Review of Literature
Chapter - 2

Review of Literature

A large number of traditional fermented soy bean products have been consumed for thousands of years in Asian countries and have reached progressively the occidental markets. Fermentation is widely used in the food industry not only to improve the sensory characteristics of product, but also to eliminate certain undesirable constituents, make nutrients more accessible while preserving and even improving the nutritional properties (Nair, 2004).

Soybean (Glycine max) is economically the most important bean in the world, providing vegetable protein for millions of people and ingredients for hundreds of chemical products (Wynstra, 1986). Soybean provides a plentiful and inexpensive supply of protein and calories. Bressani and Elias (1968) suggested that the nutritive quality of soy bean protein is the best those available from plant sources. However, some soy products have limited consumer acceptability due to undesirable off flavours (Kanda et al., 1976 and Pinthong et al., 1980).

Messina et al. (1994) reported that high consumption of soybean and soybean related products has been suggested to contribute to a reduction in the risk of breast cancer in epidemiological studies. Scalabrini et al. (1998) and Shin et al. (2000) found that the soy beans contain various oligosaccharides including raffinose and stachyose are α-galactosides that may cause a gastrointestinal discomfort to the consumers. Raffinose and stachyose may cause flatulence.
This drawback along with the disagreeable bean flavours has often limited the consumption of soy bean as the raw material (Thananunkul et al., 1976).

Raffinose and stachyose are $\alpha$-galactosides of sucrose comprising three and monomer units respectively and are non-digestible in the gut due to the absence of $\alpha$-galactosidase in the human intestinal mucosa. Consequently, intact oligosaccharaides pass directly into the lower intestine where they are metabolized by bacteria that possess this enzyme, resulting in the production of gases (Tsangalis and Shah, 2004). Wang et al. (2002, 2003 and 2004) investigated that the purpose of fermentation is to remove the undesirable beanie taste.

Rickert, Meyer and Murphy (2004) found that depending on the pH and temperature conditions, a fermentation process may dramatically modify the content and the composition of these bioactive compounds. Haumann (1984) reported that the soymilk is a traditional oriental food beverage that is growing in popularity in the United States and the world. It commonly characterized as having a beany, grassy or soy flavour, which reportedly can be improved by lactic acid fermentation as in yoghurt like products (Mital et al., 1974 and Pinthong et al., 1980). Due to its extraordinary nutritive value and health characteristic, soymilk has become very interesting food. It is very rich source of highly valuable proteins, unsaturated fatty acids, soluble and insoluble dietary fibers, and isoflavones whose presence in every day diet is very important (Bozanic, 2006).
Many approaches have been used like heat treatment either before or during initial processing to destroy lipoxidase which causes the production of undesirable flavours during oxidation of lipids (Mital and Steinkraus, 1976). Wang et al. (1998) reported that complete removal of lipids from soy milk is also being tried as an alternative. Another possible approach is use of fermentation to modify and improve flavour. Fermentation is widely used in the food industry not only to improve the sensory characteristics of a product, but also to eliminate certain undesirable constituents, make nutrients more accessible while preserving and even improving the nutritional properties. The approach has been quite successful with fungal fermentation such as those used in the production of soy sauce, Miso and Indonesian Tempeh.

Fermented foods are associated with good bacteria referred to as probiotics (Patricia et al., 2002). Probiotics were beneficial bacteria in that they favourably after the intestinal microflora balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection (Reid, 1999 and Patricia et al., 2002).

People with flourishing intestinal colonies of beneficial bacteria are better equipped to fight the growth of disease causing bacteria (Reid et al., 2003 and Holzapfel and Schillinger, 2002). Holzapfel and Schillinger (2002) reported that the examples of probiotics that have found application in probiotic products include some strains of Lactobacillus genera (L. Plantarum, L.rhamnosus, L.acidophilus, L.reuteri, L.gasseri and L.amylovorus; Bifidobacterium genera (B.adolescentis, B.longum and B.bifidum); and Enterococcus (E.faecalis and E.faecium)
Bai et al. (1999) reported that the soy protein is the best among plant protein and is inferior to animal protein in being sufficient in sulfur containing amino acids. The protein content of fermented foods is found to be higher than that of milk and found to be easily digestible than milk due to partial breakdown of protein such as casein by the fermenting bacteria. The bacterial enzymatic hydrolysis may enhance the bioavailability of protein and fat (Fernandes et al., 1987) and increase the production of free amino acids, short chain fatty acids, lactic acid, propionic acid and butyric acid are also produced by lactic acid bacteria (Rombeau et al., 1990 and Rolfe, 2000).

Rajalakshmi and Vanaja (1967) and Alm (1982) found that the fermentation of food with lactic acid bacteria increases folic acid in the fermented milk products yoghurts, bifidus milk and kefir. Similarly niacin and riboflavin levels in yoghurt are increased with fermentation (Deeth and Tamime, 1981). During the preparation of cultured foods or in the digestive tract the organism improve the digestability of some dietary nutrients (Alm, 1982).

De Simone (1986) and O’sullivan et al. (1992) stated that the yoghurt has shown to have a strong inhibitory effect on the growth of the coli form bacteria in the stomach and duodenum of human infants. However, the usual starter organism in yoghurt, Lactobacillus delbruekii sub species bulgaricus and streptococcus thermophilus are not bile tolerant and do not colonize the gut. They persist and shed in stool only as they are ingested. So recently bacteria such as Lactobacillus acidophilus and Bifidobacterium longum which can colonize the gut and these organisms have been included in yoghurt and fermented milk.
Soy milk based yoghurt namely soy yoghurt or sogurt, is produced by the fermentation of soymilk using lactic acid bacteria. Because of its beany flavour, insufficient acidity, hard and coarse texture (Granata and Morr, 1996). In addition to this beneficial effect of lactic acid fermentation of soymilk reported that soy yoghurt would offer several distinct nutritional advantages over milk yoghurt to the consumers (Lee et al., 1990).

Bifidobacteria are predominating members of the endogenous intestinal flora in human, these organisms are reported to exert beneficial effect including the activation of the immune system, reduction of serum cholesterol and inhibition of the growth of potential pathogens that may cause infections in the host (Holzapfel et al., 2001 and Ishibashi and Yamazaki, 2001).

Hori (1983) found that the bifidobacteria accounts for 92% of the intestinal flora in breast fed infants and 20% in bottle fed or weaned infants. The presence of high numbers of bifidobacteria in the infants and adult colon seems to be desirable and can be influenced by dietary supplement with bifidobacteria (Hughes and Hoover, 1991 and Rasic, 1983).

It also indicated that lactobacillus ferment functional material which having anti obesity and antimicrobial effects (Herreros et al., 2005 and Gonzalez et al., 2007). However, probiotic bacteria must be resistant to the acidity, bile, pancreatic enzymes. High acidity in the stomach and high concentration of bile components in the proximal intestine are the first host factors, which affect strain selection and adhesion.
Hydrochloric acid resistance may play a more important role during passage of the bifidobacteria through the stomach (Conway et al., 1987). Gurr (1987) and Gilliland (1990) found that the incorporation of the intestinal species *Lactobacillus acidophilus* and *Bifidobacterium* species into fermented milk products. The probiotics are generally related to inhibition of pathogenic species, reducing the risk of colon cancer, increasing the immune response and decreasing concentration of cholesterol in blood plasma. Ventling and Mistry (1993) stated that the use of MRS broth and ultra filtered milk to improve growth conditions for the different strains of Bifidobacteria, particularly for the manufacture of products that contain Bifidobacteria.

Bifidobacteria are generally characterized as gram positive, non-spore forming, non-motile, catalase negative anaerobes (Sgorbati et al., 1995). Soymilk is a good medium for growing *Bifidobacterium* because it contains oligosaccharides that are fermented by most of the strains belonging to this genus (Liu, 1997 and Scalbrini et al., 1998). They were first isolated and described in 1900 by Tisser who described them as rod shaped, non gas producing, anaerobic microorganism with Bifid morphology, isolated from the feces of breast fed infants, which he termed *Bacillus bifidus* (Gomes and Malcata, 1999).

Soymilk contains various oligosaccharides including raffinose and stachyose that may cause a gastrointestinal discomfort to consumers (Scalabrini et al., 1998 and Shin et al., 2000). It was observed that soymilk could support the growth of *Lactobacillus acidophilus* or *Streptococcus thermophilus* with *Bifidobacterium infantis* or *Bifidobacterium longum* (Wang et al., 2002).
Clark and Martain (1994) and Clark et al. (1993) reported that the Bifidobacteria may be selected that tolerate low pH of the stomach and survive the effects of bile produced by the small intestine of the humans. Bifidus milk is poorly acidified due to the slow growth of bifidobacteria in milk. Thus, prolonged incubation at a temperature favourable for bacteria growth (37°C) together with slow acidification rate must be increase the risk of proliferation of fast growing undesirable microorganism (Misra and Kulia, 1992 and Fonden and Holgersson, 1985).

Tempeh is a fermented product made from soybeans that have been soaked and cooked and to soften those (Astuti et al., 2000) like sour dough bread, tempeh requires a starter substance which is added to the substance, which is added to the cooked beans. Frias et al. (2008) stated that the Tempeh is a protein store house, and it contains all the essential amino acid, and protein has the same quality as that meat or poultry. Hence it is an excellent replacement or substitute for animal protein. Next to protein, tempeh is an excellent source of essential fatty acids and B vitamins (Irene. T.H. Liem et al., 1977).

Tempeh is absolutely cholesterol free and high in soluble dietary fibre. It is high in isoflavones, and is a good source of folic acid and low in sodium concentration. An important feature about tempeh is that, it is easy to digest because of the fermentation carried out by the mold Rhizopus oligosporous used (Alan M. Jurus and Walter J. Sunderberg, 1976).

Varzakas (1986) observed that the fermentation process breaks down the complex protein found in soy beans and make tempeh easily digestable than
other non fermented soy products, or whole soy beans. So it is an excellent source of protein for people with gastrointestinal upsets. Tempeh production involved the use of fungal starter culture strains of *Rhizopus species*. The most acceptable include *R. oligosporous, R.oryzae, Rhizopus stolonifer* etc (Wiesel et al., 1997), but the most acceptable of them all is *Rhizopus oligosporous*. Strains of both *R. oligosporous* and *R. oryzae* have high proteolytic capacities while *Rhizopus stolonifer* are less active (Baumann et al., 1991). Heseltine et al. (1963) isolated many fungi from different lots of tempeh made in Indonesia and found that only *Rhizopus* could make tempeh in pure culture fermentation.

In humans in case of recurrent *Clostridium difficile* colitis, a successful treatment was obtained by using *Lactobacillus rhamnosus* (Gorbach et al., 1987). Another antimicrobial substances resembling microcin was isolated from *Lactobacillus* species strain GG from faeces. (Biller et al., 1995).

*Lactobacillus* acidophilus effectiveness in diarrhoea caused by salmonella and shigella among children (Gorbach et al., 1990). A recent in vivo study used milk fermented with *Lactobacillus casei* and *Lactobacillus acidophilus* as immunological agents in the prevention of gastrointestinal infection due to the *Salmonella typhimurium* in mice which showed augmentation of resistance to *Salmonella* (Perdigon and Alvarez, 1992).

Antimicrobial activity of bifidobacteria was first noticed by Tisser (1899), who described various types of antagonistic effects of *B.bifidum* against *E.coli*. Iya Iiyich Mechnnikov (1908) theorized that heavy consumption of yoghurt was responsible for the usually long life spans of Bulgarian peasants. *Lactobacillus*
acidophilus strain was found to adhere to cultured human polarized intestinal cells. This strain against Helicobacter pylori in in vitro and in vivo (Cocconnier et al., 1998). Silvia et al. (1993) observed that the Lactobacillus acidophilus can be considered as a potential bio therapeutic agent in view of inhibition of entero pathogens by L.acidophilus.

A possible relationship between disease resistance and tempeh consumers due to antibiotic production (Wang et al., 1969). Probiotic bacteria can control various enteric pathogens such as Salmonella typhimurium, Shigella dysentriae, Clostridium difficile, Campylobacter jejuni and Escherichia coli. They may also provide important protection against urogenital pathogens such as Gardnerella vaginalis, Bacteroides bivius and Candida albicans (Suskovic et al., 2010).

Soy milk has desirable characteristics as an ingredient for making soy yoghurt because of its high solid content and viscosity. Bifidobacterium as a culture and in combination with the lactic acid bacteria utilized flatulent sugars, both raffinose and stachyose completely making soy yoghurt and frozen soy yoghurt factor free. This is the first report the complete elimination of these sugars from soy product by lactic acid fermentation (Tuitemwong et al., 1993).

Bezkorainy (2001) found that the bifidobacteria produce both acetic acids as primary metabolites in the molar ratio of 3:2. Glucose is degraded characteristically by the fructose-6-phosphate shunt metabolic pathway. Milk and yoghurt have similar mineral compositions, calcium are more bio available from yoghurt than from milk. Yoghurt also has been lactose and more lactic
acid galactose, peptides free aminoacids and free fatty acid then milks (Rasic et al., 1978).

There are currently about 30 known Bifidobacterium species that inhibit the human intestinal tract are rather distinct from those that the intestines of animals (Mitsuoka, 1984). Among the probiotic effects attributed to lactic acid bacteria, the assimilation of cholesterol is of particular interest for reducing the absorption of dietary cholesterol into the blood (Gilland et al., 1985).

The ability of lactic acid bacteria (LAB) to ferment the available carbohydrates in a growing medium varies with strains. Matsuoka et al. (1968) found that S. thermophilus produced a greater amount of acid in soymilk than Lactococcus lactis and L. delbrueckii ssp. bulgaricus.

Mital et al. (1974) also reported that certain organisms such as S. thermophilus, L. acidophilus, L. cellobiosis and L. plantarum which utilize sucrose, exhibited significant growth and produced substantial amounts of acid in soymilk.

Components of soybean such as isofavones, trypsin inhibitor, saponin and phytic acid have been found to exhibit antimitagenic and anti tumourial activities (Weed et al., 1985 and Rao and Sung, 1995). As fermented soymilk is rich in phenolic aglycones, which more active and more readily taken up than their glycosides, increasing the free phenolic content of soy based food through microbial bio processing may positively affects its medicinal and nutritional value (Izumi.T. Piskula et al., 2000).
Bifidobacterial strains exhibiting antagonistic activity towards certain types of psychrotrophic microorganism such as *Bacillus cereus*, *Pseudomonas*, *Yersinia* and *Listeria* are especially important as these microorganisms even at low levels in food pose a significant spoilage and public health threat (O’Riordan and Fitzgerald, 1998). Bifodobacteria are considered beneficial microorganisms and thought to create conditions unfavourable to the growth of pathogens such as *Salmonella* (Isolauri et al., 2001).

*Rhizopus oligosporous* has been reported to produce 4 to 5 antibacterial compounds during soy bean tempeh fermentation (Nowak & Steinkraus, 1988). It can inhibit the growth of *Aspergillus flavus* and *Aspergillus parasiticus* (Nout, 1989). Kobayashi et al. (1992) reported that the antibacterial protein has been purified from *Rhizopus oligosporous*, with activities against *Bacillus* species.

There is an increasing interest in the research of antimicrobial peptides produced by lactic acid bacteria because of their potentials use as antimicrobial agents for improving the safety of food products (Yildirim and Johnson, 1997). Mixed culture of yoghurt is known to coagulate more quickly than single culture. In this case, both types of bacteria grew faster, strengthening their inhibition properties against the test bacteria (Helander et al., 1992). Soymilk yogurt is made by fermenting pasteurized soymilk with *Lactobacillus bulgaricus* or other bacteria in a way similar to that used to make cow milk yogurt. Soy yogurt has isoflavonoid content higher than that of soy milk, of about 80 mg/100 g, with glucosides as the main form of isoflavones (Wang and Murphy, 1994).
Probiotic bifidobacteria are also useful in the management of gastrointestinal infections (Salminen et al., 1999). The lactic acid in yoghurt decreases pH, so inhibiting diarrhea causing pathogenic bacteria, which generally cannot survive at low pH. *L.bulgaricus* and *S.thermophilus* inhibit *S.aureus* and *S.typhimurium* with inhibition zones of 3 mm and 1.5 mm respectively (Jenie and Ekarini, 1995).

Beneficial effects conferred by probiotic microorganisms include inhibition of pathogenic bacteria such as *Salmonella*, *Shigella*, *Pseudomonas* and *Helicobacter* (Servin, 2004 and Sgouras et al., 2004). *Lactobacillus acidophilus* exhibit antagonistic activity against a variety of pathogens. In vitro studies have demonstrated inhibition of various bacteria such as *Helicobacter pylori* (Khedkar et al., 1993), *Yersinia pseudotuberculosis* (Bernet et al., 1994 and Cocconnier et al., 1993), *Salmonella typhimurium* (Bernet et al., 1994 and Cocconnier et al., 1993) and *Shigella sonnei*. Apella et al. (1992) noted that a mixture of *L. acidophilus* and *L. casei* produced an enhanced inhibitory capacity greater than single strains.

Tempeh also contains antibacterial substance that can protect against infectious disease such as diarrhoea (Nowak and Steinkraus, 1988). The fungus also produces phenolic compounds that inhibit the growth of pathogenic bacteria such as *Helicobacter pylori* (Correria, et al., 2004 b). Kobayashi et al. (1992) purified that the antibacterial protein from *R.oligosporous*, with activities against *Bacillus sp.* (especially against *Bacillus subtilis*), *Staphylococcus aureus* and *Streptococcus cremoris*. 
Microbiological studies during tempeh production have also implicated the presence of Gram positive bacteria. Non adherence to strict aseptic techniques in the traditional method of tempeh production could be responsible for the presence of coagulase positive *Staphylococcus* species (Aderibigbe and Oseboun, 2006).

Hussain *et al.* (2003) identified that the anti-diabetic and anti-hypertensive potential of aqueous extracts of soybean enriched for phenolic content by sprouting or bio processing by dietary fungus. Soybean is rich source of isoflavonoid phenolics especially genistein and diazidein which have been shown to have numerous potential health benefits.

Only few reports exist on specific antifungal and anti yeast compounds produced by LAB. Fungi are frequent cause of spoilage in foods; besides, they represent a considerable danger for human health by producers of mycotoxins. Most strains from *Candida* family are particularly dangerous in this respect *Penicillium commune* commonly spoils hard cheese while different *Fusarium* species often produce mycotoxins in cereal grains. Yeasts *Candida parapsilosis* and *Debaryomyces lansenii* spoil yoghurt and fermented dairy products (Harris *et al.*, 1989).

Animal studies have further shown prevention of infection produced by *Escherichia coli* and *Listeria monosytogens* (Nader de Macias *et al.*, 1993), *Shigella sonnei*, when micro fed milk fermented with *L.acidophilus* and *L.casei*. 
Antioxidants are believed to play a very important role in the body defense system against Reactive Oxygen Species (ROS), which are harmful by products generated during normal cell aerobic respiration (Feria et al., 2005 and Lin and Tang, 2007). The radicals may cause oxidative damage by oxidizing bio molecules and results in cell death and tissue damage (Kehrer, 1993). Atherosclerosis, cancer, emphysema, cirrhosis and arthritis have been correlated with oxidative damage (Kehrer, 1993 and Jacob, 1994). Therefore, oxidative damage plays a significant pathological role in human diseases.

Natural antioxidants in plants are related to three major groups; carotenoids, vitamins and phenolics (Helliwell, 1996 and Nijveldt et al., 2001). Phenolic compounds are plant derived antioxidants that possess metal-chelating capabilities and radical scavenging properties (Bocco et al., 1998 and Duan et al., 2006).

Bagchi et al. (2000) stated that intake of food derived antioxidants might reduce oxidative damages of the body. Epidemiological studies had showed that consumption of soy bean derived products could reduce cancer occurrence, osteoporosis and cardio vascular diseases in humans (Friedman and Brandon, 2001 and Han et al., 2004). Fermenting soy milk with lactic acid bacteria considerably increases its health value. Because of greater antioxidative actions (Wang et al., 2006).

Some antioxidant components such as isoflavones and peptides had been identified in soy bean foods (Liu et al., 2005 and Wang et al., 2008) and
increasing interest is paid for antioxidant peptides (Baydar et al., 2007 and Rajapakse et al., 2005).

Soy beans contain phenolic compounds that exhibit antioxidative activity (Murakami et al., 1984 and Drumm et al., 1990). However, ingestion of antioxidative supplements, or foods containing antioxidants, may reduce the oxidative damage on the human body (Lin and Yen, 1999).

Several traditional fermented soybean foods including miso, natto, tempeh, sufu and douche had been proven to have antioxidant activity (Chen et al., 2007 and Gibbs et al., 2004 and Zhu et al., 2008).

Masakomatsu et al. (1997) recorded that the new potent antioxidant was isolated from tempeh and was identified as 3-hydroxyanthranilic acid (HAA). HAA eliminated free radicals and inhibited the formation of fatty acid hydro peroxide in vitro, suggesting that HAA would serve as an antioxidant in the initial reaction in lipid oxidation systems. As long as HAA is taken as a component of tempeh, not in large doses as a chemical it may possibly act as a pro oxidant rather than an antioxidant invivo.

Esaki et al. (1996) identified that the Tempeh contains potent antioxidants due to the conversion of isoflavanoid compounds present in soy beans in the 6, 7, 4-tri hydroxyl isoflavone known as factor and more active antioxidant enzyme superoxide dismutase.
Wang *et al.* (2006) reported that fermentation with lactic acid bacteria and bifidobacteria, individually and in combination increased the antioxidative activity increased as the fermentation started. Neelam Khetarpaul (2005) identified that the antioxidants from tempeh which retard the development of rancidity but will keep for only one or two days.

The beneficiary role of yoghurt may be further enhanced by the supplementation of *Lactobacillus* and *Bifidobacterium* sp., resulting in a product termed AB yoghurt (Shah, 2000). *L. acidophilus*, *Bifidobacterium* sp. and *L. casei* are considered probiotic organisms since they are believed to exert beneficial health effects in the host by modulating the intestinal microflora (Schrezenmeir and De Vrese, 2001).

Besides their desired health properties, probiotics should meet several basic requirements for the development of marketable probiotic products including their survival and activity in the product, and stability during storage of the product. In addition, probiotics should not adversely affect the taste or aroma of the neither product nor acidification during the shelf life of the product (Heller, 2001).