

REVIEW OF LITERATURE

Extensive review of available literatures of some common soybean-fermented foods across the world has been mentioned below. Table A shows a comprehensive list of some familiar fermented legume (soybean and non-soybean) products of the world.

Table A. Some fermented legume products of the world

Fermented Product	Substrate	Nature and use	Country	Microorganisms
<i>Bekang</i>	Soybean	Alkaline, sticky, paste; Side dish.	India	<i>Bacillus</i> spp.
<i>Chungkokjang</i>	Soybean	Alkaline, sticky; Condiment, soup	Korea	<i>Bacillus</i> spp.
<i>Dawadawa</i>	Locust bean	Alkaline, sticky; Condiment, soup.	Ghana	<i>Bacillus</i> spp.
<i>Dhokla</i>	Bengal gram	Mild acidic, spongy; Steamed, snack	India	LAB, yeasts
<i>Doenjang</i>	Soybean	Alkaline, paste; soup.	Korea	Moulds
<i>Douchi</i>	Soybean	Alkaline, sticky; Condiment, soup.	China	<i>Bacillus</i> spp., moulds

Continued (Table A)

<i>Kecap</i>	Soybean (black)	Syrup; seasoning agent	Indonesia	Mould
<i>Khaman</i>	Bengal gram	Mild acidic, spongy; Breakfast food	India	LAB
<i>Kinema</i>	Soybean	Alkaline, sticky; Curry, soup	India, Nepal, Bhutan	<i>B. subtilis</i> , <i>Enterococcus faecium</i>
<i>Maseura</i>	Black gram	Dry, ball-like, brittle; Condiment	Nepal, India	Bacilli, LAB, yeasts
<i>Meitauza</i>	Soybean	Liquid; drink	China, Taiwan	Mould
<i>Meju</i>	Soybean	Alkaline, paste; seasoning agent	Korea	Mould
<i>Miso</i>	Soybean	Alkaline, paste; Soup	Japan	Mould
<i>Natto</i>	Soybean	Alkaline, sticky; Side dish, breakfast	Japan	<i>Bacillus natto</i>
<i>Oncom</i>	Peanut/groundnut	Alkaline, solid cake; Roasted or fried	West Java	Mould
<i>Pepo-ke</i>	Soybean	Alkaline, sticky; Side dish	Myanmar	<i>Bacillus</i> spp.

Continued (Table A)

<i>Soya-sauce</i>	Soybean	Alkaline, liquid; Seasoning	Worldwide	Mould
<i>Sufu</i>	Soybean curd	Mild-acidic, soft; Side dish	China, Taiwan	Mould
<i>Tauco</i>	Soybean	Alkaline, paste; Soup	Indonesia	Mould
<i>Tempe</i>	Soybean	Alkaline, solid; Fried cake, side dish	Indonesia (origin), The Netherlands, Japan, USA	<i>Rhizopus oligisporus</i> , <i>Klebsiella pneumonia</i>
<i>Thua-nao</i>	Soybean	Alkaline, paste, dry; Soup	Thailand	<i>Bacillus</i> spp.
<i>Turangbai</i>	Soybean	Alkaline, sticky; Curry, soup	India	<i>Bacillus</i> spp.
<i>Ugba</i>	African oil bean (<i>Pentaclethra macrophylla</i>)	Alkaline, flat, glossy, brown in color; Side dish	Nigeria	<i>Bacillus</i> spp.
<i>Wari</i>	Black gram	Ball-like, brittle; Condiment	India	LAB, yeasts

Chungkookjang

Chungkookjang (or *jeonkukjang*, *cheonggukjang*) is an ethnic fermented soybean food of Korea (Shon *et al.*, 2007). During the preparation of *chungkookjang*, boiled soybeans covered with matting made of straws are put on an ondol, Korean under-floor heating. After 3-day fermentation, the soybeans are broken, mixed with soybean powder, ground with salt and dried in the sun (Nagai and Tamang, 2010). The fermentation period of *chungkookjang* is longer than that of *natto*, which makes its colour darker than that of *natto*. Such a longer fermentation period causes production of ammonia. It is consumed as soup by Koreans. Species of *Bacillus* isolated from naturally fermented *chungkookjang* are *B. subtilis*, *B. licheniformis* and *B. amyloliquefaciens* (Tamang *et al.* 2002; Choi *et al.*, 2007, Kwon *et al.*, 2009), *B. subtilis* subsp. *chungkookjang* (Park *et al.*, 2005), *B. megaterium* (Shon *et al.*, 2009). *Enterococcus faecium* is also present in *chungkookjang* (Yoon *et al.*, 2008). *B. subtilis* isolated from *chungkookjang* produces viscous polyglutamate and fibrinolytic activity (Lee *et al.*, 1991). Hara (1990) also isolated *B. subtilis* from *chungkookjang* and shown that the plasmid was homologous to pUH1. These studies indicate the microorganism which contributes to *chungkookjang* fermentation is *B. subtilis* (*natto*). However, *B. licheniformis* B1, which was isolated from soil in Korea, is known to produce *chungkookjang* with a good quality (Lee *et al.*, 1999; Choi *et al.*, 2007). Phylogenetic analyses

were carried out on selected strains of *B. subtilis* isolated from three Asian fermented soybean foods- *kinema*, *chungkookjang* and *natto*, and found the highest similarities with the *B. subtilis* type strain (Tamang *et al.*, 2002). Kim *et al.* (1996) reported that the fibrinolytic enzyme (CK) purified from *bacillus* sp. strain CK 11-4 showed thermophilic, hydrophilic, and strong fibrinolytic activity. Shon *et al.* (2007) reported that *B. megaterium* SMY-212 is suitable fermenting strain to promote the antioxidant and free-radical scavenging activities in *chungkookjang*. In cellular defense system, scavenging of free radicals is an important issue affiliated by utilization of both exogenous and endogenous antioxidants because the increase in production of free radicals has been reported to cause damage to cell membranes, enzymes, DNA, lipids, and proteins, impairing their function (Gu *et al.*, 1998). Although the body possesses defense mechanisms as enzymes and antioxidant nutrients (Halliwell *et al.*, 1995), continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them and cause irreversible oxidative damage (Tseng *et al.*, 1997). Increase in total phenol content has also been reported in *chungkokjang* (Shon *et al.*, 2007). It has been reported that the seed coat of black soybean had higher polyphenol (total phenol contents) content than that of yellow soybean (29.0 ± 0.56 and 0.45 ± 0.02 mg/g, respectively), So it suggests that black soybeans

may be more effective in inhibiting low density lipoprotein (LDL) oxidation than yellow soybeans because of total phenol contents in its seed coat (Takashi *et al.*, 2005). Super high molecular weight PGA (poly- γ -glutamic acid) has been reported to be synthesized by *B. subtilis* subsp. *chungkokjang*, isolated from *chungkokjang* (Park *et al.*, 2005). Kubota *et al.*, (1993) reported that the PGA biopolymer have molecular weight ranging from 100 to over 1,000 KDa. Stereochemical structures of microbial γ -PGA can be divided into three types: a homopolymer of d-glutamic acid (γ -d-PGA), a homopolymer of l-glutamic acid (γ -l-PGA), and copolymer of random combinations of d-/l-glutamic acid (γ -dl-PGA).

Dawadawa

Dawadawa is a non-salted, fermented food prepared from African locust beans (*Parkia biglobosa* Welw. Ex Oliv.), a perennial tree legume, commonly consumed in the Savannah regions of West Africa (Campbell-Platt, 1980; Eka, 1980; Odunfa, 1985). Although *dawadawa* is used as a seasoning for soup and stew, it contributes to protein intake for African people. *Dawadawa* is well known in Ghana, similar product is called *iru* in Nigeria, *kinda* in Sierra Leone and *soumbala* in Burkina Faso (Achi, 2005, Azokpota *et al.*, 2006). *Daddawa*, a protein and fat-rich flavorsome ingredient is used as a good condiment and eaten with sorghum or

millet-based dumplings and porridges (Campbell-Platt, 1980; Odunfa, 1986).

Daddawa preparation is still a traditional family art done in homes. In the traditional method of its preparation, the dried pods are boiled for 16-24 h to soften the tough testa and cotyledons. The seeds are put in a mortar, pressed with feet to remove the softened testa; sand or other abrasive agents may be added. The cotyledons are washed and boiled again for 1-2 h. Excess water is drained off. The beans are spread on a calabash tray and covered with a cloth. The beans are fermented naturally for 2-4 days. Wood ash may be mixed to reduce the odor. Sometimes, millet flour may be added. The fermented bean mass is sun-dried, and then used loose, or shaped into balls or pyramids and stored in the traditional earthenware pots (Campbell-Platt 1980; Odunfa 1981, 1985, 1986).

The microorganisms involved in fermentation of *dawadawa* are *Bacillus subtilis*, *B. licheniformis*, *B. pumilus*, *B. megaterium*, *Leuconostoc mesenteroides*, *L. dextranicus*, and relatively low number of *Staphylococcus* spp. and *Micrococcus* spp. The presence of *Bacillus subtilis*, *B. licheniformis* and *Staphylococcus* spp. was reported in *dawadawa* (Odunfa 1981, 1986). Antai and Ibrahim (1986) reported the presence of *Leuconostoc mesenteroides* and *L. dextranicus* in almost equal proportion with the *Bacillus* spp. in *dawadawa*. However, Ogbadu and Okagbue (1988) could not find

any of these lactics during *daddawa* production. They found that the species of *Bacillus* responsible for *daddawa* production were variable, and reported *B. subtilis*, *B. pumilus* and *B. licheniformis* from six separate fermentations. Osinowo *et al.* (1990) reported *B. subtilis*, *B. cereus*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes* from *daddawa*. Cooking and fermentation of the locust beans (which is indigestible and poisonous to human) reduce the toxic substance and increase the digestibility. During fermentation, the temperature and pH of the beans increased from 25°C and 7.0 at 0 h to 45°C and 8.1 at 36 h, respectively (Odunfa, 1981). *Daddawa* contains 20-50% moisture, and per 100 g dry matter: 40-45 g protein, 30-40 g fat, 10-15 g carbohydrate, 3-7 g fibre, 3-6 g ash, 2.1-2.3 MJ (500-600 kcal) energy, 300 mg Ca, 550 mg P, 40 mg Fe, 0.05 mg thiamine, 0.6 mg riboflavin, 2 mg niacin and 0.9 µg folic acid (Campbell-Platt, 1987). The fatty acids in both unfermented and fermented beans were linoleic, oleic, stearic, palmitic and a trace of arachidonic acids. The fatty acids in both unfermented and fermented beans were linoleic, oleic, stearic, palmitic and a trace of arachidonic acids. The major fatty acid was linoleic acid which is an essential fatty acid (Odunfa and Adesomoju 1986). The quantities of the flatus-forming oligosaccharides decreased significantly during the first 24 h of fermentation and this decrease was attributed to the activities of α - and β -galactosidase which hydrolyzed the oligosaccharides to

reducing sugars (Odunfa, 1983). Thiamine and riboflavin content increased during fermentation (Eka, 1980). Meerak *et al.* (2008) reported γ -polyglutamic acid (PGA)-producing *Bacillus* isolated from Ghanaian *dawadawa* which included *B. subtilis*, *B. amyloliquefaciens*, *B. pumilus* and *B. licheniformis*.

Dhokla

Dhokla is an ethnic fermented spongy-textured product of Gujarati in 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. Dry seeds of Bengal gram and white polished rice are washed and soaked for 5-10 h. It is then ground, salt and water is added to make a thick paste. The slurries are left for natural fermentation in a warm place (30 °C-32 °C) for 8-10 h. The fermented batter is poured into a greased pie tin and steamed in the open rather than in a covered *idli* steamer. After 10-15 minutes of steaming *dhokla* is ready for consumption. It can be consumed directly “out-of-hand” following steaming or the cakes may be deliciously flavored with fried mustard seeds and chopped coriander leaves. The unflavored cakes are eaten with chutney and/or *sambar*, a thin spiced soup of *dhal* and vegetable. It is an important source of protein and calories in the diet and nutrition of South Indians. *Lactobacillus mesenteroides* and *Streptococcus faecalis* are essential and responsible for leavening of batter and

acid production 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).

Doenjang

Doenjang is a naturally fermented soybean paste of Korea similar to Japanese *miso* (Min and Kim, 1990). It is light grayish-brown and slightly chunky from the small proportion of uncrushed soybeans it contains. To prepare *doenjang*, cooked soybeans are pounded and mashed in a mortar, shaped into balls, wrapped in rice straw, and hung under rafters until each ball is covered with a white bloom of natural mold. Next the balls are crushed and mixed with salt and water to form *meju*, sometimes with the addition of sesame seeds or leaves, and placed in a earthenware container of 1-10 gal capacity. The fermentation period generally lasts for 6 months. It is used as seasoning or consumed as soup in Korea with boiled rice.

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



Douchi

Douchi is a traditional, salt-fermented soybean product of China. It has been used as a food seasoning and for pharmaceutical purposes since before the Han dynasty (206 BC) (Tamang and Samuel, 2010). In *Ben Cao Gang Mu* (Chinese Materia Medica), which was written by Li Shizhen at Min dynasty (1368-1644 A.D.), recorded some healthy functions of *douchi*, such as enhancing the appetite, promoting digestion, preventing asthma and etc. (Bao, 1985). Even today, *douchi* is still added to some Chinese traditional medicines (Zhang *et al.*, 2006). Four types of *douchi* are produced in China: *Mucor*-fermented *douchi*, *Aspergillus*-fermented *douchi*, *Rhizopus*-fermented *douchi*, and *Bacillus*-fermented *douchi* (Zhang *et al.*, 2007).

Douchi is prepared from soybeans by pretreatment and a two-step fermentation process (primary and secondary fermentation) as described by Kang (2001). *Douchi* is prepared from soybeans by pretreatment and a two-step fermentation process (primary and secondary fermentation) as described by Kang (2001). The soybeans are soaked in water and boiled for about 1 h but for bacterial-type *douchi*, the beans only need to be boiled for 30-40 min. The second step is *Qu*-making, where the boiled beans are inoculated with *Aspergillus oryzae* (0.3%) or *Mucor* strain spores (0.5%) or simply incubated at high temperature (over 25°C) for 3-4 days to harvest matured *qu*. In

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

Three types of *douchi* are fermented by *Mucor*, *Bacteria* and *Aspergillus* strains respectively. Among them *Aspergillus-type douchi* is the earliest, and has been the most popular type in China (Bao, 1985). The presence of two biogenic amines viz. cadaverine and putrescine leads to histamine toxicity in *douchi* by inhibiting histamine-metabolising enzymes, such as diamine oxidase and histamine methyl transferase (Arnold and Brown, 1978; Bjeldanes *et al.*, 1978; Lehane and Olley, 2000). *Douchi* produces angiotensin I-converting enzyme (ACE) inhibitors with the potential to low blood pressure (Zhang *et al.*, 2006). Te Li *et al.* (2007) reported that the anti- α -glucosidase activity was exhibited by *douchi qu* fermented with *Aspergillus oryzae*, and this activity

becomes highest at 5.0 % and 7.5 % salt levels. *Bacillus subtilis* isolated from Chinese fermented soybean seasoning produced bacteriocin (Zheng and Slavik, 1999). Changes in isoflavone isomer distribution were found to be related to β -glucosidase activity during *douchi* fermentation, which was affected by NaCl supplementation (Wang *et al.*, 2007b). Wang *et al.* (2007a) reported that DPPH and ABTS radical scavenging activity of *douchi* extracts increased significantly during the prefermentation ($p < 0.05$) but the activity decreased during the *douchi* fermentation due to high salt addition.

Kinema

Kinema is an ethnic bacilli-fermented, sticky soybean food with a slight ammonia flavor, consumed in the Darjeeling hills and Sikkim of India, eastern Nepal and some parts in Bhutan (Tamang *et al.*, 1988; Tamang, 2001). *Kinema* is one of the oldest cultural foods of the non-Brahmin Hindu Nepali (Tamang, 2010a).

During traditional preparation of *kinema*, the small-sized 'yellow cultivar' soybean dry seeds are preferred for preparation of *kinema* (Tamang, 2001), whereas in Nepal dark brown local varieties of soybean seeds are selected for making *kinema* (Nikkuni, 1997). Soybeans are washed, soaked overnight, and soaked soybeans are taken out and put into the container with fresh water, and boiled for 2-3 h until they are soft. Excess water

is drained off and the cooked soybeans seeds are filled into the wooden mortar locally called *okhli*, and are cracked lightly by a wooden pestle locally called *muslo* to split the cotyledons. This practice of cracking cooked seeds of soybeans is observed only during *kinema* production unlike *natto* and *chungkokjang*, probably to increase the surface areas for speed fermentation by aerobic spore-forming bacteria. About 1 % of firewood ash is added directly to the cooked soybeans and mixed thoroughly to maintain the alkaline condition of the product. Soybean grits are placed in a bamboo basket lined with locally grown fresh fern called *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 kitchen. During summer, the fermentation time may require 1-2 days while in winter it may require 2-3 days.

Species of *Bacillus* isolated from *kinema* include *B. subtilis*, *B. licheniformis*, *B. cereus*, *B. circulans*, *B. thuringiensis* and *B. sphaericus* (Sarkar *et al.*, 1994, 2002; Tamang, 2003]. However, *B. subtilis* is the dominant functional microorganism in *kinema* (Sarkar and Tamang, 1994; Tamang and Nikkuni, 1996). Besides bacilli, lactic acid bacterium is identified as *Enterococcus faecium*, and two types of yeasts are *Candida parapsilosis* and *Geotrichum candidum* were also isolated from *kinema* samples (Sarkar *et al.*, 1994). Hara *et al.* (1995) suggested that the plasmid pKNH of

kinema Bacillus might be responsible for γ -PGA production in *kinema* fermentation. Total amino acids, free amino acids and mineral contents increased during *kinema* fermentation, and subsequently enriched the nutritive value of the product (Sarkar and Tamang, 1995; Nikkuni *et al.*, 1995; Sarkar *et al.*, 1997b; Sarkar *et al.*, 1998; Tamang and Nikkuni, 1998). Degradation of oligosaccharides has also been reported in *kinema* (Sarkar *et al.*, 1997a). Nout *et al.* (1998) reported that the traditional way of making *kinema* and its culinary use is safe. Pulverized starter using *B. subtilis* KK-2:B10 was developed for *kinema* production (Tamang, 1999). Free fatty acid content of the *Bacillus*-fermented *kinema* was 140% less than that of naturally fermented one (Sarkar and Tamang, 1995). Foodborne pathogens were also present like *Bacillus cereus* exceeded 10^4 cfu/g, Enterobacteriaceae and coliforms exceeded 10^5 cfu/g in few samples of *kinema* (Nout *et al.*, 1998). Sarkar *et al.* (1998) reported that incubation of soybeans at 37° C for 48 h, when mixed with *Bacillus subtilis*, caused an increased concentration of both thiamine (B₁) and riboflavin (B₂). The presence of *Enterococcus faecium* had no detectable effects on the growth of the *Bacillus*, proteolytic activity, ammonia production or final pH of *kinema* fermentations (Owens *et al.*, 1993). *Kinema* has many health-promoting benefits due to high content of Group B saponin (Omizu *et al.*, 2011). The lipids identified in soybeans are campesterol,

stigmasterol and β -sitosterol, which increased during fermentation by 50-61% (Sarkar *et al.*, 1996). Moktan *et al.* (2008) evaluated the antioxidant activities of methanolic extract of *kinema*, fermented with *B. subtilis* and cooked non-fermented (CNF) soybean by *in vitro* methods namely stable DPPH- scavenging activity, Fe^{3+} -reducing power, Fe^{2+} -chelating activity, and activity in linoleic acid emulsion system. Total phenol content of *kinema* was 144% higher than that of CNF soybean (3.3mg/g dry weight) and at all the tested concentration *kinema* was found to be a better free radical scavenger, which increased in a time and dose dependent manner than the CNF soybean (Moktan *et al.*, 2008). Increase in total phenol content from 0.42 mg GAE (gallic acid equivalent)/g in boiled soybean to 2.3 mg GAE/g in *kinema* has been observed (Tamang *et al.*, 2009).

Maseura

Maseura is an ethnic fermented black gram or green gram product of Nepali living in the Himalayas (Tamang, 2010a). It is a cone-shaped hollow, brittle and friable product. It is a partially fermented legume based savoury prepared from black gram and colocasia tuber by traditional sun drying and an alternative cabinet drying. *Maseura* is similar to North Indian *wari* and South Indian *Sandige* (Soni and Sandhu, 1990a; Dahal *et al.*, 2005).

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

Biochemical and nutritional evaluation of *maseura* of Nepal was reported by Dahal *et al.* (2003). Species of bacteria present in *maseura* are *Lactobacillus fermentum*, *Lb. salivarius*, *Pediococcus pantosaceus*, *P. acidilactici*, *Enterococcus durans*, *Bacillus subtilis*, *B. mycoides*, *B. pumilus* and *B. laterosporous*; yeasts are *Saccharomyces cerevisiae*, *Pichia burtonii*, *Candida castellii*, and *C. versatilis*; and molds are species of *Cladosporium*, *Penicillium*, and *Aspergillus niger* (Dahal *et at.*, 2003; Chettri and Tamang, 2008). Moisture content of dried *maseura* is about 8-10 %, protein (18-20

%), carbohydrate (67-70 %), and minerals (Dahal *et al.* 2003). Increase in soluble protein, amino nitrogen, non-protein nitrogen, thiamine and riboflavin has been observed in *maseura* (Dahal *et al.*, 2003).

Miso

Miso is a semi-solid fermented food made from soybeans, rice or barley, and salt is mainly used for preparing *miso*-soup in Japan (Ebine, 1989; Mullin, 2005). The most popular *miso* in Japan is rice *miso*, which is prepared by mixing cooked soybeans with *koji* (steamed rice on which *Aspergillus oryzae* is cultured), salt and a small amount of water is added to control the moisture level. The mixture is then allowed to ferment (Ebine, 1989). Carbohydrates, proteins and lipids are hydrolysed by the enzymes produced by *Aspergillus oryzae* during *miso* fermentation (Shibaki and Hesseltine, 1962; Mochizuki and Imai, 1982; Ebine, 1989). Halo-tolerant yeasts and halophilic lactic acid bacteria to developing the flavor of *miso* by producing alcohols and organic acids (Mochizuki and Imai, 1982; Ebine, 1989). Saltiness of *miso* is very strong in the first stage of fermentation but becomes mild during fermentation progress (Ebine, 1989). This phenomenon is called “Shio-nare” and it plays an important role in *miso*-soup making. The value of hardness of cooked soybeans reached 500g after 2 h of cooking. Usually, the desired hardness of soybeans is

said to correspond approximately to 500g to 600g (Mochizuki and Imai, 1982). Nikkuni *et al.* (1996) reported that *Lactobacillus fructivorans* L-1 causes swelling of packaged processed *miso* by its heterofermentation producing carbon dioxide gas and ethanol.

Aspergillus oryzae, *A. sojae*, *A. kawachii*, *A. shirousamii*, and *A. awamori* have been widely used as the starter in preparation of *koji* in Japan for production of *miso* and *shoyu* (Kitamoto, 2002; Matsushita *et al.*, 2009) and in later stage of fermentation, *Zygosaccharomyces rouxii* and/or *Candida versatilis* are added to the mixture (Sugawara, 2010). Microbial composition in *miso* is almost the same as those for *shoyu*; however, the degree of hydrolysis is much lower in *miso* than in *shoyu*, and the product is a solid paste.

Natto

Natto is a Japanese fermented soybean food, characteristic of odor of ammonia and fatty acids and viscous polymer of glutamate. It is *B. subtilis* (*natto*)-fermented soybean food eaten in breakfast (Nikkuni, 1997, Kubo *et al.*, 2011). It is gray to tan in colour, and has a strong and persistent unique flavour, sometimes associated with a noticeable odour of ammonia (Steinkraus, 1983; Ohta, 1986). *Itohiki-natto* (sticky *natto*) is produced by fermenting whole cooked soybeans with *Bacillus subtilis* and accounts for more than the total production of the other two major types of

natto. *Itohiki-natto* was traditionally consumed by the Buddhist monks and also by the farmers during winters (Ohta, 1986). Japanese *natto* is the only bacilli-fermented soybean food which is now produced by commercial mono-culture starter *B. natto*, first isolated from naturally fermented *natto* by Sawamura (Sawamura, 1906). *B. natto* differs from *B. subtilis* due to biotin requirement, production of polyglutamate, possession of 5.7-kb and 60-kb plasmids (Hara *et al.*, 1983; Nagai *et al.*, 1997), and insertion sequences (Nagai *et al.*, 2000; Kimura and Itoh, 2007). Commercial *natto* starter strains are classified as *B. subtilis natto* closely related to the laboratory strain *B. subtilis* Marburg 168, which has about 4,100 protein-encoding genes in a 4,215 kbp genome (Nishito *et al.*, 2010). *Natto* has a high nutritional value, improved digestibility and an appreciable amount of certain vitamins, produced as a result of fermentation (Standal, 1963; Reddy *et al.*, 1986; Steinkraus 1983; Ohta, 1986). *Natto* is a good source of fibre and free fatty acids (Ohta, 1986). Hayashi and Nagao. (1975) reported that conversion of bacterial cells to spores during preservation increases the nutritive value of *natto*. It contains 50-65% moisture, and per 100 g dry matter: 45-55 g protein, 23-28 g fat, 10-15 g carbohydrate, 4-6 g fibre, 5-10 g ash (higher, if salt added), 2.0 MJ (470-490 kcal) energy, 300 mg Ca, 300 mg P, 1200 mg K, 15 mg Fe, 0.1 mg thiamine, 0.6 mg riboflavin, 1.3 mg niacin, 60 µg β-carotene and 20 mg vitamin C

(Campbell-Platt, 1987). Iwai *et al.* (2002) suggested that water – soluble *natto* fractions might help to prevent arteriosclerosis, as they appear to reduce lipid peroxidation and improve lipid metabolism. The fatty acid composition of *natto* and soybeans does not differ significantly. *Natto* was kept in good conditions at 35° C with an inoculation of 10⁴~10⁶ cells/g or at 40° C. 10²~10⁴ cells/g of starter, judging from the appearance, color tone and hardness at 18~20 fermentation hours (Matsumoto *et al.*, 1993). The crude α -amylase and β -galactosidase produced by *Bacillus subtilis* strain exhibited maximal activities at 135° C and 65° C, respectively, and were also found to be significantly stable at elevated temperatures (Konsoula and Liakopoulou-Kyriakides, 2007).

The riboflavin content of *natto* increased after fermentation (Arimoto, 1961). *B. subtilis* can secrete many extra cellular enzymes such as protease, amylase, γ -glutamyltranspeptidase (GTP), levansucrase, etc. (Ogawa *et al.*, 1991; Urushibata *et al.*, 2002). Cloning of DNA fragment from *B. natto* enhances the production of the extracellular proteases and levansucrase of the host *B. subtilis* cells (Nagami and Tanaka, 1986). Several strains of *B. subtilis* (*natto*) have been selected with superior functional properties such as high γ -GTP, thrombolytic, elastase activities and high viscosity for *natto* production (Nagai *et al.*, 1997; Chang *et al.*, 2000). *Natto* has been reported to have strong antioxidative function (Iwai *et al.*, 2002). Yuki *et al.* (1994) reported that

nattokinase, one of the proteases found in *natto*, is the only fibrinolytic enzyme in *natto*. The viscous material in *natto* is γ -PGA (polyglutamic acid), containing D- and L-glutamate in varying proportions depending on the amount of Mn^{2+} ions and the kinds of amino acids (Hara *et al.* 1986). *Bacillus subtilis* (*natto*) produces a unique capsular polymer of glutamic acid with γ -peptide linkage, poly- γ -glutamic acid (PGA) and the most striking feature of the γ PGA produced by *B.subtilis* (*natto*) include its very large molecular mass of over 10^5 Da and the presence of both L- and D- glutamic acids (Leonard *et al.*, 1958; Saito *et al.*, 1974). Saito *et al.* (1974) found that *natto* mucin is composed of 58% γ -polyglutamic acid and 40% polysaccharide. In *B.subtilis* (*natto*) earlier studies claimed that γ -glutamyltranspeptidase (γ GTP) which is encoded by the small plasmid pUH1(5.8 kb), catalyses the γ PGA synthesis (Hara *et al.*, 1983), but the other evidences argued against the participation of γ GTP, in γ PGA synthesis in vivo (Koehler and Thorne, 1987; Troy, 1979). However, Nagai *et al.* (1997) have also reported that plasmid gene is not required or involved in γ PGA production and γ PGA producing *B. subtilis* without a plasmid could be isolated from its natural habitat. Some of the flavour originates from the hydrolysis of soya protein to peptides and amino acids (Ohta, 1986). Low concentration of Mn^{2+} favors the L-isomer whereas high concentration results in a polymer containing upto 93 % of the D-isomer (Hara *et al.*, 1982). Urushibata *et al.*

(2002) reported that the *ywsC* gene encodes PGA synthetase (EC 6.3.2), a crucial enzyme in PGA biosynthesis. The genes for polyglutamate production, *pgsBCA*, were cloned, sequenced and expressed in *Escherichia coli* clone cells (Ashiuchi *et al.*, 1999). Tsukamoto *et al.* (2000) reported that intake of fermented soybean (*natto*) increases serum levels of MK-7 and γ -carboxylated osteocalcin in normal individuals. Meerak *et al.* (2007) reported that strains isolated as bacteria producing PGA from *natto*-like fermented foods in various Asian countries were divided into two clusters identified as *B. subtilis* and *B. amyloliquefaciens*. Suzuki and Tahara (2003), reported that the gene *ywtD*, located immediately downstream of the PGA operon, codes for γ -glutamyl hydrolase that degrades PGA into two hydrolysed products, a high molecular-mass product (F-1, with nearly 100% L-glutamic acid) and a low-molecular-mass product (F-2, with heterogeneity of D- and L-glutamic acid). PGA hydrolase is a unique enzyme that cleaves γ -glutamyl bond between D- and L-glutamic acid, recognizing adjacent L-glutamic acid toward the N-terminal region of PGA (Chunhachart *et al.*, 2006).

Oncom

Oncom is an ethnic fermented peanut or groundnut cake-like product of Indonesia, mostly in West Java. It is prepared by fermenting a soaked, cooked substrate consisting of peanut

(groundnut) press-cake as the major ingredient, along with solid waste of tapioca and solid waste of *tahu*, using a mixed culture of microorganisms with *Rhizopus* or *Neurospora* species (Winarno *et al.*, 1973). Traditionally, two kinds of *oncom* are produced in Indonesia, *oncom hitam* (black *oncom*) and *oncom merah* (orange *oncom*). When fermentation is carried out by strains of mold belonging to the genus *Neurospora* the product is called red *oncom*; if *Rhizopus oligosporus* is used, the resulting product is called black *oncom*. *Neurospora intermedia*, *N. crassa* and *N. sitophila*, were reported from *oncom* (Ho, 1986). *Neurospora sitophila* and *N. crassa* have been identified as typical molds in *oncom* (Winarno *et al.*, 1973). It is consumed as a side dish, either in the form of deep fat-fried slices, in the form of small portions in the soups, or in the other forms.

Sufu

Sufu is a traditional Chinese salt-fermented soybean curd, highly flavored and resembles a soft creamy cheese-like product which can be used in a similar way as cheese (Steinkraus, 1996). Literally *sufu* (*furu*) means 'molded milk' and *tosufu* (*dou-fu-ru*) means 'molded soymilk'. Due to its numerous dialects used in China, *sufu* has appeared in literature under many different names such as *sufu*, *fu-ru*, *dou-fu-ru*, *tou-fu-ru* etc. It is indicated in Chinese Materia Medica that *tofu* was invented by Liu An (179 BC

to 122 BC), king of Weinan (Steinkraus, 1996). Production of *sufu* has started during Han dynasty in China (Shi and Ren, 1993). The first historical record mentioned that *sufu* process was carried out in the Wei dynasty (220-265 AD) (Hong, 1985). It has been one of the most popular highly flavored side dishes consumed in China for many centuries.

Sufu is produced by various processes in different localities of China (Wang and Du, 1998). Four steps are usually involved in making *sufu*; (1) preparing *tofu* by salt precipitation from boiled soymilk, (2) preparing *phetze* (*pizi*) by spray-inoculation of diced *tofu* with a pure culture starter and fermentation at a temperature of 15-20^o C for 5-15 days. (3) salting (salt is spread between the layers of *pehtze* for 6-12 days) and (4) ripening in a dressing mixture. The most common dressing mixture used consists of *angkak*, alcoholic beverage, salt, sugar, bean paste and spices and sometimes even essence for flavor. Three types of *sufu*-red, white and yellow are prepared in Taiwan (Yuan, 1994). *Sufu* is consumed as an appetizer or side dish mainly with breakfast rice or steamed bread.

The pure culture starters mainly consist of molds - mucoraceae (*Actinomucor*, *Mucor* and *Rhizopus*) or bacteria - *Micrococcus* and *Bacillus* spp. Most *sufu* contains considerable levels of antimicrobial NaCl (5-15 %) and ethanol (1-7 %) that could prevent the survival and growth of pathogens, it is also

known that the endospore-forming rods such as *Bacillus* spp. and *Clostridium* spp. vary greatly in their salt tolerance (Brewer, 2000). Chou and Hwan, (1994) reported that addition of ethanol to the brine solution for ageing resulted in the free fatty acids in the *sufu* product. *Actinomucor repens*, *A. taiwanensis*, *Mucor circinelloides*, *M. hiemalis*, *M. racemosus*, and *Rhizopus microsporus* variety *microsporus* have been isolated from starter culture used in commercial *pehtze* fermentation for *sufu* production (Han, 2003). Diversity of lactic acid bacteria was reported in fermented brines used to ferment *tofu* into *sufu*, which included *Enterococcus hermannienseis*, *Lactobacillus agilis*, *Lb. brevis*, *Lb. buchneri*, *Lb. crispatus*, *Lb. curvatus*, *Lb. delbrueckii*, *Lb. farciminis*, *Lb. fermentum*, *Lb. pantheris*, *Lb. salivarius*, *Lb. vaccinostercus*, *Lactococcus lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Leuconostoc carnosum*, *Leuc. citreum*, *Leuc. fallax*, *Leuc. lactis*, *Leuc. mesenteroides*, *Leuc. pseudomesenteroides*, *Pediococcus acidilactici*, *Streptococcus bovis*, *S. macedonicus*, *Weissella cibaria*, *W. confusa*, *W. paramesenteroides* and *W. soli* (Chao *et al.*, 2009). The microbiological composition of *sufu* indicates that its manufacturing processes and recipes prevent growth of fungi and enterobacteriaceae (Han *et al.*, 2001).

Tempe

Tempe is a non-salted, solid substrate fermented soybean food of Indonesia, which is fermented by fungi unlike other many non-salted fermented soybeans. Fresh *tempe* has a clean, mushroomy or nutty odour (Nout and Rombouts, 1990). It is not consumed raw, but heated to develop meat-like flavour by frying spiced and salted slices in oil, by boiling with coconut milk in soups, by stewing, by roasting spiced kebabs, and in peppered ground pastes (Shurtleff and Aoyagi, 1979; Soewito, 1985). On deep frying, the flavour of *tempe* becomes nut-like and peppery, due to the presence of free fatty acids (Steinkraus, 1985). Most cultivars of yellow-seeded soybeans are suitable for *tempe*, in contrast to black-seeded ones (Sharma and Sarbhoy, 1984).

Traditionally, soaked, briefly boiled and hand-dehulled beans are inoculated with small pieces of *tempe* (starter) from a previous fermentation, wrapped in banana leaves which also serve as a source of inoculum, and left at room temperature for 1-2 days (Wang, 1986). Addition of lactic ($\leq 0.5\%$) or acetic ($\leq 0.25\%$) acid during hydration to control microbial spoilage has been suggested (Usmani and Noorani, 1986; Wadud *et al.*, 1988). Emphasis has been given to the importance of acid fermentation or artificially acidifying the beans, because the mould is proteolytic, and deamination following hydrolysis releases ammonia, causing the pH to rise. Acidifying the beans during soaking to pH ≤ 4.30 yields

tempe of good quality in which bacilli and Enterobacteriaceae could not be detected. The acidification during soaking can be controlled by recycling part of the soak water from a previous batch as an inoculum, contributing to the shelf-life and safety of *tempe* (Nout *et al.*, 1987). Cooking by steaming for at least 30 min at 100°C (Dijen and Hesseltine, 1979) or by boiling in excess water for 2-3 h (Winarno and Reddy, 1986) serves the purpose of partial cooking which facilitates fungal penetration and human digestion (Nout and Rombouts, 1990). Partial cooking of soybeans destroys trypsin inhibitors (Albrecht *et al.*, 1966), inactivates some undesirable factors such as phytic acid and flatus-causing oligosaccharides (Wang *et al.* 1979), leaches out a heat-stable and water-soluble mould inhibitor (Wang and Hesseltine, 1979; Dijen and Hesseltine, 1979), destroys contaminating bacteria that interfere with fermentation, releases some of the nutrients required for mould growth (Steinkraus, 1983), and destroys the bitter soya taste (Nout *et al.*, 1985). Addition of approximately 2% w/w maize starch, rice flour or cassava starch helps to absorb the remaining moisture, stimulates fungal growth and results in better *tempe* firmness (Nout and Rombouts, 1990). The inoculum for *tempe* fermentation can be obtained from dried and pulverized *tempe* of previous batch ('*tempe-to-tempe*'), mould grown and air dried on leaves of *Hibiscus spp.*, *Tectona grandis*, *Bambusa sp.* or *Musa paradisiaca*, locally referred to as 'usar' or 'laru' (Dijen and

Hesseltine, 1979) sold on Indonesian markets. Studies carried out by Steinkraus *et al.* (1960) resulted in pure culture fermentation. The most popular strain is *Rhizopus oligosporus* NRRL 2710 which grows at 30-42 °C (Steinkraus, 1983; Hesseltine, 1985). The optimum relative humidity during *tempe* preparation was reported as 60-65% (Usmani and Noorani, 1986), 75% (Wadud *et al.*, 1988) and 90% (Steinkraus, 1985). As the mould begins to grow rapidly during *tempe* fermentation, the temperature of the fermenting beans rises from 5 to 7° C above the incubation temperature. As a result of protein metabolism, pH increases from 4.5 (0 h) to 6.0 (26 h at 28°C, 18 h at 38°C) and 7.0 (48 h at 28°C, 30 h at 38°C), levelling off towards pH 7.5 to 8.0. During fermentation, there is increase in total soluble solids, soluble nitrogen and free amino acids, whereas total nitrogen remains fairly constant (Steinkraus *et al.*, 1960; Wang and Hesseltine, 1966; Wang *et al.*, 1968).

Rhizopus microsporus is a functional mould for fermentation of *tempe* with varieties *microsporus*, *oligosporus*, *rhizopodiformis*, *tuberosus* and *chinensis* (Nout and Kiers, 2005; Jennessen *et al.*, 2008). *Klebsiella pneumoniae*, *K. pneumoniae* subsp. *ozaenae*, *Citrobacter freundii*, *Pseudomas fluorescens*, *Enterobacter cloacae* as vitamin B₁₂-producing bacteria, and LAB: *Lactobacillus plantarum*, *Lb. fermentum*, *Lb. reuteri* and *Lb. lactis* are important microorganisms in naturally fermented *tempe* (Denter and Bisping, 1994; Feng *et al.*, 2005). *Rhizopus oligosporus* produces two

proteolytic enzyme systems, one with an optimum activity at pH 3.0 and the other at 5.5, both having maximum activity at a temperature of 50-55 °C; maximum proteolytic activity was attained at 72-96 h at 32°C (Wang and Hesseltine, 1965). *Rhizopus oligosporus* possesses a strong lipolytic activity, hydrolyzing over one-third of neutral fat of soya beans after 72 h fermentation at 37 °C. Lipolysis yields predominantly linoleic acid, besides oleic, palmitic, linolenic and stearic acids (Wagenknecht *et al.*, 1961). The free fatty acids, particularly oleic, linoleic and linolenic acids are associated with non-specific antitryptic activity (Winarno and Reddy, 1986). *Rhizopus oligosporus* derives much of its energy from oleic acid (Nout and Rombouts, 1990). This was supported by findings of Paredes-Lopez *et al.* (1987) who reported a 50% reduction of oleic acid in bean *tempe*. Carbohydrates of soya beans, especially raffinose and stachyose cause flatulence (Nout and Rombouts, 1990). Total flatus factors are reduced from 16.5 mg/g to 2.0 mg/g soya beans (Winarno and Reddy, 1986). Steinkraus, (1983) observed that stored *tempe* does not develop rancidity because of its content of 6,7,4'-trihydroxyisoflavone, an antioxidant produced by the mould. *Rhizopus* spp. is used in solid substrate fermentation (SSF) for the production of *tempe*, made of cooked soybean (Nout and Rombouts, 1990). *Rhizopus oligosporus* NRRL 2710 produced an antibiotic active against a number of Gram positive bacteria including *Staphylococcus aureus* and

Bacillus subtilis. It is still the most popular soyfood in Indonesia (Winaro and Reddy, 1986). Carbon dioxide (5-10 % v/v) inhibited the growth of *Rhizopus* spp. at non-limiting levels of oxygen. The optimum conditions were temperature 40° C, water activity (a_w) 0.995 and a gas composition of air for the growth of *Rhizopus* spp. on a model medium (Nout and Han, 2000). Mostly *Rhizopus microsporus* var. *microsporus* and var. *oligosporus* are used in the manufacture of *tempe*. These two strains were able to grow at low (0.5% v/v) oxygen levels, but the mycelial density was rather low (Nout and Han, 2000). At oxygen level as low as 0.2% (v/v) mycelial growth of *Rhizopus oligosporus* still occurred but was insufficient for *tempe* making and carbon dioxide had a stimulatory effect at 5-10% (v/v) but became inhibitory at levels exceeding 20% (v/v) (De Reu *et al.*, 1995). Thanh *et al.* (2007) reported that the shelf-life of spores in *tempe* starter is related to the physiological state of spores being sub-lethally damaged; a mechanism of physiological state transitions of *R. oligosporus* sporangiospores is proposed. Keuth and Bisping (1993) reported the formation of riboflavin, nicotinic acid, nicotinamide and vitamin B₆ by *Rhizopus* strains but did not produce physiologically active vitamin B₁₂. Ashenafi and Busse (1989) reported that the lowering of pH in fermenting beans by *Lactobacillus plantarum* plays an important role in destroying the test organisms like *Salmonella infantis*, *Enterobacter aerogenes* and *Escherichia coli*.

Nout *et al.* (1987) reported that soybean acidification, either by biological or by chemical means, is essential to inhibit *Bacillus cereus* growth during the *tempe* fermentation. Protease activity and production of free ammonia were detected at the earliest stages of *tempe* fermentation and during the first phase of mycelial senescence, amount of crude lipid and glycerol decreased in the absence of fungal growth, possibly due to the activity of enzymes released from senescent mycelium (Ruiz-Teran and Owens, 1996). Tanaka *et al.* (1985) reported that high level of sanitary practice and good refrigeration should be maintained microbiological safety of *tempe*. Nout *et al.* (1987) also reported that simple recycling process can result in predictable acidification during soaking of soybeans, contributing to the shelf-life and safety of *tempe*. Areekul *et al.* (1990) reported that *Klebsiella pneumoniae*, the bacteria contaminated during the process of *tempe* production, was responsible for the vitamin B₁₂ production. *Rhizopus oryzae* possesses strong lipase activity and caused the hydrolysis of over one-third of the neutral fat of the soybean during the three-day fermentation (Wagenknecht *et al.*, 1961). *Tempe* contains 25-65% moisture, and per 100 g dry matter: 45-55 g protein, 15-25 g fat, 15-25 g carbohydrate, 3-7 g fibre, 5-10 g ash, 1.8-1.9 MJ (430-460 kcal) energy, 400 mg Ca, 400 mg P, 25 mg Fe, 0.4 mg thiamine, 0.7 mg riboflavin, 6 mg niacin, 0.3 mg pantothenic acid, trace vitamin B₁₂ and 50 µg vitamin A (Campbell-Platt, 1987). Murata *et*

al. (1967) reported that during the *tempe* fermentation, fiber, free amino acids, riboflavin, vitamin B₆, nicotinic acid and pantothenic acid were increased but no large differences in protein and ash content between *tempe* and unfermented soybeans.

Thua-nao

Thua-nao is an ethnic fermented non-salty soybean food of northern Thailand. It is generally available as a dried paste, and is used as a flavouring agent in vegetable dishes. In some areas, the product itself is an item of diet (Sundhagul *et al.*, 1972). In the traditional method of its preparation, dry whole soybeans are washed and boiled in excess water for 3-4 h till they can be crushed between fingers. Excess water is drained off and the cooked beans are transferred to a bamboo basket lined with banana leaves. The basket is covered with banana leaves. The beans are left at room temperature for 3-4 days to undergo natural fermentation, and are considered properly fermented when they are covered with a sticky, viscous material, accompanied by pungent odor of ammonia replacing the beany flavour. The beans change from light brownish yellow to greenish brown colour (Sundhagul *et al.*, 1972). After fermentation, the raw *thua-nao* is mashed lightly into paste and added with salt, garlic, onion and red pepper. The paste is wrapped in banana leaves and cooked by steaming before eating (Sundhagul *et al.*, 1972). The protein

contents were 16.9 and 36.8%, and the fat contents were 7.4 and 14.8% for paste and chips, respectively (Sundhagul *et al.*, 1972). A low cost, protein-rich food, called 'ferm-soya-mix' in powder form, ready to eat with long shelf-life under normal conditions has been developed by blending *thua-nao* powder with flavouring agents and a small proportion of high grade fish meat (Sundhagul *et al.*, 1972). *Thua nao* is used as a seasoning and an ingredient of soup, curry and stir-fried vegetables (Okada, 2008).

Sundhagul *et al.* (1972) reported that gram-positive, spore-forming, rod-shaped bacterium, *Bacillus subtilis* is responsible for the fermentation of *thua-nao*. The initial bacterial load of 10^3 cells/g cooked beans was increased to 10^{10} cells/g *thua-nao*. The increase was rapid during the first two days. During fermentation, the pH increased from 6.3 to 8.6 in the second day and remained relatively unchanged afterwards. Inatsu *et al.* (2006) reported that *B. subtilis* produced protease, amylase, subtilisin NAT (nattokinase) and PGA in *thua nao*. PGA from bacilli are usually produced as mixture with molecular weight ranging from 10 to 1,000 KDa due to PGA depolymerase activity (Abe *et al.*, 1997). Generally the molecular weight of PGA is dependent on many factors and can decrease as fermentation increases, owing to enzyme that catalyses hydrolytic breakdown of PGA (Goto and Kunioka, 1992; King *et al.*, 2000). It is said to serve as a substitute for fermented fish. *Thua-nao* has been reported as a potential

resource of food-processing enzymes and health-promoting compounds by Inatsu *et al.* (2006). Visessanguan *et al.* (2005) reported that *Bacillus subtilis* inoculated *thua-nao* showed an increased proteolysis of soybean as *B. subtilis* released predominant active proteinases of molecular weight of 40,000 and 29,000 kDa. Enzyme γ -glutamyl hydrolase (28 kDa) purified from Thai *thua-nao* degraded γ -polyglutamic acid to a hydrolysed of only about 20 kDa (with D- and L-glutamic acid in a ratio of 70:30), suggesting that the enzyme cleaves the γ -glutamyl linkage between L- and L-glutamic acid of γ -polyglutamic acid (Tahara *et al.*, 2006; Chunchachart *et al.*, 2006). PGA-producing *Bacillus* strains isolated from Thai *thua-nao* including *B. subtilis* IFO3022, does not require biotin for growth and the *thua-nao* plasmids were found to be strongly hybridized with *natto* plasmid, pUH1, which encodes the γ -glutamyltranspeptidase (γ -GTP) gene responsible for PGA production in *B. subtilis* (*natto*) (Hara *et al.*, 1986). *B. subtilis* chungkookjang (Ashiuchi and Misono, 2001), *B. licheniformis* NK-03 (Cao *et al.*, 2010) and *B. subtilis* RKY3 (Jeong *et al.*, 2010) requires the presence of glutamic acid to produce γ -PGA, while *B. subtilis* C1 (Shih *et al.*, 2005), *B. subtilis* TAM-4 (Ito *et al.*, 1996) and *B. licheniformis* A35 (Cheng *et al.*, 1989) does not require the presence of glutamic acid to produce γ -PGA. *B. subtilis* strains isolated from fermented foods were capable of producing protease and has the potential for application in the production of

hypoallergenic fermented rice-noodle with high nutrient availability (Phromraksa *et al.*, 2009). Methanolic extracts of *thua-nao*, exhibited antioxidant and free radical scavenging properties and it was also found that there was a strong relationship between total phenolics content and antioxidant activity (Dajanta *et al.*, 2011).

Ugba

Ugba is a fermented product of leguminous seeds of African oil beans (*Pentaclethra macrophylla* Bentham) (Obeta, 1983). It is a flat, glossy, brown in color. During the preparation of *ugba*, leguminous seeds of African oil beans (*Pentaclethra macrophylla* Bentham) are boiled in water for 4-12 h to remove the fibrous seed coat. The cotyledons are sliced, washed, boiled for 1-2 h, and then soaked in water overnight to remove bitter components. It is then drained for 1 h in basket lined with banana leaves and wrapped on *ororompo* (*Mallotus oppositifolius* Mull) leaves and fermented naturally for 4-5 days. Longer the fermentation more strongly flavored *ugba* results. Less fermented (about 5 days) *ugba* is eaten directly. It is usually eaten as side dish in Nigeria. Bacteria isolated from fermentation of *ugba* are *Bacillus*, *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Alcaligenes*, and *Citrobacter* (Obeta, 1983). During fermentation, proteolytic, amylolytic and lipolytic enzyme activities are increased.

Wari

Wari is an ethnic fermented black gram product of northern India and Pakistan. These are dried, hollow, brittle, spicy and friable balls, 3-8 cm in diameter and 15-40 g in weight. Waries are used as condiments or adjuncts in cooking vegetables, legumes or rice (Batra, 1986; Soni and Sandhu, 1990a).

In the traditional method of *wari* preparation, black gram [*Vigna mungo* (L.) Hepper] are soaked in water for 6-12 h, dewatered, dehulled and ground on a stone mortar into a smooth, mucilaginous paste. The dough is mixed with inoculum from a previous batch, salt and typical spices like asafoetida (*Ferula foetida* Regel), caraway (*Carum curvi* L.), cardamom [*Elettaria cardomomum* (L.) Moton], clove [*Syzygium aromaticum* (L.) Merr. and Perry], fenugreek (*Trigonella foenum-groecum* L.), ginger (*Zingiber officinale* Rosc.) and red pepper (*Capsicum annum* L.). The mixture is allowed to ferment at room temperature for 1-3 days and hand-moulded into balls. After air-drying for 2-8 days on bamboo or palm mats, waries are turned over for further drying (Batra and Millner, 1976; Soni and Sandhu, 1990b).

Batra and Millner (1974, 1976) isolated two types of yeasts including *Candida krusei* and *Saccharomyces cerevisiae* from waries. Later on, although a wide variety of yeasts and lactic acid bacteria were found to be associated with waries, only the

combination of *Hansenula* sp. and *Leuconostoc mesenteroides* was found responsible for their production (Batra, 1986). Soni and Sandhu (1989) observed the occurrence of bacteria (10^9 - 10^{12} /g) in all the market and laboratory-made samples, but only 55% of the samples contained yeasts (0 - 10^7 /g). *Leuconostoc mesenteroides* was most abundant and present in all the market samples, followed by *Candida vartiovaarai*, *Kluyveromyces marxianus*, *Trichosporon beigelii*, *Candida krusei* and *Hansenula anomala*. Laboratory-made samples were found to contain comparatively higher bacterial load (10^{10} - 10^{12} /g) while less yeast load (0 - 10^6 /g) in 45% of the samples. The microbial load of 1.3×10^{10} /g unfermented dough increased to 6.5×10^{12} /g at the end of fermentation. Among the bacteria, *Leuconostoc mesenteroides*, *Lactobacillus delbrueckii*, *Lb. fermentum*, *B. subtilis* and *Flavobacter* spp., and among the yeasts, *Trichosporon beigelii*, *Saccharomyces cerevisiae*, *Candida krusei*, *Pichia membranaefaciens* and *Hansenula anomala* predominated the initial stages of fermentation. With the progress in fermentation, most of the microorganisms, except *Leuconostoc mesenteroides*, *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and *Trichosporon beigelii*, disappeared. There was the production of acid and gas resulting in the fall in pH from 5.65 to 3.20 and rise in volume from 200 to 420 ml. Fermentations brought about an increase in total acids from 0.50 to 1.50%, soluble solids from 7.8 to 14.7%, non-protein

nitrogen from 0.20 to 0.68%, soluble nitrogen from 0.95 to 1.50%, free amino acids from 9.79 to 45.15 mg/g and proteolytic activity from 4.82 to 6.04 IU/g. On the other hand, the level of reducing sugars and soluble protein decreased from 13.69 to 4.34 mg/g and 50.52 to 17.40 mg/g, respectively. Amylase activity increased initially, but declined thereafter. *Wari* fermentation also brought about an appreciable rise in water-soluble B vitamins including thiamine, riboflavin and cyanocobalamine (Soni and Sandhu, 1989, 1990b).