Ethnic Fermented Foods and Beverages of Vietnam

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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.

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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

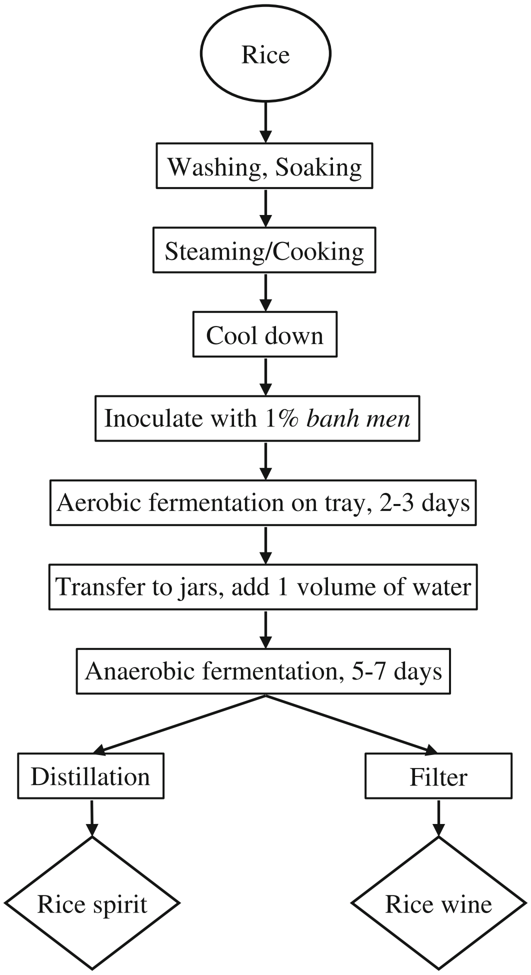

Fig. 15.2 Schematic presentation of typical technology for production of rice spirit and rice wine

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

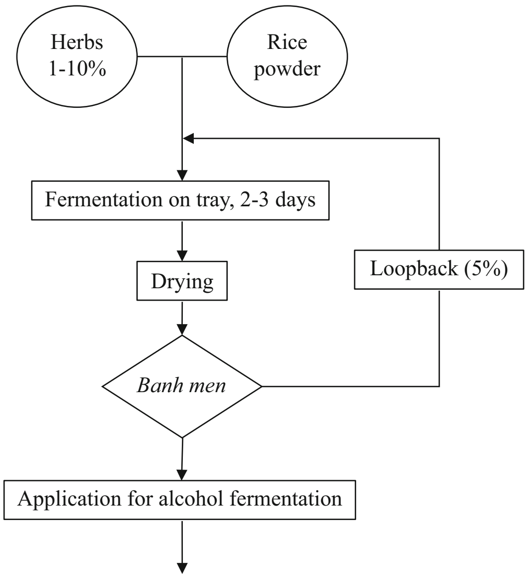

Fig. 15.3 Schematic presentation of typical *banh men* starter production technology

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

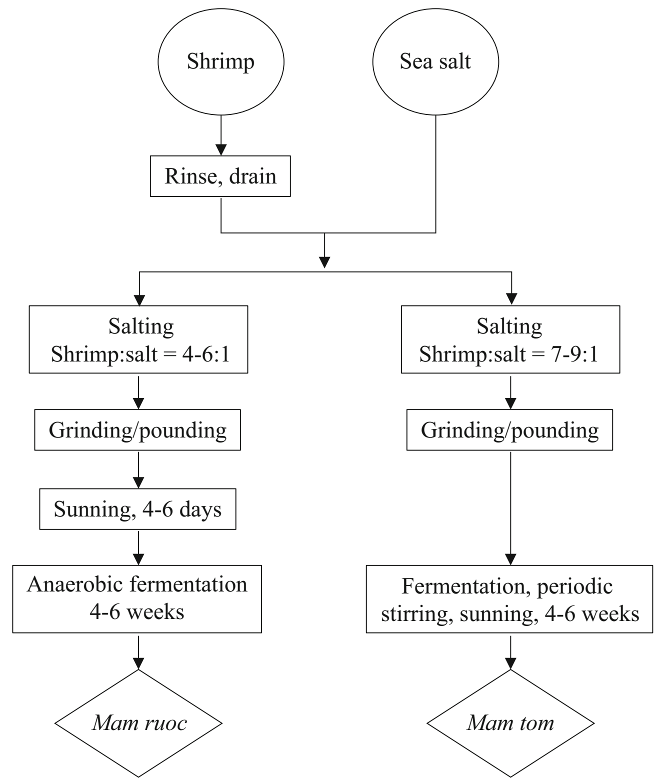

Fig. 15.8 Schematic procedures for production of *mam ruoc* and *mam tom* type of shrimp pastes

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.

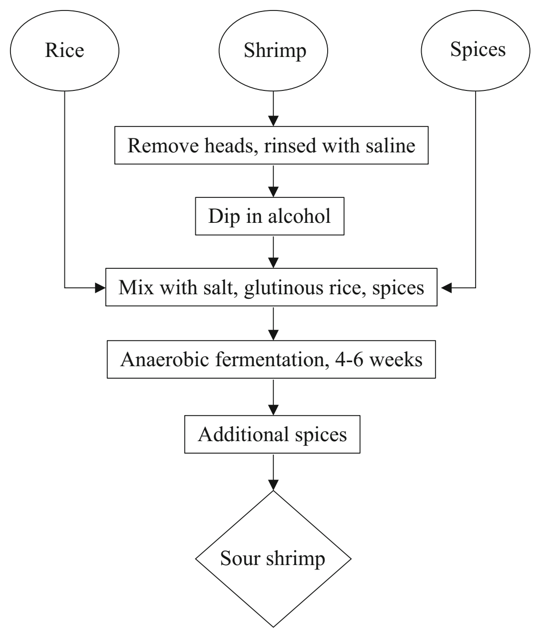

Fig. 15.9 Typical scheme of procedures for production of Hue sour shrimp

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).

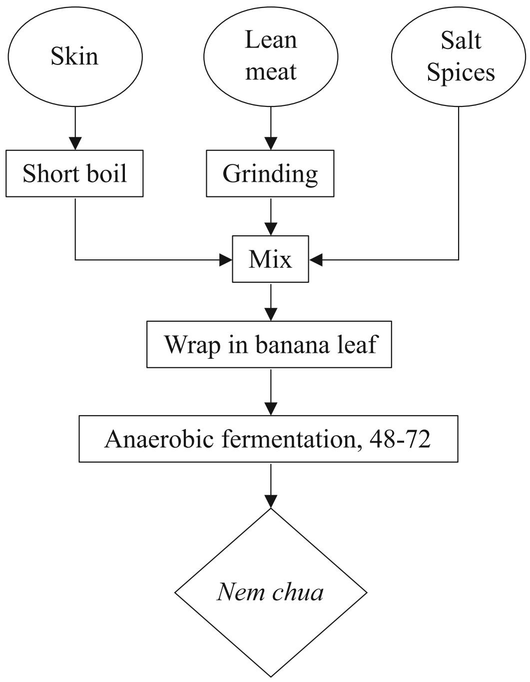

Fig. 15.11 Typical scheme of procedures for production of *nem chua*

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-

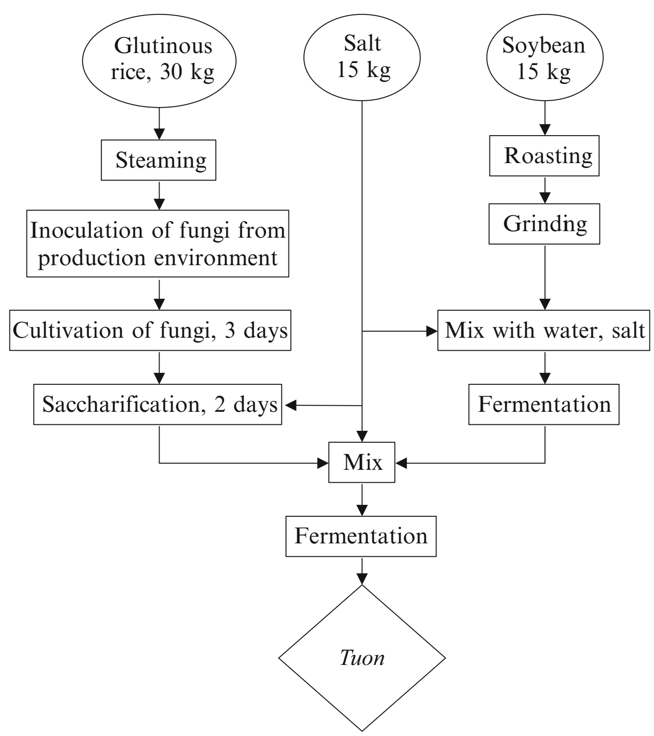

Fig. 15.13 Typical scheme of procedures for production of *tuong* from glutinous rice and soybean

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).

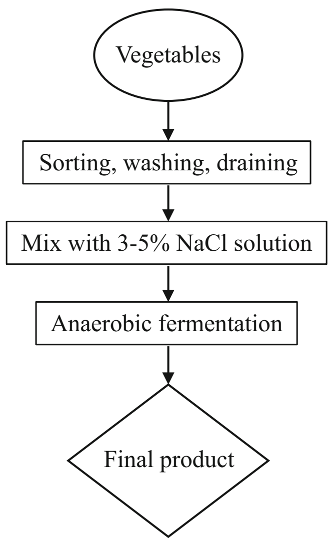

Fig. 15.15 Typical scheme of procedures for production of fermented vegetables

Table 15.9 Chemical and microbiological indices of fermented vegetables (Nguyen Thi Viet Anh, unpublished data)

		Indices				
Plant species	Place of sample	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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