

REVIEW OF LITERATURE

Canned or frozen foods are too expensive or not easily available for majority of people living in underdeveloped and developing countries, acid fermentation combined with salting remains one of the most practical methods of preserving and often enhancing the organoleptic and nutritional quality of fresh vegetables (Steinkraus, 1996). The knowledge of the art of pickling vegetables (fermentation) is a process of preservation of foods that is lost in antiquity (Battcock and Azam-Ali, 1998). It may have been developed in Asia as suggested by Pederson (1979) or in Mediterranean (Hulse, 2004) but until more evidence is available its origin will remain obscure (Steinkraus, 1996). In any event, this method of food preservation has been used for many centuries and is one of the important methods of food preservation still in use for vegetables and fruits where production by canning, drying or freezing is not the method of choice (Vaughn, 1985).

Record of acid-fermented vegetables has been well documented by several authors (Fleming, 1982; Steinkraus, 1996; Battcock and Azam-Ali, 1998). The Chinese labourers working on the Great Wall were eating acid-fermented mixed vegetables in the 3rd century B.C. (Pederson, 1979). Centuries ago, the Koreans developed kimchi made from acid-fermented Chinese cabbage, radish, and other ingredients (Lee, 1994a). Similarly, in the Western world, cabbage was fermented to sauerkraut and cucumbers to pickles (Pederson and Albury, 1969).

Table A shows a comprehensive list of fermented vegetable products of the world. Review of available literature on some common LAB-based fermented vegetables was illustrated.

Table A. LAB-based fermented vegetable products of the world

Product	Raw material	Functional LAB	Country	Reference
<i>Cucumber pickle</i>	Cucumber	<i>Leuc. mesenteroides</i> , <i>P. cerevisiae</i> , <i>P. acidilactici</i> , <i>Lb. plantarum</i> , <i>Lb. brevis</i>	European countries, USA, Canada	Vaughn (1985)
<i>Gundruk</i>	Leafy vegetables	<i>Lb. plantarum</i> , <i>Lb. casei</i> , <i>Lb. fermentum</i> , <i>P. pentosaceus</i> .	India, Nepal	Karki <i>et al.</i> (1983d)
<i>Jeruk</i>	Fruits and vegetables	<i>Leuconostoc</i> , <i>Lactobacillus</i> , <i>Pediococcus</i> , <i>Streptococcus</i>	Malaysia	Merican (1996)
<i>Kimchi</i>	Chinese cabbage, radish	<i>Leuc. mesenteroides</i> , <i>E. faecalis</i> , <i>P. cerevisiae</i> , <i>Lb. plantarum</i> , <i>Lb. brevis</i>	Korea and China	Cheigh and Park (1994)
<i>Mesu</i>	Bamboo shoots	<i>P. pentosaceus</i> , <i>Lb. brevis</i> , <i>Lb. plantarum</i>	India, Nepal	Tamang and Sarkar (1996)
<i>Naw-mai-dong</i>	Bamboo shoots	<i>Leuc. mesenteroides</i> , <i>P. pentosaceus</i> , <i>Lb. fermentum</i> , <i>Lb. buchneri</i> , <i>Lb. brevis</i>	Thailand	Dhavises (1972)
<i>Olives</i>	Olive	<i>Lb. plantarum</i> , <i>Lb. brevis</i> , <i>Lb. casei</i> , <i>E. faecium</i>	USA, Spain, Portugal, Peru, Chile	Randazzo <i>et al.</i> (2004)
<i>Pak-gard-dong</i>	Leafy vegetable	<i>Lb. plantarum</i> , <i>Lb. brevis</i> , <i>P. cerevisiae</i>	Thailand	Mingmuang (1974)

Product	Raw material	Functional LAB	Country	Reference
<i>Pak-sian-dong</i>	Leaves of <i>Gynandropis pentaphylla</i>	<i>Leuc. mesenteroides</i> , <i>Lb. fermentum</i> , <i>Lb. buchneri</i> , <i>P. pentosaceus</i> <i>Lb. plantarum</i> , <i>Lb. brevis</i>	Thailand	Dhavises (1972)
<i>Sauerkraut</i>	Cabbage	<i>Leuc. mesenteroides</i> , <i>P. cerevisiae</i> , <i>Lb. plantarum</i> , <i>Lb. brevis</i> , <i>Leuc. fallax</i>	European countries, USA, Canada	Pederson and Albury (1969), Breidt <i>et al.</i> (1995)
<i>Sayur asin</i>	Mustard cabbage and similar vegetable	<i>Leuc. mesenteroides</i> , <i>P. pentosaceus</i> , <i>Lb. confusus</i> , <i>Lb. plantarum</i>	Indonesia	Puspito and Fleet (1985)
<i>Sinki</i>	Radish tap-root	<i>Lb. fermentum</i> , <i>Lb. brevis</i> , <i>Lb. plantarum</i>	India, Nepal, Bhutan	Tamang and Sarkar (1993)
<i>Soibum</i>	Bamboo shoot	<i>Leuc. mesenteroides</i> , <i>Lb. coryniformis</i> , <i>S. lactis</i> , <i>Lb. brevis</i> , <i>Lb. delbruckii</i>	India	Giri and Janmejay (1987)
<i>Suan-cai</i>	Vegetables	<i>Lb. harbinensis</i>	China	Miyamoto <i>et al.</i> (2005)
<i>Sunki</i>	Turnip	<i>Lb. plantarum</i> , <i>Lb. brevis</i> , <i>P. pentosaceus</i>	Japan	Itabashi (1986)

Cucumber pickle

Cucumber pickle is produced and consumed in North America and Europe (Fleming, 1984). Pickling cucumbers are harvested while still immature, washed and are either dry salted or brined (Etchells *et al.*, 1973). Most of the companies in USA do not brush-wash the fruit before brining although washing has been suggested for use in controlled fermentation procedures (Fleming *et al.*, 1988). Maintenance of structural integrity of cucumbers during fermentation is greatly dependent on chemical and physical properties of the fresh fruit, including size, maturity, cultivar, and physiology (Fleming, 1984). Microbial fermentation is not limited to the cover brine, as once thought (Etchells *et al.*, 1968). Lactic acid bacteria have been shown to enter and proliferate within brined cucumber (Daeschel and Fleming, 1981). Sugar content and buffering capacity of cucumbers are important in determining the extent to which LAB can ferment before inhibited by low pH (Fleming, 1984). Cucumbers contain about 2 % fermentable sugars (Handley *et al.*, 1983) consisting essentially of glucose and fructose (Fleming *et al.*, 1988) and traces of sucrose (Handley *et al.*, 1983). Glucose and fructose are readily fermented by LAB during cucumber fermentation (Phar *et al.*, 1977), however, differential utilization of glucose and fructose by the LAB have also been reported (Lu *et al.*, 2001).

Three kinds of cucumber pickles are produced viz. fresh pack (also known as fresh cure, home style and by other names) which at most, are held in salt brine for up to 2 days, then packed in tin cans or glass jars and pasteurised; salt stock pickles from which a variety of

processed products are produced; and fermented dill pickles (Fuller and Dull, 1983). The fresh pack pickles undergo a marginal fermentation, whereas the other two undergo a complete fermentation (Vaughn, 1985). The initiation of the fermentation by *Leuconostoc mesenteroides* at lower temperature and salt concentration was verified by Pederson and Ward (1949), Pederson and Albury (1950). The other species of LAB, *Pediococcus cerevisiae*, *P. acidilactici*, *Lb. brevis* and *Lb. plantarum* predominate in cucumber fermentation at 5-8 % brines (Etchells *et al*, 1975). *P. cerevisiae* and *Lb. brevis* are less resistant to salt than *Lb. plantarum* so sometimes they are absent in the higher salt concentrations (30° Saltometer) brine (Pederson and Albury, 1961). *Lb. plantarum* is the most salt resistant species and it terminates the fermentation (Pederson and Albury, 1961). But it is severely retarded at salt concentration of 40° Saltometer and above and little if any growth occurs above 45° Saltometer (Vaughn, 1985).

Vigorous activity in the cover brine by coliform bacteria, obligate halophiles, heterofermentative LAB, and fermenting species of yeast is associated with gaseous fermentation and resulting bloater spoilage (Fleming *et al.*, 1992). Even homofermentative LAB such as *Lb. plantarum* and *P. cerevisiae* produce sufficient CO₂ when combined with CO₂ from cucumber tissue, to form bloater formation in brined cucumbers (Fleming *et al.*, 1973). *Lb. plantarum* and *P. pentosaceus* decarboxylate malic acid to yield CO₂ and lactic acid (Daeschel *et al.* 1984; McFeeters *et al.*, 1984). Malic acid is a natural constituent of pickling cucumbers (McFeeters *et al.*, 1982). Procedures have been developed to produce

and isolate non-malate decarboxylating mutants of *Lb. plantarum* (McFeeters *et al.*, 1984; Daeschel and Fleming, 1987). Purging fermenting cucumber brines with nitrogen has been shown to be effective in preventing bloater formation (Etchells *et al.*, 1973; Fleming *et al.*, 1973, 1975). Butyric acid spoilage of brined cucumbers was found to occur after an apparently narrow primary fermentation by LAB (Fleming *et al.*, 1989). *Clostridium tertium* was identified as contributing to spoilage (Fleming *et al.*, 1992). At completion, the total acidity of fermented cucumber may be as high as 0.9 % (as lactic acid) and have a pH value as low as 3.3 if oxidative yeasts have been kept inactive (Vaughn, 1985).

Gundruk

Gundruk is a non-salted and fermented leafy vegetable product which has been one of the major appetizers for the people of Nepal (Dietz, 1984) and also of India particularly in the Darjeeling hills and Sikkim (Tamang *et al.*, 1988).

In the traditional method of gundruk preparation practiced in Nepal (Karki, 1986), fresh leaves of mustard, radish and cauliflower are wilted 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). Karki *et al.* (1983d) studied the microorganisms of gundruk prepared in Nepal. They found that gundruk fermentation was initiated by *Lb. cellobiosus* (= *Lb. fermentum*) instead of *Leuconostoc mesenteroides* as in other fermented vegetable products

and was followed by *Pediococcus pentosaceus* and finally by *Lb. plantarum*. *Lb. casei*, *Lb. casei* subsp. *pseudopantarum* were also isolated from gundruk of Nepal (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 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 (Karki *et al.*, 1983b).

Mustard vegetable gundruk comprised of cyanides (15.7 %), isothiocyanates (8.5 %), as the main flavour components, followed by alcohols (12.3 %) and esters (4.1 %). Phenyl acetaldehyde (6.4 %) was the only aldehyde identified in mustard leaf gundruk. Cauliflower gundruk consisted of alcohols (50 %) as the major component followed by cyanides (6.5 %), isothiocyanates (6.1 %) and esters (3.2 %) (Karki *et al.*, 1983a). The levels of iron and calcium are high while carotenoids are reduced by more than 90 % probably during sun drying (Diez, 1984).

Jeruk

Jeruk are homemade fermented pickles indigenous to many races in Malaysia, which are prepared from common fruits and vegetables (Merican, 1996). Among the common vegetable used are gherkins and cucumbers, ginger, onion, chili, bamboo shoot, mustard leaves, etc. Fruits such as mango, papaya, nutmeg, etc. are used for pickling.

Low-income rural people consume large quantities of jeruk because pickling is an inexpensive way to preserve surplus food. Pickled vegetables are prepared like fresh vegetables; pickled fruit is eaten as a relish especially by children and expectant mothers because of the sweet-sour flavour. *Leuconostoc*, *Lactobacillus*, *Pediococcus* and *Streptococcus* have been isolated from fermenting cucumber and mustard leaf pickles (Kiam chye) produced in Malaysia (Merican, 1996).

Kimchi

Kimchi is the generic name given to a group of lactic acid fermented vegetable foods of Korea (Cheigh and Park, 1994). It is a unique vegetable product of long tradition in Korea considered as a main side-dish served along with cooked rice and other dishes (Lee, 1994a). Kimchi is closely related to sauerkraut but differs in having less acid and being carbonated (Lee, 1986; Mheen *et al.*, 1996). More specific names are used for these pickled vegetables depending on the raw material, processing methods, seasons and localities. For example, Tongbaechu-kimchi and Bossam kimchi are based on Korean cabbage; Kakduggi, Dongchimi, chonggyak-kimchi are based on Korean radish; and oisobaegi

and oiji are based on cucumber (Mheen *et al.*, 1996). Korean cabbage and radish are the major substrates while garlic, green onion, zinger, leaf of mustard, hot pepper, parsley pear, chestnut and carrot are minor ingredients. Additional minor ingredients are pickled shrimp, frozen pollack, oyster, small octopus and seasonings, table salt, monosodium glutamate, chengyak (a type of seaweed), pear, etc. The ratio of major to minor ingredients varies depending on the households and makers, the range being 70 to 90:30 to 10. A recipe for typical Tongbaechu-kimchi includes 100 g Korean cabbage, 2 g garlic, 2 g green onion, 2 g powdered hot pepper, and 0.5 g ginger. Although the proper combination of minor ingredients is reported to be the key to good-tasting kimchi, the most important factor seems to be the salt concentration. Salting of cabbage can be done at 5 to 7 % salt concentration for 12 hour or in 15 % saline solution for 3 to 7 hour, followed by rinsing and draining. Optimum salt concentration during kimchi fermentation is approximately 3 % and is adjusted by experience at the household level (Yu and Chung, 1974).

Production methods of kimchi differ depending on the variety of kimchi and the ingredients used, as well as whether production is home-based or industrial-scale (Yu and Chung, 1974; Koo and Choi, 1990; Han *et al.*, 1993; Mheen *et al.*, 1996). However, the principal process described by Cheigh and Park, (1994) consists of pretreatment, brining, blending of ingredients, and fermentation. Chinese cabbage or Oriental radish may be treated in various ways before brining (Lee, 1986). Pretreatment include grading, washing and cutting. Other materials also are graded, washed and cut or chopped for the blending and fermentation

steps. Pretreated cabbage or radish is brined at various salt concentrations by either dry salt or brine solution. Brine-treated and rinsed ingredients (cabbage, radish, cucumber, etc.) are blended together with dry salt and a premixture of chopped or sliced minor ingredients (spices, seasonings, salt-pickled fishes, and other vegetables) according to the recipes (Lee, 1986). The blend is then fermented under the appropriate conditions. Accordingly, the preparation methods, as well as the ingredients, significantly affect the biochemical, microbiological, and nutritional characteristics (Cheigh and Park, 1994).

Fermentation of kimchi in the home is usually done at ambient temperature (Mheen *et al.*, 1996). Using a 3 % salt concentration, the optimum fermentation period is one day at 30° C, 2-3 days at 20° C, 12-15 days at 10° C and 30-60 days at 5° C. The initial pH of 5.5 to 5.8 falls to an optimum of 4.5 to 4.2 and drops further to pH 4.0 if fermentation is too prolonged (Song *et al.*, 1966). Optimum total acidity (as lactic acid) is 0.4 to 0.75 %, rising to 1.0 % if over-ripening occurs and becoming 1.5 to 2.0 % at the spoilage stage (Lee and Yang, 1970). Studies on the microbial composition of kimchi using conventional methods of isolation and phenotypic identification has been done by Lim *et al.* (1989), Park *et al.* (1990) and Shin *et al.* (1996). Some important species of LAB thought to be responsible for kimchi fermentation are *Leuc. mesenteroides*, *Leuc. pseudomesenteroides*, *Leuc. lactis*, *Lb. plantarum*, *Lb. brevis*, *E. faecalis*, *P. cerevisiae*, (Kim and Whang, 1959; Kim and Chun, 1966; Mheen and Kwon, 1984). LAB isolates from kimchi were re-examined using a battery of polyphasic methods including 16S rRNA sequencing and DNA-DNA

hybridization leading to the discovery of several novel species: *Leuconostoc kimchii* (Kim *et al.*, 2000), *Lb. kimchii* (Yoon *et al.*, 2000), *Weissella kimchii* (Choi *et al.*, 2002), *Weissella koreensis* (Lee *et al.*, 2002) and *Leuc. inhae* (Kim *et al.*, 2003). *Leuc. geladium*, *Leuc. gasicomitatum* and *Lb. sakei*, *Weissella koreensis* were elucidated by using 16S RNA gene analysis (Kim and Chun, 2005). The main microorganisms responsible for ripening are *Leuc. mesenteroides* but *Lb. plantarum* is responsible for excessive acidification in the later stages (Whang *et al.*, 1960; Kim and Chun, 1966). Han and Yang (1994) found that *Leuc. mesenteroides* subsp. *mesenteroides* and *Leuc. paramesenteroides* were dominant at lower temperature (5°-10° C) while at higher temperature (15°-25° C) *Lb. plantarum* was dominant. Addition of pepper and ginger stimulated the fermentation while onion, garlic and green onion inhibited the fermentation of kimchi (Han and Yang, 1994). Fermentation at lower temperatures (5 to 15° C) is recommended for obtaining acceptable kimchi (Mheen and Kwon, 1984; Lim *et al.*, 1989; Lee *et al.*, 1992). As the pH falls to 4.0, *Lb. plantarum* become predominant (Mheen and Kwon, 1984). The mixed strains are more effective than a single strain to produce better organoleptic quality (Lee and Kim, 1988). Optimum pH and acidity for the best taste of kimchi is 4.2 and 0.6 % (as lactic acid), respectively (Lee, 1994b). Reducing sugars decreases as fermentation progresses (Lee and Lee, 1965).

Kimchi blended at low temperature (15° C) with a 3 % salt concentration and fermented slowly at -1° C can be kept for more than 3 months at this same low temperature. Kimchi is packed in polyethylene



film bags and heat treated at low temperature (60° C for 20 min). Kimchi prepared this way can be stored at 4° C for more than 3 months with a constant acidity (near 0.57 %) and good eating quality (Lee and Yang, 1970). Growth inhibition of pathogenic bacteria in kimchi has been reported by Chung *et al.* (1967), Choi and Beuchat (1994).

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, 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). Kimchi is an important source of B group vitamins, carotene, and ascorbic acid. These are already present in the raw materials; however, some vitamins particularly those of the B group (Lee *et al.*, 1960) and ascorbic acid (Lee *et al.*, 1984), may be synthesized during the fermentation process. The total free amino acids increase from about 300-600 mg/100g in kimchi, thereby glutamic acid, alanine, valine, leucine, lysine and arginine being the most important ones and their concentrations being closely related to the contents of non-volatile organic acids, free amino acids and pH (Hawer *et al.*, 1988). The amount of nitrite and secondary amines in kimchi is very low compared to those in sausage and fish (Yim *et al.*, 1973).

Kimchi is known to improve digestion, prevent constipation, control intestinal flora, and have antimutagenic, anticarcinogenic, and other pharmaceutical functions (Park and Cheigh, 1992; Lee, 1997).

Mesu

Mesu, a non-salted fermented bamboo shoot product, has traditionally been consumed by the people in the bamboo-growing regions of the Darjeeling hill and Sikkim in India (Tamang *et al.*, 1988). Tamang and Sarkar (1996) reported *Pediococcus pentosaceus*, *Lb. brevis* and *Lb. plantarum* from samples of mesu collected from markets of Kalimpong, Darjeeling and Sikkim.

Mesu is commonly used as a pickle by mixing it with salt, mustard oil and green chillies. While fresh mesu has a shelf-life of only about a week, its pickle can be stored for a year or more (Tamang and Sarkar, 1996). Proximate composition of mesu was: moisture 94.1 %, pH 4.2, acidity 0.75 %, ash 10.9 % DM (dry matter basis), protein 17.1 % DM, fat 2.4 % DM (Tamang, 1992).

Naw-mai-dong

Naw-mai-dong is a traditional fermented bamboo shoot (*Bambusa arundinacea* Willd.) product of Thailand (Dhavises, 1972). The sweeter species of bamboo such as *Bambusa burmanica* Gamble and *Dendrocalamus asper* Back. are also used as raw materials (Boon-Long, 1986).

During preparation, young bamboo shoots are harvested, woody and defective portions are removed from the shoots by trimming. After washing, shoots are sliced and mixed with salt at 2 % level. The bamboo shoots are boiled in water and the bitter liquor is discarded. These are then packed into earthen jar, covered with plastic sheet and weight kept at

the top. Fermentation is complete in 3–4 weeks at room temperature. Functional microorganisms identified from naw-mai-dong are *Leuc. mesenteroides*, *Lb. fermentum*, *Lb. buchneri*, *Lb. brevis* and *P. pentosaceus* (Dhavises, 1972). Titratable acidity reaches to 1.0-1.2 % as lactic acid (Dhavises, 1972).

Olives

Olive fermentation is practiced in southern parts of Europe, the Middle East, North Africa and California in USA (Campbell-Platt, 1987). Olive pickling for table use, practiced as an art in the missions, on the ranches, and in the homes from many years, was not of much commercial importance until about 1900 (Vaughn, 1985). The response of three Sardinian olives cultivars to processing as table olives was evaluated and characterized from the marketing, technological and chemical-physical point of view and brined with 8 % NaCl (Piga *et al.*, 2001). Cruess (1958) reported around 1900 it was independently discovered by Professor Bioletti and Mrs. Freda Ehmann that ripe olives, after preliminary treatment with lye (sodium hydroxide solution) to destroy the bitter glucoside oleuropein, could be canned and preserved by heat in much the same way as other foods. Total world production has increased linearly in the last 35 years at an annual rate of 1.5-2 % reaching over 9,00,000 tonnes in 1994-95 (Garrido Fernandez *et al.*, 1997). The disposition of California olives for products in approximate order of importance include black-ripe and green-ripe canned olives (whole and pitted), Spanish-style, Sicilian-type, Greek-type including brined and salted-cured fruits and oil

(Vaughn, 1985). Fresh or direct cure black olives also are processed and canned throughout the harvest period of up to 2-5 months (Vaughn, 1985).

Because of lack of processing space, the remainder of the olives destined for processing has to be stored in salt brine prior to making them into canned ripe olives, which undergo lactic acid fermentation (Vaughn, 1985). Thus, there are four brine fermentations including 'storage', Sicilian-type, Spanish-type and Greek-type brined olives. The 'storage' and Sicilian-type fermentations may be considered to be identical, for the fruits are placed directly in brine with no prior lye treatment whereas the Spanish-type olives are lye treated to destroy most of the bitterness, washed to remove some of the alkali, and then brined in which they undergo lactic fermentation (Fernández-Diéz, 1983). Physiochemical characteristics, i.e. NaCl concentration, external temperature, etc. are responsible for changes in the microbial flora during fermentation (Randazzo *et al.*, 2004). The use of NaCl and the progressive pH decrease select lactic acid microflora that increase from 1 % of the total microbial population in the fresh brine to 80 % after few days (Robinson, 1988). Indigenous LAB change spontaneously during natural fermentation and at the end of the process *Lactobacillus* species are involved, mainly *Lb. plantarum* (Fernandez Gonzalez *et al.*, 1993; Harris, 1998). Using PCR/RFLP and by partial sequence analysis Randazzo *et al.*, (2004) found *Lb. casei* to be the most dominant species in naturally fermented Sicilian green olives. The brined Greek-type olives are placed in high salt brine, which, on equilibration, may be too strong for the lactic acid

bacteria to ferment the olives. Attempts have been made to use lactic starter culture in olives by Fleming and McFeeters (1981). *P. cerevisiae* and *Lb. plantarum* have been used in pure culture or controlled fermentation of olives (Etchells *et al.*, 1966).

Leuc. mesenteroides and streptococci were found in low salt fermentations of Sevillano variety (Vaughn *et al.*, 1943). While *Lb. plantarum* dominated the final stage of fermentation, *P. cerevisiae* was isolated from Spanish-type brines in the last part of the initial stage of fermentation and the first phases of the intermediate stage of fermentation, then it declined rapidly (Vaughn, 1985). Preparation of starter culture for fermentation of Spanish-type olive has been described by Vaughn *et al.* (1943). The total acidity of the final product is 0.7-1.0 % as lactic acid, and pH of 3.8-4.2 (Campbell-Platt, 1987). The most important control of protecting olives in fermentation, whether storage, Sicilian-type or Spanish-type brines, is the maintenance of anaerobic conditions (Vaughn, 1985). Control of the oxidative moulds and yeasts is mandatory to prevent destruction of desired acidity, but only recently has become recognized as a problem by the industry as a whole (Mrak *et al.*, 1956; Balatsouras and Vaughn, 1958; Vaughn *et al.*, 1969).

The coliform bacteria are responsible for most of the blister and gassy spoilage of fermenting olives (Foda and Vaughn, 1950). The species of *Bacillus polymyxa* and *B. macerans* (Gililand and Vaughn, 1943; Vaughn, 1954) and yeasts *Saccharomyces* and *Hansenula* (Vaughn *et al.*, 1972) also cause gassy spoilage in olives.

Olives contain 60-70 % moisture and per 100 g dry matter: 2-4 g protein, 25-45 g fat, trace amount of carbohydrate, 12-18 g fibre, 15-35 g ash, 15-20 g NaCl, 250-400 kcal energy, 5000-9000 mg Na, 300 mg K, 200 Ca, 80 mg Mg, 50 mg P, 3 mg Fe, 0.8 mg Cu, 100 mg S, 2000-7000 mg Cl, trace amount of thiamine, riboflavin, 0.5 mg niacin, 0.07 mg vitamin B₆, 0.07 mg pantothenic acid, 150 µg (black olives)-700 µg (green olives) carotene, vitamin C and D nil (Campbell-Platt, 1987).

In a study by Ruiz-Barba *et al.* (1994) on bacteriocin-producing *Lb. plantarum* LPCO10 and its non-bacteriocin-producing, bacteriocin-immune derivative, *Lb. plantarum* 55-1, were evaluated separately for growth and persistence in natural Spanish-style green olive fermentations. Their results indicated that *Lb. plantarum* LPCO10 may be useful as a starter culture to control the lactic acid fermentation of Spanish-style green olives (Ruiz-Barba *et al.*, 1994).

Antimicrobial substances that inhibited other strains of the genera *Lactobacillus*, and some foodborne pathogens including *Listeria monocytogenes*, *Staphylococcus viridans*, *Escherichia coli* and *Salmonella* was produced by *Lb. plantarum* Zn 42, *Lb. plantarum* Zn 50 and *Lb. plantarum* Zn 52 isolated from Portuguese olive brines (Cordeiro *et al.*, 2002), and also reported the antimicrobial activities of LAB isolated from fermented table olives (Rubia-Soria *et al.*, 2006).

Pak-gard-dong

Pak-gard-dong is the fermented vegetable product of Thailand prepared from leaf of mustard (Boon-Long, 1986). During its preparation,

leaves of black mustard are collected, defective leaves are removed, washed and wilted in the sun, 2.5 % salt are added into the wilted leaves and packed into a container and left for 12 hours. After removing salt water, 3 % sugar is added and fermented at room temperature. Fermentation is completed in 3-5 days (Boon-Long, 1986).

Mingmuang (1974) reported *Pediococcus pentosaceus*, *Lb. brevis*, and *Lb. plantarum* as the major microorganisms involved in the fermentation of pak-gard-dong.

Pak-sian-dong

Pak-sian-dong is a very common pickle leafy vegetable of Thailand (Dhavises, 1972). It is prepared from leaves of pak-sian (*Gynandropis pentaphylla* DC.). The fresh vegetable is thoroughly cleaned with water and then spread out in the air or under the sun to lose water until the sample is distinctly flacid. It is then mixed with water, salt, and sugar and kept in a tightly covered container. To reduce bitter flavour, the leaves are sometimes soaked in water and salt overnight. The liquor is thrown away and fresh water and sugar are added. Usually raw cane sugar or palm sugar is preferred to refined sugar because it enhances the flavour of the finished product. Pak-sian-dong is ready in 72 hours. At this stage the pH of the liquor is 3.9 and acidity between 0.7 to 0.8 %. Dominant functional microorganisms in pak-sian-dong are *Leuc. mesenteroides*, *Lb. fermentum*, *Lb. buchneri*, *Lb. plantarum*, *Lb. brevis* and *P. pentosaceus* (Dhavises, 1972).

Sauerkraut

Sauerkraut or sauerkohl is a German term for 'sour cabbage' (Pederson, 1979). It is the salted and fermented cabbage product produced and consumed with main meals in Germany, Switzerland, Central Europe, USA, Canada and former USSR (Vaughn, 1985; Campbell-Platt, 1987).

Sauerkraut is prepared from shredded white cabbage (Vaughn, 1985). Mature, sound heads of cabbage are trimmed to remove the outer green, broken, or dirty leaves, then the cores are cut by reversing cores that leaves the cut core in the head. The head of cabbage is then sliced into long, finely cut shreds of 3-5 mm x 5-7 cm size. The cut cabbage is then brined (containing 2.25-2.5 % salt) in the tank. Once the tank has been filled to proper level it is closed and the fermentation has started. At the time of filling to the desired level the shreds are covered with a sheet of plastic large enough to cover much more than the area in the top of the tank. The plastic sheeting is placed against the top of the slaw with the edges draped over the sides of the vat to form an open bag. Then water or preferably salt brine is placed in the bag so that the weight of the added solution forces the shredded cabbage down into the brine until the uppermost shreds are covered. This method of weighting the cover provides nearly anaerobic conditions once carbon dioxide begins to be formed by fermentation (Vaughn, 1985).

The fermentation of sauerkraut by a sequence of flora (Pederson, 1930a, 1930b) has been confirmed by Holtman (1939, 1941), Murray (1940), and Stamer *et al.* (1971). Pederson (1930a) found that the

fermentation was initiated by the heterofermentative species *Leuc. mesenteroides* and was followed by heterofermentative rods and finally by homofermentative rods and cocci. Additional studies by Pederson and Albury (1954, 1969) firmly established that the *Leuc. mesenteroides* initiated the lactic fermentation of sauerkraut. It is now accepted that the sauerkraut fermentation is initiated by *Leuc. mesenteroides*, a gas-forming species whose initiation of growth is more rapid than other lactic bacteria and is active over a wide range of temperatures and salt concentrations. The CO₂ replaces air and creates an anaerobic atmosphere conducive to prevention of oxidation of ascorbic acid and darkening the natural colour of the cut cabbage and also stimulates the growth of many lactic acid bacteria (Vaughn, 1985).

At 7.5° C, fermentation is very slow, only *Leuc. mesenteroides* grow slowly attaining an acidity of about 0.4 % in about 10 days, and an acidity of 0.8 % to 0.9 % acid in a month (Pederson and Albury, 1969). At 18° C with 2.25 % salt, a total acidity of 1.7 to 2.3 % will be attained in about 20 days. At higher temperatures, i.e. 23° C, the rate of fermentation will be greater so that a brine acidity of 1.0 to 1.5 %, may be attained in 8 to 10 days. *Lb. plantarum* and *Lb. brevis* grows actively and completes fermentation in approximately in one month. At 32° C, a similar acidity will be reached in about 8-10 days with most of the acid being lactic acid produced by the homofermentative bacteria *P. cerevisiae* and *Lb. plantarum* (Pederson and Albury, 1969).

Lb. brevis produces a red pigment under certain conditions which may result in discoloration 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).

The potential of sauerkraut production in India was studied by Mukherjee *et al.* (1996), and 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 volatiles in sauerkraut are acetyl, isoamyl alcohol, n-hexanol, ethyl lactate, cis-hex-3-ene-1-ol and allyl isothiocyanate (Lee *et al.*, 1976).

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, 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). Pederson *et al.* (1939) found that ascorbic acid value of kraut during active fermentation equaled to those of original fresh cabbage. Kraut showed a slow loss of vitamin C during vat storage after completion of fermentation (Pederson and Albury, 1969). Marked changes were observed in some of the minor lipid components of cabbage during sauerkraut fermentation (Vorbeck *et al.*, 1963). Dawson and Herrington (1967) found that purified phospholipase of cabbage slowly attach a lecithin suspension at pH 5.4 and some of the hydrolysis products were utilized by the bacterial cells during growth. The unsaponifiable fractions remain relatively stable during fermentation of sauerkraut (Hill and Mattick, 1966). The characteristic

flavour of cabbage and closely related vegetables is usually associated with the sulfur-containing constituents, particularly the glucosides (Steinkraus, 1996). Edmond and Lewis (1926) found that the sulfur content of different varieties varied from 0.075-0.341 %. Mild flavoured sauerkraut blends well with meats, fruits, other vegetables, spices and other foods without imparting a strongly distinctive flavour (Pederson and Albury, 1969).

The 16S rRNA sequence of an unknown *Leuconostoc* originally isolated from sauerkraut was investigated by reverse transcription revealed a new species, *Leuc. fallax*, which represents a new albeit peripheral line within the genus *Leuconostoc sensu stricto* (Schillinger *et al.*, 1989; Martinez-Murcia, 1991).

Breidt *et al.* (1995) proposed a paired starter culture system for sauerkraut fermentation consisting of a nisin-producing strain of *Lactococcus lactis* and a nisin-resistant strain of *Leuc. mesenteroides*. They concluded that nisin produced *in situ* or added to brined cabbage can direct the progression of the species in the resultant fermentations, by preventing the growth of naturally present LAB.

Yoon *et al.* (2006) determined the suitability of cabbage as a raw material for production of probiotic cabbage juice by lactic acid bacteria (*Lb. plantarum* c3, *Lb. casei* A4, and *Lb. delbrueckii* D7). They reported that fermented cabbage juice could serve as a healthy beverage for vegetarians and lactose-allergic consumers.

Sayur asin

Sayur asin is an indigenous fermented mustard cabbage leaves of Indonesia (Puspito and Fleet, 1985). Mustard cabbage leaves are wilted, rubbed or squeezed with 2.5–5 % salt. As in Hum choy preparation, the liquid from boiled rice is added to provide fermentable carbohydrate to assure sufficient acid is produced during the fermentation. The fermentation is initiated by *Leuc. mesenteroides* and *Lb. confusus* and later dominated by *Lb. plantarum* and *P. pentosaceus*. The pH falls from 6.5 to 4.2 in 8 days of fermentation (Puspito and Fleet, 1985).

Sinki

Sinki, a non-salted fermented radish tap root product, is traditionally consumed as a base for soup and as a pickle in some north-eastern states of India, in Nepal and a few places of Bhutan (Tamang *et al.*, 1988).

Tamang and Sarkar (1993) studied the microbiology of sinki samples collected from the Darjeeling hills and some parts of Sikkim and reported the presence of *Lb. fermentum*, *Lb. brevis*, and *Lb. plantarum*. Fresh sinki had 93 % moisture content while dried sinki had 21 % moisture. This reduced moisture level increases the shelf-life of the product, for which it can be stored for several months (Tamang and Sarkar, 1993). The proximate composition of sinki was determined by Tamang (1992): pH 4.4, acidity 0.8 %, protein 14.6 % (dry matter) DM, fat

2.5 % DM, ash 11.5 % DM, Ca 120.5 mg/100 g DM, K 443.1 mg/100 g DM, Fe 18 mg/100 g DM.

Soibum

Soibum is an indigenous fermented bamboo shoot product of Manipur (Pravabati and Singh, 1986). It is prepared from the succulent bamboo shoots of *Bambusa tulda*, *Dendrocalamus giganteus* and *Melocana bambusoides* (Bhatt *et al.*, 2005). Giri and Janmey (1987) had reported the presence of *Leuc. mesenteroides*, *Lb. brevis*, *Lb. coryniformis*, *Lb. delbrueckii* and *Streptococcus lactis* and yeasts *Candida*, *Saccharomyces* and *Torulopsis* in soibum. Microorganisms from 'soibum exudates' involved in microbial bioconversion of phytosterol were isolated and identified as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus coagulans* and *Micrococcus luteus* (Sarangthem and Singh, 2003). During fermentation of soibum, degradation of protein and gain of free amino acids was reported (Giri and Janmejy, 1994; 2000).

Suan cai

Suan cai is a traditional Chinese fermented vegetables (Miyamoto *et al.*, 2005). Taxonomical analysis of two genetically distinguished *Lactobacillus* strains isolated from suan cai, which formed L-lactate from glucose, were facultative heterofermentative, and had a DNA G+C content of 53-54 mol %. Based on DNA-DNA hybridization analysis, Miyamoto *et al.* (2005) proposed the new species be named *Lb. harbinensis* sp. Nov., isolated from suan cai of China.

Sunki

Sunki is a non-salted and fermented vegetable product prepared from the leaves of turnip in Japan (Battcock and Azam-Ali, 1998). Sunki is eaten with rice and miso (fermented soybean) soup. During its preparation, turnip is boiled, inoculated with 'zumi' (a wild small apple), dried sunki from the previous year and allowed to ferment for 1-2 months. Sunki is produced under low temperature (in winter season). Microorganisms involved include *Lb. plantarum*, *Lb. brevis*, *Lb. buchneri*, *E. faecalis*, *B. coagulans* and *P. pentosaceus* (Itabashi, 1986).