2

Review of literature

2.1. Legume-based traditional fermented foods

Legume-based fermented foods, prepared mainly with the intention of adding variety and nutritional quality to an otherwise monotonous vegetarian diet, are practiced worldwide (Table 1) since prehistoric time.

2.1.1. Dawadawa

In many of the West African countries, a fermented product of seeds from the African locust bean tree (*Parkia biglobosa* Benth.) is widely used as a condiment for preparing soups and stews. Dawadawa is the name designated by the Hausa Nigerian tribe for fermented locust beans. Similar fermentations have been characterized throughout Africa, with local adaptation in the form of raw material selection or (post) processing. These regional versions are often given local names such as 'daddawa' in Northern Nigeria, 'kinda' in Sierra Leone, 'soumbala' in Burkina Faso and Mali, 'iru/dorowa/ogini-gala' in the Savannah areas of Nigeria, 'soumbara' in Ivory Coast, 'nététou' in Senegal, and

Table 1. Some popular legume-based traditional fermented foods consumed worldwide

Product name	Country or area	Substrate/ ingredient	Functional microbiota	Type of fermentation[a]	Appearance and mode of consumption	Reference
Dawadawa	West Africa, Nigeria	African locust bean	*Bacillus subtilis, B. licheniformis, B. pumilus*	SSF; N	Flavouring agent, soup and stew ingredients	Odunfa (1981, 1985a); Ogbadu and Okagbue (1988a,b)
Dhokla	India	Bengalgram and rice	*Leuconostoc mesenteroides, Lactobacillus fermentum, Pichia silvicola*	SSF; N	Steamed soft cake, snack	Desai and Salunkhe (1986); Joshi *et al.* (1989); Ramakrishnan (1979)
Dosa	India	Blackgram and rice	*Leuconostoc mesenteroides, Enterococcus faecalis, Lactobacillus fermentum, Saccharomyces cerevisiae, Debaryomyces hansenii*	SSF; N	Breakfast food	Soni *et al.* (1985, 1986); Soni and Sandhu (1989b, 1990b)
Idli	India, Sri Lanka	Blackgram and rice	*Leuconosctoc mesenteroides, Lactobacillus fermentum, Lb. delbrueckii, Enterococcus faceaclis, Galactomyces geotrichum, Candida holmii, C. glabrata, C. sake, C. tropicalis, Pichia anomala*	SSF; N	Steamed spongy cake, snack	Ramakrishnan (1979); Steinkraus *et al.* (1967)
Kinema	India, Nepal	Soybean	*Bacillus subtilis*	SSF; N	Paste, made to thick curry, side dish	Batra and Milner (1974); Sarkar *et al.* (1994)
Masayura	India, Nepal	Blackgram	*Leuconostoc mesenteroides, Pedoiococcus cerevisiae*	SSF; N	Dried, hollow, brittle, and friable, balls; condiment or adjunct	Dahal *et al.* (2003); Gajurel and Baidya (1979); Tamang (1992)
Meitauza	China, Tiawan	Soybean press-cake	*Actonomucor elegans, Mucor meitazua*	SSF; N	Cake, fried or cooked, side-dish	Kronenberg and Hang (1984); O'Toole (1999)
Miso	Japan, China	Soybean, rice and other cereals	*Aspergillus oryzae. Torulopsis etchellsii, Lactobacillus* sp.	SSF; TS	Paste, soup base or seasoning agent	Hesseltine (1965); Shibasaki and Hesseltine (1962); Shurtleff and Aoyagi (1983)
Natto	Japan	Soybean	*Bacillus subtilis*	SSF; PS	Mucilaginous snack, used as meat substitute	Fukushima (1979); Ohta (1986)
Okpehe	Nigeria	African locust bean	*Bacillus subtilis, B. pumilus, B. megaterium*	SSF; N	Flavouring agent, soup and stew ingredient	Odibo *et al.* (1992); Omafuvbe *et al.* (1999)
Oncom (ontjom)	Indonesia	Peanut press-cake	*Neurospora intermedia,* less often *Rhizopus oligosporus*	SSF; TS	Cake, deep-fried or roasted; side dish or soup ingredient	Sastraatmadja *et al.* (2002); Shurtleff and Aoyagi (1979a)
Papad (papdam)	India, Pakistan, Bangladesh	Blackgram	*Saccharomyces* spp.	SSF; N	Deep-fried or roasted; snack or condiment	Batra and Milner (1974); Shurpalekar and Venkatesh (1975)

Product name	Country or area	Substrate/ ingredient	Functional microbiota	Type of fermentation[a]	Appearance and mode of consumption	Reference
Soumbala	Africa	African locust beans	*Bacillus subtilis, B. licheniformis, B. pumilus*	SSF; N	Flavouring agent, soup and stew ingredient	Odunfa (1986); Sarkar *et al.* (2002)
Soy sauce	East and Southeast Asia	Soybean and wheat	*Aspergillus oryzae or A. soyae, Lactobacillus* spp., *Zygosaccharomyces rouxii*	SSF; SmF; TS	Brown salty liquid, seasoning agent	Nunomura and Sasaki (1993)
Sufu	China, Taiwan	Soybean whey curd	*Actomnomucor elegans, Mucor hiemalis, M. silvaticus, M. subtilissimus*	SSF; N	Paste, condiment	Han *et al.* (2001); Su (1986); Wang and Hesseltine (1970)
Tempe	Indonesia and vicinity	Soybean	*Rhizopus oligosporus*	SSF; TS	White, mould-penetrated and covered cake, stewed or deep-fried; side dish or snack, or soup ingredient	Nout and Rombouts (1990); Shurtleff and Aoyagi (1979b)
Thua-nao	Northern Thialand	Soybean	*Bacillus subtilis*	SSF; TS		Leejeerajumnean *et al.* (1997a, b); Sundhagul *et al.* (1972)
Ugba (ogiri)	Nigeria, West and Central Africa	African oil bean or castor oil bean, or melon or sesame seed	*Bacillus subtilis, Micrococcus luteus, M. roseus, Bacillus sp.*	SSF; N	Dark brown balls, salad ingredient or flavouring agent in soups, stews and sauces	Isu and Njoku (1997); Odunfa and Oyeyiola (1985)
Wadi	India, Pakistan, Bangladesh	Blackgram	*Leuconostoc mesenteroides, Enterococcus faecalis, Lactobacillus fermentum, Bacillus subtilis, Saccharomyces cerevisiae, Pichia membranaefaciens, Candida vartiovaarai, Kluyveromyces marxianus, Trichosporon beigelii, C. kursei, Hansenula anomala*	SSF; N	Dried, hollow, brittle, spicy and friable balls or cones spicy; condiment or an adjunct	Batra (1986); Batra and Milner (1974, 1976); Soni and Sandhu (1989b, 1990)

[a]SSF, solid-state fermentation; SmF, submerged fermentation; N, natural and/or back-sloped fermentation; TS, traditional undefined starter; PS, pure culture starter (based on Nout *et al.*, 2007)

'kpalugu' in parts of Ghana. Dawadawa is a culinary product that can be used to enhance or intensify meatiness in soups, sauces and other prepared dishes. It is considered as one of the most important food condiments in the entire West and Central African Savanna regions (Odunfa, 1986; Sarkar *et al.*, 2002; Steinkraus, 1996).

Seeds of the locust bean tree are the traditional substrate for the production of Nigerian dawadawa. Occasionally, other materials such as soybean, groundnut and African yam bean (*Sphenostylis stenocarpa* Harms) have also been used to replace the African locust beans. Dawadawa production is a multistep process which does not include the formal inoculation step (Amadi *et al.*, 1999; Ogbadu and Okagbue, 1988a; Wokoma and Aziagba, 2001).

In the traditional method of dawadawa preparation, the dried seeds of African locust bean tree are washed and boiled in a covered container for 18-24 h with occasional renewal of water to swell the seeds and soften the very tough seed coats which are then removed by pounding and flushing. 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 heap (10-15 cm-thick) of cooked cotyledons on a calabash is then covered with leaves and sackcloth and allowed to ferment for 3-4 days at ambient temperature (25-35°C) till the beans become covered by a sticky mucilaginous layer and develop a strong odour. Wood ash is sometimes mixed to reduce the odour. Millet flour is often added. The fermented mass is air-dried in the sun or hot shade, where the beans darken further to dark brown, and are then used loose or shaped into balls or pyramids for convenient handling and stored into traditional earthenware pots. To extend shelf-life, salt is added in some areas. Sun-drying facilitates stabilization of the final product through reduction of moisture. A 40-60 g dawadawa ball may be used by a single family for up to two weeks or more, depending on personal tastes and quantities used (Beaumont, 2002; Campbell-Platt, 1980, 1987; Odunfa, 1981, 1985a, 1986; Sarkar *et al.*, 2002; Steinkraus, 1996).

The optimal time-temperature for fermentation was 36 h at 35°C and 48 h at 40°C. The traditional method of dawadawa fermentation was improved by cooking beans in pressure cooker for 75-90 min to remove seed coats, cleaning, washing, further cooling with the addition of starter culture for 1 h, placing on a flat calabash and fermenting at 30°C for 18 h. It is likely that dawadawa fermentation begins when the softened, washed and dehulled beans are placed in perforated trays and covered. This cover most likely provides a moisture trap and possibly serves as a source of inoculum (Odunfa and Adewuyi, 1985; Osinowo *et al.*, 1990).

Predominant microorganisms isolated and/or characterized from dawadawa include *Bacillus subtilis, Bacillus pumilus, Bacillus licheniformis* and *Staphylococcus saprophyticus* (Ikenebomeh, 1989; Odunfa, 1981; Ogbadu and Okagbue, 1988a, 1988b; Ogbadu *et al.*, 1990). Antai and Ibrahim (1986) reported the presence of *Leuconostoc mesenteroides* and *Leuconostoc dextranicus* in almost equal proportion with the *Bacillus* spp. in dawadawa. However, Ogbadu and Okagbue (1988b) could not find any of these lactics during dawadawa production. They found that the species of *Bacillus* responsible for dawadawa production were variable, and reported *B. subtilis, B. pumilus* and *B. licheniformis* from six separate fermentations. Osinowa *et al.* (1990) reported *B. subtilis, Bacillus cereus, Pseudomonas aeruginosa* and *Enterobacter aerogenes* from dawadawa.

Bacteria required for the fermentation appeared to be incidental to both the process and raw materials. Indigenous flora was likely carried over from fermentation to fermentation through sieves, trays and bags which were repetitively used in dawadawa production. Contamination of spores from the local building/environment might also contribute to the fermentation microflora (Beaumont, 2002).

Changes over the course of 2-4 days-fermentation period included increase in pH, temperature and moisture from near neutral to 8.0, 25°C to 45°C and 43% to 56%, respectively (Odunfa, 1986). The fatty acids in both nonfermented and fermented beans were linoleic, oleic, stearic, palmitic and a trace of arachidonic acids. The major fatty acid was linoleic acid, an essential fatty acid (Odunfa and Adesomoju, 1986). The amino acid profile of fermented *Parkia* beans was similar to that of raw beans, with a small decrease in essential sulphur-containing amino acids and a large decrease in the nonessential aspartic and glutamic acids (Fetuga *et al.* 1973). However, Odunfa (1986) reported a five-fold increase in free amino acid content. Glutamate concentration also increased almost five-fold (11.9 mg $(100\ g)^{-1}$ dawadawa) (Odunfa, 1985b). 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; Leung *et al.* 1968; Platt, 1964).

Flavour properties of dawadawa are most likely due to its amino acid content, in particular glutamate which contributes to flavour enhancement as well as peptides and aroma volatile constituents. Volatiles may of course be directly produced during fermentation or may evolve as a result of the effect of heat on amino acid and fatty acid constituents of dawadawa (Beaumont, 2002). During fermentation, postfermentation processing and in-home cooking applications, it is likely that several volatile aroma molecules are generated which reflect the characteristic flavour for which dawadawa is known. Evidence for the participation of indigenous enzymes and biota in the development of the flavour of dawadawa was presented by Ikenebomeh *et al.* (1986). They demonstrated that both autoclaved (sterile) and γ-irradiated (which destroyed indigenous biota) beans were unable to develop the characteristic aroma of dawadawa. Additionally, the increase in pH observed during formation of dawadawa was absent, implicating that active microbial metabolism is required in order to bring about the changes observed in locust beans during fermentation.

Dawadawa contains 20-52% 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 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).

2.1.2. Dhokla

Dhokla, a steamed product with appealing taste, colour, flavour and spongy texture, constitutes one of the categories of the Gujarati dishes known as 'farshana'. Because of its organoleptic attributes, nutritional quality and improved digestibility, dhokla is well accepted by young children, adults and patients with digestive disorder, and can be ranked as one of the popular indigenous fermented foods of India (Ramakrishnan, 1979).

The amazing thing about the dhokla is its versatility; it can be prepared with different ingredients, with different proportions being used at various places (Lobo, 1986). Traditionally dhokla is prepared by overnight fermentation of a mixture Bengalgram dal (dehusked split beans) flour and rice batter at room temperature. Desai and Salunkhe (1986) were of opinion that the prime ingredients used in the preparation of dhokla are polished rice and Bengalgram dal, however, both of these ingredients can be substituted for i.e. suji, coarsely ground meal of wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), or kodri (*Paspalum scrobiculatum* L.) for rice, and soybean cotyledons, split peas, redgram, or moth beans for Bengalgram. The recipes usually made in Gujarati homes call for equal part by volume of Bengalgram, rice, mung and blackgram (Lobo, 1986).

Traditionally, the ingredients are soaked separately in tap water for 8-10 h at room temperature and coarsely ground to thick batter using a stone mortar. These are then mixed and allowed to ferment overnight (12-14 h) at room temperature (28-30°C). Fermented batter is poured onto a greased tray, steam-cooked for 10-15 min and cut into diamond shape. A mixture of peanut or sesame oil with brown hot cracked seeds of mustard and sesame, chilli, pepper, ginger and curry leaves is poured over the steamed pieces of dhokla as seasonings, and garnished with coriander leaves (Desai and Salunkhe, 1986; Mahajan and Chattopadhyay, 2000; Ramakrishnan, 1979).

During dhokla fermentation, there is an almost two-fold rise in the batter volume and a decline in pH from 5.2 to 4.5. The microorganisms involved in the fermentation are *Lactobacillus fermentum*, *Leuconostoc mesenteroides* and *Pichia silvicola* (up to 10^7 cfu g^{-1}) (Joshi *et al.*, 1989). The lactic acid bacteria contribute lactic acid and acetoin, imparting a sour taste and a pleasant flavour. The yeast produces folic acid and raises the volume of the batter, imparting sponginess to the product (Aidoo *et al.*, 2006; Kanekar and Joshi, 1993).

Dhokla serves a vital source of protein, calories and vitamins. Several studies have indicated that dhokla can be used as a supplementary diet to treat young children suffering from protein calorie malnutrition, patients with digestive disorder, and to improve the nutritional status of pregnant women (Ramakrishnan, 1979).

2.1.3. Dosa

Dosa is a thin, fairly crisp, fried and highly seasoned griddled pancake-like staple food made from fermented batter of legume-cereal mixture. Dosa is relished mainly as a breakfast food along with chutney (a batter containing fresh ground coconut and spices) and sambar (a thin spiced soup prepared from redgram dal, vegetables, tamarind juice, salt and herbs). Though indigenous to southern part of India and many places of Sri Lanka, due to its acceptable organoleptic properties dosa is being prepared and consumed as a snack food throughout India (Aidoo *et al.*, 2006; Soni and Sandhu, 1999; Soni *et al.*, 1985, 1986).

Traditionally, dosa is prepared by overnight (10-12 h) soaking of equal quantities of rice and blackgram dal separately. The soaked ingredients are ground to a fine slurry by adding 2.0-2.5 parts (w w^{-1}) water and mixed together. About 0.8-1.0% salt is added to the batter, and then allowed to ferment at room temperature (28-30°C) for 12-16 h. A bit of freshly fermented batter ('backslop') is often added to newly ground batter. The fermented batter is then spread on a hot greased griddle where it assumes the form of a crisp pancake (Soni and Sandhu, 1999).

Both bacteria and yeasts, whenever present, increased significantly with the progress in fermentation resulting in acidification and leavening. During fermentation, *Leuconostoc mesenteroides* was the most commonly encountered bacterium followed by *Enterococcus faecalis*, *Lactobacillus fermentum*, *Bacillus amyloliquefaciens*, *Lactobacillus delbrueckii*, *Bacillus subtilis*, *Pediococcus cerevisiae*, *Bacillus polymyxa* and *Enterobacter* sp. *Saccharomyces cerevisiae* is the most predominant yeast involved in the fermentation, followed by *Debaryomyces hansenii*, *Pichia anomala*, *Trichosporon beigelii*, *Oosporidium margaritiferum*, *Trichosporon pullulans*, *Kluyveromyces marxianus*, *Candida kefyr* and *Candida krusei*. Both the ingredients of dosa i.e. rice and blackgram have been found to contribute the microbiota responsible for the fermentation (Soni and Sandhu, 1990a; Soni *et al.*, 1986).

The fermentation of dosa batter causes a decline in pH from 5.1 to 4.1 and an increase in volume, soluble solids and reducing sugars. Total nitrogen and proteins do not vary significantly

although nonprotein nitrogen and total acids increase from 0.7 to 1.3% and 0.4 to 0.9 %, respectively. Soluble nitrogen and free amino acids also exhibit a rise from 0.5 to 1% and from 10 to 18 mg g^{-1}, respectively, during fermentation. Amylase activity shows an early rise (31 to 39 IU g^{-1}) and finally declines to 33 IU g^{-1}. Water-soluble vitamins, including thiamine (B_1), riboflavin (B_2) and cyanocobalamine (B_{12}) also increase significantly with the progress in dosa batter fermentation (Soni and Sandhu, 1989a; Soni *et al.*, 1985).

2.1.4. Idli

Idli, a steam-cooked product of fermented blackgram-rice mixed batter, is prepared and widely consumed throughout India and many places of Sri Lanka. An early history of its use dates back to 1100 AD (Ramakrishnan, 1979). It, along with chutney and sambar, is relished mainly as a breakfast food . The ever-increasing popularity of idli is due to its appealing sour flavour, spongy texture, easy digestibility and nutritional qualities with improved balance of carbohydrates and proteins (Aidoo *et al.*, 2006; Nout and Sarkar, 1999: Nout *et al.*, 2007).

Traditionally, idli is made from naturally fermented batter prepared from a mixture of either parboiled or polished rice plus blackgram dal. The proportion of rice and dal ranges from 1:4 to 4:1 (Radhakrishnamurthy *et al.*, 1961; Reddy *et al.*, 1986; Steinkraus *et al.*, 1967). The substrates are washed and soaked separately in tap water for 8-10 h at room temperature. While rice is coarsely ground in a stone mortar, dal is ground to a smooth mucilaginous paste. Salt is added to taste (~ 8.0 g kg^{-1} batter), and the mixture is kept into a covered container and left overnight in a warm place. The fermented batter, with significant leavening (2 to 3-fold increase in the original volume) and pleasant acid flavour, is poured into the cups of idli pan, and steamed until the starch is gelatinized and the idli cakes are soft and spongy with a honeycomb structure inside (Aidoo *et al.*, 2006; Nout and Sarkar, 1999: Nout *et al.*, 2007; Steinkraus, 1996; Venkatasubbaiah *et al.*, 1985).

2.1.5. Kinema

Kinema is a nonsalted, flavoursome, naturally fermented alkaline food, traditionally consumed mainly by the non-Brahmin Nepalis. It is now popular among the Lepchas and Bhutias who call it 'satlyangser' and 'bari', respectively (Sarkar and Tamang, 1995; Sarkar *et al.*, 1994; Tamang *et al.*, 1988).

Kinema is popular for its characteristic nutlike flavour and high protein content (480 g kg^{-1} dry weight) (Sarkar *et al.*, 1994, 1996). It is also dried, fried in edible oil and mixed with salt, onion and chillies to produce pickle. The deep-fried kinema is used as an adjunct to staples such as rice (Tamang *et al.*, 1988). Per capita daily consumption of kinema was recorded as 3.3 g in the Darjeeling hills with annual production of 326 tonnes, and 2.2 g with annual production of 326 tonnes in Sikkim during 1997-98 (Yonzone and Tamang, 1998). Kinema resembles Japanese natto, West African dawadawa and Thai thua-nao (Nout *et al.*, 1998).

The method of kinema preparation was reported briefly by Batra and Milner (1976), Batra (1986) and Tamang *et al.* (1988). In the traditional method of its preparation, yellow seeded soybeans are cleaned, washed, soaked in water overnight (12-20 h) at ambient temperature (10-25°C), and excess water is drained off. Soaked beans are cooked by boiling in an open cooker until they can be crushed easily between the finger tips, the water removed and crushed lightly by a wooden pestle to dehull the seeds. A small amount of firewood ash is often added. The soybean grits, containing teared hulls, are then wrapped with fresh fern (*Athyrium* sp.), *Musa paradisiaca* L. or *Leucosceptrum canum* Sm.

(sometimes replaced by broad leaves from other plants, such as *Macaranga pustulata* King ex Hook.f., *Ficus hookeriana* Corner or *Bauhinia vahlii* Wight & Arn.), covered with sackcloth, and kept in a bamboo basket above an earthen oven in kitchen to ferment for 1-3 days. The desired state of fermentation is indicated by the formation of a typical kinema flavour dominated by ammonia. Kinema shows long stringy threads when touched with fingers; the longer the thread, better is the quality of kinema. Fresh kinema keeps for 2-3 days during summer and a maximum of one week in winter. The shelf life kinema is often lengthened to one month by drying in the sun or by keeping on earthen ovens in kitchens (Sarkar *et al.*, 1993).

Kinema is a naturally fermented product. No deliberate inoculum is used during the traditional method of preparation. While *Bacillus subtilis* was the most dominant bacterium isolated from raw soybeans, kinema contained *B. subtilis* as well as *Enterococcus faecium*. In addition, yeasts such as *Candida parapsilosis* and *Geotrichum candidum* were found in 50-80% of market samples (Sarkar *et al.*, 1994). The high prevalence of *B. subtilis* and *E. faecium* in kinema indicates their possible involvement in its production. However, the *Bacillus*-fermentation of soybeans to produce kinema was quite unaffected by the presence of *E. faecium* in terms of proteolytic activity, ammonia production and final pH of the fermentations (Sarkar *et al.*, 1993). *B. subtilis*, therefore, is the sole organism carrying out the kinema production; the members of accompanying flora are merely opportunists (Sarkar and Tamang, 1994). The initial count of *B. subtilis* increased significantly from 10^5 cfu g^{-1} of soybean at 0 h to 10^8 cfu g^{-1} kinema produced at 48 h (Sarkar and Tamang, 1995). Monoculture fermentation of soybean by *B. subtilis* produced kinema with superior organoleptic attributes (Sarkar and Tamang, 1994). The fermentation processes as well as the acceptability of kinema were improved further by fermenting sterilized beans with *B. subtilis* at 45°C which led to more desirable fermentation within a much shorter period, compared to the traditional fermentation process (Sarkar and Tamang, 1995).

The pH and moisture content of kinema range between 7.7-8.1 and 60-63%, respectively. Kinema contains per 100 g dry matter: 47-49 g protein, 16-18 g fat, 27-30 g carbohydrate, 6-7 g ash, 2.4-3.0 g free fatty acids (as linoleic acid), 100 mg tritable acids (as lactic acid) and 1.9-2.0 MJ energy (Sarkar and Tamang, 1995; Sarkar *et al.*, 1994).

Studies on the lipid profiles of soybean during kinema production revealed that the formation of kinema led to an overall increase in fatty acid levels over the raw beans (9% for saturated and 6% for unsaturated fatty acids). The content of individual fatty acids, except for palmitic and stearic acids, was higher in kinema than that in raw beans. Free fatty acids liberated from soybeans in kinema also play an important role in the development of flavour. However, nonspecific inhibitory activity of free fatty acids could diminish the nutritional quality of kinema. Fermentation also increased about 20-30% crude lipids and 58% phytosterols of the raw substrate (Sarkar *et al.*, 1996).

B-group vitamin content of kinema was generally higher than that in unfermented beans. Soaking of beans led to a significant decrease in thiamine content, but no change was observed in riboflavin content. The remarkable decrease in thiamine content may be due to increased thiaminase activity or complex formation. Cooking, on the other hand, had no significant influence on thiamine content, although riboflavin and niacin levels increased. *Bacillus*-fermentation (at 37°C for 48 h) enhanced thiamine, riboflavin and niacin levels by 45, 71 and 23%, respectively. These levels declined by 31, 18 and 74%, respectively, in the presence of *E. faecium*, indicating that this bacterium uses readily available vitamins for growth and metabolism. Since thiamine is susceptible to heat, kinema prepared at 45°C led to a 33% decrease in levels of this vitamin compared to that prepared at 37°C. Riboflavin and niacin are heat stable, and no significant changes in their concentrations during fermentation at the elevated temperature were noted (Sarkar *et al.* 1998).

Compared to raw soybean, kinema contained significantly lower levels of minerals. The concentrations of calcium, iron, phosphorus and potassium in kinema are similar to those reported in natto (Ohta, 1986). Potassium showed the largest reduction (8.3 times) during kinema production. Water discarded during kinema preparation (after soaking and cooking) may be responsible for the six-fold depletion in mineral levels (Sarkar *et al.*, 1998). In traditional practices, spring water is used for soaking raw beans, and mineral content of traditional kinema may not be less than that observed and may even be higher in some instances than in raw beans. However, mineral content of kinema is influenced by the mineral content of the water used for soaking raw soybean. Importantly, levels of toxic elements such as cadmium, nickel and lead were below the detection limit. Despite such large losses in minerals during processing, kinema still contains appreciable quantities of calcium, magnesium, phosphorus and potassium (Sarkar *et al.*, 1998).

In a study on foodborne pathogens in kinema, Nout *et al.* (1998) found *Bacillus cereus* exceeding 10^4 cfu g^{-1} product in 33% of the market samples. Enterobacteriaceae and coliforms exceeding 10^5 cfu g^{-1} were found in 67% of the samples. *Escherichia coli* exceeding 10^5 cfu g^{-1} was found in 13% of the samples. *Staphylococcus aureus* was not detected in kinema. Though diarrhoeal type enterotoxin was produced by many *B. cereus* strains, it was inactivated during frying kinema in oil (Nout *et al.*, 1998).

Interspecific as well as intraspecific relationships among the microbial components, particularly *Bacillus* and related genera, from indigenously fermented soybean (kinema) and locust bean (soumbala) were characterized. RAPD-PCR fingerprint analysis showed a high level of genomic diversity among the *B. subtilis* isolates. Profiles of different carbon source fermentation and functionality (based upon estimations of pH, free amino nitrogen and stickiness) of *B. subtilis* isolates were associated with their genotypic and phenotypic profiles (Sarkar *et al.*, 2002).

2.1.6. Masyaura

Masyaura, a savoury made from blackgram dal, is a dried, hollow, brittle, and spongy friable ball of 5-10 cm in diameter, and shares similarity with North Indian Punjabi wadi and South Indian sandige. It is usually prepared in cottage or home-scale level, and used as a condiment or adjunct in cooking vegetables, or mixed with curry to make soups and served with rice as a side dish (Dahal *et al.*, 2003; Gajurel and Baidya, 1979; Tamang, 1992).

In India, consumption of masyaura is confined to a few places in Sikkim and Darjeeling hills of West Bengal. In Darjeeling hills, the average consumption rate of masyaura during 1996-97 was about 17%, whereas Sikkim had only 11% average consumption rate of masyaura during the same period (Yonzone and Tamang, 1998).

Traditionally, blackgram dal is the primary substrate for masyaura, occasionally supplemented with starchy roots or tubers of colocasia (*Colocasia esculenta* (L.) Schott), dioscorea (*Dioscorea alata* L.), radish (*Rhaphanus sativus* L.) or ash gourd (*Benincasa hispida* (Thunb.) Coqn.), depending upon their availability (Karki, 1986; Tamang, 1992). Blackgram dal is washed and soaked in water overnight (16 h). Seeds are drained and dehulled by hand-pressing. The hulls are flown off. Seeds are ground to a thick paste which is then added with turmeric (*Curcuma longa* L.) powder and shredded roots of dioscorea (Tamang, 1992) or colocasia tuber or ash gourd or radish, mixed properly in a ratio of 1:1, and allowed to ferment at room temperature for 1-2 days (Karki, 1986). The fermented batter is then hand-moulded into balls or cones (5-10 cm in diameter) and sun-dried on bamboo trays for 3-5 days depending upon the weather conditions (room temperature, 27°C; humidity, 65%) (Dahal *et al.*, 2003). Masayura has a long shelf-life (about one year) (Tamang, 1992).

Sun-drying of moulded paste favours microbial growth; the load of mesophilic aerobes and lactic acid bacteria increased significantly from 10^8 and 10^7 to 10^9 and 10^9 cfu g^{-1}, respectively. The results also indicated that lactic acid bacteria, accounted for 90% of the total flora during masyaura production. *Pediococcus pentosaceus* (75%) and *Pediococcus acidilactici* (10%) were the major lactic acid bacteria recovered from masyaura, followed by *Lactobacillus* spp. It is likely that colocasia shreds used in the preparation and handling might have contributed to the initial inoculum of the product. Enumeration of yeasts and moulds from masyaura revealed that *Saccharomyces cerevisiae* and *Candida versatilis* are the major yeasts involved in the fermentation, whereas the principal moulds involved are *Cladosporium* spp., *Penicillium* spp. and *Aspergills niger*. However, the mould count was almost constant up to 3 days and later increased to 10^5 cfu g^{-1} after 4 days (Dahal *et al.*, 2003).

From the nutritional point of view masyaura is a good source of protein (18-20%), carbohydrate (67-70%) and minerals. The soluble proteins, nonprotein nitrogen and B-vitamins increased during drying of masyaura. The changes in proximate composition observed were probably the result of microbial activities especially of lactic acid bacteria in the dough involving fermentation, thus accounting for the two-fold rise in total acidity and reduced pH (6.1-5.4). Increase in the levels of soluble protein, amino nitrogen and nonprotein nitrogen was presumably the result of the production of proteolytic enzymes by the fermenting microbes and the enzymatic hydrolysis of insoluble polymers under acidic conditions. The rise in various vitamin levels especially thiamine and riboflavin during the preparation appeared to be the ability to produce vitamins from simple precursors. Increase in total acidity in the product also helps in enhancing the shelf-life of the food. The *in vitro* digestibility of protein and starch was higher in sun-dried masyaura which may be due to fermentation and breakdown of proteins during drying (Dahal *et al.*, 2003).

2.1.7. Meitauza

Meitauza is made by solid-state fermentation of okara (insoluble carbohydrate residue that is left over after the production of soymilk or tofu) (Kronenberg and Hang, 1984; O'Toole, 1999). Okara is ground, steeped, strained and formed into round cakes of 10-14 cm in diameter and 2-3 cm thick at the middle and 1-1.3 cm thick at the edges. The cakes are placed in a vessel and left to ferment with moderate aeration until, after 10-15 days, they are covered with white mycelium of *Actinomucor elegans*. The moulded cakes are then sun-dried. Meitauza is served either fried in vegetable oil or cooked with vegetables as a flavouring agent (Nout *et al.*, 2007).

Changes in proximate composition of okara during fermentation were characterized by the liberation of ammonia, an eight-fold increase in nonprotein nitrogen and production of acid protease, and a rise in pH from 5.5 to 7.5 (Kronenberg and Hang, 1984). This product has also been used as a model for studying solid-state fermentations (Kronenberg and Hang, 1985). Their studies showed that mycelial growth, and not water loss or crust formation, caused increased firmness during fermentation.

2.1.8. Miso

Miso is one of the most typical kinds of traditional soybean fermented foods of Japan. Similar kind of fermented soy pastes are known as chiang in China, jang or doenjang in Korea, taoco in Indonesia and tao chieo in Thailand. In addition to soybean and salt, most of these products also contain cereals such as rice or barley (Minamiyama and Okada, 2003). As an all-purpose seasoning with a rich, hearty, often meat-like flavour and aroma, miso can be used (often like a meat stock or bouillon) in the

preparation of soups, sauces, gravies, dips, dressings and many other foods. As a result of the current healthier trend in dietary habits, there has been a recent increase in the popularity of miso and its processed products (Ebine, 1990).

The origin of miso is not clear, although most scholars agree that its earliest progenitor came from either China or Korea along the arrival of Buddhism in the 6th Century AD. Others trace the origin of miso to the northeastern provinces, known as the 'miso heartland', of Japan itself, where archeological evidence indicates the early mastery of fermentation. The first records of miso date from the Nara period (AD 710-784).

Miso is fermented using *Aspergillus oryzae* and *Zygosaccharomyces rouxii*. Shibasaki and Hesseltine (1962) described the traditional method of miso preparation, using a two-part fermentation. In the first part, steamed grains (typically rice or barley, but in some cases soybean) are inoculated with the mould and incubated for about 48 h to make koji, which serves as a source of enzymes. In the second part, koji is mixed with cooked soybean, salt, water and seed miso, packed into large vats and fermented for 6-18 months.

Heat-treated rice and/or soybean are used to prepare shinshu or rice-soybean miso. After the initial solid-state fermentation dominated by *A. oryzae*, salt (38% of the original weight of dry soybean) is mixed with the koji. The mixture is inoculated with *Z. rouxii*. Traditionally, sound miso from a previous batch is used to inoculate the koji-soybean-salt mixture prior to fermentation. Although halophilic yeasts such as *Candida versatilis* may be present, only *Z. rouxii* produces the desired metabolites for an acceptable product. Depending on the concentration of salt added and the period of fermentation, final product can be categorized as white miso, soybean miso, yellow or brown miso etc. (Nout *et al.*, 2007).

Three basic types of miso can be recognised (Hesseltine, 1965; Shibasaki and Hesseltine, 1962): 'kome miso' (made from beans and rice), 'mugi miso' (made from beans and barley) and 'mame miso' (made from soybeans alone), sold as 'hatcho miso' in the United Kingdom. For the production of kome and mugi misos, the koji is made by soaking polished rice overnight in water, steaming it for about an hour, then inoculating it with mould spores. Incubation is for 50 h at 35°C with thorough stirring at least twice. The koji is then mixed with an appropriate mixture of soaked, cooked soybean, barley or rice, and salt. Even in the case of mame miso, only a portion of the beans is moulded. The precise proportions of beans and grain used, and also the extent to which the beans are cooked, depend on the type of miso being made (Wood, 1977).

Shibasaki and Hesseltine (1962) reported high microbial counts in the early stages of miso production and their decline during later stages. Lactic acid bacteria, ranging from 10^3 to 10^6 cfu g^{-1}, were present as the indigenous microbiota on the nonsterilized miso samples (Onda *et al.*, 2002). Some salt-tolerant (halophilic) lactic acid bacteria and yeasts are involved in the miso fermentation (Collins *et al.*, 1990; Nout *et al.*, 2007; Okada, 1996; Yoshii, 1995). Strains of halotolerant *Enterococcus faecalis* are predominant early in the fermentation process and play roles in acid production and maintenance of the bright colour (the so-called 'sae' effect) of miso. During the ripening of miso, haloplilic strains of *Tetragenococcus halophila* predominate and atributte some important functions, like acid production (decrease in pH), production of preferable flavour (the so-called 'shio-nare' effect and 'oshi-aji' effect), masking of offensive flavour and induction of yeast growth (Yoshii, 1995).

Strains of *Bacillus subtilis, Pediococcus acidilactici, Micrococcus* sp., *Lactobacillus plantarum, Lactobacillus fructivorans* and *Clostridium* sp. are known to be undesirable bacterial contaminants in miso, which adversely affect its quality (Ito and Ebine, 1967; Nikkuni *et al.*, 1996; Yoshii, 1995). The

predominant growth of *B. subtilis* in miso koji causes irregular koji (also called 'nebari-koji' and 'natto-koji'), and causes the off-flavour and dark-colouration during fermentation (Yoshii, 1995), resulting in the unacceptable organoleptic quality of the final product. Pasteurization ('hi-ire') and addition of ethanol ('aruten') are general practices to control the growth of contaminants in miso products. However, addition of alcohol to miso is not cost-effective (Miyasaka, 1992). Onda *et al.* (1999) isolated a bacteriocin-producing lactic acid bacterial strain (*Enterococcus* sp. GM005) from miso. However, bacteriocin-producing lactic acid cocci were widespread at high frequencies in miso and were identified as *Enterococcus durans* and *Enterococcus faecium*. They are supposed to play important roles in preventing the growth of undesirable bacteria, such as *B. subtilis*, *P. acidilactici*, *L. plantarum* and *L. fructivorans*, and act effectively as safe biopreservatives in miso (Onda *et al.*, 2002).

Miso is considered as a health-promoting functional food, offering protection against gastrointestinal disorders, cancers of breast, stomach and colon, and cholesterol-associated and degenerative diseases (Minamiyama and Okada, 2003). *In vitro* studies on experimental animals have shown that miso has a protective effect against radiation injury and appearance of cataract (Gotoh *et al.*, 1998a; Ito, 1991; Watanabe *et al.*, 1991), and reduces the risk of liver, stomach and mammary tumours, and colonic aberrant crypt foci (Gotoh *et al.*, 1998a; Masaoka *et al.*, 1996; Watanabe *et al.*, 1997). Miso contains a variety of biologically active substances. Two of the isoflavones, genistein and diadzein, are known to have a diverged biological activities and to be present in significant amounts (0.2-0.9 g kg^{-1}) in miso as compared with other soy products (Fukutake *et al.*, 1996; Manach *et al.*, 2004). Although it has been reported that genistein and daidzein might play an important role in preventing several type of cancer (Adlercreutz, 1997), Hirota *et al.* (2000) are of opinion that 8-hydroxygenistein recovered from miso might inhibit proliferation of cancinogenic cells rather than genistein and daidzein. They further isolated and elucidated the structure of DPPH· (1,1-diphenyl-2-picryl-hydrazyl radical)-scavengers from miso, and studied their DPPH·-scavenging activities, and antiproliferative activities towards cancer cell lines. Miso is a useful agent for chemoprevention of MNU (N-methyl-N-nitrosourea)-induced rat mammary cancer, and is expected to have an excellent antitumour effect, especially when used in combination with tamoxifen (Gotoh *et al.*, 1998b).

In miso, the moisture content is 44-52%, protein is 8-19%, carbohydrate is 6-13% and fat is 2-10%, depending upon the ratio of soybean, rice and barley used as ingredients. During fermentation and aging of miso, soybean protein is digested by proteases produced by *A. oryzae* in the koji. Amino acids and their salts, particularly sodium glutamate, contribute to flavour. Miso contains 0.6-1.5% acids, mainly lactic, succinic and acetic. Esters of ethyl and higher alcohols with fatty acids in soybean lipid are important in giving miso its characteristic aroma. Up to 35% of the initial lipid content is degraded into fatty acids; the extent of maturation can be conveniently monitored by the levels of fatty acid ethyl ester (Yamabe *et al.*, 2004). Furanones, HEMF (4-hydroxy-2 (or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone) and HDMF (4-hydroxy-2,5-dimethyl-3(2H)-furanone), produced by *Z. rouxii* have been identified as important flavour components in miso. Miso also contains B-vitamins (riboflavin and cyanocobalamin) as a result of yeast fermentation (Nout *et al.*, 2007).

2.1.9. Natto

Natto is a popular soybean fermented alkaline food in the Japanese diet. It is gray to tan in colour, and has a strong and persistent unique flavour, sometimes associated with a noticeable odour of ammonia (Ohta, 1986; Steinkraus, 1983). Natto is usually eaten as is with shoyu (soy sauce) or with spicy mustard (Fukushima, 1979; Kiuchi *et al.*, 1976), or eaten with boiled rice and often used as a flavouring agent in cooked meat, vegetables and sea foods (Ohta, 1986).

Traditionally, three major types of natto are known to prepare, 'yukiwari-natto' and 'hama-natto' are koji (*Aspergillus oryzae*)-based products, while the 'itohiki-natto' (sticky natto), which is more common, is a *Bacillus*-fermented product. Itohiki-natto, generally referred to as natto, is popular in the eastern Kanto region (Tokyo). It is produced by fermenting whole cooked soybean with *Bacillus subtilis* and accounts for more than the total production of the other two major types of natto. The less common yukiwari-natto is prepared by mixing itohiki-natto with rice koji and salt, and then aging. Hama-natto is prepared by using the koji mould *Aspergillus oryzae* (Kiuchi *et al.*, 1976). Itohiki-natto was traditionally consumed by the Buddhist monks and also by the farmers during winters (Ohta, 1986). Japanese domestic soybeans of small (up to 5.5 mm in diameter) and uniform size with white to pale yellow hilum, thin and smooth seed coat and a high carbohydrate content are preferred for natto preparation (Ohta, 1986).

To prepare natto traditionally, soybeans are soaked overnight and boiled until tender. Excess water is drained off and the beans are allowed to partially air dry over bamboo trays for 20 min. The beans are put into shallow paper, and containers are stacked one above the other in large wooden boxes, covered with straw-mats and placed near ovens to ferment at approx. 36°C for one day (Standal, 1963). The straw used as wrapping material of cooked soybeans, before pine-wood sheet came into use, contained *B. subtilis* (USDA, 1958).

The use of modern technologies, such as the use of *B. subtilis* as a starter culture was developed after the 1920s (Ohta, 1986). The cooked beans are inoculated with spores of *B. subtilis* and tumbled in a barrel until the organisms are well distributed (Ohta, 1986; USDA, 1958). The beans are wrapped in paper-thin sheets of pine wood (USDA, 1958) or plastic package (Hesseltine and Wang, 1967). Polysterene foams are also in use. They are fermented for 18-20 h (Hesseltine and Wang, 1967; Ohta, 1986; USDA, 1958). The most favourable conditions for natto production are created by inoculating cooked beans with 10^8-10^9 *B. subtilis* spores ml^{-1}; equivalent to 0.5-1% substrate at 45°C, mixing thoroughly and fermenting at 40-43°C for 6 h (Sakurai, 1960). The best quality natto can be produced by incubating the inoculated beans at 40°C and 85% relative humidity for 12-16 h (Takahashi and Shimakawa (1976).

Yabe (1984) was the first to study the microorganisms involved in natto production. Sawamura (1906) identified the fermenting organisms as *Bacillus natto* in natto. Gordon *et al.* (1973) considered this species to be a synonym of *Bacillus subtilis* (Ehrenberg) Cohn. However, not all strains of *B. subtilis* are suitable for making good natto (Hesseltine, 1983, 1986). Hayashi (1977) and Ohta (1977) mentioned *B. natto* SB 3010 as the most suitable strain for natto production. The unique feature of the natto strain is the formation of a sticky viscous material which gives natto its unique character (Ohta, 1986).

Natto is also characterized by the presence of a sticky paste on its surface. When stirred, the paste increases in volume, becomes stickier and is held together like a spider web by gossamer-like threads. Natto mucin is composed mainly of an acidic glycopeptide and contains 61.5% sugars, 2.8% hexosamine, 4.1% total nitrogen, 2.9% amino-nitrogen and 20.4% uronic acid (Hayashi *et al.*, 1971). Ishikawa *et al.* (1972) examined the characteristic spinnability of a natto mucin solution and found that the mucin contained 22% fructan and 78% poly-DL-glutamic acid with δ-peptide linkage (δ-PGA). Saito *et al.* (1974) found that natto mucin is composed of 58% δ-PGA and 40% polysaccharide. Production of δ-PGA in the natto strains of *B. subtilis* is regulated by *comQXPA* quorum-sensing system and is genetically unstable because of translocation of IS*4Bsu1* into the *comP* gene at a high frequency (Nagai *et al.*, 2000). The IS*4Bsu1* is widely distributed among *B. subtilis* strains in other similar soybean-fermented foods, such as kinema, Thai thua-nao, Chinese douchi, Korean chungkuk-jang, and Burmese

chine pepoke (Inatsu *et al.*, 2002). Natto mucin can absorb 5,000 times its weight in water, and this remarkable property is used in cosmetics and wrapping of food products.

Natto has a characteristic pungent but pleasant aroma. The unique flavour of natto was thought to be related to its diacetyl content (Obata, 1959). Sulphur-containing compounds derived from cooked soybeans and pyrazines formed during fermentation are the main contributors to the characteristic natto odour. The sulphur compounds include 4-ethyl-2-methylthiazole, 3,5-dimethyl-1,2,4-triothiolane and thialdine. The pyrazines present at the highest concentrations include tetramethyl, trimethyl and 2,5-dimethyl derivatives (Owens *et al.*, 1997). Kosuge *et al.* (1962) identified tetramethyl pyrazine as the flavour component in natto. They also established that some of the free fatty acids, like butyric and isovaleric acids produce an undesirable odour in natto. Some of the flavours originate from the hydrolysis of soy protein to peptides and amino acids (Ohta, 1986). The nature of free amino acid profile in natto is similar to that of kinema (Nikkuni *et al.*, 1995).

During 18 h-fermentation of natto, the dry matter and total nitrogen content remained fairly constant at 95.5-96.1% and 7.2-7.5%, respectively; soluble nitrogen and aminonitrogen increased from 1.3 to 3.1% and 0.3 to 0.6%, respectively; ammonia nitrogen increased from 0.02 to 0.2%, whereas reducing sugars decreased from 13.4 to 11.8% (Sakurai, 1960). However, Hayashi (1974a,b,c,d) found 4% increase in total nitrogen in natto over total nitrogen of raw soybeans. This was because *B. subtilis* could fix dinitrogen. Natto is rich in essential amino acid content, compared to soybean (Sano 1961). The fatty acid compositions of natto and soybean do not differ significantly. The predominant fatty acid in natto is linoleic acid followed by oleic, linolenic and stearic acids (Goto, 1974). The riboflavin content, however, increased after fermentation (Arimoto, 1961).

Natto is prized for its high nutritional value and improved digestibility, and appreciable amount of certain vitamins, produced as a result of fermentation (Ohta, 1986; Reddy *et al.*, 1982; Standal, 1963; Steinkraus, 1996). Natto is a good source of fibre and free fatty acids (Ohta, 1986). Hayashi and Nagao (1975) reported the conversion of bacterial cells to spores during preservation increases the nutritive value of natto. It is the only food in the category of alkaline fermentations that has been industrialized.

Natto contains 50-60% 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 MJ 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).

2.1.10. Okpehe

Okpehe (okpiye) or ogiri-okpei or kpaye is an alkaline fermented condiment, prepared from the seeds of *Prosopis africana* Taub. It imparts pleasant aroma and flavour to soups and stews, and commonly used as a food condiment by Igbos of Eastern Nigeria and Igalas of Northern Nigeria (Odibo *et al.*, 1992; Oguntoyinbo *et al.*, 2001; Omafuvbe *et al.*, 1999).

During the traditional method of okpehe preparation, the seeds are boiled for 6-7 h. Seed coats are removed and the cotyledons, after washing, are boiled for 30 min. On cooling, the cooked cotyledons are wrapped with plantain (*Musa sapientum* L.) leaves and allowed to ferment for 3-5 days at room temperature (28-30°C) with intermittent exposure (2-3 h) of the packet to sunlight. At the end of fermentation, the fermented seeds are mashed with a pestle, moulded into small balls and sun-dried to obtain okpehe (Odibo *et al.*, 1992).

Several species of bacteria, especially *Bacillus spp., Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter cloacae, Klebsiella pneumoniae, Lactobacillus plantarum* and *Micrococcus* spp. were recovered from the fermenting and fermented mass of okpehe. Furthermore, okpehe purchased from local markets gave a similar flora in addition to the presence of *Pediococcus* sp., *Escherichia coli* and *Enterococcus faecalis* (Achi, 1992; Odibo *et al.*, 1992). *Bacillus* spp. were most dominant in fermented okpehe with an average count of 10^7-10^9 cfu g^{-1}. They were identified as *Bacillus subtilis* (36%), *Bacillus pumillus* (20%), *Bacillus licheniformis* (16%) and *Bacillus megaterium* (13%) (Oguntoyinbo *et al.*, 2001). However, *E. cloacae* and *K. pneumoniae* made significant contributions to the microbial ecology during okpehe fermentation. *Lactobacillus* spp. were present in low numbers towards the end of fermentation. Variations in the important microbial groups show that *Bacillus* spp. were most prevalent and occurred until the end of fermentation (Achi, 1992). The occurrence of *E. coli* and *E. faecalis* from the market samples is an index of poor sanitary handling of the food condiment by the seller (Odibo *et al.*, 1992).

The potentiality of *Bacillus* spp. and *Enterococcus* spp., isolated from traditional okpehe fermentations as starter cultures for the fermentation of *P. africana* seeds for okpehe production, was studied by Oguntoyinbo *et al.* (2007a,b). Although monoculture fermentation using only *B. subtilis* strain BFE 5372 produced okpehe with good sensory characteristics, the growth of *Bacillus cereus* was detected after 48 h of fermentation. However, mixed culture fermentation with the combination of bacteriocin-producing starter *B. subtilis* (BFE 5301) and the nonbacteriocin-producing *B. subtilis* BFE 5372 produced a product with good sensory characteristics in which growth of *B. cereus* was delayed. The bacteriocin, identified as subtilisin, was heat-stable at 100°C for 10 min and exhibited the highest activity at pH values lower or equal to pH 6.0, but was sensitive to proteolytic enzymes. Other combinations of mixed culture fermentations did not yield organoleptically acceptable products (Oguntoyinbo *et al.*, 2007a,b).

Okpehe fermentation involved a rise in pH, temperature, moisture content and total free amino acids, while titratable acidity and reducing sugar decreased gradually after 24 h and 72 h of fermentation, respectively (Omafuvbe *et al.*, 1999). Temperature, pH and titratable acidity varied with time and were influenced by the metabolic activities of the microorganisms (Achi, 1992). The pH of the fermenting seeds decreased from 6.3 (0 h) to 5.0 (96 h), while the temperature and moisture content increased from 32-38°C and 30-40%, respectively (Odibo *et al.*, 1992). The fatty acids identified in both nonfermented and fermented products (okpehe) were linoleic, oleic, stearic and palmitic. Oleic acid was present in large proportion as a free fatty acid, and linoleic acid in appreciable amounts in fermented sample (Sanni *et al.*, 1993). The total nitrogen in the fermented mash decreased, while a high proteinase activity as well as an increase in the amounts of amino acids were observed. Amylase activities also increased culminating in a peak at 72 h before stepping down. Soluble sugars showed a fluctuating pattern, whereas lipase activity was low in the fermenting mash (Sanni, 1993).

The moisture content of okpehe from different markets varied from 41 to 60%, while the protein content ranged from 33 to 35%, fat content 1.2 to 1.4%, fibre 1.3 to 1.6%, ash 3.1 to 4.4%, starch 13.6 to 14.2% and pH value 6.8 to 7.8 (Oguntoyinbo *et al.*, 2001).

With a view to obtain an adequate understanding of the wild strains of *B. subtilis* isolated from okpehe, investigation was carried out for their phenotypic diversity and functionality such as the production of enzymes, δ-PGA and bacteriocin. It was believed that the data thus generated would enable the selection of appropriate starter cultures for fermentation that will aid complete hydrolysis of the complex composition of the vegetable proteins and further achieve consistency of a product free of pathogenic and spoilage organisms. Moreover, application of such information could aid the

maximization of functionality of wild strains, particularly during the industrial take-off of condiment production (Oguntoyinbo *et al.*, 2007b).

2.1.11. Oncom

Onchom (formerly called 'ontjom', now spelled 'oncom') is a traditional Indonesian fermented food, and a close relative of okara tempeh, except that the fermentation is done with *Neurospora* (rather than *Rhizopus*), which envelops the cakes with a brilliant orange mycelium. Made and consumed for centuries, but only in West Java and especially in Bogor, the food is known there as onchom merah, onchom bereum or onchom tahu (Sastraatmadja *et al.*, 2002; Shurtleff and Aoyagi, 1979a).

The raw materials of oncom are mainly the wastes of agricultural products. Traditionally, oncom is produced by mixing soaked peanut press cake with starchy ingredients (such as cassava residues or tapioca flour) and soybean curd (15:5:1). The mixture is steamed for 1 h and inoculated, after being cooled, with pre-growing fungal mycelium, usually *Neurospora sitophila, Neurospora intermedia, Mucor* sp. and *Rhizopus* sp. The inoculated dough is moulded to form brick-shaped pieces that are incubated for 24-48 h in banana leaves at ambient temperature (25-30°C) (Djien, 1986; Sastraatmadja *et al.*, 2002). Oncom hitam (black oncom) and oncom merah (yellow-red oncom) contain different mycoflora. The merah type contains mainly *Neurospora*, whereas hitam is dominated by *Rhizopus* spp. *Rhizopus* sporangiospores are black due to melanoids, while carotenoids form the basis of the orange-yellow colour of *Neurospora* (Nout *et al.*, 2007).

Oncom flavour has been described as fruity and somewhat alcoholic; after frying, mince-meat or almond flavour can be detected. The enzymic activities (lipases and proteases) provoke an increase in free fatty acid content and degradation of proteins, resulting in improved protein digestibility which is relevant for consumers with digestive disorders (Nout *et al.*, 2007). Soy oncom, made with soybean inoculated with *Neurospora sp.*, resembles tempeh except that the flavour was more nutlike. Fermentation causes no significant change in water content or pH of oncom. Total nitrogen content increased slightly, probably because of evaporation of nonnitrogen volatile compounds formed during fermentation. Soluble nitrogen content, however, increases in different degrees, depending on the fermenting mould species (Sastraatmadja *et al.*, 2002).

Low salt O-miso was prepared from koji fermentation of soybean-oncom (S-oncom) and okara-oncom (O-oncom). Strong activities of DPPH·-scavenging, superoxide anion-scavenging and antimutagenic activities were detected in both ethanolic (70%) and aqueous extracts of *N. intermedia*-fermented seasonings. These activities are attributable to the higher contents of isoflavone-aglycones and melanoidines present (Matsuo, 2004a, 2006; Matsuo and Takeuchi, 2003). The hydrophilic antimutagenicity of defatted-oncom (D-oncom) against N-nitrosodimethylamine was investigated (Matsuo, 2004b). Aqueous extract of D-oncom had a stronger antimutagenicity than that of defatted soybean, about one-sixth of that of ascorbic acid. The main antimutagenic fraction of D-oncom was anionic and stable against heating at 37°C, and could be involved in the reduction of oxidative stress by scavenging superoxide anions (Matsuo, 2004b).

Oncom is the only food for human consumption produced by fermentation with the *Neurospora* spp. (Sastraatmadja *et al.*, 2002). Instead of using dry spore-based starters, starters for oncom are propagated and maintained by mycelial growth in a kind of fed-batch solid-state fermentation kept active by the processor. Although very little controlled experimentation has been done on this fermentation, it is presumed that a method of vegetative propagation is needed because *Neurospora* spores have limited viability when stored dry and have poor germination ability (Nout *et al.*, 2007).

2.1.12. Papad

Papad constitutes an important legume-based traditional food adjunct. It is made from legume-based dough often containing other farinaceous materials along with added salt, spices and condiments. This thin, usually circular, wafer-like product is used to prepare curry or is eaten by itself as a crackly snack or appetizer with meals after roasting or deep-frying in vegetable oil (Aidoo *et al.*, 2006; Bhattacharya and Narasimha, 1999).

Traditionally, blackgram dal flour or its blend with Bengalgram, lentil or mung dal flour is hand-kneaded with a small quantity of peanut oil, common salt (~ 8%, w w^{-1}), papad khar or sajji khar (saltworts produced by burning a variety of plant species or from highly alkaline deposits in the soil) and water, and then beaten or pounded with a cylindrical stone device into a stiff dough. The dough (sometimes with a backslop and spices added) is left to ferment for 1-6 h. The fermented dough is shaped into small balls which are rolled into thin, circular flat sheets (10-24 cm in diameter and 0.2-1.2 mm in thickness) and generally dried under shade to 12-17% (w w^{-1}) moisture content (Aidoo *et al.*, 2006; Shurpalekar, 1986).

Extensive work on proximate composition, storage, packaging and quality control of papads in India has been reported (Kulkarni *et al.*, 1996; Manan *et al.*, 1988; Pruthi *et al.*, 1984; Shurpalekar 1986). Though not much is known about the functional microbiota of papad, the possible role of *Candida krusei* and *Saccharomyces cerevisiae* during the preparation of papad has been suggested by Shurpalekar (1986). The occurrence of *Bacillus cereus* in 21% of the papad samples collected from different retail sources suggested the potential risk to the consumers (Roy *et al.*, 2007). Since papad is roasted or deep-fried in refined vegetable oil before consumption, the heating step may lower down, if not eliminate completely, the count of these undesirable bacteria.

Papad contains 10-15% moisture and per 100 g dry matter 15-30 g (or even higher with higher proportion of legume) protein, 2-8 g fat, 40-55 g carbohydrate, 12-15 g fibre, 6-8 g ash, 1.2-1.5 MJ energy, 300 mg Na, 100 mg K, 100 mg Ca, 300 mg Mg, 15 mg Fe, 0.7 mg Cu, 3.0 mg Zn, 0.25 mg thiamine, 0.15 mg riboflavin, 1.7 mg niacin and 75 μg folic acid (Campbell-Platt, 1987).

2.1.13. Soy sauce

Soy sauce (Japanese 'shoyu') is light to dark brown liquid produced from fermented soybean with meatlike salty flavour. It is used as a table condiment, and is probably the man's oldest prepared seasoning. Traditionally made in the Orient, this savoury seasoning source is now widely produced and used in European as well as American cuisine (Kataoka, 2005; Nout *et al.*, 2007; Nunomura and Sasaki, 1993). Soy sauce could be classified into Japanese-type and Chinese-type. In Japanese-type soy sauce, soybean and wheat are used with the ratio 1: 1, whereas less wheat is used in the Chinese-type (Nunomura and Sasaki, 1993). In Japan, several distinguished types of soy sauce are being used viz., koikucji, usukucji, tamari, saishikome and shiro soy sauces, all having characteristic colour and flavour (Kataoka 2005; Nout *et al.*, 2007). About 83% of all soy sauce products consumed in Japan are koikuchi shoyu. The annual production of soy sauce in Japan has been about 1 million kilolitres in recent years. Japan exports soy sauce to more than 110 countries (Kataoka, 2005).

Soy sauce is prepared by digesting mould-cultured soybean-wheat koji, in presence of about 17-19% sodium chloride and concurrently fermenting them by lactobacilli and yeasts. After 6-8 months, the mash is pressed and the liquid part obtained is pasteurized to make the final product. The characteristic flavour-aroma formation in the soy sauce depends on the manner of production employed

as well as raw materials and strains of microorganisms used. The raw ingredients for soy sauce are soybean, wheat and brine (Nunomura and Sasaki, 1993).

There are two specific fermentation stages involved in soy sauce production, the first being an aerobic koji fermentation. Seed ('tane') koji is produced by culturing single or mixed strains of *Aspergillus oryzae* or *Aspergillus sojae* on either steamed, polished rice or a mixture of wheat bran and soybean flour. Seed koji is added to a soybean/wheat mixture at a concentration of 0.1-0.2% and fermented into what is then simply called 'koji'. The second stage is an anaerobic moromi or salt mash which undergoes lactic acid bacteria and yeast (*Zygosaccharomyces rouxii*) fermentation. In the moromi fermentation, *Tetragenococcus halophila* initially proliferates and produces lactic acid, which lowers the pH to 5.5 or less. This is followed by the growth of acid-tolerant yeasts, notably *Z. rouxii*, which produce about 3% alcohol and several compounds that contribute to the characteristic aroma of soy sauce. Although *Z. rouxii* is the dominant moromi yeast, other yeasts such as *Candida versatilis* and *Candida etchellsii* also produce phenolic compounds which contribute to soy sauce aroma (Nout *et al.*, 2007).

Production of koji is essentially a solid-state fermentation. The two main groups of enzymes produced by *A. oryzae* during koji fermentation are carbohydrases (α-amylases, amyloglucosidase, maltase, sucrase, pectinase, β-galactosidase, cellulase, hemicellulase and pentosan-degrading enzymes) and proteinases, although lipase activity has also been reported. These enzymes hydrolyze carbohydrates to sugars and proteins to amino acids and low molecular weight peptides. These soluble products are essential for yeast and bacterial activities during the moromi fermentation (Aidoo *et al.*, 1994; Chou and Rawn, 1995).

Nearly 300 flavour compounds have beee identified in Japanese soy sauce (Kataoka, 2005; Nunomura and Sasaki, 1992). *Z. rouxii* produces flavour compounds, including alcohol, glycerol, esters and furanones. Furanones (HEMF) produced by *Z. rouxii* and *Candida* spp. give Japanese-type soy sauce with its characteristic flavour (Nunomura and Sasaki, 1992). This compound is also reported to have antitumour and antioxidative properties (Lee, 1996). Notwithstanding its high salt content, soy sauces require pasteurization and adequate bottling for their preservation (Nout *et al.*, 2007).

Soy sauce has been expected to possess an ability to inhibit the deterioration of food and has been used as natural preservatives. Soy sauce with *p*-hydroxybutyl benzoate as preservative killed foodborne pathogens such as *Escherichia coli, Shigella flexneri, Salmonella typhi, Salmonella paratyphi A, Salmonella enteritidis* and *Vibrio cholerae* within 6 h (Kataoka, 2005). The antimicrobial activity of soy sauce is mainly based on the combined effects of NaCl, ethanol, pH, preservatives and temperature (Yamamoto *et al.*, 1978).

Soy sauce increases the appetite, imparts a delicious flavour and promotes digestion. It contains relatively high levels (17-19%) of salt and is used to enhance the flavour of meats, seafoods and vegetables. Typical ranges in other characteristics are: 4.6-4.8 pH, 0.5-2.5 g total nitrogen, 0.2-1.1 g formol nitrogen, 3.8-2.0 g reducing sugars, and traces to 2.2 ml ethanol per 100 ml (Nout *et al.*, 2007).

2.1.14. Sufu

Sufu or fu-ru is a fermented soybean food that originated in China. It is a soft creamy cheese-type product made form cubes of soybean crud (tofu) by the action of a mould which is consumed as an appetizer or a side dish, e.g. with breakfast rice or steamed bread (Steinkraus, 1996; Su, 1986). This fermented product with its characteristic flavour adds zest to the bland taste of the rice and flour diet and has been widely consumed by Chinese people as a salty appetizer for many centuries, which can be used in a similar way as cheese (Steinkraus, 1996).

Sufu is a name of the product that first appeared in the literature (Wang and Hesseltine, 1970). Literally, sufu means 'moulded milk' and tosufu (dou fu-ru) means 'moulded soymilk'. Because of numerous dialects used in China and difficulties of phonetic translation from Chinese into English, sufu has appeared in literature under many different names. The following synonyms for sufu in the literature have been found: sufu, tosufu, fu-ru, dou-fu-ru, tou-fu-ru, toe-fu-ru, jiang-dou-fu, fu-yu and foo-yue. Sufu is also known as tofuyo, nyu-fu and fu-nyu in Japan (Yasuda and Kobayashi, 1989), chao in Vietnam, ta-huri in the Philippines, taokaoan in Indonesia and tao-hu-yi in Thialand (Beuchat, 1995). The names confuse Western people as well as the Chinese. Officially, sufu should be named 'furu' or 'doufuru' in Chinese (Han *et al.*, 2001). It has been one of the most popular highly flavoured side dishes consumed in China for many centuries and is becoming popular in Chinese shops all over the world. Sufu products are manufactured both commercially and domestically in China.

Manufacture of tofu (soybean curd) began during the era of the Han dynasty. The 'Ben-Cao-Gang-Mu' (Chinese Materia Medica), compiled by Li Shi-zhen in 1597, indicated that tofu was invented by Li An (179 BC to 122 BC), the king of Weinan (Shi and Ren, 1993; Steinkraus, 1996). However, it is not known when sufu production began. Due to the long history and incomplete written records, no attempt was made to search for its origin. The first historical record mentioned that the sufu process was carried out in the Wei Dynasty (220-265 AD) (Wang and Du, 1998). It became popular in the Ming Dynasty (1368-1644), and there are many books describing sufu processing technologies (Zhang and Shi, 1993).

Preparation of sufu is a centuries-old household activity involving natural fermentation which can be produced by various processes in different localities of China (Wang and Du, 1998). However, the mould-fermented sufu is the most popular type (Han *et al.*, 2001). Four steps are normally involved in making this type of sufu: (1) preparing tofu, (2) preparing pehtze (pizi) by fungal solid-state fermentation of tofu using *Actinomucor elegans*, (3) salting of pehtze and (4) ripening in dressing mixture (Aidoo *et al.*, 2002). Traditionally, the process of preparing sufu starts with the production of soymilk. Dehulled soybeans are washed and soaked overnight in water, drained off the excess water, and ground in a stone mill. The slurry is diluted and pressed to obtain soymilk. Next, a coagulation step is carried out by adding salts (calcium or magnesium sulphate) or acid in order to obtain a precipitate of mainly soy protein and entrapped lipids. This is collected and pressed to obtain sheets of tofu (soybean curd) of the required moisture content and firmness.

To obtain pehtze (fresh bean curd overgrown with mould mycelia) cubes (dices) of tofu are placed in wooden trays, the bottom of which is made of bamboo strips loosely woven together. The loaded trays are piled up and covered with straw for natural inoculation and kept at 15-20°C for 5-15 days. Luxuriant mycelial development over the tofu usually results in the production of pehtze. Pehtze contains about 74% water, 12% protein, and 4.3% lipid. After flattening the mycelium to form a protective skin on the cubes, those are submerged in a salt-saturated solution and kept for 6-12 days. During this period, the pehtze adsorbs about 16% of the salt. The salted pehtze is washed with water and then transferred for ripening. For the ripening, alternate layers of salted pehtze and dressing mixture (consist of angkak, alcoholic beverage, salt, sugar, bean paste and spices) are packed into jar at the ratio of about 2:1. The mouth of the jar is wrapped with sheath leaves of bamboo, sealed with clay and aged for about 6 months for further maturation. The main functions of the maturation mix are preservation, flavouring and colouring. Preservation of sufu is achieved by a combination of salt and alcohol (rice beer may be used), whereas angkak and other ingredients impart specific flavour and colour to the product (Han *et al.*, 2001, 2004).

According to the colour and flavour, sufu can be classified into four types, i.e. red sufu, white sufu, grey sufu and others, which are mainly based on the different ingredients of dressing mixtures in the ripening (Han *et al.*, 2001). Depending upon the desired flavour and colour, pehtzes may be submerged in salted, fermented rice or soybean mash, fermented soybean paste or a solution containing 5-12% sodium chloride, red rice and 10% ethanol. Red rice and soybean mash impart a red colour to sufu. Use of brine containing high levels of ethanol results in sufu with a marked alcoholic bouquet. The major functions of the moulds in this process are the formation of a protective layer of mycelial biomass surrounding the pehtze cubes and, most importantly, to release several enzymes that are responsible for the partial degradation of the protein, fibre and lipid fractions in pehtze during the maturation. This degradation results in softening of the texture, solubilization of constituents and accumulation of flavour-enhancing compounds, such as glycine and glutamic acid (Han *et al.*, 2004; Liu and Chou, 1994).

The fungal genera involved were *Actinomucor*, *Mucor* and *Rhizopus* spp., all belonging to Mucorales. *A. elegans*, *Actinomucor taiwanensis*, *Mucor* spp. and *Rhizopus spp.* are used as pure starter cultures in the manufacture of Chinese furu or sufu (Benjamin and Hesseltine, 1957; Chou *et al.*, 1988; Han *et al.*, 2001; Su, 1986). *Mucor sufu* and *Mucor wutingqiao* have been mentioned as popular starter cultures. Nevertheless, most of *Actinomucor* and *Mucor* species only grow well at 20-30°C; so it is hard to produce sufu during hot summer (Hu and Zhao, 1998a). Hu and Zhao (1998b) and Deng *et al.* (1996) screened mutants, made by using conventional mutagens and isolated strains of *Mucor* sp. M_{263} and *Mucor* sp. H_4, which could grow at 30-40°C and ensure sufu production all year round; however, the author did not provide information about the stability and safety of these mutants.

Sufu has a higher content of protein-nitrogen than other oriental soybean food, such as miso and natto (Su, 1986). Nontraditionally, soybean milk, tofu and sufu have the same importance to the people of Asia as cow's milk and cheese do the people of the Western Hemisphere. Asians prefer the salt-coagulated bean curd, not only because it has the desired texture, but also because it serves as an important source of calcium (Wang and Hesseltine, 1970; Zhao, 1997).

Sufu contains per 100 g fresh weight: 58-70 g moisture, 12-17 g crude protein, 8-12 g crude lipid, 0.2-1.5 g crude fibre, 6-12 g carbohydrate, 4-9 g ash, 100-230 mg Ca, 150-300 mg P, 7-16 mg Fe, 0.04-0.09 mg thiamine, 0.13-0.36 mg riboflavin, 0.5-1.2 mg niacin, 1.7-22 μg cyanocobalamin and 460-750 kJ energy (Su, 1986; Wang and Du, 1998).

In spite of their differences in colour and flavour, most types of sufu have a similar proximate composition. Glutamic acid and aspartic acid were the most abundant amino acids found in red sufu and grey sufu. Yen (1986) reported that the average amine contents (mg g^{-1}) in 15 samples of commercial sufu from Taiwan, China were: cadaverine, 0.039; histamine, 0.088; β-phenylethylamine, 0.063; putrescine, 0.473; tryptamine, 0.150; and tyramine, 0.485. Tyramine and putrescine were the major amines found and these might have a potential harmful effect on human beings if levels are very high.

Subunits of soybean protein have not been detected in sufu, and most proteins are degraded into peptides and amino acids (Han, 2003). It has been reported that sufu contains 22 esters, 18 alcohols, 7 ketones, 3 aldehydes, 2 phenols and other volatile compounds (Hwan and Chou, 1999). The esters contribute a characteristics flavour to red sufu (Ho *et al.*, 1989).

Sufu fermentation converts soybean isoflavones from the glucosides in tofu into the corresponding aglycones under hydrolysis by α-glucosidase (Yin *et al.*, 2004). Isoflvones are referred to as phytoestrogens due to their estrogenic activities in soybean and soybean foods (Bickoff *et al.*, 1962).

2.1.15. Tempe

Tempe (tempeh) is one of the most important mould-fermented soybean products of Indonesia. Fresh tempe of food quality has a clean, compact, grayish-white and sliceable mass of cooked particles of raw material covered, penetrated and held together by matrix of dense, cottony, nonsporulated fungal mycelia. The major desirable aspects of tempe are its attractive mushrooomy or nutty odour and texture (Mital and Garg, 1990; Nout and Rombouts, 1990; Shurtleff and Aoyagi, 1979b).

The keeping quality of tempe is short at ambient temperature. Therefore, it is consumed the same day (Mital and Garg, 1990). Irrespective of storage temperature fresh tempe eventually turns brown, the beans become visible due to senescence of the fungal mycelium, the material softens and ammonical odours emerge (Nout *et al.*, 1985). In Indonesia, such tempe is referred to as 'tempe bosok' (= ripe tempe). Although tempe bosok is unacceptable for frying or stewing purposes, it is used in Indonesian kitchen to produce strongly flavoured cookies (mendol) (Nout and Rombouts, 1990).

The traditional tempe production is a household art. Raw soybeans are washed and soaked overnight in water, followed by wet dehulling and hull separation. The hulls are removed manually and the loosened hulls are floated away with water (Mital and Garg, 1990; Nout and Rombouts, 1990). Spontaneous and uncontrolled fermentations take place during the soaking of soybeans. Acidifying the beans during soaking at pH <4.3 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. The dehulled beans are then boiled with excess water for varying lengths of time. Cooking by steaming for at least 30 min at 100°C or by boiling in excess water for 2-3 h facilitates fungal penetration and human digestion. The bitter soy taste disappears in less than 15 min at 95 °C. Partial cooking of soybeans destroys trypsin inhibitors, inactivates some undesirable factors such as phytic acid, and flatus-causing oligosaccharides, leaches out a heat-stable and water-soluble mould inhibitor, destroys contaminating bacteria that interfere with fermentation, releases some of the nutrients required for mould growth and destroys the bitter soy taste. Cooked cotyledons are then spread quickly on plaited bamboo trays ('tampah') in thin layers in order to allow the water to drain and evaporate from the surface of the soybeans. Addition of approximately 2% (w v^{-1}) maize starch, rice flour or cassava starch helps to absorb the remaining moisture, stimulates fungal growth and results in better tempe firmness. These are mixed with the starter or inoculum. The inoculum for tempe fermentation can be obtained from dried and pulverized tempe of previous batch ('tempe-to-tempe'), mould grown on dried leaves (of *Hibiscus similes* Blume, *Hibiscus tiliaceus* L., *Tectona grandis* L.f., *Bambusa* spp. or *Musa paradisiaca* L.), locally referred to as 'usar' or 'laru' or ragi (2.5 cm in diameter) containing the tempe mould and a variety of microorganisms, sold on Indonesian markets. The inoculated cotyledons are then wrapped tightly in leaves and allowed to ferment at room temperature until the soybeans are completely moulded (Albrecht *et al.*, 1966; Djien and Hesseltine, 1979; Mital and Garg, 1990; Nout *et al.*, 1985, 1987; Steinkraus, 1996; Wang and Hesseltine, 1979; Wang *et al.*, 1979).

Traditionally, large leaves are preferred as ideal material for wrapping cooked beans for fermentation (Steinkraus, 1996). Because tempe takes the shape of its fermentation container, the product with irregular surface developed when wrapped and fermented with rough-surfaced leaves. However, smooth polythylene sheets, metallic or hard plastic boxes give tempe with straight edges and smooth shiny surfaces. For better shape and texture, use of perforated plastic bags or tubes to allow access of oxygen for the mould, is not less common (Martinelli and Hesseltine, 1964). Plastic bags for tempe fermentation have also been widely adopted in Indonesia (Wang and Hesseltine, 1979). Steinkraus *et al.* (1960) used a covered stainless steel cake pan.

Incubation takes 50-20 h at 25-37°C. The higher the incubation temperature, the more rapidly *Rhizopus oligosporous* grows (Martinelli and Hesseltine, 1964). The optimum relative humidity during tempe preparation was reported as 60-65% (Usmani and Noorani, 1986a), 75% (Wadud *et al.*, 1988) and 90% (Steinkraus, 1985). As soon as the bean cotyledons are overgrown completely by the mould and knitted into a compact cake, tempe is harvested and cut into cubes (2.5 cm x 2.5 cm). It is then directly transported to the market or preserved by boiling in brine, steaming, canning, dehydration or deep frying (Djien and Hesseltine, 1979; Winarno, 1985; Winarno and Reddy, 1986)

Though it is generally accepted that fungal (*Rhizopus*) growth is essential for tempe preparation, the most frequently occurring fungi were *Rhizopus oryzae, R. oligosporus, Mucor indicus, Trichosporon beigelii, Clavispora lusitaniae, Candida maltosa* and *Candida intermedia* (Nout *et al.*, 1987). In addition, lactic acid bacteria and Enterobacteriaceae, at the level of 10^8-10^9 cfu g^{-1}, were found in fermenting tempe (Mulyowidarso *et al.*, 1990). The role of these 'accompanying' flora of bacteria and yeasts to the properties of tempe is only partly understood (Winarno and Reddy, 1986). Most probably, they contribute in the development of flavour and enhance the chemical composition through substrate modifications and synthesis of vitamins.

Studies carried out by Steinkraus *et al.* (1960) and Hesseltine *et al.* (1963) resulted in pure culture fermentation. However, the most desirable strain is *R. oligosporus* NRRL 2710. The characteristics which make this strain most suitable for tempe production are its ability to grow rapidly at 30-40 °C, ferment sucrose, high proteolytic and lipolytic activities, produce strong antioxidants and impart pleasing flavour and aroma (Steinkraus, 1996). However, other strains, including CBS 338.62 or NRRL 5905 can be used to make good tempe (Nout and Rombouts, 1990).

Since use of pure culture starters for large-scale industrial purpose is too expensive and time-consuming, semi-pure culture starters are preferred for the cost-effective production of tempe (Djien, 1985). Semi-pure culture starters are prepared by growing a pure culture of *Rhizopus* strain on traditionally cooked or steamed substrate, mostly rice (Djien, 1985) or soybeans (Usmani and Noorani, 1986b). The dry starters contain mould as well as bacteria (Djien, 1985). Instead of leaving it to chance, attempts were made to prepare mixed culture starters with simultaneous growth of lactic acid bacteria and *R. oligosporous* by adding 1% sour soak water to the boiled substrate. The resulting tempe was of superior quality (Tuncel *et al.*, 1989). Mixed pure cultures of *R. oligosporous* and *Klebsiella pneumoniae* are used to produce vitamin B_{12}-containing tempe (Areekul *et al.*, 1990)

The temperature of the fermenting beans rises by 5-7°C above the incubation temperature as the mould begins to grow rapidly during tempe production. 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), leveling off towards pH 7.5 to 8.0. During fermentation, there is increase in total soluble solids, soluble nitrogen and free amino acids, while total nitrogen remains fairly constant (Steinkraus *et al.*, 1960; Wang and Hesseltine, 1966).

R. oligosporus possesses a strong lipolytic activity, hydrolyzing over one-third of neutral fat of soybeans after 72 h-fermentation at 37°C. Lipolysis yields predominantly linoleic acid (Wagenknecht *et al.*, 1961). The free fatty acids, particularly oleic, linoleic and linolenic acids are associated with nonspecific antitryptic activity (Winarno and Reddy, 1986). *R. oligosporous* derives much of its energy from oleic acid (Nout and Rombouts, 1990). This supported the findings of Paredes-Lopez *et al.* (1987) who reported the 50% reduction of oleic acid content in bean tempe.

Carbohydrates of soybeans, especially raffinose and stachyose cause flatulence (Mital and Garg, 1990; Nout and Rombouts, 1990). Shallenberger *et al.*, 1967) observed substantial hydrolysis of

stachyose during tempe production by *R. oligosporous*. The fermentation reduced total flatus factors from 16.5 to 2.0 mg g^{-1} soybean (Winarno and Reddy, 1986). Protein-bound starch decreases the digestibility of soy protein; during prolonged fermentation (48-72 h), starch decreases from 0.4 to 0.1% (dry weight) with the formation of some unidentified carbohydrates (Van der Riet *et al.*, 1987). Phytic acid present in soybean hinders mineral absorption in intestinal tract. During steaming and fermentation of raw soybean, phytic acid is degraded into myo-inositol and orthophosphates and inorganic phosphate (Sutardi and Buckle, 1985). Sudarmadji and Markakis (1977) observed 22% reduction in phytic acid during tempe production. They attributed it to phytase activity of *R. oligosporous*. Since phytic acid has a strong chelating effect, its degradation was expected to improve the bio-availability of Ca, Mg, Zn and Fe (Nout and Rombouts, 1990). Zinc availability of tempe was 1.2 times better than of boiled beans (Moeljopawiro *et al.*, 1988).

The vitamin content of soybean increases substiantially during fermentation (Mital and Garg, 1990). Tempe has been reported to possess two-fold increase in riboflavin, seven-fold increase in niacin, and 33-fold increase in B_{12} (Steinkraus *et al.*, 1961; Truesdell *et al.*, 1987; Van Veen and Steinkraus, 1970). Except for thiamine which was reduced by approx. 50%, there were two to four-fold increase in all the other vitamins including riboflavin, nicotinic acid, panthothenate, pyridoxine, folate, cyanocobalamin and biotin (Ginting and Arcot, 2004; Murata, 1985; Okada *et al.*, 1983).

Substances with antioxidant activities are produced in soybean tempe (Winarno and Reddy, 1986). Tempe showed a remarkably stronger antioxidative activity than natto (Esaki *et al.*, 1996). Gyorgy *et al.* (1964) isolated 6,7,4'-trihydroxyisoflavone which is a potent antioxidant in lipid/aqueous systems. A potent antioxidant, 3-hydroxyantranilic acid (HAA), isolated from tempe possessed the antioxidative effect on both soybean oil and powder (Esaki *et al.*, 1996). HAA increased during *R. oligosoporous*-fermentation and reached a maximum content (500 mg kg^{-1} dried matter) after 3 days of fermentation. HAA is the principal antioxidant responsible for the atuoxidative stability of dried tempe (Esaki *et al.*, 1996).

The typical flavour of a fermented product depends upon metabolites such as pyridine derivatives, diacetyl, acetoin, 2,3-butylene glycol, valeric acid etc. (Mittal and Garg, 1990). According to Whitaker (1987), the beany flavour would be released from the proteins to which they are bound as a result of proteolysis.The flavour of tempe prepared at 31°C included the original soybean components and newly formed 3-methylbutanol, acetoin, aectic acid, methylcarbinol, 2,3-butanediol and iso-valeric acid (Moroe, 1985).

Wang *et al.* (1969, 1972) reported that *R. oligosporus* NRRL 2710 produced a heat-stable antimicrobial substance active against a number of Gram positive bacteria including *S. aureus, Bacillus subtilis, Clostridium perfringens,* and *Clostridium sporogenes*. The only Gram negative bacterium sensitive to this substance was *K. pneumoniae*. The compound contains polypeptides whose activity was neither influenced by pepsin nor by *R. oligosporous* protease but slightly decreased by trypsin and peptidase. However, it is readily inactivated by protease (Mital and Garg, 1990; Wang *et al.*, 1969, 1972). 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 energy, 400 mg Ca, 400 mg P, 25 mg Fe, 0.4 mg thiamine, 0.7 mg riboflavin, 6.0 mg niacin, 0.3 mg pantothenic acid, trace of vitamin B_{12} and 50 μg vitamin A (Campbell-Platt, 1987). *In vivo* tests on protein efficiency ratio (PER) and the digestibility of tempe confirmed that rats do not utilize protein from tempe any better than from cooked substrate (Wang, 1986; Zamora and Veum, 1988; Agosin *et al.*, 1989). However, Girija Bai *et al.* (1975) and Winarno and Reddy (1986) reported that mixed soybean-groundnut tempe gave a better net protein utilization (NPU) and PER than soybean protein.

2.1.16. Thua-nao

Thua-nao, common in northern Thailand, is sold as paste or as dried disk, dark brown in colour with a quite different aroma from natto. The dried paste is used as a flavouring agent in vegetable dishes (Leejeerajumnean, 2000; Sundhagul *et al.*, 1972).

Traditionally, soybeans are washed and boiled in an aluminum bowl over wood-fire for 7 h or more to soften the seeds. The excess water is drained off and the seeds are spread in bamboo baskets lined with fresh fronds of *Thelypteris subelata* (Bak.) K. Iwats.. The fern fronds are made into a cylinder-shaped bar and put in the centre of the bamboo basket which is then covered with banana leaves or with a layer of polypropylene mesh and covered with a thick polyethylene bag to provide warmth and a humid atmosphere, and kept at room temperature for 3-4 days to undergo natural fermentation (Leejeerajumnean, 2000; Sundhagul *et al.*, 1972).

The fermented product appears greenish brown in colour with a slightly viscous covering and pungent odour of ammonia. After fermentation, the raw thua-nao is ground into paste and added with salt, garlic, onion and red pepper. The paste is wrapped in small portions with banana leaves and cooked either by steaming or over an open fire before eating (Sundhagul *et al.*, 1972).

The cookedthua-nao paste, for its high moisture content, is kept for about two days at room temperature. Sometimes, the paste is moulded manually into circular-shaped balls of approx. 3 cm in diameter, and pressed between a plastic and a glass sheet to produce circular, thin and flat disks of about 10 cm in diameter and 2 mm in thickness. The disks are then sun-dried for a day. The dry product can be kept for up to 6 months (Leejeerajumnean, 2000).

The fermenting organism for thua-nao has been identified as *Bacillus subtilis* and *Bacillus megaterium;* the former being predominant species present from the beginning to the final product (Leejeerajumnean *et al.*, 1997a; Sundhagul *et al.*, 1972). The initial bacterial load of 10^3 cells g^{-1} cooked beans increased to 10^{10} cells g^{-1} thua-nao. The increase was rapid during the first two days, but remained relatively unchanged afterwards. Lactic acid bacteria was found after 24 h of fermentation and reached 10^6 cfu g^{-1} at the end. Mould was detected after 48 h of fermentation. No enterococci or yeasts were detected during the course of fermentation.

During fermentation, the pH increased from 6.3 to 8.6 in the second day and remained relatively unchanged afterwards (Sundhagul *et al.*, 1972). Production of volatile compounds in thua-nao during fermentation has been studied by Chairote and Kobayashi (1987). Esters, pyrazines, carbonyl compounds, and various other compounds contributed to the aroma of thua-nao (Leejeerajumnean *et al.*, 1997b).

The moisture level of thua-nao at 62% remained relatively constant. The protein contents of thua-nao paste and chips were 16.9 and 36.8%, and the fat contents were 7.4 and 14.8% for paste and chips, respectively. A low cost, protein rich food, called 'ferm-soy-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).

2.1.17. Ugba

Ugba is the native (Ibo) name of a fermented product made from African oil bean (*Pentaclethra macrophylla* Benth.) seed. It is produced and eaten particularly among certain ethnic groups in the southeastern States of Nigeria as a food supplement, generally mixed with other foods like tapioca, boiled yam,

boiled maize, fish meat, with palm oil, pepper and other additives, like salt and 'akanwu' (potash). As a condiment, it is taken as delicacy or added to soup and sauces as flavour enhancer and consumed as a meat analogue especially by the poor. As salad, ugba serves as an indispensable dessert or appetizer in most traditional ceremonies of Ibo. Besides its informal consumption, ugba is an essential food item for various traditional ceremonies, consumed by all socio-economic groups (Isu and Njoku, 1997; Mabajunwa *et al.*, 1998; Sanni *et al.*, 2002).

Production of ugba like other fermented foods in sub-Saharan Africa, particular in rural areas, is still an age-old family art. Traditionally, African oil bean seeds are boiled in water for 4-5 h to soften the hard brown shell. The shells are broken to remove the kernels which are soaked overnight in water over a low flame and smouldering wood. The seeds are allowed to cool, washed several times and soaked in water again for 6 h. They are then cut into long narrow slices of approx. 5 cm long. The sliced beans are either packed into baskets lined with blanched banana leaves or wrapped in small packets (approx. 50 g portions) using blanched leaves of *Mallotus oppositifolius* Mull.Arq. or banana leaves, transferred into an earthen ware pot and covered with a jute bag. The processed beans are allowed to ferment at room temperature (~ 30°C) for 3-5 days (Odunfa and Oyeyiola, 1985; Isu and Abu, 2000; Sanni *et al.*, 2000, 2002).

Alternatively, toasting the oil bean seeds in hot (100°C) sand for 30 min helps in efficient dehulling. The toasted seeds are sliced to 1 mm, and further boiling for 30 min followed by 2 h soaking aids in removal of bitter tastes. This process is shorter and labour- and fuel-efficient, and achieves the same quality of ugba after fermentation as the much more cumbersome, fuel-wasting and time-consuming traditional technique, and saving up to 2 days (Sokari and Wachukwu, 1997). Optimizing the process parameters, such as relative humidity, temperature and thickness of the packing material to 80%, 35°C and 70 μm high density polyethylene (HDPE), respectively, Isu and Njoku (1998) showed that the combined application of these optimal conditions resulted in better ugba when compared with the traditionally fermented products in terms of pH, texture, amino-nitrogen and viable count.

Bacillus spp. dominated the microbial population during ugba production, constituting over 95% of the total microbial density during the first 3 days of fermentation. Although several microorganisms (*Staphylococcus* spp., *Micrococcus* spp. and *Lactobacillus* spp.) were isolated form ugba, only the *Bacillus* spp. were believed to be important in the biochemical transformations necessary for ugba production. *Bacillus* spp. that fermented African oil bean seeds to ugba were identified as *Bacillus coagulans, Bacillus macerans, Bacillus megaterium, Bacillus pumilus* and *Bacillus subtilis*. However, market samples of ugba revealed the occurrence of *Bacillus licheniformis* and *Bacillus brevis*, along with *B.subitis, B. pumilus, B. megaterium* and *Bacillus polymyxa* (Sanni *et al.*, 2000). Mbajunwa *et al.* (1998) reported the occurrence of *B. sutilis*, along with *B. cereus, Pseudomonas chlororaphis, Micrococcus roseus* and *Staphylococcus saprophyticus* in ugba. However, no yeast and mould was recovered from ugba throughout the fermentation (Isu and Njoku, 1997; Mbajunwa *et al.*, 1998).

B. subtilis has been selected as the single starter culture for the fermentation of the African oil bean into ugba. *P. chlororaphis*, nevertheless, has a high potential to ferment African oil bean since the ugba produced with it had good texture and preferred aroma but the green colour it imparts into the ugba mash makes its presence in ugba very objectionable (Mbajunwa *et al.*, 1998). The use of other bacterial isolates, such as *Staphylococcus* spp. and *Micrococcus* spp, however, resulted in products with undesirable sensory qualities (Isu and Njoku, 1997). The ugba produced by using *B. subtilis* had the best preferred texture, aroma and colour with an overwhelming overall acceptability. Dominance and persistence of *B. subtilis* from the onset to the end of fermentation indicates *B. subtilis* as the main organism responsible for the production of ugba (Mbajunwa *et al.*, 1998).

Using starter cultures of *B. subtilis* cells and spores in association with cowpea granules, Isu and Abu (2000) studied on the improvement of indigenous method of ugba production. The starter cultures resulted in increase in protease activity (from 2.8 mg N min^{-1} to 51.6 mg N min^{-1}) in 48 h, while changes in the protease activity due to natural fermentation were gradual and increased from 3.0 mg N min^{-1} to 38 mg N min^{-1}, after 5 days of fermentation. Consequently, the corresponding increase in amino-nitrogen content of ugba produced by the starter cultures after 48 h was significantly higher (18.5 mg N (100 g)$^{-1}$ dry matter) than the maximum amino nitrogen content (12.5 mg N (100 g)$^{-1}$ dry matter) of ugba obtained by the natural process. Ugba produced by the starter cultures were well accepted and compared favourably with the natural product

Ugba has been reported to be nutritious, with crude protein content that includes 20 essential amino acids (Achinewhu, 1983). Mba *et al.* (1974) showed that although ugba is low in its content of sulphur-containing amino acids, it is rich in lysine. Fermentation brings about nutritionally better product than the raw seeds (Achinewhu, 1986; Enujiugha, 2000; Enujiugha and Olagundoye, 2001), and the enzyme systems, especially amylases and protease, aid in breaking down the seed macromolecules (Enujiugha *et al.*, 2002). The main biochemical process that takes place during ugba production is hydrolysis of seed protein resulting in about seven-fold increase in amino-nitrogen content and an increase in pH from 5.9 to 8.2 (Achinewhu, 1983; Isu, 1995; Njoku and Okemadu, 1989). In addition, ugba has been shown to contain significant amounts of thiamine, ribovlavin and other important vitamins and minerals (Achinewhu and Ryley, 1986).

Free fatty acids, such as linoleic (67.6%), oleic (22.6%), palmitic (7.0%) and stearic (2.0%), along with traces of lauric, myristic and capric acids were identified in 72 h-fermented ugba. However, previous studies on raw African oil bean seeds have shown that linoleic acid is the major fatty acid followed by oleic acid (Enujiugha, 2003). Ugba contains 40-45% moisture, and per 100 g dry matter: 14.8-19.8 g carbohydrate, 18.0-35.4 g crude protein, 38-44 g crude fat, 6.0 g ash, 5.6 g crude fibre and 0.1-1.2 g titratable acids (as lactic acid),. The total amino nitrogen content of ugba was found to be 13.7 mg N g^{-1}, which is about 7 times more than that of unfermented seeds (Enujiugha, 2003; Isu and Njoku, 1997; Sanni *et al.*, 2000).

2.1.18. Wadi

Wadis (warries) or Punjabi warries are dried, hollow, brittle, spicy friable balls or cones (3-8 cm in diameter and 15-40 g in weight) and used as spicy condiment or adjunct for cooking vegetables, grain legumes or rice. Traditionally consumed in the States of Punjab and West Bengal, wadis are now popular in many places in India, Pakistan and Bangladesh (Aidoo *et al.*, 2006; Batra, 1986; Nout *et al.*, 2007; Soni and Sandhu 1989b, 1990b, 1999).

Traditionally, wadis are prepared by soaking dal, generally of blackgram, in water for 6-12 h, draining, grinding into a smooth soft dough and fermenting for 1-3 days at 20-27°C, with or without spices, but with salt and backslop added. Spices, when added, include asafoetida (*Ferula asafetida* H.Karst.), caraway (*Carum carvi* L.), cardamom (*Elettaria cardomomum* Maton), clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry), fenugreek (*Trigonella foenum-graecum* L.), ginger (*Zingiber officinale* Roscoe) and red pepper (*Capsicum frutescens* L.). In an alternative method, the dough is combined with shredded wax gourd, ash gourd or winter melon and whisked vigorously until it becomes light and fluffy due to incorporation of air. The fermented or whisked dough is hand-moulded into cones or balls (3-8 cm in diameter), deposited on bamboo or palm mats smeared with oil and sun-dried for 4-8 days. Dried wadi can be stored in an airtight container for future use. The surface of the cones or balls

becomes covered with a mucilaginous coating which retains the gas formed during fermentation within them. Wadis look hollow with many air pockets and yeast spherules in the interior and have a characteristic surface crust (Aidoo *et al.*, 2006; Batra, 1981; Batra and Millner, 1974, 1976; Nout *et al.*, 2007; Soni and Sandhu, 1999).

Several bacteria and yeasts constituting the natural flora of blackgram, spices and surroundings are associated with wadi production. The development and prevalence of microbiota are affected by the seasons; summer being more favourable for bacteria, and winter for yeasts (Soni and Sandhu, 1999). Soni and Sandhu (1989b) observed the occurrence of 10^9-10^{12} cfu bacteria g^{-1} sample; however, only 55% of the samples contained yeasts (0-10^7 cfu g^{-1}).

Two types of yeasts, including *Candida krusei* and *Saccharomyces cerevisiae* were isolated from wadis (Batra and Millner, 1974, 1976). Later on, although a wide variety of yeasts and lactic acid bacteria were found to be associated with wadi, only the combination *of Hansenula* sp. and *Leuconostoc mesenteroides* was found responsible for their production (Batra, 1981, 1986). *L. mesenteroides* was most abundant and present in all the market samples, followed by *Enterococcus faecalis, Lactobacillus fermentum* and *Bacillus subtilis*, whereas *S. cerevisiae* and *Pichia membranifaciens* were the most abundant yeasts, found in all the positive samples, followed by *Candida vartiovaarae, Kluyveromyces marxianus, Trichosporon beigelii, C. kursei* and *Pichia anomala* (Soni and Sandhu, 1989b). Bacteria and yeasts generally occur in the nonfermented paste of wadi in the range of 10^6 and 10^3 cfu g^{-1}, which increases to the levels of 10^7 and 10^6 cfu g^{-1}, respectively, as fermentation progresses (Batra, 1981).

Laboratory-made samples were found to contain a higher bacterial load (10^{10}-10^{12} cfu g^{-1}) than yeast load (0-10^6 cfu g^{-1}). The microbial load of 13 cfu g^{-1} nonfermented dough increased to 10^{12} cfu g^{-1} at the end of fermentation. Among the bacteria, *L. mesenteroides, Lactobacillus delbrueckii, L. fermentum, B. subtilis* and *Flavobacter* spp., and among the yeasts, *T. beigelii, S. cerevisiae, C. krusei, P. membranifaciens* and *P. anomala* dominated the initial stages of fermentation. With the progress in fermentation, most of the microorganisms, except *L. mesenteroides, L. fermentum, S. cerevisiae* and *T. beigelii* disappeared (Soni and Sandhu, 1989b).

Lactic acid bacteria are mainly responsible for acidification of dough, a condition which favours the growth of yeasts and leavening. Production of acid and gas during wadi production results in a decrease in pH from 5.6 to 3.2, an increase in total acid (as lactic acid) from 0.5% to 1.5% and a two-fold increase in dough volume. Fermentation brings about a significant increase in soluble solids, nonprotein nitrogen, soluble nitrogen, free amino acids and water soluble B-vitamins including thiamine, riboflavin and cyanocobalamin in finished product. Enzymatic activity of amylase and proteinase also increased to some extent during wadi production. Most of these changes cause improvement in digestibility and nutritional value. Increase in total acidity during fermentation helps in extending shelf-life of the product (Nout *et al.*, 2007; Soni and Sandhu 1990b, 1999).

2.2. Influence of fermentation on antioxidative properties

Antioxidants in foods have recently attracted special interest because these can protect the human body from free radicals which cause oxidation of biomolecules leading to cell death and tissue damage resulting in various common maladies, such as atherosclerosis, cancer, emphysema, cirrhosis and arthritis (Jacob, 1994; Kehrer, 1993). Food-derived antioxidants not only have corresponding beneficial effect on human health (Lin and Yen, 1999), but can also retard rancidity and oxidative deterioration of foods caused by atmospheric oxidation and thus protect oils, fats and fat-soluble components and other nutritive ingredients present in foods.

Besides plants, microbial sources have been shown to be a potential means of producing natural antioxidants (Ishikawa, 1992). However, its isolation did not become a focus of research until the early 1980s, although Forbes *et al.* (1958) and Meisinger *et al.* (1959) established a relationship between antioxidants and microorganisms. Since this early work, a vast number of compounds and microorganisms have been characterized. The antioxidative activity of soybean fermented products such as miso, tempe and natto, inoculated with *Aspergillus oryzae, Rhizopus oligosporus* and *Bacillus subtilis*, respectively, was significantly higher than that of nonfermented steamed soybean (Berghofer *et al.*, 1998; Santiago *et al.*, 1992). Dried tempe powder has sometimes been used as an antioxidant by covering freshly caught fish with it. György *et al.* (1964) reported tempe to be very stable to rancidity development and identified 6,7,4'-trihydroxyisoflavone as an antioxidant from tempe. Moreover, several oriental fermented foods, including furu or sufu (Ren *et al.*, 2006), douchi (Wang *et al.*, 2007); soybean koji fermented with various filamentous fungi (Lin *et al.*, 2006); rice koji fermented with *Aspergillus candidus* (Yen *et al.*, 2003), monoscal adlay (*Monascus*-fermented Chinese pearl barley), showed the enhancement of antioxidative activities during fermentation(Tseng *et al.*, 2006).

The antioxidant activity has been attributed to various mechanisms, among which are total phenolic content, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical-scavenging (Yildirim *et al.*, 2001).

2.2.1. Total phenol content

Phenolic compounds are secondary metabolites, and in part, are produced as a result of the plants' interaction with the environment (Synder and Nicholson, 1990). They possess metal-chelating capabilities and radical-scavenging properties (McCue and Shetty, 2003).

After fermentation, the concentrations of phenolic compounds were reported to increase in soybean (McCue and Shetty, 2003), fava beans (Vattem and Shetty, 2002) and cranberry (*Vaccinium* sp.) pomace (Randhir *et al.*, 2004). The total phenolic contents of methanolic extract of various soybean koji, depending on the starter organisms, ranged between 23.7 and 45.7 mg gallic acid equivalent (GAE) g^{-1} extract, and were higher than that of the nonfermented soybean extract (Lin *et al.*, 2006). Similarly, Tseng *et al.* (2006) reported that the total phenol contents of methanolic extracts of monascal adaly products were higher than those of uninoculated adlay products. The antioxidant activity of the methanolic extract from peanut hulls correlated with its contents of total phenols (Yen *et al.*, 1993). Therefore, increase in total phenolic contents after fermentation might explain enhanced antioxidant properties in various soybean koji extracts (Lin *et al.*, 2006) and adlay products (Tseng *et al.*, 2006).

2.2.2. Free radical-scavenging activity

Free radical scavenging is the main mechanism by which antioxidants act in foods. Several methods have been developed in which the antioxidant activity is assessed by the scavenging of synthetic radicals in polar organic solvents, e.g. methanol at room temperature. Those used include $DPPH^{\cdot}$ which is a stable nitrogen-centered, lipophilic free radical that is widely used in evaluating the antioxidant activities in a relatively short time as compared to other methods.

Aspergillus-soy koji exhibited a nine-fold higher scavenging effect compared to cooked nonfermented soybeans; the scavenging activity of the methanolic extract of soy koji, at 20 mg ml^{-1}, varied from 55 to 100% depending on the starter organism (Lin *et al.*, 2006). While extracts of furu

exhibited 20-50% of free radical-scavenging activity (Ren *et al.*, 2006), a higher scavenging activity (25-79%) was observed at 200-800 μg ml^{-1} in rice koji (Yen *et al.*, 2003). In monascal adlay products, at 1-10 mg ml^{-1}, the scavenging abilities of methaonolic extracts ranged from 81 to 94% (Tseng *et al.*, 2006). Similarly, various monascal rice products at 1-10 mg ml^{-1} scavenged 93-98% of DPPH· (Tseng *et al.*, 2003). According to Wang *et al.* (2007), the scavenging activity of the douchi extract, expressed as trolox equivalent antioxidative capacity (TEAC) on DPPH· and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) significantly increased from 0.6 to 7.2 and from 19.6 to 45.1, respectively, during fermentation. However, the TEAC of the douchi extracts were influenced significantly by the salt contents and showed negatively correlated with this parameter (Wang *et al.*, 2007).

2.2.3. Reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Mier *et al.*, 1995). The reducing capability of the substance is generally associated with the presence of reductones which have been shown to exert antioxidant activity by breaking the free radical chain by donating a hydrogen atom, and indicates that these compounds can reduce the oxidized intermediates of lipid peroxidation processes, and act as primary and secondary antioxidants (Duh, 1998; Yen and Chen, 1995).

The reducing power of the methanolic extracts of soybean koji increased to a certain extent, and then leveled off despite further increases in concentration (Lin *et al.*, 2006). There are reports on similar findings of the enhanced reducing power of fermented beans and bean products (Berghofer *et al.*, 1998; Chung *et al.*, 2002; Wang *et al.*, 2004, 2007; Yang *et al.*, 2000). Monascal adlay products too displayed higher reducing power than uninoculated adlay products (Tseng *et al.*, 2006). According to Yang *et al.* (2000) the intracellular antioxidants, peptides of the starter organism and their hydrogen-donating ability may also contribute to the increased reducing ability. The increased reducing power observed may be due the formation of reductants that could react with free radicals to stabilize and terminate radical chain reactions during fermentation (Yang *et al.*, 2000). The reducing power of the 50% ethanolic extracts of douche, a traditional salt-fermented soybean product of China, were increased significantly during the first two weeks of postfermentation stage, and the level of salt content did affect the reducing power of the extracts which decreased with the increase in salt content. However, the effects of salt contents on the reducing powers were not significant after three weeks (Wang *et al.*, 2007).

2.2.4. Metal-chelating activity

Transient-valency metal ions, Fe^{2+} in particular, are the most important pro-oxidants found in food system, which affect both the speed of auto-oxidation and the direction of hydroperoxide decomposition that leads to the deterioration of food (Gordon, 1990; Pokorny, 1987; Yamaguchi *et al.*, 1998). They cannot be completely removed by normal food processing. Minute traces of copper and iron, and to a lesser degree manganese and cobalt, therefore, are important promoters of lipid oxidation.

Many metal-chelating substances, such as salts of phytic acid, phospholipids and oxalates present in foods may have a dramatic effect on increasing the oxidation stability through blocking the pro-oxidant metal ions, and thus limiting the formation of chain initiators by preparing metal-assisted homolysis of hydroperoxides (Ang and Hamm, 1986). Amino acids and peptides are considered as typical metal-chelating agents (Fujimoto *et al.*, 1984). The antioxidant activity of histidine-containing peptides is thought to be related to their metal-chelating ability as well as to lipid-radical trapping

potential of the imidazole ring (Murase *et al.*, 1993; Uchida and Kawakishi, 1992). The metal-chelating characteristic of natural phenolics is also an important factor in their antioxidant activities (Chen and Ahn, 1998; Nardini *et al.*, 1995).

The chelating ability increased with the increase in concentration of metal. At 1 mg ml^{-1}, chelating abilities of methanolic extracts from *Monascus*-fermented polished adlay and dehulled adlay on Fe^{2+} were 45% and 27%, respectively. The chelating ability increased to 96 and 98%, respectively, at 20 mg ml^{-1} (Tseng *et al.*, 2006). Similarly, in their earlier findings, Tseng *et al.* (2003) reported that the chelating abilities of methanolic extracts of *Monascus*-fermented polished rice and dehulled rice on Fe^{2+} at 1 mg ml^{-1} methanolic extract were 34% and 32%, respectively, which increased to 69-92% when the concentration of the extract was increased to 10 mg ml^{-1}. Though monascal adlay products showed higher chelation of Fe^{2+} than the uninocualted adlay products, the chealting abilities of various *Monascus*-fermented rice products were better than those of *Monascus*-fermented adlay products.

While investigating the chelating ability of methanolic extracts of soybean koji towards ferrous ions, Lin *et al.* (2006) found that at all the tested concentrations soybean koji exhibited a higher Fe^{2+}-chelating effect than the extract of nonfermented steamed soybean. The Fe^{2+}-chelating ability of soybean increased by 2 to 7-fold after fermentation. However, at the same dosage, methanol extract of soybean kojis exhibited 84% or more chelating activity, regardless of the nature of functional moulds (Lin *et al.*, 2006). This further demonstrated the influence of fermentation on the enhancement of Fe^{2+}-chelating ability of soybean.

2.2.5. Lipid peroxidation inhibitory activity

Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Specially, linoleic and arachidonic acids are the targets of lipid peroxidation which leads to rapid development of rancid and stale flavour and is considered as a primary mechanism of quality deterioration in lipid foods and oils (Guntensperger *et al.*, 1998; Yu, 2001). Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical (Gao *et al.*, 2000). Hydroxyl radicals eliminate hydrogen atoms from the membrane lipids which result in lipid peroxidation (Ordonez *et al.*, 2006). Hence, inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities.

Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT), are added in food during processing to suppress lipid peroxidation. Because of the possible toxicities of the synthetic antioxidants, increasing attention has been directed toward natural antioxidants (Naimiki, 1990). Recently, various extracts of plants have provoked interest as sources of natural antioxidant. Moreover, they offer an effective way to prevent the development of various off-flavour and undesirable compounds that result from lipid peroxidation in foods (Wang *et al.*, 1998).