tokyo and "*ama*" means sweet taste. The color of this type is deep, dark brown, according to the soybeans steamed deeply and the high ratio of rice *koji* (twofold of soybean amount). "*Edo-ama-miso*" is used as seasoning for cooking of seafood (fish, shellfish) and meat (beef, horse, etc.). Soybean *miso* is produced and consumed in the middle area of Japan, that is, Chubu area, including Nagoya. The characteristic of this type is salty and astringency, rather than sweet, taste. The people living in this area prefer this *miso*, and they use it, for instance, as seasoning in the soup of Japanese noodles and sauce of fried breaded pork cutlet, in addition to *miso* soup. Barley *miso* is produced and consumed in the Kyushu area. It includes both the sweet type and salty type and is used mainly in *miso* soup.

9.2.3 Biochemistry, Nutritional Composition, and Functionality

Koji mold secretes several kinds of enzyme responsible for degradation of starch, protein, and lipid, i.e., amylase, protease, and lipase. Therefore, the contents of *miso* include amino acids, peptides, glucose and related oligosaccharide, glycerol, fatty acids, lactic acid (from lactic acid bacteria), and salt (Watanabe 2009a). There are several studies going on for the functionality of *miso* against human health. Some studies are based on the results of in vitro tests, and some are on the epidemiological studies. During recent 50 years, the production of *miso* decreases about half according to the decrease of consumption (413 k ton on 2013) (Japan Federation of *Miso* Manufacturers Corporation 2014b). Those functional studies of *miso* hopefully affect to increase the consumption of *miso*.

9.3 *Shoyu*: Soy Sauce

9.3.1 Outline

Shoyu (soy sauce) is a liquid seasoning made from soybeans, wheat, and salt water by brewing using fermentation technology. It is one of the basic seasonings in Japanese cuisine. Although the seasoning named "soy sauce" is widely used in ethnic cuisine of East Asian countries, its material and manufacturing method are different from each culture and country. Japanese soy sauce is known as Japan's taste that has been exported to more than 100 countries (Japan Soy Sauce Association 2012). On the other hand, Chinese soy sauces are primarily made from soybeans, with relatively low amounts of other grains. They can be roughly split into two classes: brewed or blended. For making the brewed-type one, the *teien kotai hakko* method is mainly used. The method is characterized by high temperature (about 45 °C) and short-term fermentation (about 20 days) and is quite different from the Japanese soy sauce production method (Hayashi 1988).

There are five types of *shoyu* roughly classified by the features, "*koikuchi shoyu*" (common soy sauce), "*usukuchi shoyu*" (light-colored soy sauce), "*saishikomi shoyu*" (re-fermented soy sauce), "*tamari shoyu*" (tamari soy sauce), and "*shiro shoyu*" (extra-light-colored soy sauce), as shown in Fig. 9.6 (Soy Sauce Information Center

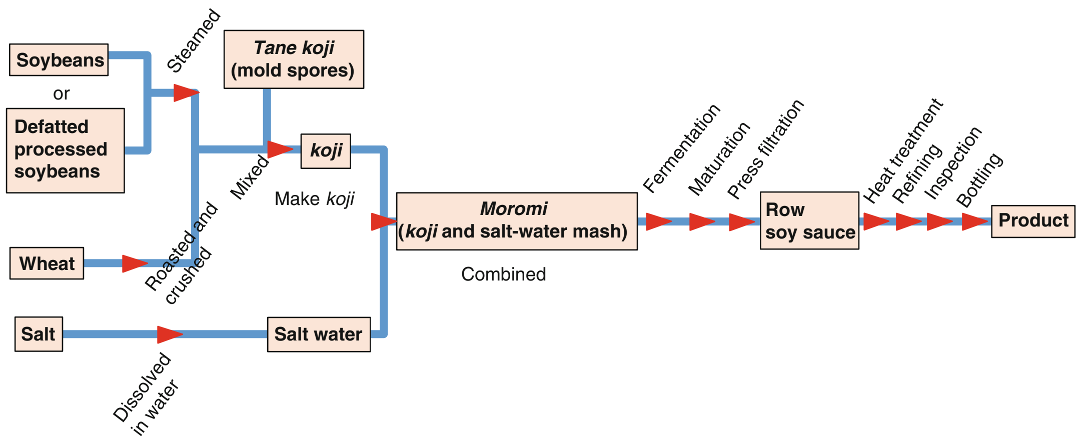

Fig. 9.7 Soy sauce manufacturing process (Soy Sauce Information Center 2014)

2014; Ministry of Agriculture, Forestry and Fisheries, Japan 2014).

Each individual *shoyu*'s features is described in detail at the final section of this chapter. There are also three different types of production method. First one is "*hon-jozo*" (regular fermenting method), next one is "*kongo-jozo*" (mixed fermenting method), and last one is "*kongo*" (mixture method) (Ministry of Agriculture, Forestry and Fisheries 2014).

9.3.2 Methods of Preparation

The method of preparation of *shoyu* manufacturing process by *hon-jozo* of *koikuchi shoyu*, which is the most popular in Japan, is described as follows (Fig. 9.7, Soy Sauce Information Center 2014):

1. Material processing step: Soybeans are soaked in water and cooked over a pressure until they are swollen. After roasting, wheat is crushed. This thermal denaturation treatment is used to facilitate the decomposition of the raw materials by *koji* mold such as *A. oryzae* and *A. sojae*.
2. *Koji*-making step: The cooked soybeans and crushed wheat are mixed in similar amounts and by adding the *koji* mold thereto and are cultured for 3–4 days under high humidity. The resulting culture is referred to as the *shoyu-koji*.
3. Fermentation step: After breaking lumps of the *shoyu-koji*, salt water is added to the *shoyu-koji* and mixed, and then it is transferred to charge in the brewing tank. The *shoyu-koji* and salt-water mash is called *moromi*. While stirring according to the situation, it is matured for a few months to a year in the tank. During maturation, enzymes from *shoyu-koji* break down protein and starch from grains to amino acids, a low molecular peptide, and glucose, respectively. In addition, first *shoyu* lactic acid bacteria and then *shoyu* yeast are grown in the mash. The resulting organic acid such as lactic acid, ethanol, and various aromas which are essential components in the taste and fragrance of soy sauce are produced by their action.
4. Squeezing step: By a "squeeze cloth" made of durable material such as nylon, the mixture is weighted and wrapped (*moromi*) to separate the solid and liquid. The resulting clear liquid is referred to as "*kiage-shoyu* (raw soy sauce)."
5. Heat treatment and refining step: The raw soy sauce obtained in the previous step is heated in order to add with the characteristic soy sauce aroma as well as sterilize the microorganisms. The precipitation which is insolubilized by heat denaturation is allowed to settle, and the insoluble is removed by filtration to obtain a clear liquid. After component adjustment, the soy sauce is packed in containers.

Table 9.2 Nutrition composition of different type soy sauces per 100 g (Ministry of Education, Culture, Sports, Science and Technology, Japan (2014) Food Composition Database)

Soy sauce name	Energy (kcal)	Moisture (g)	Protein (g)	Lipid (g)	Carbohydrate (g)	Ash (g)
Koikuchi shoyu	71	67.1	7.7	0.0	10.1	15.1
Usukuchi shoyu	54	69.7	5.7	0.0	7.8	16.8
Saishikomi shoyu	102	60.7	9.6	0.0	15.9	13.8
Tamari shoyu	111	57.3	11.8	0.0	15.9	15.0
Shiro shoyu	87	63.0	2.5	0.0	19.2	15.3

9.3.3 Mode of Consumption and Culinary

Shoyu is used in a wide variety of food and cuisines. This includes, of course, Japanese cuisine, which was added to UNESCO's Intangible Cultural Heritage list in 2013. In general, *shoyu* is used for "*tsuke-kake* use (dipping-sprinkling)" and "*ni-taki* use (boiling-cooking)." "*Tsuke-kake* use" means that in the menu, such as *sushi*, *sashimi*, and *tofu*, we directly dip or soak them in a small amount of *shoyu*. On the other hand, "*ni-taki* use" means that in the menu, such as *niku-jaga* or *nimono* dish, we boil or cook meat, fish, or vegetables with *shoyu*. For another use, it is also widely used as "*tsuyu*, the base sauce for noodle or *tempura* (fried fish and vegetables)." These findings show that *shoyu* is an essential condiment for the Japanese cuisine.

9.3.4 Microorganisms

Microorganisms which relate to *shoyu* manufacture can be roughly divided into *koji* mold, *shoyu* yeast, and *shoyu* lactic acid bacteria (Nakadai 2006a, b, c, 2007a, b). First *A. oryzae* and *A. sojae*, which belong to *Aspergillus* genus and have yellow spores, are known as *koji* molds (Nakadai 2006a). Both the genomes of the representative strains have been decoded (Machida et al. 2005; Sato et al. 2011). Although their genome sequence resembles that of *A. flavus*, an aflatoxin-producing microorganism, their genome information has demonstrated that the aflatoxin synthetic pathway of *koji* molds is incomplete (Nakadai 2006b; Matsushima et al. 2001). We have known two types of *shoyu* yeast. One is *Z. rouxii*, called main fermentative yeast, which mainly produces ethanol and 4-hydroxy-2 (or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF) and the main soy sauce aroma (Nakadai 2006c). The others are *C. versatilis* and *C. etchellsii*, called ripening yeasts, that mildly produce various aromas like 4-ethyl guaiacol and 4-ethylphenol in the late fermentation (Nakadai 2007a). *Shoyu* lactic acid bacteria, although there is a diversity of catabolism of sugar, are only known as *T. halophilus* (Nakadai 2007b).

9.3.5 Biochemistry or Nutritional Composition

The component of *shoyu* is made up of water, salt, amino acids, glucose, organic acids, and alcohols. The nutritional component-specific composition is shown in Table 9.2. In spite of the liquid, *shoyu* moisture content is as low as less than 70% (Ministry of Education, Culture, Sports, Science and Technology, Japan 2014).

9.3.6 Functionality and health benefits (Soy Sauce Information Center 2014)

Deodorizing: *Shoyu* has the effect of masking odor. *Shoyu arai* (rinsing with soy sauce) is a Japanese food preparation technique used to neutralize the raw smell of fish and meat.

Heat cooking effect: The sugar and amino acid content in *shoyu* reacts with each other when heated, in what is called an amino carbonyl reaction. This creates a unique fragrance.

Bacteriostatic effect: *Shoyu* contains salt and organic acids that help to prevent the growth of bacteria such as *E. coli* bacteria and even reduce them.

Contrasting effect: By adding a little *shoyu* to complete a dish, the sweetness of the flavors is accentuated. We call this the "contrasting effect."

Salty mitigation effect: Add a few drops of *shoyu* to anything salty, like pickles that have been pickled for too long, and the organic acids will help to soften the saltiness.

Synergistic effect: The glutamic acid in *shoyu* and the inosinic acid in dried bonito flakes, *katsuobushi*, work together to create a deep *umami*. We call this the "synergistic effect flavors."

9.3.7 Ethnical Value and Socioeconomy

Shoyu is produced in different regions of Japan, and each region has its own preferences and history of manufacture, producing unique characteristics (Soy Sauce Information Center 2014). *Koikuchi shoyu* is a common soy sauce which accounts for approximately 84 % of all *shoyu* consumed in Japan. It is a perfectly balanced seasoning with regard to flavor, color, and aroma. *Usukuchi shoyu* was born in the Kansai area, and this *shoyu* is lighter in color. *Usukuchi shoyu* contains about 10 % more salt than *koikuchi shoyu* and is used to preserve and enhance the natural color and flavor of food. *Tamari shoyu* is characterized by the grist which is almost soybeans. This soy sauce is also characterized by its thick, *umami*-rich flavor and distinctive aroma. Mainly manufactured in the Chubu area, it is used both in cooking and in making other products, such as rice crackers. *Saishikomi shoyu* is a specially produced *shoyu* that is produced between the San'in area and Kyushu. Using *kiage-shoyu* instead of the salt water, the fermentation step is the most unique step in this soy sauce. Rich in color, flavor, and aroma, this *shoyu* is used mainly as a table condiment. *Shiro shoyu* is characterized by the grist which is almost not soybeans but wheat. This *shoyu* is the lightest in color of all *shoyu*, with a distinctive aroma, mainly produced in the Hekinan district of Aichi Prefecture in the Chubu area.

9.4 *Natto*: Fermented Soybean

9.4.1 Outline

Natto is a non-salted fermented soybean, which has been produced and consumed in Japan for over a thousand years (Table 9.1 and Fig. 9.8A). *Natto* is characterized by the offensive odor of ammonia and branched short-chain fatty acids and a very viscous polymer, polyglutamate (PGA) (Fig. 9.8B). Although *natto* is eaten usually with cooked rice (Fig. 9.8E), it is also used as an ingredient in many dishes (*sushi*, soup, etc.) these days. *Natto* inherits nutritional advantages from soybeans, and moreover *B. subtilis* (*natto*), a sole fermentative microorganism of *natto*, produces some functional substances (Nagai 2015). Noteworthy substances are nattokinase, which is a serine protease activating a human fibrinolytic system, and vitamin K, which is essential to bone metabolism and blood coagulation. On the other hand, oligosaccharides, which favor the growth of lactic acid bacteria in intestines, in soybeans are consumed by *B. subtilis* (*natto*) during fermentation. Instead, *B. subtilis* (*natto*) itself seems to enhance the growth. *B. subtilis* (*natto*) also inhibits the growth of some pathogenic microorganisms. *Natto* was a highly localized foodstuff restricted to Japan, but now *natto* comes to be accepted as worldwide foodstuff, maybe because of its health-promoting benefits.

9.4.2 Methods of Preparation, Mode of Consumption, and Culinary

Before modernization of *natto* fermentation, *natto* was produced by packing boiled soybeans in bags of rice straws (Fig. 9.8F). *Natto* bacteria, *B. subtilis* (*natto*), habit in rice straws, and the bacteria grow on the soybeans packed in rice straws for 2 or 3 days, depending on ambient temperature, and ferment them to *natto*. Rice straws are used as both a source of *natto* bacteria and packing material. At the

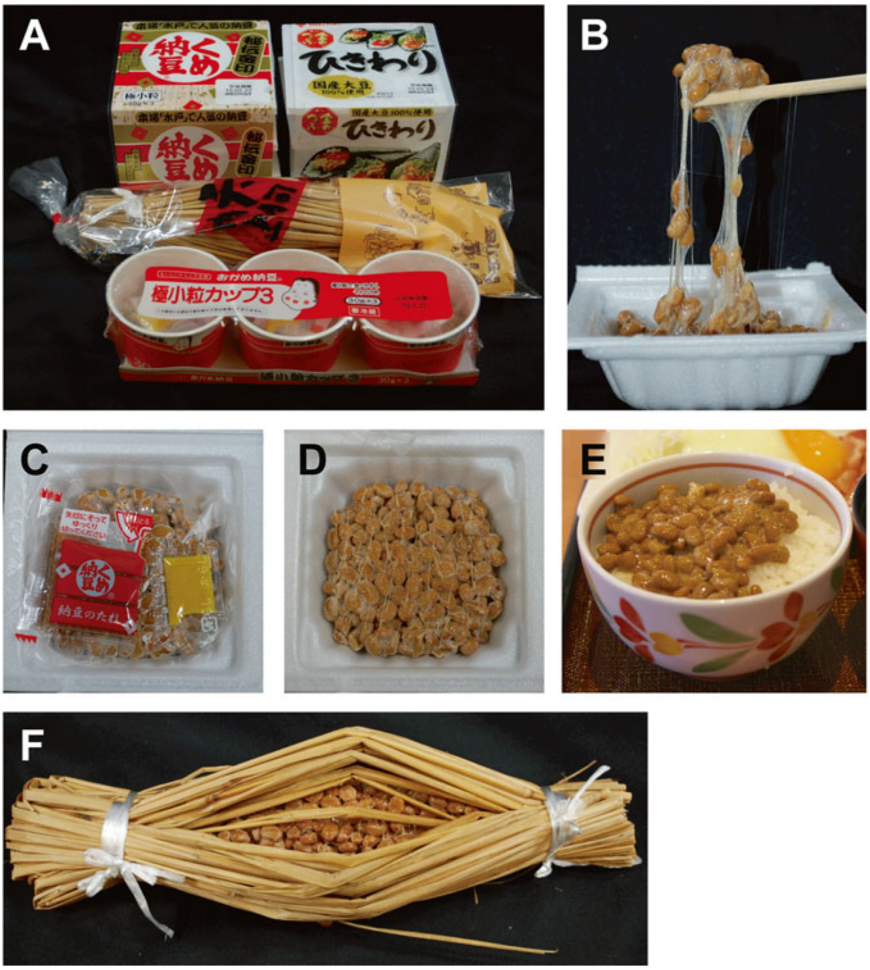

Fig. 9.8 *Natto*. (**A**) *Natto* products. (**B**) Viscous polymer, polyglutamate, of *natto*. (**C**) Inside a container. Pouches of seasoning are commonly attached on a vinyl sheet. (**D**) *Natto* under the sheet. (**E**) *Natto* on cooked rice. (**F**) *Natto* in an old-fashioned package. This is for souvenirs and *B. subtilis* (*natto*) is inoculated to boiled soybeans in the sterilized rice straws

present day, the rice straws are replaced with a clean polymer container (Fig. 9.8C, D), and a spore suspension of *B. subtilis* (*natto*) is inoculated onto boiled soybeans. The fermentation is carried out under controlled conditions (Fig. 9.9, Watanabe 2009b). *Natto* is eaten with cooked rice in many cases, especially at breakfast (Fig. 9.8E). *Natto* is also used as an ingredient in *sushi*, soup, pizza, spaghetti, and other dishes.

9.4.3 Microorganisms

Natto is fermented only by *Bacillus subtilis* (gram-positive, spore-forming, rod-shaped, aerobic bacteria), which was formerly named as *B. natto* by Sawamura (Sawamura 1906). Because typical strains of *B. subtilis* (e.g., a Marburg strain) cannot make *natto* at all, a group of *natto*-fermenting *B. subtilis* strains are often referred to as *B. subtilis* (*natto*) for industrial reasons (Nagai 2015).

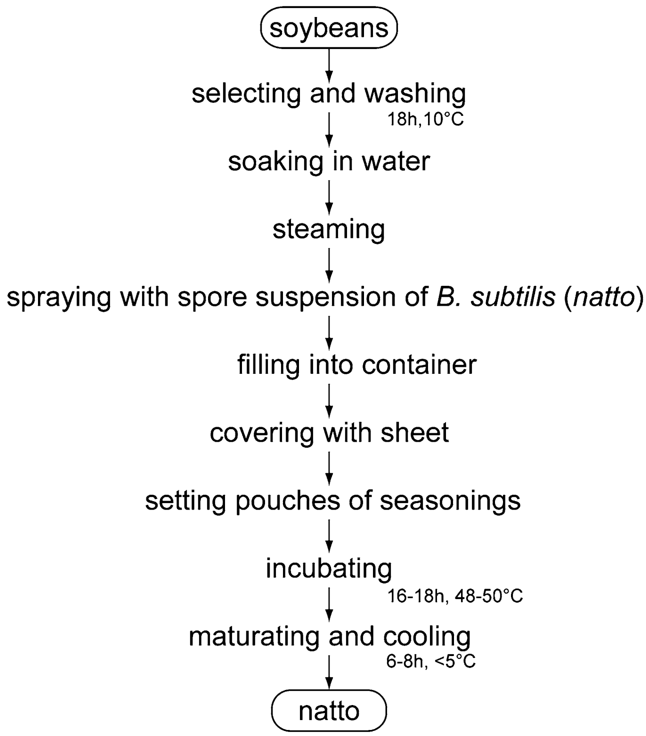

Fig. 9.9 A flowchart of natto fermentation

9.4.4 Biochemistry and Nutritional Composition

Nutritional composition per 100 g of *natto* is as follows: energy 200 kcal, water 59.5 g, protein 16.5 g, lipid 10.0 g, carbohydrate 12.1 g, and ash 1.9 g (Ministry of Education, Culture, Sports, Science and Technology, Japan 2014). The composition is almost equal to that of boiled soybeans. A prominent increase during fermentation from boiled soybeans is observed in a content of vitamin K (from 7 to 600 μg). As the high content of vitamin K inhibits the action of warfarin, an anticoagulant, patients administered with warfarin should not take *natto* (Kudo 1990). The most remarkable property of *natto* is production of PGA (Fig. 9.8b). The molecule is composed of both D- and L-glutamate, which are linked between an amino group and a γ-carboxyl group. PGA is estimated to be 10^6 Da (Nagai et al. 1997). The genes for PGA production were cloned in *E. coli* and sequenced, revealing that the PGA-producing enzyme exists as a membranous enzyme complex composed of three polypeptides (Ashiuchi et al. 1999). *B. subtilis* (*natto*) produced many kinds of enzymes, in which proteases are the most important because of their contribution to the taste of *natto*. Moreover, it is worth noted that the enzymes decompose allergen proteins in soybeans, resulting in the reduction of the allergic risk in soy proteins (Yamanishi et al. 1995).

9.4.5 Functionality and Health Benefits

Besides nutritional components, *natto* has a wide variety of functional compounds, which are produced by *B. subtilis* (*natto*) or inhered from soybeans (Nagai 2015). And nattokinase and probiotic effects of *natto* are attracting more attention of consumers. From *natto*, a urokinase-like enzyme was discovered and named nattokinase. The enzyme is a serine protease produced by *B. subtilis* (*natto*), and the function of nattokinase might be due to activation of a human fibrinolytic system rather than to direct decomposition of fibrin in blood vessels (Sumi 1991). Many reports on antibiotic activities of *natto* have been published since 1931, when *Salmonella* was successfully cured in a patient by oral administration of *natto*. One candidate of the antibiotics is dipicolinic acid contained in spores of *B. subtilis* (*natto*). *Natto* or *B. subtilis* (*natto*) has also probiotic activities. Oligosaccharides in soybeans favor the growth of lactic acid bacteria, but the oligosaccharides are consumed during *natto* fermentation. Instead, *B. subtilis* (*natto*) itself functions as probiotics in intestines (Hosoi et al. 1999).

9.4.6 Ethnical Value and Socioeconomy

It is thought that *natto* was already consumed around 1000 AD when the earliest description on *natto* appeared in Japanese classic literature (Watanabe 2009b). There are several legends on the origin of *natto*: the most famous one says that boiled soybeans packed in a bag woven with rice straws turned to *natto* on the back of a troop horse in a civil war (1083–1087 AD) which occurred in the northern area (Tohoku area, Fig. 9.1) of Japan. This might be supported by the fact that *natto* is consumed more in the northern area (Tohoku area in Fig. 9.1) of Japan than in the southern or western areas (Kansai area or Kyushu Island), though the regionality is dissolving today. *Natto*-like fermented soybeans using *Bacillus* sp. are produced in many areas of Asia (Nagai and Tamang 2010). Though viscous polymer often was not seen on the final products in the areas, PGA-producing strains were isolated from the products, the existence of which might indicate that *natto* and these foods are essentially the same. In 2005, 236,000 tons of *natto* were produced in Japan, soybeans of which were mainly imported from the USA, Canada, and China (Watanabe 2009b). The price of one container of *natto* (40 g) is about 30 yen and that of *natto* made of domestic soybeans is higher in Japan.

9.5 *Su*: Vinegar

9.5.1 Outline

Su is a vinegar in Japanese which is composed of fermented and synthetic vinegar. In general, fermented vinegar is usually produced from cereal or fruit, while, on the other hand, synthetic vinegar is generally produced from glacial acetic acid. In Japan, cereal vinegar is mainly consumed, and rice vinegar is the main category of cereal vinegar. Rice vinegar is defined as cereal vinegar using rice more than 40 g/l. Recently "rice black vinegar" is newly divided from "rice vinegar." Rice black vinegar is defined as rice vinegar using rice more than 180 g/l, and color of that vinegar should be changed to bark or black via fermentation and maturation. Here, we describe the rice vinegar, especially the rice black vinegar (Yanagida 1987).

Rice vinegars are usually made directly from rice and *koji*, molds cultured on the steamed rice, and they are produced in stainless or rarely wood tanks without mixing and aeration (Fig. 9.10). Saccharification and alcoholic fermentation were usually separated from acetic acid fermentation in rice vinegar production (Entani 2001). On the other hand, Fukuyama pot vinegar is made in pot. Fukuyama pot vinegar is one of the most traditional types of rice black vinegar in Japan produced for more than 200 years in Kagoshima Prefecture in Kyushu, the southeasternmost large island in Japan. Here, saccharification, alcohol fermentation, and acetic acid fermentation are conducted in one pot (Fig. 9.11) (Yanagida 1990, 2001).

9.5.2 Methods of Preparation

Fermentation of Fukuyama pot vinegar is conducted in loosely capped pots laid on the ground of open-air fields (Fig. 9.11). Here we describe the production process and the fermentation mechanism of Fukuyama pot vinegar. At first, steamed rice, rice *koji*, and water were put into a pot, and then floating *koji*, well-dried rice *koji*, is scattered onto that surface. Then a roughly

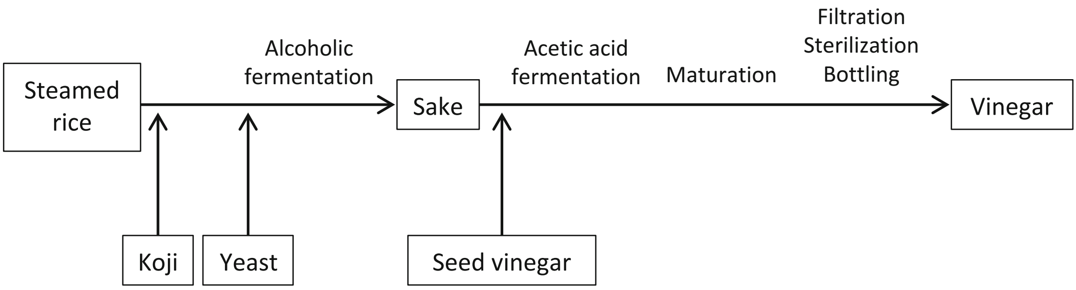

Fig. 9.10 Flow sheet of rice vinegar production

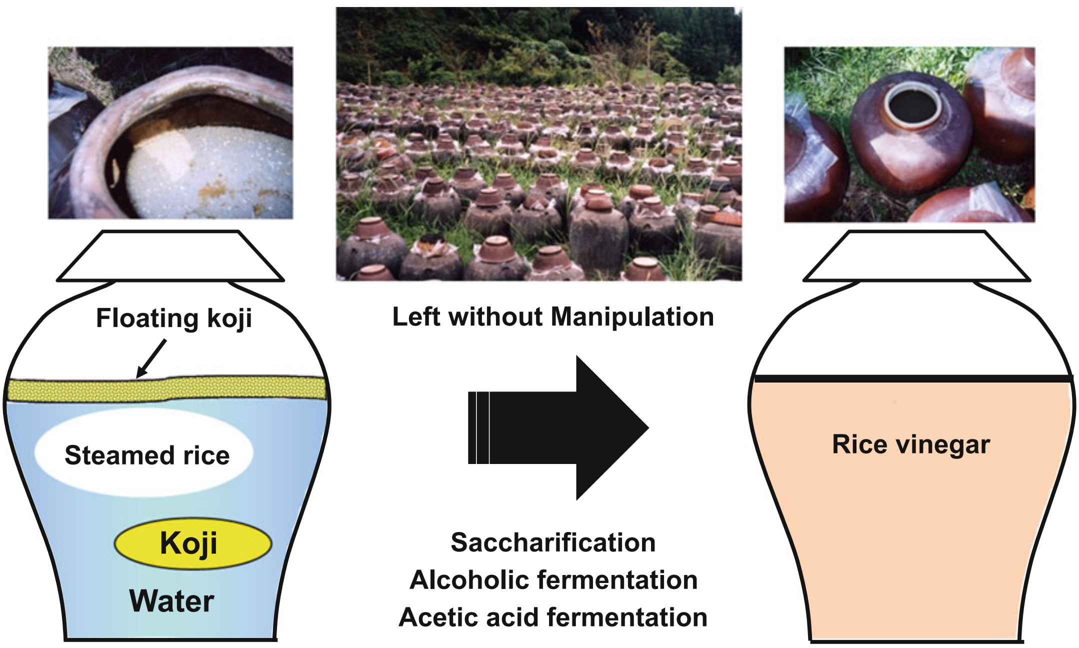

Fig. 9.11 Fermentation of Fukuyama pot vinegar

capped pot was laid on the plane for more than 90 days without specific manipulation, and rice vinegar with brown color is completed. Alcoholic fermentation is conducted in an anaerobic environment, and acetic acid fermentation is conducted in an aerobic environment. However, there were no additional microorganisms and specific manipulation for controlling the complex fermentation of Fukuyama pot vinegar. Rice vinegars in Japan are usually made from rice and *koji*, and saccharification and alcoholic fermentation processes are usually separated from acetic acid fermentation processes (Fig. 9.10). On the other hand, in Fukuyama pot vinegar, saccharification, alcoholic fermentation, and acetic acid fermentation processes proceed sequentially and partly in parallel in one pot (Fig. 9.11) (Koizumi et al. 1987). Here, floating *koji*, well-dried rice *koji*, described above plays an important role in Fukuyama pot vinegar. Floating *koji* covers the surface of fermentation mixture, inhibiting the formation of pellicle by acetic acid bacteria. After alcoholic fermentation, floating *koji* settles out spontaneously, and pellicle of acetic acid bacteria was formed on the fermentation mixture. Therefore, the settling out of floating *koji* brought about the natural transition of anaerobic alcoholic fermentation to aerobic acetic acid fermentation. Therefore, spontaneous settling out of floating *koji* is the most important point in the particular fermentation process of Fukuyama pot vinegar (Koizumi et al. 1988).

Increase of the weight of floating *koji* or change of the interfacial activity of fermentation mixture or other reasons would induce the settling out of floating *koji*; however, the mechanism of the settling out of floating *koji* is unclear. This should be clarified in the future.

9.5.3 Mode of Consumption and Culinary

Vinegar is usually used for flavoring, controlling microorganisms, and lowering pH. Rice vinegar is usually used for seasoning the *sushi* rice and also used in various Japanese dishes. Fukuyama pot vinegar is usually drunk directly for health and also can be used in various Japanese dishes (Kanie 1990).

9.5.4 Microorganisms

A. oryzae is used for producing *koji* and *S. cerevisiae* is used for alcoholic fermentation in rice vinegar (Koizumi et al. 1988). *Acetobacter aceti* or *A. pasteurianus* are usually used for rice vinegar fermentation (Ameyama and Ohtsuka1990). In addition, lactic acid bacteria would also contribute to the rice vinegar fermentation (Entani and Masai 1985a). In Fukuyama pot vinegar, *A. oryzae* is also used for producing *koji* and *S. cerevisiae* also contributed to alcoholic fermentation (Furukawa and Katakura 2012; Haruta et al. 2006). In addition *A. aceti* or *A. pasteurianus* also contributed to acetic acid fermentation. There were various lactic acid bacteria, such as *Lactobacillus*, *Lactococcus*, and *Pediococcus* species, which are detected from the mush of Fukuyama pot vinegar, and contribution of lactic acid bacteria to lactic acid fermentation would be important for Fukuyama pot vinegar fermentation to controlling the contamination in the early phase of fermentation (Furukawa et al. 2008a, 2008b, 2013, 2014; Okazaki et al. 2010; Koizumi et al. 1996).

9.5.5 Biochemistry Composition

Total nitrogen content, mainly amino acid, and organic acid content, mainly lactic acid and pyroglutamic acid (Tayama 2012), of Fukuyama pot vinegar are generally higher than other rice vinegars. These characteristic properties of chemical composition would bring about the higher *umami* and balmy acid taste of Fukuyama pot vinegar (Koizumi et al. 1987; Entani and Masai 1985b).

9.5.6 Functionality and Health Benefits

It was indicated that vinegar has some health functions: inducing saliva secretion and supporting digestion, recovery from fatigue, repressing elevation of blood sugar level, and promoting calcium absorption (Tayama 2012). Rice vinegar and Fukuyama pot vinegar also have the above health functions, and Fukuyama pot vinegar would have some other health functions (ibid.).

9.5.7 Ethnical Value and Socioeconomy

Fukuyam pot vinegar is now produced just in Japan, but it is thought to have handed down from surrounding countries sometime between the fourth and nineteenth century (Furukawa et al. 2008b). Fukuyama pot vinegar is produced in Kyushu Island, the southeasternmost large island in Japan, and there were resembling vinegars fermented in pots in the west side of Kyushu Island. Fukuyama pot vinegar is produced in small pots and this contributes to the risk distribution. Fukuyama pot vinegar production is so economic because it needs no specific manipulation in its fermentation process, and therefore, only the Fukuyama pot vinegar survived as a commercial product in Kyushu also in Japan (Higashi 1981).

Fig. 9.12 Variety of homemade *nuka-zuke*

9.6 *Tsukemono*: Fermented Vegetables

9.6.1 Outline

Tsukemono is pickled vegetables of Japan which includes two categories: traditional fermented vegetables and unfermented ones. Fermented vegetables with salt and seasonings were traditionally used to extend shelf life and enhance taste and flavors. Due to development of cold chain and health considerations, salt concentration becomes lower, and fermentation is omitted from the production process in most of commercially supplied *tsukemono*. Nevertheless, traditional fermented vegetables and homemade fermented vegetables are still consumed at the tables in Japan. In this section, the traditional fermented vegetables are described (Miyao 2002; Koizumi 2000). Most of the vegetables, even some fruits, are used for *tsukemono*, for example, Japanese radish (*daikon*), cucumber, eggplant, and Chinese cabbage (*hakusai*), and these are often named as seasoning materials for pickling (Table 9.1). One of the representative seasoning materials is a *nukadoko*, which is a pasty mixture of rice bran, salt, spices, and water. Vegetables pickled in *nukadoko* are called *nuka-zuke* (Fig. 9.12). Fermented *nukadoko* contains various lactic acid bacteria which produce lactic acid, flavors, and, in some cases, antibacterial substances called bacteriocins (Irisawa et al. 2014; Kato et al. 2014; Nakayama et al. 2007; Ono et al. 2014). Several fermented products made from local vegetables specified for *tsukemono* are named from the name of the vegetables (Table 9.1). During pickling process, vegetable cells are killed in the presence of salt, and self-digested cellular materials are fermented by lactic acid bacteria, resulting in good taste, flavors, and acidification to prevent the products from spoilage bacteria.

9.6.2 Methods of Preparation

The methods to make *tsukemono* vary from a simple salting to more complicated processes involving preparation of vegetables and of fermented materials such as *koji*, *miso*, and *shoyu*. To make *nuka-zuke*, vegetables are washed and directly pickled in *nukadoko*. *Nukadoko* is made from rice bran, salt (10–15 wt.% of rice bran), seasonings (dried kelp, red pepper, Japanese pepper), and water (100 wt.% of rice bran). It is important to mix the *nukadoko* to stabilize the microflora and to balance anaerobic fermentation by lactic acid bacteria with aerobic fermentation by yeasts. It takes more than a week to activate microbial growth and to mature the *nukadoko* (Ono et al. 2015). Domestic preparation of *takuan-zuke and hakusai-zuke* is illustrated in Fig. 9.13. In the case of food manufacturers, fresh vegetables are at first brined in the presence of ca. 20 wt.% of NaCl (Miyao 2002). Figure 9.14 shows *takuan-zuke* in a food manufacturer, for example. Fresh *daikon* are washed, pickled, and weighted for several months. During fermentation, *daikon* turns to yellow, the specific color of *takuan*, by chemical change of an isothiocyanate component (Matsuoka et al. 2002, 2008). Brined *daikon* are washed, cut, desalted to suitable salt concentration, and packed with seasonings. Other than *shio-zuke*, brined vegetables are desalted and pickled with various seasonings or materials such as *miso*, *sake*, lee, etc. (Table 9.1). Most products are packed in plastic bags or containers, followed by sterilization, and put on the market.

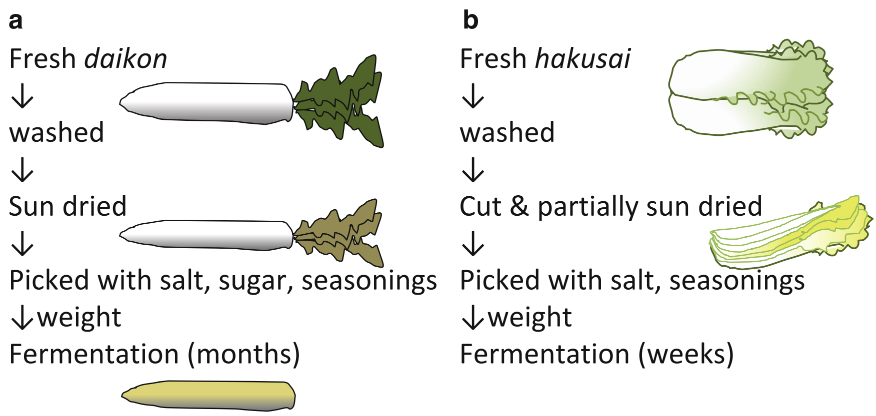

Fig. 9.13 Preparation of *takuan-zuke* (**a**) and *hakusai-zuke* (**b**). (**a**) *Daikon* roots are washed and partially sun dried until being flexible. Dried *daikon* roots are pickled with salt (4–6 wt%) with sugar (1–4 wt%), rice bran (10–20 wt%) and seasonings and pressed under a weight (ca 200 wt% of *daikon*) for more than a month. (**b**) Fresh *hakusai* are washed, cut into 1/2 or 1/4, partially dried under the sun, and pickled with salt (3 wt%) and seasonings (red pepper, dried kelp, citrus peels), followed by being pressed under a weight for several days

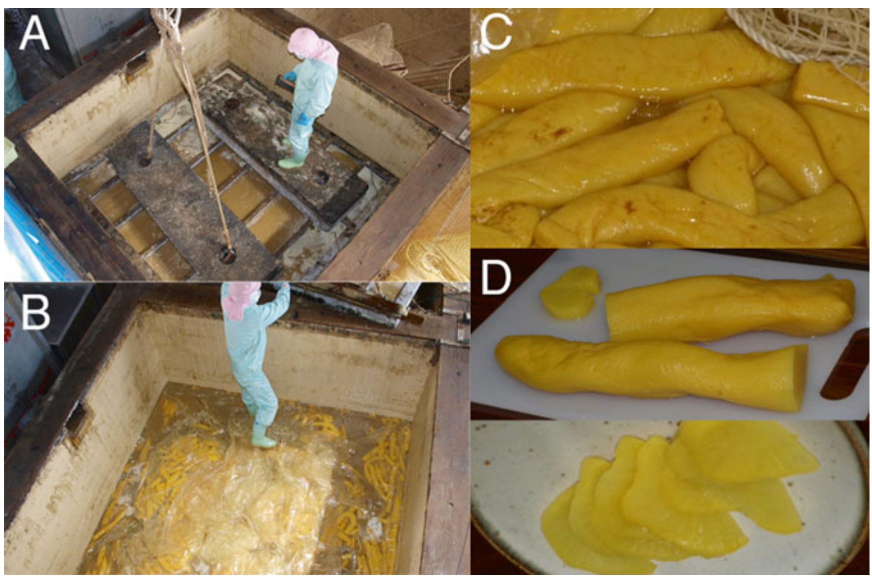

Fig. 9.14 *Takuan-zuke* in a food manufacturer. (**A**) *Daikon* are brined and weighted for several months. (**B**) Under a wooden lid, a bunch of *takuan* is kept in brine. (**C**) Without coloring materials, daikon has turned yellow by chemical reaction of an isothiocyanate. (**D**) *Takuan-zuke* with bright yellow color

9.6.3 Mode of Consumption and Culinary

A set of rice, *miso* soup, and a small dish of *tsukemono* is served practically in every traditional meal with other side dishes or after drinking *sake* (Fig. 9.15). Japanese eat rice as their staple diet. Salty *tsukemono* is necessary when eating cooked rice without taste and is valued for their unique flavors. *Tsukemono* is a kind of ready-to-eat food without cooking and is served with Japanese tea as a snack. Some fermented vegetables, such as *hakusai-zuke*, *takuan-zuke*, and *sunki-zuke*, are sometimes used for stir-fries.

Fig. 9.15 A typical set of rice, *miso* soup and *tsukemono*

9.6.4 Microorganisms

Microorganisms involved in fermented vegetables are mainly lactic acid bacteria (Table 9.1). For example, *L. brevis* (Nakagawa et al. 2001; Ogihara et al. 2009; Shinagawa et al. 1996), *L. namurensis* (Sakamoto et al. 2011; Kato et al. 2014), *L. acetotolerans* (Nakayama et al. 2007; Sakamoto et al. 2011), *L. sakei* (Nakagawa et al. 2001; Ogihara et al. 2009; Oyaizu and Ogihara 2009), *L. curvatus* (Nakagawa et al. 2001; Ogihara et al. 2009; Oyaizu and Ogihara 2009; Shinagawa et al. 1996), *L. collinoides* (Nakagawa et al. 2001), *L. fructivorans* (Nakagawa et al. 2001), *L. casei* (Nakagawa et al. 2001), *L. casei* subsp. *pseudoplantarum* (Shinagawa et al. 1996), *L. delbrueckii* (Endo et al. 2008), *L. fermentum* (Endo et al. 2008; Nakagawa et al. 2001), *L. plantarum* (Endo et al. 2008; Ogihara et al. 2009; Ono et al. 2014; Shinagawa et al. 1996), *L. lactis* (Nakagawa et al. 2001), *L. mesenteroides* (Nakagawa et al. 2001; Oyaizu and Ogihara 2009; Shinagawa et al. 1996), *L. carnosum* (Oyaizu and Ogihara 2009), *L. citreum* (Nakagawa et al. 2001), *Pediococcus pentosaceus* (Nakagawa et al. 2001; Zhao et al. 2012), and *Pediococcus damnosus* (Nakagawa et al. 2001) have been reported. Further, new species, *L. kisonensis* sp. nov., *L. otakiensis* sp. nov., *L. rapi* sp. nov., *L. sunkii* sp. nov. (Watanabe et al. 2009), *L. delbrueckii* subsp. *sunkii* subsp. nov. (Kudo et al. 2012), and *L. furfuricola* sp. nov. (Irisawa et al. 2014) have been found in Japanese fermented vegetables. Recent metagenomic approach will provide the information of ecosystem in fermented products (Ono et al. 2014, 2015; Sakamoto et al. 2011). Depending on the seasoning materials, yeasts such as *D. hansenii*, *S. cerevisiae*, *S. servazzii*, and *Z. rouxii* are also involved in the fermentation (Kato et al. 1991; Miyao 2002; Yamagata and Fujita 1974).

9.6.5 Biochemistry and Nutritional Composition

Tsukemono had been eaten as vegetable source to intake vitamins and dietary fiber before fresh vegetables are available in markets year-round. As *tsukemono* is made from raw vegetables in the presence of salt, and nutritional components are relatively conserved. In the case of *nuka-zuke*, vitamins from *nukadoko*, including rice bran and fermented products of bacteria, are accumulated in fermented vegetables (Table 9.3). Other nutritional compositions are depending on the vegetables and the maturing period.

9.6.6 Functionality and Health Benefits

Mortality due to stroke and intake of salt is highest in the Tohoku area (northern part of Honshu Island). Causal relationship between intake of salty foods including *tsukemono* and unhealthy effects has been pointed out (Yamada et al. 2003). On the other hand, *tsukemono* is one of the sources to intake nutrients from vegetables containing dietary fibers and vitamins more than original raw vegetables. Although health benefits and risks of fermented vegetable are still debatable, fermented vegetables are important elements in *Washoku* (Japanese cuisine) that are thought to be an exceptionally well-balanced and healthy diet. Besides nutrients from vegetables, components involved in functionality are lactic acid bacteria and their metabolites such as

Table 9.3 Contents of vitamins in raw and pickled (*nuka-zuke*) vegetables

	Daikon/radish		Turnip		Cucumber		Eggplant	
Vitamins	Raw	*Nuka*	Raw	*Nuka*	Raw	*Nuka*	Raw	*Nuka*
Vitamin K (μg)	tr	1	0	0	34	110	10	12
Vitamin B1 (mg)	0.02	0.33	0.03	0.45	0.03	0.26	0.05	0.1
Vitamin B2 (mg)	0.01	0.04	0.03	0.05	0.03	0.05	0.05	0.04
Niacin (mg)	0.2	2.7	0.6	3.2	0.2	1.6	0.5	1
Vitamin B6 (mg)	0.05	0.22	0.07	0.42	0.05	0.2	0.05	0.15
Vitamin B12 (μg)	0	0	0	0	0	0	0	tr
Folic acid (μg)	33	98	49	70	25	22	32	43
Pantothenic acid (mg)	0.11	0.43	0.23	1.11	0.33	0.93	0.33	0.67
Biotin (μg)	0.3	–	1	–	1.4	1.2	2.3	–
Vitamin C (mg)	11	15	18	20	14	22	4	8

Food Composition Database (http://fooddb.mext.go.jp/)

γ-aminobutyric acid. Lactic strains isolated from fermented vegetables have distinct properties from strains from dairy products (Nomura et al. 2006). Functionality of so-called plant-derived LAB has been examined (Takii et al. 2013; Waki et al. 2014; Suzuki et al. 2014a, b; Higashikawa et al. 2010; Zhao et al. 2012; Yamamoto et al. 2011). Depending on the activity of glutamate decarboxylase expressed from LAB, the concentration of γ-aminobutyric acid is increased in some types of fermented vegetables (Setoguchi et al. 2005; Ueno et al. 2007; Fukai and Tukada 2007; Watanabe et al. 2009).

9.6.7 Ethnical Value and Socioeconomy

The first historical record of *tsukemono* was written in the eighth century, and varieties of *tsukemono* were mentioned frequently in various literatures in the tenth century (Koizumi 2000). Local distinctive fermented vegetables are produced from Hokkaido (the northernmost islands) to Okinawa (the southernmost islands), with a latitude range of 46–20°N. Local specific vegetables with specific seasoning materials provide distinctive products, which characterize local food culture. One of these products, *kobutakana* in Unzen (Nagasaki Prefecture (Pref.)), was selected as "Precidia" of the "Ark of Taste" project. A unique salt-free fermentation in Japan (Watanabe et al. 2009; Kudo et al. 2012; Endo et al. 2008), *sunki-zuke*, is made from a turnip in Kiso (Nagano Pref.), which is similar to Gundruk in India (Karki and Itoh 1988). *Suguki-zuke* is also made from a turnip in Kyoto Pref. and is different from *sunki-zuke* in fermentation in the presence of salt. *Washoku* is characterized as the sense of the beauty of nature and of the changing seasons expressed at the table. Local fermented vegetables are also important elements to express the local characteristics and seasons. Salted cherry blossoms, the national flower of Japan, are served with boiled water instead of tea at a betrothal ceremony and at a wedding.

9.7 Fermented Seafoods

9.7.1 Outline

A variety of fermented seafoods are produced in Japan; only representative products are introduced here, but please refer to previous monographs for additional information (Fujii 1992; Fukuda et al. 2005). Japanese fermented seafoods are divided into three major types based on the fermentation procedure, namely, salted type, pickled type, and molded type (Tables 9.1 and 9.4). The salted fermented foods include fish sauce and salted fish, which are typically made from simply fish and salt (more than 10 %). Fermentation of these foods involves the digestion of fish materials by

Table 9.4 Summary of fermentation types of Japanese fermented seafoods (aquatic foods)

Fermentation type	Name of products: General name	Name of products: Product name	Raw materials	Salt concentration	Fermentation period	Contribution in fermentation: Self-digestion	Contribution in fermentation: Microorganisms	References
Salted type	*Gyosho* (fish sauce)	*Ishiru*	Squid	Approx. 20 %	>1 year	◎	△	Ishige and Ruddle (1990)
		Shottsuru	Sand fish	>20 %	>1 year	◎	△	Fujii (1992)
	Shiokara (salted fish)	*Ika-shiokara*	Squid	Approx. 10 %	2–3 weeks	◎	△	Fujii (1992)
		Shutou	Organ of skipjack tuna	>20 %	1–2 months	◎	△	Fujii (1992)
		Konowaka	Intestine of sea cucumber	<5 %	2–3 days	◎	△	Fukuda et al. (2005)
	Kusaya	*Kusaya*	Amberstripe scad, flying fish, etc.	3–8 %	>100 years	×	○	Fujii (1992)
Pickled type	*Nare-zushi* (fermented sushi)	*Funa-zushi*	Crucian carp	2–4 %	2 years	△	◎	Fujii (1992)
		Izushi	Salmon, etc.	<4 %	1–2 months	△	◎	Fujii (1992)
	Nuka-zuke (pickled seafood with rice bran)	*Heshiko*	Mackerel	>7 %	>7 months	○	○	Kosaka and Ooizumi (2012)
		Fugonoko-nuka-zuke	Pufferfish ovaries	10–15 %	2 years	○	◎	Kobayashi et al. (1995)
Molded type	*Fushi* (boiled, smoke-dried, and molded fish)	*Katsuobushi*	Skipjack tuna	<1 %	>3 months	×	△	Fujii (1992)
		Sababushi	Mackerel and its relatives	<1 %	> 3 months	×	△	Fukuda et al. (2005)

self-digestion and microorganisms. The predominant microorganisms are gram-positive cocci including halophilic lactic acid bacteria. Their organoleptic features are a salty taste, preferable with a fishy odor, and strong *umami* quality. Pickled fermented foods are made from raw fish materials combined with carbohydrates such as cooked rice or bran. Representative products include *funa-zushi*, *izushi*, and rice bran pickles. They are typically processed by lactic acid fermentation with bacteria. They are distinctly characterized by their sour taste and flavor, occasionally resembling cheese. The shelf life and quality stabilization period are shorter than those of salted products owing to the low salt concentration (except for salted rice bran pickles). Furthermore, there are boiled, smoke-dried, and molded fish (*fushi*), referred to as *katsuobushi*, that are made from dried smoked bonito and mold starter. The mold starter functions in the digestion of lipids and dehydration. The unique aromatic flavor derived from phenolic compounds resulting from the smoking step is also a distinct characteristic of *katsuobushi*. The soup stock extracted from sliced *katsuobushi*, called *dashi*, is the essence of Japanese cuisine (Fujii 1992; Fukuda et al. 2005).

9.7.2 Fish Sauce

Fish sauce is a liquid condiment made by the long-term fermentation of fish and salt. Japanese fish sauce is made from many types of small fish including shellfish, sandfish, sardines, squid, and others. The salt concentration is approximately 20 % or more, and the fermentation period is longer than 1 year for traditional fish sauce (Fujii 1992). The history of fish sauce production in Japan is older than that of soy sauce; it was a popular condiment until the distribution of soy sauce (Ishige and Raddle 1990). The organoleptic features of fish sauce are salty and strong *umami* qualities and preferably a (sometimes unique) fishy odor. To improve the fishy smell and to reduce the fermentation period, mold starter, called *koji*, is added to fish materials during fish sauce processing (Funatsu et al. 2000). Recently, fish sauce production in Japan has increased dramatically as a measure to reduce fishing industry waste by making full use of fish materials, although the production of traditional fish sauce is decreasing. The current production volume is estimated at approximately 6100 tons per year in Japan (Funatsu 2013).

The fermentation mechanism is the digestion of fish materials using self-digestion enzymes and microorganisms, resulting in the accumulation of chemical compounds that affect taste and flavor. The accumulation of amino acids and peptides that are associated with the typical fish sauce taste results from a self-digestion enzyme secreted from fish materials (Yamashita et al. 1991). As shown in Fig. 9.16, during fermentation, bacterial counts and total nitrogen, as well as amino acids and peptides, increase; hence, the pH decreases owing to lactic acid accumulation. Halophilic lactic acid bacteria, mainly *T. halophilus*, are dominant during fermentation in a variety of Japanese fish sauces (Fujii 1992; Dicks et al. 2009). Changes in the bacterial flora of Japanese fish sauce during fermentation have been studied previously (Fukui et al. 2012). The bacteria play an important role in the accumulation of lactic acid during fish sauce fermentation, though they have little effect on the digestion of fish materials or the production of amino acids via enzymes. The basic quality of fish sauce is stable as long as an appropriate salt concentration is used. However, the accumulation of biogenic amines, including histamine and tyramine, sometimes occurs during fish sauce production. Certain tetragenococcal strains are histamine producers (Satomi et al. 2011, 2012).

9.7.2.1 *Shiokara*

Salted fish, *shiokara* in Japanese, refers to fish that is mixed with salt and aged. A representative product is a salted squid; approximately 20,000 tons per year are produced in Japan (Ministry of Agriculture, Forestry and Fisheries 2012). A variety of fish is used in addition to squid, including the organs of skipjack tuna, sea cucumber intestines, salmon kidneys, and others. The processing method is simple, as

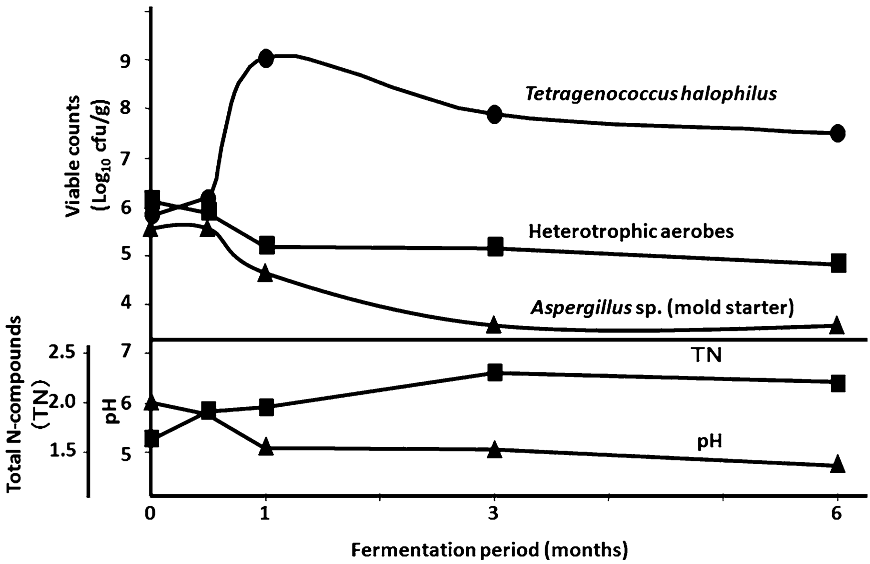

Fig. 9.16 Changes in microorganisms counts and chemical compounds during fish sauce fermentation. The fish sauce was made from deep sea smelt and fermented with mold starter at ambient temperature (*Upper graph*, ● *T. halophilus*, ■ Heterotrophic aerobes, ▲ *Aspergillus* sp. (mold starter); *Below graph*, ■ Total nitrogen-compounds, ▲ pH)

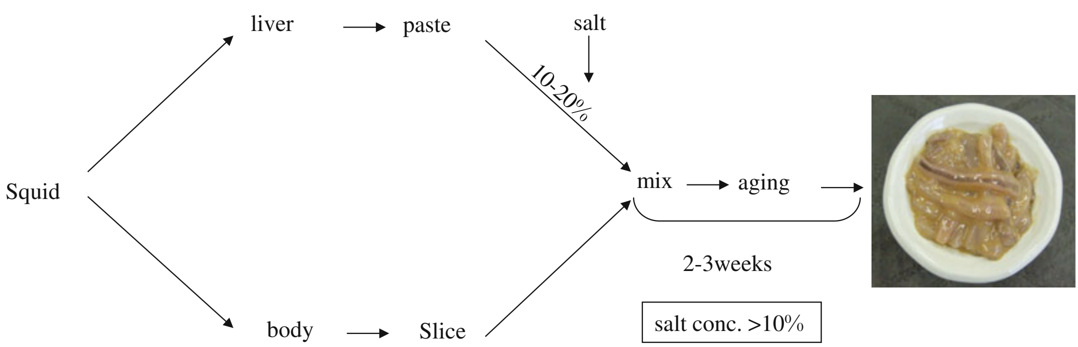

Fig. 9.17 Procedures of *Ika-Shiokara* processing in traditional method

described in Fig. 9.17. The salt concentration is approximately 10 % for traditional products, which are stored in ambient temperatures. The aging period is 10–20 days. The optimal time for consumption after aging is within several weeks without refrigeration. Recently, consumers have demanded seafood with a reduced salt concentration. Therefore, most of the salted fish sold in Japan contains only 5 % salt or less, though it is necessary to keep the products in cold or freezing conditions during distribution and storage (Fujii 1992).

For production, fish materials are digested using self-digestion enzymes, resulting in the accumulation of chemical compounds that influence the taste and flavor (Fujii et al. 1994). The accumulation of amino acids and peptides related to the typical salted fish taste is provided by the self-digestion enzymes secreted from fish materials, and squid liver is particularly important for providing proteolytic enzymes during salted squid production. Therefore, the roles of microorganisms are not significant with respect to taste, raising questions regarding whether salted fish can be considered a fermented food or not. However, *Staphylococcus* spp. have been isolated from salted squid, and the bacteria have been implicated in the production of the distinct salted squid smell (Fujii et al. 1994). Since this question is still not resolved, the words "aged" and "aging" will be used instead of fermentation.

9.7.2.2 *Kusaya*

Kusaya is a salt-dried fish made from flying fish, amberstripe scad, and mackerel scad in the Izu Islands. The unique characteristics of *kusaya* are attributed to the brine used in its production. This brine has been successively used for a long time, and some are said to have been used for more than 100 years, which is called *kusaya* gravy (Fujii 1992). Thus, various microbial metabolites have accumulated, giving the gravy unique features and flavors. *Kusaya* gravy is rich in nutrients for bacteria; it contains 3–8 % NaCl (depending on the manufacturer), volatile nitrogen compounds, and low oxygen concentrations (Fujii 1992; Satomi et al. 1997). The bacterial community is complex, indicating that it cannot be applied to specific fermentation uses (Satomi et al. 1997; Takahashi et al. 2002). Owing to its turbid brown color, high viscosity, offensive smell, and other such properties, there have been concerns regarding its safety from a public health standpoint. However, it was revealed that the gravy is able to suppress the growth of some pathogenic bacteria (Fujii et al. 1990). Although the fermentation mechanisms have not been elucidated, the compounds associated with the unique taste and odor of this gravy could result from bacterial metabolites of fish body exudates during the soaking procedures. Many of new bacterial species, mainly *Oceanospirillaceae* species, have been isolated from the gravy (Satomi and Fujii 2014).

9.7.2.3 *Nare-Zushi* (Fermented *Sushi*, Pickled *Sushi*, and Matured *Sushi*)

Nare-zushi is fermented seafood in which fish raw materials are pickled with rice or other carbohydrate materials. Common fish used to produce *nare-zushi* are crucian carp, mackerel, salmon, sandfish, and others (Fujii 1992). Two types of *nare-zushi* are produced in Japan, one with a long-term fermentation represented by *funa-zushi* and one with a short-term fermentation represented by *izushi*. *Funa-zushi*, which is made from crucian carp carrying eggs, is particularly famous for its use in typical pickled *sushi*. The traditional preparation of *funa-zushi* is as follows: a crucian carp carrying eggs is used as a raw material, the scales and internal organs are removed, the fish is cured in saturated saline for approximately 1 year, the fish is washed with water, cooked rice is packed into the fish abdomen, and it is fermented for an additional year. To consume the final product, the cooked rice that adheres to the fish is removed. The fish and fish eggs are edible. Other *nare-zushi* products such as *izushi* have similar features, though the fermentation period is shorter than that of *funa-zushi* and taste is more general. Production and consumption volumes are decreasing owing to changes in palatability and a decreasing supply of crucian carp. However, pickled *sushi* products made with other fish are still familiar in each district in Japan. The sour taste and flavor are distinct characteristics, and it occasionally resembles cheese. The chemical characteristics of *funa-zushi* are as follows: 2–4 % salt concentration, pH 3.7–3.8, and lactate, acetate, formate, and propionate as the dominant organic acids. The shelf life and quality stabilization period are shorter than those of salted fermented seafoods owing to the low salt concentration (except for salted rice bran pickles). The predominant microorganisms are lactobacilli, streptococci, and pediococci, which are involved in lactic acid fermentation. Therefore, the fermentation mechanism is considered typical lactic acid fermentation, and rice can be utilized as the carbon source. In the curing step, which involves the soaking of fish in saturated brine, halophilic lactic acid bacteria such as *T. halophilus* have been isolated. Such foods have a hygiene issue related to botulism poisoning owing to the anaerobic conditions inside the tubs used during fermentation.

Fig. 9.18 Photograph of tubs for *nuka-zuke* fermentation

9.7.2.4 *Nuka-Zuke*

Rice bran pickles called *nuka-zuke* are produced by the aging of salted fish with rice bran at ambient temperatures for over 6 months (Fujii 1992). Common fish used for *nuka-zuke* are mackerel, sardine, herring, and others. The traditional preparation of *nuka-zuke* is as follows: using whole fish, the scales and internal organs are removed, the fish is cured in saturated saline for approximately 1 week, the fish is washed with water, rice bran is packed into the fish abdomen, and it is fermented in a tub for an additional 6 months, at minimum (Fig. 9.18). To consume the final product, the rice bran that adheres to the fish is removed. The salt concentration is approximately 10 %. The organoleptic features of *nuka-zuke* are salty and strong *umami* qualities and preferably a (sometimes unique) fishy odor with rice bran flavor. In some districts, *nuka-zuke* is used as an ingredient in soup, because it can provide the salt. Recently, *nuka-zuke* production has increased dramatically with the promotion of the consumption of locally produced foods in Japan. The scale of this industry is large, valued at approximately 6 million dollars in Japan.

Like fish sauce fermentation, fermentation of *nuka-zuke* involves the digestion of fish materials using self-digestion enzymes and microorganisms. The accumulation of amino acids and peptides related to the typical *nuka-zuke* taste is attributed to the self-digestion enzymes secreted from fish material and rice bran. Halophilic lactic acid bacteria, mainly *T. halophilus*, are the dominant bacteria during fermentation in these foods (Kosaka and Ooizumi 2012). The bacteria play an important role in production of aromatic components during fermentation, though their ability to digest fish material and produce amino acids using enzymes is not significant (Kosaka et al. 2012). The basic quality of *nuka-zuke* is stable as long as the salt concentration is appropriate. Furthermore, the accumulation of biogenic amines, including histamine and tyramine, sometimes occurs during *nuka-zuke* production. Certain tetragenococcal strains are histamine producers (Satomi et al. 2012).

Unusual raw materials are used for *nuka-zuke* in some regions, such as the pufferfish ovaries, called *fugunoko-nuka-zuke* (fermented pufferfish ovaries in rice bran). Pufferfish ovaries contain a deadly poison, tetrodotoxin, so detoxification is an important step. Therefore, the fermentation period lasts up to 4 years. The predominant bacteria are *Tetragenococcus* spp., similar to other rice bran pickles. Since all *nuka-zuke* products contain a large amount of salt (more than 10 %), the fermentation properties are similar to those of salted-type products. During fermentation, lactic acid accumulates in a tub with fish and rice bran, which can result in the proliferation of halophilic lactic acid bacteria (Kobayashi et al. 1995).

9.7.2.5 *Fushi*

Katsuobushi (boiled, smoke-dried, and molded skipjack tuna) is a representative *fushi* product made from skipjack tuna. It is an indispensable ingredient in Japanese cuisine. The history of *fushi* production is very old; prototypes of the products have been dated to about 1500 years ago based on an ancient document. The present form of the molded products is very similar to those that were developed about 300 years ago in Japan (Fujii 1992). Interestingly, similar fermented foods are produced in the Maldives; the origin is unclear. The product is solid, has a low moisture content (approximately 15 % or less), and is extremely hard, causing dryness with protein vitrification (Fig. 9.19). In addition to bonito, mackerel and its relatives are used as raw materials. The production of *fushi* is currently estimated at 90,000 tons per year, of which 32,000 tons is

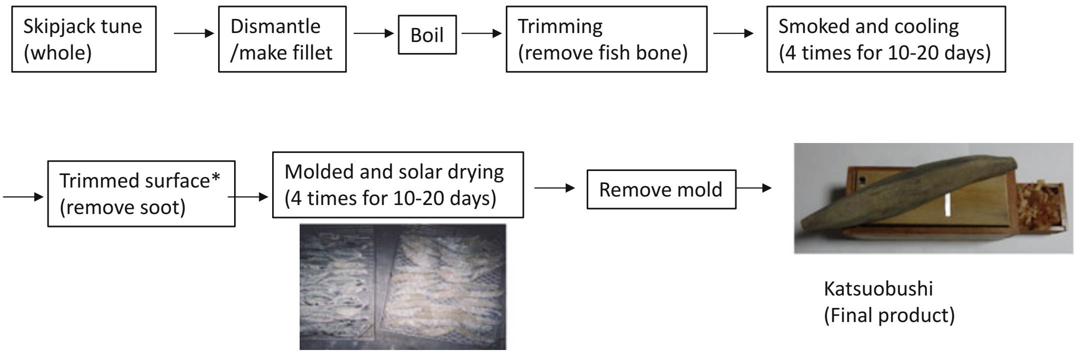

Fig. 9.19 Procedures of ktsuobushi processing in traditional method. * when manufactures does not carry out molded step, smoke-dried fillet is final products

dried bonito in Japan (Ministry of Agriculture, Forestry and Fisheries 2012). *Fushi* including *katsuobushi* is sometimes used for soup stock, rather than food; it is sliced into thin sections and the taste and aromatic components are extracted by boiling water. Soup extracted from *katsuobushi*, called *dashi* in Japanese, contains many aromatic components and a large amount of inosinic acid that enhances the taste of glutamate (Fujii 1992). Since the salt concentration is very low (<1 %), *dashi* alone does not provide a clear taste. However, *dashi* with glutamate and salt is a strongly preferred taste with *umami*. As described in Fig. 9.19, a smoking procedure is involved in processing. The unique aromatic flavor of *katsuobushi* is derived from phenolic compounds that result from the smoking step. A unique final step in *katsuobushi* processing is molding using various *Aspergillus* species, though dried fish without the molding step are also produced as a commercial item in Japan. The purpose of this step is to reduce the lipid and moisture contents of the products, which suppresses the risks of lipid oxidation and microbiological spoilage caused by high water activity. The strains used in the molding step are able to degrade lipids, but have no proteolytic activity. The molding step can make *dashi* taste more sophisticated, refined, and sharp compared with simple dried fish prepared without molding. As was mentioned above, since the materials have already been boiled, dried, and smoked before being molded, the risk of bacterial degradation is low and the fermentation is unremarkable. However, products that are treated by molding during the final processing step are regarded as fermented seafoods.

9.8 *Sake*: Rice Wine

9.8.1 Outline

The first meaning of "*sake*" given by many Japanese dictionaries is "a general term for alcoholic beverage." The second is "a transparent beverage brewed from rice by a Japanese original method." But, we use the second meaning of *sake* in most cases in present Japan. I think that this situation occurred because *sake* has continued to be a typical alcoholic beverage in Japan through many years. In other countries, they prefer the second meaning of *sake*. The *sake* of the second meaning is described in this section.

Sake is suited to be enjoyed with dishes rather than by itself. Many Japanese like to eat and drink in a Japanese tavern, "*izakaya*," where they can eat various cooking including home cooking and drink various beverages including not only *sake* but also other alcoholic beverages and soft drinks. In contrast to a pub in England, an *izakaya* attaches great importance to dishes in general. This interesting form of restaurant, *izakaya*, is considered to be produced by the characteristic of *sake*. *Sake* is often heated before drinking because its aroma and taste change according to temperature. It is called "*kan*." The Japanese have several special terms for *kan*, such as "*hitohada-kan*" (at about 35 °C; body temperature) and

Table 9.5 Technical terms used in *sake* brewing (Ishikawa 2002)

Technical term	Meaning
Toji	The master of a sake brewery. The worker is called "*kura-bito*"
Koji (rice *koji*)	Steamed rice in which *koji* mold, *Aspergillus oryzae*, is cultured
Moto	A yeast mash made from a nutritious mixture of rice, *koji*, and water. It is also called "*syubo*" that means "mother of *sake*." *Moto* has high acidity to increase the yeast content. There are some kinds of *moto*, such as *ki-moto*, *yamahai-moto*, and *sokujo-moto*, which are made by different methods
Moromi	A mixture of *moto*, *koji*, steamed rice, and water
Shikomi	The process of mixing materials
Shibori	The process of filtering *moromi* after cultivating
Hi-ire	The process of sterilizing *sake* by heating. A trouble that a stored *sake* is decayed is called "*hi-ochi*"

"*atsu-kan*" (at about 50 °C). "*Hiya*" means the room temperature. In recent Japan, persons who enjoy "*hana-bie*" (at about 10 °C; temperature at the cherry blossom) or "*yuki-bie*" (at about 5 °C; temperature on snow) are increasing. *Sake* has been a central player of Japanese food culture for a long time and will remain so in the future.

9.8.2 Technical Terms in *Sake* Brewing

Several technical terms are used in *sake* brewing, and some of them are also used in the general public of Japan because *sake* has been loved by the Japanese for a long time. The typical terms are summarized in Table 9.5.

9.8.3 Method of Preparation

Sake is brewed from rice. Beer is also brewed from grains but there is a great difference in brewing processes between *sake* and beer. In the case of beer, alcoholic fermentation starts after saccharification finishes. In the case of *sake*, saccharification and alcoholic fermentation progress simultaneously as shown in Fig. 9.20. In more detail, amylases from *koji* convert rice starch into glucose and yeasts convert the glucose into alcohol and carbon dioxide in the same container. Therefore, the glucose concentration during brewing of *sake* changes more slowly than beer. "*Sandan-jikomi*" is the standard in *sake* brewing. In this method, materials are added into *moto* three separate times. It was developed for the purpose to keep the yeast content and not to increase undesirable bacteria.

If enzymes are active when *sake* is stored, a sweetness and an unpleasant smell gradually increase (Ishikawa 2002). If a kind of lactic acid bacterium, "*hi-ochi-kin*," increases, *sake* becomes cloudy. Therefore, *sake* is usually heated to about 60 °C to deactivate enzymes and to pasteurize before maturing. This process is called "*hi-ire*."

Some people think *sake* "the fresher, the better" because some recent advertisements frequently use "*sibori-tate*" that means fresh. But, maturing is an important process for *sake* as well as other alcoholic beverages. To add a calm, smooth, and mild taste to *sake*, it is usually stored in a tank for less than 1 year. The *sake* that was stored for more than 1 year is called "*chouki-chozo-shu*," which has more unique aroma and taste.

9.8.4 Microorganisms

There is a proverb, "First *koji*, the second *moto*, and the third *tsukuri*." This expresses important processes for making *sake*, that is, making *koji* (Fig. 9.21, left) is the most important, making *moto* (Fig. 9.21, right) the second, and making *moromi* the third. In making *sake*, controlling microbes is essential. *Koji* mold, *A. oryzae*, is used for making *koji* (Ishikawa 2002). The Brewing Society of Japan authorized *koji* mold as the national microbe of Japan in 2006 (Brewing Society of Japan 2006). A good *koji* has high enzyme activities, that is, a great amount of

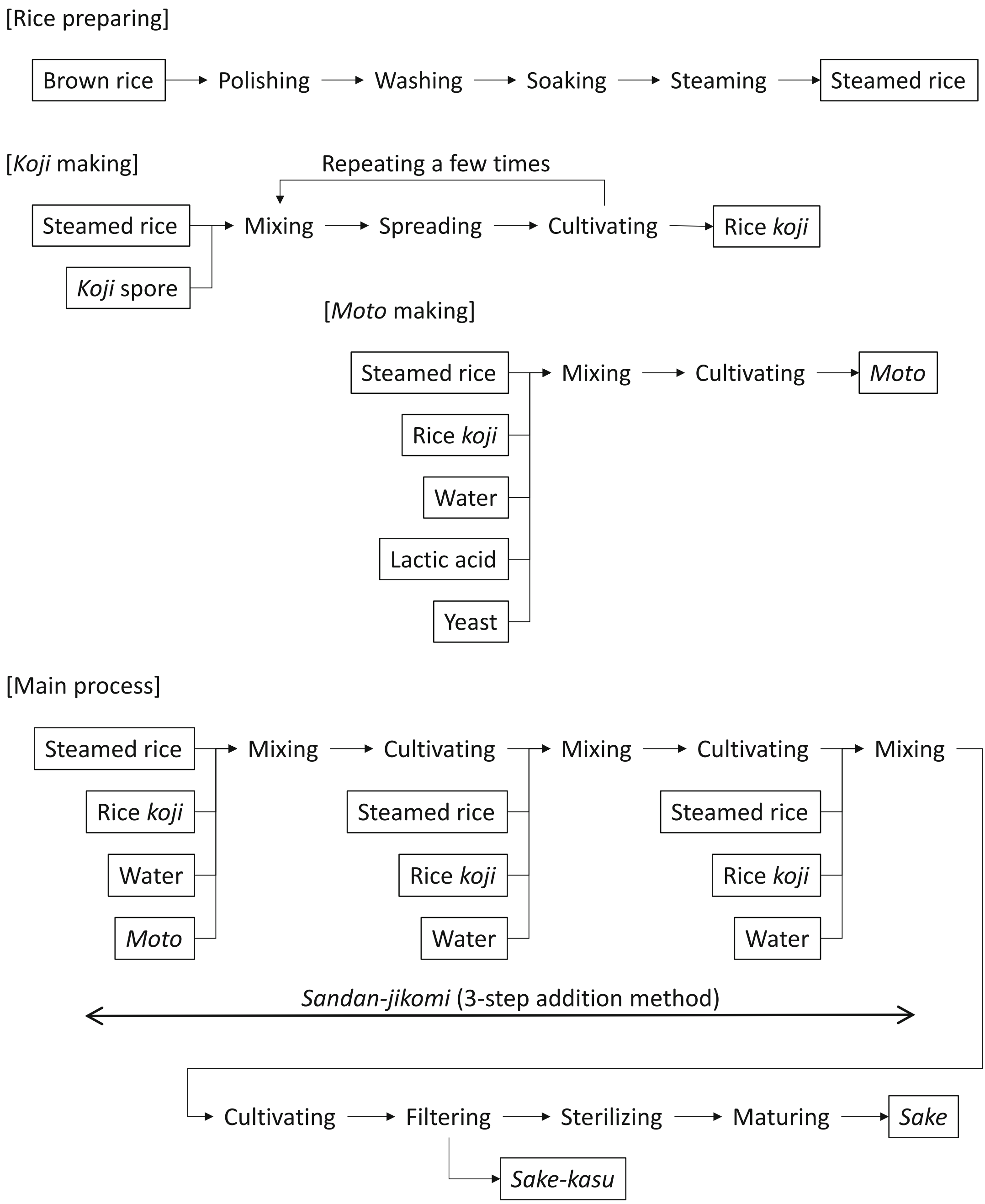

Fig. 9.20 A process for making *sake*

amylases and proteases are accumulated in *koji*. For making a good *koji*, *toji* takes extreme care to prepare the steamed rice because it is a medium of *koji* mold and has a considerable effect on the final *koji*. To control the water content of rice with an accuracy of 1 %, washing rice, soaking rice in water, and straining water from rice are precisely carried out by all the *kura-bitos* in accordance with *toji*'s cues in a traditional *koji* making.

Fig. 9.21 Photographs of making *sake*. The left and right images show the making of *koji* and *moto*, respectively. This *sake* brewery attaches importance to a traditional and handmade method (Courtesy of INABA SHUZOU, Tsukuba, Japan)

In making *moto*, yeast, *S. cerevisiae*, is cultured in an acidic medium including water, *koji*, and lactic acid (Ishikawa 2002). Lactic acid is added to prevent the increase of undesirable microorganisms. The *moto* made by this method is called "*sokujo-moto*." Before developing *sokujo-moto*, a traditional *moto*, "*ki-moto*," was used. The method to make *ki-moto* needs more time and labor. By this method, however, undesirable microorganisms are skillfully removed by the action of nitrate-reducing bacteria and lactic acid bacteria. The method is reasonable from the point of the present biology. Several *sake*s made from *ki-moto* are on the market recently because they are expected to have complex aroma and taste produced by many kinds of microorganisms (National Research Institute of Brewing, Japan 2007).

9.8.5 Nutritional Composition

At the point of nutritional composition, *sake* is distinctive among alcoholic beverages. Table 9.6 shows the contents of protein, carbohydrate, and minerals contained in *sake*, *shochu*, beer, wine, and whisky. *Sake* contains protein and carbohydrate more than the other alcoholic beverages (Ministry of Education Culture, Sports, Science and Technology, Japan (2014) Food Composition Database). This indicates that *sake* has a richer taste and is closer to soup than the other alcoholic beverages.

Table 9.6 Comparison of nutritional components among alcoholic beverages (Food Composition Database 2014)

	Protein (g/100 g)	Carbohydrate (g/100 g)	Mineral (g/100 g)
Sake (*junmai-ginjo*)	0.4	4.1	0
Shochu (*tan-shiki*)	0	0	0.1
Beer (pilsner)	0.3	3.1	0
Wine (red)	0.2	1.5	0.3
Whisky	0	0	0

9.8.6 Health Risks and Benefits

The World Health Organization indicates many risks increased by alcohol, especially heavy episodic drinking (World Health Organization 2014). The risks include health consequences for drinkers, socioeconomic consequences for drinkers, harms to other individuals, and harms to society at large. For example, harms to other

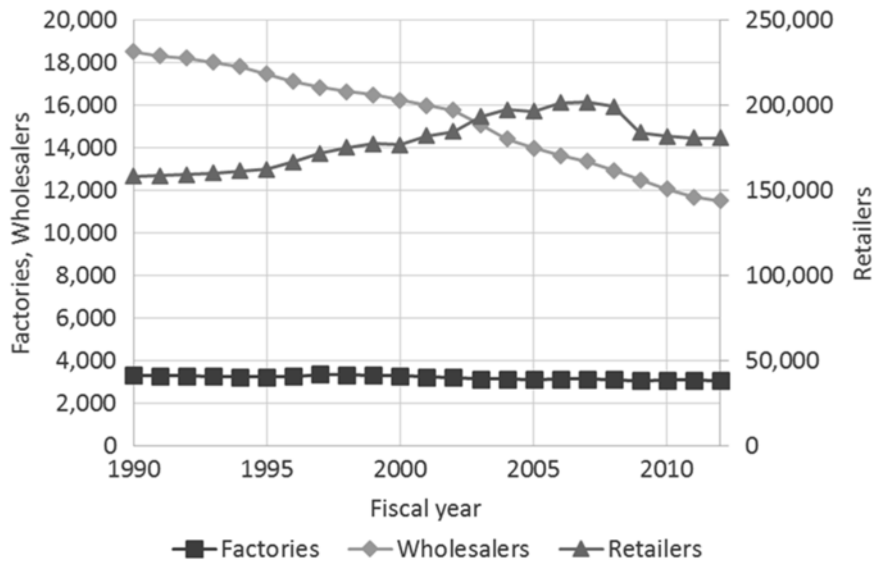

Fig. 9.22 A trend of the numbers of factories, wholesalers, and retailers of alcoholic beverages licensed by NTA. The graph is drawn based on a statistical report by NTA (National Tax Agency, Japan 2014)

individuals can be intentional, e.g., assault or homicide, or unintentional, e.g., a traffic crash, workplace accident, or scalding of a child. If alcoholic beverages including *sake* are appropriately drunk, however, many persons really feel some benefits such as increased appetite and relaxation and improvement of human relations. In addition, moderate drinking reduces the risk of ischemic heart disease statistically (Roerecke and Rehm 2012). It is recently reported that some components included in *sake* are related to control of hypertension, prevention of diabetes, skin whitening, and so on (Imayasu 1999; Imayasu and Kawato 1999a, b).

9.8.7 Ethnical Value and Socioeconomy

Most of the productions of alcohol beverages in Japan were recorded in comparison with other countries because of a tax on alcohol (World Health Organization 2014). In consequence, various statistical data about alcoholic beverages published by the National Tax Agency (NTA), Japan, are available and reliable. A tax on alcohol played a critical role in modernization of Japan (National Tax Agency, Japan 2014). The ratio of a tax on alcohol to the total of national taxes was higher than 30 % in fiscal year 1902. The ratio gradually decreased with industrialization and it became lower than 3 % in fiscal 2012. The amount of a tax on alcohol was 1350 billion yen, whereas those of an income tax, a corporate tax, and a tax on tobacco were, respectively, 13,993 billion yen, 9758 billion yen, and 1018 billion yen in fiscal 2012.

NTA licenses corporations for making or selling alcohol beverages. The number of licensed factories has not markedly changed after fiscal 1990 as shown in Fig. 9.22 (National Tax Agency, Japan 2014). The number of licensed wholesalers has decreased constantly from fiscal 1990 to fiscal 2012. The number of licensed retailers peaked at 201,874 in fiscal 2007. The categories of stores at which Japanese buy alcohol beverages have changed drastically only in about 20 years as shown in Fig. 9.23 (National Tax Agency, Japan 2006, 2010, 2014). More than 80 % of alcohol beverages were sold by ordinary liquor stores in fiscal 1990. In this 20 years, the ratio of the volume sold by liquor stores has decreased rapidly. In contrast, supermarkets and mass retailers have expanded their powers during the same period.

Fig. 9.23 Compositions of retailers in different business categories. The graph is drawn based on statistical reports by NTA (National Tax Agency, Japan 2006, 2010, 2014)

9.8.8 Consumption

The consumption of *sake* shows a chronic tendency to decrease in Japan as shown in Fig. 9.24 (National Tax Agency, Japan 2014). The ratio of *sake* to the total alcoholic beverages was more than 70 % in fiscal 1970 but that was about 6 % in fiscal 2012 (National Tax Agency, Japan 2014). There are similar tendencies of wine in France and Italy and beer in Germany and Belgium (World Health Organization 2014). It is socio-economically interesting that the consumption of many traditional alcohol beverages in the original countries has been decreasing in common. But, it is a serious fact for the *sake* industry in Japan.

To overthrow the present situation, many persons involved in sake are continuing their efforts, for example, species improvement of rice for sake; selection of excellent yeast for making sake from flowers, etc.; development of new kinds of sake such as "chouki-chozo-shu" and sparkling sake; training sommeliers for sake, "kikisake-shi"; and reducing or eliminating the trade barriers by international negotiations. The export of sake from Japan has been increasing slightly but steadily for some years (National Tax Agency, Japan 2014).

9.9 *Shochu* and *Awamori*: Spirits

9.9.1 Outline

Shochu is produced through continuous distillation or single distillation. Singly distilled *shochu* will be discussed in this section. *Shochu* is an alcoholic drink made from the distillation of "*moromi*," the product of the fermentation of *koji*, and other base ingredients such as sweet potatoes or grains. Depending on the base ingredients used, the different varieties such as sweet potato *shochu*, barley *shochu*, and rice *shochu* are each made in Kagoshima/Miyazaki Prefectures, Oita Prefecture, and Kumamoto Prefecture, respectively. *Shochu* products containing 25 % alcohol are generally sold in the market. The annual consumption of *shochu* is 468,000 kl in 2014 (Jyokai Times 2015). Of this, sweet potato *shochu* comprises 208,000 kl, and barley *shochu* comprises 193,000 kl, which together make up 85 % of the total amount (Jyokai Times 2015). *Shochu* can be drunk in many ways such as straight, on the rocks, or mixed with about 50 % hot or cold water. It is generally drunk during meals. *Awamori* is a type of *shochu* that is made from the base ingredients of just black *koji* and water

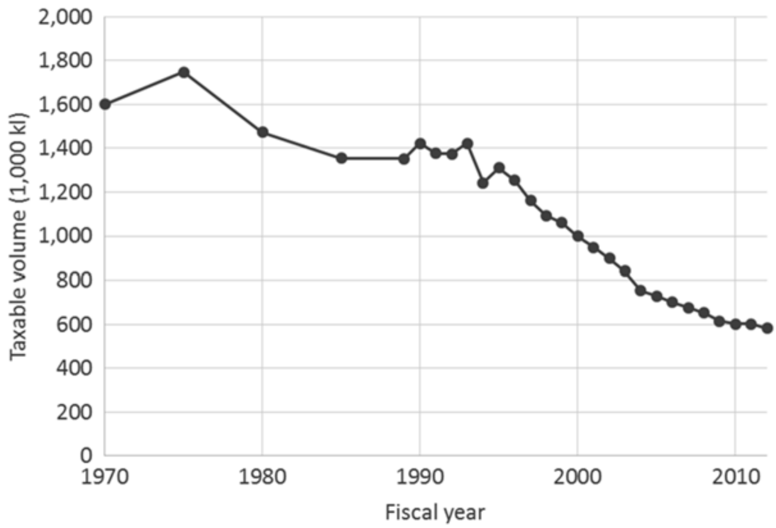

Fig. 9.24 A trend of taxable volume of *sake* in Japan. The graph is drawn based on a statistical report by NTA (National Tax Agency, Japan 2014)

and is produced in Okinawa Prefecture. *Awamori* has a longer history than *shochu*, and each year 23,000 kl is consumed (Jyokai Times 2015). *Awamori* that is stored in earthenware pots for long periods of time and allowed to mature after distillation is called "*kusu*" and is characterized by a gorgeous vanilla aroma.

9.9.2 Microorganisms Involved in *Shochu* Production

Koji mold, a kind of *Aspergillus* fungus, is classified into three types depending on the color of the conidia: yellow (*A. oryzae*, Photo 9.1 top), black (*A. luchuensis*, Photo 9.1 left), and white (*A. kawachii*, Photo 9.1 right) (Table 9.1). Yellow is used in the production of *sake*, *miso*, and soy sauce; black and white are used to make *shochu*. Black and white are characterized by producing citric acid and acid-resistant enzymes. Yellow was used in *shochu* production until 1910, but because it was difficult to keep *moromi* at low temperatures in the southern Kyushu region (the main production region of *shochu*), putrefaction was common. In 1910, Gen-ichiro Kawachi succeeded in isolating the citric acid-producing black *koji* mold from *moromi* used for the production of Okinawan *awamori*. The use of this *koji* lowered the pH of the *moromi*, thus preventing putrefaction and increasing the yield by 20–30 % (Kagoshima prefecture *sake* brewers cooperative 1940). Black *koji* then spread to all regions of Kagoshima Prefecture by 1919. In 1918, white *koji* mold was isolated as a variant of black and was highly evaluated for producing *shochu* with a soft aroma and taste, which led most makers to use the *koji* after 1945. For this reason, black became unpopular for a time, but as the demand for unique sweet potato *shochu* grew, in the 1980s, it underwent a revival. Black *koji* had been long used in the production of *awamori*.

The pH of the primary *moromi* is around 3 and the fermentation temperature sometimes exceeds 35 °C. For this reason, the yeasts used in *shochu* must have superior acid resistance and thermostability. All yeasts used in *shochu* production belong to *S. cerevisiae* (Table 9.1).

9.9.3 Base Ingredients and Processing of Ingredients

Any base ingredients may be used for the production of *shochu* except for the ingredients stipulated by the Liquor Tax Act (grain that has been allowed to germinate, fruits, and sugar). Rice and barley are mainly used as the *koji* base ingredients. The main base ingredients include a wide variety of foods such as sweet potatoes, brown sugar, rice, barely, milk, and green tea. The base ingredients of *awamori* are rice *koji* made from rice and black *koji*.

Fig. 9.25 Production process of shochu

9.9.4 The Production Process of *Shochu* and *Awamori*

Authentic *shochu* is produced through two-step fermentation, in which the *koji* (primary fermentation) and base ingredients (secondary fermentation) are fermented stepwise. This production method was established in 1914 in Kagoshima and had spread to all regions of Japan by around 1940. The production process of *shochu* and fermentation mixture formulation are shown in Fig. 9.25 and Table 9.7, respectively. *Awamori* does not undergo the secondary fermentation stage.

9.9.5 *Koji* Production

The production of *shochu* starts with the creation of *koji*. The base ingredients are mainly rice or barley. These ingredients are washed and steamed, and then *koji* spores are sprinkled on these base ingredients (a process called *tanetsuke*), which are then left to sit for about 2 days to allow the *koji* mold to grow. After *tanetsuke* is performed, the temperature of the *koji* is maintained at 37–38 °C for 26–28 h. During this period, the *koji* is grown to sufficient levels to produce enzymes necessary for the production of *shochu*. Then temperature is controlled to approximately 35 °C, at which the *koji* can produce citric acid, which is essential for the production of *shochu*. The *koji* is ready approximately 43 h after *tanetsuke*. The role of *koji* is to (1) produce acid-resistant enzymes that will break down starch, proteins, and lipids; (2) produce citric acid that will inhibit the growth of unwanted bacteria, ensuring safe fermentation; and (3) create the flavor of the *shochu*. The production of citric acid is particular to *koji* used in the making of *shochu*, in contrast to yellow *koji* (*sake koji*, *miso koji*, soy sauce *koji*, etc.)

9.9.6 Primary Fermentation Stage

Because the main production regions of *shochu* are located in warm areas, there is a high risk of contamination by unwanted bacteria such as acid-forming bacteria. However, because the citric acid in the *koji* lowers the pH of the *moromi* to around 3, the growth of unwanted bacteria can be inhibited. The primary fermentation uses *koji* and water, and then yeast is added and saccharification and fermentation occur at the same time over a 5–6-day period at about 30 °C, thus allowing the yeast to sufficiently grow to create the primary *moromi*. In the case of *awamori* production, distillation is performed after primary fermentation has continued for about 20 days.

9.9.7 The Secondary Fermentation Stage

Secondary fermentation is the process of adding base ingredients and water to the primary *moromi*. The base ingredients of sweet potatoes, brown sugar, or barley are used as the *moromi* for sweet potato *shochu*, brown sugar *shochu*, and barley *shochu*, respectively. The secondary *moromi* has a pH ranging from 4 to 4.5 and thus is environment suitable for the growth of unwanted bacteria, but because the yeast concentration of the *moromi* ranges from 4 to 8×10^7/g immediately after fermentation and the produced alcohol concentration is approximately 4 %, fermentation occurs without the contamination of unwanted bacteria. The fermentation temperature is approximately 25 °C and is not allowed to exceed 32 °C. The secondary *moromi* is created after

Table 9.7 Fermentation mixture formulation of *shochu* and *awamori* (Kayashima et al. 1991)

Type	Raw material	Primary fermentation stage	Secondary fermentation stage	Total
Sweet potato *shochu*	Rice *koji* (kg)	100	–	100
	Sweet potato (kg)	–	500	500
	Water (L)	120	280	400
Rice *shochu*	Rice *koji* (kg)	100	–	100
	Rice (kg)	–	200	200
	Water (L)	120	360	480
Awamori	Rice *koji* (kg)	100	–	100
	Water (L)	170	–	170

10–14 days have passed and the alcohol level ranges from 14 % to 18 %.

9.9.8 The Distillation Stage

After the secondary *moromi* has finished the fermentation process, single distillation is performed in a pot still to create unrefined alcoholic drink ranging from 36 % to 45 % alcohol. There are two types of single distillation pot still: atmospheric distillation pot stills, which are used for sweet potato and brown sugar *shochu* and *awamori*, and vacuum distillation pot stills, which are used for grain-type *shochu*, such as barley and rice *shochu*. The distillation equipment is generally made from stainless steel, but may also be made from iron, bronze, or wood.

Atmospheric Distillation: This method generally uses steam that is directly blown into the *moromi*. With regard to distillation time, steam is blown into the *moromi* for about 30 min until *shochu* is produced, and the amount of steam is then controlled so that the distillation process is completed about 180 min later. At the end of distillation process, the alcohol level of distilled liquid ranges from 8 % to 10 %. Part of the taste and aroma of *shochu* is produced through thermal reactions during the distillation process.

Vacuum Distillation: This method of distillation was introduced in the 1970s. *Shochu* produced through vacuum distillation is highly regarded for its softness. The *moromi* is distilled in a vacuum maintained at approximately 100 Torr and kept at a temperature ranging from 40 to 50 °C.

9.9.9 Maturation Stage

The *shochu* is matured in stainless steel containers, earthenware jars, or oak barrels. Immediately after the distillation process, the distilled alcohol generally has an irritating odor (gas odor) and harsh taste and is clouded by the oily components; therefore, it is necessary to eliminate the gas odor and filtrate the oily components during storage. The gas odor is mainly composed of aldehydes and sulfur compounds. The oily components are mainly composed of chemicals such as ethyl linoleate and ethyl palmitate. Sweet potato *shochu* is allowed to mature for 2–4 months, and other types of *shochu* and *awamori* are generally matured for 1 year or longer. After the unrefined alcohol is filtered, matured, and blended, it is generally mixed with water and sold as 25 % alcohol drinks.

9.9.10 The Ingredients and Flavor of *Shochu* and *Awamori*

Besides ethanol and water, authentic *shochu* includes other ingredients such as higher alcohols, fatty acid esters (oily components), volatile organic acids, and minerals, but these ingredients only comprise approximately 0.2 % of the total volume. However, these trace constituents have an important meaning for the production of *shochu* and *awamori*. The differences in flavor depending on the type of *shochu* (sweet potato *shochu*, brown sugar *shochu*, etc.) are all due to these trace constituents. The oily components soften the strong taste of the *shochu*, making it mild, and thus are essential in contributing to the physical taste of the *shochu*.

The characteristic aroma of sweet potato *shochu* is reported to be created through monoterpene alcohols such as linalool, α-terpineol, citronellol, and geraniol, isoeugenol, rose oxide, and β-damascenone (Ota et al. 1990; Kamiwatari et al. 2005; Takamine et al. 2011). However, very little of these ingredients are included in rice or barley *shochu*. Monoterpene alcohols and β-damascenone are said to possess healing effects, and these ingredients that are particular to sweet potato *shochu* are thought to be important for creating a relaxing feeling after an evening drink of sweet potato *shochu*. Mixing with hot water especially brings out the aroma, sweetness, and healing effects of sweet potato *shochu*. When the maturation period of *awamori* becomes longer, the vanilla aroma of the drink becomes stronger. This is due to the ferulic acid esterase produced from *koji* that liberates the ferulic acid from the cell walls of the rice during the fermentation process. During the distillation process, it is converted into 4-vinylguaiacol and then into vanillin during the maturation stage.

9.9.11 Drinking *Shochu* Increases Thrombolytic Activity

Figure 9.26 shows a fibrinolytic activity in plasmin. One hour after intake of five types of

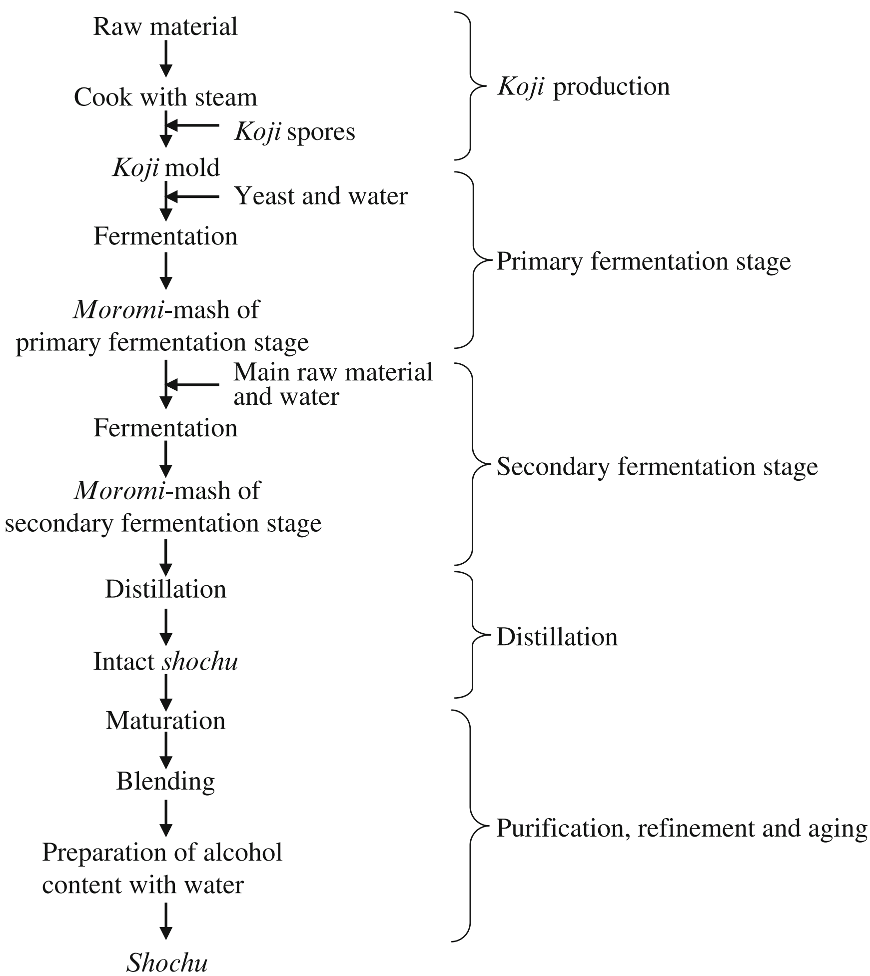

Fig. 9.26 Effect of alcohol beverages on fibrinolytic activity

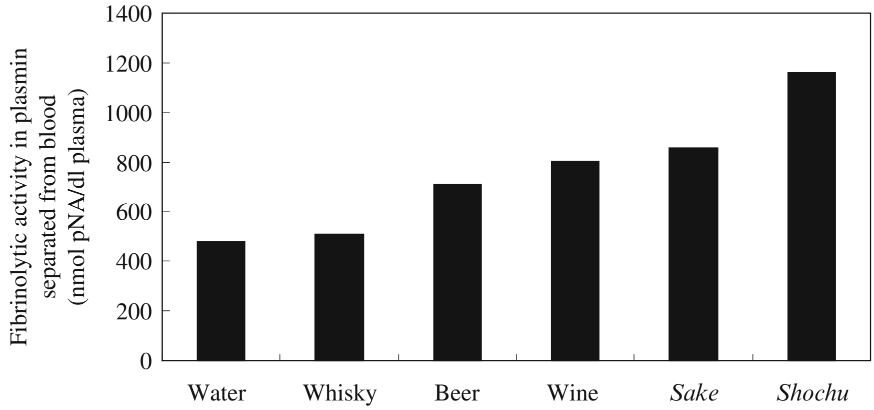

Photo 9.1 Three types of *koji* for *shochu* and *awamori*

alcohol beverages, plasmin was separated from blood and subjected to enzyme assay. The results show that when *shochu* was drunk, a significant increase in fibrinolytic activity was noted.

From its specific molecular weight, substrate specificity, and immunological properties, the enzyme was thought to be urokinase-type plasminogen activator and its precursor (Sumi 2001).

9.10 Conclusion

Japanese cuisines have evolved to make eating cooked rice more enjoyable. Fermented foods play a central role in Japanese taste and have become indispensable to Japanese food culture. In these days, not only for the importance as the taste of Japan, these fermented foods also get the attention of Japanese consumers for their health-promoting functions, e.g., prevention of radiation injury, cancer, and hypertension with *miso* (Watanabe 2013; Watanabe et al. 2006); angiotensin I-converting enzyme inhibitory peptides in *shoyu* (Nakahara et al. 2010); activation of fibrinolytic system by nattokinase (Sumi 1991); alleviation of hypertension by *natto* (Kim et al. 2008); enhancement of NK-cell activity and improvement of bowel symptoms by so-called plant-derived LAB in *tsukemono* (Takii et al. 2013); promotion of intestinal absorption of calcium (Kishi et al. 1999) and reduction of body weight, body fat mass, and serum triglyceride levels (Kondo et al. 2009) by *su*; and so on.

The manufacturing skills required for Japanese characteristic fermented foods have been handed down, including a lot of improvements to build up the present style, from generation to generation for several hundred years or even more than a thousand years by masters in manufacture or homemakers at home. In the recent time, development of microbiology and incipient biotechnology and industry made it possible to produce various fermented foods stably in large scale. However, small numbers of consumers choose the products made by traditional small-scale manufacturers, because those of mass production tend to be less characteristic. This may be why as many as about 1500 *shoyu*, 1000 *miso*, and 1500 *sake* factories continue to produce their products, although the numbers are in decline.

It is expected hereafter that the fermentation process whereby microorganisms produce many desirable properties from various raw materials in traditional fermentation procedures can be elucidated in detail by the application of advanced technology such as omics analysis that can also lead to the production of a new generation of Japanese traditional fermented foods, having characteristics such as being rich in remarkable tastes and flavors, having more health-promoting functions, being adaptable to the preference of worldwide people, being adaptable to Halal certification, etc.

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Ethnic Fermented Foods and Beverages of Cambodia

10

Sota Yamamoto

10.1 Introduction

Cambodia occupies an area of approximately 180,000 km^2 in the South-Central Indochina peninsula and belongs to Monsoon Asia. The landscape of Cambodia is characterized by a low-lying central plain that is surrounded by uplands and low mountains. The drainage systems of the Mekong and the Tonle Sap Rivers join near Phnom Penh before entering the Vietnamese delta. Cambodia is a multiethnic society of Khmer and non-Khmer ethnic groups, such as Khmer Khe (Khmer Khork), Suoy, Kuy, Brao, Krung, Kravet (Kavet), Lun, Tampuan, Phnong, Kachok (above ethnic groups belong to the Mon-Khmer language family), Jarai (Austronesian), and Lao (Tai-Kadai), mainly living in the north-east part of Cambodia (Center for Advanced Study 2009). Because of the diverse natural environment and ethnic groups, there are many fermented foods and beverages made from rice, fish, palm, etc., in Cambodia. Among these fermented foods and beverages, fermentation starters (*koji* cakes) in the form of a hard ball made from rice are notable for their variations.

10.2 Fermentation Starters

10.2.1 Local Names and the Basic Production Process

Local names for fermentation starters in Cambodia are shown in Table 10.1. Khmer people called starters "(*mae*) *dombae*" or "*mae sra*" (meaning a starter of liquor), and Khmer Khe also called them "*dombae*." Brao, Krung, Kravet, Lun, and Tampuan called them "*buh*" or "*puh*," which are very similar to the local names used by the Katu, Ngeh, Ta'oi, and Alak ("*bu*" or "*pu*") in Laos (Yoshida 1993). These local names seem common for Mon-Khmer people in the Annam Mountains. Local names, such as "*praa*" in Kachok, "*pooy*" in Jarai, and "*paeng*" in Lao, seem to be cognates, but the others ("*krrow*" in Suoy and "(*d*)*rry*" in Phnong) are of unknown origin. There are many variations of fermentation starters in Cambodia (Fig. 10.1). The smallest one is 3 cm in diameter and 1.9 cm in thickness, and the biggest one is 16 cm in diameter and 6.3 cm in thickness. Oblateness varies from 0 to 0.7.

Some Khmer people started to produce starters just after the Pol Pot era of 1975–1979 (Yamamoto and Matsumoto 2011). This was either because they could not produce them during that period or they tried to make money by producing them after that period. In general, Khmer people initially produce starters mainly for the purpose of selling them or selling the rice or palm liquor made with

S. Yamamoto (✉)
Research Center for the Pacific Islands, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-8580, Japan
e-mail: sotayama@cpi.kagoshima-u.ac.jp

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them. Some people learn techniques from their parents or relatives, but the others learn them from nonrelatives; some of them are Vietnamese. Some even pay money or gold pieces to learn. Some people have stopped producing fermented starters more than 15 years previously because cheap starters made in Vietnam or China appeared in the markets (Fig. 10.2) or they had other sources of cash income.

Table 10.1 Local names for fermentation starters in Cambodia

Ethnic group	Local names
Mon-Khmer	
Brao	*buh*
Kachok	*praa*
Khmer	(*mae*) *dombae*, *mae sraa*
Khmer Khe	*dombae*
Kravet	*buh*
Krung	*buh*
Lun	*buh*
Phnong	(*d*)*rry*
Suoy	*krrow*
Tampuan	*buh*, *puh*
Austronesian	
Jarai	*pooy*
Tai-Kadai	
Lao	*paeng*

Unlike the Khmer, minority people have been producing starters for many years. However, some minority people have recently stopped producing starters because of the tedious nature of starter production (especially pounding the rice and plants), difficulty in collecting plants for starters, and easy access to markets where cheap starters are available, although many people complain that rice wine or liquor made with Vietnamese or Chinese starters give them bad headaches or stomach problems. Minority people learn the techniques of production from their parents or relatives. The production of fermentation starters among minority people is typically for the purpose of home consumption of rice wine or liquor.

The basic production process of fermentation starters in Cambodia is shown in Fig. 10.3. The process is classified into the following four types, depending on whether rice liquor and old starters are used (Yamamoto and Matsumoto 2011). Plants used in the process are shown in Table 10.2.

Fig. 10.1 Many kinds of homemade starters collected in Cambodia (the length of the left pen is 15 cm)

Fig. 10.2 Starters imported from Vietnam or China are sold in local markets

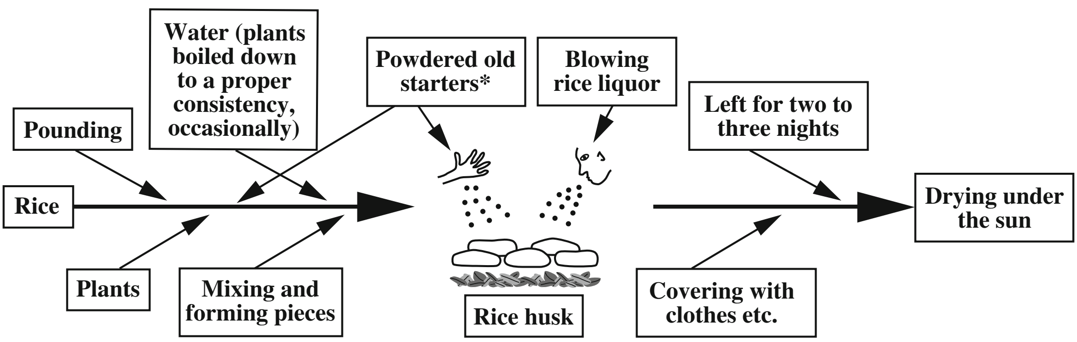

Fig. 10.3 Schematic diagram of the production process of fermentation starters in Cambodia. Type I, use of both rice liquor and old starters; type II, use of old starters without rice liquor; type III, use of rice liquor without old starters; and type IV, no use of rice liquor or old starters. *Only scattering onto the new starters (Subtype 1 of types I and II); only mixing with rice powder (Subtype 2 of types I and II); both mixing and scattering (Subtype 3 of types I and II) (Modified from Yamamoto and Matsumoto 2011)

10.2.2 Type I: Use of Both Rice Liquor and Old Starters

10.2.2.1 Subtype 1: Scattering Old Starters Over New Ones

[Khmer in Banteay Meanchey]

Take 5 kg of non-sticky rice → soak it in water for 10–20 min → pound it → mix the rice powder with dried and pounded plants (*Alpinia* spp., *Capsicum* spp., *Ocimum tenuiflorum*, *Piper lolot* or *Piper sarmentosum*, *Piper nigrum*, *Piper retrofractum* and *Syzygium* spp.) and rice liquor (no water) and form it into pieces (about 50–80 pieces, 10 cm in diameter and 2–3 cm in thickness) → put new starters on rice husks → blow rice liquor onto the new starters → scatter powdered old

Table 10.2 Plants and other materials used for fermentation starters

Local names[a]	Scientific names	Plant parts
khtüm sâ:	*Allium sativum*	Bulb
rum'dé:*ng* (including *rum'dé*:*ng srôk* and *rum'dé*:*ng prei*)	*Alpinia galanga*, *Alpinia* spp.	Tuber, stem
krâva:*nh*	*Amomum kravanh*	Fruit, tuber
**sla*: (Lao)	*Areca catechu*	Root
khnaö(*r*)	*Artocarpus heterophyllus*	Leaf
sdau	*Azadirachta indica*	Bark
mré:*ch tônsa*:*y*	*Baeckea frutescens*	Root
kânh ché ba:*y da*:*ch*	*Capparis micracantha*	Root
mtés	*Capsicum annuum*, *C. frutescens*	Fruit, calyx
**thaloong* (Phnong)	*Carica papaya*	Fresh root
kôor	*Ceiba pentandra*	Dried flower
smau kântroëy	*Chrysopogon aciculatus*	Root
tep tiëru:, *teppiru*:	*Cinnamomum* spp. (*C. cambodianum*, *C. tetragonum*, etc.)	Bark
**maak phao* (Lao)	*Cocos nucifera*	Root
**pea chaa*:*b* (Tampuan) (*trathok prei* in Khmer)	*Costus speciosus*?	Root
smau krâva:*nh chru*:*k*, *smau se*	*Cyperus rotundus*	Root
chë:*ng cha*:*b*	*Dasymaschalon lomentaceum*	Root
ângkât khmau	*Diospyros bejaudi*	Root
phlèang, *phlang*	*Glycosmis pentaphylla*	Root
kôki	*Hopea* spp. (*H. odorata*?)	Root
phka chan', *pôch kak law haw*	*Illicium verum*	Dried fruit
mchul miëhs	*Ixora* spp.	Root
donka:*y*	*Lepisanthes rubiginosa*?	Stem, root, leaf
khvöt	*Limonia acidissima*	Bark
chhë 'aèm, *voër 'aèm*	Mainly *Albizia myriophylla*, including *Cinnamomum cassia*, etc.	Bark, root
smach'	*Melaleuca cajuputi*	Stem
prèah khlâ:*b*	*Mimosa* spp. (*M. pudica*?)	Root
krâvan' (or *rumduël*)	*Mitrella mesnyi*	Stem
nhô	*Morinda* spp.	Root
**phrit* (Phnong), *bhrit* (Brao)	*Musa* spp.	Root, pericarp
law haw (*pôch kak*)	*Myristica fragrans*	Dried fruit
thnam' chuëk	*Nicotiana tabacum*	Dried leaf
mrèahs pröw	*Ocimum tenuiflorum*	Stem, leaf
smau' phluk	*Panicum repens*	Root
**phoo kiau* (Lao)	*Piper betle*	Dried leaf
chaplu:	*Piper lolot* or *Piper sarmentosum*	Root
mré:*ch*	*Piper nigrum*	Dried fruit
déi phléi	*Piper retrofractum*	Dried fruit
rumdenh miëhs	*Prismatomeris tetrandra*	Root
âmpöw	*Saccharum officinarum*	Stem, leaf
kâkâhs	*Sindora siamensis*	Fruit

(continued)

Table 10.2 (continued)

Local names[a]	Scientific names	Plant parts
**yar hua* (Lao)	*Smilax glabra*	Root
**phratandang* (Krung), *chophkrong* (Kravet), *phiapheh phishang* (Kachok)	*Solanum* spp.	Fruit, leaf, root
klam' pu:, *khan' phlu*:	*Syzygium aromaticum*	Bud
chumpu:	*Syzygium jambos* or *S. malaccense*	Heartwood, root, leaf
lolook	The nest of *lolook* (*lolook*: a kind of turtledove)	–
**khua khao hor'* (Lao)	*Tinospora crispa*	–
khnhéi (including *khnhéi tek* and *khnhéi plë:ng*)	*Zingiber officinale*	Tuber
**chonrok* (Krung), *chondok* (Brao), *chondorodorok* (Lun)	Zingiberaceae (*Boesenbergia pandurata*?)	Tuber
chhë plë:ng	Unidentified	Bark
**mboong* (Krung)	Unidentified	Root, stem, leaf
**ngam* (Jarai, Tampuan), *ngamngaam* (Tampuan)	Unidentified (a climbing thorny plant, its stems are sweet)	Stem, root

Modified from Yamamoto and Matsumoto (2011)
[a]Plants used only by the minority people are marked with an asterisk with their local names, and the other plants show only the local names of Khmer, which were referred to in Kham (2004) and Dy (2000)

starters (five to six pieces) → cover with mosquito nets for three nights (a producer would also put the fruits of *Capsicum* spp. and a few pieces of charcoal on and around the mosquito nets to protect them from *app*, a kind of an evil spirit) → dry under the sun for 4–5 days.

10.2.2.2 Subtype 2: Mixing Old Starters with Rice Powder

[Lao in Steung Treng]

Take 3 kg of sticky rice → soak it in water for 2 h → pound it → mix rice powder with dried and pounded plants (*Albizia myriophylla*, *Alpinia* spp., *Areca catechu*, *Capsicum* spp., *Cocos nucifera*, *Nicotiana tabacum*, *Piper nigrum*, *Piper lolot* or *Piper sarmentosum*, *Piper retrofractum* and *Tinospora crispa*), powdered old starters, and water and form it into pieces (with a diameter of 3–4 cm) → put new starters (on rice husks occasionally; husks are not required every time) in flat baskets → blow rice liquor onto the new starters → cover with rice sacks for three nights → blow rice liquor onto the starters again, scatter the ashes of banana leaves, and cover again for three nights → dry under the sun for 2–3 days.

10.2.2.3 Subtype 3: Both Mixing and Scattering Old Starters

[Khmer in Siem Reap] (Fig. 10.4)

Take 10 kg of non-sticky rice → soak it in water for one night → grind it on a stone mortar → pour two kinds of special rice liquor (a gold necklace and a silver coin are soaked in the rice liquor separately) into a broken lump of rice, while saying "*chol meas*, *prak hau meas*, *meas hau prak* (gold enter, silver call gold, gold call silver)" → add plant A* (4.45 kg), while saying "*prei hau srok*, *srok hau prei* (wild call local, local call wild)," old starters (150 g), rice bran (100 g), and sugar (10 g) and form it into pieces (115 big pieces with 8 cm in diameter and 2 cm in thickness, each piece approximately 200 g, and 309 small pieces 3–4 cm in diameter and 2 cm in thickness, each piece approximately 50 g) → spread a mosquito net out, scatter powdered old starters (20 g) and rice husks, and place down the new starters → sprinkle plant A (400 g) on the new starters using one's fingers, spray the most potent rice liquor from the mouth over it several times, and scatter powdered old starters (70 g) → put a few planks across a bed and cover the new starters with mosquito nets for two nights → dry under the sun for 1–3 days.

*Plant A is a mixture of "*chhe prei*" (forest plants) and "*chhe srok*" (local plants). "*Chhe prei*," consisting of *Albizia myriophylla*, *Amomum kravanh*, *Capparis micracantha*, *Cyperus rotundus*, *Diospyros bejaudi*, *Glycosmis pentaphylla*, *Ixora* spp., *Mimosa* spp., *Prismatomeris tetrandra*, and other plants, is boiled down to the correct consistency, and only this liquid is used. "*Chhe srok*," consisting of *Alpinia* spp., *Capsicum* spp., *Chrysopogon aciculatus Illicium verum*, *Panicum repens*, *Piper nigrum*, *Piper retrofractum*, and *Zingiber officinale*, is dried and pounded.

Fig. 10.4 The production process of Khmer in Siem Reap. Preparing plants (**a**, **b**) and a mixture of "*chhe prei*" (forest plants) and "*chhe srok*" (local plants) (**c**). Pouring two kinds of special rice liquor (a gold necklace and a silver coin were soaked in the rice liquor separately) into a broken lump of rice, while saying "*chol meas*, *prak hau meas*, *meas hau prak* (gold enter, silver call gold, gold call silver)" (**d**). Preparing and mixing ground rice and plants (**e**, **f**), spreading a mosquito net out and scattering powdered old starters and rice husks (**g**), placing down the new starters (**h**), blowing rice liquor by mouth (**i**), scattering powdered old starters (**j**), placing a few planks across a bed and covering the new starters with mosquito nets for two nights (**k**), and the products after drying in the sun (**l**)

10.2.3 Type II: Use of Old Starters Without Rice Liquor

10.2.3.1 Subtype 1: Scattering Old Starters Over the New Ones

[Krung in Ratanak Kiri]

Take 1.25 kg of any kind of rice → soak it in water for 30 min → mix the rice with pounded plants (usually fresh, *Alpinia* spp., *Capsicum* spp., *Saccharum officinarum*, *Solanum* spp., and *Zingiber officinale*) and the supernatant solution of *Albizia myriophylla* and form it into pieces (one large piece 7 cm in diameter and 3 cm in thickness and small pieces 4 cm in diameter and 2 cm in thickness) → put the new starters directly in a flat basket → depress the centers of the starters and put one dried *Capsicum* fruit into the big pieces and powdered fruit into the small pieces to make the starters more potent (Fig. 10.5) → scatter the powder of one large piece of old starter over them, and then cover with leaves and branches of "*la thuk*" for two nights → dry under the sun for 1–3 days with some pieces of charcoal and roots of "*chonrok*" (maybe *Boesenbergia pandurata*) to avoid evil spirits.

Fig. 10.5 A *Capsicum* fruit put into a starter of Krung in Ratanak Kiri

Fig. 10.6 Depressing the centers of starters of Kachok in Ratanak Kiri

10.2.3.2 Subtype 2: Mixing Old Starters with Rice Powder

[Kachok in Ratanak Kiri]

Take 2.5 kg of non-sticky rice → soak it in water for 15 min → mix the rice with dried and pounded plants (*Capsicum* spp. and *Solanum* spp.), powder of old starters (three pieces), and water and form it into pieces 5–6 cm in diameter and 3 cm in thickness → put the new starters on rice husks in flat baskets and depress the centers of the starters (Fig. 10.6) → cover them with leaves and branches of "*kadho*" for three nights → dry under the sun and keep them over the fireplace.

10.2.3.3 Subtype 3: Both Mixing and Scattering Old Starters

[Khmer in Kampong Thom]

Take 50 kg of non-sticky rice (a floating rice variety called "*chuong*" is better because of its low price and the hardness of its grain) → soak it in water for 1–2 h → grind it on a stone mortar → mix rice with dried and pounded plants (*Albizia myriophylla*, *Alpinia* spp., *Capsicum* spp., *Illicium verum*, *Nicotiana tabacum Piper nigrum*, *Zingiber officinale*), powder from old starters (50 pieces), and salt water and form it into pieces 4 cm in diameter

→ put the new starters on rice straw, scatter the powder of old starters (500 g), and cover them with cloths for one night → remove the cloths and leave the new starters for one night → dry under the sun for 2 days.

10.2.4 Type III: Use of Rice Liquor Without Old Starters

[Tampuan in Ratanak Kiri]

Take 5 kg of sticky rice (non-sticky rice occasionally) → soak it in water for 30 min → pound rice and fresh and pounded plants (*Albizia myriophylla*, *Alpinia* spp., *Capsicum* spp., *Saccharum officinarum*, charcoal) → mix them with water and form it into pieces 7–8 cm in diameter → put the new starters on leaves and branches of "*tuum kok*" ("*donka*:*y*" in Khmer, maybe *Lepisanthes rubiginosa*), blow strong rice liquor onto them, and cover them with leaves and branches of "*tuum kok*" again for five nights → keep them in the house (no drying under the sun).

10.2.5 Type IV: No Use of Rice Liquor or Old Starters

10.2.5.1 Subtype 1: No Drying of New Starters Under the Sun and Using Leaves and Branches for Covering Starters

[Phnong in Mondul Kiri]

Take 3 kg of non-sticky rice and 2 kg of sticky rice (mixed together) → soak it in water for 30 min → pound rice and dried plant (*Albizia myriophylla*, *Morinda* spp., and charcoal., *Capsicum* spp) together → mix them with liquid from plants (*Albizia myriophylla* and *Morinda* spp. are boiled and cooled) and form it into pieces 4–5 cm in diameter and 3–4 cm in thickness → put new starters on rice husks, put "*kateh*" (cotton) onto the starters while making the wish that mold would grow like cotton on them (Fig. 10.7), and cover them with any leaves for three nights → keep them inside the house (no drying under the sun).

Fig. 10.7 Putting "*kateh*" (cotton) onto the starters of Phnong in Mondul Kiri while wishing that mold will grow on the starters like the cotton

10.2.5.2 Subtype 2: Drying New Starters Under the Sun

[Brao in Steung Treng]

Take 10 kg of any rice (sticky rice is better) → soak it in water for one night → pound rice and fresh and pounded plants (*Alpinia* spp., *Albizia myriophylla*, *Capsicum* spp., *Lepisanthes rubiginosa*, and *Musa* spp.) together → mix them with supernatant solution from *Albizia myriophylla* and form it into pieces 7–8 cm in diameter and 4–5 cm in thickness → put the new starters on rice husks on a mat and pierce the starters to hang them with bamboo threads over the fireplace later → put dried *Capsicum* fruits on the starters to make them potent and smell better and cover them with leaves of *Lepisanthes rubiginosa* for three nights → dry them under the sun for 7 days and keep them over the fireplace.

10.2.6 Two Different Lines of Production Process: "Rice Wine Culture" and "Rice Liquor Culture"

The use of sticky or non-sticky rice is not clearly classified into the four types and seems to be more related to the rice in daily use than starter production techniques. For example, the Khmer usually eat non-sticky rice while the Lao eat sticky rice. However, some people deliberately mix sticky and non-sticky rice to produce starters. It is unknown whether this technique is introduced from somewhere else or originated in the places where it is used. Some people use only broken rice for starter production because normal rice is needed for eating. Many people soak rice in water before pounding it, usually for at least several hours but sometimes for less than 1 h, although some people don't soak the rice in water.

Many Khmer people use old fermentation starters, but some of the minority people do so. It is widely believed that new starters mixed with old ones will ferment more successfully and steadily than starters used without old ones, because old starters may promote the fermentation process. This technique seems to be an advanced technique that may have different origins from the technique that does not incorporate the use of old starters. Kato et al. (2006) studied microflora of Cambodian fermentation starters and reported that amylolytic molds and filamentous yeasts are detected and two molds are identified as *Rhizopus* sp. closely related to *Rhizopus schipperae*.

Some Khmer people use rice liquor in the production process, but the minority people do not use it, with the exception of Lao, Tampuan, and Jarai. This is partly because Khmer, Suoy, and Lao people usually drink rice liquor and do not drink rice wine in daily life nowadays, but the other minority people drink rice wine until recently or still drink it. The techniques of Tampuan and Jarai people might have been influenced by the Lao.

Techniques of types I and II are derived from "rice liquor culture" because the processes used by almost all Khmer and Lao people belong to these two types. In contrast, the techniques of type IV (no use of old starters or rice liquor), which many minority people utilize, seem to be derived from "rice wine culture." Type III seems to be an intermediate between types I and IV.

Many people put new starters on rice husks (and rice straw). This technique seems to be common to all four types and is also widely recognized throughout Southeast Asia (Yoshida 1993: 153–155). However, the covering materials are quite different in "rice liquor culture" and "rice wine culture." Khmer, Suoy, and Lao people use cloths, flat baskets, and nets to cover new starters, but the other minority people usually use leaves and branches of plants, such as "*mboong*" or "*la baa*" in Krung, "*la baa*" (*Dipterocarpus tuberculatus*) in Lun, "*tuum kok*" (*Lepisanthes rubiginosa*) or banana in Tampuan, "*rlaan*" ("*sangkae*" in Khmer, *Combretum quadrangulare*) or banana in Phnong, "*dho*" in Kachok, and "*dho*" in Jarai. Mold or yeast, which is good for fermentation starters, adheres not only to rice husks and straws but also to the leaves of some plants (Ueda 1999). These results also indicate that type IV is different from types I and II and appears to be more of a prototype of the production process of fermentation starters.

Only some people among the Tampuan, Phnong, and Jarai do not utilize a process of drying starters in the sun before keeping them inside their houses. These people leave new starters, which are covered by leaves or branches, in the house for longer periods than the others, such as five to seven nights or even almost 1 month. All cases without a drying process are classified into type IV, which indicates that this technique seems to be also one of the characteristics of "rice wine culture."

10.2.7 Plants Used for Fermentation Starters in Cambodia and Neighboring Regions

The plants used for fermentation starters in Cambodia are shown in Table 10.2. The plants can be divided roughly into two categories, irrespective of the ethnic group: spices and herbs (*Capsicum* spp., *Allium sativum*, *Alpinia* spp., *Amomum kravanh*, *Cinnamomum* spp., *Piper* spp., *Zingiber officinale*, *Illicium verum*, etc.) or sweet ingredients (*Albizia* spp., *Cinnamomum* spp., *Saccharum officinarum*, and "*ngam*" or "*ngamngaam*" used among minority groups). Plants used for starters in other regions in and near Southeast Asia are also listed in Table 10.3 for comparison with those in Cambodia.

The top 10 plants used with high frequency in Cambodia originate in the Old World, with the exception of *Capsicum*, which was introduced into Asia around the sixteenth century. Spices and herbs, which are known for their antimicrobial agents and which also stimulate mold and yeast (Saono et al. 1982; Dung et al. 2005), are used because these plants are said to be "*kdaw*" (hot), "*har*" (spicy), and "*khlang*" (strong) and rice wine or liquor made with starters containing them would be hot and strong. Therefore, it is assumed that the use of *Capsicum* did not originate in one place, but rather that *Capsicum* was easily accepted as "one of the spices" in many places or cultures after its introduction into Cambodia. Some Phnong people deliberately do not use *Capsicum* for starters because they believe that they will get diarrhea if they drink rice wine made with starters including *Capsicum*. This also supports the hypothesis that the acceptance of *Capsicum* depends on the locality.

Plants such as *Albizia* spp., *Cinnamomum* spp., *Saccharum officinarum*, and "*ngam*" or "*ngamngaam*" are used in Cambodia because the people think that rice wine or liquor made with starters containing them would taste sweet. Some people mix even sugar or palm sugar with rice powder to produce starters, which is considered a modernized technique that does not incorporate plant materials. These saccharides act as nutrients that promote the growth of yeast and as the source of ethanol fermentation, which inhibits the growth of unwanted bacteria (Hayashida and Kinoshita 2004). Sweet plants, such as *Albizia* spp., *Cinnamomum* spp., *Saccharum officinarum*, and the juice of *Cocos nucifera*, are also used in other regions (Table 10.3). This technique seems to be fundamental in the production process of fermentation starters in Southeast Asia.

10.2.8 Rituals and Taboos in the Production Process

The production of fermentation starters do not differ along gender lines, but many people express the view that women usually produce the starters and men sometimes help them to collect plants or pound rice and plants. Rituals and taboos in the production process include "taboo of blood, pregnant women, and *kruu khmer* (traditional Khmer doctors)," "production by only one person or in a small room," "taboo of food," "taboo of sounds," and "use of charcoal and/or *Capsicum*."

Taboo of blood, pregnant women, and "*kruu khmer*": Menstruating women or persons who are injured and bleeding are not allowed to produce starters or visit the production place, and neither are pregnant women. Some Khmer people think that "*kruu khmer*" is not allowed to see or visit the production place because the starter production might end in failure by a curse of "*kruu khmer*." If the people mention above visited the production places by accident, the producers will make several pieces of starter with these unwelcome people to avoid production failure.

Production by only one person or in a small room: The entire production process of starters, from pounding to drying, should be done by only one person. The reason is not clear. Some Khmer and Lao people think that the process from mixing to making pieces should be done by a few people in a small room. Taboo of food (sour fruits or materials reminiscent of sourness and strong smell): Sour fruits, such as lime and tamarind, or foods retaining strong smells, such as "*pra hoc*" (fermented fish), are not allowed to be eaten or even touched by producers during the entire process or during pounding and making pieces. It is generally accepted that people connect the "sour" taste of fruits with the "sour" taste of rice wine, which means unsuccessful fermentation. Taboo of sounds (loud voice, getting angry or fighting, singing songs): Talking loudly, getting angry, fighting, and singing songs are not allowed during the period of pounding to making pieces.

Use of charcoal and/or *Capsicum*: People in Cambodia use charcoal and/or *Capsicum* fruits in rituals when they produced starters. For example,

Table 10.3 Plants used for fermentation starters in Southeast Asia and neighboring regions

Region and plants
Yunnan, China
Aconitum sp.(1)
Rhododendron sp. (1)
Scutellaria sp.(1)
Vietnam
Amomum tsao-ko(2), (3)
Asarum sieboldii (root, leaf)(2), (3)
Atractylodes macrocephala (tuber)(3)
Cinnamomum cassia (outer bark)(3)
Curcuma longa (tuber)(3)
Foeniculum vulgare (flower)(2), (3)
Glycyrrhiza uralensis (root)(2), (3)
Mentha arvensis (leaf)(3)
Myristica fragrans(2), (3)
Syzygium aromaticum (flower)(2), (3)
Laos
Areca catechu(4)
Capsicum spp.(4), (6)
Carica papaya (leaf)(4)
Cocos nucifera (bark, root)(4)
Nicotiana tabacum (leaf, root)(4)
Piper betle(4)
Saccharum officinarum (leaf, stem)(4), (5)
Solanum spp. (leaf, fruit)(4), (6)
Zingiber officinale(4)
Thailand
Zingiber officinale(7)
Malaysia
Cinnamon(4)
Piper nigrum(4)
Zingiber officinale(4)
Indonesia
Allium cepa(8)
Alpinia spp. (tuber)(8)
Capsicum spp.(8)
Cinnamomum parthenoxylon(4)
Cinnamon(8)
Citrus spp.(8)
Cocos nucifera (juice)(8)
Foeniculum vulgare(8)
Piper nigrum(8)
Saccharum officinarum(8)
Philippines
Bidens pilosa(4)
Cosmos caudatus(4)
Saccharum officinarum(4)
Zingiber officinale(9)
Taiwan
Abrus precatorius(4)
Blumea balsamifera(4)
Chenopodium spp. (seed)(4)
Chrysanthemum cf. *indicum*(4)
Lysimachia fragrand(4)
Myanmar
Allium sativum(6)
Carica papaya (leaf)(6)
Piper nigrum(6)
Solanum spp. (fruit)(6)
Zingiber officinale(10)
India, Nepal, etc.
Albizia kalkora (bark)(11)
Albizia myriophylla (bark)(12), (13), (14)
Amomum subulatum(15)
Artocarpus heterophyllus (leaf)(4)
Asclepias acida(4)
Asplenium esculentum (leaf)(4)
Buddleja asiatica (leaf)(14), (16), (17)
Capsicum spp. (fruit, leaf)(4), (14), (15), (16), (17), (18)
Cinnamomum glanduliferum (leaf, bark)(19)
Cinnamomum zeylanicum(15)
Cissampelos pareira (whole, tuber)(4), (19)
Cynodon dactylon (whole)(19)
Ficus religiosa (seed)(15)
Gaultheria sp. (leaf)(4)
Imperata cylindrica (tuber)(4)
Leucas aspera (leaf, flower)(19)
Lygodium salicifolium (whole)(19)
Madhuca longifolia (flower)(4)
Piper betle (leaf)(19)
Piper longum(15)
Plumbago zeylanica (root)(14), (16), (17)
Ruellia suffruticosa (root)(4)
Rumex sp.(4)
Saccharum officinarum(4)
Scoparia dulcis (whole)(19)
Solanum indicum (leaf, fruit)(4)
Syzygium cumini (fruit)(4)
Vernonia cinerea (leaf, flower)(14), (17), (19)
Zingiber officinale(4), (14), (16), (17), (18)

Modified from Yamamoto and Matsumoto (2011)
(1) Yamaguchi and Umemoto (1996), (2) Dung et al. (2007), (3) Dung et al. (2005), (4) Yoshida (1993), (5) Kozaki et al. (2005), (6) unpublished author data, (7) Kozaki et al. (2002), (8) Saono et al. (1982), (9) Kozaki et al. (2001), (10) Ochiai (2008), (11) Panmei et al. (2007), (12)Jeyaram et al. (2008), (13)Singh and Singh (2006), (14)Tamang et al. (2007), (15)Das and Pandey (2007), (16) Tamang et al. (1988) (17) Tsuyoshi et al. (2005), (18) Kozaki et al. (2000), and (19) Greeshma et al. (2006)

some used both charcoal and *Capsicum*, and some used only charcoal. Some people also use charcoal and/or *Capsicum* in fermented rice production rituals or when boiling rice for rice liquor. Yoshida (1993) reported the use of charcoal and *Capsicum* in starter production rituals among the Lao, Thai Dam, Thai Neua, Yao, and Khmu in Laos and the Murut and Rungus in Borneo, and the same ritual was also found in the Chin state in Myanmar (Ochiai 2008: 244). Yoshida (1993: 155–158) suggested that the use of charcoal and *Capsicum* in rituals might have been introduced into Borneo as "one set" from the continental region of Southeast Asia some time after the fifteenth century. The same use is confirmed in Cambodia, which lies between the two regions surveyed by Yoshida, seeming to support his hypothesis somewhat. However, Shimomoto (1984) reported that the Rungus used charcoal and *Capsicum* as "burning" symbols and that these two could encourage rice wine to be more "burning" (potent) in rituals, which is very similar to the idea of people in Cambodia that rice wine or liquor made with starters including "hot" and "spicy" plants will be hot and strong. Therefore, it is still unknown whether these techniques originated in one place and were dispersed to other regions (like Yoshida's hypothesis) or whether they originated in many places under certain cultural backgrounds. More studies on this ritual are necessary to discuss its origins and dispersal.

10.3 Rice Wine

10.3.1 Local Names for Rice Wine and Bamboo Tubes to Drink Rice Wine

Rice wine is mainly produced by the minority people in Northeast Cambodia. Table 10.4 shows local names for rice wine (in a jar) and bamboo tubes to drink rice wine. The following are some case studies of the production process of rice wine in Cambodia.

Table 10.4 Local names for rice wine (mainly in a jar) and bamboo tubes used to drink rice wine and rice liquor in Cambodia

	Local names	
Ethnic group	Rice wine	Bamboo tube
Mon-Khmer		
Brao	*tavee taam, tavee wan*	*hanong, sunong*
Kachok	*jyoo choom*	*jeeng*
Kravet	*wan tave*	*hanong*
Krung	*tavee tanem, tavee van*	*chunong, hanang*
Lun	*tavee taam*	*hanong*
Phnong	*rragn yang*	*dhoong*
Suoy	*kragn taak*	*chunuu*
Tampuan	*tapae tuum, tavee taam*	*chuwat, suwat, saaf at*
Austronesian		
Jarai	*pae, pae phun, pae che*	*ding, ding pae*
Tai-Kadai		
Lao	*lao hai*	*kam lao*

10.3.2 Brao in Ratanak Kiri (Fig. 10.8)

Take 3.75 kg of non-sticky rice → wash it with rice husk → steam it → mix it with six pieces of a starter (about 4 cm in diameter and 2 cm in thickness) → put it in a basket for one night covered with "*mboong*" (*Dipterocarpus* spp.) or banana leaves → put in a jar → seal the jar with ashes dissolved in water.

10.3.3 Kachok in Ratanak Kiri (Fig. 10.9)

Take 1.5–1.75 kg of non-sticky rice → stem it with rice husk → mix it with three pieces of a starter (about 5.5 cm in diameter and 3 cm in thickness) → mix it with rice husk → put it in a basket for three nights covered with "*kadoo*" → put rice husk into the bottom of a jar first → put the mixture in the jar → put rice husk on the mixture → cover it by a banana leaf → seal the jar with ashes dissolved in water → drink it 10–15 days after sealing.

Fig. 10.8 Rice wine (*tavee taam*) of Brao in Ratanak Kiri (**a**). Tube (*hanong*) *top* (**b**). Starters are kept in a bamboo container with *Capsicum* fruits as an insect repellent (**c**)

Fig. 10.9 Starters (*praa*) (*left*) and tube (*jeeng*) *top* (*right*) of Kachok in Ratanak Kiri

10.3.4 Kravet in Ratanak Kiri

Take 2.5 kg of non-sticky rice and 2.5 kg of rice husk → steam it → mix it with one piece of a starter → put in a jar → cover it by leaves of any kind of plants → seal the jar with ashes dissolved in water → drink it 5 days after sealing (a jar left over more than 1–2 months tastes much better).

10.3.5 Krung in Ratanak Kiri (Fig. 10.10)

Take 3.75 kg of non-sticky rice → wash it with rice husk → steam it → mix it with six pieces of a starter (about 4 cm in diameter and 2 cm in thickness) → put it in a basket for one night covered with "*mboong*" or banana leaves → put in a jar → seal the jar with ashes dissolved in water.

Fig. 10.10 Rice wine (*tavee van*) of Krung in Ratanak Kiri (**a**). They put mixture of steamed rice with rice husk and starters into a basket for one night covered with "*mboong*" or banana leaves (**b**). Tube (*chunong*) *top* (**c**)

10.3.6 Lun in Ratanak Kiri (Fig. 10.11)

Take 2.5 kg of non-sticky rice → wash it with rice husk → steam it → mix it with one piece of a starter (about 7 cm in diameter) → put it in a jar → cover it by a banana leaf → seal the jar with ashes dissolved in water → drink it 10 days after sealing (a jar left over more than 1 month tastes much better).

10.3.7 Phnong in Mondul Kiri

Take 3 kg of non-sticky rice → boil it → mix it with five pieces of a starter (about 4 cm in diameter and 3.4 cm in thickness) → mix it with rice husk → put rice husk into the bottom of a jar first (for making drink easy) → put the mixture in the jar → put rice husk on the mixture (for preventing invasion of insects) → cover it by a container made from a gourd → seal the rim of the gourd with ashes dissolved in water → drink it 7 days after sealing (a jar left over more than 1–2 months tastes much better).

10.3.8 Suoy in Kampong Speu

Take 5 kg of non-sticky rice → boil it → mix it with two to three pieces of a starter (about

Fig. 10.11 Rice wine in a jar (called *tavee taam*) of Lun in Ratanak Kiri. Sealing the jar with ashes dissolved in water (**a**). Opening the seal (**b**). Pouring water into the jar and drinking it with a tube (*hanong*) (**c**). You have to finish one cup of water (**d**)

6 cm in diameter and 2.5 cm in thickness) → put it in a jar → seal the jar with clay → drink it 45 days after sealing.

10.3.9 Tampuan in Ratanak Kiri

Take 1.75 kg of non-sticky rice → boil it → mix it with ten pieces of a starter → mix it with rice husk → put it in a jar → put rice husk on the mixture → cover it by plastic (they had covered it by banana leaves before) → seal the jar with ashes dissolved in water → drink it 10 days after sealing (a jar left over more than 1 month tastes much better).

10.3.10 Jarai in Ratanak Kiri (Fig. 10.12)

Take 2.5 kg of non-sticky rice → boil it → mix it with rice husk and 15 pieces of a starter (about 9.5 cm in diameter and 4.8 cm in thickness) → put it in a jar → cover it by banana leaves → seal the jar with ashes dissolved in water → drink it 15 days after sealing (a jar left over more than 1 month tastes much better).

10.3.11 Lao in Ratanak Kiri

Take 5 kg of non-sticky rice and 6–7 kg of rice husk → steam it → mix it with 15 pieces of a starter → put it in a jar → cover it by mango leaves and plastic → drink it 15 days after sealing.

Fig. 10.12 Starters (*pooy*) (*left*) and rice wine in a jar (*pae phun*) (*right*) of Jarai in Ratanak Kiri

10.4 Rice Liquor

10.4.1 Local Names for Rice Liquor

Khmer people in Cambodia mainly produce rice liquor, but some minority people (e.g., Lao) also produce it. A local name for rice liquor was "*sraa sar*" in Khmer, "*bhlong blaay*" in Kuy, "*lao lao*" in Lao, and "*tapae tuuak*" in Tampuan and Jarai. Rice wine is usually produced and consumed by each household, but rice liquor is very important as processed products by small-scale farmers in rural areas of Cambodia (Hamano et al. 2013). The following are some case studies of the production process of rice liquor in Cambodia.

Fig. 10.13 Rice liquor (*sraa sar*) of Khmer in Takaev. Filtering the product when selling

10.4.2 Khmer in Takaev (Fig. 10.13)

Take 37 kg of non-sticky rice → soak it in water for 1.5–2 h → steam it → mix it with 300 g of a Vietnamese starter (4000 Riel/kg obtained from a market) → put it in a ceramic pot (about 40 L) and leave it for 2 days → pour water into the ceramic pot and leave it for 2 days → distill the mixture (it takes 5 h) → 45 L of rice liquor (35–40°, 70,000 Riel/30 L).

10.4.3 Khmer in Battambang (Fig. 10.14)

Take 25 kg of non-sticky rice → soak it in water for 15 min → steam it → mix it with 300 g of a Vietnamese starter obtained from a market → put it in a ceramic pot (about 40 L) and leave it for 2 days → pour water into the ceramic pot and leave it for 2 days → distill the mixture (it takes 7–8 h) → 40 L of rice liquor (25–30°, 45,000 Riel/30 L, filtering the product before selling).

10.4.4 Khmer in Kampong Chhnang (Fig. 10.15)

Take 30 L of palm sugar (40,000 Riel) and 250–500 g of sticky rice → put it into a ceramic pot with 20 cm in length of a bark of *kakahs*

Fig. 10.14 Production process of rice liquor of Khmer in Battambang. Mixing steamed rice with powder of a Vietnamese starter (**a**), putting it into a ceramic pot (**b**), and leaving it for 2 days. Thereafter, putting water into the pot and leaving it for 2 days (**c**). Distilling the mixture (**d**)

Fig. 10.15 Production process of rice liquor of Khmer in Kampong Chhnang. Putting palm sugar and sticky rice into a ceramic pot with 20 cm in length of a bark of *kakahs* (*Sindora siamensis*)

(*Sindora siamensis*) and leave it for 2 days → distill the mixture (it takes 6 h) → 60 L of rice liquor (20–30°, 35,000–40,000 Riel/30 L).

10.4.5 Suoy in Kampong Speu (Fig. 10.16)

Take 1.25 kg of non-sticky rice → boil it → mix it with 30–50 g of a Vietnamese starter → put it in a ceramic pot (top rim 14 cm, 28 cm in diameter, 28 cm in height) and leave it for 2 days → pour water into the ceramic pot and leave it for 2 days → distill the mixture (it takes 1–2 h) → 1.5 L of rice liquor.

10.4.6 Kuy in Kampong Thom

Take 17 kg of non-sticky rice → boil it → mix it with a Vietnamese starter → put it in a ceramic pot and leave it for 2 days → pour water into the ceramic pot and leave it for 2 days → distill the mixture (it takes 5–7 h) → 30 L of rice

Fig. 10.16 Production process of rice liquor of Suoy in Kampong Speu. Mixing boiled rice with powder of a Vietnamese starter (**a**, **b**). Putting the mixture in a pot (**c**). Tools for distilling (**d**–**f**)

Fig. 10.17 Rice liquor (*lao lao*) of Lao in Steung Treng. Homemade starters called *paeng* (**a**). Distilling (**b**). Products (**c**)

liquor (*bhlong blaay*, about 30°, 40,000 Riel/30 L, filtering the product before selling).

10.4.7 Lao in Steung Treng (Fig. 10.17)

Take 10 kg of sticky rice (always sticky rice) → soak it in water for one night → steam it → wash it in a river to remove its stickiness → mix it with ten pieces of a homemade starter → put it in a ceramic pot and leave it for 3 days → pour water into the ceramic pot and leave it for 3 days → distill the mixture (it takes 4–5 h) → 9 L of rice liquor (*lao lao*, 5000 Riel/L).

10.4.8 Khmer Khe in Steung Treng (Fig. 10.18)

Take 4–5 kg of sticky rice → soak it in water for 1 h → steam it → mix it with six pieces of a homemade starter (about 3.5 cm in diameter and 1 cm in thickness) → put it in a ceramic pot and leave it for 3 days → pour water into the ceramic pot and leave it for 3 days → distill the mixture (it takes 3 h) → 5 L of rice liquor (for home consumption).

10.4.9 Tampuan in Ratanak Kiri (Fig. 10.19)

Take 5 kg of sticky rice (always sticky rice) → steam it → mix it with 15 pieces of a homemade starter (about 3.5 cm in diameter and 1 cm in thickness) → put it in a ceramic pot and leave it for 5 days → pour water into the ceramic pot and leave it for 3–4 days → distill the mixture (it takes 1 day) → 10 L of rice liquor (*tapae tuuak*, for home consumption).

10.4.10 Jarai in Ratanak Kiri (Fig. 10.20)

Take 3.75 kg of sticky rice → steam it → mix it with 200 g of powder of a Vietnamese starter → put it in a ceramic pot and leave it for 5

Fig. 10.18 Tools of Khmer Khe for distilling rice liquor in Steung Treng

Fig. 10.19 Tools of Tampuan for distilling rice liquor (*tapae tuuak*) in Ratanak Kiri

Fig. 10.20 Tools of Jarai for distilling rice liquor (*tapae tuuak*) in Ratanak Kiri

Fig. 10.21 Tools of Tampuan for distilling rice liquor (*tapae tuuak*) in Ratanak Kiri

days → pour water into the ceramic pot and leave it for 5 days → distill the mixture (it takes 1 day) → 4–5 L of rice liquor (for home consumption).

10.4.11 Tampuan in Ratanak Kiri (Fig. 10.21)

Take 5 kg of sticky rice → soak it in water for one night → steam it → mix it with 20 pieces of a homemade starter (about 3.5 cm in diameter and 1 cm in thickness) → put it in a ceramic pot and leave it for 5 days → pour water into the ceramic pot and leave it for 3 days → distill the mixture (it takes 1 day) → 7–8 L of rice liquor (*tapae tuuak*, 2000 Riel per bottle).

10.5 Palm Wine

Palm wine (*tek tnart chu*) is made mainly from palmyra palm in Cambodia. This is also important as processed products by small-scale farm-

Fig. 10.22 A lady selling palm wine (*tek tnart chu*) by bicycle in Kampong Chhnang (**a**). Palm wine with fried grasshoppers in Kampong Speu (**b**)

ers in Cambodia (Fig. 10.22). Producers choose a good flower bud and pinch it by a special tool (Fig. 10.23). Two or three days later, they slice the top of the bud a little bit and get the sap of palmyra palm. They collect the sap one time in the morning (6–8 a.m.) and one time in the evening (5–6 p.m.) (Fig. 10.24). At that time, they collect the container filled with the sap, slice the top of the bud again, and put a new container on the bud. Some people put barks of *popel* (*Shorea cochinchinensis*) in the containers to make the sap clear (Fig. 10.24). There are two types of fermentation process: fermenting the sap in the container while hanging on trees or using *mae tek tnart chu*. When they ferment the sap on the trees, they put several plants, such as *kakahs* (*Sindora siamensis*), *sangkae* (*Combretum quadrangulare*), and *angkraang* (*Ziziphus cambodiana*), in the containers. *Mae tek tnart chu* is made from xylems or barks of some plants such as *kakahs*, *popel*, *sangkae*, *reang* (*Barringtonia* spp.?), *nieng nuen* (*Dalbergia* spp.?), *khlong* (*Dipterocarpus* spp.?), *sdau* (*Azadirachta indica*), *kuey* (*Myxopyrum smilacifolium*?), etc. (Fig. 10.25). They burn these plants to make them dry, put them in a ceramic pot, and pour the sap into the pot. It becomes *mae tek tnart chu* for 2–3 days. Thereafter, it takes only 2–3 h to make the fresh sap become palm wine if they put the fresh sap into *mae tek tnart chu*. They can use *mae tek tnart chu* for 2–3 months.

Fig. 10.23 A tool for pinching a flower bud to get a sap in Kampong Speu

10.6 Other Fermented Foods

Sweet fermented alcoholic rice (*tapae*) is sold in local markets (Fig. 10.26). Local people boil sticky rice, mix it with fermentation starters, and leave it for 2 days (sometimes they rap the mixture with rice straw). A woman in Takaev said that “An *app* (an evil spirit) likes to drink the liquid of *tapae*, and *tapae* would go bad (unsuccessful fermentation) if the *app* touches *tapae*. Thus, I used to put fruits of chili peppers or thorny plants on the lid of a pot in which pieces of *tapae* were fermenting, to protect them from *app*” (Yamamoto and Matsumoto 2008). Fermented rice noodles called *nom banvuchock* are also

Fig. 10.24 Climbing up to the top and replace containers filled with the sap by new empty ones (**a**–**c**). They put barks of plants in the container (**d**)

Fig. 10.25 *Mae tek tnart chu* in Kampong Speu

Fig. 10.26 Sweet fermented alcoholic rice (*tapae*) sold in Takaev (*left*) and in Kracheh (light)

Fig. 10.27 Fermented rice noodles called *nom banvuchock* sold in Kampong Speu (*right*) and in Siem Reap

consumed in Cambodia (Fig. 10.27). Ikeda et al. (2010) reported the characterization of the composition and bacterial manufacturing process of *nom banvuchock*. Fermented fish or shrimp, such as *phaak* (fermented fish with sticky rice, Fig. 10.28), *pra hoc* (fermented fish), *mam* (fermented fish with roasted rice powder), *kapi* (fermented shrimp paste), and *tak trey* (fish sauce), is a very important condiment in Cambodia, and more detailed information on fermented fish in Cambodia is shown in Ishige and Ruddle (1990).

Fig. 10.28 *Phaak* (fermented fish with sticky rice)

10.7 Conclusion

There are two different lines of production process of local starters that are confirmed in Cambodia: one based on "rice wine culture," characterized by no use of rice liquor and old starters, use of leaves and branches for covering, and no drying process of starters, and one based on "rice liquor culture," characterized by the use of rice liquor (blown on) and old starters (scattered on new starters and/or mixed with rice powder) and the addition of sugar without using plant materials. The process based on "rice wine culture" seems to be an older type of starter production than that based on "rice liquor culture," suggesting that new techniques belonging to "rice liquor culture" could have infiltrated the "rice wine culture" of Cambodia. Moreover, production process of rice wine or rice liquor seems to have several different types. Further studies are needed to classify production process of rice wine or rice liquor in Cambodia based on techniques. Not only the production processes but also plant use and related rituals should be studied and compared to elucidate the origins and dispersal routes of rice wine and rice liquor in Cambodia and Southeast Asia.

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11 Ethnic Fermented Foods and Beverages of Korea

Dong-Hwa Shin, Young-Myoung Kim, Wan-Soo Park, and Jae-Ho Kim

11.1 Introduction

Korea is a peninsular country in Northeast Asia with a total area (Republic of Korea) of 99,585 km^2 and a population of 51,465 thousand (Oct. 2015). Korea has four distinct seasons and also has long indented coastlines and many rivers that provide a variety of marine and freshwater products. The climatic characteristics of Korea have promoted the customs of eating seasonal foods and storing various types of foods including fermented foods, such as fermented soybean foods, fermented vegetable foods, and fermented fish products for the next seasons. A variety of aquatic products such as small shrimp and anchovy are also available due to the geographical characteristics of Korea.

The dietary customs, tastes of people, shapes of utensils, and even the layout of kitchen are dependent on staple foods of the country. Rice, the staple food in Korea, and other grains such as barley have been major food sources of Korean people. Other food sources include different plants and vegetables, a number of meat-based foods, fish and shellfish, sun-dried salt, soybean sauce and paste, *Gochujang* (fermented soybean paste mixed with red pepper), and a variety of spices and condiments, such as red pepper, black pepper, ginger, garlic, spring onion, or scallion, are utilized.

Korea's traditional foods are famous for their rich and diverse flavors, and their culinary art has been passed on to the next generation through experience and practice.

A kind of meat sauce/paste which was present in ancient China about 3,000 years ago gradually disappeared as a new type of soybean sauce/paste was introduced to ancient Korea about 1,000 years later. Meanwhile, aquatic salted and fermented products were widely developed in Southeast Asia (Lee 1984, 1993a, b, c). Further studies

D.-H. Shin (✉)
Shindonghwa Food Research Institute,
Eunma Apt. 12- 802, 212 Samsung-ro, Kangnam-ku,
Seoul, Republic of Korea, 06284
e-mail: dhshin@jbnu.ac.kr

Y.-M. Kim
Gijang Mulsan CO.LTD, 110-401, Xi-APT. #50,
Ipbuk-ro, Kwonseon-ku, Suwon-si, Kyunggi-do,
Republic of Korea, 16369
e-mail: ymkim6321@naver.com

W.-S. Park
World Institute of Kimchi, 86, Kimchi Street, South
District, Gwangju Metropolitan City 61755, Republic
of Korea
e-mail: wspark@wikim.re.kr

J.-H. Kim
Korea Food Research Institute,
62, Anyangpangyo-ro 1201 beon-gil, Bundang-gu,
Seongnam-si, Gyeonggi-do 13539, Republic of Korea
e-mail: ricewine@kfri.re.kr

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found that a variety of fermented vegetable products as well as fermented marine products with a considerably advanced quality were developed in Northeast Asia such as ancient Korea.

Agriculture in Korea has been recognized as a life industry that becomes a basis of the dietary life in Korean people before the Christian era according to weathers and climates including geopolitical conditions. Different products are produced based on agricultural products and have been used in various ways with direct or indirect manners, while the natural condition, which represents four distinctive seasons, has been conquered. In particular, it has been used as a way of keeping and preserving foods and that affects their dietary life significantly. In addition, vegetables and marine products play a role in side dishes in the dietary life with a principal food of boiled rice aside. Moreover, there exist various fermented foods in order to give tastes and flavors to the side dishes with boiled rice.

According to these requirements, different fermented foods have been introduced using various agricultural products, such as grains, vegetables, and beans, before the Christian era, and that becomes an opportunity of presenting differences and originalities in Korean-style foods from generation to generation. In particular, different alcoholic beverages, rice cakes, meats, and fishes have been offered in memorial services based on the harvest ceremony in agricultural cultures, which allows establishments of producing techniques of various alcoholic beverages and special food products. Fermented foods have been produced based on natural phenomena at the beginning of the world and have been largely developed by various fermentation techniques through improving its quality and diversifying its products. Fermented foods in Korea have been presented as a coexistence manner together with traditional and industrial ways. These foods still have been produced as different products and have been distributed through different ways. Representative traditional fermented foods in Korea are summarized in Table 11.1.

The detailed production methods of the products and their characteristics are introduced in the following sections.

11.2 Fermented Soybean Products

Fermented soybean products mostly use soybeans as a major matrix and are a type of fermented foods based on functions of microorganisms. There are a few fermented foods, which use such functions of microorganisms based on grains or vegetables in Western dietary habits, but soybean products are also very limited. In the East, however, there are various soybean products, and fermented soybean products have been used as the most important source of seasoning in Eastern dishes. The history of producing such fermented soybean foods goes back to before the Christian era (Lee 1993a, b, c).

Fermented soybean products have been used to provide some salty and savory flavors in cooked vegetables and meats for Korean main diets of boiled rice and dishes. It has been largely used for most of Korean ethnic foods as a seasoning. Also, Korean foods represent different tastes and flavors depending on fermented soybean products used compared to other global foods. The fermented soybean products produced in Korea are largely divided into four different types, such as *Ganjang*, *Doenjang*, *Gochujang*, and *Cheonggukjang*. These products contain different main and extra ingredients through its fermentation processes and represent different seasoning methods in processing foods (Shin 2011). The most important thing in producing such fermented soybean products is the soybean, the main raw material. Soybeans are stored and distributed as dried products after harvest and represent quality differences between varieties. In addition to the quality of the main material, the fermentation process and condition determined by microorganisms significantly affect the quality and property of final products.

11.2.1 *Doenjang*

Doenjang is one of the traditional soybean-fermented products which is a paste-type product with yellowish-brown color. Also, it has the longest history of all traditional seasonings in fer-

Table 11.1 Ethnic fermented foods and alcoholic beverages of Korea

Raw material	Food name	Characteristics
Rice or other grain	*Makgeolli*	Turbid alcoholic liquor fermented by *Nuruk*, (a starter for producing traditional alcoholic beverage using powdered whole wheat kernels (Yoo et al. 2011)
		Alcohol concentration: 6–8 %
	Yakju	Based on *Makgeolli*, make it clear by sedimentation; Grain as the main ingredient. Also added medicinal herbs during fermentation
		Alcohol concentration: 10–18 % (Kim et al. 2004)
	Soju	Distilled liquor prepared by *Makgeolli*
		Alcohol concentration: 20–45 % (Ryu et al. 2015)
	Vinegar	Use *Makgeolli* as a raw material and let it go through acetic acid fermentation
	Jeungpyeong	Make dough with rice powder mixed with *Makgeolli* and let it ferment then steam
Soybean	*Ganjang*	Soy sauce, brown-colored liquid
	Doenjang	Brown-colored paste made by *Meju*
	Gochujang	Red pepper used
		Fermented spicy condiment
	Cheonggukjang	Solely soybean used and fermented by *Bacillus*
		High-temperature fermentation (40–43 °C)
Fish	*Jeot-gal* (Fermented Fish)	Names are classified by raw fish used: *Mullus*, shellfish, crustacean, edible viscera, fish meat
	Sikhae	Fishes mixed with millet and rice and let ferment
Vegetable	*Kimchi*	Lactic acid-fermented product
		Use *Baechu* (Chinese cabbage) cabbage), radish, etc as a main raw material and red pepper, fermented fish, ginger, etc. as sub-ingredients.
	Pickles	All root vegetable can be used to make pickles
		Typically radish, cucumber, carrot

mented soybean products (Shin 2011). *Doenjang* has usually been used as a source of seasonings. In addition, it has largely been cooked as a pot stew and a sauce with vegetables. *Doenjang* contains a unique fermentation flavor and a savory flavor with nucleic and amino acids.

11.2.2 Preparation Method

For producing *Doenjang*, *Meju*, a basic material, is to be made as shown in Figs. 11.1, 11.2, and 11.3.

After producing *Meju*, *Ganjang* and *Doenjang* are produced by the process presented in Fig. 11.4. In this process, some earthenwares are used in a traditional process and some plastics, stainless steels, cement tanks, etc. are also used according to purposes.

As shown in Fig. 11.4, solid and liquid contents are separated after aging. The liquid and solid contents are used to produce *Ganjang* and *Doenjang*, respectively, in a traditional method. *Doenjang* is ingested as soups in Korean tables almost everyday and has been known as an excellent protein source with various functional ingredients (Kwon et al. 2011). For seasoning the *Doenjang* soup, some garlic, onion, and red pepper powder are added to the soup according to personal tastes.

11.2.3 Microorganism

Aspergillus and *Bacillus* genera are used in commercial fermented soybean products, which requires strain management, but in ethnic fermented soybean products, various natural strains

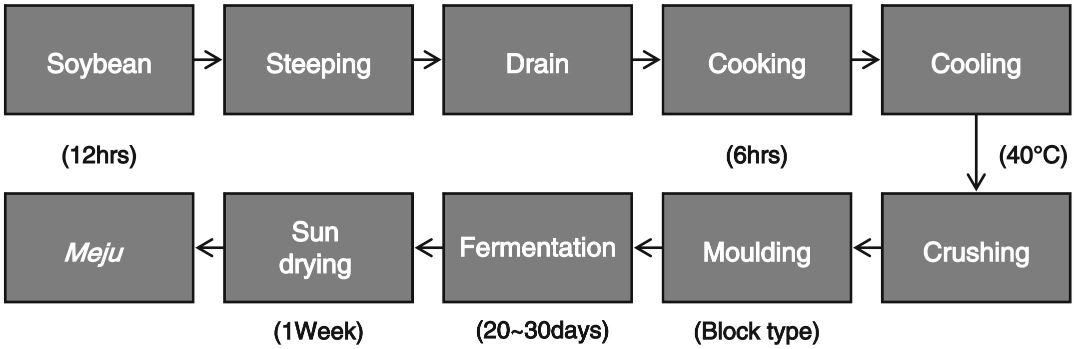

Fig. 11.1 Procedure of *Meju* preparation

Fig. 11.2 *Shape of Meju*

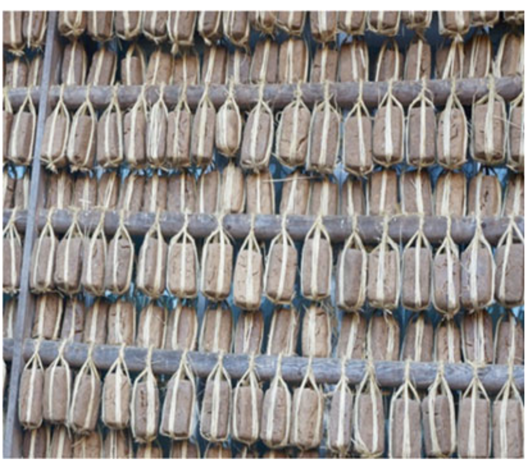

Fig. 11.3 Natural fermentation of *Meju*

and species are used. For traditional production of *Doenjang*, *Meju* (a crushed fermented soybean block for traditional soy sauce and soybean paste, Hong et al. 2015) is used as a major ingredient. The microorganisms present in fermented *Meju* are molds, *Mucor* and *Aspergillus*; bacteria, *Bacillus*, *Lactobacillus*, and *Streptococcus*; and yeasts, *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Hansenula*, and *Debaryomyces* genera (Park 2009) (Table 11.2).

11.2.4 Nutritional Composition

The major ingredient of *Doenjang* is soybean, which contains oils and proteins about 20 % and 40 %, respectively, and 12 different isomers including daidzein, genistein, and glycitein which are known as isoflavones and phytoestrogens (Shin 2011). *Doenjang* produces water-soluble flavor substances from degraded proteins, fats, and carbohydrates by different microbial enzymes during its fermentation process. General ingredients in *Doenjang* are presented in Table 11.3 (RDA 2011).

11.2.5 Functionality and Health Benefits

Doenjang represents various physiological functions through the substances newly produced during its fermentation process, in addition to various functionalities of soybeans. It has been

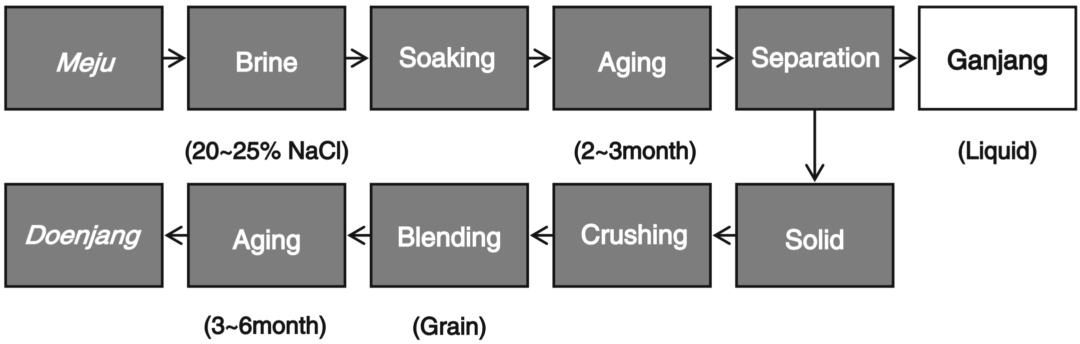

Fig. 11.4 Procedure of *Doenjang* and *Ganjang* preparation

Table 11.2 Microorganisms isolated from *Meju* (Choi et al. 1995)

Mold	*Aspergillus flavus*, *Asp. fumigatus*, *Asp. niger*, *Asp. oryzae*, *Asp. retricus*, *Asp. spinosa*, *Asp. terreus*, *Asp. wentii*
	Botrytis cinerea
	Mucor adundans, *Mucor circinelloides*, *Mucor griseocyanus*, *Mucor jasseni*, *Mucor hiemalis*, *Mucor racemosus*
	Penicillium citrinum, *Pen griseopurpureum*, *Pen. griesotula.*
	Pen. kaupscinskii, *Pen. lanosum*, *Pen. thomii*, *Pen. turalense*
	Rhizopus chinensis, *Rhi. nigricans*, *Rhi. oryzae*, *Rhi. stolonifer*
Yeast	*Candida edax*, *Candida incommenis*, *Candida utilis*
	Hansenula anomala, *Hansenula capsulata*, *Hansenula holstii*
	Rhodotorula flaca, *Rhodotorula glutinis*
	Saccharomyces sp., *Sac. exiguus*, *Sac. cerevisiae*, *Sac. kluyveri*
	Zygosaccharomyces japonicus, *Zygosaccharomyces rouxii*
Bacteria	*Bacillus citreus*, *Bac. circulans*, *Bac. amylolichemiformis*, *Bac.*
	megaterium, *Bac. mesentericus*, *Bac. subtilis*, *Bac. pumilus*
	Lactobacillus sp.
	Pediococcus sp., *Pediococcus acidilactici*

known that trypsin inhibitors, isoflavones, vitamin E, and linoleic acid, which is an unsaturated fatty acid, represent anticancer effects in the traditional *Doenjang* (Kwon et al. 2011). Also, *Doenjang* extracts increase both the glutathione S-transferase activity that contributes to detoxifying the liver and the natural killer cell activity involved in removing cancer cells (Choi et al. 1998a).

It has also been known that the contents of aglycone, which is produced during the fermentation of *Doenjang*, including genistein and daidzein, are increased during the fermentation and aging processess of *Doenjang*, and that the aglycone leads to decreased tumor weights and presents an inhibition effect of causing tumors (Table 11.4) (Son 1995; Jung et al. 2006).

In a peculiar fact, there are no anti-mutation effects in a condition that *Doenjang* is not fermented. It is estimated that there are creations and increases in bioactive substances during the fermentation process (Jung et al. 2006). The brown colored and phenolic substances produced during the fermentation and aging processes of *Doenjang* show strong antioxidant functions and increase antioxidant capabilities according to increases in the amount of free amino acids with the progress of fermentation (Lee et al. 2014). In addition, a substance that shows a function of ACE inhibitor in *Doenjang* has been identified as arginine-proline (Kim et al. 1999), and the ACE inhibition capability is increased during the fermentation process (Shin et al. 2011).

In an experiment on animals, a *Doenjang* diet positively affects lipid metabolism through inhibiting the synthesis of VLDL-C and LCL-C instead of synthesizing HDL-C and shows effects

Table 11.3 Chemical composition of the fermented soybean products

Food	Proximate compositions			Dietary fiber			Minerals		
	Water	Protein	Fat	Ash	CHO	TDF	Calcium	Phosphorus	Iron
	kcal	g	g	g	g	g	mg	mg	mg
Doenjang	54.0	13.6	8.2	12.5	11.7	–	84	208	2.5
Gochujang	44.6	4.9	1.1	8.2	43.8	–	40	90	2.2
Cheonggukjang	70.7	10.2	0.8	3.4	14.9	–	96	177	3.8
Ganjang (soy sauce)	70.4	7.7	0.3	16.7	4.9	–	38	155	0.9

Food	Minerals		Vitamin					
			A			B_1	B_2	C
	Potassium	Sodium	Valent	Retinol	β-Carotene	Thiamin	Riboflavin	Ascorbic acid
	mg	mg	RE	μg	μg	mg	mg	mg
Doenjang	647	3748	0	0	0	0.04	0.12	0
Gochujang	822	3164	408	0	2445	0.17	0.52	5
Cheonggukjang	602	961	13	0	80	0.15	0.29	0
Ganjang (soy sauce)	390	7157	0	0	0	0.02	0.08	0

Table 11.4 Antitumor activity of *Doenjang*

Sample	Tumor weight (g)	Inhibition rate (%)
Sarcoma 180 cancer cell (A) + PBS	5.8 ± 0.3^{a}	–
(A) + Fermented *Doenjang* (3 months)	5.4 ± 0.2^{a}	7
(A) + Fermented *Doenjang* (6 months)	4.7 ± 0.3^{b}	19
(A) + Fermented *Doenjang* (24 months)	3.6 ± 0.2^{c}	38

※ $^{a-c}$Duncan's multiple range test ($p < 0.05$)

of decreasing blood flow TC and improving high cholesterol (Lee 2002). It is reported that *Doenjang* shows an anticoagulation effect due to a thrombin inhibitor (Jang et al. 2004). Consumption of *Doenjang* contributes to fat burning and weight loss by promoting a β-oxidation process of fatty acids (Kwak et al. 2012).

For implementing an experiment on a human body, *Doenjang* pills, 9.9 g/day (40 g of raw *Doenjang*), are administered to subjects for 12 weeks, and the body weight and body fat of subjects are measured (Tables 11.5 and 11.6) (Cha et al. 2012). The result shows significant decreases in the body weight and body fat compared to a control group (Cha et al. 2014).

An immune control substance (KFSP) is also identified from *Doenjang* even though it is not present in soybeans. It reveals that *Doenjang* plays a role in immune control (Kim et al. 2014b), and the production of immune control substances is also verified in an experiment on animals (Lee and Hwang 1997; Jang et al. 2008).

11.2.6 Ethnic Value and Socio-economy

Doenjang is one of the essential side dishes in Korean tables almost everyday and has been positioned as an important vegetable protein source. *Doenjang* is usually ingested in the form of soups as a side dish of boiled rice. Korean takes an average of 8.8 g *Doenjang* everyday, and its annual gross domestic product and export are 621,764 tons (MFDS 2013) and 40.105 million dollars (MFDS 2013), respectively. Korean traditional foods have been propagated throughout the world due to recent Korean Wave, and preferences on *Doenjang* in foreign consumers have also been increased. Both the factory-produced

Table 11.5 Changes in body weight and composition and abdominal fat area measurements for 0 week and 12 weeks (Cha et al. 2012)

Parameters	*Doenjang* group ($n=26$)			Placebo group ($n=25$)			Absolute group difference[2]	P-value[b]
	0 week	12 weeks	Change	0 week	12 weeks	Change		
Body weight (kg)	70.2±1.8[a]	69.4±1.8	−0.8**	67.8±1.7	67.6±1.8	−0.3	0.55±0.4	<0.001
Body fat mass (kg)	22.1±1.0	21.4±0.9	−0.7**	22.1±0.9	21.8±0.9	−0.4*	0.31±0.3	<0.001
Body fat (%)	31.7±1.2	31.1±1.6	−0.6**	32.6±0.9	32.2±0.9	−0.4	0.17±0.3	0.007
Total fat (cm^2)	333.1±3.5	248.8±10.5	−84.3***	327.9±38.8	253.6±12.3	−74.4*	9.9±35.6	0.788
Visceral fat (cm^2)	74.4±4.8	65.9±3.6	−8.6***	65.8±4.0	65.3±3.4	−0.60	7.9±3.7	0.041
Subcutaneous fat (cm^2)	258.6±23.7	182.9±9.7	−75.7**	262.1±39.3	188.3±10.8	−73.8*	1.9±38.7	0.960
VSR	0.35±0.03	0.39±0.03	0.04*	0.31±0.03	0.37±0.02	0.06***	0.01±0.02	0.447

*$p<0.05$, **$p<0.01$, ***$p<0.001$: *P*-values indicate significant differences in the variables between 0 and 12 weeks, which were evaluated by paired *t*-test

[a]Values are expressed as means ± SE. *WHR* waist to hip ratio, *VSR* visceral to subcutaneous ratio

[b]Repeated measurement analyses (per protocol) after adjusting the age, gender, and BMI

[2]Values in this column represent the difference between the mean change scores of the *Doenjang* group and those of the placebo group; 95 % CIs in parentheses

Table 11.6 Autonomic nervous functions (deep breathing) of the *Gochujang* and placebo groups for 0 and 12 weeks

	Gochujang group ($n=13$)		P-value[a]	Placebo group ($n=13$)		P-value[a]	
	0 week	12 weeks		0 week	12 weeks		P-value[b]
Breathing	0.2±0.3	0.0±0.0	0.008**	0.1±0.2	0.2±0.4	0.387	0.027*
ECG	0.4±0.4	0.3±0.5	0.613	0.4±0.4	0.5±0.5	0.613	0.470
Valsalva	0.3±0.4	0.4±0.4	0.570	0.3±0.4	0.6±0.5	0.022*	0.340
Upright	0.2±0.2	0.1±0.2	0.337	0.0±0.1	0.1±0.2	0.337	0.192
Handgrip	0.0±0.0	0.0±0.0	–	0.0±0.0	0.0±0.0	–	–
T_score	1.1±0.8	0.8±0.7	0.279	0.8±0.6	1.4±1.0	0.073	0.035*

[a]Abnormal = 1, borderline = 0.5, normal = 0; All values are presented as mean ± SD

[b]Analyzed by paired *t*-test, independent *t*-test, *$P<0.05$, **$P<0.01$

and the traditional products are released to market, and some consumers prefer the traditional fermented soybean products even though there are some differences between the factory-produced and traditional products. Figure 11.5 shows pictures of *Doenjang*.

11.2.7 *Gochujang*

Gochujang uses very hot-flavored red pepper as an extra ingredient differed from other fermented soybean products, and some grains, such as rice and barley, are added to *Gochujang* for supplementing a sweet taste through saccharifying it. For producing *Gochujang*, a specific type of *Meju* that is made of mixing rice and soybeans with a ratio of 6:4 is required. Then, the mixture is steamed, formed, and fermented. The red pepper, the major extra ingredient, is originated from South America and has been introduced to Korea in the sixteenth century or theretofore (Kwon et al. 2011). *Gochujang* is a unique fermented spice only in Korea and has been deeply rooted in Korean tables.

11.2.8 Preparation Method

For producing *Gochujang*, it requires *Gochujang Meju* differed from other fermented soybean products. *Gochujang Meju* is a mixture of soybeans and starch, and it contains enzymes pro-

Fig. 11.5 Aging of *Doenjang* and its final product

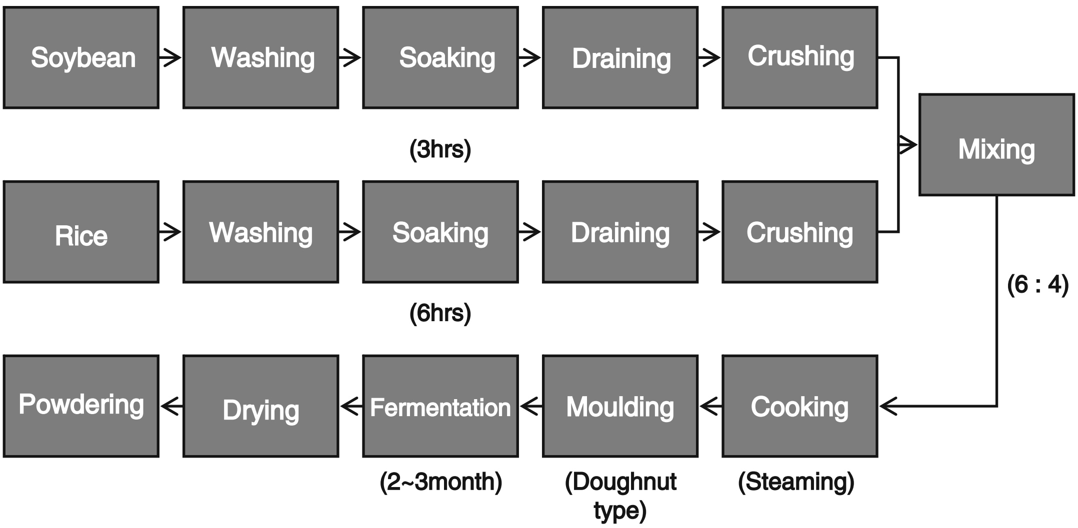

Fig. 11.6 *Gochujang Meju* preparing method

duced by naturally proliferated microorganisms according to compositions of matrices (Shin et al. 2012) (Fig. 11.6).

Gochujang has been produced by both traditional and industrial methods. The *Gochujang Meju* is used to the traditional method, but in the industrial method, Koji produced by using pure strains is used.

The *Gochujang* produced by the process presented in Fig. 11.7 is used as a spice for various foods, and a large amount of *Gochujang* is used as seasoning to Korean foods compared to other countries' spices. *Gochujang* represents a paste-type product and can be used in different types of foods like dressings, seasoned bar rice cakes, stews, and so on. *Gochujang* gives a balance to foods by its own hot-flavored taste as well as sweet and savory tastes.

11.2.9 Microorganism

Gochujang uses more diverse ingredients compared to *Doenjang*. The species and genus of microorganisms in the traditional *Gochujang*, which contains a large amount of red pepper

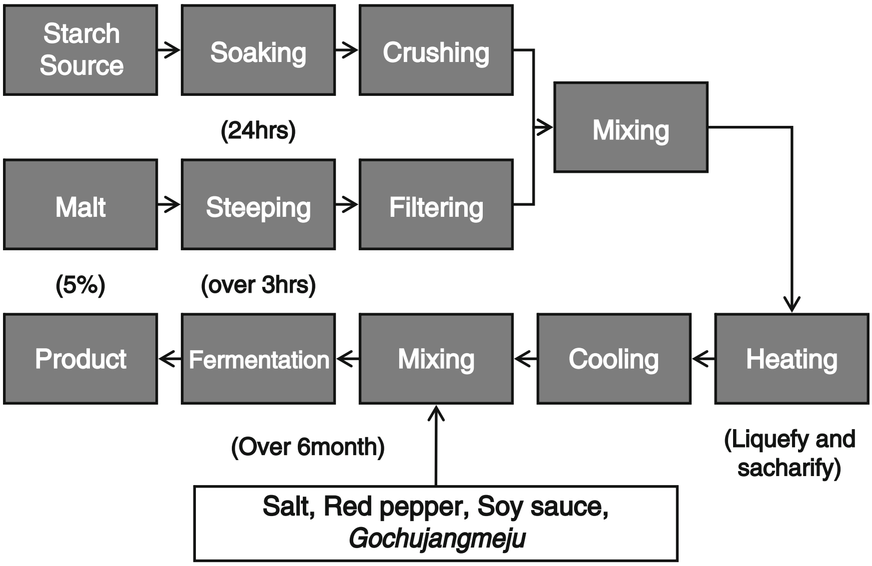

Fig. 11.7 General procedure for making *Gochujang*

powders, malts, grains, *Ganjang*, and soybeans, are largely distributed (Kwon et al. 2009).

As the factory-made *Gochujang* is processed using Koji, which is made by a pure strain, the strain is relatively simple and the predominant strain is known as *B. subtilis*. The microbial load in *Gochujang* is about 10^7–10^8/g, and the frequency of presenting the microorganisms is determined as *Bacillus velegensis*>*B. amyloliquefaciens*>*B. subtilis*, and the *Oceanobacillus* is also detected in addition to the halophilic microorganisms determined as *B. cheniformis*>*B. subtilis*>*B. velezensis*>*B. amylo-licheniformis* (Nam et al. 2012). Yeasts are *Zygosaccharomyces* and *Candida lactis*, and *Z. rouxii* shows the highest detection rate (Park 2009). *Aspergillus* is the predominant strain of molds and *Penicillium* and *Rhizopus* are also detected (Kim et al. 2013). These molds are usually proliferated on the surface of *Gochujang* instead of the inside (Kim et al. 2013).

11.2.10 Nutritional Composition

The capsaicin from red pepper, which represents its color and hot-flavored taste, and some sugars from grains significantly affect the taste and nutrition. The general ingredients of *Gochujang* (Table 11.2) consist of 44.6 % of moisture, 4.9 % of protein, 1.1 % of fat, 8.2 % of ash, 43.8 % of carbohydrate, 40 mg% of calcium, 90 mg% of phosphors, 2.2 mg% of iron, and 82 mg% of calcium (RDA 2011). Vitamins A, B_1, B_2, C, and niacin are also included. Particularly, it shows the highest content of β-carotene, 2445 μg (RDA 2011).

The content of saccharides in the nutritional composition of *Gochujang* is about 43–59 %, in which the contents of glucose and maltose are high (Shin et al. 2011). Various free amino acids are detected, and the high contents of the glutamic acid and proline affect the taste of *Gochujang*. The major component of the nucleic acids is CMP (Shin et al. 2011). The capsaicin that gives the characteristic taste of *Gochujang*, a hot-flavored taste, is included about 200–300 mg% (Choi 2004).

11.2.11 Functionality and Health Benefits

Gochujang represents different functionalities by both the capsaicin generated from red pepper and some other fermentation products.

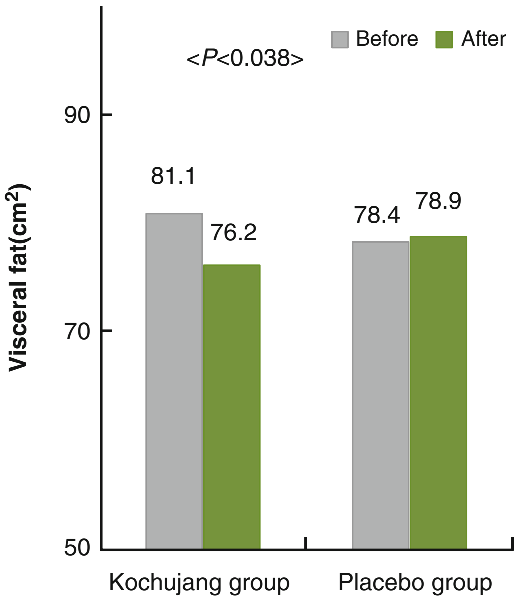

Fig. 11.8 Changes in visceral fats

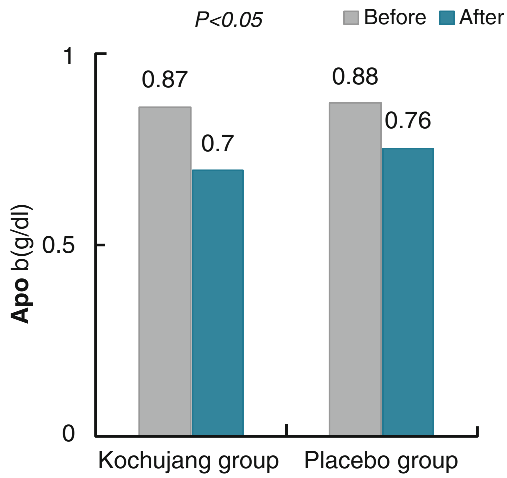

Fig. 11.9 Changes in Apo B

Functionalities in traditional *Gochujang* are improved during the fermentation process. *Gochujang* shows anticancer effects due to its antioxidant characteristics (Song et al. 2008). Inhibition effects on the proliferation of stomach cancer cells are verified (Song et al. 2008), and an antitumor effect is also identified (Park et al. 2001). Remarkable effects of a *Gochujang* diet are determined as antiobesity (Cha et al. 2012) and blood sugar control functions. In a recent experiment on administering 32 g *Gochujang* pills to overweight and obese adults for 12 weeks, the level of visceral and subcutaneous fats is significantly decreased compared with a placebo group (Cha et al. 2013) as shown in Figs. 11.8 and 11.9.

TC and LDL-C levels in blood are decreased by *Gochujang* pills (32.0 g/day) are applied (Kim et al. 2010). *Gochujang* also shows an effect of stabilizing the autonomous nervous system (Table 11.6) (Im 2013).

Consumption of *Gochujang* decreases breathing rates of hyperlipidemia patients, which represent a difference in heart bits between expiration and inspiration in repetitive deep breathing, and releases stress (Im 2013).

11.2.12 Ethnic Value and Socio-economy

Gochujang has been introduced in the latest of fermented soybean products but is remarkably increased in its use as a seasoning source that corresponds with the Korean taste preferring a hot-flavored spice of *Gochujang*. The personal daily intake of *Gochujang* is about 6.4 g, and the gross domestic product is 141,027 tons (MFDS 2013). Also, it has been exported to Japan, America, Europe, and other countries, and the amount of exports is about 9,571 tons (MFDS 2013). The exports and regions have recently been expanded. In addition, the production has been changed from one-man based businesses to enterprise scales according to changes in social conditions. Recently, its commercialization rate is recorded about 80–90 %. The rate will be increased further, and it is expected that the amount of homemade traditional *Gochujang* will be largely decreased (MFDS 2012).

Figure 11.10 shows *Gochujang Meju* and a final product.

11.2.13 *Cheonggukjang*

Cheonggukjang requires the shortest fermentation period and the highest fermentation tempera-

Fig. 11.10 Pictures of *Gochujang Meju* and a final product

ture (40–43 °C) compared to other fermented soybean products. It is a product without adding any extra ingredients compared to other fermented soybean products and has a unique flavor and a peculiar taste. Although it is similar to Japanese *Natto* and Indian *kinema*, it shows differences in production methods, applied microorganisms, and ways of intakes (Lee et al. 2011b; Tamang 2015).

11.2.14 Preparation Method

In producing *Cheonggukjang*, well-matured soybeans are prepared and washed. Then, the soybeans are fully steeped in water at 10–16 °C and are steamed and dehydrated in order to undergo fermentation (Shin 2011). The process can simply be presented in Fig. 11.11.

The fermentation of *Cheonggukjang* is performed at around 40–43 °C, and *Bacillus* strains are selectively proliferated. As the fermentation is completed within a short period of time, 1–2 days, it shows lots of adhesive and sticky substances. After completing the fermentation of *Cheonggukjang*, the preparation can be finished by adding some salts and spices including garlic. Recently, *Cheonggukjang* has been distributed as a frozen product with a specific size and shape (Shin 2011).

11.2.15 Microorganism

Cheonggukjang that has a long historic background is produced through natural fermentations and has still been produced on home or small business scales (Chung et al. 2006). In Japan, *Natto* strains were separated in 1913 by Dr. S.Sawamura and named as *Bacillus natto* (Hosoi and Kiuchi 2008). It is also known that the predominant strain of *Cheonggukjang* is *Bacillus subtilis* (Shin 2011).

Recently, new strains that can reduce the unique odor in *Cheonggukjang* significantly have been developed for business purposes (Kim et al. 2003b, c). The new strains related to *Cheonggukjang* are identified to use sucrose, fructose, and so on as a carbon source. Also, it contributes to production of adhesive and sticky substances in *Cheonggukjang* (Baek et al. 2010).

Although all vegetative cells are inactivated through the cooking process in producing *Cheonggukjang*, spore formers survive and lead to the fermentation of *Cheonggukjang* (Kwon et al. 2006). The amount of biogenic amines, which are harmful components produced during the fermentation process, can be reduced by selecting a specific strain (Cho et al. 2006a).

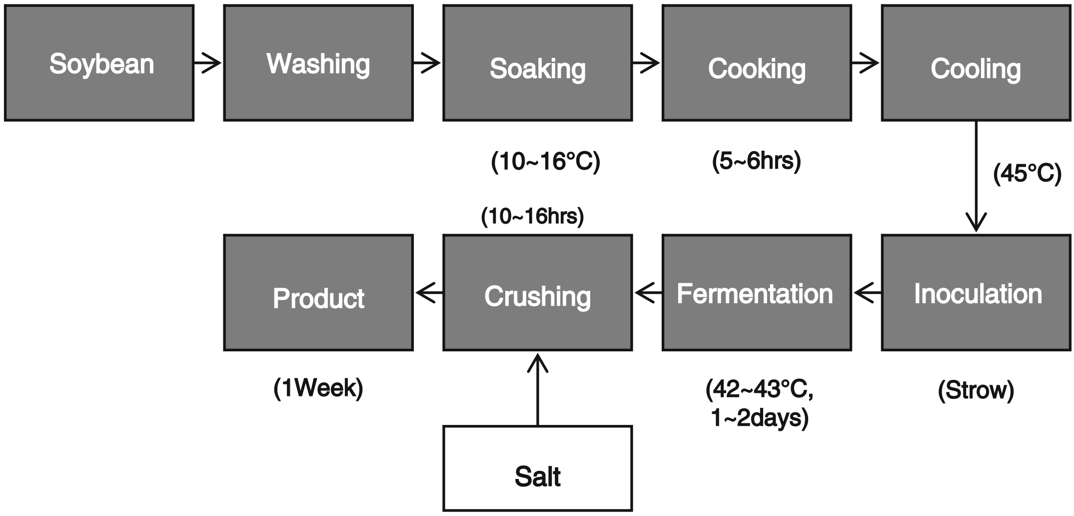

Fig. 11.11 *Cheonggukjang* preparing method

Table 11.7 Proximate composition of *Cheongkukjang*

Product	Protein (g)	Lipid (g)	Carbohydrate (g)	Fiber (g)	Ca (mg)	Fe (mg)	K (mg)	B1 (mg)	B2 (mg)
Steamed soybean	16.0	9.0	7.6	2.1	70	2.0	570	0.22	0.09
Cheongkukjang	16.5	10.0	9.8	2.3	90	3.3	660	0.70	0.56

11.2.16 Nutritional Composition

Although *Cheonggukjang* is originated from soybeans, it produces own flavor and taste with various substances after the completion of the fermentation process. Table 11.6 shows the comparison of general composition between soybeans and fermented *Cheonggukjang* (Kim and Hahm 2002) (Table 11.7).

Amino acids that remarkably affect flavors after the completion of a fermentation process are mostly produced by proteases, which is produced by the involved strains; in particular, there are huge increases in amino acids and sticky substances (Back et al. 2008). The amino acids of the highest content are glutamic acid and alanine (Back et al. 2008). *Cheonggukjang* shows an increase in pH during its fermentation; specifically shows pH over 8 after 24 h of fermentation (Joo and Oh 2009).

11.2.17 Functionality and Health Benefits

Cheonggukjang increases or newly produces different functional ingredients such as dietary fibers, phospholipids, isoflavones, phenolic acids, polyglutamic acids (gamma PGA), and saponins, through its fermentation (Sung et al. 2005). It has been known that these special ingredients shows improvements in antiobesity, blood sugar control, thrombolytic activity, blood pressure control, lipid, and intestine and immune functions (Kim et al. 2009a). The comparison of the increased and newly produced ingredients through the fermentation of *Cheonggukjang* is presented in Table 11.8 (Kwon et al. 2010).

As shown in Table 11.7, there are increases and new productions of different functional ingredients, and the digestion rate is significantly

Table 11.8 Bioactive compound content in cooked soybean and *Cheonggukjang*

Phytochemical			Steamed soybean	*Cheongkukjang*
Isoflavone	Glucoside	Daidzin	15–57	79–93
		Genistein	36–86	87–91
		Glycitein	2–6	10–12
	Aglycone	Daidzein	0.3–5	4–7
		Genistein	0.2–5	3–4
		Glycitein	0.1–0.6	11–13
Gamma-PGA			–	Ç
Ammonia			–	Ç
Protein absorption (%)			65	95

Table 11.9 Passage time of feces through large intestine before and after the ingestion of *Cheonggukjang*

Large intestine part	Before ($n=10$)	After ($n=10$)	*P*-value
Rt. colon	15.0 ± 14.3[a]	10.2 ± 11.9	0.313
Lt. colon	8.4 ± 9.4	6.9 ± 8.2	0.549
RS. colon	19.2 ± 13.4	9.1 ± 7.8	0.008**
Total colon	42.2 ± 20.1	26.2 ± 20.3	0.000***

**$p<0.01$, ***$p<0.001$
[a]Mean ± SD

increased (Kwon et al. 2010). Increases in these functional ingredients inhibit the proliferation of tumor cells and delay the segmentation of tumor cells (Kwak et al. 2002). In particular, it shows an effect of improving constipation through improving movements of intestines. That is, *Cheonggukjang* has lots of insoluble and soluble fibers, which leads to improving the viscosity of the contents of intestines (Lee and Hwang 1997; Kim et al. 2006). In addition, it reduces the passage time of feces through intestines by improving the function of the large intestine (Table 11.9) and increases the layers of beneficial enterobacteria (Kim 2009; Lae 2005). In particular, *Cheonggukjang* has many beneficial bacteria, and these bacteria promote the peristaltic motion of intestines. Also, it plays a role in prebiotics of lactic acids (Park et al. 1998a).

11.2.18 Ethnic Value and Socio-economy

Cheonggukjang is one of the fermented soybean products with a unique flavor and is usually enjoyed in winter season as a pot stew together with boiled rice. Also, it has savory tastes that are preferred by Koreans. In recent years, *Cheonggukjang* is consumed all year round (Shin 2011). Although there is little consumption in the younger generation due to its pungent odor, there are steady demands in the elders. According to developments of production technologies, there are new products that almost remove such odor, which lead to increased consumptions. The annual gross production of *Cheonggukjang* is 8,388 tons (MFDS 2013), and some products are exported (MFDS 2013). Although the recent major consumers are ethnic Koreans, there is a possibility of increasing demands of the natives through increasing consumers and improving its flavors. Figure 11.12 shows *Cheonggukjang*.

11.2.19 *Ganjang*

Ganjang has largely been used in North- Eastern Asian countries, such as Korea, Japan, and China, including some Southeast countries. It is one of the fermented soybean products and has been known throughout the world called a soy sauce. Traditional *Ganjang* in Korea is made by separating the liquid part in which the mixture of *Meju* and brine is aged for 3–4 months. The liquid part is used as an undiluted solution (Fig. 11.2). In the case of the commercially produced *Ganjang*, it uses Koji, which is made of soybeans and flour, as the major ingredient. Then, it is steeped in brine and is fermented and aged. The fermented

Fig. 11.12 Pictures of *Cheonggukjang* and cuisine

product is used as an undiluted solution of *Ganjang* after filtration. *Doenjang* is not processed in the same way.

The traditional *Ganjang* shows a savory taste because the soybean protein is dissolved into amino acids by the enzymes produced by different microorganisms in the fermentation process of *Meju*. In the case of the factory-made *Ganjang*, however, its taste is presented by dissolving the soybeans and wheat starch during the fermentation process by the enzymes usually produced by *Aspergillus* and *Bacillus subtilis* (Liu et al. 2015).

Recently, demands for factory-made products largely exceed the traditional products. The traditional products have limitedly been used in specific consumers and purposes and, its demands is limited. While there are some studies on the functionality of *Ganjang*, an effort of decreasing salt has been made at a national level in order to avoid its high salt content (Song et al. 2015).

11.3 Fermented Vegetable Products

Kimchi is a general and collective term used to call a group of fermented vegetable foods in Korea. It is a traditional Korean dish of salted and fermented vegetables, such as *Kimchi* cabbage (*Baechu*, Chinese cabbage), white radish, and cucumber. It is freely seasoned with a variety of ingredients, including red pepper powder, garlic, green onion, ginger, *jeotgal* (salted and fermented seafoods), and other possible ingredients, and then it is allowed to ferment for a period of time (Park 1994; Codex Alimentarius 2001). Over millenniums of tradition, *Kimchi* has remained a classic delight for generations in all regions of Korea, and now it is enjoyed by a growing number of *Kimchi* lovers over the world. *Kimchi* is an epitome of "slow foods." It is a food which requires maturation and fermentation, for which the right combination of earth, water, wind, and sunlight is critical. *Kimchi* is a creation brimmed with life and perfected by the motherly hand of nature. *Kimchi* is a gift from Korean forebears who have learned the art of fermentation and understood its benefits to human well-being.

The history of *Kimchi* in Korea has been thought to be more than 3,000 years old, but unfortunately, the literature record to support this could not be found in Korea (Lim et al. 2014; Kim et al. 2014a, b). The first record of salted vegetable pickles in Korea that might be an origin of *Kimchi* appeared in *Dongkukisangkukjip* written by Lee Gyu-bo in 1241 (Park 2013). In the period of Goryeo dynasty (918–1392 AD), the pickles were modified to make several types of *Kimchi* using common raw materials to fit the taste of Koreans. Until the Goryeo dynasty, the main vegetable to make *Kimchi* was a radish (Lim et al. 2014; Kim et al. 2014a, b). Records also showed that in addition to the radish, a cucumber and an eggplant were used as the main vegetables of various types of *Kimchi*. Then in the period of the Joseon dynasty (seventeenth century), *Pogi Baechu Kimchi* (whole cabbage *Kimchi*) and other types of *Kimchi* which were prepared using hot red peppers became popular (Lim et al. 2014; Kim et al. 2014a, b). The peppers were introduced to Korea in the early seventeenth century. The first record of using red pepper for *Kimchi* appeared in 1670, and salted and fermented seafoods (*Jeotgal*) were also reportedly used at this time (Park 2007, 2013).

Traditionally, natural fermentation techniques have been applied to agricultural and marine products for their long-term preservation, because those raw materials have short shelf lives in the warm and hot seasons. *Kimchi* is an excellent example of how vegetables like *Kimchi* cabbage and radish can be stored for a long time in an ordinary household, especially during the long winter season when fresh vegetables are scarce. One of the most important household events in Korea is *Kimjang*, which is a preparation of bulk of *Kimchi* in the late fall. During *Kimjang* season, Koreans make a lot of *Kimchi* at once, which is prepared by housewives with assistance of neighbors. The *Kimjang*'s culinary art has been passed on to the next generation through experiences and practices. It is a time-honored tradition for Koreans, in which they get together with family, friends, and neighbors to make and share pots of *Kimchi*. The *Kimchi*-making drives, a nationwide charity campaign unique to Korea, stemmed from this tradition. Every November, a string of *Kimchi* making and sharing events take place across the nation. *Kimchi* prepared by volunteers is delivered to the underprivileged in the hope of making the coming winter a little warmer for them. The *Kimjang* culture (*Kimjang*: making and sharing *Kimchi* in Korea) was listed in the Representative List of the Intangible Cultural Heritage of Humanity of UNESCO (Dec. 5, 2013), and its cultural value has been recognized internationally as the spirit of sharing with community, which is inherent in *Kimchi* and *Kimjang* (Lim et al. 2014; Kim et al. 2014a, b).

11.3.1 *Pogi Baechu Kimchi* (Whole Cabbage *Kimchi*)

Many types of *Kimchi* are available depending on the raw materials and processing methods as shown in Table 11.10 (Jo 2000; Seo et al. 2013). *Kimchi* cabbages and radishes are most widely used as the main vegetables for making *Kimchi*, but many other vegetables are also used depending on their availability. Since the northern part of Korea is cold and the southern part is mild in winter, the winter *Kimchi* prepared in the northern part contains less salt for preservation, whereas the one prepared in the southern part requires more salt. And people living near the sea tend to add seafoods, which are naturally abundant in that area, to *Kimchi. Kimchi* is largely classified into two main types: winter *Kimchi* and seasonal *Kimchi*. Winter *Kimchi* includes *Pogi Baechu Kimchi* (whole cabbage *Kimchi*), *Dongchimi* (whole radish *Kimchi*), *Chonggak Kimchi* (ponytail radish *Kimchi*), and *Kkakdugi* (diced radish *Kimchi*) as shown in Fig. 11.13. The winter *Kimchi* types have been prepared in early winter and consumed throughout the winter until spring vegetables are available. In recent years, however, vegetables are available year round owing to developments of new culture technologies including a greenhouse system. Thus, it is no longer necessary to prepare a large stock of *Kimchi* to last throughout the winter seasons.

There are many types of the seasonal *Kimchi* depending on the availability of seasonal vegetables. In the spring, *Nabak Kimchi* (young cabbage and sliced radish *Kimchi*) has a refreshing taste; during the summer, *Oisobagi* (cucumber *Kimchi*) and *Yeolmu Kimchi* (baby radish *Kimchi*) are the most popular; and for the fall season, *Pogi Baechu Kimchi*, *Kkakdugi*, and *Chonggak Kimchi* are favorites. In this section, *Kimchi*, mainly the *Pogi Baechu Kimchi*, which is the most representative type of *Kimchi* and consumed most in Korea, will be introduced and discussed.

Table 11.10 List of representative *Kimchi* types[1]

Vegetable families	Main parts used	Types of *Kimchi*		
		Mixed	Stuffed	Watery
Brassicaceae (18)	Leaves (12)	*Matt* (sliced cabbage) *Kimchi*, *Geotjeori* (unfermented cabbage *Kimchi*), *Gat* (mustard leaves) *Kimchi*, *Yeolmu* (young leafy radish) *Kimchi*, *Mucheong* (radish leaves) *Kimchi*, *Yuchae* (young rape leaves) *Kimchi*	*Baechu Kimchi*, *Mukeunji* (ripened cabbage) *Kimchi*, *Bossam* (stuffed wrapped) *Kimchi*	*Baek* (white) *Kimchi*, *Yeolmumul Kimchi* (young leafy radish), *Gatmul Kimchi* (mustard leaves)
	Roots (3)	*Kkakdugi* (cubed radish) *Kimchi*	*Bineul* (stuffed radish) *Kimchi*	*Dongchimi* (watery radish) *Kimchi*
	Roots and leaves (3)	*Chonggak* (ponytail radish) *Kimchi*, *Seokbakji* (sliced radish and cabbage) *Kimchi*	–	*Nabak* (watery sliced radish and cabbage) *Kimchi*
Liliaceae (5)	Leaves (4)	*Pa* (welsh onion) *Kimchi*, *Buchu* (garlic chives) *Kimchi*, *Sanmaneul* (alpine leek) *Kimchi*, *Dallae* (wild garlic) *Kimchi*		
	Roots (1)	*Yangpa* (onion) *Kimchi*		
Cucurbitaceae (3)	Fruit (3)	*Oi* (cubed cucumber) *Kimchi*, *Bak* (*Lagenaria*) *Kimchi*	*Oisobagi* (cucumber) *Kimchi*	–
Miscellaneous (15)	Fruit (3)	*Gaji* (eggplant) *Kimchi*	*Gochusobagi* (green pepper) *Kimchi*	–
	Leaves (9)	*Godeulppaegi* (*Youngia*) *Kimchi*, *Kkaennip* (perilla leaves) *Kimchi*, *Mindeulle* (dandelion) *Kimchi*, *Gom-chewi* (*Ligularia fischeri*) *Kimchi*, *Ppongip* (mulberry leaves) *Kimchi*, *Gogumasun* (sweet potato stalks) *Kimchi*, *Gochuip* (green pepper leaves) *Kimchi*, *Danggwi* (*Ligusticum acutilobum* leaves) *Kimchi*, Dureup (*Aralia* shoots leaves) *Kimchi*		
	Roots (3)	*Danggwi* (*Ligusticum acutilobum*) *Kimchi*, *Deodeok* (*Codonopsis lanceolata*) *Kimchi*, *Kkakdugi* (cubed yam) *Kimchi*		

[1]Reference: Seo et al. (2013)

Fig. 11.13 Typical types of winter *Kimchi* including (**a**) *Pogi Baechu Kimchi* (whole cabbage *Kimchi*), (**b**) *Dongchimi* (whole radish *Kimchi*), (**c**) *Chonggak Kimchi* (ponytail radish *Kimchi*), and (**d**) *Kkakdugi* (diced radish *Kimchi*)

11.3.2 Preparation Method

Many different recipes have been published, and fermentation methods have also been "invented" for making *Kimchi*. The basic taste of *Kimchi* is derived from a combination of salt, various organic acids from fermentation of vegetables, spices (including hot red pepper, garlic, ginger, and green thread onion), and seafoods, fermented (*Jeotgal*) or fresh. The principal process of making *Kimchi* is first to salt major raw materials such as *Kimchi* cabbage, radish, or cucumber under proper conditions; then to blend fruits or cereals, if necessary, spices, seasonings, and fermented seafood products (optional) to the salted major materials; and then finally to subject the blended materials to lactic acid fermentation.

A typical manufacturing process for making *Pogi Baechu Kimchi* is shown in Fig. 11.14. The basic processes for making *Kimchi* are as follows. Step 1, major ingredients such as *Kimchi* cabbage and/or radish are brined under NaCl concentration of about 8–15 % for 2–18 h, depending on the type of vegetables, at room temperatures. Step 2, the salted vegetables are washed with freshwater and drained to remove excess water. They may or may not be cut into suitable sized pieces/parts. Step 3, the prepared vegetables are mixed with seasoning mixture mainly consisting of red pepper (*Capsicum annuum* L.) powder, garlic, ginger, edible allium varieties other than garlic, and radish. These ingredients may be chopped, sliced, and broken into pieces. Typical ingredients for *Pogi Baechu Kimchi* are shown in Table 11.11. Step 4, the fresh *Kimchi* are packaged into appropriate containers and fermented at low temperatures for a few days (Cho et al. 1998a, b; Choi 2004; Codex Alimentarius 2001). These processes let the vegetables undergo a unique process of naturally mixed lactic acid fermentation. During *Kimchi* fermentation at low temperature (from 0 °C to room temperatures), lactic acid bacteria from nature can prosper and dominate other microorganisms because of producing organic acids such as lactic acid. As a result of fermentation, properly fermented *Kimchi* contains lots of lactic acid bacteria and organic acids as yogurt. Therefore, during storage and transportation of *Kimchi*, it is recommended that the temperature be kept at −3 °C to 4 °C (Choi et al. 1998a, b).

Kimchi is characterized with its palatability giving sour, sweet, and carbonated tastes and is very different from sauerkraut, which is a popular fermented vegetable product in the West (Steinkraus 1993). There are some differences in the ingredients and manufacturing methods between Korean *Kimchi* and the fermented vegetable products of other countries. During salting and washing of *Kimchi* cabbage, there is a unique process to mix all sorts of spices and supplementary ingredients such as green onions, garlic, red pepper, ginger, radish, or *Jeotgal*. While going through the processes, the salted cabbages have effectively mingled with the taste of seasoning. Salting processes, including washing and draining the salted vegetables, provide the special conditions in which non-halophile putrefactive and pathogenic bacteria seldom grow, and the crisp texture of *Kimchi* is maintained during its fermentation. Mixing all sorts of spices and supplementary ingredients will give various flavors and functionalities (Cho et al. 1998b).

In the practices to manufacture *Pogi Baechu Kimchi*, changes in several processing factors and some chemical components occurred during production as shown in Tables 11.12 and 11.13, respectively. *Pogi Baechu Kimchi* were made with raw *Kimchi* cabbages (cultivar name: *Namdo-janggun*) which were cultivated in the southern area of Korea, harvested on Dec. 7, 2014, and stored at 1 °C for 1 week after precooling for 3 days. The methods to make the *Kimchi* are as shown in Fig. 11.14 and Table 11.11. As shown in Table 11.12, the pretreatment and salting yields of raw cabbage were estimated to be 66.7 % and 84.7 %, respectively. The production yields of salted cabbages and *Kimchi* products were also estimated to be 56.6 % and 50.0 %, respectively. As shown in Table 11.13, while raw cabbages were processed to make fresh *Kimchi* products, water content was decreased from 93.1 % to 83.9 % and pH was lowered from 6.13 to 5.75. On the other hand, titratable acidity,

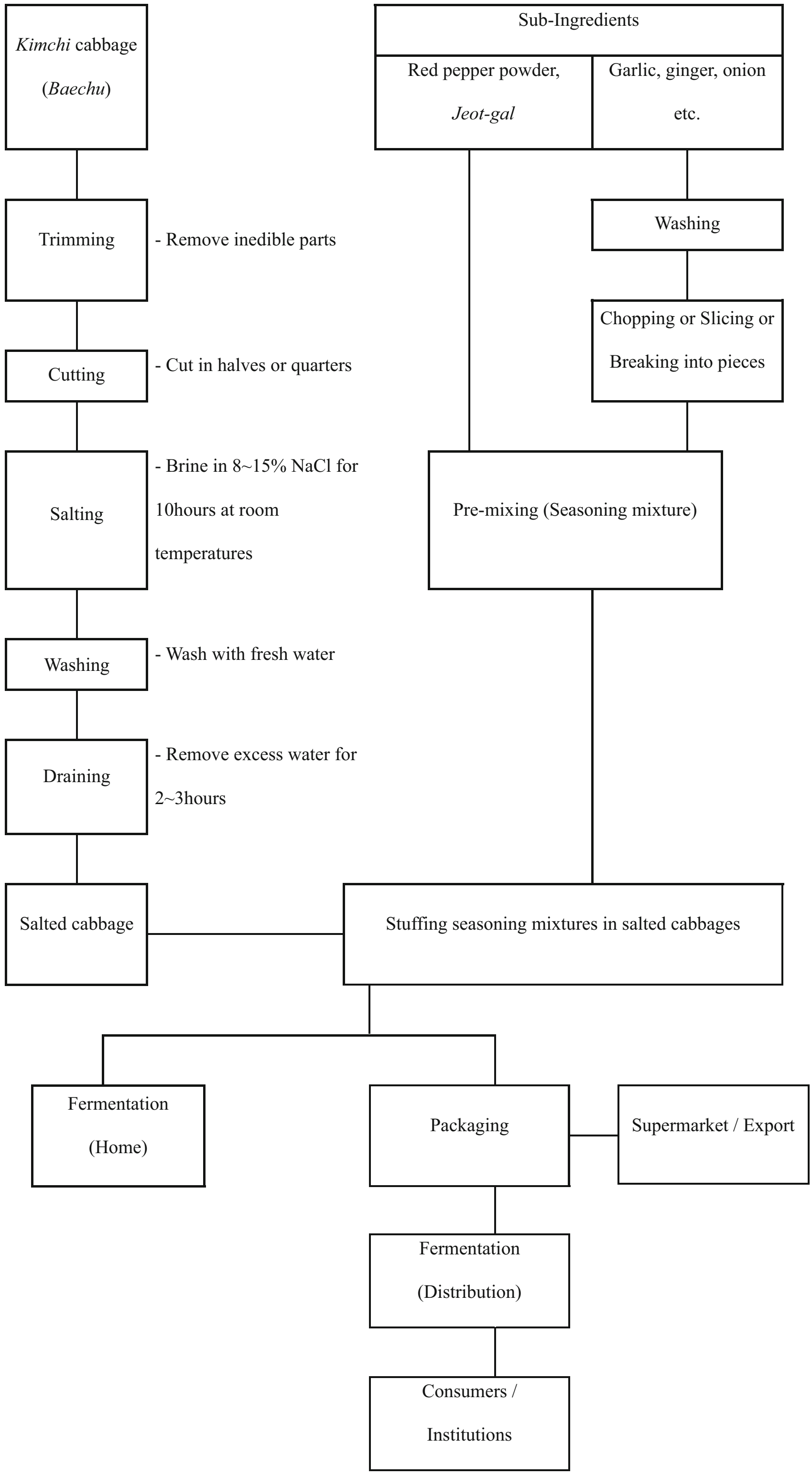

Fig. 11.14 A typical manufacturing process for making *Pogi Baechu Kimchi*

Table 11.11 Typical ingredients for *Pogi Baechu Kimchi*

Components	Descriptions	Ratio (%)
Salted *Kimchi* cabbage	Salt contents: 1.5–2.0 %(w/w)	69.2
Sliced radish		6.9
Chopped garlic		1.4
Powdered red pepper		4.2
Sliced green onion		1.7
Chopped ginger		0.6
Sliced onion		0.7
Sliced dropwort		0.1
Mixed meat extract	Boil dried walleye pollock, radish, onion, green onion, dried red pepper, and dried sea tangle in the proper amount of water	5.5
Mixed *Jeotgal* (fermented seafoods)	Mix anchovy *Jeot-gal* and shrimp *Jeotgal* according to preference	5.4
Glutinous rice paste	Make a paste of glutinous rice powder and water with a ratio of 1:10	2.3
Chopped apple		1.7
Sea staghorn	Wash and wet dried sea staghorn before use	0.3
Total		100

Table 11.12 Changes in several processing factors during production of *Pogi Baechu Kimchi*

Processing factors	(%)
Pretreatment yield of raw cabbages	66.7
Waste portion	33.3
Salting yield	84.7
Production yield of salted cabbages	56.6
Production yield of *Kimchi*	50.0

Table 11.13 Changes in some chemical components during production of *Pogi Baechu Kimchi*

Components	Raw cabbages	Salted cabbages	Fresh *Kimchi*
Water (%)	93.13	90.16	83.91
pH	6.13	6.26	5.75
Titratable acidity (%)	0.17	0.17	0.40
Salinity (%)	(Not detected)	1.52	1.77
Reducing sugars (%)	28.64	30.58	45.75

salinity, and content of reducing sugars were increased from 0.17 % to 0.40 %, from 0.0 % to 1.77 %, and from 28.6 % to 45.8 %, respectively.

11.3.3 Microorganism

Kimchi fermentation is carried out by various microorganisms presented in raw materials and ingredients in *Kimchi*. There exist small numbers of lactic acid bacteria in the raw materials, but they rapidly dominate the *Kimchi* fermentation because of anaerobic and low-temperature conditions including the presence of NaCl (1.5–3.0 %), which favor their growth. Those conditions can also suppress the growth of other bacteria. Small numbers of lactic acid bacteria such as *Leuconostoc* spp. and *Lactobacillus* spp. are presented in the initial phase of *Kimchi* fermentation, but they dominate during the fermentation. Yeasts and fungi and coliform bacteria are detected in the early stage, but they disappeared during fermentation of *Kimchi* as shown in Fig. 11.15 (Chang et al. 2011; Chung et al. 1997; Seo et al. 2013). At present, lactic acid bacteria belonging to the genera *Leuconostoc*, *Weissella*, and *Lactobacillus* are believed to be key players in the natural *Kimchi* fermentation (Kim and Chun 2005; Cho et al. 2006a, b; Park et al. 2010).

At the same time, sugars present in those ingredients are converted to several organic acids such as lactic acid by those lactic acid bacteria during the fermentation, and these acids are responsible for the taste of *Kimchi* (Kim et al. 1998). The rate of *Kimchi* fermentation is markedly affected by salt concentration and fermentation temperature, and it has been reported that optimum time for taking a sliced *Baechu Kimchi* is determined as it contained 0.6–0.8 % titratable acidity (pH 4.2), 1.5–2.5 % salt content, and high volatile organic acids (Mheen and Kwon 1984; Cho et al. 1998a, b). However, as fermentation characteristics of three main types of cabbage *Kimchi* (sliced cabbage *Kimchi*, whole cabbage *Kimchi*, and watery whole cabbage *Kimchi*) were compared in detail, a relationship between pH and titratable acidity (so-called pH-acid curve) showed quite differences according to Kimchi

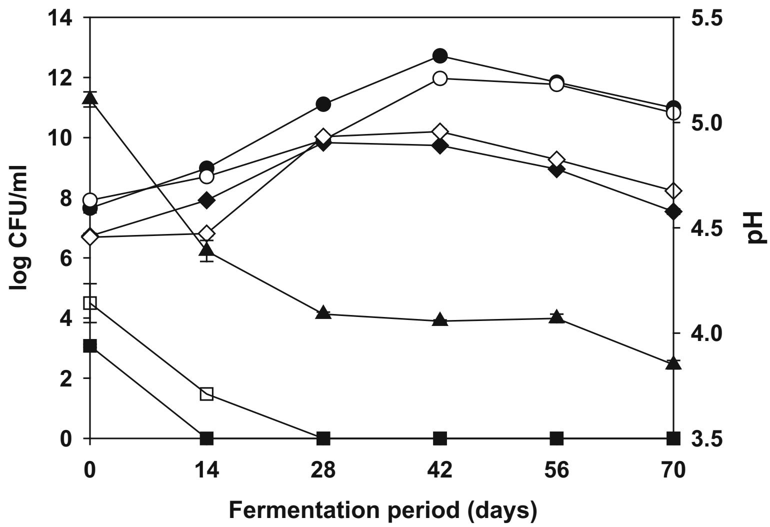

Fig. 11.15 Microbial population dynamics of *Baechu Kimchi* during the fermentation at 4 °C (Seo et al. 2013). ▲, pH; ●, aerobic bacteria; ○, lactic acid bacteria; ◆, *Leuconostoc* spp.; ◇, *Lactobacillus* spp.; ■, yeast and fungi; □, coliform bacteria

types (Park et al. 1996). The similar results were obtained from a study on three main types of radish *Kimchi* (sliced radish *Kimchi*, whole radish *Kimchi*, and watery whole radish *Kimchi*) (data not shown).

After a late period of fermentation, several unfavorable phenomena such as formation of excessive acids, degradation of pectic substances, and development of off-flavors occur due to the growth of aerobes. These changes are called an over-ripening of *Kimchi*, which is often observed in the winter *Kimchi* stored for an extended period and also in the summer *Kimchi*. The over-ripening is the most serious problem in the storage of *Kimchi*. Since the over-ripening of *Kimchi* is mainly due to the activities of microorganisms forming lactic acid, the best way to overcome this problem would be to control their growth without destroying *Kimchi* quality. On the other hand, softening of *Kimchi* is due to degradation of the pectic substances in vegetable tissues by polygalacturonase from raw materials or microorganisms in *Kimchi*.

As explained above, natural *Kimchi* fermentation with unsterilized raw materials leads to the growth of various microorganisms including the lactic acid bacteria, which results in variations in the taste and quality of *Kimchi* product. Therefore, the use of selected starter cultures is considered as an alternative for commercial production of the standardized *Kimchi* with high quality (Kim et al. 1998). Now, the potential lactic acid bacteria including *Leuconostoc mesenteroides*, *Leu. citreum*, and *Lactobacillus plantarum* have been used as microbial additives for commercial *Kimchi* production in Korea.

11.3.4 Nutritional Composition

The nutritional composition of *Kimchi* varies depending on the main ingredients. An example of the nutritional composition in a typical type of *Kimchi* is shown in Table 11.14. Sugars present in *Kimchi* are glucose, mannose, fructose, galactose, and arabinose (Ha et al. 1989). During the fermentation of *Kimchi*, sugar compounds are dissolved slowly and move to the juice from the tissue. Not only are sugars nutritionally important, but they are also the taste components. *Kimchi* also contains considerable amounts of amino acids (Hawer et al. 1988). The amino acids in *Kimchi* are its important taste components and are originated from the proteinaceous raw materials, such as seafoods including fermented anchovy, shrimp, oyster, etc. *Kimchi* with seafoods contains remarkably higher amount of glutamic acid, arginine, lysine, aspartic acid, and alanine than *Kimchi* without seafoods.

Table 11.14 An example of the nutritional composition in a typical type of *Kimchi*

Nutrients	Per 100 g of edible portion
Food energy (Kal)	32
Moisture (g)	88.4
Crude protein (g)	2.0
Crude lipid (g)	0.6
Total sugar (g)	1.3
Crude fiber (g)	1.2
Crude ash (g)	0.5
Calcium (mg)	45
Phosphorus (mg)	28
Vitamin A (IU)	492
Vitamin B1 (mg)	0.03
Vitamin B2 (mg)	0.06
Niacin (mg)	2.1
Vitamin C (mg)	21

Lipid content of *Kimchi* is very low, so it is considered as a low-caloric food (Cheigh 2004). The lipid composition of *Kimchi* is somewhat different from that of sauerkraut because *Kimchi* contains seafoods or meats which are not used in sauerkraut making. *Kimchi* is also an important source of vitamins (ascorbic acid, carotene, B complex), minerals (calcium, iron, potassium), and dietary fiber (Cheigh 2004). However, some vitamins, particularly those of the B group and ascorbic acid, may be synthesized during the *Kimchi* fermentation. Mineral contents of *Kimchi* vary depending on the recipe and the type. In general, calcium and phosphorus are major minerals in *Kimchi*. The carotene content in *Kimchi* varies depending on the amount of red pepper, carrot, and other green vegetables used (Jang et al. 1991).

11.3.5 Functionality and Health Benefits

Kimchi is recognized as a naturally fermented health food, because it contains several nutrients and physiologically functional sources such as vitamins, minerals, and phytochemicals as well as lots of live lactic acid bacteria cells (Cheigh and Park 1994). As the fermentation proceeds, various kinds of lactic acid bacteria can grow, and nutritional resources with different functionalities are generated. In fact, *Kimchi* was selected as one of the world's five most healthful foods by the *Health*, an American magazine focused on health and owned by Time Inc. in March 2006, and public and scientific interests in *Kimchi* have been increasing over the world.

The physiological properties of the microorganisms involved in *Kimchi* fermentation and the content of fermentable sugars in the raw materials affect the production of organic acids quantitatively and qualitatively. The organic acids and viable lactic acid bacteria are found to have good effects on human intestinal microflora (Lee et al. 1996; Kang et al. 2002; Park et al. 2014a). *Kimchi* contains a higher concentration and a wider range of organic acids compared with other types of fermented food, because it undergoes the two stages of initial salting and secondary seasoning.

Lactic acid bacteria isolated from *Kimchi* have antimicrobial activity against unfavorable microorganisms such as *E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, etc. (Ahn et al. 2012; Al-Delaimy and Ali 1970; Chang and Chang 2011; Davidson and Hoover 1993; Jeong et al. 2011; Kang et al. 2002; Kim 1995). In addition to this, it was reported that major lactic acid bacteria such as *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *L. fermentum*, and *L. plantarum* in *Kimchi* suppressed the mutagenicity of certain kinds of mutagens 4-NQO, MeIQ, and Trp-P-2 (Park et al. 1998a, b). In some experiments, mouse fed with *L. plantarum* isolated from *Kimchi* showed also immunostimulation effect. The active factors were presumed to be the cell wall constituents of microorganism. Moreover, it was reported that factors in fermented cabbage of *Kimchi* inhibit the synthesis of enzymes, which mediate the conversion of procarcinogens to proximal carcinogens involved in colon cancer.

Recently, therapeutic effects of lactic acid bacteria in *Kimchi* have been suggested in some viral and bacterial diseases, particularly avian influenza. This may provide a speculative explanation for the very low incidences of severe damage from SARS or avian influenza among people in Korea, which may be a result of enhanced immune function from *Kimchi* (Seo et al. 2012).

Several biologically active components including carotenes, capsaicin, chlorophylls, dietary fibers, phenolic compounds, ascorbic acid, allyl sulfides, and organic acids such as lactic acid are considered to be the active agents for the health benefits of *Kimchi* (Choi et al. 1997; Park et al. 1998a, b; Lee et al. 2008, 2011a, b, c). Therefore, *Kimchi* has been suggested to be rich in functional and bioactive substances with outstanding antiaging, anticancer, thrombolytic, and immune-boosting effects and has demonstrated many health benefits such as antioxidant, antimutagenic, anticarcinogenic, and antihypertensive activities (Cheigh et al. 2004; Choi et al. 1997; Lee et al. 2008, 2011a, b, c; Park et al. 1998a, b). Dietary fiber, ascorbic acid, carotene, and the components in the extracts of red pepper and the extracts of garlic used as *Kimchi* ingredients are assumed to suppress the formation of carcinogenic or mutagenic compounds and to inhibit mutagenicities induced by several carcinogens/mutagens (Park 1995). Also dietary fiber in *Kimchi* has an effect on preventing constipation and controlling intestinal microflora (Kim et al. 2006; Park et al. 2014a).

11.3.6 Ethnic Value and Socio-economy

Kimchi is one of the typical traditional and ethnic foods that have been made and consumed for a long time in Korea. *Kimchi* has been traditionally served as a "must" at almost every meal along with boiled rice and other dishes. *Kimchi* is prepared by fermenting the salted main ingredients, such as *Kimchi* cabbage, white radish, cucumber, and other vegetables, which is mixed with a seasoning mixture consisting of red pepper (*Capsicum annuum* L.) powder, garlic, ginger, edible allium varieties other than the garlic, and salted and fermented seafoods (*Jeotgal*), for a certain period of time (Park 1994; Codex Alimentarius 2001).

As mentioned in Table 11.10 (Jo 2000; Seo et al. 2013), there are more than 200 different types of *Kimchi*; each type represents varies and changes in ingredients and preparation methods. As diets change along with availability of diverse types of foods, new types of *Kimchi* are being formulated through the creative use of both conventional and unconventional ingredients. *Kimchi* becomes a long-savored side dish that harmonizes with not only Korean dishes but also Western staples such as steak. It also continues to grow in popularity, as it becomes a component in modern culinary creations such as *Kimchi* fried rice, *Kimchi* tacos, and *Kimchi* pizza.

Generally, Koreans used to make *Kimchi* at home. However, in recent years, there is an increasing trend of consuming the commercial *Kimchi* products made in a factory. *Kimchi* industry in Korea has grown rapidly due to the increase in domestic and overseas demand of *Kimchi*. *Kimchi* has gained international popularity since the 1988 Seoul Olympic Games, when the large number of foreign tourists visiting the country had a chance to get a taste of *Kimchi*.

The market size of *Kimchi* industry in Korea was estimated to be 1.3 billion US dollars in 2013, and the total amount of *Kimchi* consumed per year in Korea was estimated to be 1,480,000 M/T, of which 557,000 M/T (37.6 %) was produced by commercial *Kimchi* manufacturers in Korea (Park et al. 2014b). According to a survey (Park et al. 2014b), 754 *Kimchi* processing factories were operating in 2013 in Korea. The amount of *Kimchi* export has been steadily increasing since the Seoul Olympic Games (1988), and 26,000 M/T of *Kimchi* has been exported to 61 countries including Japan (74 %) in 2013 (KITA 2014). The Codex standard of *Kimchi*, which was elaborated to promote international trade, was finally adopted in the 24th Codex Alimentarius Commission held on July 5, 2001. It was the first Codex of this kind in the Northeast Asia including Korea. At the same time, the amount of *Kimchi* import to Korea has been also sharply increased, from 19 M/T in 1966 to 220,218 M/T in 2013, with China being the only country exporting *Kimchi* to Korea. According to a Korean government report in 2014, an average adult consumes 60.7 g of *Kimchi*/day, which is in declining trend.

In the future, more and more commercial *Kimchi* products will be consumed, because it is not easy to keep *Kimchi* palatable for an extended period of time. As more and more women work outside their homes, many man-hours of labor that must go into preparing *Kimchi* become an excessive burden on the Korean family. Changes in the housing situation also make *Kimchi* preparation more and more troublesome for average Korean families, mainly because the number of the average household member and the space available for storing *Kimchi* are decreasing. The increase in apartment housing poses an even greater problem in finding space for big *Kimchi* jars, which are often used for *Kimchi* storage. In addition, the winter *Kimchi* will be prepared at home in smaller batches per preparation, but with increased frequency, due to the year-round availability of vegetables and the extended shelf life of *Kimchi* stored in home refrigerators. A technological upgrading of *Kimchi*-producing plants will require a large capital investment, and, therefore, small-scale plants may gradually disappear. Globalization of *Kimchi* will be accelerated through more frequent cultural exchange between Korea and other countries around the world, which will steadily boost export of *Kimchi*.

11.4 Fermented Fish Products

Fermented fish products, *Jeot-gal*, are inherited from thousands of years and have played an important role in Korean food culture as side dishes and appetizers of staple meals and cooked rice and as essential seasoning ingredients of *Kimchi*. Fermented fish products are generally classified into three categories: *Jeotgal* (salt-fermented fish products), fish sauce, and *sikhae* (lactic fermented fish products) based on fermentation recipes and technologies; *Jeot-gals* are reclassified as plain salt-fermented *Jeot-gal* and seasoned and aged *Jeot-gal* (Kim and Kim 1991; Lee et al. 1983).

Jeot-gals have been regarded as an economic way of preserving massive productions of small pelagic fishes by use of only salt and have also been evaluated as valued food stuffs for its appetizing food rolls and nutritional properties (Lee et al. 1986). *Jeot-gals* prepared by traditional recipes are generally characterized by long-term preserved *Jeot-gals*, as plain salt-fermented products, and are stored and simply fermented and highly salted fishes. Thus, these are not generally applicable for direct eatery uses, else while seasoned and aged *Jeot-gals* are commonly consumed as direct food uses as side dishes of cooked rice for its reduced salinity and seasoning properties. Fish sauces are widely used as essential seasoning ingredients for *Kimchi* preparations and are characterized as a simple process for obtaining a clear liquid sauce in which a long-term period of salt fermentation of unfrozen fresh fishes is required at least longer than 10 months without application of microbial fermentation process.

Sikhae, traditional lactic fermented fishes, are prepared by using a mild salting condition and are differently used to ingredients for cooked grains as millet or rice, natural amylase source as malts, spices as red pepper, garlic, ginger, etc. (Lee 1993a, b, c). It is characterized as lactic fermented foods in which the salinity is lowered by below 3 % and is consumed as direct eatable side dishes for its acidic and spicy taste. Also, it is expected as health beneficial foods due to its potential biological and microbial activities and also palatable flavor and taste (Lee et al. 2003; Lee and Kim 2010).

11.4.1 Classification of Korean Fermented Fish Products

Fermented fish products are generally classified by a mixed standard according to raw materials and fermentation technologies. Unfrozen fresh fishes are ordinarily employed as raw materials for plain *Jeot-gal* and fish sauce preparation, but frozen fishes also can be used as raw materials for seasoned and aged *Jeot-gal* and *sikhae* preparations. More than 20 varieties of fermented fish products are named in combinations of raw materials plus fermentation technology as anchovy *Jeot-gal*. General classification of Korean fermented fish products are shown in Table 11.15.

11.4.2 *Jeot-gal*

Fermented fish product *Jeot-gals* could be divided into two categories: plain *Jeot-gal* and seasoned and aged *Jeot-gal* (Kim and Kim 1991). The plain *Jeot-gal* is prepared mainly for the storage purpose of massive harvest of small fishes, for which simple salting is enough for retarding microbial growths and putrefactive actions. Salinity normally stronger than 20 % is applied and kept in a fermentation tank without controlling its temperature.

Organic components as protein, fat, and carbohydrates are gradually digested into small components during the fermentation and storage of *Jeot-gal* and are storable for longer than several years (Lee et al. 1986; Lee 1993a, b, c). However, the digested components as fermented fish sauces are obtained after 6 months of the fermentation in which degradations in raw fish meats are highly related with salinity, temperature, and storage periods. For the preparation of plain *Jeot-gal*, unfrozen small fresh fishes are widely used as raw fishes, in which anchovy and sand lance are major species in industry (Kim and Kim 1991).

Meanwhile, seasoned and aged *Jeot-gals* are seasoned and fermented fish products with a major purpose of eating it directly as a side dish of cooked rice, and relatively low salinity are applied and storable for 3 months at a refrigerated condition as below 10 °C (Kim and Kim 1991; Lee 1993a, b, c). For the preparation of seasoned and aged *Jeot-gal*, varieties of fresh or thawed frozen fish meats and edible intestine and fish roes are used as raw materials with seasoning and stabilizing ingredients and spices.

Table 11.15 Classification of fermented seafoods in Korea (Kim and Kim 1991; Lee et al. 1983)

Products	Raw materials	Species of raw fish
Plain *Jeot-gal* (salt-fermented *Jeot-gal*)	Fish, *Mollusca*	1. anchovy, 2. hairtail, 3. corvenia, 4. croaker, 5. big-eyed herring, 6. squid, 7. sea arrow, 8. damsel fish, 9. gizzard shad, 10. sardine, 11. flounder
	Shellfishes	1. oyster, 2. littleneck clam, 3. mussel, 4. surf clam, 5. top shell, 6. small abalone
	Crustacea	1. shrimp, 2. oriental river shrimp, 3. blue crab, 4. Japanese swimming crab, 5. freshwater crab
	Edible viscera	1. cod gill, 2. sea cucumber intestine, 3. hairtail intestine, 4. gizzard shad intestine
Seasoned and aged *Jeot-gal*	Fish meat, fish roe	1. squid, 2. longarm octopus, 3. sea arrow, 4. herring roe, 5. walleye pollock roe, 6. cod gill, 7. sea urchin roe, 8. littleneck clam, 9. surf clam, 10. scallop, 11. ascidian, 12. big-eyed herring, 13. shrimp, 14. abalone viscera, 15. ascidian, 16. walleye pollock, intestine, 17. gizzard shad intestine
Fish sauce	Whole fishes, by-products	1. anchovy, 2. sand lance, 3. sardine, 4. icefish, 5. big-eyed herring, 6. tuna viscera, 7. shrimp, 8. squid viscera, 9. krill
Sikhae (meat)	Fish and *Mollusca*	1. flounder, 2. anchovy, 3. walleye pollock, 4. squid, 5. walleye pollock roe, 6. pollock

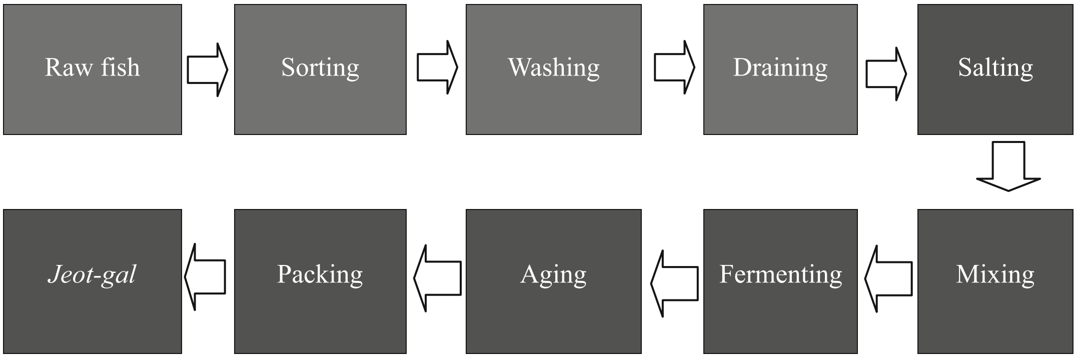

Fig. 11.16 Schematic process diagram of plain salt-fermented *Jeot-gal*

11.4.3 Preparation Method

(a) Plain *Jeot-gal*

Plain *Jeot-gals* are prepared simply salting of fresh fish and waiting fermented. Whole fishes are washed and salted with a certain salinity stronger than 20 % and are kept in a fermentation tank or container for at least 3 months. Salinities of products are dependent on species of raw fishes and fermentation temperature. In the case of *Mollusca* and *Crustacea* as raw materials, the salinity more than 20–30 % is needed to effectively control putrefactive microbial actions, and its fermentation is evenly processed at a warm condition of higher than 25 °C. A schematic process diagrams are illustrated in Fig. 11.16 (Kim and Kim 1991).

For the fermentation of crustacean like crab, soy sauce were widely used as a salting medium in place of salt to give a quick salting effect at anaerobic fermentation condition to control putrefactive microbial action. Figures 11.17, 11.18, 11.19, and 11.20 represent typical plain fermented *Jeot-gal* made of anchovy, shrimp, clam, and crab by traditional fermentation process.

Fig. 11.17 Plain anchovy *Jeot-gal*

(b) Seasoned and aged *Jeot-gal*

Seasoned and aged *Jeot-gals* are prepared by salting for raw fishes according to marinating with ingredients and spices at a relatively low salinity. Hydrolyzed starch syrups or rice syrups are widely used for desalting pre-salted raw

Fig. 11.18 Plain shrimp *Jeot-gal*

Fig. 11.19 Plain clam *Jeot-gal*

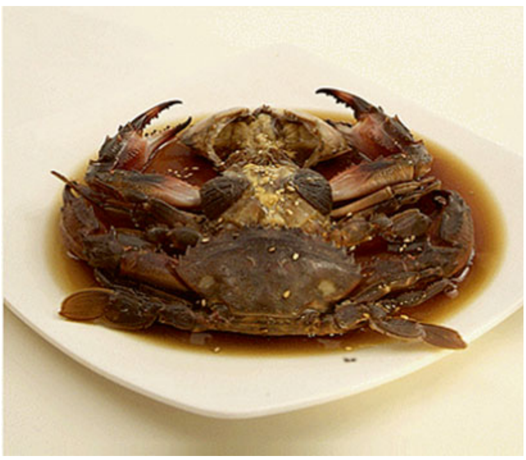

Fig. 11.20 Crab *Jeot-gal* fermented with soy sauce

materials and to control water activity. Also, different seasonings, spices, and natural colorants are applicable to improve commercial attractiveness. At least several weeks of aging are required for the matured flavor, taste, and textural stabilization of products. Mild salinity as 3–8 % is applied. Figure 11.21 represents schematic process diagrams of seasoned and aged *Jeot-gals* (Kim and Kim 1991). Two dominant items among commercial seasoned and aged *Jeot-gals* prepared by traditional process are presented in Figs. 11.22 and 11.23.

11.4.4 Microorganism

Various strains of microorganisms of proteolytic, lipolytic, and amylolytic activities were isolated and characterized from commercially produced traditional plain salt-fermented *Jeot-gals* and seasoned and aged *Jeot-gals* (Hur 1996; Ham and Jin 2002). As a result of scientific investigations, various microbes including *Micrococcus*, *Pseudomonas*, *Pediococcus*, *Sarcina*, *Leuconostoc*, *Flavobacterium*, *Bacillus*, yeast, *Halobacterium*, *Saccharomyces*, *and Torulopsis* were isolated, and *Pseudomonas*, *Flavobacterium*, *Brevibacterium*, *Sarcina*, *Leuconostoc*, *Bacillu*s, yeasts, and *Micrococcus* were reported as typically found microorganisms. Figures 11.24 and 11.25 represent changes in microbial flora and sensory properties during the salt fermentation of anchovy and shrimp *Jeot-gals* (Lee et al. 1986; Kim and Kim 1991)

With regard to bacterial distributions in salt-fermented fish products on the wholesale market, dominant strains isolated from *Jeot-gals* were reported as 35.5 % of coliform bacteria, 45.5 % of which were *Enterobacter cloacae*; 8.6 % of *Vibrio* spp., 62.5 % of which were *V. alginolyticus*; 12.9 % of *Staphylococcus* spp., 41.7 % of which were *S. lentus*; and 43 % of other bacteria with a major strains of *Aeromonas* spp., *Hafnia* spp., *Pantea* spp., *Pseudomonas aeruginosa*, and *Serratia liquefaciens* (Ham and Jin 2002)

11.4.5 Nutritional Composition

According to scientific researches, dynamic changes in biochemical compositions of the salted fish have taken place during the fermentation of *Jeotgal*. Nitrogenous compounds of low molecules as soluble nitrogen, amino nitrogen, and volatile basic nitrogens increased as a result of decompositions of protein in the raw fishes.

Nutritional components in *Jeot-gals* could be summarized as high protein and minerals, low fats, and vitamins. Based on fermentative decompositions of raw fishes and protein contents in *Jeot-gals*, it shows slightly decreases and large

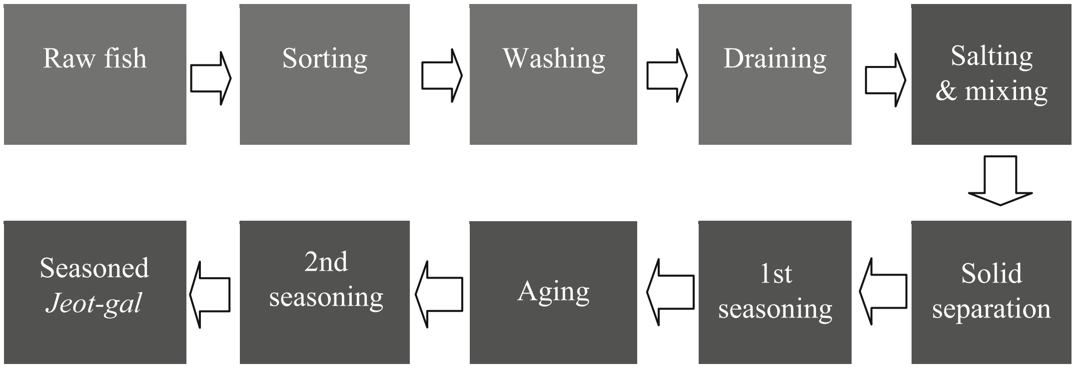

Fig. 11.21 Schematic process diagram of seasoned and aged *Jeot-gal*

Fig. 11.22 Seasoned and aged walleye pollock roe *Jeot-gal*

Fig. 11.23 Seasoned and aged squid *Jeot-gal*

increments in minerals compared to raw fishes in which potential rolls are expected as mineral sources. Some examples of a proximate composition of *Jeot-gal* are given in Table 11.16.

Figure 11.26 represents typical changes in biochemical and sensory characteristics during salt fermentation of anchovy *Jeot-gal*.

11.4.6 Functionality and Health Benefits

Jeot-gal represents different physiological functions through newly produced substances based on its fermentation process in addition to different functionalities in raw fishes. It has been known that antitumor activities of strains *Bacillus subtilis* isolated from *Jeot-gal* were reported. Lactic acid bacteria and yeast isolated from salt-fermented *Jeot-gal* reveal resistance to artificial gastric acid and bile juice (Lee et al. 2003; Kim et al. 2005).

It was also reported that culture supernatants of two *Jeot-gal* probiotic strains, *Lactococcus lactis* and *Lactobacillus plantarum*, isolated from *Jeot-gal* showed both an antioxidative activity in the range of 77–81 % and a cholesterol-lowering activity as much as 33–60 %, respectively, as shown in Table 11.17 (Kim et al. 2003a, c).

Lactobacillus strain including *Lb. brevis* strain AML 15 isolated from *Jeot-gal* showed a meaningful GABA productivity in MRS broth containing MSG (Jeon et al. 2004; Shin et al. 2007). Table 11.18 represents probiotic properties of

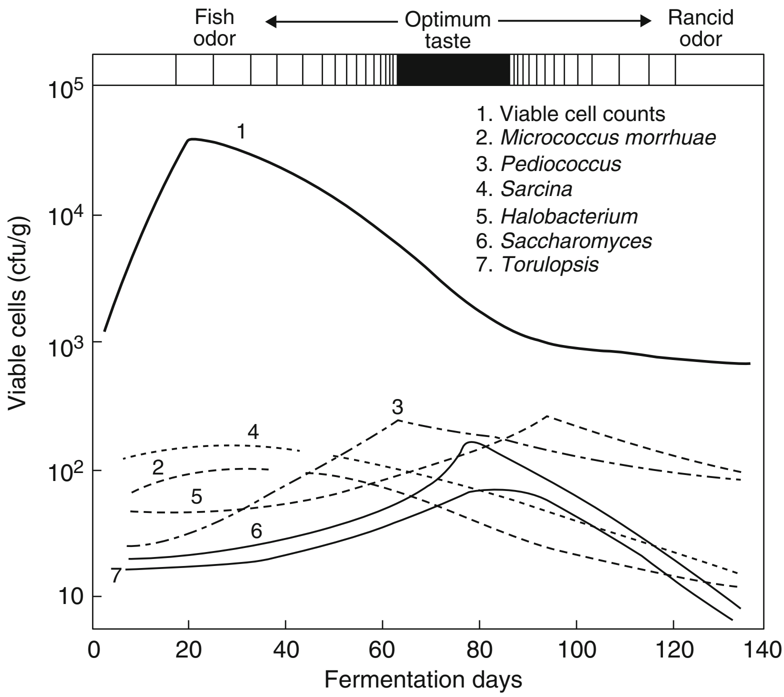

Fig. 11.24 Changes in microflora and sensory properties during the salt fermentation of traditional shrimp *Jeot-gal* (Lee et al. 1986)

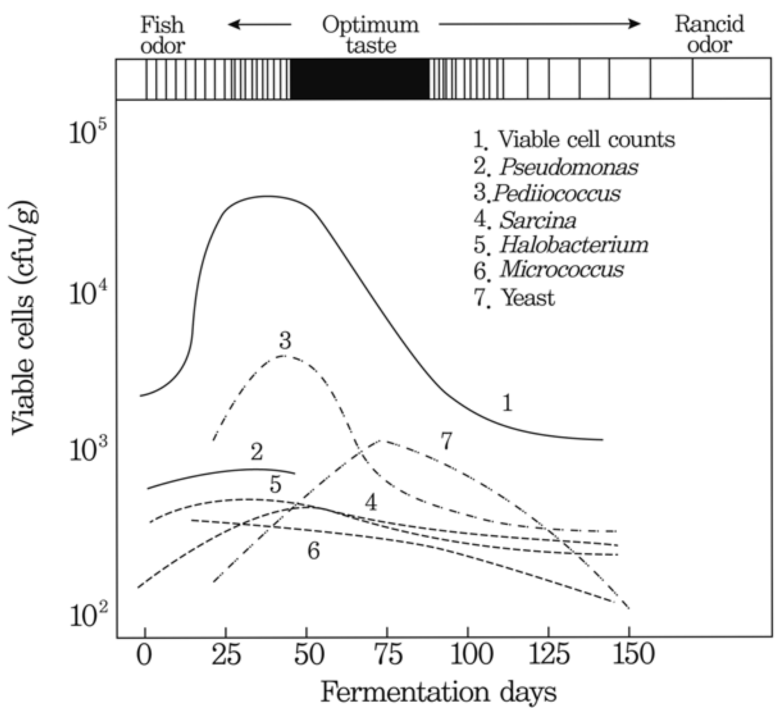

Fig. 11.25 Changes in microflora and sensory properties during the salt fermentation of traditional anchovy *Jeot-gal* (Lee et al. 1986)

Table 11.16 Some examples of a proximate composition of *Jeot-gal* (NFRDI 2009)

Composition (/100 g)	Plain *Jeot-gal*				Seasoned and aged *Jeot-gal*				
	Anchovy	Gizzard shad	Littleneck clam	Shrimp	Walleye pollock roe	Pollock intestine	Oyster	Sea arrow	Squid
Water (g)	54.4	62.8	76.5	60.7	66.0	64.3	70.2	57.2	63.9
Protein (g)	14.1	15.3	8.5	10.4	20.5	12.9	8.6	16.0	14.3
Fat (g)	11.2	6.7	1.0	0.9	3.0	3.2	2.7	2.0	0.9
Ash (g)	19.7	14.7	12.5	27.3	7.8	11.4	10.4	24.5	18.9
NaCl (g)	16.6	13.6	9.6	22.3	6.3	10.2	8.7	21.2	17.0
Carbohydrate (g)	0.6	0.5	1.5	0.7	2.7	8.2	8.1	0.3	2.0
Ca (mg)	592	466	112	871	28	99	196	110	139
P (mg)	348	322	117	229	249	109	140	239	155
Fe (mg)	5.5	8.0	16.9	6.2	1.2	1.4	8.8	5.6	1.5
Retinol (ug)	60	0	3	0	66	6	3	0	0
B_1 (mg)	0.02	0.02	0.02	0.07	0.8	0.3	0.11	0.09	0.02
B_2 (mg)	0.23	0.18	0.10	0.09	0.2	0.0	0.20	0.12	0.03
Niacin (mg)	6.3	4.1	2.0	1.2	8.9	3.3	3.0	3.4	2.3
C (mg)	0	0	1	0	0	0	1	0	0

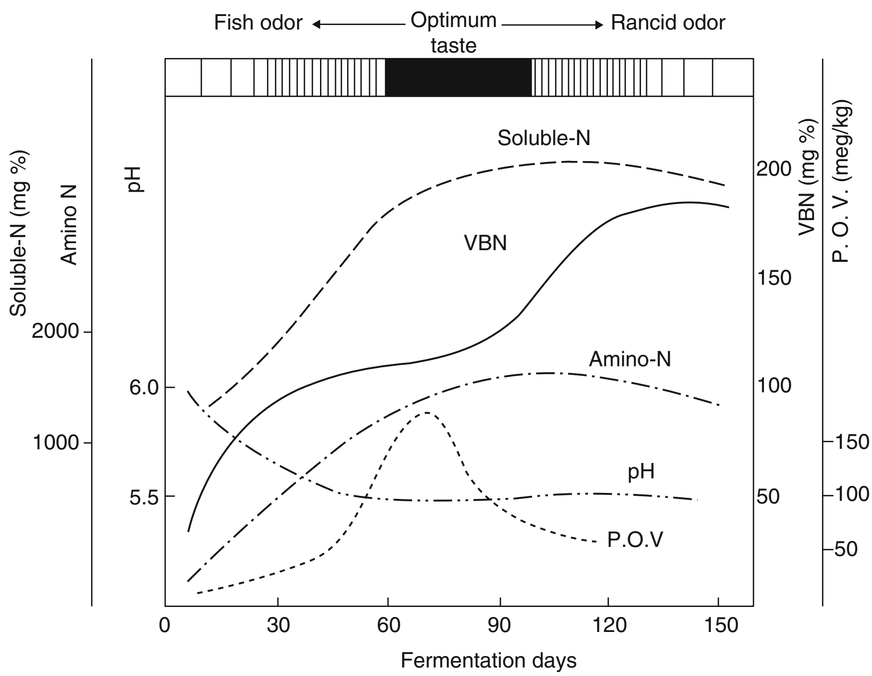

Fig. 11.26 Changes in biochemical compositions and sensory properties during the salt fermentation of anchovy *Jeot-gal* (Lee et al. 1986)

lactic acid bacteria isolated from *Jeot-gal*. Newly isolated three strains of lactic acid bacteria, ML36, ML128, and ML178, revealed to be all highly resistant against artificial gastric juice and also against nisin and major antibiotics (Kim et al. 2005).

11.4.7 Ethnic Value and Socio-economy

The annual production of *Jeot-gal* reached 35,000 tons (excluding the imported shrimp of 13,436 tons) as an equivalent value of 400 million

Table 11.17 Antioxidative effect due to the radical scavenging effect on the DPPH and inhibition of lipid peroxidation and cholesterol-lowering activity in some lactic acid bacteria and yeast isolated from *Jeot-gal* (Kim et al. 2003a, c)

Strains	DPPH	TCA	Cholesterol-lowering activity
Lactococcus lactis NK 24	78 %	3.5 %	50 %
Lactobacillus plantarum NK 181	77 %	4.6 %	60 %
Yeast strain HW 161	81 %	2.0 %	33 %

Table 11.18 Antibiotic resistances of lactic acid bacteria strains (Kim et al. 2005)

	Lactic acid bacteria strain			
Antibiotics (μg/Ml)	ML 36	ML 128	ML 178	
Nisin	0	+[a]	+	+
	25	+	+	+
	50	+	+	+
	100	–[b]	+	+
Rifamycin	0	+	+	+
	5	+	+	+
	10	+	–	+
	20	+	–	–
Streptomycin	0	+	+	+
	5	+	+	+
	10	+	+	+
	20	+	+	+
Tetracycline	0	+	+	+
	5	+	+	+
	10	+	+	+
	20	+	+	+

[a]Growth
[b]No growth

Table 11.19 Annual production of major *Jeot-gal* products in Korea (2012. M/T) (KFA 2013)

Jeot-gals	Production (1000 kg)	Proportion (%)
Anchovy	11,664	33.2
Walleye pollock roe	4977	14.1
Shrimp[a]	7358	20.9
Sea urchin	40	0.1
Oyster	581	1.6
Squid	3438	9.9
Clam	867	2.4
Walleye pollock intestine	499	1.4
Croaker	419	1.2
Others	5350	15.2
Total	35,158	100

Block letters indicate seasoned and aged
Shrimp[a]: imported amount 13,436 ton (9.4 million US dollars) were excluded

US dollars (KFA 2013). Annual consumptions of *Jeot-gals* could be estimated to be about 968 g per head. This estimation may assist the essential food as the traditional *Jeot-gal* in Korea especially considering its high salinity. Recent production statistics are given in Table 11.19 (KFA 2013).

11.4.8 Fish Sauce

Fish sauce is a type of clear liquid source obtained using long-term fermented fish mashes such as anchovy, icefish, sand lance, shrimp, or some by-products of fish processing as tunas, squid, hairtail, etc. Fish sauces are one of the major seasoning ingredients of *Kimchi* and also savory salty seasonings for cooking Korean foods like vegetable salads, fish soups, and variety of dipping sauces. For the preparation of fish sauce, certain salt concentrations higher than 20 %(w/w) are required for the effective control of microbial putrefaction during storage and distribution, and use of microbial starters is unusual. It takes 10 months at least at an uncontrolled natural temperature condition for producing fish sauces. Also, a thermal sterilization process is not generally employed to traditional fish sauces up to the present for maintaining its own flavors and tastes, but some thermal sterilization processes are recommended and legally enforced to meet the increased hygienic demands of consumers. Seasoned fish sauces are also prepared for its first fermentation of the first filtered cake of fermented fish mashes with saline water and seasonings (Kim and Kim 1991).

Figures 11.27 and 11.28 represent a commercial pack of anchovy sauces and a sand lance sauce produced by a traditional technology.

Fig. 11.27 Commercial anchovy sauce

Fig. 11.28 Commercial sand lance sauce

11.4.9 Preparation Method

For producing fish sauces, a series of process are required. According to traditional processes, fresh raw fishes are washed, drained, and thoroughly mixed with salt based on an approximate ratio of 3:1 (fish to salt, weight base) and are fermented in an anaerobic tank for more than 10 months at uncontrolled temperature. To make anaerobic fermentation conditions, wooden, ceramic, or plastic weight covers are commercially being used for pressing the surface of salted fish masses in order to fully immerse the solid into a liquid phase. Also, the temperature control for its fermentation at 15–17 °C is recommended for the development of good flavors and tastes in the products (Kim and Kim 1991).

For the preparation of fish sauces, using microbial starters is not commonly applied, and a commercial volume of fermentation tanks exceeds several tones. Sanitary control of the fermentation process is especially strengthen to meet the consumer's needs, and fine filtration of fermented mashes and blending of fish sauces are regarded as important quality control points in producing products. A schematic diagram for the production of the traditional fish sauce in Korea is represented in Fig. 11.29.

11.4.10 Microorganism

Fish sauce made of anchovy contains several strains belonging to the family of *Clostridiums*, and *Vibrio*, *Bacillus*, *Halobacterium*, *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Acinetobacter*, *Corynebacterium*, *Moraxella* sp., *and Flavobacterium* were reported in which the major strains were *Bacillus*, *Micrococcus*, and *Staphylococcus* sp. In the case of shrimp sauce, *Bacillus* sp., *Pediococcus*, *and Halophilus* were also reported as major microbes (Lee et al.1986, 2002; Hur 1996).

11.4.11 Nutritional Composition

Table 11.20 represents the proximate composition of traditional fish sauces in Korea. Major components of traditional fish sauce are nitrogenous compounds, which are the total nitrogen and amino acid nitrogen (NH_2-N) among various degradation products of fish fermentation (Cho et al. 1999; Cho et al. 2014). The mean values of salinity, total nitrogen, and amino acid from nitrogen of commercial fish sauces are 21.9 g, 1.43 g, and 518.1 mg per 100 g, respectively (Kim and Kim 1991).

11.4.12 Functionality and Health Benefits

Angiotensin-1 converting enzyme (ACE) inhibitory activities of fermented sardine sauces were

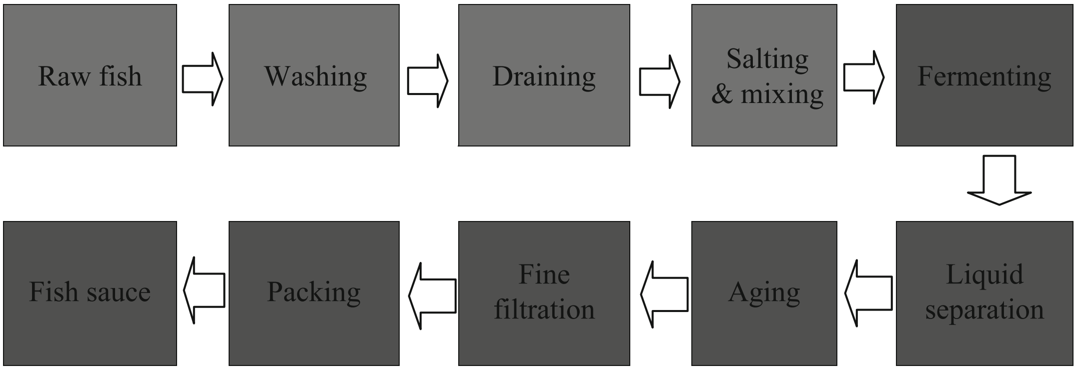

Fig. 11.29 Schematic process diagram of the traditional fish sauce in Korea

Table 11.20 Proximate composition of the Korean traditional fish sauce (NFRDI 2009)

Components	Value range	Mean
pH	5.34–6.23	5.69
Moisture (g/100 g)	66.6–71.0	68.72
NaCl (g/100 g)	20.8–23.2	21.96
Total nitrogen (g/100 g)	0.96–1.73	1.43
Acidity (m/100 ml)	11.58–24.58	18.06
NH_2-N (mg/100 g)	371.4–680.3	518.12

Table 11.21 ACE inhibition effect according to amounts of sardine sauces fermented for 90 days

Sardine sauce[a]	ACE inhibition ratio (%)		
	20 μg	50 μg	100 μg
S	9.4 (9.8)[b]	21.9 (27.0)	42.1 (43.9)
M	1.2 (14.1)	17.3 (28.1)	27.6 (50.9)
R	13.6 (13.1)	25.5 (27.3)	36.3 (42.6)

[a]Sardine sauce S, M, and R were prepared from scrap, muscle, and round of sardine, respectively.
[b]Each data in the parentheses represents ACE inhibition ratio of Sardine sauce fermented with *koji*

Table 11.22 The fibrinolytic activity of *B. subtilis* JM-1, JM-2, and JM-3 isolated from anchovy sauce (Lee et al. 2002)

Sample	Relative activity (%)
B. subtilis JM-1	60
B. subtilis JM-1	90
B. subtilis JM-1	110
Plasmin (positive control)	100
BHI broth (negative control)	0

reported. Three different types of fish sauces were prepared from scrap (S), meat (M), and round (R) of the sardine and ACE inhibitory activities of fish sauces according to fermentation processes (Yeom et al. 1993). Then, the results of the highest inhibition in the sauce M fermented with *koji* (soy sauce koji produced by inoculation of *Aspergillus oryzae* on the cooked soybean was added to the ratio of 4.5 % of sardine meat by weight of 25 % salt concentration) for 30 days were obtained, and it showed decreases in the inhibition. Also, it represented increases in the inhibition according to increments of added amounts. Related information is shown in Table 11.21 (Yeom et al. 1993).

Table 11.22 represents the fibrinolytic activity of bacterial strains isolated from the fermented anchovy sauce produced by traditional fermentation technology (Lee et al. 2002). Three bacterial strains of fibrinolytic activity were newly isolated from fermented anchovy sauce. On the basis of PCR amplification of 16S rRNA, and in comparison to BLAST DNA database, newly isolated strains were identified as *Bacillus subtilis*, and their fibrinolytic activities were 60–110 %, by which expectable the potential use as starter of biologically active fish sauces (Lee et al. 2002).

11.4.13 Ethnic Value and Socio-economy

Ethnic fish sauces are commercially produced using anchovy, icefish, sand lance, sardine, shrimp, and some fish by-products. The roles of foods in Korea are important for essential seasoning ingredients for *Kimchi* preparations and also are useful for other purposes in seasoning ingredients for cooking Korean foods. Qualities in four different commercial fish sauces in Korea have been guaranteed by the government, and its annual productions reached about 100 million US dollars (KFA 2013).

11.4.14 *Sikhae*

Traditional *sikhae* made of fishes and shellfishes presents characteristic flavors, acidic and spicy tastes, and chewable textures for its preparations combined with fermentation technologies like salt and lactic fermentations. Raw fishes including thawed frozen fishes are hygienically prepared with light salting for reducing water in the meat by use of osmotic pressure. Then seasoned with cooked grains as millet or rice are to be used as a good nutrition source for lactic fermentation, and some spices such as red pepper, garlic, and ginger are added for the enhancement of sensory qualities.

Malts are occasionally added to enhance fermentation speed and also to improve sensory acceptance. White lean fishes like flounder, walleye pollock, and squid meat are acceptable as raw fishes. The sensory quality of fermented *sikhaes* is unstable at ambient temperature for the rapid growth of acid-forming bacteria. It can be stored only for several weeks at refrigerated temperature; thus, frozen storages are recommended for long-term storage (Lee et al. 1986, 1983; Kim and Kim 1991).

11.4.15 Preparation Method

For the preparation of the traditional *sikhae*, raw fishes regardless of fresh or thawed frozen fishes are hygienically prepared as scaled, eviscerated, wash and drained, cut or filleted, and salted lightly as 3–5 %(w/w) for a while (30 min to few h) to discard excessive water in the meat using salt osmotic pressure. Then, the drained fishes are mixed with ingredients including spices and goes through fermentation of *Jeotgal*. Temperature and humidity controls are recommended for controlling its lactic fermentation. A schematic process diagram of *sikhae* is given in Fig. 11.30 (Kim and Kim 1991).

11.4.16 Microorganism

Dominant microorganisms associated with *sikhae* including flatfish *sikhae* are known to be strains of acid-forming and proteolytic activities. *Bacillus*, *Lactobacillus*, *Leuconostoc*, *Weissella*, *Streptococcaceae*, *Enterococcus*, yeasts, molds, and some Gram-negative rods are dominants, and these are not strictly influenced by temperature (Lee et al. 1983; Kim et al. 2009a, b). It has generally been known that growth patterns of acid-forming bacteria, yeast, and proteolytic bacteria show the maximized level at a specific optimum fermentation time (Lee 1993a, b, c). Figure 11.31 shows microfloral changes during the fermentation of traditional flatfish *sikhae* representing a dramatic decrease in lipolytic bacteria and peak growth in acid-forming, proteolytic bacteria and yeast at optimum level of sensory taste (Lee et al. 1983).

11.4.17 Nutritional Composition

Proximate compositions of *sikhae* made of flatfish were 64.7–66.7 % of moisture, 14.1–16.6 % of protein, 1.1–1.2 % of fat, 7.5–7.7 % of ash, and 8.7–11.0 % of carbohydrates (Lee 1993a, b, c). According to fermentation, it generally showed increased patterns in carbohydrates and ashes and represented decreased patterns in water, protein, and fat contents compared to that of raw fishes. All nutritional changes might be caused by the enzymatic decomposition of organic compounds by salting and lactic fermentations (Lee et al. 1986; Kim and Kim 1991).

11.4.18 Functionality and Health Benefits

Some probiotic and antimicrobial activities, especially effective on the growth control of

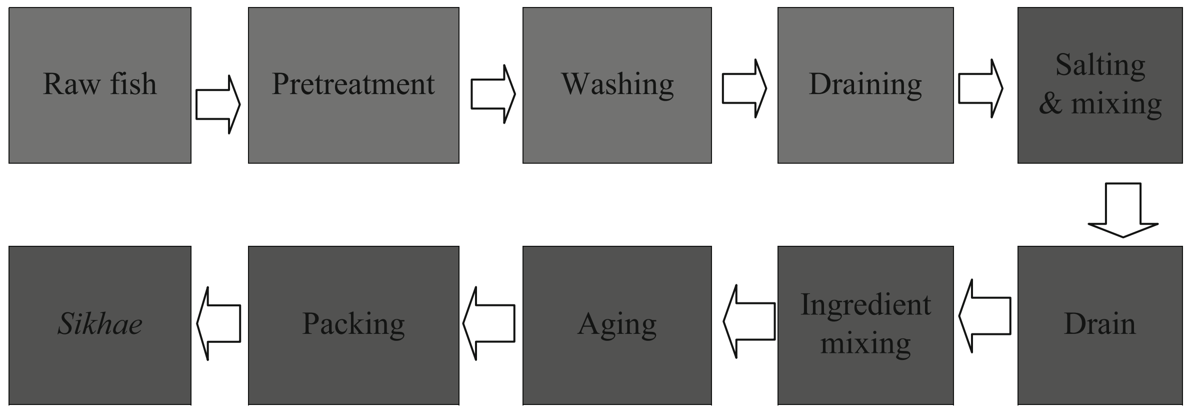

Fig. 11.30 Schematic process diagram of lactic fermented *sikhae*

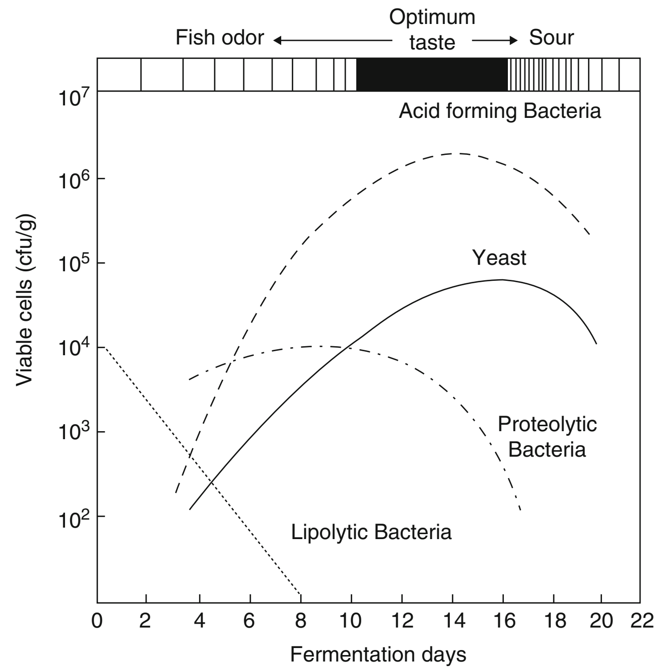

Fig. 11.31 Changes in microflora and sensory properties during the fermentation of flatfish *sikhae*

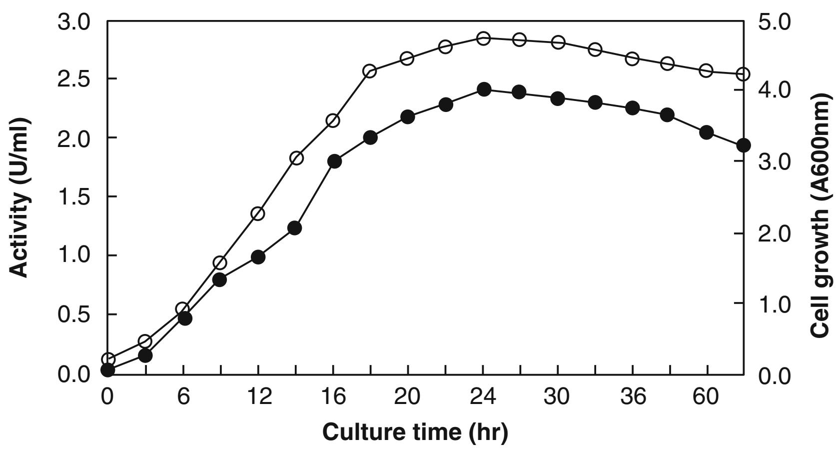

Fig. 11.32 Time course of the cell growth and cholesterol oxidase activity of *Bacillus* sp. SFF34. (1) Cholesterol medium was composed of 0.2 % cholesterol, 0.5 % yeast extract, 2.0 % glucose, 0.2 % NH_4NO_3, 0.02 % K_2HPO_4, 0.03 % $MgSO_4.7H_2O$. (2) ○-○, cell growth; ●-●, cholesterol oxidase activity

certain Gram-positive bacteria such as *Staphylococcus* sp., were reported, and their activities might be associated with the lactic acid-forming properties of *sikhae* (Lee et al. 2003, Lee and Kim 2010). Regarding the bioactivities for controlling the blood pressure, strains producing r-aminobutyric acid (GABA) have recently been of interest due to its potentials of lactic fermentation (Jeon et al. 2004). Figure 11.32 represents cholesterol degradation bacteria, which produce a remarkable amount of extracellular cholesterol oxidase and have been isolated from traditional flatfish *sikhae*. The isolated strains were identified as a strain of *Bacillus* sp. SFF34 and shown to give the maximum yield of cholesterol oxidase in the experimentally pre-

Fig. 11.33 Traditional *sikhae* made of flatfish

pared medium. Optimum culture conditions as temperature, pH, and agitation speed were 30 °C, 7.0, and 150 rpm, respectively (Kim et al. 2001).

11.4.19 Ethnic Value and Social-economy

Production and consumption of *sikhae* have been limited by regions and seasons. Also, there exist difficulties in controlling quality stability and limitations of the sensory acceptance of products. At present, two commercial items such as walleye pollock *sikha*e and flatfish *sikhae* are registered and quality assured by the government, and increasing R&D works on lactic fermented *sikhae* could be a positive alternative consumption of salted *Jeot-gal* for its low salinity and bioactivities in lactic acid-forming bacteria (Kim and Kim 1991; Lee 1993a, b, c; Kim et al. 2001, 2003a, b, c, 2005; Lee and Kim 2010). Figure 11.33 show an appearance of flatfish *sikhae* prepared by traditional methods.

11.5 Traditional Alcoholic Beverage

Korea's long history and unique culture have led to developments of its own traditional alcoholic beverages. It is quite different from Western traditional alcohols due to geographical and cultural distances, and they have some similarities with those from China and Japan. The major difference between Western and Oriental alcohols is whether to use rice or not. Fruit-based alcohols in Western countries take their flavor from the fruit, and whiskey and beer are made using barley instead of rice. More than 300 varieties have been produced in Korea. Korean traditional alcohols can be divided into three categories: *takju* (or *Makgeolli*), *Yakju*, and *Soju* (Chung 2004; Lee 2007).

Korea has created unique alcohols using *Nuruk*. *Nuruk* is similar to malt and yeast that can only be found in Korea. *Nuruk* is made of ground grains that have been moistened and packed into a mold. Once the *Nuruk* has been shaped, it is hung up to ferment for 2–4 weeks in a room. The major crop has always been rice, and most Korean traditional alcohols use rice, of both the glutinous and non-glutinous variety, which are fermented with the aid of yeast and *Nuruk*, a wheat-based source of the enzyme amylase. *Takju* is prepared by fermenting steamed rice and *Nuruk*. *Yakju* uses filtered alcohols and is then fermented with steamed rice and *Nuruk*. *Yakju* is distinguished from *takju* by its relative clarity. It is called *Soju* when it is distilled (Lee 1996).

Makgeolli is a fermented beverage that contains a small amount of alcohol. *Makgeolli* can mean "rough, coarse, or tough," but also "just filtered now." There are two different types of *Makgeolli* being distributed; sterilized *Makgeolli*, which has been sterilized before the bottling process, and draft *Makgeolli*, which is left to ferment slowly even after the bottling process without sterilization. In Korea, the most popular types of *Makgeolli* are rice *Makgeolli* made of rice and *dongdongju* in which unstrained rice floats on the surface. When drinking *Makgeolli*, make sure to shake or stir it well before drinking. The best *Makgeolli* is an intriguing blend of sweet, sour, bitter, and astringent tastes. *Makgeolli* contains various nutrients including vitamins B and C, essential amino acids, dietary fiber, and other useful physiologically active substances. It has been reported that the physiologically active substance contained in *Makgeolli* has functional properties such as improving blood circulation by lowering the cholesterol level and suppressing adipocytic differentiation (Ha et al. 2014a, b).

11.5.1 Preparation Method

Makgeolli is made with a mixture of rice, the main ingredient, and *Nuruk* used as a starter culture, to which potable water is added and then fermented for a certain amount of time at an optimal temperature. *Makgeolli* characteristically involves a two-step fermentation process in which saccharification and alcohol fermentation processes occur simultaneously.

The fermentation procedure is divided into two steps: In the first step, *Nuruk*, sterilized water, and steamed rice are mixed and fermented at 20–25 °C for 2–3 days. To prepare mash, washed non-glutinous rice is soaked for 3 h, drained for 30 min, and steamed for 45 min. After cooling it, the steamed rice and water are added to the product of the first step, and then these are mixed and fermented at 15–25 °C for 7–100 days in the second step. At the end of the fermentation, the fermentation broth is strained through a sieve to produce *Makgeolli* (Chung 2004; Lee 2007) (Figs. 11.34, 11.35, 11.36, 11.37, and 11.38).

11.5.2 Microorganism

The diversity of microbial community in *Makgeolli* being produced in a conventional method using PCR-DGGE was analyzed, and the results were shown as follows. Bacteria identified in the PCR-DGGE profile during its fermentation were 12 species as follows: 5 species of *Lactobacillus* (*L. curvatus*, *L. kisonensis*, *L. plantarum*, *L. sakei*, *and L. gasseri*), 4 species of *Pediococcus* (*P. acidilactici*, *P. parvulus*, *P. agglomerans*, *P. pentosaceus*), and *Pantoea agglomerans*, *Pantoea ananatis*, and *Citrobacter freundii* which belong to the *Enterobacteriaceae* family (Kwon et al. 2012). In addition, changes

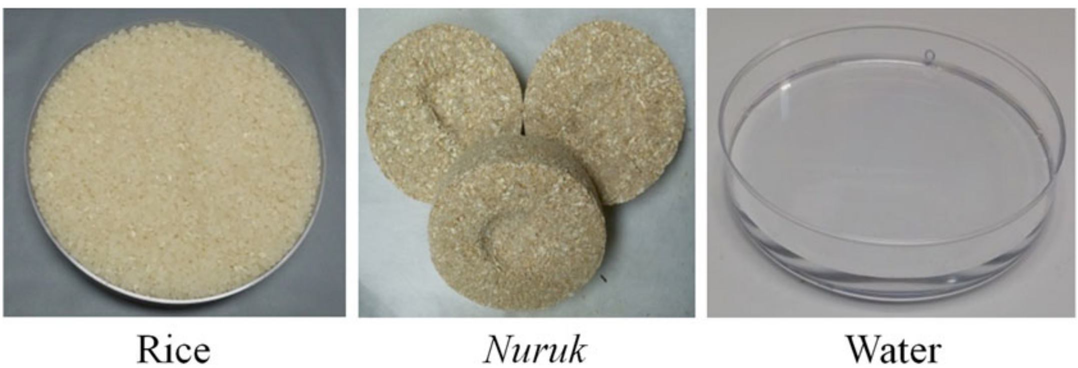

Fig. 11.34 Raw materials for *Makgeolli* preparation

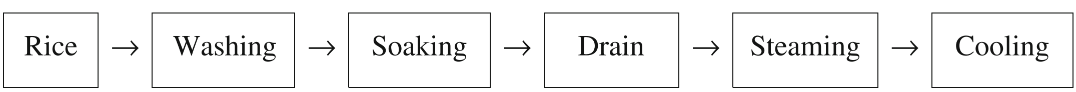

Fig. 11.35 Procedure of steaming rice

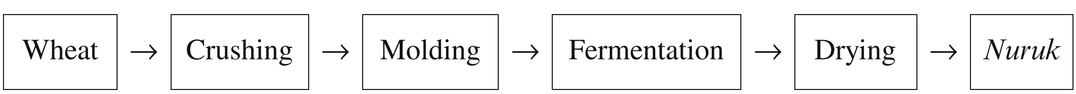

Fig. 11.36 Procedure of *Nuruk*

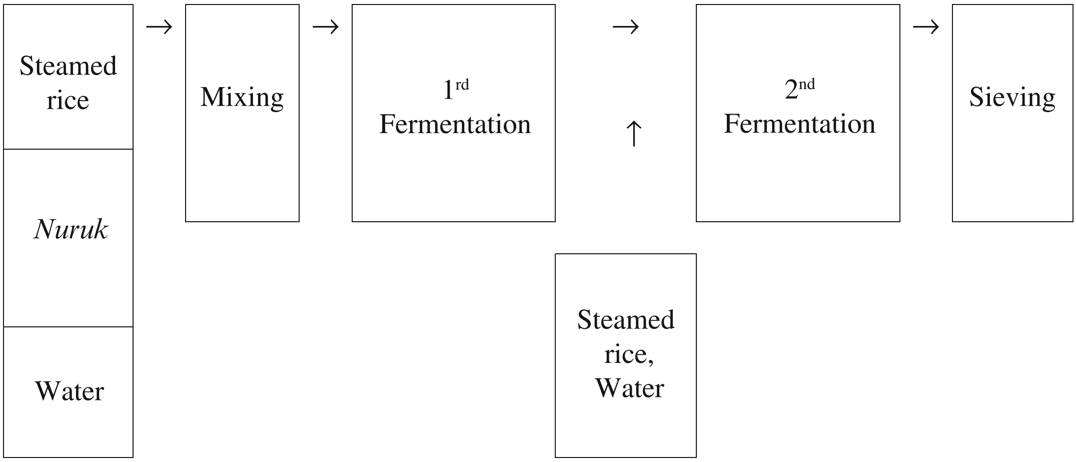

Fig. 11.37 Procedure of *Makgeolli* Preparation

Fig. 11.38 Commercial *Makgeolli*

in the bacteria during the fermentation showed a distinctive difference between before and after the second day of fermentation. Bacteria flora appears mainly in fermentation, and lactic acid bacteria are known as the product of *Makgeolli*. When isolated, 103 species of lactic acid bacteria from *Makgeolli* were identified, and 7 species such as *Lactobacillus paracasei*, *L. arizonensis*, *L. plantarum*, *L. harbinensis*, *L. parabuchneri*, *L. brevis*, and *L. hilgardii* had been reported (Jin et al. 2008; Kwon et al. 2012).

A total of 18 bands were identified from *Makgeolli* produced by traditional method, when the specimens taken during its fermentation period were analyzed by using PCR-DGGE technique, in order to observe the change of fungi (Kwon et al. 2012). It had been reported that a total of six species were identified from

PCR-DGGE profile of fungi, such as *Pichia kudriavzevii*, *Saccharomyces cerevisiae*, *Absidia idahoensis*, *Kluyveromyces marxianus*, *Saccharomycopsis fibuligera*, and *Torulaspora delbrueckii* (Kwon et al. 2012).

11.5.3 Nutritional Composition

Makgeolli contains protein ranging from 1.75 % to 1.9 %. In addition, it is a very unique alcoholic beverage in that it contains 0.8 % organic acid, acetylcholine (ACh), and vitamins such as riboflavin, as well as inositol, dietary fiber, and physiologically active beneficial components. Various kinds of vitamins and nutrients are contained evenly in *Makgeolli*, including about more than ten kinds of balanced essential amino acids, such as lysine, tryptophan, phenylalanine, and methionine (Lee et al. 2011a, b, c).

The results of generic compound analysis for *Makgeolli* and sake are presented in Table 11.23. It can be seen from the comparison that *Makgeolli* contains much more nutritional compounds such as dietary fiber, inorganic components, and vitamins than sake, which is because of the floating matter containing the fermented products as a raw material of the liquor not being filtered from the *Makgeolli*, leaving unfiltered materials in the final product.

The protein content of *Makgeolli* is about 1.7 %, and there exist a number of amino acids degraded during the fermentation process. In particular, it is also very unique that many live yeast are contained in the unpasteurized *Makgeolli*, which easily provides digestive enzymes or inorganic minerals required by the human body. *Makgeolli* is distributed with different pasteurized and unpasteurized types. The pH level of pasteurized *Makgeolli* is in the range of 3.60–4.17, whereas that of unpasteurized *Makgeolli* ranges from 3.40 to 3.77. The pH trend of pasteurized *Makgeolli* therefore displays higher levels compared to unpasteurized *Makgeolli*.

The organic acids contained in *Makgeolli* are obtained by dissolution from *Nuruk*, a Korean fermentation starter, or generated from the microbial fermentation process. The content should be between 0.15 % and 0.3 %, as percentages exceeding 0.5 % make the product undrinkable as an oxidation product (Lee et al. 2011a, b, c). An analysis of organic acid contents in commer-

Table 11.23 Nutritional composition of *Makgeolli* and sake (per 100 g edible portion)

		Makgeolli (alcohol 6 %)	*Sake* (alcohol 16 %)
Proximate compositions	Energy (Kcal)	56	133
	Water (g)	91.8	82.5
	Protein (g)	1.7	0.6
	Fat (g)	0	0
	Ash (g)	0.1	0
	Carbohydrate (g)	1.8	4.2
Dietary fiber	Total (g)	0.6	–
Minerals	Calcium (mg)	6	2
	Phosphorus (mg)	14	8
	Iron (mg)	0.1	0
	Potassium (mg)	15	5
	Sodium (mg)	6	2
Vitamins	Retinol (RE)	6	0
	β-Carotene (μg)	0	0
	Thiamin (μg)	10	0
	Riboflavin (μg)	30	0
	Niacin (μg)	300	0
	Ascorbic acid (mg)	1	0

cial *Makgeolli* in the market for both pasteurized and unpasteurized *Makgeolli* represents a similar level in the mean concentrations of total organic acids; however, their composition ratios were different. Lactic acid, acetic acid, and citric acid were detected from both pasteurized and unpasteurized *Makgeolli*, whereas tartaric acid, pyruvic acid, malic acid, and fumaric acid showed variations depending on the products (Lee et al. 2011a, b, c). In pasteurized *Makgeolli*, the highest content was citric acid, with the concentration of 2.49–5.03 mg/mL, followed by lactic acid and acetic acid, at 0.74–2.87 mg/mL and 0.21–1.09 mg/mL, respectively. In the case of unpasteurized *Makgeolli*, lactic acid showed the highest concentration overall at 0.96–5.70 mg/mL, followed by citric acid and acetic acid at 1.28–4.98 and 0.41–1.52 mg/mL, respectively (Lee et al. 2011a, b, c). Although pasteurized and unpasteurized *Makgeolli* showed similar mean concentrations of total organic acids (7.53 vs. 7.44 mg/mL), they displayed different composition ratios. In particular, lactic acid was detected at higher levels in unpasteurized *Makgeolli* compared to pasteurized *Makgeolli* (Lee et al. 2011a, b, c). The generic composition of organic acids in commercial *Makgeolli* is presented in Table 11.24.

11.5.4 Functionality and Health Benefits

Makgeolli is an alcoholic beverage of unfiltered fermented cereal, which is drunk as the entire fermented product containing the yeast or lactic acid involved in fermentation. Consumption of the entire final product is known to provide higher levels of health functionality compared to *Yakju* or *Cheongju*, which are filtered to clear liquor after fermentation. *Makgeolli* contains floating matter, including microorganism cell compounds such as yeast and lactic acid bacteria (LAB), in addition to the raw material-derived and fermentation product-derived starch polysaccharides, oligosaccharides, organic acids, and peptide, as well as various kinds of biologically active beneficial components (Lee et al. 2011a, b, c). It has relatively lower alcohol levels compared to other alcoholic beverages and does not cause gastric burden. It also contains biologically active beneficial components of many kinds, including vitamin B complex and various kinds of lactic acid, as well as inositol, acetylcholine, and riboflavin, in addition to the rich contents of protein and dietary fiber. It has been reported that *Makgeolli* contains about ten kinds of essential amino acids involved in in vivo metabolism (Lee and Shin 2011).

Makgeolli was also demonstrated to possess anticancer effects, such as growth inhibition of cancer cells and suppression of cancer cell metastasis. The component attributed to the anticancer effects, farnesol, contained about 150–500 ppb depending on the products, which is 10–15 times higher than the content in wine or beer (Ha et al. 2014a, b). In particular, some studies have suggested that there exist large amounts of farnesol in the cloudy but exist small amounts of *Makgeolli* (Ha et al. 2014a, b). Another study also presented results from the investigation of inhibiting the activation of cancer metastasis by analysis of cell motility and the activation of MMM inhibition.

Table 11.24 Composition of organic acids in commercial *Makgeolli* (mg/mL)

Makgeolli	Pyruvate	Malate	Lactate	Acetate	Citrate
A	0.07*	0.72	0.87	0.37	3.55
B	Trace	0.37	1.55	0.70	2.49
C	0.05	0.89	2.87	0.21	4.46
D	0.21	1.16	2.75	1.09	4.83
E	0.12	1.35	1.66	0.94	5.03
F	0.04	0.40	0.74	0.47	2.70
G	Trace	0.27	0.96	0.54	1.28

*Each data represents the mean of three independent experiments

The study reported that *Makgeolli* suppressed the expression of MMP-2 and MMP-9 in a dose-dependent manner, with exhibition of MMP-9-specific activities in particular (Lee and Shin 2011). Moreover, the methanol fraction of *Makgeolli* was confirmed to show dose-dependent growth inhibition of four kinds of cancer cell lines, including HepG2, B16-F10, HT29, and MCF-7. The methanol fractions particularly showed the greatest increase of QR activity measured from the liver cancer cell, HepG2, suggesting cancer prevention effects (Shin et al. 2008). In addition to the anticancer effects, *Makgeolli* has also been reported to possess anti-obesity, anti-angiogenic, and anti-inflammatory activities. According to a recently conducted study, which assessed the anti-obesity effects of *Makgeolli* using the 3T3-L1 preadipocyte cell line, two different types of *Makgeolli* presented about 20 % inhibition compared to the control group. From such results, *Makgeolli* was identified to suppress the differentiation of adipocytes by inhibiting the formation of lipid droplets to be used as the index of differentiation of adipocytes. It was also reported that no cytotoxicity was observed on the 3T3-L1 cells and differentiation-induced adipocytes (Lee et al. 2011a, b, c).

When assessing the effects of *Makgeolli Nuruk* extract on alcohol-induced gastritis/gastric ulcer using the HCl-EtOH-induced gastric damage model and the ethanol absolute-induced gastric damage model, the *Nuruk* extract demonstrated significant inhibition of gastric lesion compared to the control group, displaying about 1.37 times greater inhibition than the positive control, cimetidine. The *Nuruk* extract administration group also showed significant antiulcer effects compared to the control group after the administration of absolute alcohol in the alcohol-induced gastric damage model, and the *Nuruk* extract of *Makgeolli* appeared to be related to the increase of micro blood flow incurred by prostaglandin (Lee and Shin 2011).

Meanwhile, Shin et al. (2010) reported that the beneficial effects of *Makgeolli* and its by-products may be used to improve the lipid metabolic syndrome of menopausal women. In addition, *Makgeolli* and the by-products might improve blood homeostasis-mediated activities via anti-platelets, and *Makgeolli* and its by-products may be used as antihypertensive functional foods and nutraceuticals (Shin et al. 2010).

11.5.5 Ethnical Value and Socio-economy

Koreans had advanced farming cultures from the earlier days. As the ironware had become popular, a full-scale agricultural society had been settled together with the development of traditional alcoholic beverages made from grains. In particular, a traditional Korean alcoholic drink, *Makgeolli*, had strengthened the relationship among farmers by sharing *Makgeolli* as *Nongju*, which means the farmer's alcoholic drink, in the busy seasons. At the same time, it became a power source to shape the food and beverage culture in agricultural society. *Makgeolli*, which has been leading the food and beverage culture of Korea's agricultural society, now has been recognized not only for its traditional manufacturing method, history, and conventional food but also for the wisdom found inside the original Korean tradition. Nowadays, because of the people's interest for health, low-alcoholic beverages, such as *Makgeolli*, have become popular in Korea.

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12 Ethnic Fermented Foods and Beverage of Iran

Neda Mollakhalili Meybodi,
Maryam Tajabadi Ebrahimi,
and Amir Mohammad Mortazavian

12.1 Introduction

Iran locating in West Asia is bordered by the Caspian Sea, Persian Gulf, and Gulf of Oman. Iran's mountains however form both the political and the economic history of the country, is also surrounded by several broad basins, or plateaus with majority of agricultural and urban settlements (Pak and Farajzadeh 2007) (Fig. 12.1).

Iran has an uneven climate. In its northwest, the winters are cold with heavy snowfall and subfreezing temperatures during December and January, but it is rather mild during spring and autumn and dry and hot during the summers. In the south of Iran, however, the winters are mild; the summers are very hot with an average daily temperature above 38 °C (100.4 °F) in July. On the Khuzestan Plain, summer heat is going along with high humidity.

The majority of Iran population (around 67–80 %) consists of Iranic peoples. The largest groups in this category comprise Persians and Kurds, with smaller groups including Gilakis, Mazandaranis, Lurs, Tats, Talyshs, and Baluchs.

Approximately 30 % of Iran's total surface area is capable of farming, but they are not cultivated regarding its poor soil and inadequacy of water distribution in many areas. The variety of temperature in different parts of Iran and the diversity of climatic zones resulted in cultivation of different crops, comprising cereals, fruits, vegetables, cotton, sugar beets, sugarcane and pistachios, nuts, olives, spices like saffron (the largest producer in the world with approximately 81 % of the world's total output), raisin, tea, tobacco, and medicinal herbs.

Iranian food which is also denoted as Persian food is composed of small amounts of red meat and large amounts of grains like rice, fruits, and vegetables. The Iranian foods are mainly famed for their fresh taste and healthy properties.

Fermented foods are paying growing attention in Iran for their formation of a diversity of aromas, flavors, and textures from a single raw material. However, fermented foods are produced for a long

N.M. Meybodi
Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Research Center for Food Hygiene and Safety, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

M.T. Ebrahimi (✉)
Department of Biology, Faculty Member of Science, Islamic Azad University, Central Tehran Branch, Tehran, Iran
e-mail: m.tajabadi@iauctb.ac.ir

A.M. Mortazavian
Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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time, they received quite little consideration from the native scientific organization, and little publications have been available during these years for many products (Campbell Platt 1987; Wood 2012). Even the researches have been done are not disseminated as publications in peer-reviewed international scientific journals to be available by others. This impedes research development and does not afford support to others to instigate research in the area. Therefore, many of the foods continue to be produced by small-scale plot producers. In this chapter, the production, microbiology, and biochemistry of a wide variety of native fermented foods and beverages of Iran are described. The main Iranian fermented foods are presented in Table 12.1.

12.2 Classification of Fermented Foods and Beverages of Iran

Fermented foods can be categorized regarding different points of view, for example, the kind of microorganism used in the preparation of fermented foods is considered as a basis to classify fermented foods in Southeast Asia. However, other classification techniques are also available which are based on commodity and/or function of the food. These different classification techniques indicate the different attitude of the authors. It is worthy to note that a classification technique, which works very well in one part of the world, is not appropriate

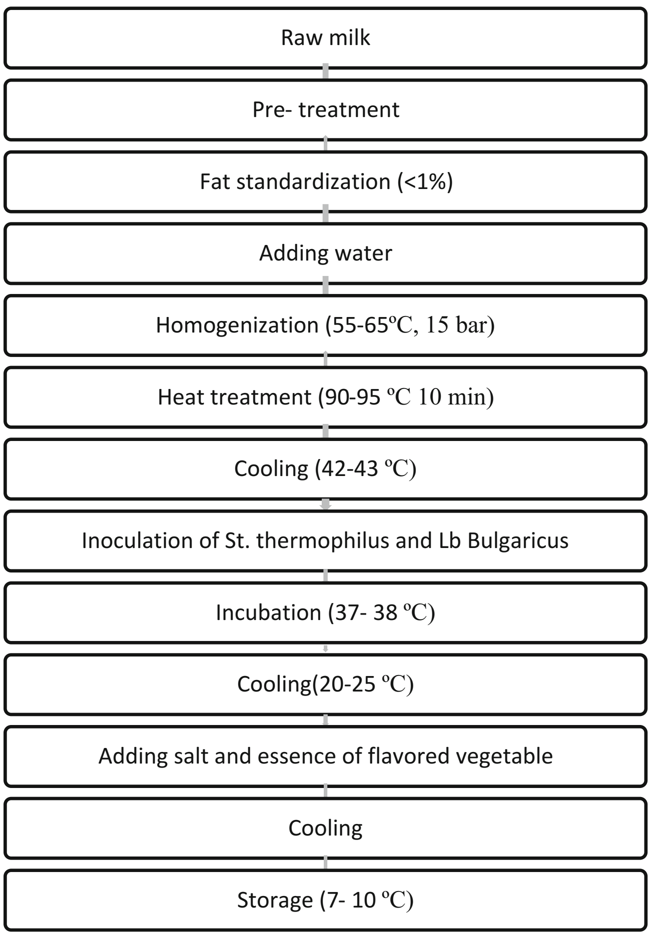

Fig. 12.1 The industrial production of *Doogh* by two methods

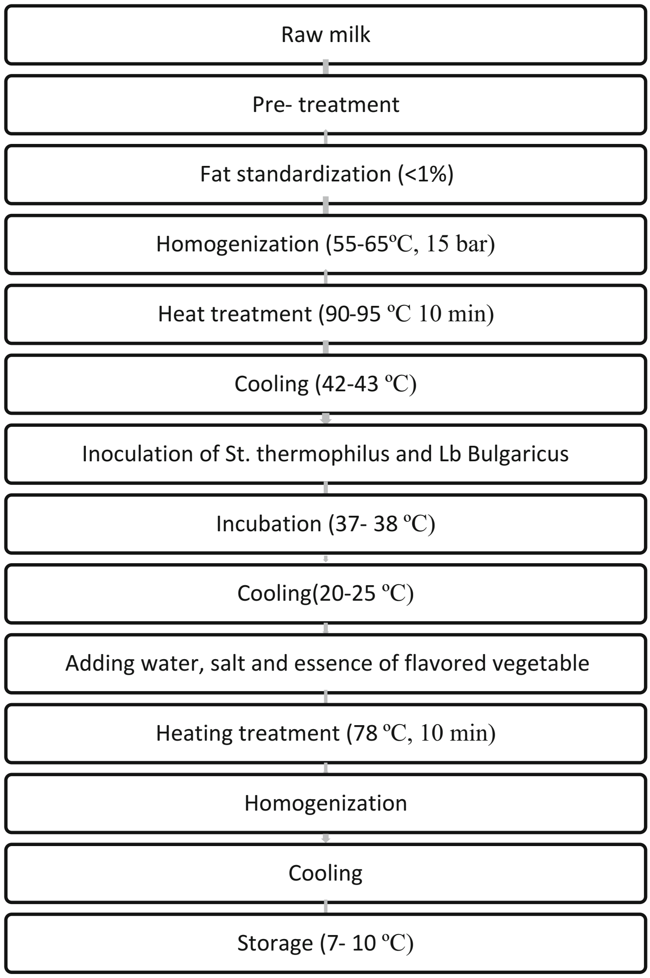

Fig. 12.1 (continued)

in other parts, and when a classification method is invented, it is difficult to disseminate the foods.

In this chapter, Iranian fermented foods are manufactured using traditional fermentation process including:

1. Dairy products
2. Cereal-based products
3. Fruit- and vegetable-based products
4. Meat based, which will be discussed regarding their ingredients, manufacturing process, microbiology, biochemistry, and nutritional points of view in the next

12.3 Fermented Dairy Products

Fermented dairy products, also recognized as cultured dairy foods, cultured dairy products, and/or cultured milk products, are dairy foods which have been fermented mainly by lactic acid bacteria like *Lactobacillus*, *Lactococcus*, and *Leuconostoc* (Wouters et al. 2002). The action of fermenting increases the shelf life of the product, along with enhancing their taste and digestibility. A range of different cultured milk products are available in Iran, namely, *cheese*, *yogurt*, *kashk*, *Doogh*, and *kefir*.

Table 12.1 Some common and uncommon ethnic fermented foods and beverages of Iran

Name of product	Substrate	Microorganisms	References
Beverages			
Doogh	Animal milk	*St. thermophilus*, *Lb. bulgaricus*	Dana et al. (2011)
Kefir	Animal milk	*Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, and *Kluyveromyces marxianus*	Oh et al. (2013)
Vinegar	Different fruits and seeds	Yeast and lactic acid bacteria (LAB) or molds and acetic acid bacteria (AAB) (the former being responsible for the alcoholic fermentation and the latter needed for the acetification)	Bhat et al. (2014)
Wine	Grape	Yeasts and bacteria. The latter can quickly turn the nascent wine into vinegar	Jackson (2008)
Dairy products			
Cheese	Animal milk	*Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. delbrueckii* subsp. *lactis*, *Lb. helveticus*, *Lb. casei*, *Lb. plantarum*, *Lb. salivarius*, *Leuconostoc* spp., *Strep. thermophilus*, *Ent. durans*, *Ent. faecium*, and *Staphylococcus* spp., *Brevibacterium linens*, *Propionibacterium freudenreichii*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Penicillium camemberti*, *P. roqueforti*	Karimi et al. (2011); Karimi et al. (2012)
Yogurt	Animal milk	*St. thermophilus*, *Lb. bulgaricus*	Han et al. (2014)
Kashk	Animal milk	*St. thermophilus*, *Lb. bulgaricus*	Soltani and Güzeler (2013); Noori et al. (2013)
Cereal-based products			
Bread	Flour, water	*Sour dough* (*Lactobacillus plantarum*, *Lactobacillus delbrueckii, and Leuconostoc mesenteroides*)	Rahnama et al. (2014); Khoshakhlagh et al. (2014)
Tarkhineh (*Tarkhowana or Doowina*)	Wheat meal (bulgur or cracked and bran-free parboiled wheat), sour doogh	*St. thermophilus*, *Lb. bulgaricus*	Mashak et al. (2014); TAJAABADY et al. (2011)
Kashk-e zard	Cereal flour (mainly wheat flour), yogurt, vegetable	*St. thermophilus*, *Lb. bulgaricus*	Mashak et al. (2014)
Fermented vegetable-based products			
Sauerkraut	Cabbage	*Leuconostoc mesenteroides*, *Lactobacillus plantarum, and L. brevis*	Owens (2014); Farnworth (2008)
Pickle	Vegetable	*Lactobacillus plantarum*, *Leuconostoc mesenteroides*	Farnworth (2008)
Processed olive	Olive	Natural of ingredients, *Lactobacillus plantarum*	Marsilio et al. (2005)
Fermented meat			
Mahyaveh	Sardines (*Sardinella sp.*) or anchovies (*Stolephorus sp.*), salt, mustard (*Brassica juncea*), water	Lactic acid bacteria (LAB)	Zarei et al. (2012)

12.4 Milk as a Medium for Microbial Growth

12.4.1 Cheese

Cheese was firstly achieved inadvertently by an Arabian merchant when he keep his milk into a waterproof pouch making of a sheep's stomach (so-called Mashk) during a long day's trip at the desert (Classen et al. 1994). The rennet existed inside the Mashk and the heat of the sun converted the milk to separated curd and whey. Before using the cultures to prepare cheese, it was made by:

– Natural souring resulting the temperature
– Adding buttermilk or sour whey
– Addition of homemade starter

About 1900, Hansen in Denmark set commercial cultures for cheese making on the market which increase the production of cheese on a wider scale.

About 2000, names are allocated to cheeses according to their origin and country, source of milk, method of production, moisture content, the used cultures inventor, and method of ripening.

To produce cheese from milk, two main stages must be considered:

1. Concentrating the casein and fat of milk via coagulating the casein by proteolytic enzymes or lactic acid
2. Whey elimination after mechanical disturbance of the coagulated casein

Using these techniques, more than 1000 cheese varieties are created today. Higher varieties of cheeses are achieved by changing different aspects of cheese like the starter culture type, additional cultures, fermentation setting, renting, cutting the curd, scalding, whey removal, formation of green cheese, salting, adding spices, and ripening. The enormous diversity of cheeses makes its production advantageous even at small cottage industry. In Iran some cheeses are produced locally which are famous in both inside and outside of country.

12.4.2 *Lighvan* Cheese: An Iranian Traditional Cheese

Despite the progress and developments in manufacturing equipment and techniques, the rapid growth in production and type of industrial cheese in Iran, *Lighvan* is still produced and preferred in different area of the country (Abdi et al. 2006). Considering the pleasing organoleptic characteristic, *Lighvan* cheese is famous and commonly consumed in Iran (Mirzaei et al. 2008). The semihard white *Lighvan* cheese produced by sheep's milk or a combination of sheep's and goat's milk (the ratio of the latter is not more than 20–30 %) is ripened in brine. Regarding the fact that *Lighvan* cheese is prepared without any starter culture addition, the native microflora is effective in determination of cheese ripening (Mirzaei 2011). The instruments used in cheese making are severely artisanal and conventional with manual manufacturing phases. *Lighvan* cheese is manufactured at *Lighvan* village which is placed in Tabriz (the northeast of Iran). However, the Food and Drug Office try to convince the cheese producers of the village to use pasteurized milk to manufacture cheese; most traditional cheese makers believe the higher pleasant taste and flavor of cheese prepared with raw milk which can be attributed to the higher proteolytic and lipolytic enzyme activities of microflora in raw milk (Kafili et al. 2009). The producing procedure of *Lighvan* cheese is summarized in Fig. 12.2.

In addition to *Lighvan*, other cheeses like *Siahmazegan* and *Talesh* are also produced in Iran wherein their high fat content and specific flavor make them famous.

12.4.3 Yogurt

In Iran yogurt is being served in more healthy state by adding some vegetables like Prangos ferulacea, pennyroyal, mint, spinach, tarragon, grilled eggplant, cucumber, and shallot. In other words, the yogurts are being flavored. Yogurt is also available in drained states in Iran which are more nutritious compared to yogurt in a same volume.

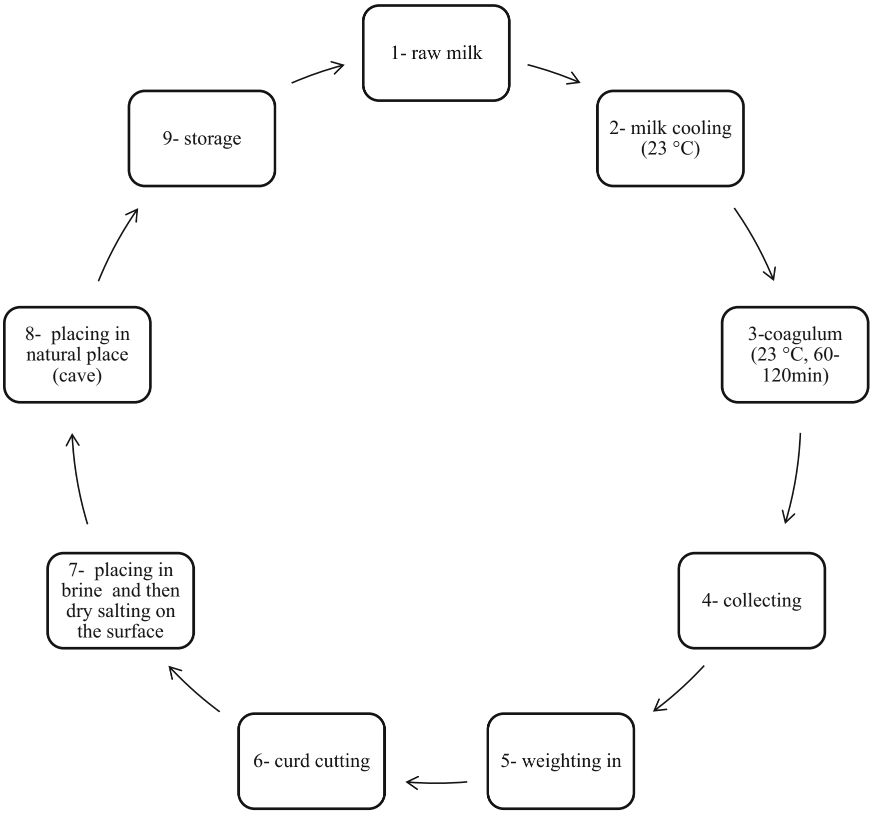

Fig. 12.2 Flow diagram of Lighvan cheese production

12.4.4 *Kashk*

Kashk is another famous and accepted fermented Iranian dairy product. It is referred to in the tenth-century Shahnameh (a Persian book of poetry). Actually, this word may derive from the word Khushk (a Persian word means drying) which denotes that this product is manufactured by drying process (Noori et al. 2013). A low-fat dried yogurt with no cereal addition is identified as *kashk* in Iran, and it is used in many Iranian traditional foods (Shiroodi et al. 2012). *Kashk* is in whitish-yellow, semiliquid, or dried form which must be soaked and softened before being used in the latter form. In fact it was prepared in dried form traditionally but industrially is in liquid form. Dried *kashk* is a thick yogurt-type product manufactured by dehydration of domestic yogurt by sun-drying during summer in the different regions of Iran (Dehkordi et al. 2014). This product can be stored at room temperature for months without nutritional value loss or spoiling (Soltani and Güzeler 2013). Some chemical composition of dried *kashk* is presented in Table 12.2.

However, dried *kashk* is a highly nutritious fermented dairy product that can be used in diets of children and pregnant and lactating women (Ebrahimi et al. 2011). It may be contaminated due to inappropriate conditions of production and storage. Recently, *kashk* is manufactured in a healthier way in industrial dairy unit in two forms of traditional liquid *kashk* and industrial liquid *kashk*. These two have been significantly imparted in dairy products used in Iran, and specific standards are settled to consider them.

Table 12.2 The microflora of kefir grain

The microflora of kefir grain	
Yeast	Bacteria
Candida kefir	*Lactobacillus kefir*
Kluyveromyces marxianus	*Lactobacillus helveticus*
Saccharomyces lactis	*L. brevis*
S. cerevisiae	*L. casei*
S. fragilis	*L. plantarum*
	Streptococcus lactis
	Leuconostoc mesenteroides
	Acetobacter aceti

Table 12.3 Chemical composition of dried kashk (an Iranian fermented dairy product)

Parameter	Mean
pH	4.27 ± 0.24
Acidity (LA %)	1.40 ± 0.29
Moisture (%)	14.21 ± 2.54
Fat (%)	9.17 ± 3.10
Protein (%)	51.74 ± 3.57
Salt (%)	9.77 ± 1.44
Ash (%)	12.25 ± 1.50

Soltani and Güzeler (2013)

12.5 The Production of Traditional Liquid *Kashk*

It is manufactured by traditionally dried *kashk*. *Kashks* are put into a large container and washed by pressurized water (given inside from the one side of the container) repeatedly. Then *kashks* are milled and mixed with drinking water, edible salt, and maybe other spices like mint. The drinking water used is four to five times higher in amounts of the *kashks*, and the proportion of edible salt is 0.5–1 %. The mixture is subsequently passed throughout a filter and poured to the separator to standardize the fat ratio and separate small particles. The mixture is then homogenized (50–55 °C, 2 bar) and heated (85 °C, 15 s). It is then cooled before being filled in glass jars. In the final step, the jars are put in special boxes and stored at 4 ± 1 °C.

12.6 The Production of Industrial Liquid *Kashk*

This *kashk* is produced by yogurt in industrial units (Soltani and Güzeler 2013). Firstly, the raw milk is checked considering its chemical and microbiological characteristic. The standardized milk (fat ratio about 0.4–0.6 %) is pasteurized (90–95 °C, 5 min) and then cooled (43 ± 1 °C). The starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) at amount 1–2 % is added to milk and incubated (37 °C, 6–7 h). At the final step, the produced yogurt is homogenized (50–55 °C, 2 bar), and its dry matter is increased to about 18–19 % using quark separator. After adding edible salt (0.8–1 %) and whey powder (1–2 %), the mixture is exposed to heat treatment (88 °C, 10 min). It is then cooled (55 ± 5 °C), packed, and stored at +4 °C.

The chemical composition of traditional and industrial liquid *kashk* is presented in Table 12.3.

12.7 *Doogh*

Doogh as an Iranian drinking yogurt type is a dairy fermented beverage which comprises the main division of daily beverage utilization in Iran (Kiani et al. 2008). *Doogh* is frequently manufactured by combination of yogurt, drinking water, salt, and essence of flavoring vegetables like thyme, mint, and oregano. Two separate methods have been used to produce *Doogh* which are presented in Fig. 12.3.

According to codex standard, *Doogh* (yogurt drinking) is defined as a traditional dairy Iranian beverage on the basis of different milk fermentation (Esfandiari et al. 2013). This product is mainly consumed in Iran and is exported to other countries like Armenia, Azerbaijan, Afghanistan, Balkans, Iraq, and, to lesser extent, central Asia and some parts of the Middle East. *Doogh* is a derivative form of Persian word "dooshidan" which means "milking." Traditionally, *Doogh* meant a beverage product which is manufactured via the dilution of yogurt after a tough agitation step, in particular sacs prepared by goat and/or sheepskin naming "Mashk." *Doogh*, as a famous Iranian dairy beverage, with a growing

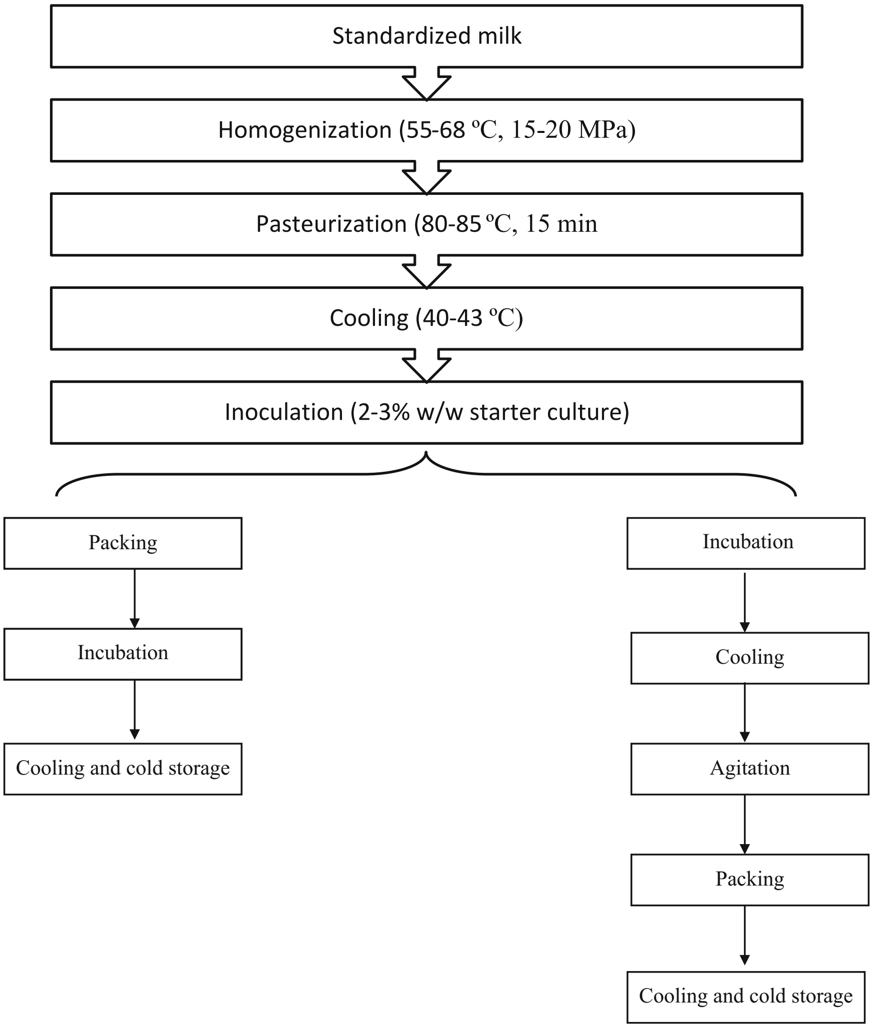

Fig. 12.3 Main processing step of set and stirred yogurt

annual intake of 13 million tons, is accepted as the traditional national drink in Iran. The reputation of *Doogh* is not only due to its notable organoleptic properties but also due to its important health benefits to humans. Regarding the ISIRI statement, *Doogh* manufactured in Iranian dairy units is classified into four groups (Soltani and Güzeler 2013):

1. Noncarbonated and unheat treated
2. Noncarbonated and heat treated
3. Carbonated and unheat treated
4. Carbonated and heat treated

Heating and carbonating treatments are mainly used after fermentation. Heat treatment is applied to prevent the starter microorganism activity and possible cross contamination and also to lengthen the shelf life of the final product.

12.8 Some Properties of *Doogh*

Fermented dairy products are usually categorized into either viscous, diluted, beverage, or carbonated products. Considering this classification, *Doogh* is placed in diluted group; however, it has been carbonated recently to make a bubbly alternative of the traditional product (Nilsson et al. 2008). *Doogh* is naturally an acidic beverage and prone to phase separation similar to other acidified milk drinks. The phase separation mainly occurred due to low pH-induced aggregation of casein (Foroughinaia et al. 2007). During the making of acidified beverages like *Doogh* by yogurt dilution, the particles of fragmented acid casein gel are separated, resulting in critical loss of stability and higher sedimentation in particles as mentioned (Lucey et al. 1999). It is worthy to consider that the edible salt addition in the production of *Doogh* makes a key role in phase separation enhancement (Köksoy and Kılıç 2003). So the consumers will shake the product before consumption. In order to inhibit this occurrence, different researches have been conducted to increase the stabilization of *Doogh* using different gums like guar (Foroughinaia et al. 2007), tragacanth (Azarikia and Abbasi 2010), gellan, and pectin (Kiani et al. 2010). The results indicated that using one gum is more effective to enhance the stability of *Doogh* compared to a combination of different gums. It has also been concluded that there is no relationship between the stabilization capability of gum and the pH range of *Doogh* produced.

12.9 Cereal-Based Fermented Products

12.9.1 Bread

Respecting the results of studied one at national level in Iran, per capita utilization of bread is about 160 kg, which is greatly higher compared to the world average (Alami et al. 2014). The quality of bread has been denoted considering different factors including:

1. Sensory characteristic like its shape, volume, taste, color, and texture
2. Nutritional values like vitamins, iron, and calcium amounts
3. Health consideration like its microbial contamination and prohibited additive addition

"Traditional Iranian breads" are mainly popular due to their diversity, taste, and quality. However, the quality of urban breads has diminished in some cases; on the other hand, because of the use of sodium bicarbonate, rural breads have high quality, since they are mainly dependent on sourdough which is rich in lactobacilli and baker's yeast). Lactic acid bacteria (LAB) which are used in sourdough as starter cultures contribute to the breads' organoleptic and protective properties against microbial spoilage (Mollakhalili Meybodi et al. 2015).

Commonly, five types of bread are produced in Iran, namely, *Sangak*, *Taftoon*, *Barbari*, *Lavash*, and *village* breads (Faridi et al. 1983) which are discussed in the succeeding text separately.

12.9.2 *Barbari* Bread

Barbari is a famous flatbread which is widely used in the northern and northwestern area of Iran (Ghanbari and Farmani 2013). It has 70–80 cm length and 25–30 cm width with a thickness of about 3.5 mm. The bread is prepared by mixing all the ingredients to reach a suitable consistency and then is fermented for about 2 h. The dough are shaped in balls and then flattened into an oval shape and rested for 20 min. Subsequently, about 0.5–1 % of a mixture containing flour, water, and oil is poured on the surface to create polished and brown surface after baking. Final proof and baking times are, respectively, about 15 and 8–12 min.

12.9.3 *Sangak* Bread

Sangak is a commonly used flatbread because of its good flavor and high nutritional value. The name *Sangak* is derivative from its way of baking on a hotbed of small pebble gravels in the oven. It is manufactured by whole wheat flour which is shaped in triangular with 0.5–1 cm thickness. In traditional *Sangak* bread ovens, the temperature is set at 250–350 °C and is prepared for only a few minutes. The dough prepared for *Sangak* bread has higher moisture content and lower consistency compared to others (Najafi et al. 2012).

12.9.4 *Taftoon* Bread

Taftoon is an Iranian flatbread manufactured by highly extracted soft white wheat flours. The bread is usually round with length of about 400–500 mm, width of 400–500 mm, and thickness of 2 mm. The ingredients of *Taftoon* bread are flour = 100 kg, water = 78–90 L, yeast = 450–650 g, and salt = 90–130 g, and its preparation method is similar to other bread (Salehifar and Shahedi 2010).

12.9.5 *Lavash* Bread

Lavash is also traditional Iranian white wheat flatbread that can be used to prepare a sandwich. The *Lavash* bread is lightly baked and can be served as an appetizer or a healthy snack (Mortazavi and Sadeghi 2013; Fazeli et al. 2004). It is oval shaped with 60–70 cm length, 30–40 cm width, and 2–3 cm thickness. The ingredients are highly extracted flour (100 %), yeast (1 %), salt (2 %), soda (0.25 %), and water (45 %) (Movahed et al. 2011). They are mixed thoroughly and fermented for 1–3 h. The dough balls are being flatted on a wooden surface using a roller. It is then heated at 200–250 °C for about 5 min.

12.9.6 *Tarkhineh*

Tarkhineh (Tarkhowana or Doowina in Kurdish) is a rare fermented cereal food (both commercially and in homes) which is traditionally prepared and consumed in the west of Iran (Kurdistan, Kermanshah, and Hamedan provinces) (Tabatabaee et al. 2012). It is prepared by soaking wheat (either bulgur or broken and bran-free partially boiled wheat) in sour *Doogh* which is then fermented for 7–10 days. Afterward, some flavoring agents like dried vegetables, salt, and spices are added to the mixture (doughlike mixture) and exposed in small pieces to sunlight to be dried. *Tarkhineh* is preferred to be consumed in Iran due to its natural taste/flavor and therapeutic effects (Ebrahimi et al. 2014). Its fermentation process mainly occurs as a result of the natural microorganisms present in the raw materials whose growth, activity, and role during the fermentation are influenced by environmental conditions, especially temperature and salt content. The microbial communities in *Tarkhineh* have been mainly identified as *L. nagelii*, *L. bifermentans*, *Leu. cermoris*, *L. fructosus*, *L. fermentum*, *L. intestinalis*, *L. agilis*, and *L. acidipiscis* (Tafvizi and Tajabadi Ebrahimi 2015).

12.9.7 *Kashk-e Zard*

Kashk-e Zard is also a cereal-based fermented product usually consumed in the southeastern part of Iran (Sistan and Baluchestan province) (Mashak et al. 2014). It is prepared by mixing cereal flour, usually wheat flour, yogurt, different vegetables, salt, and spices along with 1-week lactic and alcoholic fermentation. It is usually a combination of 65 % yogurt and 35 % wheat flour. The fermentation process occurred at two separate phases to create the final product. Firstly, wheat flour is mixed with yogurt to create a doughlike. After storing for 1 week in a closed pot and warm place, the yogurt is added again and the dough is kneaded to homogenize the product. At the end of this stage, spices and garlic are added and stored for 7–10 days for complete fermentation. Finally, the product is spread out to be dried. After drying, it is grounded to 1–3 mm granules. The products similar to this in other countries are called Tarhana (Turkey and Greece), *Kishk* (Lebanon and Egypt), *Kushuk* (Iraq), *Madeer-Oggt* (Saudi Arabia), Kichk (India), *Talkuna* (Finland), *Tahanya* (Hungary), and *Atole* (Scotland) (Mashak et al. 2014).

12.10 Fermented Vegetable

The common fermented vegetable consumed in Iran and their main characteristic are summarize in Tables 12.4 and 12.5 (Marsilio et al. 2005).

12.11 Olives

Olive (*Olea europaea* L.) is a subtropical fruit with significant economic importance. However, Mediterranean basin is the main region of olive production; some other countries like Iran are also important. It has been believed that Iran is the main center of olive production in the world considering both the origin and the diversification (Dastkar et al. 2013). To design olive fermentation process, particular biological and technological restrictions must be considered to lessen the expenses throughout the production and downstream processes. All Iranian olive fermentations occurred during these three fundamentally separate steps including pretreatment of raw material, fermentation, and product recovery.

12.12 Olive Brining and Fermentation Control

In order to control the fermentation process and the safety of final product, it is necessary to be aware of microorganisms that took part in olive fermentation. During the production of green olive, the bitterness is removed using alkaline treatment. However, the lactic acid bacteria dominate in the brine of green olives; the fermentative yeasts are present in the brines of black ones. *Saccharomyces cerevisiae* and *Candida boidinii* are, respectively, the most common recognized species in green olives and processed black olives (Arroyo-López et al. 2008).

Table 12.4 The chemical composition of traditional liquid kashk (1) and industrial liquid kashk (2)

	Mean	
Parameter	1	2
pH	4.15±0.07	3.78±0.05
Acidity (LA %)	1.82±0.13	1.54±0.10
Moisture (%)	74.56±0.19	81.41±0.15
Fat (%)	1.99±0.07	1.65±0.06
Protein (%)	13.66±0.22	8.59±0.22
Salt (%)	2.54±0.06	1.69±0.07
Ash (%)	2.83±0.06	2.30±0.08

12.13 Pickled Vegetables

Pickled vegetables prepared in households or small factories are popular in Iran (Prajapati and Nair 2008; Farnworth 2008). The vegetables pickled in Iran are carrots, cucumbers, cabbage, cauliflower, garlic, and pepper. These products are mainly consumed as appetizers and almost every meal. It is called *jeruk* in Malaysia and has been famous since very early times.

12.14 Fermented Meat

12.14.1 *Mahyaveh*

Mahyaveh is a traditional fermented fish sauce widely consumed in the southern part of Iran (Zarei et al. 2012). This product is mostly produced according to the family tradition, availability of raw materials, consumer preferences, and climatic conditions of the region. Therefore, wide variations can be seen in production methods, proportions of raw materials, and composition between retail samples from different sources. *Mahyaveh* is typically composed of sardines (*Sardinella* sp.) or anchovies (*Stolephorus* sp.),

Table 12.5 The main fermented vegetable consumed in Iran

Product	Salt concentration	Condition	The dominant microflora	Acid (%)	PH
Olive	2–3 %	Alkaline treatment, submerged	Natural of ingredients, *Lactobacillus plantarum*	0.7–1	3.8
Pickled vegetable	1.5–6.6 %	Submerged	Natural of ingredients, *Lactobacillus plantarum*, *Pediococcus cerevisiae*	0.6	3.8
Black tea	–		Natural of ingredients	–	4.9

Stamer (1975)

salt, mustard (*Brassica juncea* L.) Czern, and water. It is traditionally prepared from fresh or dried fish, which are beheaded, washed, and packed along with salt and warm water into earthen or glass jars. The jars are allowed to stand under the sun or at ambient temperature for 25–30 days. The fish–salt mixture is then mashed into slurry and filtered through a stainless steel mesh. The brown liquid portion of fermented fish is then mixed with mustard and other spices. Various spices such as cumin (*Cuminum cyminum* L.), coriander (*Coriandrum sativum* L.), fennel (*Foeniculum vulgare* Mill.) seeds, black pepper (*Piper nigrum* L.), and thyme (*Thymbra capitata* L.) are added according to the consumer preferences. After a thorough mixing, the jars are placed at ambient temperature until the desirable taste and aroma are produced, usually after 10–15 days. Zarei et al. (2012) found that the pH of *mahyaveh* from different locations was in the range of 4.89–7.55, and the concentration of NaCl was in the range of 7.48–17.1 %. Moreover, they observed histamine (2.66 g/kg) was found to be the main biogenic amine in the Iranian fish sauce (Taheri et al. 2014). The high histamine content can be related to the high levels of bacterial count, especially *Enterobacteriaceae* and lactic acid bacteria in this product.

12.15 Conclusion

Fermenting foods have been used by humans for thousands of years, in all around the world. In fact, the fermentation process is considered as a controlled spoilage in which the enzymes manufactured by microorganisms break the carbohydrates, fats, and proteins into the simpler compounds. This breakdown resulted in the production of final products which are easier to digest, have increased nutritional value, and are without toxins.

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13 Ethnic Fermented Foods of the Philippines with Reference to Lactic Acid Bacteria and Yeasts

Francisco B. Elegado, Shara Mae T. Colegio, Vanessa Marie T. Lim, Andrea Therese R. Gervasio, Maria Teresa M. Perez, Marilen P. Balolong, Charina Grace B. Banaay, and Bernadette C. Mendoza

13.1 Introduction

The Philippines is an archipelago of 7107 islands, situated above the equator, south of Taiwan, east of Vietnam, north of Indonesia, and west of the Pacific Ocean. The country has typically hot and humid weather throughout the year and generally experiences dry season from December to May and rainy season from June to November. There are four climate types in the country as described in Fig. 13.1.

There are a variety of people spread across the country with distinct languages and cultural traditions. About 95 % of the Philippine population are of Malay (Malayan and Indonesian) stock divided into nine major ethnolinguistic groups, namely, the Tagalogs, Ilocanos, Pampangueños, Pangasinense, and Bicolanos in Luzon and the Cebuanos, Boholanos, Ilonggos, and Waray-Waray of the Visayan islands and Mindanao. There are also several racial and religious minorities such as the Chinese-Filipinos (1.5 %), Spanish-Filipinos, Japanese-Filipinos, Indian-Filipinos, and ten Muslim groups (4 %) mainly in Mindanao, Basilan, and Sulu islands and numerous other small ethnic groups such as the *Aetas*, *Badjaos*, *Bagobos*, *Balangaos*, *Gaddangs*, *Higaonons*, *Ibaloi*, *Ibanags*, *Ifugaos*, *Igorots*, *Itnegs*, *Mangyans*, *Manobos*, *Maranaos*, *Sambals*, *Tagbanwas*, *Tausug*, and *Tasaday*, among others (http://www.nationsencyclopedia.com/Asia-and-Oceania/Philippines-ethnic-groups.html; http://www.ethnicgroupsphilippines.com).

The Philippines is basically an agricultural country with 40 % of the labor force devoted to farming, fisheries, livestock, and forestry, contributing to 20 % of the GDP (http://www.nationsencyclopedia.com/economies/Asia-and-the-Pacific/Philippines-agriculture.html). The main cereal crops being produced are rice and corn. In 2012, agricultural statistics showed that the other major crops produced in order of economic value are bananas, coconut, sugarcane, rubber, mango, pineapple, and cassava. The minor crops are sweet potato, coffee, onion, eggplant, calamansi, tomato, abaca (fiber), cabbage, mongo, peanut, and garlic. Total crop production is valued in 2012 at Php 797.73 billion, while fish production is valued at Php 92.29 billion (http://www.nscb.gov.ph/secstat/d_agri.asp).

F.B. Elegado (✉) • M.T.M. Perez
National Institutes of Molecular Biology and Biotechnology, University of the Philippines Los Baños, College, Laguna, Philippines
e-mail: fbelegado@hotmail.com

S.M.T. Colegio • C.G.B. Banaay • B.C. Mendoza
Institute of Biological Science, University of the Philippines Los Baños, College, Laguna, Philippines

V.M.T. Lim • A.T.R. Gervasio • M.P. Balolong
Department of Biology, College of Science, University of the Philippines Manila, Padre Faura, Manila, Philippines

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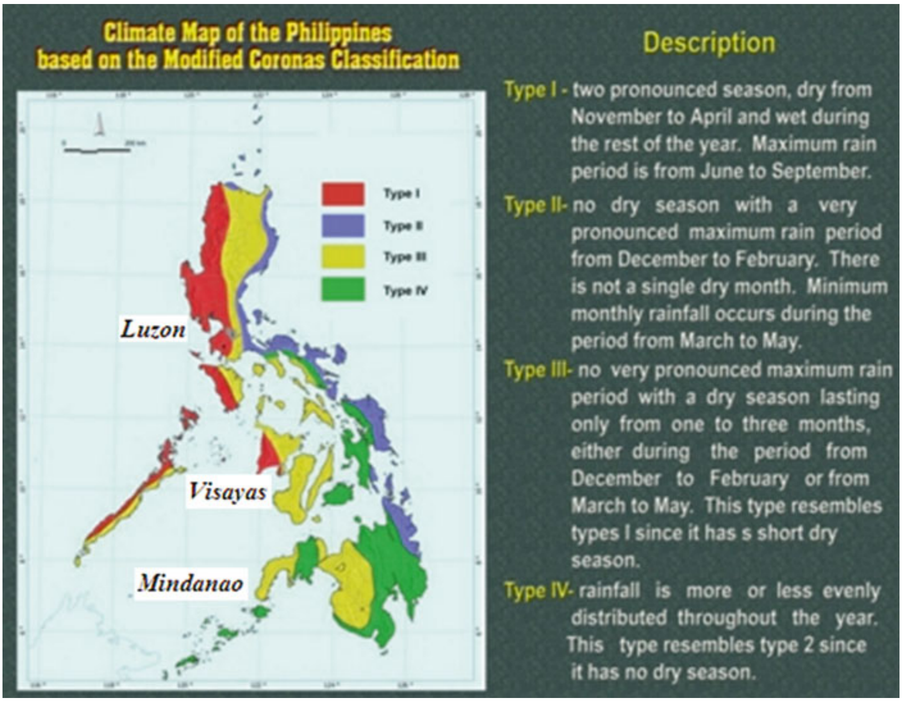

Fig. 13.1 Geographical distribution and descriptions of climate types in the Philippines (https://kidlat.pagasa.dost.gov.ph/index.php/climate-of-the-philippines)

The Philippine cuisine has evolved through its history and culture, enriched by the influences of foreign countries. Indigenous food culture started with its Malayo-Polynesian origins and then later on mixed with the food culture of Chinese and Arab traders. Major influences on food culture and cuisine were further brought about by three and a half centuries of Spanish colonization and a half century of American colonization. Recently, varying degrees of influences from Asian and European cuisines have further added to the variety and truly internationalized the Philippine food culture. A complete description of Philippine cuisines and food culture is available in the web (http://www.asian-recipe.com/philippines/philippine-cuisine.html).

13.2 Functionalities of Fermented Food

Growing public awareness of diet-related health issues has incurred the demand for naturally processed food products, particularly indigenous fermented foods and food preparations containing probiotic microorganisms (FAO/WHO 2002; Siro et al. 2008). The health-promoting properties of the fermented foods and probiotic products put them under the category of "functional food" which is defined as foods that provide health benefits beyond basic nutrition.

Traditional fermented culinary foods abound in almost all countries worldwide (Steinkraus 1995) are essential components of diets especially in

Asia (Tamang and Kailasapathy 2010). The methods of processing traditional fermented foods were initially developed in homes, and the process is normally handed down from generation to generation. With increased preference for a particular fermented food, small-scale industries ventured on their production, and they have been contributing to economic growth of many countries.

Japan has pioneered the studies of fermented foods and use of probiotic microorganisms from these foods to commercialize functional food products. The Japanese government approved the use of food for specified health use or "FOSHU" in the 1980s (Shimizu 2012; Yamada et al. 2008; Arai 2002). The probiotic industries of some countries have contributed to their economic development. The global probiotic market is projected to reach USD 46.6 billion by 2020, with Europe as the largest and Asia-Pacific region as the fastest-growing market (http://www.marketsandmarkets.com/PressReleases/probiotics.asp). Yakult and Danone are the most widely distributed probiotic drinks in Europe, Asia, Oceania, and America. For instance, approximately 32.37 million people around the world drink Yakult daily (Yakult Annual Report 2014). Japan had the highest sales volume of Yakult in 2014 which was approximately 8.96M bottles/day, while in the Philippines, its sales volume was 1.59M bottles/day (Yakult Annual Report 2014). In the USA and other countries, yogurt drink has the highest increase among the beverage products (Euro Monitor 2014).

13.3 Fermented Foods in the Philippines

Like other Asian countries, the Philippines has a rich diversity of ethnic cultures and a wide array of fermented foods produced from a wide biodiversity of indigenous raw materials and microbial resources (Sanchez 2008; Steinkraus 1995; Banaay et al. 2013). Every locality has their own fermented foods that are traditionally unique to their ethnic culture. These foods may have been adopted by other places and had gained wide acceptance. In the Philippines, there is a diversity of indigenous fermented foods – some of which are not yet even documented. These products could also vary and have certain peculiarities in different regions. They are more often found in rural communities such as rice wine (*tapuy*), sweetened rice (*binubudan*), fermented cooked rice cake (*puto*), fermented mixture of cooked rice and shrimp (*balao-balao*) or fish (*burong isda*), sugarcane wine (*basi*), coconut wine (*tuba*), distilled coconut wine (*lambanog*), palm sap vinegar (*sukang paombong*), fermented fish paste (*bagoong*), fermented fish sauce (*patis*), fermented small shrimp (*alamang*), fermented small fish (*tinabal*), cellulosic growth *of Acetobacter aceti* subsp. *xylinum* on sugary medium (*nata de coco* and *nata de piña*), fermented mustard leaves (*burong mustasa*), green mango pickles (*burong mangga*), and white soft cheese (*kesong puti*). The purposes of their production also vary, but in general, they are for culinary, preservative, and folkloric uses. Some have been prepared and packaged for commercial purposes. Most of the Philippine fermented foods have been listed by Banaay et al. (2013), and a modified list is shown below (Table 13.1). The antimicrobial functionalities, i.e., the bacteriocins of selected lactic acid bacteria from Philippine fermented foods, have also been discussed (Banaay et al. 2013; Perez et al. 2015). Steinkraus (1983, 1995) had compiled various research data on indigenous fermented foods and alcoholic beverages around the world, including selected fermented foods in the Philippines, focusing on the methods of production, microbiology, and physicochemical and nutritive changes during the course of fermentation. Sanchez (2008) further elaborated on the common fermented foods and alcoholic beverages in the country.

13.4 Geographical Distribution of Philippine Fermented Foods

The Philippines is divided into three main groups of islands, namely, Luzon, Visayas, and Mindanao (Fig. 13.1). Luzon consists of eight (8) regions, the Visayas with four (4) regions, and Mindanao with six (6) regions, totaling to 18 regions. From north to south, the names of the different regions

Table 13.1 Ethnic fermented foods and beverages of the Philippines

Local name of fermented foods	Substrates	Nature and uses	Microorganisms	Regions of consumption in the Philippines	Reference(s)
Burong mustasa	Mustard leaves, cooked rice, and/or rice washings	Side dish	*Leuconostoc mesenteroides*	Luzon	Banaay et al. (2013)
			Enterococcus faecalis		Larcia et al. (2011)
			Lactobacillus plantarum		
			Streptobacillus sp.		
			Fusobacterium sp.		
			Weissella sp.		
Burong pipino	Cucumber	Side dish	*Leu. mesenteroides*	Whole Philippines	Banaay et al. (2013)
			Lb. brevis		
			Pediococcus cerevisiae		
			Lb. plantarum		
Atcharang kangkong	Water spinach	Side dish	*Lb. plantarum*, *Weissella confusa*	Luzon, especially Bulacan	Clemente (2012)
Kesong puti	Cow/carabao milk	White soft cheese	*Lactococcus lactis*	Central and Southern Luzon, Visayas	Banaay et al. (2013)
			Enterococcus sp.		Belen (2010)
			Corynebacterium vitarumen		Sanchez (2008)
			Providencia sp.		
Balao-balao	Cooked rice, shrimp, salt	Side dish condiment	*Leu. mesenteroides*	Central and Southern Luzon	Arroyo et al. (1977)
			P. cerevisiae		Solidum (1979)
			Lb. plantarum yeasts		Sanchez (2008)
Burong isda	Freshwater or brackish water fish, rice, salt	Side dish condiment	*Lb. plantarum*	Central and Southern Luzon	Olympia et al. (1995)
			Leu. mesenteroides		Orillo and Pederson (1968)
			Leu. paramesenteroides		Sakai et al. (1983)
			E. faecalis		Sanchez (2008)
			P. cerevisiae		
			P. acidilactici		
			Micrococcus sp.		
			Streptococcus sp.		
			Lb. coryniformis		

Tinabal	Parrot fish (*tinabal molmol*), frigate fish (*tinabal mongko*), salt	Side dish, viand	*P. pentosaceus*	Visayas	Calanoga (1995)
			Streptomyces equinus		Sanchez (2008)
			Leuconostoc sp.		
			Lactobacillus sp.		
			Bacillus sp.		
			Micrococcus sp.		
			(*Staphylococcus* sp., *Pseudomonas* sp., and *Alcaligenes* sp. are present at early days of fermentation)		
Burong talangka	Small shore crabs (*Varuna litterata*)	Side dish, viand	*E. faecalis*	Central and Southern Luzon	Sanchez (2008)
			P. cerevisiae		
			L. plantarum		
Patis	Small fish, salt	Fish sauce, condiment	*Pediococcus halophilus*	Whole Philippines	Baens-Arcega (1977)
			Bacillus subtilis		Rosario and Basaran (1984)
			B. licheniformis		Sanchez (2008)
			Penicillium sp.		
Bagoong isda/guinamos	Small fish, salt	Side dish, viand	*Micrococcus* sp.		Baens-Arcega (1977)
					Olympia (1992)
					Sanchez (2008)
Bagoong alamang	Small shrimp, salt	Condiment, viand		Whole Philippines	Sanchez (2008)
					Steinkraus (1995)
Bagoong na sisi	Shell fish, salt	Condiment, viand, side dish	No report yet	Visayas and Mindanao	Banaay et al. (2013)
Dayok	Fish entrails, salt	Viand, side dish	No report yet	Mindanao	Banaay et al. (2013)
Longganisa	Ground pork, beef or chicken meat, spices, preservatives	Viand	*P. acidilactici*, *Lactococcus lactis*, *P. pentosaceus*, *Micrococcus aurantiacus*	Whole Philippines	Sanchez (2008)
Agos-os	Sweet potato and ground pig's head	Viand	*Enterococcus faecalis*	Leyte	Tan (2010)
			E. faecium		
Burong kalabi	Cooked rice, ground carabao meat	Viand	*L. plantarum*	Central Luzon	Gervasio and Lim (2007)
			P. acidilactici		

(continued)

Table 13.1 (continued)

Local name of fermented foods	Substrates	Nature and uses	Microorganisms	Regions of consumption in the Philippines	Reference(s)
Burong babi	Cooked rice, ground pork	Viand	*L. plantarum*	Central Luzon	Gervasio and Lim (2007)
			L. pentosus		Elegado et al. (2004)
			P. acidilactici		
Puto	Rice, sugar	Steamed rice cake, snacks		Whole Philippines	Sanchez (2008)
Bibingka	Rice, sugar	Baked rice cake, snacks	No report yet	Whole Philippines	
Puto balanghoy	Cassava	Steamed cassava cake, snacks	No report yet	Mindanao	
Landang	Cassava or buli palm flour	Dried jelly pellets, rice substitute	No report yet	Visayas, Mindanao	
Tapuy	Rice, glutinous rice, bubod starter	Rice wine or rice beer	*Rhizopus* sp., *Aspergillus* sp., *S. cerevisiae*	Luzon	Sanchez (2008)
Pangasi	Rice	Rice wine or rice beer	No report yet	Mindanao	
Basi	Sugarcane	Sugarcane wine	*Saccharomyces*, *Endomycopsis*	Luzon	Sanchez (2008)
			Lactic acid bacteria		Steinkraus (1983)
Tuba/lambanog	Coconut flower sap	Coconut wine/whisky	*S. cerevisiae* and other yeasts, *P. cerevisiae*, *Lactobacillus* sp.	Whole Philippines	
Suka	Sugarcane juice (*sukang Iloko*), Palm flower sap (*sukang tuba*)	Sugarcane vinegar, condiment, seasoning	Yeasts, *Lactobacillus* sp.	Whole Philippines	
			Acetobacter sp.		
Sinamak	Sugarcane juice, spices (chili, garlic, onion)	Sugarcane spicy vinegar, condiment, seasoning	No report yet	Visayas	
				Mindanao	
Pinakurat	Coconut sap (chili, garlic, onion)	Coconut vinegar, condiment, seasoning	No report yet	Luzon	
Toyo	Soybeans	Soy sauce, seasoning	*P. halophilus*	Whole Philippines	Sanchez (2008)
			E. faecalis		
			Lb. delbrueckii		
			Aspergillus sojae		
			Saccharomyces rouxii		

are as follows: CAR (Cordillera Administrative Region), Region I (Ilocos), Region II (Cagayan Valley), Region III (Central Luzon), NCR (National Capital Region), Region IV-A (CALABARZON or Cavite, Laguna, Batangas, Rizal, and Quezon provinces), Region IV-B (MIMAROPA), Region V (Bicol), Region VI (Western Visayas), Region VII (Central Visayas), Region VIII (Eastern Visayas), NIR (Negros Island Region), Region IX (Zamboanga Peninsula), Region X (Northern Mindanao), Region XI (Davao), Region XII (Soccsksargen), Region XIII (Caraga), and ARMM (Autonomous Region in Muslim Mindanao) (http://www.nscb.gov.ph/activestats/psgc/listreg.asp).

The geographical distribution of Philippine fermented foods basically depends on raw material availability (Table 13.1). There are fermented foods that are available all over the country, but regional peculiarities on the use of some of these products as food or condiments exist. In general, fish-based fermented foods like *bagoong isda* (fermented fish paste), *bagoong alamang* (fermented tiny shrimp), and *patis* (fermented fish sauce) are found all over the country. However, *bagoong isda* is produced and most often consumed as condiments in the regions of Ilocos, Cagayan Valley, and Eastern Visayas and provinces of Batangas and Pangasinan. *Tinabal* (whole fermented fish) and fermented oysters are common in the Bicol, Visayas, and MIMAROPA regions. On the other hand, *burong babi* (fermented pork), *burong kalabi* (fermented carabeef), *balao-balao* (fermented rice-shrimp mixture), and *burong isda* (rice-fish mixture) are popular in Central Luzon. Specialty snacks from steamed fermented rice like *puto* and *bibingka* (mixed with coconut) are abundant in Central Luzon, CALABARZON, MIMAROPA, Bicol, and Eastern Visayas. Fermented vegetables like *burong mustasa* (fermented mustard leaves) are often consumed in Bulacan province and CALABARZON region. *Kesong puti* (soft white cheese from carabao's milk) is a delicacy in Laguna and Nueva Ecija provinces.

There are atypical fermented foods in selected places like *agos-os* (fermented sweet potato and ground pig head mixture) in Leyte province and *puto balanghoy* (fermented cassava) and *landang* (fermented sago flour) in Mindanao. *Agos-os* has been reportedly (Tan 2010) developed by the local people of Leyte during the Japanese occupation in the 1940s. Root crops like sweet potato were abundant and pig head was cheap. Thus, this particular food preservation was used to augment food supply that was so scarce during those times. *Landang* is another fermented food material from palm starch or locally known as *sago* that is also mixed with cassava starch. This food material augments the corn or rice staple in Mindanao. Coconut milk dish is prepared using *landang*. Another fermented product which is typically found in southern Bicol and the Eastern Visayas region is "*muscovado* kefir" or fermented *muscovado* drink. We focused on the microbial characterization of fermented foods in Central Luzon and their bacteriocin-producing lactic acid bacteria and "muscovado kefir" of southern Bicol and Eastern Visayas.

13.5 Isolation of Bacteriocinogenic Lactic Acid Bacteria from Fermented Foods in Central Luzon

Nineteen fermented food preparations, either meat, starch, or plant based, were collected from different locations in four provinces in Central Luzon, the Philippines, namely, (1) San Fernando, Pampanga; (2) Mabalacat, Pampanga; (3) Capas, Tarlac; and (4) Dinalupihan, Bataan. The collected food preparations were stored in ice buckets during transport and kept at 4 °C for overnight storage to minimize growth of spoilage microorganisms prior to use. Fifty grams of each sample were homogenized in a stomacher, and from each homogenized sample, 5 g were suspended into 45 ml de Man, Rogosa, Sharpe medium and incubated at room temperature, which served as enriched samples. These samples were serially diluted tenfold up to 10^{-7}; pour plated on glucose, yeast, peptone (GYP) agar with calcium carbonate; and incubated for 24 h in an anaerobic candle jar. Colonies that showed clear zones were isolated and purified through repeated streaking. Pure colonies were characterized by Gram staining and catalase reaction test. One hundred twenty-six Gram-positive and catalase-negative isolates were selected as putative lactic acid bacteria (LAB) and were all tested for bacteriocin production using the "spot-on-lawn" assay. Briefly, *Enterococcus faecium 79* (Ef 79) and *Listeria monocytogenes* 3 (Lm 3), the indicator organisms, were grown at 37°C to stationary phase for 16–24 h (Elegado et al. 2004) to come up with a cell count of approximately 10^8 cfu/ml. Fifty microliters of the indicator microorganism were mixed into 5 mL 0.8 % soft MRS agar and overlain onto 20 ml MRS agar plates to create a double-layered MRS agar plate used as the seeded medium. Then, 10 μL of boiled supernatant of overnight MRS culture of putative LAB, also known as undiluted samples, were spotted in triplicates on the seeded medium and incubated for 24 h at 37 °C. The isolates that gave good bacteriocin activity were preserved in glycerol stocks in the biofreezer and further characterized through PCR-based screening for the bacteriocin-encoding genes using primers for lactococcin A, nisin *A*/*Z*, plantaricin *PlaS*, and pediocin *PapA* (Mackay 2005). Table 13.2 shows the primer sequences and PCR conditions used.

Sixteen out of nineteen enriched fermented food samples exhibited colonies with clearing zones on GYP agar with calcium carbonate. The colony count per g or ml samples ranged from 1.8×10^6 to 1.0×10^{10}. The LAB isolates have varied cell morphological features. Majority were short rods while others were cocci, diplococci, and a combination of short and long rods. Ten random colonies were picked from each sample and a total of 130 colonies were assayed for bacteriocin activity. Thirty isolates produced bacteriocin against *Enterococcus faecium* 79, while 26 isolates are bacteriocinogenic against *Listeria monocytogenes* The isolates with the good combined bacteriocin activity against the two indicator organisms are listed in Table 13.3.

Fourteen LAB isolates were positive for the pediocin *Pap* gene, and two isolates were positive for the plantaricin *PlaS* gene. No isolate was positive for lactococcin A and nisin A/Z genes. The bacteriocins of other isolates may be of another type. It was also elucidated that isolates 3G3 and 4B1 resist simulated gastric juice (pH 2) and simulated intestinal fluid (0.3 % bile acid). Furthermore, these isolates have good adhesion property onto the pig duodenum and middle colon. *Pediococcus acidilactici* 4E5 and 3G3 and *Lactobacillus brevis* 4B1 have been used in several application studies such as antimicrobial and anti-obesity effects (Balolong et al. 2015).

Table 13.2 List of primers and annealing temperatures for PCR screening of bacteriocin-encoding genes

Bacteriocin primer	Sequence (5–3′)	Expected size (bp)	Annealing temp (°C)	References
Lactococcin A	CAATCAGTAGAGTTATTAACATTTG	870	55	Martinez et al. (1996)
LCNA 1	GATTTAAAAAGACATTCGATAATTAT			
LCNA 2				
Nisin A/Z	CGA GCA TAA TAA ACG GC	320	55	Ward et al. (1994)
NISL'	GGATAG TAT CCA TGT CTG AAC			
NISR				
Plantaricin *PlaS*	AAY AAR YTI GCI TAY AAY ATG	380	50	Remiger et al. (1996)
α-Forward	GCY TTY AAR RAI CCY TCI CC			
β-Reverse				
Pediocin *PapA*	CTG CGT TGA TAG GCC ACG TTT CA	300	55	Motlagh et al. (1994)
Forward	GCT TCT GTA AAA ACT GTA GCC			
Reverse				

Table 13.3 Identities of LAB isolates with good bacteriocin activity

Fermented food	Sample code	Identity by 16S rRNA sequencing	Culture pH	Bacteriocin activity (AU/ml)	
				Ef 79	*Lm* 3
Burong tilapia[a]	3F2	*Pediococcus acidilactici*	3.85	100	400
	3F8	*P. acidilactici*	3.88	100	800
	3F10	*P. acidilactici*	3.53	800	3200
Burong abi[b]	3G3	*P. acidilactici*	3.77	1600	6400
	3G8	*P. acidilactici*	4.23	3200	1600
Balao-balao[c]	4B1	*Lactobacillus brevis*	4.22	800	12,800
	4E2	*P. acidilactici*	3.95	100	12,800
	4E4	*P. acidilactici*	4.45	400	12,800
	4E5	***P. acidilactici***	**4.24**	**3200**	**12,800**
	4E6	*P. acidilactici*	4.44	1600	3200
	4E10	*Pediococcus lolii*	4.29	400	800

Gervasio and Lim (2007), Perez et al. (2012)
[a]Fermented rice-*tilapia* mixture
[b]Burong babi
[c]Fermented rice-shrimp mixture

13.6 Muscovado-Based Kefir from Leyte, Philippines: Yeast and Lactic Acid Microbial Flora and Bioactivity

Kefir is an acidic drink more commonly obtained from milk fermentation. Unlike starter culture-produced yogurt or other traditionally fermented milk, milk kefir is made from kefir grains (Lopitz-Otsoa et al. 2006). Kefir grains are small clusters of microorganisms held together by a polysaccharide matrix. These are soft, gelatinous, white, cauliflower-like biological masses, comprised of protein, lipids, and soluble polysaccharides with sizes ranging from 3 to 20 mm. Yeasts and lactic acid bacteria are the major microorganisms present inside or attached onto the surface of the matrix. Studies have shown that kefir drinks can confer various health benefits such as immune system stimulation, antitumor effects, antimicrobial properties, and cholesterol reduction (Farnworth 2006). Kefir was recorded to contain vitamins, minerals, and essential amino acids. It is a good source of biotin that aids the body's assimilation of other B vitamins and thus helps in healing and maintenance. Other than animal milk, kefir beverage can be prepared by directly adding the kefir grains in other substrates like soy milk, rice, oat milk, coconut, fruit, and sugary water (Silva et al. 2009; Otles and Cagindi 2003). In Baybay, Leyte, Philippines, the locals produce a kefir beverage by the fermentation of a muscovado sugar solution using kefir grains. Muscovado sugar is dark brown, coarse, and sticky unrefined sugar with high mineral content. It retains all of the natural ingredients of sugarcane juice. Muscovado sugar is being produced in small commercial-scale technology in various provinces such as Antique, Batangas, Bukidnon, Davao del Sur, Iloilo, Negros Occidental, North Cotabato, Tarlac, and Sultan Kudarat. In the Bicol region, Leyte, and other areas in the Visayas, solid muscovado sugar are often contained in coconut shells and sold in public markets.

Microbiological and physicochemical evaluation of kefir from Leyte, Philippines, were done. Kefir grains were grown in 5 % muscovado sugar solution at room temperature (26 °C), and the major microorganisms were isolated and identified during a 3-day fermentation period. The general microbial counts, i.e., total heterotrophic microbes, yeasts and molds, and lactic acid bacteria, were estimated by conventional serial dilution and spread plating technique, using plate count agar (PCA), potato dextrose agar (PDA), and de Man, Rogosa, Sharpe agar (MRSA), respectively. Isolation of representative dominant colonies with unique colony characteristics and subsequent purification by repeated streaking on the appropriate agar medium were done. Cultural characteristics and Gram stain of the isolates were noted followed by microscopic observation to ascertain purity, cell shapes, and sizes. The bacterial isolates were also tested for catalase reaction. Molecular identification of selected yeast and bacterial isolates was done using ITS-PCR and 16S rRNA gene sequence analyses. Furthermore, antibacterial assay was conducted using modified Kirby-Bauer disk diffusion methods (Bauer et al. 1966) and direct inhibition assay using various test organisms. Mutagenicity assay using the Ames test (Ames et al. 1973) and cytotoxicity assay (MTT or thiazolyl blue tetrazolium bromide assay) (Villarante et al. 2011) were also done using crude kefir extracts.

The muscovado-based kefir drink was observed to be of consistent light-brown color throughout the period of the study (Fig. 13.2a). It had a characteristic acidic smell and sweet taste with a hint of alcohol. Kefir grains were soft, gelatinous, cauliflower-like light-brown masses that are irregular in shape and sizes ranging from 5 to 10 mm (Fig. 13.2b).

Figure 13.3 shows the microbiological counts for total heterotrophic microbial population, putative yeasts and molds, and putative lactic acid bacteria and the pH profile of the muscovado kefir from day 0 to day 3. The average heterotrophic counts on PCA were consistently 10^6 CFU/ml ($1.4–7.7 \times 10^6$) with the peak attained on the third day of fermentation. Fungal count estimates on PDA ranged from 10^4 to 10^6 CFU/ml, with declining trend during the course of fermentation. There were no mold-like colonies observed on the PDA plates even after an extended incubation of up to 7 days (data not shown). The main reason could be the low pH or other inhibitory substances produced by competing microorganisms. Lastly, the putative lactic acid bacteria count ranged from 10^5 to 10^6 CFU/ml. The lowest count was at initial sampling (day 0) and it increased through time. These microbial counts were within the range of what Miguel et al. (2010) have reported for the LAB density of milk-based kefir from several Brazilian states, the USA, and Canada which was $10^4–10^{10}$ CFU/ml. The control plates from the muscovado sugar solution had <10 CFU/ml which is expected as the water activity of muscovado is much less than that which tolerates microbial growth. Thus, it were the kefir grains that contained the significant microbial starters for the fermentation.

A total of 39 isolates from PCA, 21 from PDA, and 27 from MRSA were obtained. Successive reinoculation of single colonies was done until the isolates were purified as checked under the microscope. The pure yeastlike isolates from PDA had ovoid to coccoid to cylindrical cell shapes, and seven (7) exhibited either budding or fission and thus were selected for further characterization. Meanwhile, all but three (3) of the MRSA isolates were Gram-positive and cata-

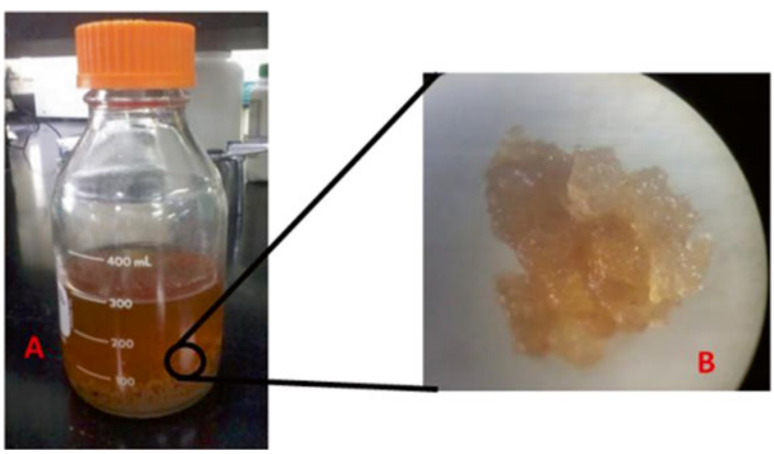

Fig. 13.2 Laboratory production of muscovado kefir (**a**) using kefir grains (**b**) (magnified 10×) from Leyte

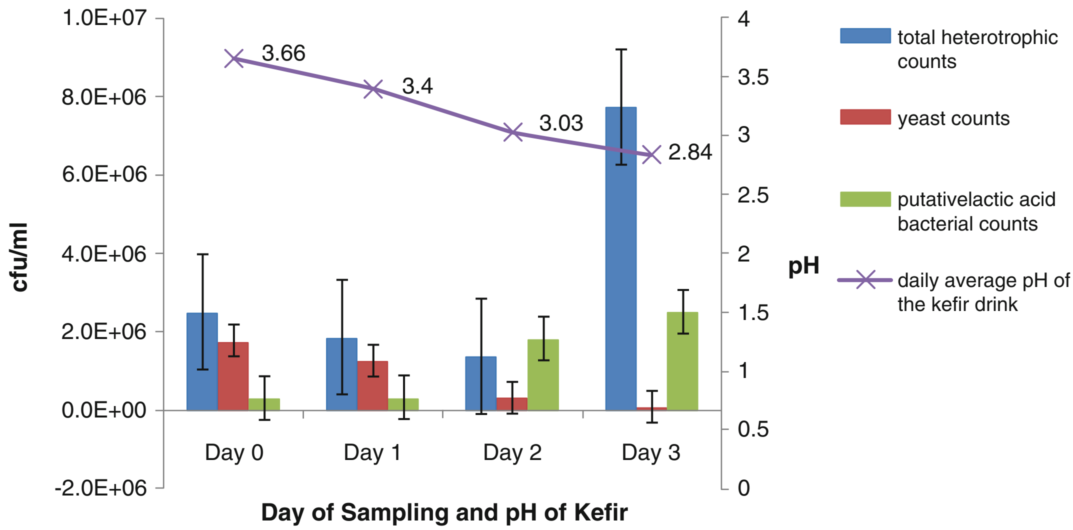

Fig. 13.3 Total heterotrophic, yeast, and putative lactic acid bacterial counts obtained during the 3-day fermentation of kefir grains in 5 % (w/v) muscovado sugar solution

lase negative, which suggested that they were either microaerophiles of facultative anaerobes. Tube fermentation tests using Durham tubes showed that 89 % were unable to produce gas and thus were considered homofermentative. Further identification using molecular techniques of selected representative isolates was subsequently done.

Genomic DNA were extracted from the selected isolates, and yeasts were subjected to ITS-PCR, while the bacterial isolates were subjected to 16SRNA sequence. The determination of sequence similarity was analyzed using the Basic Local Alignment Search Tool (BLAST) database (http://www.blast.ncbi.nlm.nih.gov/blast.cgi). Amplification of the extracted genomic DNA from the yeast isolates with ITS1 and ITS4 primers (White et al. 1990) yielded fragments that were about 450–850 bp long. The putative yeast isolates were divided into three groups depending on the size of the resulting PCR bands (Fig. 13.4). Three of the isolates had 450 bp, 11 had 650 bp, and 7 had 850 bp. Representative isolates were subjected to sequence analysis. BLAST was used to confirm the identity of the isolates. The identity of two isolates with 650 bp ITS was 99 % similar to *Lachancea fermentati*, another two isolates with 850 bp ITS were 100 % similar to *Saccharomyces cerevisiae*, and one isolate with 450 bp ITS was identified as *Candida inconspicua*. The other isolate with 450 bp ITS was 100 % similar to *Pichia manshurica*.

Based on the PCR results, 52.4 % (11 out of 21) of the putative yeast isolates had amplicon size of 650 bp, and both representatives from the group were confirmed as *Lachancea fermentati*, formerly known as *Zygosaccharomyces fermentati* which can utilize cellobiose but is not a good ethanol producer. Although the 650 bp group was the most dominant and both representative isolates were *L. fermentati*, it cannot be concluded yet that it is the dominant yeast in Philippine muscovado kefir since there are other yeast species with approximately 650 bp ITS amplicon such as *Candida famata* and *Pichia chambardii* (Fujita et al. 2001). Meanwhile, the two isolates with 850 bp ITS size were confirmed as *S. cerevisiae*, which is almost always the dominant yeast isolated from milk-based kefir grains (Leite et al. 2012). Some of the cited possible importance of the presence of *S. cerevisiae* in kefir are as follows: (i) reduction of the concentration of the lactic acid produced by LAB, (ii) removal of hydrogen peroxide by catalase activity to promote microaerophilic conditions for the LAB, (iii) production of stimulating factors for the

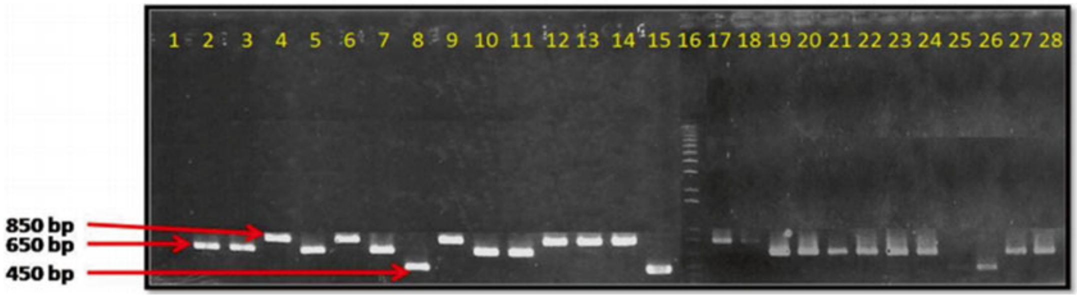

Fig. 13.4 PCR products from the amplification of the internal transcribed spacer (ITS) regions of the genomic DNA extracted from Philippine muscovado kefir yeast isolates, run in 1 % agarose gel and stained with ethidium bromide. Lane 16 shows 1 kb+ ladder

growth of LAB, and (iv) improvement of kefiran production by physical contact with LAB (Cheirsilp et al. 2003). The 450 bp-sized isolate were *C. inconspicua*, reported to be associated with wine fermentation (Li et al. 2010), and *P. manshurica*, known to produce volatile phenol (Saez et al. 2011). Figure 13.5a–c show the phylogenetic trees of the various yeast isolates constructed by neighbor joining algorithm with 1000 bootstrap replications using the Molecular Evolutionary Genetic Analysis (MEGA) 5 software. PCR amplification of the 16S rRNA gene of the extracted genomic DNA of putative LAB using 27F and 1492R primer pair (Lane 1991; Turner et al. 1999) yielded amplicons with approximately 1500 bp (Fig. 13.6). The determination of sequence similarity was analyzed using the BLAST database (http://www.blast.ncbi.nlm.nih.gov/blast.cgi).

Sequence analysis of the PCR products in all ten bacterial isolates confirmed the identity of the putative LAB isolates as *Lactobacillus paracasei* (95 % and 99 % homology), *Lactobacillus satsumensis* (96–99 %), *Lactobacillus oeni* (96 %), and *Lactobacillus ghanensis* (99 %). One isolate was however identified as 99 % similar to *Corynebacterium variabile. L. paracasei* has been commonly isolated from kefir along with the other LAB isolates. It is a known probiotic (Fuller 1989), which promotes beneficial health effects in humans and animals, including the reduction of symptoms in lactose intolerance and enhancement of the bioavailability of nutrients. It was also reported to have antagonistic actions against intestinal and food-borne pathogens (Chiang and Pan 2012). On the other hand, *L. satsumensis* has rarely been reported to be isolated from milk kefir grains. Apparently, it prefers carbohydrates as it has been previously isolated from *shochu* mashes, a traditional Japanese distilled spirit made from fermented rice, sweet potato (from where it got its name), barley, or other starchy materials (Endo and Okada 2005). Furthermore, our results provide the first report on the presence of *L. oeni* and *L. ghanensis* in kefir grains. *L. oeni* has been previously isolated from wine (Mañez-Lazaro et al. 2009). *L. ghanensis*, on the other hand, was previously associated with the fermentation of coca, which is a microbiologically complex process involving the activities of yeasts, lactic acid bacteria, and acetic acid bacteria producing the characteristic cocoa flavor (Nielsen et al. 2007). Phylogenetic trees were constructed from the sequence data to determine the relationships of the LAB isolates with species belonging to their own genus (Fig. 13.7).

Among the chosen MRSA isolates for molecular identification, *Corynebacterium variabile* was the only non-LAB. *C. variabile* has been previously reported to be part of the complex microflora on the surface of smear-ripened cheeses which contribute to the development of flavor and textural properties during cheese ripening. *C. variabile* has distinct metabolic capabilities which were associated with the utilization of alternative carbon and sulfur sources; amino acid metabolism; fatty acid degradation; catabolism of gluconate, lactate, propionate, taurine, and gamma-aminobutyric acid; and production of acetoin, butanediol, and methanethiol, which are important flavor compounds (Schröder et al. 2011).

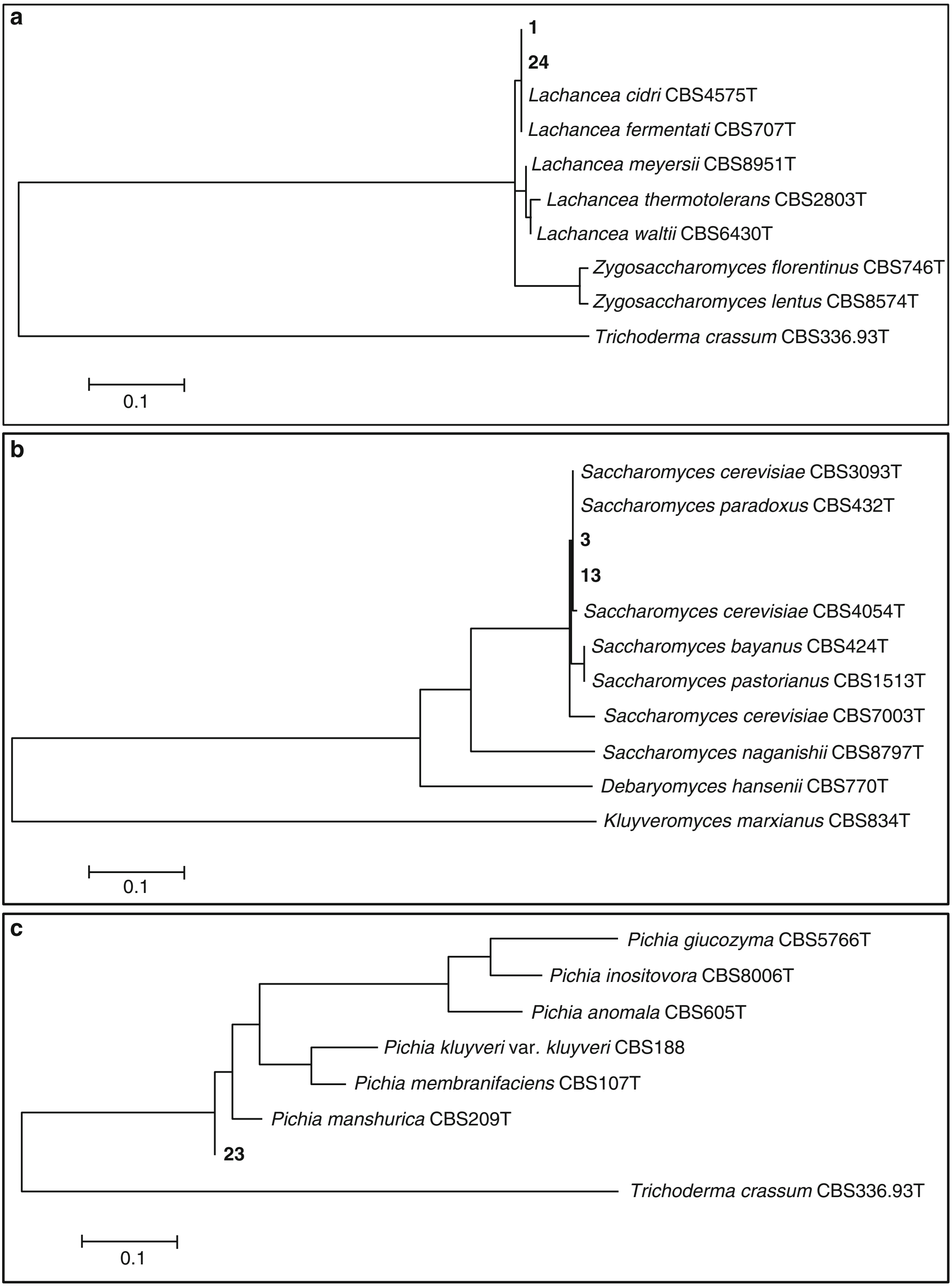

Fig. 13.5 Phylogenetic tree of *Lachancea* spp. (**a**), *Saccharomyces* spp. (**b**), and *Pichia* spp. (**c**) with yeast isolates from muscovado kefir constructed by neighbor joining algorithm with 1000 bootstrap replications using the MEGA 5 software

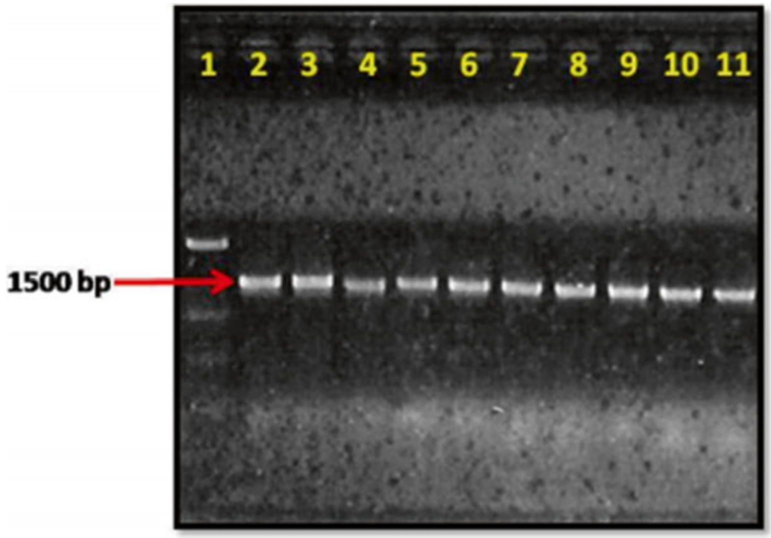

Fig. 13.6 PCR products from the amplification of the 16S rRNA gene of genomic DNA from the Philippine muscovado kefir bacterial isolates, run in 1 % agarose gel and stained with ethidium bromide. Lane 1 shows the 1 kb+ DNA ladder

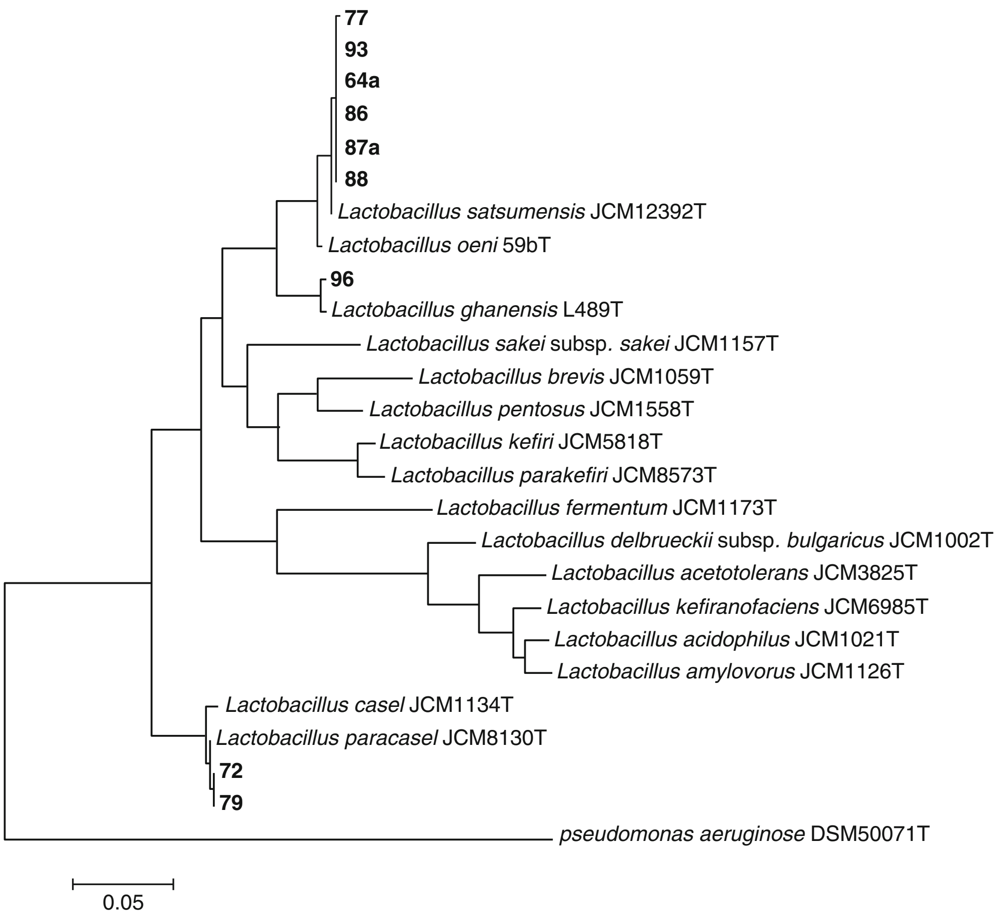

Fig. 13.7 Phylogenetic tree of *Lactobacillus* with muscovado kefir putative LAB isolates

Table 13.4 Results of the direct inhibition assay conducted on ten bacteria isolated from Philippine muscovado kefir

Kefir isolates	Zone of inhibition (diameter in mm)								
Code	Identity	*L. innocua*	*L. ivanovii*	*E. coli* (EHEC)	*K. oxytoca*	*S. marcescens*	*E. aerogenes*	*S. enteritidis*	*B. cereus*
64a	*L. satsumensis*	+	–	+++	+++	+++	++	–	+
72	*L. paracasei*	–	–	–	+	+	–	+	–
77	*L. satsumensis*	–	–	–	+	–	–	–	–
78	*C. variabile*	–	–	–	–	–	–	+	–
79	*L. paracasei*	–	–	+++	++	+	–	–	–
86	*L. satsumensis*	–	–	–	–	–	–	–	–
87a	*L. satsumensis*	–	–	–	–	+	–	–	–
88	*L. oeni*	–	+	++	+++	++	+++	+	–
93	*L. satsumensis*	–	–	–	+	++	–	–	–
96	*L. ghanensis*	–	–	+++	+	–	–	–	–
Streptomycin, 20 mg/ml	++	++	++	++	++	++	++	++	

Colegio 2013
Legend: –, isolates which did not exhibit any zone of inhibition
+, isolates which exhibited zones of inhibition which were less than those of the positive controls (22–27 mm)
++, isolates which exhibited zones of inhibition which were equal to those of the positive controls (22–27 mm)
+++, isolates which exhibited zones of inhibition which were greater than those of the positive controls (22–27 mm)

Direct inhibition assay was performed using the identified kefir bacterial isolates against *Listeria innocua* 33090, *Listeria ivanovii*, enterohemorrhagic *Escherichia coli* 10311 (EHEC), *Klebsiella oxytoca* B-1753, *Serratia marcescens* B-1748, *Enterobacter aerogenes* B-1141, *Salmonella* enteritidis, and *Bacillus cereus* 1509. Streptomycin, which caused rapid cessation of bacterial protein synthesis (Luzzato et al. 1968), served as the positive control. Among the five identified *L. satsumensis* strains, isolate 64a exhibited the greatest antibacterial activity since it inhibited the growth of 75 % of the tested pathogens as shown by the zones of inhibition, which were significantly comparable to those of the positive control (streptomycin). *L. oeni* has also shown very promising antimicrobial activity against 75 % of the test organisms. Between the two identified *L. paracasei* isolates, isolate 72 showed activity against *K. oxytoca*, *S. marcescens*, and *S. enteritidis*, while isolate 79 inhibited the growth of *E. coli* 9 (EHEC), *K. oxytoca*, and *S. marcescens. L. ghanensis* was inhibitory against *K. oxytoca* and *E. coli* (Table 13.4).

On the other hand, test assays showed that the cell-free muscovado kefir liquid extract did show any inhibitory action, mutagenic properties on *Salmonella* TA102 and TA104, nor cytotoxic effect on MCF-7, a line of breast cancer cells (Colegio 2013).

13.7 Conclusion

The Philippine archipelago is home to a variety of fermented foods distributed geographically and to the relative abundance of raw materials. The lactic acid bacteria isolated from various fermented foods gathered from public markets in Central Luzon, Philippines, were studied. Thirty-two, out of the 126 putative lactic acid bacteria (LAB), isolated from 19 fermented food preparations showed bacteriocinogenic properties to as much as 6400 AU/ml against *Enterococcus faecium* and 12,800 AU/ml against *Listeria monocytogenes* for some isolates. Two good bacteriocinogenic LAB isolates, identified by 16S rRNA as *Pediococcus acidilactici* 3G3 and *Lactobacillus brevis* 4B1, were tested to have good acid and bile tolerance, comparable to that of commercial probiotics.

The microbial load of Philippine muscovado kefir, mostly putative lactic acid bacteria, was found to be maximum when the pH was at its minimum after 3 days. On the other hand, yeast population decreased over time due to the decrease in pH. Out of the 89 colonies isolated and purified from muscovado kefir, 20 were considered as putative yeasts and the rest mostly putative lactic acid bacteria. Using ITS-PCR to identify the yeast isolates, 11 were *Lachancea fermentati*, 7 were *Saccharomyces cerevisiae*, 2 were identified as *Candida inconspicua*, and 1 was *Pichia manshurica*. 16S rRNA gene sequence analysis identified four species of lactic acid bacteria, namely, *Lactobacillus satsumensis*, *Lactobacillus paracasei* subsp. tolerans, *Lactobacillus oeni*, and *Lactobacillus ghanensis*. One isolate was confirmed as *Corynebacterium variabile*. These isolates could be very crucial in the formation of the kefir grains since they were part of the dominant bacterial microflora and they can be used as probiotics to elicit health-promoting biological functions in the host. These LAB isolates have antibacterial activities against eight test organisms in varying degrees.

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Ethnic Fermented Foods and Beverages of Indonesia

14

Ingrid Suryanti Surono

14.1 Introduction

Indonesia is the largest archipelago in the world extending some 2000 km from north to south and more than 5000 km from east to west and consists of 17,508 islands, about 6000 of which are inhabited, scattered over both sides of the equator (Fig. 14.1). The archipelago stretches over more than one tenth of the equator between Southeast Asia and Australia. The largest islands are the Kalimantan, Sumatra, Papua, Sulawesi, and Java. Indonesia lies between latitudes 11°S and 6°N and longitudes 95°E and 141°E and consists of islands (CIA 2015).

The temperature ranges between 16 and 35 °C with humidity ranging from 60 % to 98 %. There are two seasons, the rainy monsoon season which usually lasts from November through May, with the heaviest rainfall from November through March, followed by the dry season which is driest between June and September. Rainfall varies throughout Indonesia, averaging 706 mm (28 in.) yearly.

Indonesia has a population of 255,993,674 people (estimated per July 2015) and is the fifth most populous nation in the world after China, India, EU, and the United States, the majority of which are of Malay extraction. The remainder of the natives is Melanesian (in Papua and the eastern islands). There are ethnic Chinese, Indians, and Arabs concentrated mostly in urban areas throughout the archipelago. There are about 300 ethnic groups, each with cultural identities developed over centuries and influenced by Indian, Arabic, Chinese, and European sources, and 742 different languages and dialects. Major ethnic groups are Javanese (45 %), Sundanese (14 %), Madurese (7.5 %), Coastal Malays (7.5 %), and others (26 %) (Expat website Association 2015).

The agricultural sector of Indonesia comprises large plantations (both state owned and private) that tend to focus on commodities which are important export products (palm oil and rubber) and smallholder production modes that focus on rice, soybeans, corn, fruits, and vegetables. According to FAO of the United Nations (2015), the top 11 products of Indonesia in 2012 include paddy rice, palm oil, rubber, chicken, cassava, maize, coconuts, banana, palm kernels, mango, and mangosteens.

Rice is a staple in Indonesia, except in Papua and Maluku where people sustain themselves with sago, which is a type of tapioca; sweet potatoes; and cassava. Indonesian cuisine is as varied as its culture, and the food in Indonesia is as diverse as its geography with the influences from China, Europe, and even India, rich in flavors; soy-based dishes, such as variations of tofu (*tahu*) and *tempe*, are also very popular (Table 14.1).

I.S. Surono (✉)
Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jl. Jalur Sutera Barat Kav. 21, Alam Sutera Campus, Serpong-Tangerang 15143, Indonesia
e-mail: isurono@binus.edu; gridsw@yahoo.com

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Fig. 14.1 Map of Indonesia

Fermentation is one of the oldest and most economic methods in preserving the quality and safety of foods; it not only prolongs the shelf life but also reduces volume, shortens cooking times, provides better nutritional bioavailability, enhances flavor and aroma, and can be considered as a functional food that exerts health-promoting benefits (Tamang 2015). A rich variety of indigenous traditional fermented foods involving yeast, mold, bacteria, and their combination, owned by each area, are an important part of the culture, identity, and heritage and have certain distinct sensory characteristics as a result of metabolite accumulation produced by microbes involved, contributing to flavor, texture, and aroma. Traditional fermented foods and beverages are also considered as important part of diet due to its high nutritive value, digestibility, and reduced antinutrient compounds. Fermentation may assist in the detoxification of certain undesirable compounds such as toxin and antinutrients which may be present in raw foods, such as phytates, polyphenols, and tannins (Sharma and Kapoor 1996). The manufacture of fermented foods uses diverse raw materials as substrates, such as cereals, legumes, tubers, fruits, vegetables, animal such as meat and milk, and marine sources; many of them are made only on home scale in traditional methods of preparation passed on from generation to generation using relatively simple equipment at very low cost with insufficient hygienic precautions (Surono and Hosono 1994a, b).

Most of the traditional food fermentations are conducted by natural, spontaneous fermentation involving mixed beneficial microbes from staple ingredients and environmental surrounding as home industry. As a consequence, pure and single culture will not be involved; natural contamination and inconsistent quality of the product may occur due to lack of sterility and the use of natural fermentation (Nout and Sarkar 1999). Based on the substrate used, fermented foods and beverages can be classified into:

Fermented grain, cereals, and legume foods
Fermented fruits and vegetable products
Fermented milk products
Fermented fish and meat products
Fermented roots and tuber products

Table 14.1 Ethnic fermented foods and beverages of Indonesia

Foods	Substrates	Nature and uses	Microorganisms	Regions of consumption in Indonesia	References
Tempe	Soybeans	Side dish	*Rz. oligosporus*	All regions	Astuti et al. 2000
Oncom	Soybean, peanut	Side dish	*N. sitophila*, *Rz. oligosporus*	West Java	Sastraatmadja et al. (2002), Hoo (1986), Afifah et al. 2014, Sulchan and Nur (2007), and Sumi and Yatagai (2006)
Gembus	Soybean	Side dish	*Rz. oligosporus*	Central Java	Kuswanto (2004), Sulchan and Rukmi (2007), Sulchan and Nur (2007). and Fatimah (1998)
Kecap	Soybean	Condiment	*A. oryzae*, *A. sojae*, *Rz. oryzae*, *Rz. oligosporus*	All regions	Steinkraus (1995) and Judoamidjojo (1986)
Acar	Vegetables	Condiment	*Lb. plantarum*	All regions	Lennox and Efiuvwere (2013)
Sayur asin	Vegetables	Condiment	*Lb. plantarum*, *Leu. mesenteroides*, *Lb. confusus*, *Lb. curvatus*, *P. pentosaceus*	West Java	Sulistiani et al. (2014) and Puspito and Fleet (1985)
Tauco	Soybean	Condiment	*R. oligosporus*, *Rz. oryzae*, *A. oryzae*, *Lb. delbrueckii*, *Hansenula* sp.	West Java	Winarno et al. (1973)
Tempoyak	Flesh of durian (*Durio zibethinus*)	Condiment	*Ent. gallinarum* UP-9, *Ent. faecalis* UP-11, *Oenococcus*, *Leuconostoc*, *Enterococcus*, *Lactococcus*, *Pediococcus acidilactici*, *Lactobacillus*. *Leuconostoc* sp.	Sumatra	Wirawati (2002), Pato and Surono (2013), Widowati et al. (2013), and Yuliana and Garcia (2009)
Mandai	Inner part of *cempedak* or jackfruit	Condiment	*P. pentosaceus*, *Lb. plantarum*, *Lb. pentosus*	Kalimantan	Emmawati (2014), Rahayu (2003), (2010)
Brem	Cassava, glutinous rice	Snack, beverages	*Rz. oryzae*, *M. rouxii*, *A. oryzae*, *S. cerevisiae*, *Acetobacter aceti*	Central Java, Bali	Basuki (1977), Saono et al. (1984), and Aryanta (2000)
Tuak	Juice of plant	Beverages	*S. cerevisiae*, *C. tropicalis*	North Sumatra, Nusa Tenggara	Hermansyah et al. (2015)
Dadih	Buffalo milk	Beverage	*Lac. lactis* subsp. *lactis*, *Lb. brevis*, *Lb. plantarum*, *Lb. casei*, *Lb. paracasei*, and *Leu. mesenteroides*	West Sumatra	Imai et al. (1987) and Surono (2003a, b)
Urutan	Meat, pork	Side dish	*Lb. plantarum*, *Lb. farciminis*, and obligate heterofermentative lactobacilli *Lb. fermentum* and *Lb. hilgardii*. Besides, *P. acidilactici* and *P. pentosaceus*	Bali	Antara et al. (2002) and Aryanta (1998)

(continued)

Table 14.1 (continued)

Foods	Substrates	Nature and uses	Microorganisms	Regions of consumption in Indonesia	References
Peda	Fish	Side dish	*Lb. plantarum*, *Lb. curvatus*, *Lb. murinus* and *Strep. thermophilus*	Java	Rahayu (2003)
Terasi	Fish and shrimp	Condiment	*Bacillus* sp. and *Pseudomonas* sp.	Sumatra, Java	Surono and Hosono (1994a)
Telur asin	Duck egg	Side dish	*Lb. plantarum*, *Lb. casei* subsp. *rhamnosus*, *Enterococcus gallinarum*, and *P. acidilactici*	All region	Suprapti (2002) and Saputra (2013)
Tape	Cassava, glutinous rice	Snack	*Rz. oryzae*, *M. rouxii*, *A. oryzae*, *S. cerevisiae*, *E. burtonii*, *H. anomala*, and *P. pentosaceus*. *Lb. plantarum* and *Lb. fermentum*	West Java, Central Java	Aryanta (1988) and Uchimura et al. (1998)
Growol	Cassava	Snack	*Coryneform*, *Streptococcus*, *Bacillus*, *Actinobacteria Lactobacillus*, and yeast	Yogyakarta	Suharni (1984)
Gatot	Cassava	Staple food	*P. pentosaceus*, *Saccharomyces* sp. *TR7*, *Lb. plantarum* 250 Mut7 FNCC	Yogyakarta	Ichsyani (2014)

14.2 Traditional Fermented Foods of Indonesia

14.2.1 Fermented Grains/Legumes and Cereals

Tempe, *oncom*, *tauco*, and *kecap* are all Indonesian legumes and grain fermented foods. *Tempe* and *oncom* are solid fermented foods, tauco is in the form of paste or slurry, and *kecap* is a liquid fermented food. Historically, most traditional soy protein foods originated from China and were introduced later to other countries in the East and Southeast Asia (Smith 1963). *Tempe* is unique among major traditional soya foods, because it is the only fermented soya food product that did not originate from China or Japan (Shurtleff and Aoyagi 2007). Today, Japan leads to industrialization, technology development, equipment manufacture, and worldwide soybean-based food marketing.

14.3 History, Manufacture, Biochemical and Nutritional Value, and Socioeconomics of *Tempe*

14.3.1 History of *Tempe*

The word *tempe* appears to have originated in Central Java, Indonesia. It is not derived from Chinese (as other soy foods in Indonesia), and it does not start with the prefix *tau* or *tao* (as do *tauci*, *tauco*, *taugé*, *taujiong*, *tahu*, *takua*) (Astuti 1999). The earliest known *tempe* reference is found in the *Serat Centhini* manuscript and first cited in *History of Tempeh* (Shurtleff and Aoyagi 1979) and then in *The Book of Tempeh* (Shurtleff and Aoyagi 1985), so it is presumed that *tempe* existed in Java in the early 1600s. The *Serat Centhini* (the Centhini manuscript), a classic work of modern Javanese literature and a kind of encyclopedia, was probably written around 1815.

"Serat" means manuscript or work or tale. "Centhini" (also spelled "Centini") refers to a character in the book written in verse, and the information is given often very detailed on many different subjects – not just religion but also various aspects of Javanese culture and life. On one page the word *tempe* appears, indicating that tempe was produced in the early seventeenth century (Okada 1988).

Prinsen Geerligs (1896), a Dutchman, is the first to spell the word *tempeh* (with an "h" on the end) and also the first to name the *tempeh* mold as *Rhizopus oryzae*. Other authors from the Dutch use the spelling *témpé* (Gericke and Roorda 1875; Heyne 1913) or *tèmpé* (Vorderman 1902; Stahel 1946).

In 1905, Dr Kendo Saito of Tokyo Imperial University described that the main *tempeh* microorganism is *Rhizopus oligosporus* (Kendo 1905). *Tempe* was introduced to the Japanese by Dr Nakano Masahiro in 1958 and some published papers on *tempeh* were written by Japanese scientist (Nakano 1959; Ohta et al. 1964; Ohta 1965, 1971; Nakano 1967, Watanabe et al. 1971). Indonesians pronounce the word *tempe*, which is the correct spelling in Indonesian language. Van Veen (1962) reported that the attempt to introduce *tempeh* to Indian population by missionaries in Travancore, in Southern India in 1936, was not successful, since they did not have any interest in this unknown fermentation product.

14.3.2 Manufacturing Tempe, Biochemical and Nutritional Values

Tempe is legumes' fermentation with the aid of mold, *Rhizopus* sp. The hydrated, cooked, dehulled whole soybeans are fermented by *Rhizopus* sp. molds. It is a moist solid cake with a mild, pleasant taste. According to Steinkraus (1980), *tempe* is a single cell protein grown on edible substrate. *Tempe* fermentation is similar to cheese fermentation since the hydrolysis of protein and lipid occurs, flavor intensifies, and free amino acid is released (Steinkraus 1983). The traditional product is highly perishable and is usually consumed the day it is made. In industrial production, it can be preserved by drying or freezing (after blanching to inactivate the mold and its enzymes).

There are two distinct fermentation periods. The first occurs during soaking of the soybean and results in acidification by lactic acid bacterial fermentation. Second is fungal fermentation and results in mycelial growth and partial digestion of the enzymes from the mold (Steinkraus et al. 1960). In the preparation, the soybeans (*Glycine max*) are soaked overnight in three volumes of water containing 10 mL of 0.85 % lactic acid per liter of water or *Lb. plantarum*, a lactic acid-producing bacterium that can be added to the soak water in place of lactic acid. The soak water is acidified to about pH 5.0 to inhibit the growth of microorganisms which can cause spoilage, boiled, drained, cooled, and spread out on a tray, followed by mixing with a little molded *tempe* cake from a former batch or adding fermentation starter containing the spores of *Rz. oligosporus* (Fig. 14.4); Wang et al. (1975) recommended 10^6 spores per 100 g cooked soybean for optimal fermentation, then wrapped with banana leaves, and kept overnight for about for 24–36 h at room temperature until the mass is bound by cottony mycelium of the mold into a solid white cake. *Tempe* is often produced in Indonesia using *Hibiscus tiliaceus* leaves, called *usar*. The undersides of the leaves are covered in downy hairs known technically as trichomes to which the spores of *Rz. oligosporus* can be found adhering. During fermentation, there is some biochemical reaction occurred involving some enzymes; hence, *tempe* is more digestible as compared to soybean (VanVeen and Schaefer 1950). The fermentation eliminates the beany flavor of raw soybeans and gives the product a bland but attractive flavor (Hesseltine and Wang 1967). Martinelli and Hesseltine (1964) introduced the use of plastic bags as containers for *tempe* fermentation, which is perforated to provide the moderate aeration necessary for mold growth without excessive sporulation, resulting in an attractive creamy, white fresh *tempe* cake (Figs. 14.2 and 14.3). This new idea and new technology is quickly transferred to *tempe* makers in Java and becomes widely used (Fig. 14.4 and 14.5).

Fig. 14.2 *Tempe* in plastic bag

Fig. 14.3 Fermented soybean cake, *Tempe*

Fig. 14.4 Adding inoculums of *Rz. oligosporus* to the dehulled cooked soybeans (Source: Rumah *Tempe* Indonesia)

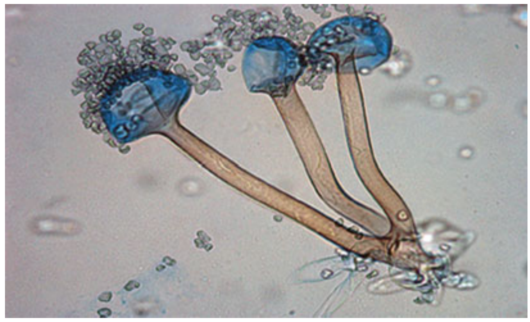

Fig. 14.5 *Rz. oligosporus* (http://www.mycology.adelaide.edu.au)

14.3.3 Microbes Involve in Tempe Manufacture

The microbes in *tempe* are complex, involving mixed culture fermentation by molds, yeasts, lactic acid bacteria, and various other bacteria. The major genus of importance is the mold *Rhizopus* with different species such as *Rz. microsporus*, *Rz. oligosporus*, and *Rz. oryzae* (Nout and Kiers 2005).

Rz. oligosporus is a species that can grow between 30 and 42 °C (optimum 25–37 °C), characterized by an inability to ferment sucrose; high proteolytic and lipolytic activity; and ability to release free ammonia after 48–72 h fermentation (Wang and Hesseltine 1965, 1979; Steinkraus 1983), grow on wheat or other cereal substrates without producing noticeable amount of organic acids due to minimal amylase activity (Wang and Hesseltine 1979), inhibit production of aflatoxin, and biosynthesize B vitamins (Murata et al. 1968).

Lactic acid bacteria play a role in the acidification of the soya beans during soaking, thereby preventing the growth of spoilage microorganisms (Ashenafi and Busse 1991; Nout et al. 1987), improving the shelf life of *tempe*. During fermentation, lactic acid bacteria grow up to 10^9 cfu/g-1 in final *tempe* products.

Samson et al. (1987) reported that the microbial load of 110 commercial *tempe* samples in the Netherlands was more than 10^7 cfu/g aerobic plate counts, predominated by Enterobacteriaceae and lactic acid bacteria. Sixty-nine percent of the samples contained yeast more than 10^5 cfu/g. Some samples also contained *Staph. aureus*, *B. cereus*, or *E. coli*. Ashenafi (1994) found high numbers of enterobacteria, enterococci, and staphylococci, whereas Mulyowidarso et al. (1991) found high numbers of *Bacillus* species in *tempe*. The contribution of bacteria and yeasts to the properties of *tempe* is in developing flavor and substrate modification and in the safety of the product (Nout and Rombouts 1990).

14.4 Biochemical Changes and Nutritional Value

During fermentation, there are some changes in the chemical composition of the soybeans. The mold, *Rhizopus* spp., produces a variety of carbohydrases, lipases, and proteases, which degrade the macronutrients into substances of lower molecular mass, with a higher water solubility. Also vitamins, phytochemicals, and antioxidative constituents are formed (Astuti et al. 2000; Nout and Kiers 2005).

Soaking, washing, dehulling, and cooking cause considerable loss of solids due to solution into the water (Steinkraus et al. 1960). Fermentation of soybeans by the *tempe* mold also causes an increase in soluble solids from 13.0 % in cooked soybeans to 27.5 % in *tempe* (Steinkraus et al. 1960) and explains the higher digestibility of *tempe* compared to plain cooked soybeans as stated by VanVeen and Schaefer (1950). During fermentation, the pH gradually increases from 5.0 to 7.5 due to ammonia production in the later stages of fermentation (Hand 1966). Fresh, properly fermented *tempe* has been reported to have a pH value of 7.25 (Ilyas et al. 1970). The strains with good amylolytic activity are unsuited for fermentation of *tempe* since they will break down starch to simple sugars which are then used to produce organic acids which will lower the pH and inhibit the growth of the mold.

The main sugars in soybean are sucrose, stachyose, and raffinose, and the last two are oligosaccharides which are considered primarily responsible for flatulence. Soaking and boiling treatment can reduce stachyose, raffinose, and sucrose at the amount of 51 %, 48 %, and 41 % of the original content, respectively (Kasmidjo 1989/1990). Shallenberger et al. (1976) reported further decrease of stachyose and sucrose during the fermentation of *tempe* which might be due to the activity of bacteria, since *Rz. oligosporus* is not able to utilize stachyose, raffinose, and sucrose. Sorenson and Hesseltine (1966) reported that glucose, fructose, galactose, and maltose supported excellent growth of the mold. On the other hand, raffinose was relatively constant.

The amino acid composition of soybeans apparently is not significantly changed by fermentation, but free amino acids and ammonia increased (Wang et al. 1968). Lysine and methionine have been found to decrease during the course of long fermentation. Wang et al. (1968) also found insignificant increase in PER of soybeans after fermentation, which might be attributed to better availability of amino acids liberated from the beans during fermentation and to better digestibility due to increase in soluble solids and nitrogen.

Hesseltine (1965) stated that the total fat (ether extractable) of tempe remained relatively constant throughout fermentation, although about one third of soybean oil was hydrolyzed due to its lipolytic activity into fatty acids by the *tempe* mold into palmitic, stearic, oleic, linoleic, and linolenic acids, with linoleic acid predominant. Linolenic acid is the only fatty acid utilized by the mold, and about 40 % of this fatty acid is used (Hesseltine 1965). The lipid content of *tempe* is lower than that of unfermented soybeans since the lipase enzyme hydrolyses triacylglycerol into free fatty acids around 40–50 % during soybean fermentation (Pawiroharsono 1997). Furthermore, the fatty acids are used as a source of energy for the mold. During *tempe* fermentation the lipid contents decrease about 26 % (Astuti 1994). A study by Graham et al. shows that the mold of *Rz. oligosporus* and *Rz. stolonifer* uses linoleic acid, oleic acid, and palmitic acid as energy sources, which rapidly decrease during fermentation, and palmitic acid, stearic acid, and linoleic acid by 63.4, 59.25, and 55.78, respectively (Astuti 1994).

Fatty acids in soybean are rich in unsaturated fatty acids (around 80 %), mainly oleic acid, linoleic acid, and linolenic acid. The concentration of oleic acid and linoleic acid increases proportionally with duration of fermentation time, but linolenic acid is decreased, and the optimal concentration is achieved on 24 h of fermentation (Wagenknecht et al. 1961).

A 30.7 % reduction of phytic acid occurred in the *tempe* fermentation (Egounlety and Aworh 2003), while Van der Riet et al. (1987) reported that phytic acid was reduced by about 65 % as a result of the action of phytase enzyme produced by *Rz. oligosporus*. Phytic acid is known as an antinutrient factor which is able to bind divalent minerals, thus lowering the mineral bioavailability. Therefore, the decrease in phytic acid has a beneficial effect on mineral bioavailability (Wang et al. 1980; Astuti 1994).

Rz. oligosporus caused almost complete destruction of phytic acid, due to phytase activity of the mold and improved bioavailability of iron (Sudarmadji and Markakis 1977; Fardiaz and Markakis 1981; Sutardi and Buckle 1985). Phytates adversely affect nutritional status by chelating minerals and making them unavailable for use by humans.

14.5 Nutritional Value of *Tempe*

Tempe contains 157 calories per 100 g, proteins (12.7 %), carbohydrates and fats (4 %), and vitamins B_1 (0.17 mg) and B_{12} (2.9 μg); it is low in cholesterol and saturated fat; is high in fiber and most B vitamins including B12; and has good-quality protein (19.5 %), comparable to protein content of meat products(Shurtleff and Aoyagi 1979; Okada 1989). On a 40 % dry solids basis (Steinkraus 1983), it contains all essential amino acids and is rich in lysine which is lacking in cereal grains, but methionine is limited (Shurtleff and Aoyagi 1979). Wang et al. (1968) found that the nutritive value of *tempe* made from a mixture of soybean and wheat was comparable to that of milk casein. *Tempe* fermentation does not alter amino acid profiles but make them bioavailable. The protein content and nutritive value make *tempe* a good substitute for meat (Steinkraus 1983). During the fermentation process, the levels of anti-nutritional constituents are decreased, and the nutritional quality and digestibility of the fermented product are improved due to the enzymatic activity of the mold (Nout and Kiers 2005). The mold also contributes to the development of a desirable texture, taste, and aroma of the product (Hachmeister and Fung 1993).

14.5.1 Antibacterial and Enzymes in *Tempe*

The high digestibility of *tempe* has been observed during World War II when prisoners suffering from dysentery were able to digest *tempe* much better than soya beans (Steinkraus 1996; Tibbott 2004). Pediatric research in Indonesia indicated that in infants, the recovery after acute bacterial diarrhea was faster when *tempe* was consumed as an ingredient of the infant food formula (Karyadi and Lukito 1996, 2000; Soenarto et al. 1997). *Tempe* showed a strong bioactivity in vitro by reducing the adhesion of enterotoxigenic diarrhea-causing *E. coli* to animal and human intestinal cells (Kiers et al. 2002; Roubos-van den Hil et al. 2009), and *tempe* intake was associated with better memory (Hogervorst et al. 2008).

Tempe has antibacterial effect against *Lb. bulgaricus*, *Strep. thermophilus*, *Bacillus* sp., and *Listeria* sp., although growth of *Lb. plantarum*, isolated from *tempe*, was not affected. No antibacterial activity of *tempe* against *E. coli* or *Salmonella* was observed (Kiers et al. 2002; Kobayasi et al. 1992; Wang et al. 1969, 1972). *Tempe* was found to possess anti-diarrhea-associated bacteria. On the one hand, *tempe* inhibits the adhesion of ETEC to intestinal cells, which can be of interest in the recovery and prevention of diarrhea in humans. On the other hand, *tempe* has antibacterial against *B. cereus* cells and spores, which can be of interest in food preservation and pathogen control. The anti-adhesion activity is caused by an interaction between ETEC and *tempe* extracts, which results in a loss of adhesion capability of ETEC to the intestinal cells (Roubos-van den Hil et al. 2009). This bioactivity is found in *tempe* derived from leguminous seeds, but not with *tempe* derived from cereals. The bioactive component(s) are released or formed during fermentation by enzymatic degradation of leguminous matter (Roubos-van den Hil et al. 2010). Fermentation with several other microorganisms also resulted in the formation of bioactive components, such as carbohydrate and arabinose, which is an important monosaccharide constituent supposed to originate from arabinan or arabinogalactan chains of the pectic cell wall polysaccharides of legumes (Roubos-van den Hil et al. 2010). *Tempe* contains enzymes with thrombolytic activity, which can digest thrombotic protein (fibrin) and which will be inactivated by heating above 65 °C (Sumi and Okamoto 2003). Aoki et al. (2003) revealed that tempe contains γ-aminobutyric acid that suppresses the elevation of blood pressure; contains dietary fiber, saponins, isoflavones, and superoxide dismutase which eliminates active oxygen; and has an anticarcinogenic effect.

14.5.2 Socioeconomy of *Tempe*

Tempe is an indigenous fermented food of Indonesia and the most extensively studied worldwide. For most of Indonesian people, *tempe* is a meat substitute, and the price is affordable for everyone. Throughout Indonesia *tempe* is consumed by people of low as well as high socioeconomic level. The *tempe* makers produce at home using 10–150 kg of soybean daily, and the producers are united in Cooperatives of Producers of *Tempeh* and *Tofu* in Indonesia. Urban population growth has stimulated a rise in the number of *tempe* processors in many cities throughout Indonesia, in response to the demand for relatively inexpensive foods. The significance of *tempe* industry as part of informal sector plays an important role. *Temp*e was formerly considered as an inferior food due to its low costs compared to other protein foods such as meats, fish, and eggs. Over the last four decades, the attitude toward *tempe* has changed, and it is now considered as inexpensive food with high nutritive values (Syarief 1997).

In 1982 a company in Southern Netherlands was making 6000–8000 lb/week of *tempe*, making it the largest *tempe* manufacturing company in the world. In early 1979, there were 13 commercial *tempe* shops in the United States, one in Canada, and four in the Netherlands (Shurtleff and Aoyagi 1979). The total sales of refrigerated *tempe* as meat alternatives in the natural food channel for the year ending August 2011 were at least $51.6 million, and 19.3 % of this was refrigerated *tempe*, while in the mainstream/mass market (including conventional supermarket chains), sales of refrigerated meat alternatives for the year ending August 2011 were at least $65.9 million, and 4.47 % of this was refrigerated *tempe* (Shurtleff and Aoyagi 2011).

In Indonesia, *tempe* is consumed as a protein-rich meat substitute by all economic groups due to its low-cost production, low price, and nutritional value (Karyadi and Lukito 1996). Outside Indonesia, *tempe* gains interest as a major protein source other than meat, especially nutritional and health functionality (Astuti 2000; Nout and Kiers 2005; Steinkraus 1996). Rumah *Tempe* Indonesia (RTI) or Indonesia *Tempe* House was launched on the 6th of June 2012 in Bogor, West Java Province, as a model of Tempe factory for the efficient, hygienic, and eco-friendly issue, implementing good manufacturing practice and good hygiene practice.

14.6 *Oncom* and *Gembus*: Microbiology, Nutritive Value, and Potential Health Benefit

Fermentation on solid waste of soybean, peanut residue, or shredded coconut residue also conducted in Indonesia produced *oncom*, *gembus*, and *bongkrek*, respectively. *Oncom* is one of the traditional fermented foods of West Java, a Sundanese ethnic cuisine of Indonesia, involving several molds and closely related to *tempe*.

Fig. 14.6 *Oncom* Bandung

Oncom is made from the by-products of tofu or peanut press cake residue after the oil has been pressed out and cassava tailings when extracting the starch (Fig. 14.6). There are two kinds of *oncom*: *red oncom* and *black oncom*. The solid by-product of tofu or peanut press cakes covered with massive coat of living conidia is called red oncom because of the glistening orange color of the conidia of the microorganisms, and the thicker the conidial layer, the higher the commercial value of the product (Sastraatmadja et al. 2002; Wood 1998).

Since *oncom* production uses by-products to make food, it increases the economic efficiency of food production. Black oncom is made by using *Rz. oligosporus* and other types of *Mucor* (Sastraatmadja et al. 2002; Wood 1998), while *red oncom* is made by involving *Neurospora*, particularly *N. crassa*, *N. intermedia* var. *oncomensis*, and *N. sitophila* (Hoo 1986). It is the only human food produced from *Neurospora*. *N. intermedia* var. *oncomensis* had bright yellow and large macroconidia in contrast to wild *N. intermedia* with pink and small macroconidia (Hoo 1986).

In the production of *oncom*, sanitation and hygiene are important to prevent bacterial or mold contamination such as *A. flavus* (which produces aflatoxin), even though aflatoxin-producing molds (*Aspergillus* spp.) are naturally present on peanut press cake when the peanut has already contaminated with the molds. *N. intermedia* var. *oncomensis* and *Rz. oligosporus* reduce the aflatoxin produced by *A. flavus* (Nout 1989). Soybean is the best substrate for growing *Rz. oligosporus* to produce *tempe*, but *oncom* has not been as thoroughly studied.

Tofu *oncom* is made from soybean residues and peanut *oncom* is based on peanut, well-known traditional fermented foods in West Java. The *oncom* cultures *N. intermedia* var. *oncomensis* had bright yellow and large macroconidia. Generally, red *oncom* is made from solid tofu waste, i.e., the soy residue after its protein has been taken for tofu making, while the black *oncom* is generally made from the peanut dregs mixed with cassava dregs or cassava powder, i.e., tapioca, in order to make a better texture and to make it more tender. Although both the substrate material is a kind of waste, its nutrient is still high enough to be exploited by human. Tofu waste still contains high nutrient values; however, most of its organoleptic properties are less preferred. Fermented *tofu* waste, i.e., red *oncom*, is preferred as food product than the waste without fermentation.

Tofu waste might contain protein similar to *tofu* and soy, although it has undergone many changes because of certain treatments during the manufacturing process of *tofu*, such as heating. The flavor of *oncom* can be described as strong, fruity, almond-like, and somewhat alcoholic, but when fried, it takes mincemeat flavor, while the alcoholic flavor which is present due to sugar degradation will vaporize and disappear. The best *oncom* in Indonesia is *oncom Bandung* (Fig. 14.6); instead of using peanut press cake, raw peanuts are used as the main ingredients.

The high nutrient content of *tofu* and its large amounts provide a significant opportunity to be used as a growth media for enzyme-producing microbes for health. *Oncom* has 187 kcal per 100 g, protein 13 %, fat 6 %, carbohydrate 22.6 %, vitamin B_1 0.09 mg, and vitamin B_{12} 3.1 μg

(Winarno 1989). *In vivo* study revealed that *red oncom* reduces the cholesterol levels of rats, suggesting potential health benefit for humans. The fibrinolytic activities in *oncom* also show potential prevention toward cardiovascular diseases. *Bacillus licheniformis* RO3 with high fibrinolytic activities was isolated from red *oncom* (Afifah et al. 2015).

Gembus is also made from solid soybean waste of *tofu* (Kuswanto 2004), fermented by *Rz. oligosporus*, involving *B. pumilus* 2.g which has high proteolytic and fibrinolytic activities (Afifah et al. 2013). *Gembus* is a variety of *tempe*, but whose substrate is different (solid tofu waste and soybean, respectively). Microbial fibrinolytic enzymes from food-grade microorganisms have the potential to be developed as additives for functional foods and as drugs to prevent or cure cardiovascular diseases (Afifah et al. 2014).

Like the soy *tempe*, *gembus tempe* contains several substances such as fiber, polyunsaturated fatty acids, ergosterol, and isoflavonoids, which may have influences on lowering the level of blood lipids (Sumi and Yatagai 2006).

Gembus tempe contains 65 calories, protein (3.41 %), carbohydrate (11.94%), fat (0.2%), calcium (143 mg), iron (0.4 mg), and vitamin B_1 (0.09 mg) (Sulchan and Nur 2007). Sulchan and Rukmi (2007) reported that *gembus tempe* contains energy (77.70 kcal), protein (4.07 g), lipid (0.23 g), total carbohydrate (14.25 g), fiber (4.69 g), ash (0.84 g), calcium (159.98 mg), phosphorus (59.69 mg), iron (0.48 mg), and water (6 %).

Gembus tempe, which is made of the solid soybean waste of tofu (Kuswanto 2004), rich in fiber (4.69 %), and contains a threefold greater level in fiber compared to *tempe* (1.40 g %). The amount of essential fatty acid content in *gembus tempe*, mainly linoleic and linolenic acid, 21.51 % and 1.82 %, respectively (Fatimah 1998).

Sulchan and Rukmi (2007) reported that *gembus tempe* did not contain cysteine, proline, and tryptophan, whereas methionine was found at 11.9 mg/100 g, in extreme contrast with *tempe* containing cysteine (70 mg/100 g) and methionine (168 mg/100 g).

Tempe bongkrek is a freshly fermented coconut press cake or shredded coconut residue by *Rz. oligosporus*. Hygienic conditions should be taken into consideration in preventing *P. cocovenenans* contamination and outgrowth of the mold that produces two toxins, toxoflavin and bongkrek acid.

Consumption of *tempe* bongkrek is associated with a food-borne human intoxication and significant numbers of deaths annually. Since 1975, *tempe bongkrek* production has been banned by local authorities for safety reasons.

Garcia et al. (1999) found that 40 % and 50 % coconut fat concentrations in the substrate (shredded coconut residue from coconut milk production) support production of 1.4 mg/g bongkrek acid, while less than 10 % coconut fat supporting growth of the *P. cocovenenans* yields no bongkrek acid. Oleic acid was most stimulatory in production of bongkrek acid (2.62 mg/g dry substrate). Lauric, myristic, and palmitic acids also stimulated production of bongkrek acid but at lower levels.

14.7 *Tauco* (*Miso*-Like Product)

Tauco is a yellow-colored saline paste, Indonesian style *miso*, made from fermented yellow soybeans and a yellow-colored saline paste with a meat-like flavor and used in Chinese and Indonesian cuisines as flavoring agent (Fig. 14.7). The name comes from its pronunciation in the Hokkien dialect of the Chinese language, and it originates from China. *Tauco* is often used as condiment and flavoring for stir-fried dishes of Indonesian cuisine traditions such as Sundanese and Javanese cuisines. Cianjur town is the center of *tauco* production.

To make *tauco*, the soybeans are soaked in fresh water, the hulls are removed, and the seeds are boiled and spread on bamboo trays to cool. Rice or glutinous rice flour is roasted until golden brown, then mixed with the seeds, and set aside for 3–5 days to ferment between hibiscus (*waroe*) leaves on flat trays, involving mold in the fermentation by *Rz. oligosporus*, *Rz. oryzae*, and *A. oryzae* followed by brine (20 %) fermentation for 20–30 days involving *Lb. delbrueckii* and *Hansenula* sp. After the second phase of fermen-

Fig. 14.7 Viscous liquid *tauco*, sweet *tauco* (*left*), salty *tauco* (*right*)

tation is completed, the brine is drained; palm sugar (25 %) is added and the mixture is cooked and stored for 24 h or placed directly into bottles. Microorganisms present in *tauco* are *A. oryzae*, *Rz. oligosporus*, *Rz. oryzae*, *Hansenula* sp., *Zygosaccharomyces soyae*, and *Lb. delbrueckii* (Winarno et al. 1973).

When the mass has molded, it is sun-dried for a few days until very hard, and the soybean *koji* for making *tauco* is used. Remove the leaves and put this mass of soybean *koji* into salt water. On the third or fourth day, add some yeast and some cane sugar syrup. Continue the soaking and fermentation in salt water for 3–4 weeks. *Tauco* is available in viscous liquid form (Fig. 14.7) or semisolid form which is obtained by sun-drying the liquid product to a final moisture content of 25 %.

14.8 *Kecap* (Soy Sauce)

In the nineteenth century, sinologist Samuel Wells Williams wrote that in China, the best soy sauce is "made by boiling beans soft, adding an equal quantity of wheat or barley, and leaving the mass to ferment; a portion of salt and three times as much water are afterwards put in, and the whole compound left for 2 or 3 months when the liquid is pressed and strained" (Williams 1848).

Kecap is an Indonesian soy sauce and usually traditionally made by small-scale producers, with little or no innovation in the process since ancient times. Black soybeans are boiled to undergo spontaneous solid-state fermentation (SSF) before being subjected to brine fermentation. After the brine is filtered, the filtrate is boiled together with caramel and spices, yielding the final product, *kecap* (Roling et al. 1994).

It is a liquid, brown-colored condiment, made by a two-stage batch fermentation which involves the biochemical activities of mold (*Rz. oryzae* or *Rz. oligosporus*), lactic acid bacteria (*Lactobacillus* sp.), and yeast (*S. rouxii*). There are two types of *kecap*, sweetened soy sauce (*kecap manis*) and salty soy sauce (*kecap asin*). According to Codex Alimentarius Commission (FAO/WHO 2004a, b), soy sauce is a clear liquid seasoning obtained by fermentation of soybean and/or by hydrolysis of soybean or other vegetable protein sources to produce soy extract which is further processed into sweet soy sauce or salty soy sauce.

Naturally brewed soy sauce is the product obtained by *A. oryzae* and/or *A. sojae* and/or *Rz. oryzae* and/or *Rz. oligosporus* as main starter(s) and cultured in either soybean or soybean and cereal grains with or without addition of bacteria and/or mold and/or yeast and/or enzyme. Non-brewed soy sauce is the product obtained by hydrolization of soybean and/or other vegetable protein by using acids or enzymes in the brine or salt water, namely, "Hydrolyzed Vegetable Protein," and classified as delicious agents (taste enhancer agents), not included in the category of soy sauce (FAO/WHO 2004a, b). While mixed soy sauce is the product obtained by brewed soy sauce and hydrolyzed vegetable protein, proportion added by brewed soy sauce is not less than 50 % (FAO/WHO 2004a, b).

Kecap is made by spreading cooked soybeans on a bamboo tray and leaving for a period to make molded soybeans (*kecap koji*). The molded soybeans are then mixed with salt solution to carry out the second stage of fermentation under 20 % brine solution for 14–120 days at room temperature (Steinkraus 1995). Then the fermented mash is filtered. To make *kecap manis*, the filtrate is mixed with palm sugar and spices, boiled for 4–5 h and filtered (Steinkraus 1995). *Kecap manis* contains 26–65 % carbohydrate, 0.3 % total nitrogen, and 3–9 % salt (Judoamidjojo 1986).

Kecap manufactured in home industry does not usually use any inoculum in *kecap koji* preparation; molds grow on the surface of cooked soybeans as the result of infection from the environment such as the air and the previously used trays (Judoamidjojo 1986; Nikkuni et al. 2002; Steinkraus 1995). Molds isolated from *kecap koji* were mostly of *Aspergillus* sp. (Judoamidjojo 1986; Nikkuni et al. 2002), and aflatoxin producers were found from Indonesian soybean *koji* samples (Nikkuni et al. 2002). According to Sadjono et al. (1992), approximately 47 % of 32 samples of traditionally fermented Indonesian *kecap* tested contained aflatoxin B_1 at more than 5 μg/kg. Therefore, the possibility of aflatoxin contamination cannot be ruled out in traditional *koji* making process, and it is thus necessary to use a pure culture starter for food safety concern.

Kecap mash prepared according to the traditional method is as follows: black soybeans (40 kg) were soaked in water overnight, boiled for about 3 h, spread on ten bamboo trays (ca. 90 cm in diameter), inoculated with 120 g of the starter culture, and left for 3 days in the koji fermentation room at room temperature by inoculated starter culture. The molded soybeans (*kecap koji*) were sun-dried for 2 days, winnowed to remove the hulls and spores, and placed in a plastic pail, and 70 L of hot water (58 °C) and 30 kg of salt were added to prepare *kecap* mash and allowed to ferment for 2 months at room temperature with exposure to sunlight.

14.8.1 Preparation of *Kecap Koji*

Kecap koji were prepared without inoculum by the conventional method in home industry, but in the factory, using the starter culture, hence, the *kecap koji* fermentation in the factory is faster than in home industry, 3 and 9 days, respectively. *Koji* culturing is in a mixture of equal amount of boiled soybeans and roasted wheat to form a grain mixture, then *Aspergillus* spores are added (the cultures are called *koji* in Japanese) (Judoamidjojo 1986). After sun-drying, the moisture contents decreased to 7–8 %.

14.8.2 *Kecap* Mash Fermentation

Kecap mashes were prepared with *kecap koji* and allowed to ferment for 2 months; the pH value of the mash reached 5.5 and contained about 21 % salt. The contents of formol nitrogen and water-soluble nitrogen increased with the fermentation time and showed higher as compared to without the starter culture. The molds involve in brewing soy sauce are *A. oryzae* and *A. sojae* (Fig. 14.8), strains with high proteolytic capacity (Maheshwari et al. 2010). *S. cerevisiae* is also involved, and the yeast will convert some of the sugars to ethanol, and further biochemical changes contribute to flavor development of soy sauce. *Bacillus* sp. may also grow in soy sauce ingredients and generate odors and ammonia, while *Lactobacillus* species will produce lactic acid and lower the pH.

14.8.3 Traditional Brine Fermentation

The *Aspergillus* sp. breaks down the grain proteins into free amino acid and protein fragments and starches into simple sugars. This amino-

Fig. 14.8 Molded soy and wheat by *A. sojae* cultures in traditional fermentation of kecap (Source https://ja.wikipedia.org/wiki/%E3%83%95%E3%82%A1%E3%82%A4%E3%83%AB:Shoyukoji.jpg)

glycosidic reaction gives soy sauce its dark brown color.

14.8.4 Brine Fermentation at Industrial Manufacturer

After 43 h SSF followed with brine fermentation for 4 months. About 8000 kg SSF material is mixed with 16,000 l brine. Final salt concentration of the brine is 15 %. Three overlapping phases occurred in brine fermentation: first, amino acid production (based on formol nitrogen production), followed by lactic acid fermentation (based on acetate and lactate production), and lastly yeast fermentation (based on ethanol and glycerol production) (Roling and van Verseveld 1996).

14.8.5 Amino Acid Production

Amino acid production started directly after the preparation of brine, and amino acids were produced during the first 3 weeks, and glutamic acid, mainly responsible for flavor, is produced during a longer period, and less than 15 % of the final glutamic acid is formed during the last 3 months and continued to increase slowly after 4 months (Roling and van Verseveld 1996). The activity of glutaminase, which is responsible for conversion of glutamine to glutamic acid, rapidly decreased but did not completely disappear. Even after formol nitrogen production had stopped, protease and leucine aminopeptidase activities were present. Therefore, the exhaustion of digestible proteins was more likely the cause of the termination of amino acid production (Roling and van Verseveld 1996).

14.8.6 Fermentation by Lactic Acid Bacteria

Staphylococci and enterobacteriaceae involved in SSF decreased rapidly after addition of salt. Only bacilli was observed after 1 week in brine solution. Numbers of salt-tolerant bacteria were high at the start of fermentation ($10^{7–8}$ cfu/ml), but dropped rapidly within 2 days before increasing again. After 1 week of brine fermentation, only the lactic acid bacterium *T. halophila* was isolated from samples and reached around 10^8 cfu/ml.

The pH of the brine dropped from 5.0–5.1 to 4.4–4.6, and concentrations of lactate and acetate increased up to 164 mM and 69 mM, respectively. Fructose completely disappeared. Lactic acid fermentation took about 2.5–3 weeks, then no further changes in lactate and acetate concentrations occurred, and the number of *T. halophila* declined (Roling and van Verseveld 1996).

14.8.7 Yeast Fermentation

Immediately after the preparation of brine, high number of yeast was observed ($10^{4–5}$ cfu/ml). Yeast fermentation in brine fermentation started after 5 days, and in some cases, after 42 days there was no yeast fermentation observed. During yeast fermentation a slight increase in yeast number, ethanol, and glycerol concentrations were observed. *Zygosaccharomyces rouxii* was the dominant yeast species. Glucose concentration dropped during the yeast fermentation, but galactose concentration remained unchanged. In brine fermentation heat-dependent browning reactions (Maillard reactions) took place during the entire period of fermentation (Roling and van Verseveld 1996).

The filtrate brine undergoes several post-fermentation treatments, such as the addition of caramelized sugar and subsequent boiling for several hours, resulting in a thick, strong brown color and evaporation of volatile compounds such as ethanol.

The organic acids formed during the growth of *T. halophila* have a preserving effect on *kecap*. Amino acids contribute to the flavor of *kecap*, directly as glutamic acid or indirectly via Maillard reactions during boiling of the mixture of brine extract and caramel (Yokotsuka 1986). The amino acid content and lactic acid concentration do not change much after 4 weeks; hence, brine fermentation for 1 month seems to be sufficient for industrial *kecap* production.

Addition of food-grade enzymes in brewing *kecap* represents continuous innovation in production methods and is also conducted by *kecap* manufacturer to speed up the fermentation. The addition of enzymes should be allowed for brewed *kecap*. In traditional *kecap* manufacturing, microorganisms are added for the sole purpose of producing enzymes that hydrolyzed soy proteins for development of the characteristic taste attributes of soy sauce. Whether produced traditionally, or added directly, enzymes carry out the same function. The fully fermented grain slurry is placed into cloth-lined containers and pressed to separate the solids from the liquid *kecap*. The isolated solids are used as fertilizer or fed to animals while the liquid *kecap* is processed further. Finally, the raw *kecap* is pasteurized to eliminate any active yeasts and molds remaining in the soy sauce and then filtered. The *kecap* can be aged or directly bottled and sold (Fig. 14.9).

In traditional practice, the liquid is extracted, clarified, and filtered before introduction of

Fig. 14.9 *Kecap manis* (sweet soy sauce)

desired taste and flavor by addition of brown sugar, spices, and certain additives (enhancer, preservatives and or coloring, and molasses); finally it is pasteurized and packaged. Sweet soy sauce in Indonesia is mostly produced by medium-large enterprise (approx. 60 %) while the remaining 40 % produced by small-medium enterprise (FAO/WHO 2004b).

Industrial manufacturers use defatted yellow soybean flakes and wheat instead of black soybeans only. SSF is well controlled and inoculated; however, brine fermentation is spontaneous and subjected to tropical weather conditions for 4 months (Wilfred et al. 1996). During brine fermentation in traditional *kecap* manufacture, amino acid production and growth of the lactic acid bacterium *Tetragenococcus halophila* (until recently known as *Pediococcus halophilus*) take place. Few or no obvious yeast fermentation is observed (Roling et al. 1994).

14.8.8 Socioeconomic Value

In Indonesia, during 2003, total production of sweet soy sauce and salty soy sauce was approximately 80.000 tons (90 %) and 31.200 tons (10 %), respectively, and the potential growth is about 3.6 % per year. As condiment, consumption of sweet soy sauce is about 0.9 l/capita/year. Besides being part of daily Indonesian cuisine, *kecap* or sweet soy sauce also served as Indonesian typical sauces for instant noodle and other products.

14.8.9 Quality Criteria of Soy Sauce

The total nitrogen should be not less than 0.4 % w/w in salty soy sauce and not less than 0.15 % in sweet soy sauce, the soluble solid contents, exclusive of added salt not less than 6 % (w/v), and the sugar content for sweet soy sauce is not less than 30 %, and salt content for salty soy sauce is not less than 10 % (FAO/WHO 2004a).

14.8.10 Food Safety Concern on Carcinogens

Soy sauce may contain ethyl carbamate, a Group 2A carcinogen (Matsudo et al. 1993). In 2001, the UK Food Standards Agency found that 22 % of tested samples contained a chemical carcinogen named 3-MCPD (3-monochloropropane-1,2-diol) at levels considerably higher than those deemed safe by the EU (0.02 mg/kg) from various soy sauces manufactured in mainland China, Taiwan, Hong Kong, and Thailand, made from hydrolyzed soy protein, rather than being naturally fermented (Hamlet et al. 2002; Crews et al. 2003). About two thirds of these samples also contained a second carcinogenic chemical named 1,3-DCP (1,3-dichloropropan-2-ol) which should not be present at any levels in food. Both chemicals have cancer potential, and the agency recommended to withdraw from shelves and avoided 3-MCPD and 1,3-DCP, chloropropanol carcinogens. The same carcinogens were found in soy sauces manufactured in Vietnam, causing a food scare in 2007. Continuous lifetime exposure to high levels of 3-MCPD could pose a health risk, and Health Canada has established 1.0 part per million (ppm) as a guideline for importers of the sauces and considered to be a very safe level (Fu et al. 2007).

14.9 Fermented Fruits and Vegetable Products

14.9.1 *Acar* Pickles

Acar is a type of vegetable pickles (Fig. 14.10) made in Indonesia, Malaysia, and Singapore, usually prepared in bulk as it easily is stored in a well-sealed glass jar in refrigerator for a week and served as the condiment for any meals. It is a localized version of the Mughlai Achaar. It is known as *atjar* in Dutch cuisine, derived from Indonesian *acar*. In Indonesia, *acar* is commonly made from small chunks of cucumber, carrot,

Fig. 14.10 Mixture of cucumber and carrot *acar*

shallot, young bamboo shoot, eggplant, chili, and occasionally pineapple and marinated in a sweet and sour solution of sugar and vinegar.

Acar is a very popular accompaniment in many of Indonesian dishes, such as *nasi goreng* (fried rice), friend noodle, *sate*, and almost all varieties of *soto*. It is very easy to prepare at home; the key to a successful *acar* is to use the freshest ingredients possible. Just like common pickles, the sour taste of vegetable acar may freshen up the meal, especially the fishy dish such as grilled fish or the rich and oily dish such as *mutton satay* to neutralize the fatty taste.

Various microorganisms are usually associated with fresh fruits and vegetables as normal flora, transit flora, spoilage, and pathogenic organisms. Cucumber comes in contact with soil insects and animals during its growth and harvest from the field, and therefore, the microbial flora will include soil microorganisms and those from contaminated irrigation water; direct contamination by wild animals, birds, and insects; and transportation with contaminated containers (Reina et al. 2002; Heaton and Jones 2007; Williamson et al. 2003).

Cucumber (*Cucumis sativus*) is one of the primary vegetables often fermented to obtain pickles involving a mixed microbial fermentation in which desirable and undesirable bacteria and fungi interact and compete during the initial stages of the fermentation. The harvested cucumbers were naturally fermented in 10 % (w/v) NaCl solution for 30 days with and without $CaCl_2$ and/or polygalacturonase (PG). $CaCl_2$ and PG treatments did not interfere with fermentation. Separately, $CaCl_2$ enhanced firmness of pickles while PG was effective in causing excessive softening. When $CaCl_2$ was present in PG-containing solutions, softening by PG was inhibited (Lennox and Efiuvwere 2013; Buescher et al. 1979).

14.9.1.1 Microbial Changes During Acar Fermentation

The common microorganisms usually isolated from cucumber are enteropathogenic bacteria, lactic acid bacteria (LAB), *Pseudomonas* spp., *E. carotovora*, and some fungi, and the heterotrophic plate counts in the produce range between 4.0×10^2 and 5.7×10^2 cfu/g-1 (Nahaisi et al. 2005).

The changes in microflora of the fermenting cucumber in brine solution were reported by Lennox and Efiuvwere (2013), with the initial counts of 4.2×10^6 cfu/ml, 1.4×10^3 cfu/ml, 5.0×10^6 cfu/ml, and 1.2×10^3 cfu/ml for lactic acid bacteria (LAB), fungi, *E. coli*, and *Salmonella–Shigella*, respectively, in the fermenting brine solution. There was sharp increase in counts of LAB and indicator organisms in the brine on the third day, and thereafter they started to decline but the indicators were finally inhibited by the 12th day. *Salmonella–Shigella* showed very slight increase on the third day but were finally inhibited by the 12th day. The fungi counts fluctuated and reached their peak on the sixth day, but were finally inhibited also by the 15th day. LAB persisted to the end of the fermentation with the total count of 2.1×10 cfu/ml. There was no growth of *Salmonella–Shigella* within the fruit during fermentation. On the third day of fermentation, only LAB and indicator organisms grew with counts of 5×10^3 cfu/g and 3×10^3 cfu/g, respectively. The indicator organisms were inhibited within the cucumber by the sixth day. The changes in the microbial flora in the brine and cucumber could have been due to the competitive nature of the microorganisms in any environment and proved to be effective in eliminating pathogenic organisms and other contaminants and also preserved the cucumber.

There is usually succession of LAB during fermentation of pickled cucumber. The epiphytic LAB which occur naturally on the surface of the cucumber initiate fermentation and effectively control the microbial ecology of the fermentation by consuming the glucose and fructose present, producing lactic acid, and lowering the brine pH which favors *Lb. plantarum*, a homofermentative, acid-tolerant LAB which takes over the fermentation and does not result in production of carbon dioxide from sugars (Lennox and Efiuvwere 2013). The lactic acid they produce is effective in inhibiting the growth of other bacteria that may decompose or spoil the cucumber due to metabolic products such as lactic acid like bacteriocins, peroxides, and peptides that can inhibit other bacteria (DeVuyst and Vandamme 1994; Sapers and Annous 2004).

14.9.2 *Sayur Asin*

Sayur asin (Fig. 14.11) is an ethnic, fermented mustard cabbage leaf (*Brassica juncea* var. *rugosa*) product from Indonesia (Puspito and Fleet 1985). Mustard cabbage leaves are sorted, washed, withered, wilted, and rubbed or squeezed with 2.5–5 % salt. Liquid from boiled rice (*air tajin*) is added to provide fermentable carbohydrate to assure that sufficient acid is produced during fermentation. Fermentation is initiated by *Leuc. mesenteroides*, *Lb. confusus*, and *Lb. curvatus* and later dominated by *Lb. plantarum* and *P. pentosaceus*. Starch degrading species of *Bacillus*, *Staphylococcus*, and *Corynebacterium* exhibited limited growth during the first day of fermentation. The yeasts, *C. sake* and *C. guilliermondii*, also contributed to the fermentation. The pH falls from 6.5 to 4.2 in 8 days of fermentation (Puspito and Fleet 1985). Lactic acid, acetic acid, succinic acid, ethanol, and glycerol are produced during 2–14 days of fermentation. Hydrolysis of starch and maltose resulted in glucose, which is utilized by microbes for their growth during fermentation.

Manufacture of fermented mustard cabbage leaves was made by addition of the salt to vegetables, allowing the growth of certain fermentable microorganisms, resulting in sensory changes bearing acidic and unique characteristics to the *sayur asin* (Chiou 2004). Epiphytic lactic acid bacteria (LAB) of which initially amounted to slightly between 10 and 1000 cfu/g plant (0.001–1 % of the total population of microorganisms) became dominant within the microorganism population in the fermented mustard cabbage as a result of the anaerobic condition on the vegetables (Daeschel et al. 1987; Azcarate and Todd 2010). After 2 days of fermentation, the lactic acid was produced at 0.8–1.5 % in 2.5 % brine and the pH reached 3.4 (Sulistiani et al. 2014). A combination of acidic conditions and salt concentrations suppressed the growth of undesirable microorganisms, hence, preserving the vegetables.

Pederson (1971) reported that 2.25–2.5 % salt allowed exclusive growth of lactic acid bacteria, suppressed the growth of spoilage bacteria, and inhibited pectinolytic and proteolytic enzymes that can cause softening and putrefaction (Swain et al. 2014); the osmosis process will draw out the water and nutrients from vegetables as growth

Fig. 14.11 *Sayur asin* on sale in the traditional market

medium, facilitating metabolism of sugar into lactic acid during fermentation. Low salt concentration such as less than 2.25 % will support the growth of proteolytic bacteria, while adding more than 10 % salt will enable the growth of halophilic bacteria and cause fermentation failure. In general the higher the salt concentration, the slower fermentation. For a short time fermentation preferably 2.5–10 % brine solution is used (Swain et al. 2014).

Sulistiani et al. (2014) reported identification of 246 lactic acid bacteria isolates from *sayur asin* based on 16S rDNA sequence data. The bacteria belong to 11 species, viz., *Lb. farciminis* (15 isolates), *Lb. fermentum* (83 isolates), *Lb. namurensis* (18 isolates), *Lb. plantarum* (107 isolates), *Lb. helveticus* (1 isolate), *Lb. brevis* (1 isolate), *Lb. versmoldensis* (3 isolates), *Lb. casei* (12 isolates), *Lb. rhamnosus* (2 isolates), *Lb. fabifermentans* (3 isolates), and *Lb. satsumensis* (1 isolate), and revealed that *Lb. plantarum* and *Lb. fermentum* are common LAB used in *sayur asin* production from Central Java, Indonesia, and have also been reported to be found in different fermented foods (Ludwig et al. 2009). These species have been characterized into different fermentation types: obligately homofermentative, facultatively heterofermentative, and obligately heterofermentative (Felis and Franco 2007), and they have been reported to be not pathogenic to human or animal (Azcarate and Todd 2010). Therefore, the sayur asin is safe for human consumption.

Several species which were not found by Puspito and Fleet (1985) have been determined by molecular identification. These include *Lb. namurensis*, *Lb. versmoldensis*, *Lb. rhamnosus* (previously known as *Lb. casei* subsp. *rhamnosus*), *Lb. fabifermentans*, and *Lb. satsumensis*. The result showed that each sample consisted of various species, predominated by *Lb. plantarum* and *Lb. fermentum*, with *Lb. fermentum* and *Lb. plantarum* being more acid tolerant and often dominating the fermentation processes of vegetables and cereals (Sulistiani et al. 2014). In another study, Swain et al. (2014) reported that *Leu. mesenteroides*, *Lb. confusus*, *Lb. curvatus*, *P. pentosaceus*, and *Lb. plantarum* have been isolated from *sayur asin*. The spontaneous fermentation of *sayur asin* involved diverse variation of lactic acid bacteria.

According to Chao et al. (2009), the variety of LAB population in the fermented mustard was influenced by different fermentation process and treatments, especially the salt concentration. During the process, the squeezed and salt treatment diffused water and nutrient out of the vegetable tissues by high osmotic pressure (Chiou 2004). In addition, the varying conditions of anaerobiosis, moisture levels, and temperature resulted in changes in the population balance and selected for spontaneous fermentation by lactic acid bacteria (Azcarate and Todd 2010). The growth of the lactic acid bacteria is also influenced by nutrient movement from plant material into the surrounding liquid (Daeschel et al. 1984).

14.9.3 *Brem*

Brem is traditional fermented food or fermented beverage, a non-distilled ethnic alcoholic drink from Indonesia prepared from glutinous rice. It is a dried, starchy, sweet–sour rice extract and is eaten as a snack. There are two types of *brem*: *brem cake* (solid), which is yellowish-white, sweet–sour snack usually eaten in Madiun, where it is prepared in blocks of 0.5 × 5 to 7 cm (Fig. 14.12), and in Wonogiri (Fig. 14.13), where it is sweet, very soluble, white, and thin circular blocks of 5 cm diameter, and *brem beverage* (liquid), which is made of rice wine from Bali and Nusa Tenggara, but mostly known from Bali (Basuki 1977).

Brem cake from Madiun (Fig. 14.12) and Wonogiri (Fig. 14.13) is believed by Indonesian

Fig. 14.12 *Brem* Madiun

Fig. 14.13 *Brem* Wonogiri

consumer to be important for stimulating the blood system. It is also reported to prevent dermatitis, probably due to the presence of significant amounts of B vitamins produced by the microorganisms. This product is consumed as a snack and is not part of the daily family diet.

All three types of *brem* are made from the liquid portions of *tapé ketan* (fermented glutinous rice). The glutinous rice is steamed and spread on the trays lined by banana leaves to cool, then 0.2 % powdered *ragi* (inoculum), the same ragi for *tape singkong* fermentation is added to the cooled rice and mixed thoroughly, incubated at room temperature (30 °C) for 3 days aerobically, the juicy rice called tape is pressed out and transferred to the fermenting jars, fermented anaerobically at room temperature for 8–10 weeks. After fermentation, the juice is siphoned carefully into sterilized bottles and stored in a cool room for aging around 8–12 months (Aryanta 1980).

During *brem* production, the filtrate of *tapé ketan* is boiled down, poured onto a table, covered with banana leaves, and left to cool at ambient temperature over 8–12 h (*brem* Madiun) or sun-dried for 1 day to produce *brem* Wonogiri (Campbell-Platt 1987).

Brem beverage is a traditional rice wine of Bali island. The process of *brem* making involves a solid-state fermentation of steamed glutinous rice by a traditional inoculum (*ragi*), extraction of the liquor, and further liquid-state fermentation without additional inoculation. The quality of this product is inconsistent due to the inconsistency of the microorganisms in ragi as a consequence of spontaneous fermentation. In the Philippines, rice wine is called tapuy, while in Japan it is called sake (Aryanta 2000).

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). Alcohol content of *brem* Bali (Fig. 14.14) is 6.1 % (Winarno 1986). *Brem* with improved *ragi* is produced which has more desirable flavor than conventionally made *brem* (Saono et al. 1984).

Brem beverage consumed and holds important use in temple ceremonies of Hinduism called Tetabuhan, an offering beverage for *Buto Kala* (lit. Kala the Giant) in order to evoke harmony.

Brem Bali *beverage* can be either white or red depending on the proportions of white and black glutinous rice used in production; it is very sweet to semisweet, yet acidic, and contains alcohol with varying degree, usually from 5 % to 14 %.

Liquid *brem* is made from fermented mash of black/white glutinous rice using a dry starter

Fig. 14.14 *Brem* Bali beverage

called *ragi tape*. Glutinous rice is soaked and drained, steamed for 1 h, and then cooled down. The cooled glutinous rice is then inoculated with *ragi tape* and amylolysis begins. A honey-like rice syrup settles in the bottom of the malting vessel. Following 3 days of conversion from the starch to sugar, yeast culture is added and alcoholic fermentation begins. Alcoholic fermentation typically goes on for 2 weeks.

14.9.3.1 Microbiological and Biochemical Changes

Aryanta (1980), Lotong (1985), and Uchimura (1998) reported the microbes in *ragi*. Aryanta (1980) reported that during the first 3 days of fermentation, the population of molds in brem (*ragi* NKL as inoculum) was 3.5 × 105 cfu/ml and decreased to 5.9×10^2 cfu/ml; the yeast was at 5.5×10^4 cfu/ml and increased to 4.9×10^6 cfu/ml, and after 2 weeks of fermentation, it then decreased until the sixth week, and no mold was found. No yeast was isolated at the eighth week of fermentation. On the third day of fermentation, bacterial count was 6.5 × 10 cfu/ml which increased until the second week (8.0×10^6 cfu/ml) and then decreased until sixth week. On the eighth week of fermentation, no bacteria were isolated (Aryanta 1980).

The sugar content of *brem* decreased during the fermentation, due to the decomposition of simple sugars into ethanol and carbon dioxide by the yeast's enzyme activity (Pederson 1971) through Embden–Meyerhof–Parnas (Aurand and Woods 1973).

During fermentation of *brem*, the pH decreased, which might be due to more production of some organic acids at longer fermentation time. After 10 weeks, the pH reach 4.0 and contained 3 % reducing sugar, 6 % ethanol, and 0.6 % total acidity (as acetic acid), due to the oxidation of ethanol to acetic acid by the activity of *Acetobacter aceti*.

14.9.4 *Tuak* (Palm Wine)

Tuak (palm wine) is one of the indigenous alcoholic beverages most widely known in North Sumatra region of Indonesia. The *Arecaceae* such as palm sap of aren (*Arenga pinnata*) and nipa (*Nypa fruticans*) called *nira*, a sweet juice with pH between 5.5–6.5 % and 80–90 % moisture content, is fermented spontaneously through the application of one or more several kinds of woodbark or root, called raru (*Xylocarpus* woodbark or a variety of forest mangosteen), into the sap water of sugar palm (*Arenga pinnata*) with the involvement of natural yeasts for 2–3 days.

The sweet taste of the palm sap is due to the presence of sugars (sucrose, glucose, fructose, and maltose). The sugar content is 12.30–17.40 %, and the reducing sugar is 0.5–1 %. In addition to sugar, the juice contains other ingredients such as protein, fat, water, starch, and ash as well as organic acids (citric, malic, succinic, lactic, fumaric) that play a role in the formation of specific brown sugar flavor (Judoamidjojo 1985). Hence, palm sap is a good medium for the growth of microorganisms such as bacteria, fungi, and yeast and needs to be preserved as soon as possible. The presence of microorganisms may spoil the palm sap which is characterized by the formation of mucus to become turbid, murky, green, white, and frothy sour taste.

In North Sumatra, *Tuak* is produced by spontaneous fermentation of the palm sap (*Arenga pinnata*) in the presence of raru wood or several kinds of woodskin or roots (like nirih – Xylocarpus woodskin or a kind of forest mangosteen) for overnight incubation. Native people in Gorontalo, North Sulawesi, called *tuak* as "bohito" in their native language (Latief and Latief 2014).

Hermansyah et al. (2015) isolated and identified culture independent method for *Candida tropicalis* from North Sumatra's *tuak* among other yeasts. The *C. tropicalis* isolate is able to utilize glucose for more rapid and higher production of ethanol at high temperature of 42 °C as compared with *S. cerevisiae*. However, the optimum temperature of *C. tropicalis* isolates is 30 °C as displayed by its ability to produce 6.55 % (v/v) and 4.58 % ethanol from 100 g/l glucose fermentation at 30 °C and 42 °C, respectively. Rahayu dan Kuswanto (1988) revealed that the alcohol content in *tuak* was 3–10 %, which depends on fermentation medium (*aren* or *nipa*) and fermentation time, and the most important thing is the natural indigenous microbes involved during fermentation. Hartanto (1997) revealed that alcohol content of tuak is quite similar to wine (6–12 %).

14.9.4.1 Social Aspect

Tuak contains alcohol; hence, Moslem community does not drink *tuak*. Non-Moslem communities in some areas in Indonesia consume *tuak* and also used it as traditional remedy and in some ritual or traditional ceremony in Sumatra and Flores islands (Ikegami 1997; Ola 2009). The ethnic tribe Batak Toba believe that *tuak* is good for new mothers after giving birth to augment their breast milk production and to remove the impurities through sweat. In North Sumatra, there is traditional ceremony to respect old generation, namely, *manuan ompu-ompu* dan *manulangi* (Ikegami 1997). Likewise, traditional ceremony called *Lewak Tapo* in Lamaholot ethnic group of Adonara island, East Flores. Latief and Latief (2014) reported that in Momala village, Gorontalo, North Sulawesi, a community called *pakua lo bohito* consumes *tuak* for making social interaction, as Japanese drink sake.

Fig. 14.15 Fermented durian, *tempoyak*

14.9.5 *Tempoyak*

Tempoyak is a traditional condiment (Fig. 14.15) made from the flesh or raw pulp (aril) of the durian fruit (*Durio zibethinus*), a kind of tropical fruit, naturally fermented at room temperature in a tightly closed container and normally prepared from excess, poor-quality or overripe fruits (Ganjar 2000). This product is popular among people living in Riau Province, Sumatra, Indonesia, as well as in Malaysia. Interestingly, even though the fresh durian pulp is fermented without any heat application, there is no record of food-borne illness caused by the consumption of tempoyak. *Tempoyak* has a long history of safe consumption, in Riau, Sumatra.

The fermentation process may involve salt or without salt, and usually low amount of salt, 1.3 %, is added to support the growth of desired lactic acid bacteria besides yeast as saccharolytic microbes. *Tempoyak* is manufactured by mixing durian pulp with salt and allowing it to ferment for 3–7 days, producing a distinctive durian smell and creamy yellow color with sour and salty taste (the sour taste dominates). *Tempoyak* is consumed with rice or added to cooking dishes as condiment (Ganjar 2000).

The initial pH of *tempoyak* is in the range of 6.62–6.83, and after 2 days of fermentation, the pH is in the range of 3.96–4.08 (Leisner et al. 2001; Merican 1977; Amin et al. 2004). The total acidity of *tempoyak* is around 3.6 % as acetic acid and the final pH value is 3.8–4.6 (Steinkrauss et al. 1996; Merican 1977). Suan (1996) reported that *tempoyak* has 2.0 % ash, 67 % moisture, 4.5 % total sugar, 2.5 % crude fiber, and 1.4 % fat.

14.9.6 Lactic Acid Bacteria Involvement During Tempoyak Fermentation

Several LAB found to be involved in the fermentation of *tempoyak* are *Lb. plantarum*, *Leu. mesenteroides* subsp. *mesenteroides*, *Streptococcus faecalis* (Ohhira et al. 1990), *Leu. mesenteroides*, *Lb. brevis*, *Lb. mali*, *Lb. fermentum* (Leisner et al. 2001), and *Lb. durianis* (Leisner et al. 2002). Wirawati (2002) isolated *Lb. plantarum*, *Lb. coryniformis*, and *Lb. casei* from tempoyak. Yuliana and Dizon (2011) found *Lb. plantarum*, *Lactobacillus* sp., *W. paramesenteroides*, and *P. acidilactici* in *tempoyak*.

In another study, Widowati et al. (2013) reported the involvement of *Oenococcus*, *Leuconostoc*, *Enterococcus*, *Lactococcus*, *Pediococcus*, and *Lactobacillus. Leuconostoc* sp. in the presence of 2 % and 4 % salt, respectively, at 20 ± 2 °C during early stages of 4 weeks fermentation, whereas heterofermentative *Lactobacillus* sp. and homofermentative *Lactobacillus* sp. dominated the bacterial population in the middle stage. At the end of fermentation, homofermentative *Lactobacillus* sp. and *Pediococcus* sp. were found during tempoyak fermentation.

While Pato and Surono (2013) isolated *Ent. gallinarum* and *Ent. faecalis*, *out of* 12 isolates classified as genus *Lactobacillus* sp. isolates and the other 32 *Enterococcus* sp. isolates from tempoyak in Riau, . Almost all LAB isolated from *tempoyak* were relatively resistant to acid as indicated by the reduction in the number of colonies between 0.76 and 2.82 log cycles at pH 3.0 after 2 h incubation. These lactic acid bacteria play an essential role in preserving raw food materials, i.e., durian, and contribute to the nutritional, organoleptic, and health properties of *tempoyak*.

Pato and Surono (2013) identified two potential probiotic strains isolated from tempoyak, *Enterococcus* sp. UP-9 and *Enterococcus* sp. UP-11, by PCR the 16S rRNA gene sequences using specific primers and sequencing of amplified region by using an automated sequencer, and showed 97 % homology to *Ent. gallinarum* and *Ent. faecalis*, and named *Enterococcus gallinarum* UP-9 and *Enterococcus faecalis* sp. UP-11, respectively. This finding is contradictory to the previous report by Leisner et al. (2000) where Lactobacillus was the dominant lactic acid bacteria in tempoyak due to its natural fermentation. *Enterococcus* sp. and *Lactobacillus* sp. were the predominant genus in tempoyak and relatively resistant to acid as the isolates were originated from tempoyak with pH 3.69. *Ent. gallinarum* UP-9 and *Ent. faecalis* UP-11 showed potential probiotic properties and were able to reduce cholesterol level by different mechanisms, namely, deconjugating taurocholic acid and cholesterol binding (Pato and Surono 2013).

Amin et al. (2004) reported that addition of 1 % salt in *tempoyak* fermentation for 10 days showed the highest lactic acid bacteria viable counts, and at 8–10 days fermentation, the viable lactic acid bacterial counts were comparable between 1 % and 2 % salt addition. The higher the salt concentration, the lower the lactic acid bacteria viable counts; however, addition of 2 % salt produced the most preferred *tempoyak* by sensory evaluation.

The involvement of lactic acid bacteria in *tempoyak* fermentation might be due to the total sugar content in durian fruit, 15–20 % (Ketsa and Daengkanit 1998), and 17 % saccharose which may favor the growth of lactic acid bacteria and yeast (Leisner 2001).

14.9.7 *Mandai*

Mandai is a fermented product made from *cempedak* (*Artocarpus champeden*) or jackfruit (*Artocarpus heterophyllus*) inner peel in brine solution, a traditional fermented food of native people in the province of Central, South, and East Kalimantan. *Dami*, inner part of peel, is the nonedible part of the fruit. The *dami* is cleaned and soaked in a 5–15 % brine solution for 2 weeks. Fermented *mandai* is seasoned and consumed as a side dish of rice. It tastes good and is savory and its texture resembles that of meat, making these foods popular. *Mandai* fermentation process is part of an effort to preserve and to utilize the waste from jackfruit consumption. *Mandai* generally can be kept for 1 year or more. Rahayu (2003) found nine isolates of lactic acid bacteria from *mandai* cempedak and identified by molecular detection as *Lactobacillus plantarum* and, *P. pentosaceus*. Emmawati (2014) reported the involvement of *Lb. plantarum* in *mandai* fermentation.

14.9.7.1 Dynamic Changes of Lactic Acid Bacteria and Biochemical Changes During Mandai Fermentation

The total viable count of lactic acid bacteria was reported to increase during *mandai* fermentation in 10 % and 15 % brine solution, but not in of 5 % brine solution. The initial viable count of lactic acid bacteria was approximately 6–7 log cfu/ml and the final viable count was in the range of 7.0–7.7 cfu/ml (Emmawati et al. 2015; Nur 2009) until day 14 of fermentation. Salt concentration significantly influences the amount of lactic acid bacteria. The higher the concentration of salt added during *mandai* fermentation, the higher the viability of lactic acid bacteria counts. Salt in the fermentation of *mandai* added environmental selection factors.

Effect of salinity on the growth of lactic acid bacteria during fermentation was also reported by Ji et al. (2007) in cabbage fermentation. Higher salt concentration will decrease the total viable count of lactic acid bacteria. Eight to 12 % brine solution will inhibit the growth of lactic acid bacteria at the beginning of fermentation and then increase at the end of fermentation. Salt affects microbial growth by reducing the availability of water in the cell. The presence of salt also lowers the reduction potential of limiting the growth of aerobic microorganisms and otherwise supports the growth of microorganisms that are microaerophilic and anaerobic (Emmawati 2014). *Mandai* fermentation in 10 % and 15 % brine solution facilitates the growth of lactic acid bacteria, as shown by higher viable counts of lactic acid bacteria. On the other hand, 5 % brine solution may facilitate other microbes to grow, and the competition for the nutrients may suppress the growth of lactic acid bacteria.

Biochemical aspects such as reducing sugar and N-total decreased to 0.240 % at day 14 and 0.159 % at 21 day, respectively. Substrates for salinity increased in the third week to 4.941 % and relatively stable. The pH value of the substrate is in the range of 3.71–6.02 (Emmawati 2014).

The dynamic changes of microbes during *mandai* fermentation was reported by Emmawati (2014) that on days 4–8 of fermentation, cocci isolates predominate the microbe population. On day 8, the viable counts of lactic acid bacteria were decreased in *mandai* fermentation. The lactic acid bacteria isolated from day 8 at 15 % brine solution of fermentation reveal that the isolates are halotolerant or halophilic cocci. However, at day 12, the cocci isolates were not found, probably due to acidic environment as a result of more metabolites produced, including organic acids, thus lowering the pH and making the condition to become acidic with the final pH in the range of 4.16–4.8.

14.9.8 Low Salt Concentration on Microbial and Biochemical Changes in Mandai Fermentation

In general, *mandai* is made in high salt concentration. Nur (2009) reported the fermentation of *mandai* in 10 % (w/v) brine solution for 14 days. Microbial succession occurred during fermentation. Yeast cells grew dominantly (2.8×10^9 cfu/g) on day 5, but bacteria were dominant at day 14 (1.1×10^7 cfu/g). The highest decrease of reducing sugar and N-total contents were 0.240 % at day 14 and 0.159 % at day 5, respectively. The pH value was varied within the range of 3.71–6.12 for the whole period of fermentation.

Biochemical parameters such as reduction sugar, N-total, pH, and salinity of substrates were changed. Reducing sugar content decreased to 0.240 % on day 14 and the levels of N-total also declined in day 5 to 0.159 %. The pH value of the substrate was in the range of 3.71–6.02 (Nur 2009).

The addition of salt to organic substrates leads to a series of spontaneous fermentation and microbial selection that leads to a succession of microbes. Salt in high concentrations can inhibit the growth of spoilage and pathogenic microbes due to the decrease in the value of water activity (a_w) and ionized salt into ions Cl toxic. Treatment with high salt on the one hand affects aroma preservation and formation and on the other hand poses a concern for the health of the consumer, especially hypertension.

Rahayu (2003) and Lindayani and Hartayanie (2013) isolated *Lb. pentosus* from *mandai* of Semarang city, which can grow at 10 °C, 45 °C, and 50 °C and at pH 4.4 in 6.5 % brine solution. *Lb. pentosus* is heterofermentative lactic acid bacteria that cannot grow at pH 9.6 and in 18 % brine solution.

14.10 Fermented Milk Products

14.10.1 *Dadih*

Dadih (*dadiah*, in native language) is an Indonesian traditional fermented milk made out of buffalo or cow's milk produced and consumed by the West Sumatran *Minangkabau* ethnic group of Indonesia (Fig. 14.16). It is one of the very popular dairy products in Bukittinggi, Padang Panjang, Solok, Lima Puluh Kota, and Tanah Datar (Surono and Hosono 1996a).

It is a significant dairy product in the diet resembling yogurt and is similar to dahi of India with a distinctive thicker consistency, smooth texture, and pleasant flavor due to its higher total solid content, higher fat content, and casein content as compared to cow's milk. *Dadih* provided safety, portability, and novelty to milk nutrients for the indigenous people in West Sumatra.

Fig. 14.16 *Dadih* product

The higher protein content in buffalo milk results in custard-like consistency at the end of fermentation. In addition, higher fat content enriches the flavor developed in the *dadih* products. A good-quality *dadih* is firm with uniform consistency and has a creamy-white color, pleasant aroma, and acidic taste with smooth and glossy surface; its cut surface is trim and free from cracks and air bubbles.

Dadih and *dahi* are Indonesian and Indian yogurts, respectively, which seem to share the same root word. The body and texture of yogurt depend largely on the composition of milk employed in its manufacture, whereas the manufacture of *dadih* and *dahi* is simpler than Western-type yogurt, without any starter cultures involved (Surono and Hosono 2011). *Dadih* is served at weddings and during inauguration of an honorable title "Datuk" in West Sumatra during the ethnic tradition or "adat" ceremony. Generally, *dadih* is consumed during breakfast with rice after adding sliced shallot and chili (*sambal*), or it is mixed with palm sugar and coconut milk, being served as a topping of steamed traditional glutinous rice flakes, a corn flake-like product, called *ampiang dadih*.

14.10.1.1 Manufacturing of *Dadih*

The manufacturing method of *dadih* is quite similar to the *dahi* of India, except for the heat treatment of raw milk and the starter cultures being incorporated. In *dahi* making, the raw cow or buffalo milk, or a combination of both, is pasteurized and then fermented using leftover *dahi* from the previous lot as starter cultures (Indian Standard Institution 1980). In Indonesia, *dadih* is a homemade product by the traditional way, involving the milk of water buffaloes without any heat application to buffalo milk while manufacturing. The milk is neither boiled nor inoculated with any starter culture. The fresh unheated buffalo milk is placed in bamboo tubes covered with banana leaves, incubated at the ambient temperature (28–30 °C) overnight, and allowed to ferment naturally until it acquires a thick consistency (Akuzawa and Surono 2002).

14.10.1.2 Important Lactic Acid Bacteria During Fermentation

The buffalo milk was poured into bamboo tubes and kept overnight at room temperature, stimulating the mesophilic indigenous LAB derived from the fresh raw milk to dominate and grow, allowing natural fermentation. Consequently, the fermentation of *dadih* is much longer than yogurt, 24 and 4 h, respectively, due to different types of LAB involved in the fermentation process at the incubation temperature, 28–30 °C and 45 °C, involving mesophilic cultures and thermophilic cultures, respectively, besides thicker consistency of *dadih*. Bamboo tube is hygroscopic and aided in keeping the product from wheying off.

The milk is fermented by indigenous LAB of the buffalo milk. Its natural fermentation provides different strains of indigenous lactic bacteria involved in each fermentation (Akuzawa and Surono 2002). The natural indigenous LAB observed in *dadih* could be derived from the bamboo tubes, buffalo milk, or banana leaves involved in milk fermentation, and buffalo milk has been observed to contribute the most, while bamboo tubes, banana leaves, and personal hygiene practice may also contribute.

Various indigenous lactic acid bacteria (LAB) involved in the *dadih* fermentation may vary from time to time, from one place to another due to the natural fermentation without any starter culture involved (Surono 2000; Akuzawa and Surono 2002). Interestingly, with minimum hygiene practice implemented, there was no product failure and no food poisoning reported among people consuming *dadih*. Instead, the older generation believes that consuming *dadih* may provide a beneficial effect to their health. Some *dadih* LAB have antimutagenicity, hypocholesterolemic properties, anti-pathogenic properties, and immunomodulatory properties (Surono and Hosono 1996a, b; Pato et al. 2004; Surono et al. 2011).

Hosono et al. (1989) reported that *Leu. paramesenteroides* predominates in *dadih* fermentation, responsible for producing aromatic compounds such as diacetyl, acetic acid, and other volatile compounds. Surono and Nurani (2001) found that *Lactobacillus* sp., *Lactococcus* sp., and *Leuconostoc* sp. were dominant in *dadih*. Surono (2003b) reported that among 20 colonies of *dadih* LAB isolated from Bukittinggi, West Sumatra, five strains were identified as *Lac. lactis* subsp. *lactis*, three strains of *Lb. brevis*, and three each of *Lb. plantarum*, *Lb. casei*, *Lb. paracasei*, and *Leu. mesenteroides*. Fresh *dadih* contains 4.3×10^8 cfu/g, dominated by lactic acid bacteria, which was 4.0×10^8 cfu/g. *Lc. cremoris*, *Lc. lactis*, *Lb. casei* subsp. *casei*, and *Lb. casei* subsp. *rhamnosus* were also found. Several strains belonging to *Ent. faecalis* subsp. *liquefaciens* were also found in *dadih*, indicating that the way of manufacturing dadih did not implement good hygiene practices, since microbes belong to the *Enterococci* group (Hosono et al. 1990). Surono and Nurani (2001) reported that the total viable lactic acid bacteria count was in the range of 1.42×10^8–3.80×10^8 cfu/g in *dadih* originated from Bukittinggi and Padang Panjang area of West Sumatra.

Diverse microbes have been observed to involve in *dadih* fermentation due to traditional way of *dadih* manufacture. Surono et al. (1983) reported the involvement of yeast-like fungi at

1.1×10^7 cfu/g, identified as *Endomyces lactis*, which is commonly found in dairy products. Imai et al. (1987) reported that the major bacterial species responsible for *dadih* fermentation were *Lb. casei* subsp. *casei* and *Lb. plantarum*. Microbial isolates of *dadih* have also been reported to exhibit probiotic attributes.

14.10.1.3 Biochemical Changes During Buffalo Milk Fermentation

A consortium of LAB, which could be homofermentative and heterofermentative natural starter cultures producing lactic acid, with the involvement of beta-galactosidase from lactic starter cultures, results in coagulation of buffalo milk beginning at pH below 5.0 and completing at 4.6 (Surono (2003a). Texture, body, and acid flavor of *dadih* owe their origin to lactic acid produced during fermentation.

Small quantities of flavor compounds are generated through carbohydrate catabolism, via volatile fatty acids, ethanol, acetoin, acetic acid, butanone, diacetyl, and acetaldehyde. Homolactic starter cultures in *dadih* such as lactobacilli, lactococci, pediococci, and streptococci yield lactic acid as 95 % of the fermentation output. Heterolactic starter cultures, such as *Lb. brevis*, *Lb. fermentum*, and *Leuconostoc* sp., contribute to flavor compounds. There are two important roles of lactic acid in *dadih* manufacture, which helps to destabilize the casein micelles and gives the *dadih* its distinctive and characteristic sharp acidic taste.

During fermentation, natural LAB multiply viable counts of 10^5–10^9 cfu/g (Judoamidjojo et al. 1983; Hosono et al. 1989; Surono and Nurani 2001) and occupy about 1 % volume of *dadih* product. These LAB cells contain cell walls, enzymes, nucleic acids, cellular proteins, lipids, and carbohydrates. Beta-galactosidase activity contributes a major conversion of lactose into LAB in *dadih*, which is beneficial for lactase-deficient people.

14.10.2 Probiotic Bacteria Isolated from *Dadih*

Dadih and several dairy products have been reported to consist of probiotic bacteria, which when consumed alive and in an adequate amount confer health benefit to the host (FAO/WHO 2002). The older generation believes that consuming *dadih* may provide a beneficial effect to their health. This fact has inspired more exploration of the powerful indigenous LAB involved during dadih fermentation, excluding the contaminants and the pathogens from the milk itself as well as environmental surroundings.

Collado et al. (2007a) reported that all the five strains of *dadih* origin showed good adhesion property, and the most adhesive was *Lb. plantarum* strain IS-10506. All LAB strains isolated from *dadih*-fermented milk were able to significantly reduce the adhesion levels of all the pathogens tested. *Lb. plantarum* IS-10506 and *Ent. faecium* IS-27526 had the highest inhibition abilities. The inhibitory, competitive, and displacing properties against pathogens were also observed. Hence, the two strains are promising candidates for future probiotics.

Furthermore, Surono et al. (2010) reported a significant increase of viable fecal LAB of rats after 3 days of administration with *Lb. plantarum* IS-10506 and *Lb. plantarum* IS-20506 at 1.2×10^{10}–1.6×10^{10} cfu/g each, by 3.25–3.5 and 0.35–0.65 log cycles, respectively, and continued the increment after 7 days, by 1.8–2.0 and 2.1–2.3 log cycles, respectively. The abilities of *dadih* LAB isolates in detoxifying mutagens (Hosono et al. 1990; Surono and Hosono 1996a, b) and cyanobacterial toxins have been reported. The mutagen absorbed and bound to the cell wall, while the cyanobacterial toxin was being metabolized (Surono et al. 2008, 2009; Nybom et al. 2008).

Many researchers reported hypocholesterolemic activity of *dadih*. Hosono and Tono-oka (1995) reported that *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* R-43 and R-22 of dadih origin showed high-cholesterol-binding abilities, 33.91 % and 29.73 %, respectively. Surono (2003b) reported that *Lc. lactis* subsp. *lactis* strain IS-10285 and IS-29862 possess taurocholate-deconjugating abilities. Pato et al. (2004) found that rats fed with fermented milk made from *Lc. Lactis* subsp. *lactis* strain IS-10285 showed significant ($p<0.05$) lower total bile acids in serum. All these attributes show *dadih* as potential health-benefiting product. The probiotic properties of several strains isolated from *dadih* may provide the evidence on how strong the indigenous LAB derived from the fresh raw buffalo milk are in combating the contaminants, both spoilage bacteria and pathogens, during the spontaneous fermentation of *dadih* (Collado et al. 2007b).

Surono et al. (2011) reported significantly increased total salivary secretory IgA (sIgA) level and bodyweight of children ($p<0.05$) compared to placebo in a pilot randomized controlled trial on *Ent. faecium* IS-27526 isolated from *dadih* on children supplemented with lyophilized *Ent. faecium* IS-27526 (2.31×10^8 cfu/g/) in 125 ml ultrahigh-temperature low-fat milk for 90 days. Changes of total salivary sIgA levels were significantly higher in underweight children supplemented with probiotic, while weight gain was observed significantly in children with normal bodyweight supplemented with probiotic.

In a 90-day randomized double-blind placebo-controlled pre-post trial , Surono et al. (2014) has been conducted on Indonesian children aged 12–24 months supplemented with microencapsulated *Lb. plantarum* IS-10506 of *dadih* origin, at 10^{10} cfu/g as probiotic, and 20 mg zinc sulfate monohydrate (8 mg zinc elemental) showed significant increase of fecal sIgA in probiotic group ($p<0.01$), and in probiotic and zinc group ($p<0.027$), as compared to placebo group. Changes of serum zinc concentration in the combination of probiotic and zinc group showed the highest elevation after supplementation. Supplementing probiotic *Lb. plantarum* IS-10506 and zinc for 90 days resulted in a significant increase of humoral immune response as well as improved zinc status of the young children (Surono et al. 2014).

14.11 Fermented Fish, Meat, and Egg Products

14.11.1 *Ikan Peda* (Fish Pickled with Salt)

Lightly salted fermented fish are mostly produced in Southeast Asia (Ishige 1993). Fish salted and packed in Thailand and then shipped to Malaysia and Indonesia accidentally underwent fermentation because the fish were still not completely dried. These fish developed a distinct flavor upon arriving in Indonesia and were named *pedah Siam* (Van Veen 1965).

Ikan peda (Fig. 14.17) is a wet fermented fish made from mackerel (*Scomber kanagurta*) or *Kembung* fish (*Rastrelliger neglectus*) mixed with 20–30 % salt fermented in two steps. In the primary fermentation, the eviscerated fish are fermented with high salt concentration (25 %, w/w) in a vessel and in sealed container for 3 days. The fish are then washed, drained, and piled in wooden boxes, putting banana leaves between the fish; then they are sprinkled with 30 % salt, covered with banana leaves, and fermented for another 1 week or longer, and an aroma characteristic of *ikan peda* is developed. Then the *ikan peda* is dried in the air (Putro 1993).

Rahayu (2003) isolated *Lb. plantarum*, *Lb. curvatus*, *Lb. murinus*, and *Strep. thermophilus*

Fig. 14.17 *Ikan peda*

from *ikan peda*. During fermentation, enzymes derived from the fish and halophilic bacteria, including lactic acid bacteria, are involved which produce lactic acid and preserved the fish. According to Van Veen (1965), the best *ikan peda* has moisture content of 44–47 %, 7–14 % fat, 21–22 % protein, and 15–17 % NaCl.

Ikan peda is described as being fatty, partly dried salty fish with reddish brown color, moist and slightly pasty with a flabby texture, and a specific flavor, which is cheesy, tasty, salty, and often mixed with mild rancid flavor. Prolonged storage during retailing will facilitate the development of rancid flavor accompanied by a change in meat color to reddish brown (Van Veen 1965; Hanafiah 1987).

14.11.2 *Terasi* (Shrimp Paste)

Terasi is one of the salty fermented products that undergo alkaline fermentation, made from fish and/or shrimp which is in the form of paste (Fig. 14.18). *Terasi* and *tauco* serve similar roles as condiments due to the presence of glutamic acid and its specific flavor.

Terasi is an indigenous fermented food in Indonesia, salted fish or shrimp, mixed with 2–5 % salt, dried repeatedly, ground into a fine paste, and allowed to ferment naturally for a period of several weeks until the desired flavor has developed, followed by sun-drying for 1–3 days. It has a flavor reminiscence of ripe cheese. Closely related products are the Filipino *Bagoong*, Burmese *Ngapi*, Malaysian *Belacan*, and Thai *Kappi*. The most important center of its manufacture is Bagansiapiapi, North Sumatra (Surono and Hosono 1994a).

14.11.2.1 Manufacturing *Terasi*

Terasi in general is prepared as follows: The shrimp or fish are mixed with salt at10 % level on the fishing boats and then spread out on the floor. Further salt is added at 5 %, and the product is dried in the sun for about 1–3 days, occasionally turned over to decrease the moisture content from about 80 % to 50 % and also to minimize off flavor. The resulting mass is minced, pressed tightly into wooden tubs to exclude the air and allow to ferment 1–4 weeks (Surono and Hosono 1994a).

14.11.2.2 Chemical Composition of *Terasi*

The protein content of *terasi* was 25.42 g/100 g, with glutamic acid as dominant amino acid, 17.73 g/100 g. The pH of *terasi* was 7.53, with a salt content of 16.75 g/100 g, a fat content of 6.11 g/100 g, and a carbohydrate content of 1.94 g/100 g. Its moisture content was 37.41 % (Surono and Hosono 1994a).

14.11.2.3 Microbial and Biochemical Changes of *Terasi*

Surono and Hosono (1994a) reported that acid-producing *Bacillus* sp. and *Pseudomonas* sp. were dominating the microbial population involved during *terasi* fermentation. On the other hand, *Kurthia gibsonii* and *Sporolactobacillus*

Fig. 14.18 (**a**) *Terasi* fermentation, (**b**) *Terasi* in block

inulinus represented minority of aerobic bacteria population in terasi, while *Micrococcus* sp. had the ability to utilize protein but poor in utilizing carbohydrate.

According to Surono and Hosono (1994a), during the early stage of *terasi* fermentation, the pH drops to 4.5 and most *Pseudomonas* sp. failed to grow. Since *terasi* are manufactured without any concern on good hygiene practice, *Pseudomonas* sp. may represent contaminants during mixing, drying, and packaging of terasi. This kind of bacteria grows well in proteinaceous food and actively spoils seafoods.

In the presence of 10 % salt, during fermentation, the species *Bacillus*, *Pediococcus*, *Lactobacillus*, *Micrococcus*, *Sarcina*, *Staphylococcus*, *Clostridium*, *Brevibacterium*, *Flavobacterium*, and *Corynebacterium* were decreased. At the beginning of terasi fermentation, there was an increase in total bacteria dominated by lactic acid bacteria, micrococci, and bacilli, which decrease at the end of fermentation. The endogenous enzymes from *B. subtilis* and *B. coagulans* together with the enzymes derived from intestines of the fish hydrolyzed the protein (Surono and Hosono 1994b). The bacterial enzymes were mainly responsible for the deamination and decarboxylation of amino acids to form lower fatty acids and amides, producing characteristic flavor of *terasi*. The combination of salt and the microbial degradation products of protein, fat, and carbohydrate may contribute to the taste and aroma of the *terasi* (Aryanta 2000).

The endogenous proteolytic enzymes, rather than bacteria, are responsible for the hydrolysis of fish muscle, prior to the bacterial activity. Furthermore, the halophiles could possibly hydrolyze short-chain fatty acids; hence, the presence of esterase (C4) and esterase lipase (C8) activities in all of the bacteria presented in *terasi* starter supports the hypothesis that bacterial enzymes hydrolyze the fat, producing low-molecular-weight fatty acids that are responsible for the cheesy odor Surono and Hosono (1994b).

The total halophilic count of *terasi* was 1.1×10^5 cfu/g, dominated by halophilic *Bacillus* sp. *B. pumilus* is the dominant species throughout the fermentation. Other bacteria responsible for early stage of fermentation were *B. coagulans*, *B. megaterium, and B. subtilis*, while in the later stage of fermentation, *B. licheniformis*, *M. colpogenes*, *M. roseus*, *M. varians*, and *Staphylococcus* sp. were found in *terasi* (Surono and Hosono 1994a).

Surono and Hosono (1994a) reported that *terasi* starter was composed of *B. brevis*, *B. pumilus*, *B. megaterium*, *B. coagulans*, *B. subtilis*, and *M. kristinae* in the proportion of 39.1 %, 26.1 %, 8.7 %, 8.7 %, 8.7 %, and 8.7 %, respectively. All of the microflora found in *terasi starter* were salt tolerant, as shown by their capability to grow on agar plates in the presence of 10 % NaCl. The dominant flora were *Bacillus* sp., which are halophilic and aerobic, grow in the temperature range of 10–50 °C, and have esterase (C4) and esterase lipase (C8) activities. None of the isolates had the ability to produce gas from glucose, even though most of the isolates could produce acid from glucose (Surono and Hosono 1994a). Only *B. pumilus*, *B. coagulans*, and *M. kristinae* had the ability to hydrolyze long-chain fatty acids. *Terasi* has a typically characteristic aroma of cheese and ammonia. The cheesy odor is produced by low-molecular-weight fatty acids, and the ammoniacal odor is due to the presence of amines and ammonia (Dougan and Howard 1975).

T. muriaticus strain was found and reported to produce histamine in fermented fish in Japan and Thailand. Viable cell lactic acid bacterial counts in *terasi* were 10^4–10^6 cfu/g. All the isolates were catalase negative, were Gram-positive cocci, and were able to grow at 15 % NaCl and classified into two types: the *Tetragenococcus halophilus* group and the *T. muriaticus* group as revealed by a restriction fragment length polymorphism (RFLP) analysis and sequencing of the 16S rRNA gene (Kobayashi et al. 2000, 2003).

14.11.3 *Urutan* (Traditional Balinese Pork Sausage)

Urutan (Fig. 14.19) is usually prepared to celebrate the Galungan Day, which is a special holy day for Hindus in Bali. A day before the feast, Balinese people slaughter pigs to prepare tradi-

Fig. 14.19 *Urutan*, fermented pork sausage (Picture courtesy of Prof. Nyoman Semadi Antara)

tional foods. Due to the excessive fresh pork available, it needs to be preserved to prolong the shelf life of the meat which can then be consumed on the following days or weeks. Two common Balinese practices to preserve the pork meat are by drying *dendeng* and fermenting *urutan*.

Urutan is a Balinese traditional fermented sausage, which is made of chopped lean pork and fat which are mixed with spices (garlic, turmeric, aromatic ginger, chili, and pepper), sugar, and salt. The mixture is filled into cleaned pig intestine and fermented spontaneously and then sun-dried for 2–5 days (Aryanta 2000).

Urutan has a different microbial ecology, compared with other fermented sausages, and this is primarily due to spontaneous fermentation which occurs during the drying process, and the quality of the product varies from time to time, from place to place. The use of spices and high-temperature fermentation is of special interest in relation to the characteristics of lactic acid bacteria involved in the process, and their distribution plays an important role in food fermentation, responsible for the characteristic flavor development, and contributes to the preservation of the fermented product. The pork meat inside the casing became more compact and solid after fermentation. Traditionally, *urutan* was manufactured without any addition of nitrite and/or nitrate, and the color became dark red at the end of fermentation. The products were dried under the sun, and the juice (mixture of oil and water) was dripped so that the casing becomes dry and wrinkled (Antara et al. 2002).

14.11.3.1 Microbial and Biochemical Changes During Urutan Fermentation

The total viable lactic acid bacterial counts were remarkably increased from the initial count of 1.72×10^5–1.84×10^8 cfu/g on day 1 and then decreased to 4.98×10^7 cfu/g at days 2–5 (Antara et al. 2002). The results also showed that lactic acid bacteria predominated the total microbial population. The sharp reduction of pH on the first day of fermentation affected the growth of Enterobacteriaceae, which were not detected at day 2 of fermentation. Soluble protein decreased at the first day of fermentation. Total acidity increased significantly after day 1 of fermentation, and it remained constant until the end of the process. The carbon sources in the product primarily sugar and spices were utilized by homofermentative lactic acid bacteria and converted into lactic acid as the lactic acid bacteria grow and multiply during fermentation. There was no butyric acid detected, and only small amounts of succinic and propionic acids were produced (Antara et al. 2004).

Antara et al. (2002) found that in *urutan*, 77.5 % of bacteria are lactobacilli, and the other 22.5 % are pediococci. Further molecular identification by 16S rDNA sequence revealed that *Lb. plantarum*, *Lb. farciminis*, and obligate heterofermentative lactobacilli *Lb. fermentum* and *Lb. hilgardii*. Besides, *P. acidilactici* and *P. pentosaceus* were also detected (Antara et al. 2002). Microbial succession occurred during fermentation of *urutan*, dominated at initial growth by *Lb. plantarum*, predominated during 2 days of fermentation, then followed by *P. acidilactici*, and finally, *Lb.*

farciminis was found to be predominant at the last stage of fermentation (Antara et al. 2004).

P. acidilactici was found after 2 days and slightly decreased until the end of fermentation. On the other hand, *Lb. farciminis* was distributed in an increasing manner from day 2 until the end of fermentation process. The heterofermentative lactobacilli (*Lb. fermentum* and *Lb. hilgardii*) were not significantly detected during the whole fermentation, only detected in small number on the third and fifth days, respectively (Antara et al. 2004). Most of lactic acid bacteria present in *urutan* during fermentation were identified as the homofermentative type, which produced mostly lactic acid and a small amount of acetic acid as well as carbon dioxide (Aryanta 1998).

The rapid growth of LAB during the first day of fermentation of urutan is in agreement with those reported for salami; the LAB in this product decreased after 2 days of fermentation and remained constant until the end of the process (Antara et al. 2002), in contrast to the LAB growth in salami whereby the LAB population starts to increase on the first day, reaching a maximum of 7 days, and remains constant until the end of the ripening period (Coppola et al. 1998, 2000). The difference of LAB growth in urutan might be due to the use of local types of spices, especially aromatic ginger and turmeric, and the high concentration of garlic suppressed the growth of LAB in *urutan* (Antara et al. 2002).

Lb. plantarum dominating the total lactic acid bacterial population during fermentation makes *urutan* different from salami where *Lb. sake* and *Lb. curvatus* were present as the dominant species (Coppola et al. 2000). The presence of *P. acidilactici* in *urutan*, which was also distributed significantly, could be due to the high fermentation temperature. This species is widely used for rapid fermentation with temperature above 30 °C (Ordonez et al. 1999). The existence of *P. acidilactici* as bacteriocin producer plays an important role for the succession. The bacteriocin produced in this product inhibited the growth of *Lb. plantarum and P. pentosaceus*, but the growth of *Lb. farciminis* was stimulated by the intrinsic condition of urutan after 3 days until the end of fermentation.

The heterofermentative lactobacilli were only existent at the final stage of fermentation as shown by the lactic acid produced, and the absence of gas in *urutan* indicated that the role of the heterofermentative lactobacilli was not significant since, as reported, the heterofermentative lactobacilli (*Lb. fermentum* and *Lb. hilgardii*) were not significantly detected during the whole fermentation. The existence of these species was only in small number on the third and fifth days, respectively (Antara et al. 2002).

The denaturation of protein and the absence of bubbles from CO_2 made the texture of *urutan* more compact and solid (Antara et al. 2002). Inconsistent of quality and risk of fermentation failure are the common problems on traditional fermented products which are produced under spontaneous natural fermentation indicating off-odor development. Antara et al. (2004) reported that at pH 4.3–4.5, urutan (Balinese dry fermented sausage) using mixed LAB as the starter culture might inhibit the growth of Clostridia.

14.11.3.2 *Telur Asin* (Salted egg)

Telur asin (salted egg) is an alkaline-fermented ethnic food in Indonesia (Fig. 14.20). Alkaline-fermented foods constitute a group of less-known food products that are widely consumed in Southeast Asia and African countries. Traditional processing of *telur asin* is carried out by coating method. The fresh duck eggs are coated with a muddy paste containing ash or red brick powder and salt at 1:1, allowed to ferment in a jar for 15–20 days, and the telur asin can be preserved for 2–3 weeks (Margono et al. 2000). Suprapti (2002) reported that the use of ash as coating agent will produce *telur asin* with pale yellow and grayish surrounding the yolk, while the use of red brick powder produces *telur asin* with reddish yolk.

In alkaline-fermented foods, the protein of the raw materials is broken down into amino acids and peptides; ammonia is released during the fermentation, raising the pH of the final products and giving the food a strong ammoniacal smell in spontaneous fermentation by mixed bacteria cultures.

Fig. 14.20 Boiled *telur asin*, *telur asin* covered with muddy ash and salt, smoked *telur asin*

Saputra (2013) reported that *Lb. plantarum*, *Lb. casei* subsp. *rhamnosus*, *Enterococcus gallinarum*, and *P. acidilactici* have been isolated from *telur asin* and inhibited the pathogen, especially *E. coli and Staph aureus by organic acids produced and* bacteriocin produced by *P. acidilactici. Telur asin* may also be smoked, producing rich unique flavor of salty and smoked taste.

14.12 Fermented Roots and Tuber Products

14.12.1 *Tapé*, *Growol*, and *Gatot*

Tapé is a sweet and sour fermented food with an alcoholic flavor, prepared from glutinous rice or cassava or other cereals by using *ragi starter* in Indonesia (Campbell-Platt 1994). It is eaten as dessert or delicacy in Indonesia. There are various starchy substrates used to prepare *tapé*, such as cassava (*tapé singkong*), glutinous rice (*tapé ketan*), maize (*tapé jagung*), and millet (*tapé cantel*). The raw material is washed, soaked, steamed, cooled to room temperature on a woven bamboo tray, sprinkled with *ragi* powder, packed in small banana leaves, and fermented for 2–3 days at room temperature, and a soft juicy mass of *tapé* is produced (Saono et al. 1977). *Tapé singkong* is deep-fried in coconut oil and baked before consumption. The alcohol content ranged from 3 % to 8.5 % v/v (Cronk et al. 1977). The glutinous rice lipids are hydrolyzed during *tapé ketan* fermentation (Cronk et al. 1979).

Tape is a traditional fermented food in Indonesia and also in Malaysia, the Philippines, and Vietnam, made from raw material containing starch and *ragi*, the microbial starter. *Ragi* is a mixture of rice flour, spices, sugar cage, water, and yeasts and used as inoculums in alcoholic fermentation in natural fermentation. Aryanta (1988) isolated *Rz. oryzae*, *M. rouxii*, *Asp. oryzae*, *S. cerevisiae*, *E. burtonii*, *H. anomala*, and *P. pentosaceus* from *ragi* (NKL brand) obtained from Denpasar. On the other hand, Winarni (1988) isolated some amylolytic molds and yeasts from four types of local commercial *ragi. P. pentosaceus* is the acid producer commonly found in ragi and most abundantly isolated from newly collected ragi samples in Indonesia, followed by a small number of *Lb. plantarum* and *Lb. fermentum* strains (Uchimura et al. 1998).

Tape singkong (Fig. 14.21) is a fermented steam cassava by *ragi tape* starter, involving *Amylomyces rouxii* and *E. burtonii*, *S. cerevisiae*, *Rhizopus* sp., and *Hansenula* sp. (Steinkraus 1983; Hassan et al. 1986; Suliantari and Rahayu 1990).

Fig. 14.21 *Tape singkong* (fermented cassava)

The fungi *Rz. arrhizus* var. *rouxii* strain TT is able to utilize starch and convert it to alcohol, producing soft, juicy, sweet tape which lack sourness, and 1.88 % (w/v) ethanol was produced after 72 h of fermentation (Hassan et al. 1986). The yeast is inoculated together with the fungi to be able to do the fermentation, while inoculating yeast only, the fermentation does not occur.

14.12.1.1 Biochemical Changes

The fermentation process of *tape singkong* has two main stages: the conversion of starch into simple sugars carried out by amylase producers (molds and yeasts) and the conversion of sugars into alcohol and acids carried out by certain yeasts.

Esters are also formed due to the reaction between alcohol and acids which contribute to the sweet–sour taste with a mild alcoholic flavor of the product (Djien 1972; Karim and Hassan 1986). The pH of *tape singkong* decreased from an initial value of 5.65–5.15 during the first 4 days of fermentation, while total acidity increased from 3.5 mg/100 g to 5.2 mg/100 g. During the first 4 days of fermentation, reducing sugar increased from 7.9 % to 16.0 % and decreased to 12.73 % after this time (Rahayu 1980).

Tape singkong is sweet, slightly sour, and aromatic, but too much acid content in *tape* is undesirable. The nutrient contents of *tape singkong* are as follows: 0.5 % protein, 0.1 % fat, 42.5 % carbohydrate, and 56 % moisture (Winarno 1983).

Mixed cultures of *Streptococcus*, *Rhizopus*, and *Saccharomycopsis* produce aroma in *tapé*, whereas *Sm. fibuligera* produces α-amylase and *Rhizopus* sp. produces glucoamylase (Suprianto et al. 1989).

Growol is fermented raw cassava tubers, whereas *gatot* is fermented dried cassava tubers involving lactic acid bacteria. These products are popular in certain part of Java. The manufacture of fermented tubers is by soaking the peeled raw cassava for growol and dried cassava for gatot for several days until the tubers become soft.

Growol is a traditional fermented food made from cassava which has sour taste, only found in Yogyakarta area, especially Kulon Progo and the surrounding area. The peeled and sliced cassava was soaked in the water for 4 days, drained, and crushed before being steamed.

The fermentation occurs naturally; *Coryneform*, *Streptococcus*, *Bacillus*, and *Actinobacteria* grow at the beginning of the fermentation followed by *Lactobacillus* and yeast until the end of fermentation. Suharni (1984) reported that lactic acid bacteria dominated and found at 1.64×10^8 cfu/g. *Lactobacillus casei* subsp. *rhamnosus* TGR2 isolated from *growol* produces extracellular metabolites which remain stable at room temperature and has resistance to heating at 98 °C for 30 min, at pH 3–8 (Rahayu 1995).

Gatot, dried cassava, is a traditional fermented food in Gunung Kidul, Yogyakarta. Cassava is peeled dried to get the typical black color of *gatot* as a staple food. Ichsyani (2014) reported that *P. pentosaceus* and *Saccharomyces* sp. *TR7* isolated from *gatot* have amylolytic activity and able to reduce cyanide in cassava. *Lb. plantarum* 250 Mut7 FNCC also has been isolated from *gatot* (Harmayani et al. 2001) and showed the ability in suppressing bacteroides (Sari 2014).

14.13 Conclusion

Traditional fermented foods are mostly carried out involving mixed cultures. The preparation and consumption of traditional foods are the traditional wisdom of the people in each area, which have fostered a distinct food culture of the people. Fermented foods are known to be not only nutritious but also healthy foods. The Indonesian biodiversity of microbes involved in the traditional fermentation which may vary from time to time, from place to place, may give opportunity to the exploration of the beneficial effects of the microbes, enzymes involved, and the metabolites as functional foods, such as probiotics, some enzymes in combating the metabolic syndrome or degenerative diseases, and even for producing ethanol as biofuel to support human welfare.

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Ethnic Fermented Foods and Beverages of Vietnam

15

Vu Nguyen Thanh and Nguyen Thi Viet Anh

15.1 Introduction

Vietnam is located on the eastern Indochina Peninsula and covers a total area of *ca.* 331,210 km^2. The country is bound by the South China Sea to the east and south, Laos and Cambodia to the west, and China to the north (Fig. 15.1). On the map, the country has the S-shape with distance of 1650 km from north to south and 50 km wide at the narrowest point. The northern part of the country consists mostly of highlands and the Red River Delta. The Annamite Range covered with tropical forest lies along the narrow middle part of the country and lefts over a small arable area of highland plateaus and coastline. The southern part is the lowland Mekong River Delta.

Because of its geography, the climate in Vietnam varies greatly from north to south. The northern regions have a humid subtropical climate. Because it is close to the Tropic of Cancer, winter in the northern part is cooler. The temperatures range from 5 °C in the winter to more than 37 °C in the summer. The climate in the southern regions is predominantly tropical savanna with high humidity and distinct wet and dry seasons. Seasonal temperatures vary only a few degrees, usually in the 21–28 °C range.

V.N. Thanh (✉) • N.T. Viet Anh
Food Industries Research Institute,
301 Nguyen Trai, Thanh Xuan, Hanoi, Vietnam
e-mail: thanh@firi.ac.vn

Vietnam is a multiethnic country with 54 distinct groups, each with its own language, lifestyle, and cultural heritage. In 2014, the total population of Vietnam was estimated at 90.7 million. Kinh (Viet) is the largest ethnic group which accounts for more than 86.8 % of the population and follows by Tay 2.0 %, Thai 1.8 %, Muong 1.5 %, Khmer 1.4 %, Chinese 1.1 %, Hmong 1 %, and others 4.2 %. With about 67 % of the population living in the rural area, Vietnam is still an agricultural country (GSOV 2015).

Similar to other Southeast Asian countries, Vietnam has long been a predominantly agricultural civilization based on wet rice cultivation. With production yield of 44.0 million tons per annum, Vietnam ranks among five world's largest rice producers (FAOStat 2013). Due to a relatively wide range of temperature, Vietnam has diverse variety of cultivars. Besides the cultivars characteristic for tropical climatic zone, relatively cold winter in the northern part of the country allows the cultivation of plant species more typical for the temperate zone. Livestock farming in Vietnam was limited in the past and the supply of meats and milk products was scant. With a dense network of river, lakes and a long coastline of 3444 km, aquaculture and fishery provide the population with fish, crustaceans, and mollusks as alternatives to meat and milk products. Besides, peanuts and beans are also widely used as the source of protein. The specificity of agriculture has a direct impact on the Vietnamese

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Fig. 15.1 Location of Vietnam among Southeast Asian countries

cuisine. Traditionally, typical meal of Vietnamese people consists of rice, vegetables, and fermented products derived from fish/shrimp.

Production of foods and beverages by means of traditional fermentation has long history and represents an important piece of human development. In the modern time, traditional fermentation does not lose the value and still occupies a special position in our daily life (Marshall and Mejaa-Lorao 2012). Traditional fermentation is a reliable way for food storage. The products have been proven through centuries for the safeness, organoleptic quality, nutritional values, and health benefits. Traditional products and methods of manufacturing are integrated part of our culture tradition, reflecting local and national identities. Traditional fermentation could be considered as technological and cultural heritage of each country.

Vietnam has long been an agricultural country with rice, corn, cassava, and soybean as the major commodities. With more than 3400 km of coastal line, fishery has been an important part of the country's economy. Locating in the tropics, the climate of Vietnam is hot, humid, and favorable for microbial growth. High microbial diversity and growth rate also means fast pace of agricultural and fishery product deterioration. Traditional fermentation at first instance was meant to preserve the nutritional values of agricultural products and latter to diversify food products and culinary ingredients. Alcohol fermentation of rice, lactic acid fermentation of vegetables and meat, and hydrolysis of plant and animal proteins are the main types of traditional fermentation in Vietnam. Some of the most famous products are rice liquor of Lang Van, Mau Son, and San Lung; soy sauce of Ban, Cu Da, and Nam Dan; fish sauce of Phu Quoc and Cat Hai; pickle shrimp of Hue; fermented meat of Thanh Hoa and Phu Tho; shrimp paste of Hau Loc; etc. These distinctive products and manufacturing techniques are of economic significance and local and national pride. However, traditional fermentation in Vietnam is not free of concerns. These types of foods and beverages are manufactured in small scale, mainly at household level in a poor hygiene condition. Production methods are relatively simple with little or no improvements over the years. Management and production procedures are undocumented and often based on personal experiences. With few exceptions, the fermentation processes are spontaneous and initiated by microorganisms from the substrate and/or from environment.

With 54 ethnic groups, each with own language and culture tradition, the diversity of traditional fermentation in Vietnam is immense. Understanding of the traditional fermentation processes is needed in order to preserve and to improve the technologies and traditions. From economic point of view, new product development based on traditional processes would have higher chance of being accepted while require less technological investment. Microorganisms associated with traditional fermentation processes are generally regarded as safe and could be utilized for different purposes beyond their initial applications. This chapter presents an overview of Vietnam traditional fermentation with emphasis on production techniques and microorganisms involved (Table 15.1).

15.2 Alcohol Fermentation of Rice and Starch-Containing Materials

In Vietnam, traditional alcohol fermentation of rice has centuries of history. Before the introduction of beer, this had been the main way of obtaining alcoholic drinks. Even now, traditional production of alcohol from rice still makes up 80 % of the total national distilled liquor production. A vast network of small breweries is producing alcohol across the country. There is at least one of such breweries in nearly every village. Besides, alcohol production can be the main activity of a whole village. Even if the definition of brand names and trademarks did not exist in Vietnamese culture, the liquors produced by villages with centuries of tradition such as Lang Van (Bac Giang), Dai Lam (Bac Ninh), Lang Ngau (Hanoi), Kim Son (Ninh Binh), Lang Voc (Ha Nam), Phu Le (Ben Tre), Bau Da (Binh Dinh), San Lung, Ban Pho (Lao Cai), Mau Son (Lang Son), and Go Den (Long An) have long been known for the taste and quality. Although alcohol can be produced from different starch-containing materials such as rice, corn, cassava, sweet potatoes, etc., rice is still the most common due to wide availability and acceptance. Being one of the world's largest rice producers and the countries where rice cultivation originated, Vietnam possesses a rich assortment of rice varieties and so the rice liquors produced from. Also, with 54 distinct ethnic groups, there is high variation in methods for alcohol production. Despite of the differences, breweries share the same production principle where starter (*banh men*) containing a relatively stable microbial community is used. *Banh men* provides microorganisms necessary for breaking down of starch and subsequent fermentation of the released monomers to ethanol. By loopback inoculation, *banh men* is passed from generation to generation and has become the cultural and technological heritage of the country.

Table 15.1 Ethnic fermented foods and beverages of Vietnam

Foods	Substrates	Nature and uses	Microorganisms	Regions	Reference
Banh men	Rice, herbs, inoculum	Starter culture for alcohol production	*Rhizopus oryzae* (=*Amylomyces rouxii*), *R. microsporus*, *Mucor indicus*, *M. circinelloides*, *Saccharomycopsis fibuligera*, *Saccharomyces cerevisiae*, *Issatchenkia orientalis*, *Pichia anomala*, *Candida tropicalis*, *P. ranongensis*, *Clavispora lusitaniae*, *Pediococcus pentosaceus*, *Lactobacillus plantarum*, *Lb. brevis*, *Weissella confusa*, and *W. paramesenteroides*	All regions	Lee and Fujio (1999), Dung et al. (2006), and Thanh et al. (2008)
Fish sauce	Fish, salt	Hydrolysis products of fish, liquid sauce	*Bacillus*, *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Lactobacillus*, *Clostridium*	All regions	Noguchi et al. (2004) and Uchida et al. (2004)
Shrimp paste	Shrimp, salt	Hydrolysis products of shrimp, semisolid product	*Bacillus*, LAB	All regions	Dinh et al. (2015)
Sour shrimp	Shrimp, rice, salt, spices	Lactic acid fermentation, partial hydrolysis of shrimp	LAB, *Bacillus*, *Clostridium*, coliforms	Hue	Nguyen Thi Viet Anh et al. (2010b)
Nem chua	Pork, sugar, garlic, spices	Lactic acid fermentation product of meat	*Lb. plantarum*, *Pediococcus pentosaceus*, *Lb. brevis*, *Lb. farciminis*, *C. haemulonii*, *C. halonitratophila*, *C. maltosa*, *C. parapsilosis*, *C. sake*	All regions	Le Thanh Mai et al. (2011) and Do Thi Thuy Le et al. (2014)
Tuong	Glutinous rice, soybean, salt, water	Microbial hydrolysis products of rice, soybean	*A. oryzae*, *B. subtilis*	Northern Vietnam	Nguyen Thi Viet Anh (2010)
Fermented vegetables	Vegetables, salt	Lactic acid fermentation of vegetables	*Lb. fermentum*, *Lb. pentosus*, *Lb. plantarum*, *Lb. brevis*, *Pd. pentosaceus*, *B. subtilis*	All regions	Inatsu et al. (2005) and Doan Thi Lam Nguyen et al. (2013)

15.2.1 Method for Production of Alcohol from Starch

The typical ethnic alcohol production process of Vietnam is presented in Fig. 15.2. Starch-containing material such as rice, corn, cassava, etc. is gelatinized by soaking in water and followed by cooking or steaming. The cooked starch material with moisture content of around 50–60 % is spread on tray or shallow basket to cool down. Powdered starter (*banh men*) is sprinkled over and mixed well. The amount of starter used is about 1 % of the initial starch material. The mixture is placed on top of a plastic sheet and kept with the thickness of around 10–20 cm, covered with cloth to ensure aeration. After about 2 days of incubation at an ambient temperature, fungal and yeast growth could be judged visually and by the aroma formation. The fermentation mixture becomes moisture, coherent, and ready for anaerobic fermentation. The fermentation mash is then transferred into a jar and an equal volume of water is added. The jar is covered with a plastic sheet and headspace is kept at minimum to reduce aeration. After 5–7 days of fermentation, when most of the starch is consumed judging by viscosity reduction and flotation of rice spent, the mash is ready for distillation. At this

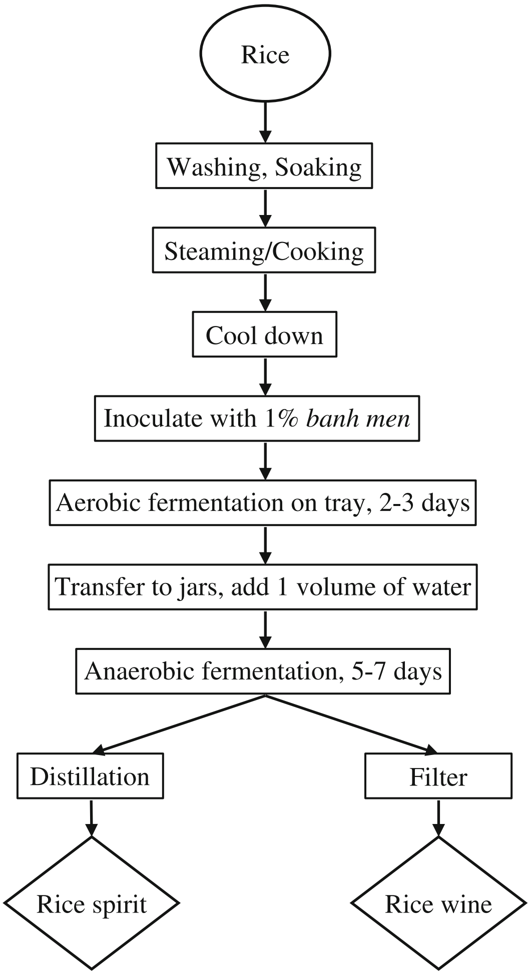


Fig. 15.2 Schematic presentation of typical technology for production of rice spirit and rice wine

moment, ethanol content in the broth reaches 10–12 % (v/v); the fermentation mash is acidic (pH 3.6–4.3) and with low residual glucose concentration (less than 1 %, typically) (Dung et al. 2007). The whole fermentation process is temperature dependent, and the operator decides the lengths of each step based on appearance and aroma. The optimum temperature for alcohol fermentation is around 28–32 °C. When fermentation is completed, the broth is transferred in to distillation bowl and heated slowly. Distillator made of bronze is of favorite choice. The upper part is sealed and connected with a tube that passes through a water tank for condensation. Traditional operators usually differentiate three distillation fractions. The first fraction having alcohol content of around 55–65 % (v/v) and with unpleasant flavor is not suitable for consumption. The second fraction containing 35–45 % (v/v) alcohol with mild and pleasant aroma is collected and stored. The last fraction having sour taste and low alcohol content is usually combined with the first fraction for redistillation. Rice spirit after distillation is usually stored in closed vessels for several months to obtain the best quality. A thin film of polymerized matters may be formed on the surface is discarded. Longer storage is not typical. For production of rice wine, the fermentation mash is homogenized mechanically and fortified with alcohol to avoid acidification. Rice wine can be obtained directly in the form of milky suspension or as a clear liquid after clarification and filtration.

15.2.2 Alcohol Fermentation Starter (*Banh Men*)

The use of alcohol fermentation starter (*banh men*) in Vietnam is quite different from the use of *koji* in Japan or *daqu* in China. *Banh men* serves as microbial inoculum rather than the source of enzymes as in the case of *koji* and *daqu*. The amount of *banh men* inoculum is usually small comparing with the fermentation mash (about 1 %). The quality and the yield of rice alcohol are predicated by the quality of *banh men*. It is often believed that mixing of different types of *banh men* would improve fermentation performance. *Banh men* is made from uncooked dough of rice, water, and a variety of herbs and spices. This dough is inoculated with starter from the previous batch and shaped into small balls or flattened tablets, which are incubated at around 30 °C for about a week. During this period, the niche microflora develops and, simultaneously, the tablets dehydrate. These tablets can be stored at ambient temperatures without significant loss of viability for at least 6 months (Aidoo et al. 2006). The outline of the preparation method for *banh men* is presented in Fig. 15.3.

For dough preparation, wet milling is often used and believed to be better than dough

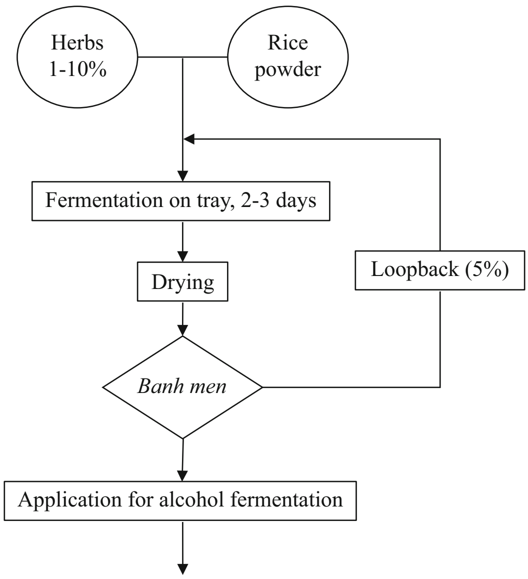


Fig. 15.3 Schematic presentation of typical *banh men* starter production technology

obtained from dry rice powder. The recipes for herb and spice ingredient are highly secretive and vary among breweries. They may contain up to 46 different plant species. One of the available recipes is presented in Table 15.2. The role of oriental herbs and spices in *banh men* is often assumed to suppress unwanted microorganisms and contribute to the organoleptic quality of rice spirit (Hesseltine et al. 1985; Dung et al. 2006). Taken that most of the herbs used in *banh men* are rich in terpenoid compounds as well as the quantity applied is sufficiently high (1–10 %), the assumption on microbial suppression is most likely credible (Nguyễn Văn Hiệu 1992). The secrecy concerning herbal ingredient might due to the intention of keeping the uniqueness of the produced goods among breweries rather than advantage in fermentation efficiency. Until now, there is no direct evidence for the selective pressure of oriental herbs on the microbiota of *banh men* as well as its contribution to the taste and aroma of the final product. In mountainous area, instead of herbs, plant leaves rich in essential oils are used for starter production. The type of starter is referred as *men la*. The role of plant leaves in *men la* is most likely similar to the herbs in *banh men*. Besides the herb/leaf variation, there are no significant differences in the microbial compositions between two types of starter (author's (VNT) observation).

As mentioned above, rice dough containing herbs and *banh men* inoculum is made into small balls or flatten cakes of 3–5 cm in diameters and placed on tray covered with rice husk (Fig. 15.3). The rice husk is not the source of microbes but to keep the dough not to stick to the tray and to ensure aeration and heat exchange. The trays are usually kept on top of each other with good distances to avoid overheating (as a result of microbial growth and biochemical reactions). This makes *banh men* different from Chinese *daqu*, where accumulated heat could reach to 60 °C, causing detrimental effect on mesophillic microorganisms (Zheng et al. 2014). The time span for microbial growth in *banh men* tablets is short since the substrate is dried out after 2–3 days. *Banh men* of good quality has a soft, porous inner part and covered by a thin light yellow-brownish "skin" with light aerial growth of mucoraceous fungi. Under microscope one can observe yeast cells, fungi mainly in the form of chlamydospores, and intact starch particles (author's (VNT) observation). The microbial load of *banh men* is not high. Typically, each gram of *banh men* contains 10^3–10^6 cfu/g of mold, 10^6–10^7 cfu/g of yeast, and 10^3–10^6 cfu/g of lactic acid bacteria (Lee and Fujio 1999; Dung et al. 2007).

15.2.3 Microbiology of Alcohol Fermentation from Starch

Banh men contains a complex and sustainable microbiota. It is generally accepted that the microbiota consists of starch degraders, such as *Amylomyces rouxii*, *Rhizopus oryzae*, *Mucor indicus*, *M. circinelloides*, *Saccharomycopsis fibuligera*, *Hyphopichia burtonii*, and alcohol fermenters, mainly *Saccharomyces cerevisiae*, *Pichia anomala*, and *Candida* spp. Bacteria are often regarded as contaminants and do not contribute significant role to the fermentation process (Lee and Fujio 1999). The list of microorganisms associated with *banh men* is summarized in Table 15.3. A typical image of

Table 15.2 List of herb species used for *banh men* and amount used

Plant species	Vietnamese name	Common name, plant part	Ratio
Cinnamomum loureiroi	Quế chi	Saigon cinnamon bark	2.5
Illicium verum	Hoa hồi	Star anise fruits	2.5
Amomum tsaoko	Thảo quả	Cao guo fruits	2.5
Asarum heterotropoides	Tế tân	Wild ginger (whole plant)	1.5
Eugenia caryophyllata	Đinh hương	Clove flowers	0.3
Elettaria cardamomum	Đậu khấu	Green cardamom fruits	1.2
Kaempferia galanga	Địa liền	Aromatic ginger rhizome	1.5
Homalomena occulta	Thiên niên kiện	*Homalomena* rhizome	1.0
Acorus gramineus	Thạch xương bồ	*Acorus gramineus* rhizome	0.6
Cimicifuga foetida	Thăng ma	*Cimicifuga foetida* rhizome	6.5
Glycyrrhiza uralensis	Cam thảo	Chinese licorice root and stolons	0.3
Ocimum gratissimum	Hương nhu	Clove basil (whole plant except roots)	0.2
Angelica dahurica	Bạch chỉ	*Angelica dahurica* root	0.4
Ligusticum wallichii	Xuyên khung	*Ligusticum wallichii* rhizome	0.2
Capsicum frutescens	Rễ ớt	Chili root	0.5
Alpinia officinarum	Lương khương	*Alpinia officinarum* rhizome	1.0
Cymbopogon citratus	Củ sả	Lemongrass	0.3

Nguyễn Văn Hiệu (1992)

Table 15.3 Microorganisms involved in production of alcohol from starch materials

Microorganisms	Source	Note
R. oryzae, *M. indicus*, *M. circinelloides*, *A. rouxii*, *S. cerevisiae*, *Hyphopichia burtonii*, *Sm. fibuligera*, *P. anomala*, and *Candida* sp.	Lee and Fujio (1999)	Based on 12 *banh men* samples; bacteria were not studied; identification based on morphological and physiological properties
A. rouxii, *A.* aff. *rouxii*, *R. oryzae*, *R. oligosporus*, *S. cerevisiae*, *C. glabrata*, *P. anomala*, lactic acid bacteria	Dung et al. (2006)	Identification of representative strains of technological interest
A. rouxii, *R. oryzae*, *R. microsporus*, *Absidia corymbifera*, *Sm. fibuligera*, *S. cerevisiae*, *I. orientalis*, *P. anomala*, *C. tropicalis*, *P. ranongensis*, *Cv. lusitaniae*, *Pd. pentosaceus*, *Lb. plantarum*, *Lb. brevis*, *W. confusa*, and *W. paramesenteroides*	Thanh et al. (2008)	PCR-mediated DGGE of 52 starter samples, identification of bands using DNA sequencing

isolation plate of fungi and yeasts from *banh men* on malt agar is shown in Fig. 15.4.

Although several fungi found in *banh men* are capable of hydrolyzing rice starch, *A. rouxii* is the most notable. *A. rouxii* was first described by Calmette in his comprehensive study on *banh men* (mentioned as "levure chinoise") dated back to 1892 (Calmette 1892). Calmette considered the fungus as the major agent causing liquefaction of rice starch. By using the purified fungus and commercial yeast, he could obtain 340 g of absolute alcohol from 1000 g of rice instead of 180 g when using *banh men* (Calmette 1892). The fungus was also successfully utilized for upgrading of *banh men* for production of rice wine from purple glutinous rice (Dung et al. 2006).

The taxonomy of *A. rouxii* is however not simple. Recent study of *Mucorales* based on ITS sequences has placed all of the following species as synonyms: *A. rouxii*, *R. achlamydosporus*, *R. arrhizus*, *R. boreas*, *R. delemar*, *R. chiuniang*, *R. javanicus*, *R. oryzae*, *R. maydis*, *R. niveus*, *R. peka*, and *R. tonkinensis* (Walther et al. 2013). In early literature, *A. rouxii* was also known as *Chlamydomucor rouxii* (Calmette) Went and

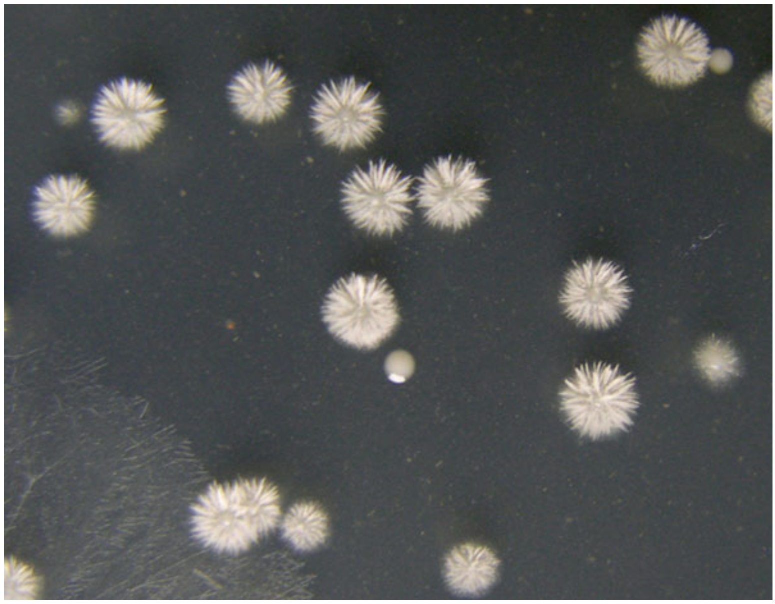


Fig. 15.4 Typical mycological composition of *banh men* on isolation media (malt agar). *Amylomyces rouxii*, spreading filamentous growth (*low left corner*); *Saccharomycopsis fibuligera*, filamentous colony with restricted growth, the major type in the photo; *Saccharomyces cerevisiae*, shiny colony in the center

Prin. Geerligs, *Chl. oryzae* Went and Prin. Geerligs, *Chl. rouxianus* (Calmette) Wehmer, *Chl. javanicus* Yamazaki, and *R. chlamydosporus* (Went and Prin. Geerligs) Boedijn. Officially, all of these species names are now under *R. arrhizus*. It should be noted that strains designated as *M. rouxii* are not *A. rouxii* and might relate to *M. indicus* or *M. circinelloides* even if there was confusion in the past (Walther et al. 2013).

The name *A. rouxii* represents a special phenotype of *R. arrhizus* that widely occurs in Asian amylolytic starters (Kito et al. 2009). It is characterized by intensive formation of large chlamydospores and abortive sporangia and the lack of rhizoids, stolon, and black-pigmented sporangia. *A. rouxii* can utilize sucrose, maltose, but not glycerol. Under anaerobic condition, *A. rouxii* produces abundant hyphae with chlamydospores but no yeast cells (Hesseltine et al. 1985). *A. rouxii* itself is a heterogeneous group. *A. rouxii* strains isolated from *banh men* produce malic acid but not lactic acid, while strains from *look-pang* and *ragi-tape* produce majorly lactic acid (Kito et al. 2009). The separation of the two groups is supported well by RFLP and DNA sequencing data (Kito et al. 2009; Dolatabadi et al. 2014a, b). Based on this observation, *A. rouxii* strains should be placed under two varieties of *R. arrhizus*, namely, lactic acid-negative strains from *banh men*, under *R. arrhizus* var. *delemar*, and lactic acid-producing strains from *look-pang* and *ragi-tape* under *R. arrhizus* var. *arrhizus*. Considering that *A. rouxii* was first described based on strain isolated from *banh men*, it should be noted that the designation of CBS 438.76, a lactic acid-producing strain (*R. arrhizus* var. *arrhizus*) isolated from *look-pang* as neotype for *A. rouxii*, was not a right choice (VNT's opinion) (Zheng et al. 2007). The phenotypic convergence (lack of black sporangia) in strains isolated from different types of starters could be due to independent mutations accumulated as the results of technological selection pressure (Kito et al. 2009). During the production of the starter tablets, short thrive follows by dehydration, and storage would facilitate the selection of substrate growth with resting chlamydospores rather than the formation of aerial hyphae and sporangia.

Besides being an important component in Asian amylolytic starters, *R. arrhizus* and its synonyms (notably *R. oryzae*) are the major pathogen (70 % cases) causing mucormycosis that occurs in immunocompromised patients because of diabetic ketoacidosis, neutropenia, organ transplantation, and/or increased serum levels of available iron. The high-affinity iron permease FTR1 is a key virulence factor required for *R. oryzae* pathogenesis (Ibrahim et al. 2010).

R. microsporus is the second most frequent mucoraceous fungi isolated from *banh men*. Similar to the case of *A. rouxii*, the domesticated form of *R. microsporus* having reduced sporulation activity and was treated as a separate species *R. oligosporus* or a variety (*R. microsporus* var. *oligosporus*). However, this variety along with others (*azygosporus*, *chinensis*, *oligosporus*, *microsporus*, *rhizopodiformis*, and *tuberosus*) is not supported genetically by matting compatibility and sequencing data of ITS, ACT, and 1-α (TEF) genes (Dolatabadi et al. 2014b). Strains of *R. microsporus* can produce rhizoxins, potent antimitotic macrocyclic polyketide toxins. However, it was revealed that the fungus cannot produce toxin by itself and the toxin is produced by bacteria of the genus *Burkholderia* that live inside the fungal cells (Partida-Martinez et al. 2007). Alarming was the fact that the bacteria have been found in *R. microsporus* CBS 111563, originally isolated from *banh men*. When the strain was tested for *sufu* and *tempe* production, considerable amounts of rhizoxins could be detected in the final product (Rohm et al. 2010). It is not clear how prevalent is the toxigenic phenotype of *R. microsporus* from *banh men*.

Among fungi of the genus *Mucor* found in *banh men*, *M. circinelloides* is most prevalent (Lee and Fujio 1999). *M. circinelloides* is a dimorphic species and could form both filamentous and yeastlike growth. Besides amylolytic activities, strains of *M. circinelloides* are capable of degrading cellulosic materials (Wei et al. 2013). Inferring strains from *banh men* to *M. circinelloides* based on morphological observation should be accompanied with caution. ITS sequences indicated that strains identified morphologically as *M. circinelloides* represent a group of species. Currently at least four distinct genetic groups were detected, namely, *M. circinelloides* f. *circinelloides*, *M. circinelloides* f. *griseocyanus*, *M. circinelloides* f. *lusitanicus*, and *M. circinelloides* f. *janssenii* (Walther et al. 2013). The taxonomy of *M. circinelloides* awaits revision, and each of the genetic groups would have a separate specific status. Surprisingly, *banh men* nearly lacks of *Aspergillus*, *Trichoderma*, and *Penicillium*, the most common saprotrophic fungi in indoor environment (Haleem Khan and Mohan Karuppayil 2012). Hesseltine et al. (1985) found that *Mucor*, *Amylomyces*, and *Rhizopus* isolated from amylolytic starters grew well under anaerobic conditions, especially when CO_2 was supplied. Based on this observation, it was postulated that the selective development of mucoraceous fungi in starters is due to the following factors: (1) the selective action of spices, (2) anaerobic conditions, and (3) the presence of CO_2 (Hesseltine et al. 1985).

Besides amylolytic mucoraceous fungi, yeast *Saccharomycopsis fibuligera*, an active amylase producer, is also commonly found in *banh men. Sm. fibuligera* produces extracellular α-amylase, glucoamylase, and raw starch-digesting glucoamylase (Chi et al. 2009). Alpha-amylase of *Sm. fibuligera* has molecular weight of 54 kDa and is encoded by *ALP1*. The enzyme is not adsorbed by starch (Chi et al. 2009). In the medium, *Sm. fibuligera* often produces extracellular C_{14}-C_{18} 2-D-hydroxy fatty acids in the form of needle-shaped crystals (Kurtzman et al. 1973). There is no information on the possible effect of these compounds on microbial community of *banh men* as well as the quality of the fermentation products although it seems quite obvious. Kurtzman et al. (1973) informed that the compounds had little or no antibacterial activity. However, their test was restricted to common enteric pathogens (Kurtzman et al. 1973).

Fermentative yeasts *Saccharomyces cerevisiae*, *Wickerhamomyces anomalus*, *Issatchenkia orientalis*, and *Clavispora lusitaniae* were common in *banh men*. Among these, *S. cerevisiae* is the most prevalent. Although *S. cerevisiae* is recognized as the most important ethanol producer in *banh men*, the information regarding strains isolated from *banh men* is scant. It is known that *S. cerevisiae* strains isolated from *ragi* and *sake* are genetically related and distant from wild strains or strains utilized for wine fermentation (Liti et al. 2009). It is not clear if *S. cerevisiae* from *banh men* also belongs to the same group.

Most of the works on *S. cerevisiae* isolated from *banh men* still limited to alcohol production efficiency (Dung et al. 2006). Given the importance of *S. cerevisiae* in alcohol fermentation and aroma formation, further study on genetic and biology of these strains should be conducted in order to improve traditional fermentation.

Ascosporic yeast *Wickerhamomyces anomalus* (formerly known as *Pichia anomala*) could be found in *banh men* using both isolation and culture-independent methods (Thanh et al. 2008). *W. anomalus* is a regular component in several types of Asia-Pacific alcohol fermentation starters (Haard et al. 1999; Limtong et al. 2002; Sujaya et al. 2004). The yeast can accumulate up to 5 % (w/v) ethanol in broth culture (Limtong et al. 2002). *W. anomalus* strains are known to produce killer toxin, a glycoprotein that can inhibit the growth of other yeasts (Schneider et al. 2012). *W. anomalus* (*P. anomala*) has long been utilized as biocontrol agent for apples (Haïssam 2011). Taken the high frequency of occurrence, it is interesting to know if *W. anomalus* killer toxin would play any role in shaping up the relatively stable microbiota of *banh men. P. anomala* produces a spectrum of small volatile compounds, such as ethyl acetate, ethyl propanoate, phenyl ethanol, and 2-phenylethyl acetate (Passoth et al. 2006). These volatile compounds might contribute to the aroma of rice liquor.

Bacterial community of *banh men* has received little attention and is often regarded as contaminant and unwanted (Haard et al. 1999). By using PCR-mediated DGGE, it was shown that the bacterial microflora of *banh men* was highly variable in species composition and dominated by lactic acid bacteria (LAB). The most frequent LAB were *Pediococcus pentosaceus*, *Lactobacillus plantarum*, *Lb. brevis*, *Weissella confusa*, and *W. paramesenteroides*. Species of amylase-producing *Bacillus* (*Bacillus subtilis*, *B. circulans*, *B. amyloliquefaciens*, *B. sporothermodurans*) and acetic acid bacteria (*Acetobacter orientalis*, *A. pasteurianus*) have also been detected (Thanh et al. 2008). One of the major differences between the fungal and bacterial microbiota of *banh men* is the consistency. Whereas fungal microflora showed little variation between samples, the bacterial community exhibited a rather "spontaneous" species composition. This might be due to different selection pressure exercises on these two communities by the various processes (Thanh et al. 2008). Although with species variation, the constant presence of functional bacterial groups (starch degrader, lactic acid, and acetic acid producers) does not rule out the role of bacteria in *banh men*, especially in the formation of organoleptic quality of the final product.

15.2.4 Cultural, Economic, and Health Aspects of Alcohol Fermentation

Traditional alcohol production and consumption is an indispensable part of Vietnamese culture. Rice liquor is served to honor the visitors or to mark occasions. It is used during agricultural fests, ritual observances of marriage, funeral, and ceremonies in honor of ancestors or local spirits. The rice liquor is prized for being a local product and because it satisfies local values and tastes. There is a common perception that regular drink of rice liquor in small amount can be health beneficial. Production of rice liquor has an important economic position, especially in rural areas. Alcohol production gives the women additional working opportunities and contributes to the family income. Besides the liquor for sale or consumption, fermentation by-products are sometimes considered as the only surplus. The spent grain and wort is utilized as feed for pig. Animal manures and waste are often fed to anaerobic digesters for obtaining biogas fuel.

According to the Ministry of Industry and Trade, in 2007, villagers in Vietnam produce 260 million liters of rice liquor by means of traditional fermentation or roughly 4.5 times higher than the one produced by Vietnamese factories (MOIT 2013). Rice alcohol is the favorite choice for people with low income. According to a survey conducted in 83 sites covering 15 provinces, rice liquor is produced mainly in small operations (66 % had two or fewer workers). The production is a heritage family business as 94 % of the operators used family members as labors, 89 % received the skill from the family members, and 70 % have more than 10 years of experience (Pham Xuan Da 2009b). Rice is the type of the main raw material for alcohol production and accounts for 90 % of the cases, among that 50 % used regular rice and 40 % used glutinous rice. Corn, sweet potatoes, and cassava are used by 10 % of the operators. The rate of rice substitution depends on the agriculture condition in each area. In the southern part of the country where the rice hub is located, the rate of rice substitution accounted for only 4 %, while in the north the rate was 18 % (Pham Xuan Da 2009a). The making of alcohol starter is no longer a private business of the breweries but largely commercialized. While in the north, 57 % breweries still prefer to use their own starter, in the south 82 % prefer to buy it from the market. The distribution network for rice liquor is still primitive and that explains the vast network of breweries across the country. Around 70 % of the points of sale are located at the production sites and 24 % at nearby areas. Only 6 % are being sold beyond the production areas (Pham Xuan Da 2009a).

The average alcohol concentration of the samples collected at 83 breweries was 35.5 ± 6.8 % (v/v). The methanol concentration was relatively low and met acceptable standard (90.4 % of the samples). Methanol is considered as one of the most toxic compounds associated with alcoholic drinks. Hydrolysis product of pectin is the main source of methanol in alcoholic beverages. Rice has relatively low pectin content and so the methanol concentration in rice liquor. Traditional distillation technique cannot separate methanol from ethanol. Methanol concentrations in different distillation fractions are similar and in the acceptable range (Nguyen Kim Dong et al. 2012). Acetaldehyde is a by-product of alcohol fermentation. It is an important volatile compound in rice liquor and contributes to the sherry-like aroma with the sensory threshold of 100–125 mg/L (Zoecklein et al. 1995). Acetaldehyde is also a toxic and carcinogenic compound. Acetaldehyde concentration in traditional alcohol fermentation is relatively high (83–266 mg/L in the fermentation broth) (Nguyen Kim Dong et al. 2012). However, it has low boiling temperature (20.2 °C) and could be easily removed by means of distillation. It was shown that with traditional distillation technique, the first 20 % of the distillate contained 480 mg/L acetaldehyde but then reduced to below 50 mg/L in subsequent fractions (VNT's calculation from Nguyen Kim Dong et al. 2012). Similarly, the first 20 % of the distillate contained rather high amount of isobutanol (2500 mg/L) and isopentanol (2000 mg/L), but in the remaining 80 %, the concentrations of these alcohols fell below 150 mg/L. In opposite, furfurans and organic acids came out in the last 20 % of the distillate. Esters, on the other hand, are the group of compounds with different boiling temperatures. They can be concentrated in the very first or last fractions (Nguyen Kim Dong et al. 2012). Most breweries are aware of quality differences between distillation fractions. Unsuitable fractions are usually subjected to redistillation or sometimes substitution for low-grade liquors. Vietnam standard for distilled spirits (TCVN 7043: 2013) factually only regulates the methanol content and at relatively high level (2000 mg/L of absolute ethanol). Thus, most of the traditional rice liquors would meet the requirement. However, it is well known that over high concentration of congeners would cause headache and hangover and be harmful to the customers. More work to be done in order to standardize traditional alcohol fermentation while keeping the diversity.

15.2.5 Diversity of Alcohol Fermentation

Vietnam has 54 ethnic groups, each with own language and culture tradition, and this reflects on the diversity of alcoholic products and production methods thereof. The fermentation techniques may be altered depending on the type of raw material, specific climate condition, and intended use of the final products. There are three major product categories that could be listed: (a) distilled spirit, (b) wine (undistilled), and (c) partially fermented snack. Distilled spirit is the main type of traditional alcoholic drink in Vietnam. It can be made from a variety of starch-containing raw materials and each, in turn, results in specific taste and aroma. Spirit made from glutinous rice is widely accepted. Glutinous rice produces more aroma and slightly higher alcohol yield than regular rice, although it is also more expensive. In the densely populated river delta areas, where rice is the major cultivar, spirit made from rice is popular. *Banh men* is the starter type used in these areas. The typical brands of rice liquor produced are Lang Van (Bac Ninh province) and Bau Da (Binh Dinh province). Due to variations in distillation techniques, Lang Van offers liquors with 30–35 % alcohol, while Bau Da offers much stronger liquor with alcohol content of around 50 % (Lachenmeier et al. 2009).

In mountainous areas of Vietnam, corn and *men la* starter are routinely used by people of ethnic minorities to make alcoholic drinks. The distiller used by the highland people has a rather unique design. It has a concave chilling lid, and instead of leading the steam out of the boiler for condensation, the process takes place inside the boiler on the chilling lid. A wooden bridge put inside the boiler traps the falling condensate and leads the liquid out for collection. The combination of above mentioned factors (corn, *men la*, and unique distillation technique) results in low alcohol yields but some of the very fine type of liquors. Corn liquor produced by people of Quan Ba village located at the very northern tip of Vietnam is typical for such drink. Slight modification of fermentation technique could be observed in the mountainous area of Mau Son (Lang Son province). Due to the cool weather condition (average temperature of 15.5 °C), the fermentation process takes up to 25 days to complete, or roughly three times longer than average. After inoculation of cooked rice with *men la*, the mixture is fermented with aeration for 2 days and then in a close vessel for 10 days. After this, water is added and anaerobic fermentation is carried out for another 10–13 days before it can be distilled.

The use of cassava for alcohol fermentation is not popular since it gives low-quality spirit. Fermentation of cassava is generally restricted to industrial processes or for obtaining of cheap (and low grade) liquor. Otherwise, fermentation of cassava is similar to that for rice liquor. Rice wine is produced from glutinous rice. Purple glutinous rice is favorable for the color appearance and unique taste. Rice wine can be milky or clear depending on whether or not filtration is applied. Due to relatively low alcohol content (10–12 % v/v) and high microbial load in fermented product, rice wine is often fortified with alcohol to prevent acidification. Rice wine is usually served along with foods. Listed in wine category, *ruou can* (literally translated as “tube wine”) produced by highland people is an exotic example. For production of *ruou can*, glutinous rice is inoculated with *men la* and mixed with a quantity of rice husk. The mixture is then transferred to a slender ceramic jar that has been partially filled with rice husk. The open mouth of the jar is then sealed with ash and husk mixture. Fermentation is carried out for about a month. For consumption, thin bamboo tubes are inserted through the fermented mass and directed to the husk layer. People use the tubes to drink the wine. Rice husk, thus, plays a role in aiding filtration. *Ruou can* has a bitter alcoholic, sweet, and refreshing taste. The wine is served during ceremonies and for highly respected guests.

Ruou nep is a sweet and light alcoholic snack obtained by partial fermentation of glutinous rice (Fig. 15.5). It is produced by inoculating cooked glutinous rice with alcohol starter and let fermentation to take place in aerobic condition for 2–3

Fig. 15.5 *Ruou nep*, a sweet and bitter alcohol fermentation snack made from glutinous rice

days. The liquid formed as the result of starch hydrolysis is then mixed with fermented rice grains and served directly. This is the only type of alcohol-containing snack that can be consumed ubiquitously regardless of the age and gender. Traditionally, *ruou nep* is served during the Doan Ngo fest (May 5, lunar calendar). Currently *ruou nep* has become increasingly popular. *Ruou nep* mixed with yogurt and ice is a nice snack in the hot summer days. Both ordinary glutinous rice and purple glutinous rice can be used for making *ruou nep*, although the latter is of preference. In the northern part of the country, regular alcohol starter (*banh men*) is used for making of *ruou nep*. In the south, a special type of starter, *men ngot* (literally translated as "sweet yeast") is utilized for the same purpose. To our knowledge, *men ngot* is similar to *banh men* but having no or reduced density of *Saccharomyces* yeast cells (author's (VNT) unpublished data).

15.3 Traditional Fermentation of Fishery Products

With a coastal line of 3444 km and a dense network of rivers and lakes across the country, fishery has long been an important part of Vietnam's economy. Currently, Vietnam is one of the world's leading exporters of fish and fishery products. Traditional fermentation of fishery products is indispensable in providing ingredient for Vietnamese cuisine. According to the Ministry of Agriculture and Rural Development (MARD) statistics, annually around 240,000 tons of fishery products are used for fabrication of fish sauce, shrimp paste, and other fermented products. Among that, fish sauce accounts for 85 % of the raw material use. The total revenue of the fishery fermentation industry is estimated at 200 million USD. Similar to other traditional fermentation processes, production of fish sauce and shrimp paste is mainly based on small-scale operations. If the country has 63 industrial enterprises operating in the field, there are more than 1900 small-scale cottage operators, producing fermented fishery products across the country. Although this contributes to the product diversity, there is a serious concern of sanitation standard. Fermented fishery products could be classified into two categories: (a) proteolytic products from salted fish (fish sauce) similar to *nampla* (Thailand), *shottsuru* (Japan), and *patis* (Philippine) and (b) lactic acid fermentation product from fish and shrimp, similar to *narezushi* (Japan) and *pla chom* (Thailand).

15.3.1 *Nuoc Mam*

Fish sauce (*nuoc mam*) has been produced in Vietnam for centuries. It is an amber-colored liquid extracted from the fermentation of fish with sea salt. It has salty taste, sweet umami of protein hydrolysate, and specific aroma of fermented fish. Fish sauce is indispensable Vietnamese culinary ingredient. It is used as dipping sauce, marinated ingredient, and additive for soup. Fish sauce has been used as a high calorie and nutritional drink for fisherman. Fish sauce is produced mainly by small-scale manual operators, mostly at household level. The production is located along the coastline from the north to the south of Vietnam. The method of production varies at different areas and results in unique products for each region. Famous brands include Phu Quoc, Nha Trang, Phu Yen, Phan Thiet, Dong Hoi, Hau Loc, Cua Lo, Cat Hai, and Van Don.

15.3.1.1 Method of Production

Production of fish sauce is a complex microbiological and biochemical process. It includes proteolysis of protein into peptides and amino acids, partial decomposition of amino acids, and formation of secondary metabolites. The process may take from 6 to 18 months to complete, depending on the weather condition and product type. Although sharing the same principle, the method for fish sauce production is highly variable between regions. For the good quality of fish sauce, fresh fish is required. Fish need to be rinsed and drained then mixed with salt. The amount of salt used is in the range from 10 % to 30 % depending on the customs and fish quality.

In general, operators in the northern part of the country use more salt than in the southern regions. For example, in Cat Hai, each part of salt is used for eight to ten parts of fish but in Phu Quoc, the ratio is 1:3. If the fish is not fresh, more salt is required (often 20–30 % of the fish weight). The climate condition in the south is more favorable for fish sauce production than in the north. Fish sauce production in the south is often carried out in larger scale. After mixing with salt, the mass in transferred to huge wooden vessels of 2.5–8 m^3 previously filled with a layer of salt and then topped with salt to cover the fish. A bamboo or wooden rack is placed on top and weighted down with rocks to keep the fish from floating when the liquid is extracted from the fish because of osmotic pressure and protein hydrolysis. The vessels are covered to avoid dust, animals, and evaporation. After 2–4 days, the vessel is drained through a hole located at the bottom for collection of partially hydrolyzed liquid (*nuoc boi*) and to deflate the content. After closing the hole, the content is fermented further for 7–12 months. Clear, amber-colored liquid with specific aroma formed inside the vessel is collected as first-grade fish sauce. After drainage, the remaining part is added with the partially hydrolyzed liquid (*nuoc boi*) and salt and fermented for collection of second-grade fish sauce.

In the north, where the weather condition is often unstable and with lower cumulative temperature, fish sauce production is often carried in smaller scale using lower salt concentration. Instead of large wooden vessels, relatively smaller jars of clay are used. Drainage procedure is not applied. Jars containing mixture of fish and salt are placed under direct sunlight for about 3 months. From time to time, the jars are open and stirred to air out and let the fish exposed to sunlight. The periodic sunning procedure ensures acceleration of hydrolysis and maturation. Low concentration of salt perhaps also helps in promoting microbial growth. After 6–7 months, when the liquid fraction becomes clear and reddish amber in color and has fragrant aroma, the fermentation is completed. First-grade fish sauce is collected from the bottom through a tube or siphon placed in the middle of the jar to avoid mixing layers. Second- and third-grade fish sauces are obtained by adding water and salt to cover the fish remain and fermented for another 2–3 months each time.

Fish sauce after collection will need to be filtered to remove suspended particles and let stand for about 2 weeks to air out of strong odor before bottling. Fish sauce of second and third grades often needs to be boiled. Operators may decide to mix different grades of fish sauce to obtain an average product. The fish spent can be used for animal feed. Images from the fish sauce production scene are presented in Figs. 15.6 and 15.7.

15.3.1.2 Chemical and Microbial Indices of Fish Sauce

Fish sauce production is spontaneous fermentation and decomposition processes. Its quality is affected by environmental and climate conditions, raw materials, and method of production. There is a large variation in terms of properties, composition, and organoleptic quality among regions. In general, fish sauces of northern provinces have lighter color and low nitrogen and salt content. In the central and southern provinces, fish sauce is reddish brown in color and with higher salt and amino acid concentrations and strong specific aroma. Basic chemical and microbiological indices of fish sauce samples collected from different provinces are presented in Table 15.4. Fish sauces from Vietnam have relatively high content of total nitrogen, amino acids, and nucleic acids and are equivalent to those of Thailand and Japan but quite different from fish sauces of Myanmar, Laos, Korea, and China. Acetic acid fermentation was observed in fish sauces of Myanmar and China, while fish sauces of Vietnam have low content of organic acids. Pyroglutamate and lactate have been detected at relatively high concentrations in fish sauces from Vietnam (Park et al. 2001). Most of fish sauce samples were positive in coliforms, however, at

Fig. 15.6 Fish sauce production in Vietnam. (**a**) Fermentation in wooden vessels; (**b**) Fermentation in jars of clay; (**c**) Fermentation jar with central pipe for liquid collection. (**d**) Stirring of fermentation mass; (**e**) Filtration of fish sauce; (**f**) Commercial fish sauce products

the densities lower than the tolerable limit. Following bacteria have often been isolated from Vietnamese fish sauces: *Bacillus*, *Micrococcus*, *Staphylococcus*, and *Streptococcus*. Salt-tolerant strains with high protease activity and aroma production capability were isolated from Vietnamese fish sauce. They belong to the genera *Bacillus*, *Clostridium*, and *Lactobacillus* (Noguchi et al. 2004; Uchida et al. 2004).

15.3.2 Shrimp Paste (*Mam Ruoc/Mam Tom*)

15.3.2.1 Method For Shrimp Paste Production

Shrimp paste is made from *Acetes* shrimp, mainly *Acetes japonicus*. There are two types of shrimp paste in Vietnam, namely, *mam ruoc* and *mam tom*. *Mam ruoc* is reddish in color and with

Fig. 15.7 Large-scale production of fish sauce and drainage system

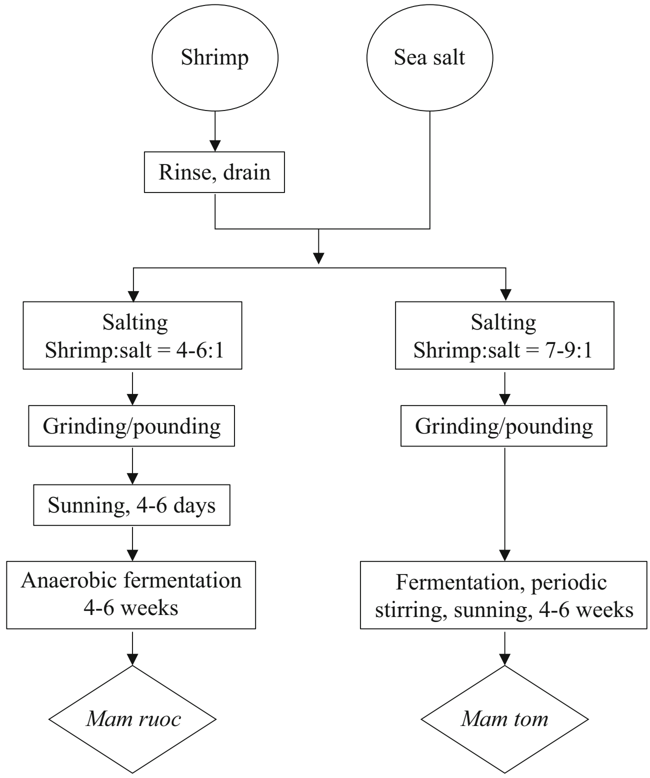
Table 15.4 Basic chemical and microbiological indices of fish sauce samples collected at different provinces of Vietnam

Sample	NaCl, g/l	Total nitrogen, g/l	Amino acid, g/l	Aerobic count, log cfu/g	Coliform	Source
Nam O, Da Nang[a]	260–290	22–25	15–16	3.5–4.2	+	a
Thanh Binh, Quang Nam[a]	280–295	21–25	13–16	3.3–4.3	+	a
Phu Thuan, Hue[a]	250–295	20–28	14–18	3.3–3.9	+	a
Trieu Phong, Quang Tri[a]	167–252	21–34	14–21	3.0–4.2	+	a
Dong Hoi, Quang Binh[a]	146–194	28–31	18–19	3.0–4.0	+	a
Bo Trach, Quang Binh[a]	251–290	22–30	14–18	3.5–4.3	+	a
Ky Anh, Ha Tinh[a]	197–249	19–38	11–25	3.0–3.7	+	a
Cam Xuyen, Ha Tinh[a]	160–219	29–31	19–22	3.6–4.2	+	a
Cat Hai, Hai Phong[b]	nd	18–34	11–21	2.0–3.0	+	b
Cua Hoi, Nghe An[c]	220–265	25–33	19–23	3.3–4.0	+	c
Phu Quoc, Kien Giang[c]	250–286	30–36	20–24	3.0–4.0	+	c
Phu Yen[d]	255–269	21–32	13–19	2.0–3.0	+	d

nd no data
[a]Nguyen Thi Viet Anh (2005)
[b]Nguyen Thi Viet Anh (2011)
[c]Nguyen Thi Viet Anh (2012a)
[d]Nguyen Thi Viet Anh (2012b)

specific sweetness of hydrolyzed protein and fragrant aroma, while *mam tom* is grayish dark brown and with strong fishy smell. The production methods for *mam ruoc* and *mam tom* are slightly different (Fig. 15.8). For *mam ruoc* production, after being caught, small shrimps are unloaded and fish and impurities are removed. It was then rinsed, drained, and mixed with sea salt at the ratio of 4–6 shrimps to 1 salt. The mixture is then homogenized mechanically by grinding or pounding. The paste is placed on bamboo tray and dried under sunlight for 4–5 days. The shrimp

Fig. 15.8 Schematic procedures for production of *mam ruoc* and *mam tom* type of shrimp pastes



paste is then filled to the jars of clay and topped with a thin layer of sea salt, covered tightly, and fermented in anaerobic condition for 4–6 weeks. The obtained product is reddish, fine, paste-like in texture, salty sweet, and with pleasant aroma.

Mam tom is prepared in similar manner but salt concentration used is significantly lower (shrimp to salt ratio of 4–6:1 for *mam ruoc* and 7–9:1 for *mam tom*). After grinding/pounding, the paste is transferred directly to the jars for fermentation (without partial drying). Instead of strict anaerobic fermentation, for *mam tom* production, periodic stirring and sunning is performed. The obtained product is more fluidly and has strong odorous smell.

15.3.2.2 Chemical and Microbiological Indices of Shrimp Paste

Differences in method of production affect not only the organoleptic properties of shrimp paste but also nutritional values. *Mam ruoc* has higher total solid content and more nutritious. Some important chemical and microbiological indices of *mam ruoc* and *mam tom* are presented in Tables 15.5 and 15.6. Microorganisms isolated from shrimp paste belong to the group of lactic acid bacteria and *Bacillus*. *Bacillus* strains isolated from shrimp paste demonstrated high fibrinolytic activity (Dinh et al. 2015).

15.3.3 Sour Shrimp

15.3.3.1 Sour Shrimp Production Method

Sour shrimp is a popular product in the central part of Vietnam. The most famous brand is sour shrimp of Hue, the old capital of Vietnam. Sour shrimp is used as dipping sauce or cooking ingredient or can be served directly with meat and vegetables. The product is made from fresh shrimp, glutinous rice, salt, and spices (galangal, garlic, chili, roasted rice powder) by means of fermentation. During the fermentation, starch from glutinous rice and shrimp proteins are partially decomposed to sugars and peptides.

Table 15.5 Chemical and microbiological indices of *mam ruoc* (Nguyen Thi Viet Anh 2005)

Samples	NaCl, %	Protein, %	Amino acid, %	Amino acid: protein, %	Aerobic count, log cfu/ml	Coliforms, log cfu/ml	*Clostridium*
Nam O, Da Nang	10–13	3.7–4.1	1.8–2.6	43–68	4–5	2	–
Phu Thuan, Hue	16–20	2.7–4.1	1.6–2.6	55–62	4–5	2–3	v
Trieu Phong, Quang Tri	10–14	4.5–5.6	2.1–3.0	47–55	4–5	2–3	–
Dong Hoi, Quang Binh	11–15	3.6–5.9	2.0–2.4	40–58	4	2–3	v
Bo Trach, Quang Binh	11–16	3.7–4.4	1.9–2.4	44–57	4–5	2–3	v
Ky Anh, Ha Tinh	15–16	3.4–4.1	1.3–1.5	35–36	5	2–3	–
Cam Xuyen, Ha Tinh	14–19	3.2–4.2	1.3–1.6	32–49	5	2–3	v

Table 15.6 Chemical and microbiological indices of *mam tom* (Nguyen Thi Viet Anh, unpublished data)

Mam tom sample	NaCl, %	Protein, %	Amino acid, %	Aerobic count, log cfu/ml	Coliforms
Cat Hai, Hai Phong	16,5	3,6	1,6	10^4	+
Tinh Gia, Thanh Hoa	17,1	3,2	1,4	10^4	+
Hau Loc, Thanh Hoa	19,5	3,1	1,5	10^3	+

Lactic acid bacteria convert sugar into lactic acid. The final product is chili hot, salty-sour with complex aroma of fermented protein and spices. Similar products are *zucgal* of Korea, *balao balao* of the Philippines, and *kung chom* of Thailand.

Shrimps used for fermentation should be fresh and with relatively soft shell (*Metapenaeus ensis*, typically). Shrimps are headed, rinsed with saline, drained, and briefly dipped in alcohol to kill germs and for the reddish color to develop. It is then mixed with cooked glutinous rice, galangal, garlic, chili, roasted rice powder, and 8–10 % salt. The mixture is fermented in anaerobic conditions for about 4–6 weeks. The product is then supplemented with variety of spices depending on brand before bottling. The typical scheme for production of sour shrimp and the final product are presented in Figs. 15.9 and 15.10, respectively.

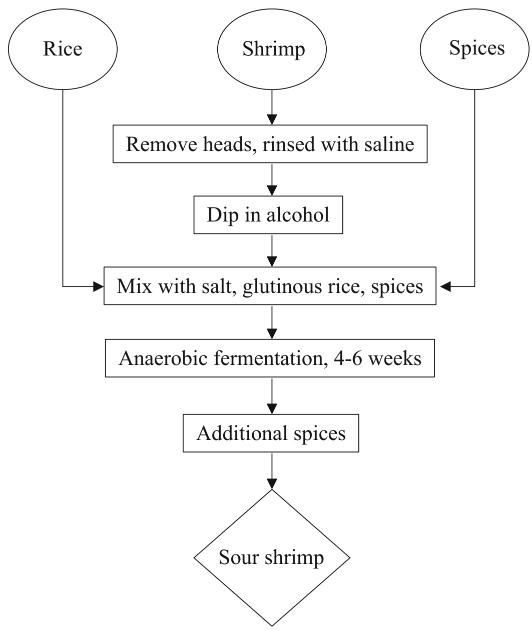


Fig. 15.9 Typical scheme of procedures for production of Hue sour shrimp

15.3.3.2 Microbiological and Chemical Indices of Sour Shrimp

Production of sour shrimp is a manual process and without the use of pure culture. Fermentation is spontaneous caused by microorganisms associated with substrates or introduced from the environment. The basis of sour shrimp fermentation is the hydrolysis of rice starch and shrimp protein and lactic acid fermentation. Although fermentation is spontaneous, the products are safe for consumption. Some important chemical and microbiological indices of sour shrimp are presented in

Table 15.7. The fermentation is directed toward lactic acid fermentation by the presence of relatively high salt concentration, the pretreatment procedures with saline and alcohol, as well as the selective effect of spices (galangal, garlic, and chili). During the fermentation, lactic acid bacteria thrive from day 4 and gradually reduce in viable count after 2 weeks. Bacteria of the genus *Bacillus* occur along with lactic acid bacteria. *Clostridium* and coliforms are abundant during the first week of fermentation but reduce afterward (Nguyen Thi Viet Anh et al. 2010b). Lactic acid produced during fermentation inhibits the growth of spoilage bacteria. Furthermore, many lactic acid bacteria can produce bacteriocin that may suppress the growth of undesirable microorganisms. The initial step of fermentation is very important since several unwanted salt-tolerant bacteria, such as *Staphylococcus aureus*, *Halobacterium salinarum*, and *Bacillus*, could thrive. Attempts of introducing lactic acid bacteria in the form of starter culture gave positive effect. Concerning the aroma of sour shrimp, 2-furancarboxaldehyde and 2-furanmethanol are considered as important contributors (Nguyen Thi Viet Anh et al. 2010b).

Fig. 15.10 Commercial Hue sour shrimp

15.4 Fermented Meat -*Nem Chua*

15.4.1 Production Method

Nem chua is a popular lactic acid fermentation product of uncooked meat. The famous brands are Uoc Le (Hanoi), Phung (Hanoi), Vinh Yen (Quang Ninh), Thanh Hoa, Dong Ba (Hue), Ninh Hoa (Khanh Hoa), Lai Vung (Dong Thap), etc. *Nem chua* from each region has a unique taste and flavor, but, perhaps, *nem chua* of Thanh Hoa is the most famous. *Nem chua* usually is dipped with chili sauce and served directly. It is a nice snack for beer. Sometimes, *nem chua* is fried, used as ingredient for cooking or for mixing with salad. *Nem chua* is similar to *som mou* of Laos, *nham* of Thailand, and *tocino* of the Philippines.

At different regions, the making of *nem chua* is slightly different but all base on spontaneous lactic acid fermentation. The most general way of making *nem chua* is presented in Fig. 15.11. For making of *nem chua*, fresh meat is ground and mixed with salt, sugar, pepper, and garlic. It was then wrapped in a piece of banana leaf along with a guava leaf fragment and let fermented at 28–32 °C for about 2–3 days. The final product is solid

Table 15.7 Chemical and microbiological indices of sour shrimp (Nguyen Thi Viet Anh et al. 2010b)

	Chemical indices						Microbiological indices				
Sample	pH	Acid, %	Total N, g/kg	N amin, g/kg	N NH4, g/kg	NaCl, %	Total count, log cfu/g	LAB, log cfu/g	Yeast/fungi	Coliforms, log cfu/g	*E. coli*
#1	4.5	0.18	12.50	9.38	2.17	9.3	4.8	3.3	–	1.2	–
#2	4.9	0.08	11.83	8.68	2.45	7.7	4.8	3.2	–	1.3	–
#3	4.6	0.11	12.11	8.30	2.66	7.1	5.0	3.3	–	0.9	–
#4	4.3	0.18	12.60	9.38	1.94	8.1	4.2	3.9	–	1.0	–
#5	4.3	0.25	12.97	9.24	1.02	7.8	4.2	3.0	–	0.8	–
#6	4.6	0.17	10.50	8.40	1.32	8.9	5.4	3.6	–	1.3	–
#7	4.5	0.19	11.00	8.90	2.03	8.5	5.2	3.9	–	1.2	–
#8	4.6	0.14	11.20	8.40	2.15	9.0	4.9	3.3	–	0.7	–
#9	4.4	0.15	10.80	8.70	1.89	9.2	5.3	3.9	–	1.2	–

in texture, pink colored, and with pleasant aroma of fermented meat and garlic. *Nem chua* can be served directly without cooking (Phan Thanh Tam and Pham Cong Thanh 2007) (Fig. 15.12).

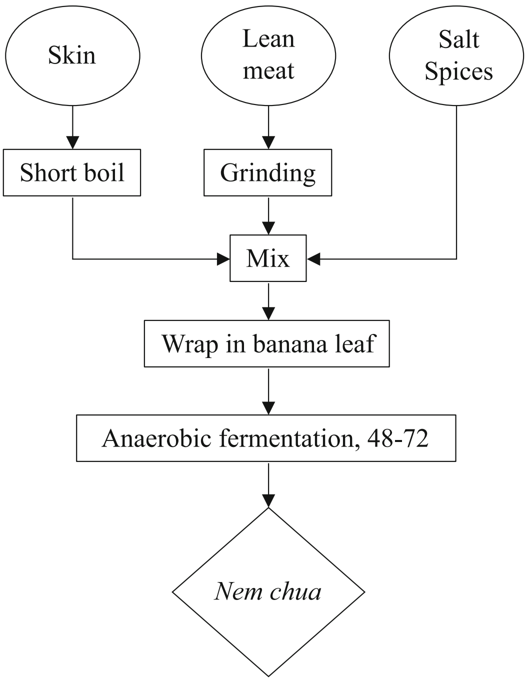


Fig. 15.11 Typical scheme of procedures for production of *nem chua*

15.4.2 Microbiological and Chemical Indices of *Nem Chua*

Production of *nem chua* is usually in small-scale work using manual labor. *Nem chua* fermentation is a spontaneous process. The final product has pH in the range from 4.3 to 4.6. Lactic acid bacteria are dominant with more than 80 % of the total count. *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *L. brevis*, and *L. farciminis* are frequently found in *nem chua*. The most prevalent is *Lactobacillus plantarum* (Le Thanh Mai et al. 2011). During the first day of fermentation, active growth of yeast is observed. The total number of yeast may reach 5.10^5 cfu/g. Most frequent species are *Candida haemulonii*, *C. halonitratophila*, *C. maltosa*, *C. parapsilosis*, and *C. sake*. At the end of fermentation, the number of yeast reduces to hardly detectable level (Do Thi Thuy Le et al. 2014). Although being very popular, there is some real concern about the hygienic standard of *nem chua*. The main risk associates with the possible contamination of pig parasite, notably the tapeworm *Taenia saginata*. Short-term lactic acid fermentation would not be sufficient to kill the parasite. Sickness caused by the tapeworm is severe and may be fatal. Furthermore, VNT also has observed that during the first day of fermenta-

Fig. 15.12 *Nem chua* wrapped in banana leaf (*left*) and final product (*right*)

tion, depending on samples, yeast microflora of *nem chua* may be dominated by the pathogenic yeast *Malassezia furfur.*

15.5 Fermented Product from Rice and Soybean - *Tuong*

15.5.1 Production Method

Tuong is a popular sauce made from soybean and glutinous rice. The famous brands are Ban (Hung Yen), Cu Da (Hanoi), Kha Do (Vinh Phuc), and Nam Dan (Nghe An). *Tuong* is used as dipping sauce for vegetable and meat and for cooking. Production of *tuong* has long history and especially popular in northern part of Vietnam. Few decades ago, there was a vessel of around hundred liters of *tuong* in nearly every average family in the Red River Delta area. *Tuong* is produced by combination of two substrates that had previously been fermented in separated processes. Glutinous rice is hydrolyzed by fungal enzyme and soybean is hydrolyzed and fermented by the action of bacteria. The mixture of two substrates is fermented further to form the final product. The method for production of *tuong* is presented in Figs. 15.13 and 15.14. For production of *tuong*, glutinous rice is soaked in water for 5–6 h and then cooked by steaming. The cooked rice is spread in a layer of 3–5 cm on a bamboo tray for cooling and for fungi to develop. Usually fungal inoculation is not needed since the working environment and tools are densely loaded with fungal spores from the previous batches. It could be easily observed that inner wall and step door surface of the manufacturing workshop are greenish because of *Aspergillus oryzae* spores. Although some operators do use rice from previous batches for inoculation, most are not aware of the fungal source. After about 3 days of incubation with periodic mixing, rice granules are covered with fungal mycelia. It was then wetted with water containing 2 % of salt at the ratio of 5:1 and transferred to a closed vessel for saccharification. Due to the biochemical reactions, the temperature inside the container may reach 50–60 °C. Mixing is required if the container is overheated. In winter time, the container is covered with mats for insulation. Surprisingly, the opera-

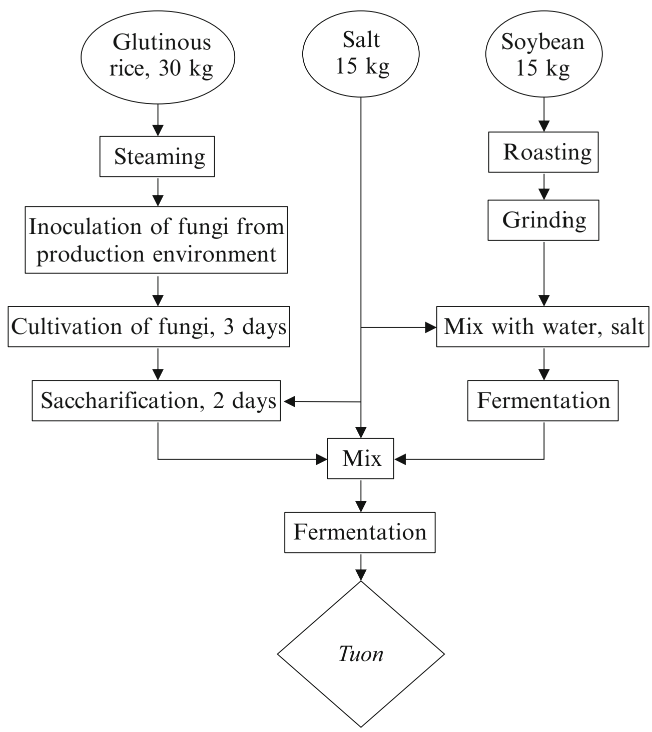


Fig. 15.13 Typical scheme of procedures for production of *tuong* from glutinous rice and soybean

Fig. 15.14 Production of *tuong*. (**a**) Mixing of moldy glutinous rice on bamboo tray; (**b**) racks for cultivation of mold; (**c**) jar of clay for fermentation of *tuong*; (**d**) final product

tors do know the optimum temperature for enzymatic hydrolysis of starch. After about 2 days, due to the action of fungal amylase, the rice granules are partially hydrolyzed and become sweet. It is now ready for mixing with fermented soybean.

In a separate action, soybeans are washed, roasted, grinded, and transferred into a jar of clay. Five portions of saline water are added for fermentation. For acceleration, about 10 % of liquid from the previous batch may be added. After a week of fermentation, the liquid fraction is discarded, and the soybean suspension is mixed with salt and the partially hydrolyzed rice. The fermentation is carried out with periodical mixing and sunning for about 3 months. The final product is amber colored, salty sweet, and with specific aroma of fermented soybean.

15.5.2 Chemical and Microbiological Indices of Tuong

Some important chemical and microbiological parameters of popular *tuong* brands are presented in Table 15.8. Significant variation was observed between different brands and within different manufacturers of the same brand. Because of poorly controlled fermentation, aflatoxin was detected in several samples. Morphologically, *Aspergillus flavus*, a toxin-producing fungus, is difficult to differentiate from *A. oryzae*. In fact, the two are considered to be conspecific. *A. oryzae* is regarded as a domesticated form of *A. flavus* (Gibbons et al. 2012). Esters, butyric acid, isobutyric acid, pyrazine, and maltol are the important compounds contributing to the aroma of *tuong* (Nguyen Thi Viet Anh et al. 2010a).

Table 15.8 Chemical and microbiological indices of different *tuong* brands (Nguyen Thi Viet Anh 2010; Nguyen Thi Viet Anh et al. 2010a)

Brand	Sample	Indices									
		pH	Acid, g/l	Total N, g/l	N amin, g/l	Sugar, g/l	NaCl, g/l	OD	Aflatoxin	LAB, log cfu/ml	Total count, log cfu/ml
Ban	1	4.2	7.0	5.6	4.2	139	101	3.9	+	5.8	7.9
	2	4.2	6.2	6.7	5.3	145	118	4.8	–	7.4	8.5
	3	4.1	7.1	5.9	4.9	142	118	4.2	–	7.1	8.1
	4	4.5	5.6	4.9	4.6	145	125	2.4	–	6.9	8.0
	5	4.2	6.0	4.9	4.1	138	124	2.0	–	7.4	8.6
	6	4.2	6.0	6.9	6.1	141	100	2.2	–	7.3	8.4
	7	4.3	7.1	5.2	4.5	138	111	1.8	–	7.9	7.9
Cu Da	8	4.5	5.2	4.9	4.3	142	85	2.2	+	5.8	7.2
	9	4.5	5.8	7.5	6.3	148	91	2.2	+	5.1	7.3
	10	4.5	5.1	5.8	4.7	143	101	2.6	–	5.9	6.9
	11	4.3	6.0	5.1	4.5	141	111	2.4	–	5.7	6.8
Nam Dan	12	4.2	7.2	5.7	4.3	127	152	2.6	+	5.4	6.8
	13	4.4	5.0	4.6	3.8	132	165	2.1	–	5.4	6.8
	14	4.5	5.8	5.3	4.5	133	158	2.4	–	nd	nd

+ positive, – negative, *nd* no data

15.6 Fermented Vegetables

15.6.1 Production Method

Vietnam is an agricultural country with more than 70 % of the population relying on agriculture for livelihood. With rather high variation in seasonal temperatures, Vietnam has a vast variety of tropical and subtropical vegetables. Similar to some other Asian countries with hot weather, sour vegetables are often included in the meal of Vietnamese people. Fermented vegetables can be served directly along with protein and lipid-rich dishes or used as ingredient for cooking of fish, soup, etc. These lactic acid fermentation products are similar to *kimchi* of Korea, *takana zuke* of Japan, *burong prutas* of the Philippines, and *sayur asin* of Indonesia.

The method for preparation of fermented vegetables is rather simple. Fresh vegetables are sorted and washed and the excess water is removed by draining. In some cases, vegetables are partially dried to increase the dry matter content. Several types of vegetables can be used in mixture for fermentation. For enhancing the flavor and taste, spices may be added. It is then transferred to a vessel containing 3–5 % saline solution. A bamboo grid may be put on top to keep the vegetables submerged. For acceleration of fermentation, small amount of sugar may be added. Anaerobic fermentation is carried out for several days until the product is ready for consumption. Vegetable fermentation is a spontaneous process and relied on the presence of lactic acid bacteria in the substrates. Introduction of fermentation fluid from the previous successful batch may improve the rate of fermentation. A typical scheme for production of fermented vegetables is presented in Fig. 15.15.

15.6.2 Chemical and Microbiological Indices

Important chemical and microbiological parameters of popular types of fermented vegetables are shown in Tables 15.9 and 15.10. Fermented vegetables of the northern areas have relatively lower salt concentration and are similar to the products of Japan (Inatsu et al. 2005). Comparing with plant leaves, higher salt concentration is used for tubers and fruits. Since fermentation of vegetables is a spontaneous process, quality variation is high. Samples collected in the markets showed large discrepancy in pH and bacterial load. In some cases, even products with no sign of fermentation have also been detected. Besides lactic acid bacteria, commercial samples may contain coliform, yeast, and fungi (Cao Hoang Lan et al. 2013; Le Thanh Mai et al. 2011). Lactic acid bacteria occurred in fermented products often were the ones associated with the fresh plant materials (Inatsu et al. 2005). *Lactobacillus fermentum* was found to be dominant in fermented mustard greens (*Brassica juncea*); meanwhile *Lb. pentosus* was found the most common in fermented eggplant (*Solanum macrocarpon*) (Doan Thi Lam Nguyen et al. 2013). Bacterial compositions of fermented mustard greens were as follows: *Lactobacillus fermentum* (57 %), *Lb. pentosus* (24 %), *Lb. plantarum* (17 %), *Pediococcus pentosaceus* (1 %), and *Lb. brevis* (1 %). Besides *Lb. pentosus*, *Bacillus subtilis* was frequently found in fermented eggplant. *B. subtilis* produces biological active compounds that kill gram-negative bacteria (Doan Thi Lam Nguyen et al. 2013). Mustard greens and African eggplant are the main plant species used for production of fermented vegetables in Vietnam. Species of lactic acid bacteria found in fermented vegetables have been shown to produce bacteriocin against pathogens such as *Listeria monocytogenes. Lactococcus lactis* can produce nisin (Inatsu et al. 2005). Application of starter culture may significantly accelerate fermentation of vegetables (Ho Phu Ha et al. 2011. *Lactobacillus* isolated from fermented cucumber could produce GABA in the media containing glutamic acid. These isolates can be used for production of GABA-rich functional foods (Ha Thi Phuong et Nguyen Thi Viet Anh 2012).

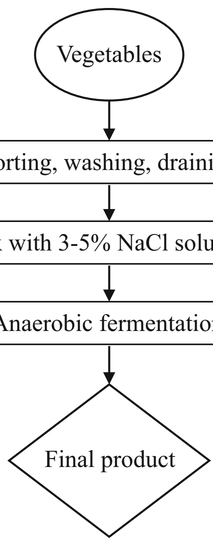


Fig. 15.15 Typical scheme of procedures for production of fermented vegetables

Table 15.9 Chemical and microbiological indices of fermented vegetables (Nguyen Thi Viet Anh, unpublished data)

Plant species	Place of sample	Indices				
		pH	Salt, %	Aerobic, log cfu/g	LAB, log cfu/g	Coliforms
Brassica juncea (mustard greens)	Hanoi, Ha Nam, Bac Giang	3.5–5.5	0.8–2.2	4–8	5–8	2–5
Solanum macrocarpon (African eggplant)	Hanoi, Ha Nam, Bac Giang, Nghe An, Quang Binh	3.2–6.5	1.2–2.8	5–8	4–8	2–5
Artocarpus heterophyllus (jackfruit)	Nghe An, Ha Tinh, Quang Binh	4.8–6.4	1.5–3.0	3–6	2–5	2–3
Allium fistulosum (Welsh onion)	Hanoi, Thanh Hoa, Nghe An	3.2–5.2	0.7–1.8	4–8	3–8	2–3
Brassica oleracea (cabbage)	Hanoi	3.8–6.4	0.3–0.5	6–8	5–8	2–4
Allium chinense (Chinese onion)	Hanoi, Nghe An, Ho Chi Minh city	3.5–5.6	0.4–1.4	5–7	4–7	2–3

Table 15.10 List of microorganisms found in fermented vegetables

Plant species	Gram (–) bacteria	Lactic acid bacteria	Source
Brassica juncea (mustard greens)	*Citrobacter freundii*, *Klebsiella oxytoca*, *Proteus vulgaris*	*Lb. curvatus*, *Lb. fermentum*, *Lb. pentosus*, *Lb. plantarum*, *Lb. mesenteroides*, *Pd. pentosaceus*	Inatsu et al. (2005)
Solanum macrocarpon (African eggplant)	*K. pneumoniae*, *Providencia rettgeri*	*Lb. acidophilus*, *Lb. brevis*, *Lb. delbrueckii*, *Lb. pentosus*, *Lb. plantarum*, *Lactococcus lactis*, *Leuconostoc lactis*	Inatsu et al. (2005)
Artocarpus heterophyllus (jackfruit)		*Lb. plantarum*, *Lb. acidophilus*	Nguyen Thi Viet Anh (2010)
Allium fistulosum (Welsh onion)	*Enterobacter cloacae*, *K. pneumoniae*, *Prov. rettgeri*	*Lb. curvatus*, *Lb. delbrueckii*, *Lb. pentosus*, *Lb. plantarum*, *Lc. lactis*, *Leuconostoc mesenteroides*, *Pd. pentosaceus*	Inatsu et al. (2005)
Brassica oleracea (cabbage)	*Cit. youngae*, *Ent. amnigenus*, *Ent. cloacae*, *K. oxytoca*	*Lb. plantarum*, *Leuc. citreum*, *Leuc. mesenteroides*, *Pd. pentosaceus*	Inatsu et al. (2005)
Allium chinense (Chinese onion)	*Cit. freundii*, *Cit. youngae*, *K. pneumoniae*, *Prov. alcalifaciens*	*Lb. acidophilus*, *Lb. pentosus*, *Lb. plantarum*, *Lc. lactis*, *Leu. mesenteroides*	Inatsu et al. (2005)

15.7 Conclusion

Traditional fermentation has a special position in daily life of Vietnamese people. It provides indispensable foods, beverages, and ingredients for Vietnamese cuisine. The patterns of traditional fermentation in Vietnam are similar to that of Thailand, Indonesia, and the Philippines. Most of the fermentation processes are spontaneous and safety standard is of concern. Despite the success of industrialization in food industry, traditional fermentation demonstrates a tenacious and conservative vitality. Understanding of the traditional fermentation processes is needed in order to preserve and to improve the technologies and traditions.

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