# 2. REVIEW OF LITERATURE

Many of the traditional fermented foods of Darjeeling hills and Sikkim have similarities with several fermented foods of the Orient, Africa and Europe (Table 2). Since most of the foods under investigation had no written record, mainly the literatures of their similar products were reviewed.

## 2.1. FERMENTED LEGUMES

### 2.1.1. Kinema

'Kinema' is a Nepali name which has so far been erroneously spelt as 'kenima' (Batra and Millner 1976; Hesseltine 1979; Ramakrishnan 1979; Batra 1986; Campbell-Platt 1987). Although kinema is popular in Nepal, Darjeeling district of West Bengal and Sikkim in India, its antiquity is unknown. It is produced in low lying warm valleys of the area. Kinema is deep fried and used as an adjunct to staples such as rice. Uncooked kinema has a strong ammoniacal odour, but when fried, it has a pleasant nutty flavour (Batra 1986). The method of preparation of kinema was reported briefly by Batra and Millner (1976) and Batra (1986). Whole soya beans are washed, soaked for 24 h, cooked for 2-6 h, cooled to about 40°C, wrapped with broad leaves and left to ferment at 35-45°C for 48-72 h. At the end of fermentation, the beans become covered with a thick, white, mucilaginous coating (Batra 1986). Two rod-shaped, acid-producing bacteria at a level of 2.2-26 x $10^6$/g dry weight of kinema were recovered (Batra and Millner 1976; Batra 1986). One of the rods appeared as *Bacillus subtilis* (Batra 1986).

Kinema contains 45-65% moisture, and per 100 g dry matter: 45-55 g protein, 25-30 g fat, 10-15 g carbohydrate, 4-7 g fibre, 5-8 g ash and 2.0-2.1 MJ (490-510 kcal) energy (Campbell-Platt 1987).

**Table 2.** Traditional fermented foods similar to those common in Darjeeling hills and Sikkim

| Name | Area or country | Substrate | Microorganism | Use of food | Reference |
|---|---|---|---|---|---|
| Similar to kinema | | | | | |
| Natto | Japan | Soya beans | *Bacillus subtilis* | Eaten with shoyu or boiled rice | Sakurai (1960); Ohta (1986) |
| Thua-nao | North Thailand | Soya beans | *Bacillus subtilis* | Dried paste and chip | Sundhagul *et al.* (1972) |
| Tou-shi | China | Soya beans | *Bacillus* sp. | Seasoning | Yokotsuka (1985) |
| Tu-su | China | Soya beans | Unknown | | Ohta (1986) |
| Tempe | Indonesia | Soya beans | *Rhizopus oligosporus* | Fried, meat substitute in soup | Hesseltine *et al.* (1963); Steinkraus (1983a); Nout and Rombouts (1990) |
| Daddawa | West Africa | African locust beans | *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilus* | Condiment | Campbell-Platt (1980); Odunfa (1981, 1985a); Ogbadu and Okagbue (1988) |

| Name | Area or country | Substrate | Microorganism | Use of food | Reference |
|---|---|---|---|---|---|
| Similar to masayura | | | | | |
| Wari | North India and Pakistan | Black gram | *Candida krusei*, *Saccharomyces cerevisiae*, *Leuconostoc mesenteroides*, *Lactobacillus fermentum*, *Streptococcus faecalis* | Spicy condiment | Batra and Millner (1974, 1976); Sandhu *et al.* (1986) |
| Similar to gundruk | | | | | |
| Sauerkraut | Germany, Switzerland and Central Europe | Cabbage | *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Pediococcus cerevisiae* | Acidic shredded cabbage; used as a side dish | Stamer (1975); Pederson (1979) |
| Kimchi | Korea | Chinese cabbage, Oriental radish | *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Pediococcus* sp. | Mildly acidic carbonated vegetable; used as a side dish | Mheen *et al.* (1983) |

| Name | Area or country | Substrate | Microorganism | Use of food | Reference |
|---|---|---|---|---|---|
| Similar to mesu | | | | | |
| Naw-mai-dong | Thailand | Young bamboo shoot | *Leuconostoc mesenteroides*, *Pediococcus cerevisiae*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermentum* | Pickle | Dhavises (1972) |
| Similar to shel roti | | | | | |
| Jalebi | India, Pakistan and Nepal | Wheat flour | *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Hansenula anomala*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus fermentum* | Syrup-filled confection | Batra and Millner (1974); Batra (1981, 1986); Ramakrishnan (1979) |
| Similar to dahi | | | | | |
| Yoghurt | Worldwide | Milk | *Streptococcus thermophilus*, *Lactobacillus bulgaricus* | Soft gel, acidic, non-alcoholic savory | Rašić and Kurmann (1978); Oberman (1985) |

| Name | Area or country | Substrate | Microorganism | Use of food | Reference |
|---|---|---|---|---|---|
| Similar to murcha | | | | | |
| Ragi | Indonesia | Rice flour, herbs and spices | *Amylomyces rouxii*, *Mucor circinelloides*, *Rhizopus* sp., *Candida* spp., *Saccharomycopsis malanga*, *Pediococcus pentosaceus* *Saccharomycopsis fibuligera* | Starter | Saono *et al.* (1974); Hesseltine and Ray (1988); Hesseltine and Kurtzman (1990) |
| Chinese yeast | China and Taiwan | Rice flour, herbs and spices | *Amylomyces rouxii*, *Mucor* sp., *Rhizopus* sp., *Saccharomycopsis fibuligera*, *Saccharomycopsis malanga* | Starter | Hesseltine *et al.* (1988); Hesseltine and Kurtzman (1990) |
| Bubod | Philippines | Rice flour, herbs and spices | *Amylomyces* sp., *Mucor* sp., *Rhizopus* sp., *Saccharomycopsis fibuligera*, *Saccharomyces cerevisiae* | Starter | Tanimura *et al.* (1977); Del Rosario (1980); Hesseltine and Kurtzman (1990) |

| Name | Area or country | Substrate | Microorganism | Use of food | Reference |
|---|---|---|---|---|---|
| Loogpang | Thailand | Rice flour, herbs and spices | *Amylomyces* sp., *Mucor* sp., *Absidia* sp., *Rhizopus* sp., *Aspergillus* sp., *Saccharomycopsis fibuligera* | Starter | Pichyangkura and Kulprecha (1977); Hesseltine and Kurtzman (1990) |
| Similar to jnard | | | | | |
| Pachwai or bakhar | India | Rice flour | *Hansenula anomala*, *Endomycopsis fibuligera*, *Amylomyces rouxii*, *Mucor fragilis*, *Rhizopus arrhizus* | Alcoholic drink | Batra (1986) |
| Chiang or lugri | Tibet and Nepal | Barley flour | *Saccharomyces cerevisiae*, *Saccharomyces uvarum* | Mildly alcoholic drink | Batra (1986) |

### 2.1.2. Natto

Natto is a popular soya bean fermented 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 (Steinkraus 1983a; Ohta 1986). Itohiki-natto (sticky natto) is produced by fermenting whole cooked soya beans 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).

Natto is eaten as is with shoyu (main name for soya sauce in Japan) or with mustard, often in breakfast and dinner (Kiuchi *et al.* 1976; Fukushima 1979) or eaten with boiled rice and often used as a flavouring agent in cooked meat, vegetables and sea foods (Ohta 1986). Japanese domestic soya beans of small and uniform size with white to pale yellow hilum and smooth seed coat are preferred for natto preparation (Ohta 1986).

In the traditional method of natto preparation, soya beans are soaked overnight and boiled until tender. 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 containers covered with wax paper, and the containers are stacked one above the other in large wooden boxes, covered with straw-mats, and placed near ovens to ferment at approximately 36°C for one day (Standal 1963).

The straw used as a wrapping material of cooked soya beans, 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 (USDA 1958; Ohta 1986). The beans are wrapped in paper-thin sheets of pine-wood (USDA 1958) or plastic package (Hesseltine and Wang 1967). Polystyrene foams are also in use (Ohta 1986). They are fermented at 40-45°C for 18-20 h (USDA 1958; Hesseltine and Wang 1967; Ohta 1986).

The most favourable conditions for natto production are created by inoculating cooked beans with *B. subtilis* spores $10^8$-$10^9$ /ml, equivalent to 0.5-1.0% substrate at 45°C, mixing thoroughly and fermenting at 40-43°C for 6 h (Sakurai 1960). Takahashi and Shimakawa (1976) reported that the best quality natto can be produced by incubating the inoculated beans at 40°C and 85% relative humidity for 12-16 h.

Yabe (1894) was the first to study the microorganisms involved in natto production. Sawamura (1905) identified the fermenting organism 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 1983b, 1986). Hayashi (1977) and Ohta (1977) mentioned *Bacillus natto* SB 3010 as the most suitable strain for natto production. The unique feature of *B. natto* is the formation of a sticky viscous material which gives natto its unique

characteristics (Ohta 1986).

Throughout the fermentation period of 18 h, the dry matter and total nitrogen remained fairly constant at 95.5-96.1% and 7.2-7.5%, respectively; water-soluble nitrogen and amino nitrogen increased from 1.26 to 3.13% and 0.07 to 0.6%, respectively; ammonia nitrogen increased from 0.02 to 0.2%; whereas reducing sugars decreased from 13.4 to 11.8%, all expressed on dry matter basis (Sakurai 1960).

Sakurai (1960) compared the starting soya beans (cooked, steamed and surface-dried, but without fermentation) with natto being fermented for 6-8 h. It was found that dry matter and total nitrogen remained fairly constant at 95.5-96.1% and 7.2-7.5%, respectively. Soluble nitrogen increased from 0.89 to 2.88% as a result of proteolytic activity of the organism. Reducing sugars decreased from 13.81 to 11.46% (6 h) and 11.09% (8 h). The ash content decreased slightly from 5.15 to 5.10% (6 h) before an increase to 5.23% (6 h), all expressed on dry matter basis. While the fat content remained relatively constant, total acid (as lactic) increased from 0.42 to 1.17% (8 h) and pH dropped from 6.48 to 6.10 after 6 h and then increased to 6.20 after 8 h. However, Hayashi (1974a,b,c,d) found a 4% increase in total nitrogen in natto over total nitrogen of the raw soya beans. This was because *B. natto* could fix dinitrogen. Natto is rich in essential amino acid content, compared to soya beans (Sano 1961).

The fatty acid composition of natto and soya beans does 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 mucilage is composed mainly of an acidic glycopeptide and contains 61.5% sugars, 2.8% hexosamines, 4.1% total nitrogen, 2.9% amino-nitrogen and 20.4% uronic acid (Hayashi *et al.* 1971). Saito *et al.* (1974) found that natto mucin is composed of 58% γ-polyglutamic acid and 40% polysaccharide. Ishikawa *et al* . (1972) examined the characteristic spinnability of a natto mucin solution and found that the mucin contained 22.1% fructan and 77.6% poly-DL -glutamic acid which had high viscoelasticity, and was spinnable due to formation of network structures of randomly coiled poly-DL-glutamic acid through intermolecular H-bondings in the presence of fructan.

The unique flavour of natto was thought to be related to diacetyl content (Obata 1959). Kosuge (1962) identified tetramethyl pyrazine as the flavour component in natto. He also established that some of the free fatty acids, like butyric and isovaleric acids produce an undesirable odour in natto. Am ammonia-like odour is directly involved in the quality of natto flavour (Ohta 1986). Soya beans with high carbohydrate content produce less ammonia than those with a lower carbohydrate content. Some of the flavour originates from the hydrolysis of soya protein to peptides and amino acids (Ohta 1986).

Natto has a high nutritional value, improved digestibility and an appreciable amount of certain vitamins, produced as a result of fermentation (Standal 1963; Reddy *et al.* 1982; Steinkraus 1983a; Ohta 1986). Natto is a good source of fibre and free fatty acids (Ohta 1986). Hayashi and Nagao (1975) reported that conversion of

bacterial cells to spores during preservation increases the nutritive value of natto.

Natto contains 50-65% moisture, and per 100 g dry matter: 45-55 g protein, 23-28 g fat, 10-15 g carbohydrate, 4-6 g fibre, 5-10 g ash (higher, if salt added), 2.0 MJ (470-490 kcal) energy, 300 mg Ca, 300 mg P, 1200 mg K, 15 mg Fe, 0.1 mg thiamine, 0.6 mg riboflavin, 1.3 mg niacin, 60 µg β-carotene and 20 mg vitamin C (Campbell-Platt 1987).

### 2.1.3. Thua-nao

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

The cooked thua-nao paste, for its high moisture content, is kept for only about two days at room temperature. On the other hand, thua-nao chips can be prepared by cutting raw thua-nao paste into thin chips and then sun-drying, and kept for several months (Sundhagul *et al.* 1972).

The fermenting organism for thua-nao has been identified as *Bacillus subtilis*. The initial bacterial load of $10^3$ cells/g cooked beans was increased to $10^{10}$ cells/g thua-nao. The increase was rapid during the first two days. During fermentation, the pH increased from 6.3 to 8.6 in the second day and remained relatively unchanged afterwards. The moisture level at 62% remained relatively constant. Chemical determination of thua-nao paste and chips showed that they had high protein and fat contents. The protein contents were 16.9 and 36.8%, and the fat contents were 7.4 and 14.8% for paste and chips, respectively (Sundhagul *et al.* 1972).

A low cost, protein-rich food, called 'ferm-soya-mix' in powder form, ready to eat with long shelf-life under normal conditions has been developed by blending thua-nao powder with flavouring agents and a small proportion of high grade fish meat (Sundhagul *et al.* 1973).

### 2.1.4. Tou-shi

Tou-shi, shi-tou-shi or shi is a soya bean fermented food commonly consumed in China. In the traditional method of its preparation, soya beans (yellow or black) are cooked, cooled, placed in a pile on a straw-mat, covered with straw and fermented at 25-30°C for 1-2 days. Tou-shi is mixed with minced ginger and salt, and then tightly packed into jars. After aging for one week, they are ready

for consumption (Yokotsuka 1985).

On the basis of microorganisms employed, tou-shi can be classified as *Aspergillus oryzae*, *Mucor* or *Bacillus* type (Yokotsuka 1985).

### 2.1.5. Tempe

'Tempe kedele', usually referred to as 'tempe', is one of the most important traditional fermented soya bean foods in Indonesia. Fresh tempe has a clean, mushroomy or nutty odour (Nout and Rombouts 1990). It is not consumed raw, but heated to develop meat-like flavour by frying spiced and salted slices in oil, by boiling with coconut milk in soups, by stewing, by roasting spiced kebabs, and in peppered ground pastes (Shurtleff and Aoyagi 1979; Soewito 1985). On deep frying, the flavour of tempe becomes nut-like and peppery, due to the presence of free fatty acids (Steinkraus 1983a).

Most cultivars of yellow-seeded soya beans are suitable for tempe, in contrast to black-seeded ones (Sharma and Sarbhoy 1984). Traditionally, soaked, hand-dehulled and briefly boiled beans are inoculated with small pieces of tempe from a previous fermentation, wrapped in banana leaves which also serve as a source of inoculum, and left at room temperature for 1-2 days (Wang 1986b).

At present, most wet dehulling of soya beans is mechanized in the Indonesian traditional process, using simple electric-driven disc dehullers. After removal of hulls, hydration is carried out by autoclaving, boiling, steaming or by overnight soaking (Nout and Rombouts 1990). Addition of lactic ($\leq$0.5%) or acetic ($\leq$0.25%) acid during hydration to control microbial spoilage has been

suggested (Usmani and Noorani 1986; Wadud *et al*. 1988). Emphasis has been given to the importance of acid fermentation or artificially acidifying the beans, because the mould is proteolytic, and deamination following hydrolysis releases ammonia, causing the pH to rise. Above pH 7.0, sufficient free ammonia is released to kill the mould and a lower initial pH allows a longer fermentation time before ammonia is liberated (Steinkraus 1983a).

During the traditional tempe manufacturing process, spontaneous and uncontrolled fermentations of soya beans take place during the soaking period prior to mould fermentation. Acidifying the beans during soaking to pH $\leq$4.30 yields tempe of good quality in which bacilli and Enterobacteriaceae could not be detected. The acidification during soaking can be controlled by recycling part of the soak water from a previous batch as an inoculum, contributing to the shelf-life and safety of tempe (Nout *et al*. 1987).

Cooking by steaming for at least 30 min at 100°C (Djien and Hesseltine 1979) or by boiling in excess water for 2-3 h (Winarno and Reddy 1986) serves the purpose of partial cooking which facilitates fungal penetration and human digestion (Nout and Rombouts 1990). Partial cooking of soya beans destroys trypsin inhibitors (Albrecht *et al*. 1966), inactivates some undesirable factors such as phytic acid (Chang *et al*. 1977; Toma and Tabekhia 1974) and flatus-causing oligosaccharides (Wang *et al*. 1979), leaches out a heat-stable and water-soluble mould inhibitor (Wang and Hesseltine 1979; Djien and Hesseltine 1979), destroys contaminating bacteria that interfere with fermentation, releases

some of the nutrients required for mould growth (Steinkraus 1983a), and destroys the bitter soya taste (Nout *et al*. 1985).

Following cooking, the beans should have no excess moisture on them, beacause the presence of free water on the cotyledons favours bacterial growth and spoilage during or following the mould fermentation (Steinkraus 1983a). The use of basket centrifuges for removal of boiling water from the cotyledons has been advised by Shurtleff and Aoyagi (1980). Addition of approximately 2% w/w maize starch, rice flour or cassava starch helps to absorb the remaining moisture, stimulates fungal growth and results in better tempe firmness (Nout and Rombouts 1990).

The inoculum for tempe fermentation can be obtained from dried and pulverized tempe of previous batch ('tempe-to-tempe'), mould grown and air dried on leaves of *Hibiscus* spp., *Tectona grandis*, *Bambusa* sp. or *Musa paradisiaca*, locally referred to as 'usar' or 'laru' (Djien and Hesseltine 1979) sold on Indonesian markets or ragi (2.5 cm in diameter) containing the tempe mould and a variety of microorganisms also sold on Indonesian markets.

Studies carried out by Steinkraus *et al*. (1960) and Hesseltine *et al*. (1963) resulted in a pure culture fermentation. The most popular strain is *Rhizopus oligosporus* NRRL 2710 which grows at 30-42°C (Steinkraus 1983a; Hesseltine 1985a). But use of pure culture starters for large scale industrial purpose is too expensive and time-consuming (Djien 1985). Therefore, 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 soya beans (Usmani and Noorani 1986). 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. oligosporus* 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. oligosporus* and *Klebsiella pneumoniae* are used to produce vitamin $B_{12}$-containing tempe (Areekul *et al.* 1990).

Large leaves, used traditionally, are excellent for wrapping beans for fermentation (Steinkraus 1983a). But, rough-surfaced leaves result in tempe with irregular surfaces, because tempe takes the shape of its fermentation container. Smooth polythene sheets, metallic or hard plastic boxes give tempe with straight edges and smooth shiny surfaces. An interesting development was the use of plastic bags or tubes perforated at 0.2-1,3 cm intervals to allow access of oxygen for the mould (Martinelli and Hesseltine 1964). Plastic bags for tempe fermentation have also been widely adopted for use in Indonesia (Wang and Hesseltine 1979). Steinkraus *et al.* (1960) used covered stainless steel cake pans.

Incubation takes 80-22 h at 25-37°C, respectively. The higher the incubation temperature, the more rapidly *R. oligosporus* grows (Martinelli and Hesseltine 1964). The optimum relative humidity during tempe preparation was reported as 60-65% (Usmani and Noorani 1986), 75% (Wadud *et al.* 1988) and 90% (Steinkraus 1985).

As 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).

As the mould begins to grow rapidly during tempe fermentation, the temperature of the fermenting beans rises from 5 to 7°C above the incubation temperature. As a result of protein metabolism, pH increases from 4.5 (0 h) to 6.0 (26 h at 28°C, 18 h at 38°C) and 7.0 (48 h at 28°C, 30 h at 38°C), leveling off towards pH 7.5 to 8.0. During fermentation, there is increase in total soluble solids, soluble nitrogen and free amino acids, whereas total nitrogen remains fairly constant (Steinkraus *et al.* 1960; Wang and Hesseltine 1966; Wang *et al.* 1968).

*Rhizopus oligosporus* produces two proteolytic enzyme systems, one with an optimum activity at pH 3.0 and the other at 5.5, both having maximum activity at a temperature of 50-55°C; maximum proteolytic activity was attained at 72-96 h at 32°C (Wang and Hesseltine 1965).

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

Carbohydrates of soya beans, especially raffinose and stachyose cause flatulence (Nout and Rombouts 1990). During fermentation, there is rapid removal of hexoses and slow hydrolysis of stachyose (Shallenberger *et al*. 1967). Total flatus factors are reduced from 16.5 to 2,0 mg/g soya beans (Winarno and Reddy 1986). Protein-bound starch decreases the digestibility of soya 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).

Tempe has been reported to contain nutritionally important amount of vitamin $B_{12}$ (Steinkraus *et al*. 1961; van Veen and Steinkraus 1970; Liem *et al*. 1977; Winarno 1979; Truesdell *et al*. 1987). Except for thiamine which was reduced by approximately 50%, all other vitamins including riboflavin, nicotinic acid, pantothenic acid, pyridoxine, folic acid, cyanocobalamine and biotin increased significantly (Shurtleff and Aoyagi 1979; Okada *et al*. 1983; Murata 1985).

Murata (1977) attributed the improved nutritive value of tempe to stabilization of the oil by antioxidants produced during the fermentation and synthesis of B vitamins. Steinkraus (1983a) observed that stored tempe does not develop rancidity because of its content of 6,7,4'-trihydroxyisoflavone, an antioxidant produced by the mould.

According to Whitaker (1987), the beany flavour would be released from the proteins to which they are bound as a result of proteolysis. Flavour components of the boiled soya beans included mannitol, esters (ethyl palmitate and ethyl linoleate) and free

fatty acids (palmitic, stearic, oleic, linoleic, linolenic). Tempe prepared at 38°C had a stronger odour than that prepared at 31°C. The flavour of tempe prepared at 31°C included the original soya bean components and newly formed 3-methylbutanol, acetoin, acetic acid, methylcarbinol, 2,3-butanediol and isovaleric acid (Moroe 1985).

*Rhizopus oligosporus* NRRL 2710 produced an antibiotic active against a number of Gram positive bacteria including *Staphylococcus aureus* and *Bacillus subtilis*. The only Gram negative bacterium sensitive to the antibiotic was *Klebsiella pneumoniae* (Wang *et al.* 1969).

Work on protein efficiency ratio (PER) and the digestibility of tempe (Wang 1986b; Zamora and Veum 1988; Agosin *et al.* 1989) confirmed that rats do not utilize protein from tempe any better than from cooked substrate. However, Girija Bai *et al.* (1975) and Winarno and Reddy (1986) reported that mixed soya beans-groundnut tempe gave better net protein utilization (NPU) and PER than soya bean protein .

Tempe-like products could also be made from wheat, rice, other cereal grains and their various combinations (Hesseltine *et al.* 1967).

Tempe contains 25-65% moisture, and per 100 g dry matter: 45-55 g protein, 15-25 g fat, 15-25 g carbohydrate, 3-7 g fibre, 5-10 g ash, 1.8-1.9 MJ (430-460 kcal) energy, 400 mg Ca, 400 mg P, 25 mg Fe, 0.4 mg thiamine, 0.7 mg riboflavin, 6 mg niacin, 0,3 mg pantothenic acid, trace vitamin $B_{12}$ and 50 µg vitamin A (Campbell-Platt 1987).

### 2.1.6. Daddawa

Daddawa or iru is a fermented food produced by natural fermentation of the cotyledons of African locust bean (*Parkia biglobosa* Welw. ex Oliv.), a perennial tree legume, commonly consumed in the Savannah regions of West Africa (Campbell-Platt 1980; Eka 1980; Odunfa 1985a). Daddawa, a protein and fat-rich flavoursome ingredient, is used as a good condiment and eaten with sorghum or millet-based dumplings and porridges (Campbell-Platt 1980; Odunfa 1986).

Daddawa preparation is still a traditional family art done in homes. In the traditional method of its preparation, the dried pods are boiled for 12-24 h to soften the tough testa and cotyledons. The seeds are put in a mortar, pressed with feet to remove the softened testa; sand or other abrasive agents may be added. The cotyledons are washed and boiled again for 1-2 h. Excess water is drained off. Seeds are spread in calabash trays in layers of about 10 cm deep or in a hole in the ground. They are left at 25-35°C for 3-4 days. Wood ash may be mixed to reduce the odour. Sometimes, millet flour may be added. The fermented bean mass is sun-dried, and then used loose, or shaped into balls or pyramids and stored in the traditional earthenware pots (Campbell-Platt 1980; Odunfa 1981, 1985a, 1986).

Odunfa and Adewuyi (1985) studied the optimization of process conditions for daddawa production and found the optimal time/temperature for fermentation were 36 h at 35°C and 48 h at 40°C.

Osinowo *et al*. (1990) improved the traditional method of daddawa fermentation by cooking beans in pressure cooker for 75-90

min to remove seed coats, cleaning, washing, further cooking with the addition of starter culture for 1 h, placing in flat calabash and fermenting at 30°C for 18 h.

The presence of *Bacillus subtilis*, *B. licheniformis* and *Staphylococcus* spp. was reported in daddawa (Odunfa 1981, 1986). Antai and Ibrahim (1986) reported the presence of *Leuconostoc mesenteroides* and *L. dextranicus* in almost equal proportion with the *Bacillus* spp. in daddawa. However, Ogbadu and Okagbue (1988) could not find any of these lactics during daddawa production. They found that the species of *Bacillus* responsible for daddawa production were variable, and reported *B. subtilis, B. pumilus* and *B. licheniformis* from six separate fermentations. Osinowo *et al.* (1990) reported *B. subtilis*, *B. cereus*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes* from daddawa.

During fermentation, the temperature and pH of the beans increased from 25°C and 7.0 at 0 h to 45°C and 8.1 at 36 h, respectively (Odunfa 1981). The fatty acids in both unfermented and fermented beans were linoleic, oleic, stearic, palmitic and a trace of arachidonic acids. The major fatty acid was linoleic acid which is an essential fatty acid (Odunfa and Adesomoju 1986). The amino acid pattern of fermented *Parkia* beans was similar to that of raw beans, with a small decrease in essential sulphur-containing amino acids and large decrease in the non-essential aspartic and glutamic acids (Fetuga *et al.* 1973). 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 $\alpha$- and $\beta$-galactosidase which hydrolyzed the oligosaccharides to reducing sugars (Odunfa 1983). Thiamine and

riboflavin content increased during fermentation (Platt 1964; Leung *et al*. 1968; Eka 1980).

Daddawa contains 20-50% moisture, and per 100 g dry matter: 40-45 g protein, 30-40 g fat, 10-15 g carbohydrate, 3-7 g fibre, 3-6 g ash, 2.1-2.3 MJ (500-600 kcal) energy, 300 mg Ca, 550 mg P, 40 mg Fe, 0.05 mg thiamine, 0.6 mg riboflavin, 2 mg niacin and 0.9 µg folic acid (Campbell-Platt 1987).

### 2.1.7. Wari

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

In the traditional method of wari preparation, black gram [*Vigna mungo*(L.) Hepper] dhals are soaked in water for 6-12 h, dewatered, dehulled and ground on a stone martar into a smooth, mucilaginous paste. The dough is mixed with inoculum from a previous batch, salt and typical spices like asafoetida (*Ferula foetida* Regel), caraway (*Carum carvi* L.), cardamom [*Elettaria cardomomum* (L.) Moton], clove [*Syzygium aromaticum* (L.) Merr. and Perry], fenugreek (*Trigonella foenum-groecum* L.), ginger (*Zingiber officinale* Rosc.) and red pepper (*Capsicum annuum* L.). The mixture is allowed to ferment at room temperature for 1-3 days and hand-moulded into balls. After air-drying for 2-8 days on bamboo or palm mats, waries are turned over for further drying (Batra and Millner 1976; Batra 1981; Soni and Sandhu 1990).

Batra and Millner (1974, 1976) isolated two types of yeasts including *Candida krusei* and *Saccharomyces cerevisiae* from waries. Later on, although a wide variety of yeasts and lactic acid bacteria were found to be associated with waries, only the combination of *Hansenula* sp. and *Leuconostoc mesenteroides* was found responsible for their production (Batra 1981, 1986).

Sandhu and Soni (1989) observed the occurrence of bacteria ($10^9$-$10^{12}$/g) in all the market and laboratory-made samples, but only 55% of the samples contained yeasts (0-$10^7$/g). *Leuconostoc mesenteroides* was most abundant and present in all the market samples, followed by *Streptococcus faecalis*, *Lactobacillus fermentum* and *Bacillus subtilis*. *Saccharomyces cerevisiae* and *Pichia membranaefaciens* were the most abundant yeasts, found in all the positive samples, followed by *Candida vartiovaarai*, *Kluyveromyces marxianus*, *Trichosporon beigelii*, *Candida krusei* and *Hansenula anomala*. Laboratory-made samples were found to contain comparatively higher bacterial load ($10^{10}$-$10^{12}$/g) while less yeast load (0-$10^6$/g) in 45% of the samples.

The microbial load of 1.3 x $10^{10}$/g unfermented dough increased to 6.5 x $10^{12}$/g at the end of fermentation. Among the bacteria, *Leuconostoc mesenteroides*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Bacillus subtilis* and *Flavobacter* spp., and among the yeasts, *Trichosporon beigelii*, *Saccharomyces cerevisiae*, *Candida krusei*, *Pichia membranaefaciens* and *Hansenula anomala* predominated the initial stages of fermentation. With the progress in fermentation, most of the microorganisms, except *Leuconostoc mesenteroides*, *Lactobacillus fermentum*, *Saccharomyces cerevisiae*

and *Trichosporon beigelii*, disappeared. There was the production of acid and gas resulting in the fall in pH from 5.65 to 3.20 and rise in volume from 200 to 420 ml. Fermentations brought about an increase in total acids from 0.50 to 1.50%, soluble solids from 7.8 to 14.7%, non-protein nitrogen from 0.20 to 0.68%, soluble nitrogen from 0.95 to 1.50%, free amino acids from 9.79 to 45.15 mg/g and proteolytic activity from 4.82 to 6.04 IU/g. On the other hand, the level of reducing sugars and soluble protein decreased from 13.69 to 4.34 mg/g and 50.52 to 17.40 mg/g, respectively. Amylase activity increased initially, but declined thereafter. Wari fermentation also brought about an appreciable rise in water-soluble B vitamins including thiamine, riboflavin and cyanocobalamine (Sandhu *et al*.1986; Sandhu and Soni 1989; Soni and Sandhu 1990).

## 2.2. FERMENTED VEGETABLES

### 2.2.1. Gundruk

Gundruk, a non-salted and fermented leafy vegetable product, has been one of the major appetizers for the people of Nepal since a long time back (Karki *et al*. 1983d). It is cooked in water and served as a side dish (Dietz 1984).

In the traditional method of its preparation, fresh leaves of mustard [*Brassica juncea* (L.) Czern.], radish (*Raphanus sativus* L.), cauliflower (*Brassica oleracea* L. var. *botrytis* L.) and rape (*Brassica campestris* L.) are left for wilting for 2-3 days. The leaves are shredded, pressed into an earthen jar and covered with lukewarm (30-35°C) water. After fermentation at 16-20°C for 5-7 days, the leaves are removed from the jar and sun-dried (Karki *et al*. 1983d; Karki 1986).

In the samples of gundruk from Nepal, the microflora, represented by lactic acid bacteria, contained *Lactobacillus plantarum*, *L. casei* ssp. *casei*, *L. casei* ssp. *pseudoplantarum*, *L. cellobiosus* and *Pediococcus pentosaceus*. During fermentation, heterofermentative *Lactobacillus cellobiosus*, instead of *Leuconostoc mesenteroides* as in other fermented vegetable products, initiates the fermentation and is followed by homofermentative *Pediococcus pentosaceus* and finally *Lactobacillus plantarum* (Karki *et al.* 1983d).

The pH and acidity (as lactic) in gundruk were 4.0-4.3 and 0.8-1.0%, respectively (Karki *et al.* 1983d). In gundruk, almost 90% of the organic acids consisted of lactic and acetic acids. Besides, citric and malic acids were found in lower concentrations (Karki 1986). The level of palmitic, oleic, linoleic and linolenic acids was much higher in mustard leaf gundruk compared to those in the unfermented vegetables (Karki *et al.* 1983c). In mustard gundruk, free amino acids, particularly glutamic acid, alanine, leucine, lysine and threonine remarkably increased with the corresponding decrease in asparagine, glutamine, histidine and arginine, indicating the influence of fermentation. Proline content in mustard vegetable was greater than that in cauliflower gundruk or mustard gundruk. This may be due to the wilting of vegetables prior to fermentation (Karki *et al.* 1983b).

The main flavour components developed during the fermentation of mustard leaves are cyanides (15.7%), isothiocyanates (8.5%) followed by alcohols (12.3%) and esters (4.1%). Phenylacetaldehyde (6.4%) was the only aldehyde identified in mustard leaf gundruk

(Karki *et al.* 1983a).

According to Dietz (1984), vitamin A is lost during sun-drying of gundruk.

### 2.2.2. Sauerkraut

Sauerkraut or sauerkohl is a German term for 'sour cabbage', which is generally prepared from shredded white cabbage. It is eaten with main meals in Germany, Switzerland, Central Europe, USA, Canada and USSR (Pederson 1979; Campbell-Platt 1987).

For the preparation of sauerkraut, cabbage (*Brassica oleracea* L. var. *capitata*) is trimmed, washed, shredded (3-5 mm x 5-7 cm), placed in barrels with 2.0-2.5% salt, distributed evenly and packed tightly in layers, covered, sealed and allowed to ferment at 16-22°C for 1-2 weeks, followed by gradual reduction in temperature to 0-5°C at the end of one month (Stamer 1975; Frazier and Westhoff 1978; Pederson 1979; Steinkraus 1983b; Vaughn 1985).

Pederson (1930a,b) determined the sequence of microorganisms that develop in a typical sauerkraut fermentation. Subsequent studies by Pederson and Albury (1954, 1969) and Stamer *et al.* (1971) established that *Leuconostoc mesenteroides* initiates fermentation in the shredded cabbage over a wide range of temperature and salt concentration, producing carbon dioxide and lactic acid, followed by predominance of *Lactobacillus brevis* and *Lactobacillus plantarum*. If the fermentation temperature is higher, *Pediococcus cerevisiae* develops and contributes to acid production. While low salt concentration (1.0%) favours the growth of heterofermentative lactics including *Leuconostoc mesenteroides* and

*Lactobacillus brevis*, a higher concentration (3.5%) of salt favours the growth of homofermentative lactics including *Pediococcus cerevisiae* and *Lactobacillus plantarum*.

The fermentation was very slow at 7.5°C, producing 0.8-0.9% acidity (as lactic) in a month, but rapid at 23°C, producing 1.0-1.5% acidity in 8-10 days and more rapid at 32°C, producing 1.8-2.0% acidity in 8-10 days (Pederson and Albury 1969). Higher temperature may result in inferior quality and dark kraut (Pederson 1979). The optimum temperature of about 18°C at 2.25% salt was recorded for sauerkraut fermentation (Parmele *et al.* 1927; Marten *et al.* 1929; Pederson and Albury 1969). During fermentation, carbohydrates are converted to lactic and acetic acids, ethanol, carbon dioxide, mannitol and dextran (Pederson 1979).

Mukherjee *et al.* (1977) observed the loss of total nitrogen from 0.23% (30 days) to 0.12% (120 days), loss of total ash, and gradual increase of crude fibre during sauerkraut production.

The major amount of the volatiles in sauerkraut is accounted for by acetal, isoamyl alcohol, n-hexanol, ethyl lactate, cis-hex-3-ene-1-ol and allyl isothiocyanate. Only the two latter compounds have been identified as major constituents of fresh cabbage (Lee *et al.* 1976).

*Lactobacillus brevis* produces a red pigment under certain conditions which may result in discolouration or darkening of sauerkraut (Stamer *et al.* 1973). The growth of pigmented yeast may be the cause of kraut defect or 'pink kraut' (Brunkow *et al.* 1925). In fact, anaerobiosis helps to eliminate aerobic growth of moulds and yeasts in sauerkraut (Pederson and Albury 1969).

Sauerkraut contains 35-45% moisture, and per 100 g dry matter: 3-5 g protein, trace amount of fat, 15-20 g carbohydrate, 25-30 g fibre, 35-45 g ash, 15-25 g NaCl, 0.3-0.4 MJ (70-100 kcal) energy, 150 mg Ca, 2 mg Fe, 0.1 mg thiamine, 0.15 mg riboflavin, 0.7 mg niacin, 50 µg carotene and 50-70 mg ascorbic acid (Campbell-Platt 1987).

### 2.2.3. Kimchi

Kimchi is the general name given to a group of fermented acid vegetable foods with a long tradition in Korea. More specific names are used for pickled vegetables depending on the raw material, processing method, season and locality. It is a side dish served along with cooked rice. Kimchi is closely related to sauerkraut, but differs in having less acid and being carbonated (Mheen *et al.* 1977; Lee 1986).

For the preparation of kimchi, Oriental radish (*Raphanus sativus* L.), Chinese cabbage (*Brassica chinensis*), cucumber (*Cucumis sativus* L.) or other vegetables are mixed with small amount of onion, chilli, pepper, garlic, ginger and 4-6% salt or brine. The mixture is packed into a large earthenware vessel. Fish, shrimps or oysters may be added and fermented at 10-18°C for 5-20 days (Mheen *et al.* 1977; Lee 1986).

Kimchi contains *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Lactobacillus brevis*, *Lactobacillus plantarum* and *Pediococcus cerevisiae* (Kim and Whang 1959; Kim and Chun 1966; Mheen and Kwon 1979). Whang *et al.* (1960) isolated *Achromobacter*, *Flavobacterium* and *Pseudomonas* spp. from kimchi. Ha (1960) observed

that few yeasts and moulds appear in the later stages of fermentation, causing softening of the product. Pathogenic bacteria present on the ingredients disappear during fermentation (Soh 1960; Chung *et al*. 1967).

At the time of fermentation, the initial pH of 5.5-5.8 falls to pH 4.2-4.5 (Song *et al*. 1966). The optimum acidity of kimchi is 0.4-0.8% (as lactic), while higher acidity makes it unacceptable (Lee and Yang 1970; Mheen *et al*. 1977). Kimchi produced at 6-7°C contained more lactic and succinic acids but less oxalic, malic, tartaric, malonic, maleic and glycolic acids than that produced at 22-23°C (Kim and Rhee 1975). Lee and Lee (1965) reported a decrease in reducing sugars during fermentation.

Using a 3% salt concentration, the optimum period of fermentation was one day at 30°C, 2-3 days at 20°C, 12-15 days at 10°C, and 30-60 days at 5°C (Yu and Chung 1974; Mheen *et al*. 1977). Vitamins including thiamine, riboflavin, cyanocobalamine and niacin reached their highest levels (twice the initial level) when kimchi had the most palatable taste, and decreased when kimchi became too sour (Lee *et al*. 1960). Kimchi produced following inoculation with *Propionibacterium freudenreichii* van Niel ssp. *shermanii* contained 102 µg cyanocobalamine, whereas non-inoculated fermentation had 47 µg cyanocobalamine per 100 g substrate (Ro *et al*. 1979). Vitamin C and carotene decrease upon ripening (Lee *et al*. 1960; Lee and Lee 1965; Song *et al*. 1966).

Kimchi contains 75-95% moisture, and per 100 g dry matter: 10-30 g protein, 3-10 g fat, 30-50 g carbohydrate, 5-10 g fibre, 10-20 g ash, 1.0-1.4 MJ (250-330 kcal) energy, 20-300 mg Ca, 250-600 mg P, 2-11 mg Fe, 0.15-0.7 mg thiamine, 0.2-1.0 mg riboflavin, 3-40

mg niacin, 100-300 µg cyanocobalamine and 75-450 mg vitamin C (Campbell-Platt 1987).

### 2.2.4. Naw-mai-dong

Naw-mai-dong, the pickle obtained by fermenting young shoots of bamboo [*Bambusa arundinacea* (Retz.) Roxb.] is common in Thailand. Those of the sweeter species, such as *Bambusa burmanica* Gamble and *Dendrocalamus asper* Back. are also used as raw materials (Boon-Long 1986).

In the traditional method of its preparation, bamboo shoots are boiled in water and the bitter liquor is discarded. They are then sliced (2-3 mm x 1.5 cm), mixed with 2% salt, packed into a narrow-mouthed earthen jar, weighted down and fermented at room temperature for 3-4 weeks (Dhavises 1972; Yanasugondha 1977; Boon-Long 1986).

Naw-mai-dong contained 1-1.2% lactic acid (Boon-Long 1986). *Pediococcus cerevisiae* predominates at the early stages of fermentation. *Lactobacillus plantarum* comes to predominate after 6 h and *Lactobacillus brevis* predominates at the final stage (Dhavises 1972).

## 2.3. FERMENTED CEREAL PREPARATION

### 2.3.1. Jalebi

Jalebies are pretzel-like syrup-filled confections, prepared from deep-fried fermented wheat-flour batter. These are consumed throughout India, Nepal and Pakistan (Batra and Millner 1974, 1986; Ramakrishnan 1979). They have been known in these areas since 1450 A.D. and are probably of Arabic or Persian origin (Gode 1943).

Jalebies are prepared by mixing wheat flour with dahi (curd), adding water in it and leaving overnight at room temperature. The thick leavened batter is squeezed through an embroidered hole (about 4 mm in diameter) in thick and durable cotton cloth, and deposited as continuous spirals into hot fat. After about one min, when the spirals become light brown, these are removed from fat with a sieved spatula. Excess fat is drained away, and the jalebies are immediately immersed into sugar syrup for 1-2 min. Often rose (*Rosa indica*Lour.) or kewda (*Pandanus tectorius* Soland. ex. Parkinson) water and orange food colour are added to the syrup (Ramakrishnan 1977, 1979; Batra 1981, 1986).

Ramakrishnan (1977, 1979) reported the presence of *Lactobacillus fermentum* ($6 \times 10^8$/g), *L. buchneri* ($3.2 \times 10^8$/g), *Streptococcus lactis* ($6 \times 10^8$/g), *S. faecalis* ($6 \times 10^8$/g) and *Saccharomyces* sp. in fermented jalebi batter. But, Batra (1981, 1986) found *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *S. faecalis*, *Saccharomyces bayanus*, *Saccharomyces cerevisiae* and *Hansenula anomala* in fermented jalebi batter. During fermentation at 28°C, the bacterial and yeast counts increased from $3.26 \times 10^5$ to $12.6 \times 10^6$/g and $9.4 \times 10^4$ to $6 \times 10^6$/g, respectively. At 19°C, while the bacterial count was lowered to $1 \times 10^6$/g, there was no change in the count of yeasts.

During fermentation, the pH decreases from 4.4 to 3.3 and the volume of the batter increases by 9%. Amino nitrogen and free sugar contents decrease during fermentation (Ramakrishnan 1977, 1979).

Jalebi contains 32-38% moisture, and per 100 g dry matter:

4-7 g protein, 15-20 g fat, 75-78 g carbohydrate, 2-4 g fibre, 2-3 g ash, 1.9-2.0 MJ (460-480 kcal) energy, 2 mg Na, 90 mg K, 70 mg Ca, 10 mg Mg, 1 mg Fe, 0.1 mg Cu, 0.5 mg Zn, 0.17 mg thiamine, 0.03 mg riboflavin, 2.0 mg niacin, 14 µg folic acid, retinol, carotene, vitamin C and D (Campbell-Platt 1987).

## 2.4 FERMENTED DAIRY PRODUCTS

### 2.4.1. Dahi

Dahi, a major adjunct to the daily diet in India, Pakistan, Nepal, Bangladesh and Sri Lanka, is the result of action of lactic acid bacteria on cow's or buffalo's milk. It resemples plain yoghurt in appearance and consistency, and differs in having less acidity (Batra and Millner 1976; Mital 1977; Shuaib and Azmey 1977; Ekmon and Nagodawithana 1977).

In the traditional method of its preparation, milk is boiled, cooled, inoculated with previous batch of dahi and kept at ambient temperature for 8-12 h for setting (Verma and Mathur 1986).

Laxminarayana *et al.* (1952a,b) observed that dahi from north India is firm and sweet to mildly sour in taste, with a preponderance of streptococci over lactobacilli, whereas dahi from south India is soft and acidic, with more lactobacilli than streptococci. In the eastern part of India, misti dahi (sweetened dahi or payodhi) is very popular (Ghosh and Rajorhia 1987, 1990).

Laxminarayana *et al.* (1952b), Ranganathan *et al.* (1964), and Ramakrishnan (1979) isolated *Lactobacillus bulgaricus*, *L. acidophilus*, *L. helveticus*, *L. casei*, *L. brevis*, *Streptococcus thermophilus*,

*S. lactis, S. cremoris and S.faecalis* from dahi.

Ranganathan *et al.* (1964) observed that in the microflora of dahi *S. thermophilus* constitutes 50% of toal streptococci with *S. faecalis* and *S. lactis* next most numerous, and *L. bulgaricus* constitutes 70% with *L.* casei and *L. brevis* next most numerous. A mixed culture of *S. thermophilus* and *L. bulgaricus* produced greater amount of acid than mixed culture of *S. thermophilus*, *S. lactis* ssp. *diacetylactis* and *S. lactis* (Sharma and Jain 1975). However, *S. lactis* ssp. *diacetylactis* imparted desirable flavour to dahi by producing higher amount of diacetyl and volatile acid than *S. thermophilus* and *S. cremoris* (Baisya and Bose 1975).

Ghosh and Rajorhia (1990) found that a mixture of various strains of *S. lactis*, *S. diacetylactis*, *S. cremoris* and *Leuconostoc* sp. was most appropriate for production of misti dahi from buffalo's milk containing 18% milk solids and 14% sucrose.

Batra and Millner (1976) isolated yeasts from dahi of Punjab and identified them as *Candida krusei*, *Trichosporon* sp. and *Torulopsis* sp.

A good quality dahi has a pH 4.6-5.0 (Rao and Dastur 1955) and acidity 0.8-1.0% as lactic (Srinivasan and Banerjee 1946). During fermentation, there is increase in non-protein nitrogen and dialyzable nitrogen, but decrease in protein nitrogen and ammonia nitrogen (Venkatappaiah and Basu 1956; Verma and Mathur 1986).

Rao and Basu (1962) found that a mixed culture of *L. bulgaricus* and *L. cremoris* decreased thiamine, riboflavin and nicotinic acid content, whereas single cultures of *S. lactis* and *S. cremoris* raised the thiamine concentration from 2 to 20% over that of milk

during dahi fermentation. Boman and Dalal (1956) observed the increase of riboflavin, folic and folinic acid content during dahi fermentation.

Dahi contains 85-88% moisture, 3.2-3.4% protein, 5-8% fat, 4.6-5.2% lactose, 0.7-0.75% ash, 0.5-5.2% lactic acid, 0.12-0.14% Ca and 0.09-0.11% P (Laxminarayana *et al.* 1952b).

### 2.4.2. Yoghurt

According to some sources, yoghurt originated in Asia (Oberman 1985). To produce yoghurt, milk from cow, goat, sheep, buffalo or camel is heated to 88-95°C to pasteurize, homogenized and cooked to 42-47°C, before addition of 2-5% lactic starter culture. It is then fermented for 3-6 h until desired acidity obtained and setting yoghurt into soft gel (Campbell-Platt 1987).

The essential microflora in yoghurt consists of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

A proportion of 1:1 of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* is considered to be optimum for flavour and texture production (Vedamuthu 1982), but 1:5, 1:10 or 2.1:1.2 are also favourable (Rašić and Kurmann 1978).

The natural yoghurt (without addition) contains 85-90% moisture, and per 100 g dry matter: 30-35 g protein, 7-15 g fat, 43-48 g carbohydrate, 6-8 g ash, 1.6-1.8 MJ (380-420 kcal) energy, 1700 mg K, 1400 mg Ca, 120 mg Mg, 1000 mg P, 0.6 mg Fe, 0.3 mg Cu, 4 mg Zn, 1300 mg Cl, 0.35 mg thiamine, 1.8 mg riboflavin, 1 mg niacin, 7 mg potential niacin from tryptophan, 7 µg free folic acid, 14 µg total folic acid, 0.3 mg vitamin $B_6$, trace vitamin $B_{12}$, 60 µg retinol, 35 µg carotene, trace vitamin D, 3 mg vitamin C and

0.2 mg vitamin E (Campbell-Platt 1987).

### 2.4.3. Chu-ra

Chu-ra, a fermented milk product, is traditionally consumed in Tibet, Nepal and northeast India. During its preparation, yak's milk is heated, curd separated by filtration through a cloth, moulded into rectangular (20-40 cm x 15 cm x 15 cm) loaves and left to ferment at low temperature for several days. The loaves are sliced, and the slices are strung on yak hair twine, and allowed to sun dry (Batra and Millner 1976).

## 2.5. STARTER CULTURES

### 2.5.1. Murcha

Murcha, a starter culture, has erroneously been referred to as a rice beer as well (Ray 1906; Batra and Millner 1974, 1976; Hesseltine *et al.* 1988). Murcha is a small rice starch cake, about 4-6 cm in diameter and available in the central and eastern Himalayas. In addition to rice starch, the cakes may also contain berries, roots and leaves of wild native plants (Ray 1906). Hutchinson and Ram-Ayyar (1925) reported the presence of several efficient saccharifying fungi, namely *Aspergillus oryzae*, *Endomycopsis burtonii*, *Mucor javanicus*, *M. prainii* and *Rhizopus cambodja* in murcha. Batra and Millner (1974) isolated *R. arrhizus*, *M. fragilis*, *M. rouxianus*, and the yeast *Hansenula anomala* var. *schneggii* from murcha. Batra (1981) reported the presence of *E. fibuligera* and *Amylomyces rouxii* in addition to those mentioned earlier (Batra and Millner 1974).

The presence of *Mucor* and *Rhizopus* in murcha samples of Nepal was confirmed by Hesseltine (1983b) and Hesseltine *et al.* (1985, 1988). However, *Amylomyces* was consistently absent in those studies, because of the prevailing low temperature in Nepal. Bacterial count of 2 x $10^8$/g, yeast count of 6 x $10^8$/g and mould count of 2.8 x $10^8$/g were found in murcha samples of Nepal (Hesseltine *et al.* 1988). The lactic acid bacteria in murcha samples of Nepal included mostly *Pediococcus pentosaceus* and few *Streptococcus faecalis* (Hesseltine and Ray 1988). *Saccharomycopsis fibuligera* represents the dominant starch-degrading yeast in murcha, associated with less predominance of *Saccharomyces* and *Pichia* (Hesseltine and Kurtzman 1990).

### 2.5.2. Ragi, Chinese yeast, bubod and loogpang

The starter preparations go under a variety of names, such as ragi in Indonesia, Chinese yeast or chiu-chu in China and Taiwan, bubod in Philippines and loogpang in Thailand (Hesseltine *et al.* 1988). Except for the Thai loogpang in which the organisms are grown on bran, the predominant forms of the ragi type starters are small (3-6 cm), round and flattened cakes of rice flour (Djien 1977; Pichyangkura and Kulprecha 1977). The use of Chinese yeast was described as early as 531 A.D. in China (Yamazaki 1932).

The starter cultures are made by mixing rice flour with various spices such as ground garlic, black pepper, ginger etc. Some wild herbs are also blended. Water is added to make a thick paste which is kneaded into small flattened cakes. Powdered old starter cultures are sprinkled over the cakes, placed on baamboo tray, fermented at 25-30°C for 2-5 days, and then sun-dried

(Macfadyen 1903; Saono *et al*. 1974, 1982; Tanimura *et al*. 1977; Yeoh 1977; Djien 1977, 1986).

Spices such as garlic, lengkuas, ginger and kapulaga added to ragi may inhibit development of undesirable microorganisms (Soedarsono 1972).

Ragi is used to make tapé, a fermented food of Indonesia. Tapé is a syrup-like product made from glutinous rice (Hesseltine *et al*. 1988). Chinese yeast is used to make Chinese dessert lao-chao (Hesseltine 1983b) and Shaohsingchui, an alcoholic Chinese beverage (Yamazaki 1918). Bubod is used to make basi (sugarcane wine) and binobodan (rice wine) in Philippines (Del Rosario 1980). Loogpang is used to make tapé-like products and rice wine preparation in Thailand (Pichyangkura and Kulprecha 1977).

Ishimaru and Nakano (1969) found *Streptococcus faecalis* and *Lactobacillus plantarum* in ragi and obtained bacterial count as high as $10^{10}$ in 24 h-old culture. Djien (1972) found the presence of *Amylomyces rouxii*, *Endomycopsis chodati*, *Mucor rouxii* and *Rhizopus* sp. in ragi of Indonesia, and concluded that combination of *Amylomyces* and *Endomycopsis* resulted in good tapé fermentation; while others being unimportant. Toyota and Kozaki (1978) studied the bacteria in ragi and identified them as *Pediococcus pentosaceus*. Hadisepoetro *et al*. (1979) reported the presence of a mould *Zygorhynchus*, two yeasts including *Candida* and *Torulopsis* and a lactic acid bacterium, *Pediococcus* in ragi. The counts of mould, yeasts and bacteria were 3.2-4.0 x $10^4$, 5.6-14 x $10^6$ and 3.0-18 x $10^4$, respectively. The presence of *Pediococcus pentosaceus* and *Streptococcus faecalis* was confirmed by Hesseltine and Ray (1988).

Saono and Basuki (1978, 1979) found that the yeasts isolated from ragi had no proteolytic activities, whereas the mould isolates had amylolytic activities.

Hesseltine *et al.* (1988) examined viability of *Amylomyces rouxii*, *Mucor* and *Rhizopus* in ragi. While *Amylomyces rouxii* could survive remarkably well when kept at room temperature in a dry state, there was considerable reduction in number of *Mucor* and *Rhizopus* with long period of storage.

Suprianto *et al.* (1989) reported the active microorganisms to be *Rhizopus* sp., *Saccharomycopsis* sp. and *Streptococcus* sp. in tapé fermentation. The presence of *Saccharomycopsis fibuligera* and *Saccharomycopsis malanga* was reported in ragi (Hesseltine and Kurtzman 1990).

According to Hesseltine *et al.* (1988), four genera of moulds including *Mucor*, *Amylomyces*, *Chlamydomucor* and *Rhizopus* are involved in Chinese yeast. Hesseltine and Kurtzman (1990) reported the presence of *Saccharomycopsis fibuligera* and *S. malanga* in Chinese yeast with less numbers of *Saccharomyces*, *Pichia* and *Candida*.

Bubod contains *Amylomyces, Mucor, Rhizopus* and *Saccharomyces* (Tanimura *et al.* 1977). The microbial count in bubod ranged from $2.1 \times 10^3$-$2.3 \times 10^7$ for moulds, $7.4 \times 10^4$-$3.0 \times 10^7$ for yeasts and $2.9 \times 10^5$-$4.7 \times 10^7$ for lactic acid bacteria (Del Rosario 1980). Hesseltine and Kurtzman (1990) reported the presence of *Saccharomycopsis fibuligera* in bubod.

Loogpang from Thailand contains *Amylomyces*, *Rhizopus*, *Aspergillus*, *Mucor* and *Absidia* (Pichyangkura and Kulprecha 1977).

## 2.6. FERMENTED BEVERAGES

### 2.6.1. Millet beverage

In old literatures (Hooker 1854; Risley 1894; Gorer 1938), there are mentions of fermented millet or marwa beverage of Darjeeling hills and Sikkim. The beverage is also known as 'chang' by the Sikkimese (Risley 1894) and 'chi' by the Lepchas (Gorer 1938). 'Thumba', the fermented beverage common in Darjeeling, Sikkim and Nepal has been reported by Hesseltine (1965, 1979) and Batra and Millner (1976). *Endomycopsis fibuliger* has been found in thumba of West Bengal (Hesseltine 1979).

### 2.6.2. Rice beverage

Rice beer or pachwai or murcha or bakhar is probably the most widely consumed fermented beverage in rural Asia. It is prepared by mixing a starter culture with the cooled rice gruel and fermented for 24 h or longer. The beer is decanted and the residue is used as a meal. the fermentation is carried out by several mucoraceous species or *Aspergillus oryzae* that convert starch to sugars, which are fermented by *Hansenula anomala*, *Endomycopsis fibuligera* and *Amylomyces rouxii*. The ethanol content is 3% and the odour of ethylacetate is discernible (Batra and Millner 1976; Batra 1981, 1986).

### 2.6.3. Barley beverage

Chiang or lugri, a barley-based fermented beverage, is a mild alcoholic, thick, transluscent, foamy drink with a sweet-sour taste and somewhat aromatic flavour. It is consumed without

additional carbonation and is usually neither aged nor filtered. The chiang of south-western Tibet, along the Nepal border is sour and aged during storage for one month. Chiang is consumed in this area with addition of 2-3 g of yak butter which floats onthe top of beverage (Batra and Millner 1976; Batra 1986).

In the traditional method of its preparation, during late March through May high quality grains from previous year is soaked overnight, dewatered, spread in gunny sacks, incubated for 2-5 days in a warm place and allowed to dry gradually. the grain is further air-dried in the sun, then coarsely ground and mashed. The mash is boiled, cooled, mixed with unmalted crushed grain and fermented for 3-6 days in a cool place. The starter inoculum comes either from the unmalted grain and flowers of diverse plants that are added, or from the portion of beer added to the mash from a previous batch. *Saccharomyces cerevisiae* and *S. uvarum* are found to occur in chiang or lugri (Batra 1986).