CHAPTER 2

LITERATURE REVIEW

The application of functional foods have been widely accepted and increasingly used over the world. The term ‘functional food’ also is known as designer foods, pharmafoods or nutraceuticals. Functional foods are part of foods or modified or formulated foods that claim to have specific physiological or nutritional effects to promote health above the basic nutrition (Schneeman, 2000). The principally vital roles of functional food are to reduce the risk of diseases and to enhance specific physiological functions. The principles of functional foods are in agreement with Hippocrates’s highlight since 2000 years ago as ‘‘Let food be your medicine and medicine be your food”(Dijsselbloem et al., 2004). A large portion of functional foods from natural sources are derived from microbes and plants. Live microorganisms with beneficial effects on health (also defined as probiotics) and beneficial ingredients from natural sources are used to approach the strategic development of functional foods (Stanton et al., 2005).

2.1 Probiotic lactic acid bacteria

Probiotics are commonly defined as the viable microorganisms and microbial food supplements that exhibit the beneficial effects on the consumer health by improving intestinal microbial balance (Salminen et al., 1998; Saarela et al., 2000).

Probiotics have been considered to have potential health-promoting benefits as biotherapeutic agents (Begley et al., 2006). Thai Food and Drug Administration or Thai FDA (2008) described the probiotics definition as live microorganisms that when administered in adequate amounts effect on the host beneficial health. Although, live microorganisms were generally recommended that health-promoting active as probiotics, non-viable bacteria were included in the probiotic definition (Ouwehand and Salminen, 1999). Initially, several microbes have been used unintentionally in food production such as dairy products and fermented vegetables. In recent years, there has been a renewed interest in microbial uses due to, apart from improving food flavour, their beneficial aspect in health restoration and disease treatment. Several microorganisms, under the name of "probiotics", have been proposed and used in a wide range of clinical trials, ranging from diarrheal disease to cancer prevention. The criteria used to select among potential probiotics are related to the acid and bile tolerance, production of antimicrobial substances, cholesterol metabolism, production of useful enzymes, and safety for food and clinical use (Ouwehand et al., 1999). Lactic acid bacteria (LAB) have a long and safe history of application and consumption (Holzapfel et al., 1995; Caplice and Fitzgerald, 1999). The LAB group has the most claims to be selected among potential probiotics. LAB also play an important role in the food fermentation without any problems. They predominate as the natural microbiota and were applied as traditionally starter culture for the fermented food in order to use as protective cultures against microbial pathogens and spoilage microorganisms. They are not only to provide, or maintain, or to improve the safety but to qualify acceptability worldwide of fermented products. They produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, vitamins, or useful enzymes (Leroy and Vuyst, 2004). LAB have also been shown to have functional probiotic properties to promote host health and relief disease symptoms.

The LAB have been approved by FDA to Generally Recognized as Safe (GRAS) status (Stiles and Holzapfel, 1998; Mogensen et al., 2002). They belong to the group of Gram-positive bacteria, catalase-negative, facultative or anaerobic cocci or rod, and non-spore forming that united by a constellation of morphology, metabolic profile, and phylogical characteristics. They are generally mesophilic growth but can grow at temperatures as low as 5°C or as high as 45°C. Similarity, while the majority of strains grow at pH 4.0-4.5, some are active at pH 9.6 and others at pH 3.2. All LAB produce lactic acid from hexose and since they lack functional heme linked electron transport chains and a functional Krebs' cycle, they obtain energy via substrate level phosphorylation. The produced lactic acid may be L(+) or, less frequently, D(-) or a mixture of both. It should be noted that D(-) lactic acid is not metabolized by humans and is not recommended for infants and young children (Caplice and Fitzgerald, 1999).

The boundaries of the LAB group have been subjected to some controversy, but there has been general agreement that the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* from the core of the group (Axelsson, 1998). The members of genera *Bifidobacterium* and *Lactobacillus* have been the most associated with several probiotic effects on humans and animals (Park et al., 2007). The representative LAB species were declared as probiotics by FDA and Thai FDA include in Table 2.1. These lactobacilli and other LAB have provided and used by the food industry have a long history without any harmful effects from both cells and their metabolites.

Table 2.1 Lists of probiotic lactic acid bacteria with documented history of use in human food (modified from Mogensen et al., 2002)

Used since	Group/Genera/Species	Used in/ Major application(s)
	***Bifidobacterium* sp.**	
1970	*Bifidobacterium bifidum*	Probiotic ingredient in fermented milk
1980	*Bifidobacterium animalis*	Probiotic properties. Fermented milk
1980	*Bifidobacterium breve*	Probiotics in fermented milks and infant formula
1980	*Bifidobacterium infantis*	Probiotics in fermented milks and infant formula
1980	*Bifidobacterium lactis* (*B. animalis*)	Fermented milks with probiotic properties. Common in European fermented milks
1980	*Bifidobacterium longum*	Fermented milks with probiotic properties
1991	*Bifidobacterium adolescentis*	Probiotic properties. Fermented milk

Table 2.1 (Continued)

Used since	**Group/Genera/Species**	**Used in/ Major application(s)**
	***Lactobacillus* sp.**	
1950	*Lactobacillus acidophilus*	Probiotics and fermented milk
1962	*Lactobacillus johnsonii*	Probiotics and biopreservation
1970	*Lactobacillus paracasei*	Probiotics and probiotic cheese
1980	*Lactobacillus gasseri*	Probiotics and fermented milk
	Lactobacillus reuteri	Probiotics
	Lactobacillus rhamnosus	Probiotic culture and starter
1988	*Lactobacillus crispatus*	Probiotics
1996	*Lactobacillus salivarius*	Probiotics and cheese fermentation
<1996	*Lactobacillus zeae* (=*L. casei* subsp. *casei*/*L. rhamnosus*)	Probiotics and cheese production
	***Enterococcus* sp.**	
1980	*Enterococcus faecium*	Probiotics, fermented milk and cheese fermentation
1982	*Enterococcus durans*	Human probiotics. Cheese and sour dough fermentation

2.2 Functional aspects of probiotic LAB

The domain factors of probiotic LAB survival and growth are important to protect and promote the host health. The criteria used to claim among potential probiotics are related to acid and bile tolerance, production of antimicrobial substances, cholesterol metabolism, production of useful enzymes, and safety for food and clinical use (Ouwehand et al., 1999). Probiotics have been defined in many different ways, depending on our understanding of the mechanisms of their effects on the health and well being of humans. Some criteria used to select the probiotics were varied on the objectives of applications.

2.2.1 Survival in low pH

The pH environment of host stomach varies from 2 to 8 due to the consumed food. Probiotic LAB taken orally must first survive to transit through the stomach acid and digested enzyme before they can pass through the intestinal tract (Corzo and Gilliland, 1999). Therefore, the probiotic strains which could tolerate the acidic conditions indicated the existence in the low pH of stomach. The survival in the low pH environment of probiotic LAB is also provides the ability to face acid stress in fermented food products. This may increase shelf life in fermented foods.

2.2.2 Survival in bile acids

The most abundant human bile acids is cholic acid (3α, 7α -12α-trihydroxy-5-β-cholic acid). The bile acids are synthesized in the liver form cholesterol and are conjugated by amide linkage to glycine or taurine to be glycocholic acid, taurocholic acid, glycodeoxycholic acid and taurodeoxycholic (Zarras and Vogl, 1999). These conjugated form are secreted from the gall bladder into the duodenum in the range between 500 and 700 mL/d (Dunne et al., 2001). Bile acids both conjugated and deconjugated form have been shown to have inhibitory effect against intestinal aerobic and anaerobic bacteria such as *Escherichia coli*, *Klebsiella* sp., *Enterococcus* sp., *Clostridium* sp. and lactobacilli group (Dunne et al., 2001). The deconjugated bile acids are more great inhibition than conjugated bile acids. The probiotic LAB taken orally have to reach the stress condition of the upper intestinal and colonic host with contain high concentration of bile (Corzo and Gilliland, 1999). Thus, probiotic bacteria necessary be able to tolerate the human bile condition. In *vitro* bile tolerance assessment of probiotic LAB is extensively measured the resistant to oxgall, a product derived from bovine bile, with concentration ranged 0.15-0.30% (w/v) (Morelli, 2000; Sirilun et al., 2010).

2.2.3 Amylolytic, proteolytic and lipolytic activity

The probiotic strains demonstrate the amylolytic or amylase activity, proteolytic and lipolytic activities that provided to utilize starch, protein and lipid from food intake, respectively. The utilized activity may demonstrate the host's digestive system and nutrient degradation during food processing. Moreover, probiotics may compete utilize nutrients against consumed by pathogenic microorganisms (Essid et al., 2009).

2.2.4 Production of inhibitory substances

The inhibitory substances from probiotic LAB were produced as several metabolites against microbial pathogens or food spoilage microorganisms. De Waard et al. (2002) demonstrated that the antimicrobial activities of *Lactobacillus* strains were broad inhibitory spectrum, against yeast and bacteria both of gram-negative and gram-positive. The activity might be owing to their activities related to the amount of bacteriocins from lactic acid bacteria that are active against a number of microorganisms at the optimum pH (De Waard et al., 2002). In general, the antimicrobial activity of lactobacilli may be due to organic acids, short chain fatty acids (Kuwaki et al., 2002), hydrogen peroxide (Caplice and Fitzgerald, 1999), bacteriocins (Testa et al., 2003) or other inhibitory substances from LAB metabolites (Caplice and Fitzgerald, 1999). The inhibitory agents produced by probiotic LAB varied among strains.

2.2.5 Effect of antibiotic used on probiotic LAB

The oral intake of the antibiotics for prevention and treatment of microbial infection may affect the intestinal microbiota such as causing an excessive infections or overgrowth of microorganisms with antibiotic resistance. Over growth of the undesirable microorganisms may cause diarrhea, stomatitis, intestinal inflammation or systemic infection. The problems from super infection of resistance intestinal pathogens were abundantly observed among immunocompromised patients. The antibiotic susceptibility is the one criteria of safety evaluations in probiotic claimed. This property concerned that potential probiotics, which used living organisms, does not deliver a host of antibiotic resistant genes with the risk of transferring the genes in many probiotic bacteria and other pathogenic bacteria (Salminen et al 1998; Klein et al., 2000; Zhou et al., 2005). However, some probiotic LAB strains have been demonstrated to resist some antibiotics, may provide the balancing of normal intestinal microflora in antibiotic-associated diarrheal patients (Salminen et al., 1998). Bansal and Garg (2008) reported that associated probiotics in intestinal disorder therapy can reverse the effects produced by overgrowth of opportunistic pathogens like *Clostridium difficile* or *Klebsiella oxytoca* by reducing the changes in bowel habits, stool consistency and decreasing diarrhoea duration. Probiotic LAB association during or after antibiotics therapy may support the stability of microflora and maintain the colonization resistance against gastro-intestinal pathogens.

2.2.6 Adhesion properties

The gastrointestinal tract, especially the small intestine, is the flux of digesta washes out through the tract. Any bacterium was unable to withstand the flow unless by attaching itself to the intestinal surface (Morelli, 2000). Consequently, bacterial adhesion is considered important as one of the selection criteria for probiotic strains. The adhesion is considered an important property of probiotic strains, comparable with viability and metabolic activity. Adhesion ability of probiotics is the first step of colonization. The adherence property of probiotics can prolong beneficial health of the host and against invasion of the host by enteropathogenic bacteria via the tolerance peristaltic movement of intestine, washing off of lumen content (Saarela et al., 2000). The adhesion of LAB strains on the surface of host cell may be due to several ways such as 1) strains bound specific interactions mediated by adhesion molecule on surface like a lock-and-key interaction (Beachey, 1981; Busscher and Weerkamp, 1987), and 2) carbohydrates on the bacterial cell wall appeared to be partly responsible for the interaction between the bacteria and the extracellular adhesion-promoting factor (Coconnier et al., 1992). Moreover, Schillinger et al. (2005) reported that the hydrophobic potential of strains differed considerably. Hydrophobicity plays a key role in the first contact between a bacterial cell and mucus or epithelial cells. Adhesion of lactobacilli depends on the strain, environment and physical nature of involved surface. Chauvière et al. (1992) demonstrated that *L. acidophilus* can inhibit diarrheagenic *E. coli* adherence to human colorectal carcinoma (Caco-2) cells in a concentration dependent manner.

2.2.7 Cholesterol-lowering property

Hypercholesterolemia is considered as a major risk factor for the development of coronary heart disease (Pereira et al., 2003). Although therapeutic drugs are available to relieve this problem, they are often expensive and can have side effects. One of the health-promoting benefits of probiotics is their ability to reduce blood cholesterol (Gilliland, 1985; Taranto et al., 2003; Lim et al., 2004; Liong and

Shah, 2005a; Begley et al., 2006). Several studies have suggested that humans and animals origin isolated *Lactobacillus* spp. or their containing foods influence cholesterol levels in laboratory media or living organisms (Taranto et al., 2003). Several studies indicated that *Lactobacillus* species were able to reduce cholesterol via several mechanisms (Gilliland et al., 1985; Brashears et al., 1998; Liong and Shah, 2005a; Liong and Shah, 2005b). The cholesterol-lowering effect of lactobacilli from broth culture media and blood serum is by several means. Firstly, cholesterol reduction related to bile salt hydrolase (BSH) activity due to deconjugate bile acids by BSH lead to decrease cholesterol absorption in the intestine. Secondly, cholesterol is reduced by the lactobacilli assimilation or incorporation into the cellular membrane during cells growth (De Smet et al., 1995; Corzo and Gilliland, 1999; Lim et al., 2004; Liong and Shah, 2005a; Begley et al., 2006; Sirilun et al., 2010). Thirdly, some substances of lactobacilli produced during growth can be absorbed to inhibit cholesterol synthesis in the body (Corzo and Gilliland, 1999). Fourthly, lactobacilli such as *L. acidophilus* can remove cholesterol from media is due to the disruption of the cholesterol micelles caused by bile salt deconjugation and precipitation of cholesterol with the free bile salts as the media pH dropped from acid production during growth (Brashears et al., 1998).

Gilliland et al. (1985) have suggested that *L. acidophilus* RP32 from fecal of pigs can grow well in the presence of bile and assimilate cholesterol from a laboratory growth medium. Thus, this species has the potential to inhibit increased serum cholesterol of pigs. Human intestinal isolated *L. casei* can remove cholesterol from media by means of the destabilisation and co-precipitation of cholesterol micelles (Walker and Gilliland, 1993; Brashears et al., 1998). *L. acidophilus* ATCC 43121 can incorporate cholesterol and can remove cholesterol from media into the cellular membrane during growth (Noh et al., 1997).

2.3 Proposed mechanisms of action and health effects of probiotics

Recently, focus on probiotic foods has attended to have the following health propositions: (1) attach to host epithelial tissue (2) acid and bile tolerance (3) balancing gut health (4) improving body's natural defenses, and (5) lowering blood cholesterol (Kaur et al., 2002; Ouwehand et al., 2002). The proposed mechanisms of the functional probiotics are concluded in Figure 2.1.

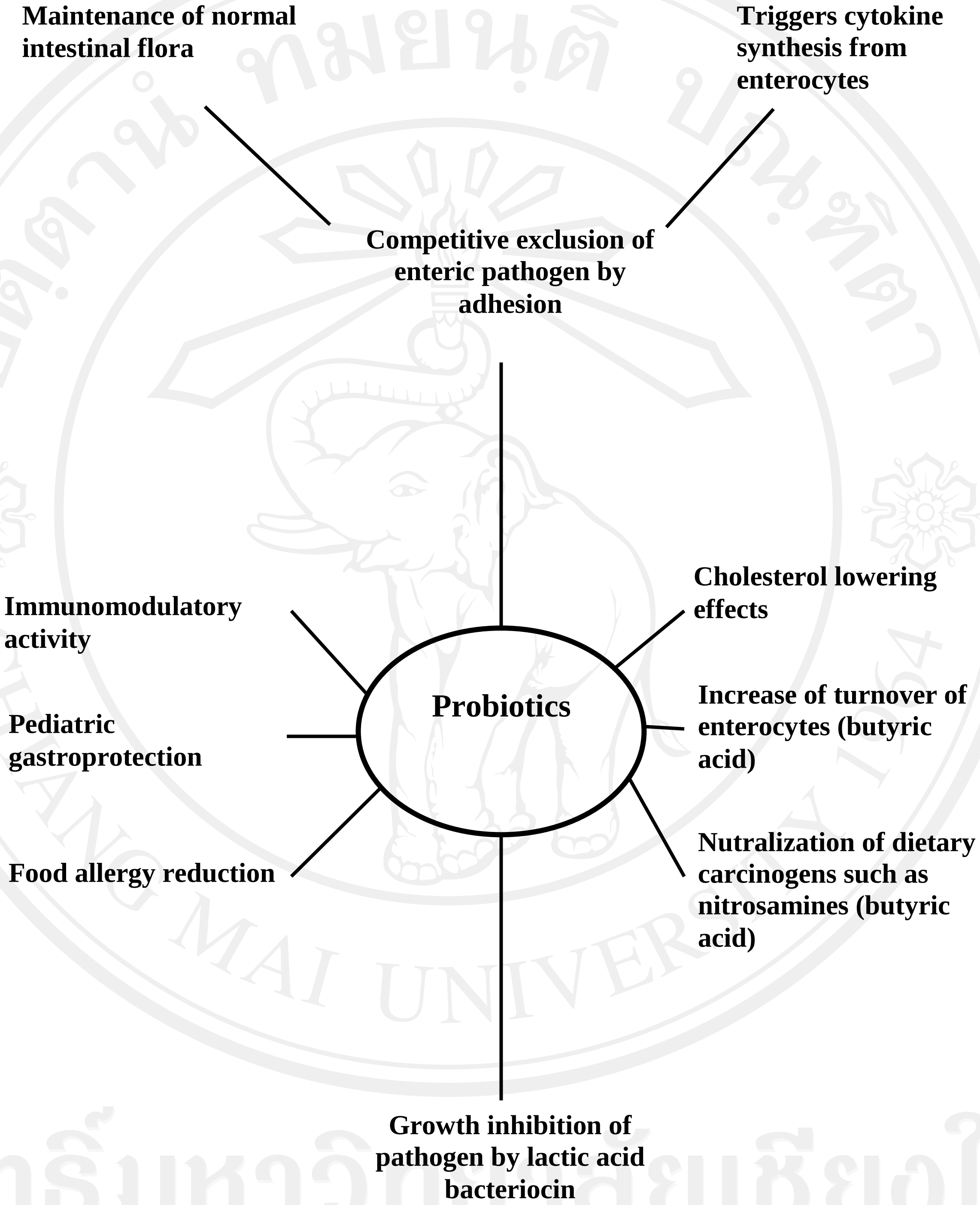


Figure 2.1 Purported mechanisms of functional probiotics (adapted from Saito, 2004).

Health benefits of probiotic LAB are very strain specific; therefore, the use of probiotics should be investigated for there possible benefits and actions for host health. There is no universal strain that provide all proposed functional properties, not even strains of the same species. Some of health benefits of probiotic LAB have been well documented and established are lists below.

2.3.1 Alleviation of lactose intolerance

Lactose will be hydrolyzed by β-galactosidase (or lactase) in the brush border membrane of the mucosa of the small intestine into monosaccharides. The lactase hydrolyzed-monosaccharides, glucose and galactose, are readily absorbed in the blood stream (De Verse et al., 2001; Vasiljevic and Shah, 2008). In case of lactose intolerant or lactose malabsorption, clinical symptoms are accompanied such as flatulence, nausea, bloating, abdominal pain and diarrhoea (Vasiljevic and Shah, 2008). LAB can alleviates the lactose intolerant symptoms in lactose hydrolysis enzyme deficient individuals. It was reported that fermented products containing LAB, in case fermented milk and yoghurt, showed increasing lactase activity in the small intestine (Parvez et al., 2006), improving absorption and lactose tolerance (Vasiljevic and Shah, 2008).

2.3.2 Prevention and reduction of diarrhoea

Probiotics LAB have been shown to have significant benefit for treatment and prevention of diarrhoea, antibiotic-assocoated diarrhoea (D’Souza et al., 2002), travellers' diarrhoea (Isolauri, 1991), young children's diarrhoea (Oberhelman et al., 1999), rotavirus caused diarrhoea (Saavedra et al., 1994; Guandalini et al., 2000), *Clostridium dificile* and other bacterial caused diarrhoea (Vasiljevic and Shah, 2008). The administration of probiotics in the antibiotic associated diarrhoea is required to restore the balance of the intestinal microflora. Several possible mechanisms of probiotic LAB alleviate or ameliorate diarrhoea symptoms have been explained. LAB are known to release various metabolites that exert synergistic effects on diarrhoeal caused pathogens (De Waard et al., 2002). Probiotics can modify the receptor in epithelial cells via excreted soluble factors to inhibit the adhesion of rotavirus and bacterial pathogens (Parvez et al., 2006; Vasiljevic and Shah, 2008). Furthermore, probiotics may improve mucosal barrier function to prevent the attachment of enteropathogens and modulate the immune system response both to anti-inflammatory and pro-inflammatory derections (Vasiljevic and Shah, 2008).

2.3.3 Inhibition of *Helicobacter pylori* infection

H. pyroli is an intestinal pathogen, long-term infection that leads to chronic gastritis, peptic ulcer and increase the risk of gastric malignacies (Suerbaum and Michetti, 2002). The therapy of *H. pylori* infection is combined both antibiotics used and a proton pump inhibitor. These ways of therapy presented very expensive treatment with several side effects including antibiotic-associated diarrhoea and antibiotic resistance induction in likelihood (Vasiljevic and Shah, 2008). Probiotics do not demonstrate to eradicate *H. pyroli*, but they are able to reduce the bacterial load and inflammation of host. The applications of probiotics in the field of *H. pyroli* and intestinal pathogenic infection have been proposed for improving eradication rate and tolerability, for balancing gastrointestinal microflora, and for compliance of multiple antibiotic regimens used for the infection (De Verse, 2002; Parvez et al., 2006). There are some evidences regarding the mechanisms of probiotics LAB to affect on *H. pyroli* via antimicrobial substances, enhancing gut barrier function and competition for adhesion sites (Vasiljevic and Shah, 2008).

2.3.4 Prevention of inflammatory bowel disease

Inflammatory bowel disease (IBD), Crohn's disease and ulcerative disease, is characterized by inflammation, ulceration and abnormal narrowing of the gastrointestinal tract. The results of these diseases present abdominal pain, diarrhoea and gastrointestinal bleeding (Kugathasan et al., 2007; Prisiandaro et al., 2009). Chronic diseases, ulcerative colitis and Crohn's disease, presented by deteriorating and relaxing diseases, affected individuals to the progression of colorectal cancer later (Vasiljevic and Shah, 2008). Some probiotic LAB have been suggested that associated to the therapies may benefit patients with IBD which by improve the intestinal mobility, initiate epithelial cell homeostasis and relieve constipation possibly through a reduction in gut pH (Vanderpool et al., 2008; Prisiandaro et al., 2009). Crohn's disease is predominantly a mucosal Th1-driven immune response which initial increase in interleukin (IL)-12 express, then interferon (IFN)-γ and tumor necrosis factor (TNF)-α are released. In contrast, ulcerative colitis is predominantly a mucosal Th2-driven immune response with pro-inflammatory cytokines production (Kugathasan et al., 2007). From the immune response observations, several probiotic LAB strains such as *L. rhamnosus* GG were demonstrated to modulate the immune system by downregulating TNF-α induced production and increase secretory IgA levels in the gut with affected to inflammatory diseases. On the other hand, *L. reuteri* was related to the up-regulation of the levels of the anti-inflammatory nerve growth factor (Vasiljevic and Shah, 2008).

2.3.5 Nutrient bioavailability

Probiotic LAB are known to release various enzymes which exert synergistic effects on digestion some dietary nutrients, alleviating symptoms of intestinal malabsorption, producing organic acids to decrease the intestinal content pH and inhibit enteropathogens. Some LAB hydrolytic enzymes may increase the bioavailability of fat and protein and enhance other metabolites such as free amino acids, short chain fatty acids (SCFA), organic acids. These metabolites may protect against enteropathogen and pathological changes in the colonic mucosa (Parvez et al., 2006). Enzyme hydrolyses like β-glucosidase, which produced from some LAB hydrolyses plant glycosides into a nonsugar residue (aglycone). The aglycone may be aroma compound or health related compound (Pyo et al., 2005; Yin et al., 2005; Otieno et al., 2006a). Bedides, the β-glucosidase-producing LAB has a potential to hydrolyse the bitter glucoside (oleuropein of olives) to debitter by fermentation (Leal-Sanchez, 2003). Moreover, the β-glucosidase hydrolyses the isoflavone glucosides developing aglycones, which are compounds with anticancer effects (Messina and Wood, 2008).

2.3.6 Control of blood cholesterol

Human cardiovascular disease is the most important problem in many countries due to hypercholesterol. High levels of total blood cholesterol or other blood lipids are strongly associated with increased incidences of human cardiovascular diseases and colon cancer (Kim et al., 2008; Sirilun et al., 2010). Cholesterol as small as 1 mmol higher than the normal cholesterol level has been shown to increase the risk of coronary heart disease and coronary death by approximately 35% and 45%, respectively (Liong and Shah, 2005a). Reduction of total serum cholesterol of 1% can lower the risk of coronary heart disease by 2 to 3% (Pereira et al., 2003). Probiotic LAB have been evaluated for their effect on serum cholesterol levels. The hypocholestereolemic effects of probiotics might be exerted via various possible mechanisms including assimilation by growing cells, binding cholesterol to the cell membrane surface (Liong and Shah, 2005a; 2005b), and producing bile salt hydrolase (BSH) to deconjugate bile (precursor of cholesterol synthesis) into free bile salts that enhance precipitation or excretion via fecal route (Begley and Gahan, 2006). Moreover, free bile salts due to deconjugated enzymes presented poor solubilize of lipids, which limit their absorption in the gut leading to further decrease of serum lipid levels (Vasiljevic and Shah, 2008).

Some of the functional aspects and the potential health benefits of probiotic strains as shown in Table 2.2.

Table 2.2 Some of the established and potential health benefits of probiotic organisms (adapted from Vasiljevic and Shah, 2008)

Health effect	**Mode of action**
Scientifically established	
Alleviation of lactose intolerance	Delivery of intracellular β-galactosidase into human gastrointestinal tract.
Prevention and reduction of symptoms of rotavirus and antibiotic associated diarrhoea	Competitive exclusion Translocation/barrier effect Improved immune response
Potential	
Bioavailability of vitamins and minerals	Lowering the pH colonic contents may increase the bio-availability of calcium. Some probiotics produce B-vitamins
Treatment and prevention of allergy (atopic eczema, food allergy)	Translocation/barrier effect Immune exclusion, elimination and regulation

Table 2.2 (Continued)

Health effect	**Mode of action**
Reduction of risk associated with mutagenicity and carcinogenicity	Metabolism of mutagens Alteration of intestinal metabolic activity Normalization of intestinal permeability Enhanced intestinal immunity
Hypocholesterolemic effect	Deconjugation of bile salts
Inhibition of *Helicobacter pylori* and intestinal pathogens	Competitive exclusion Improvement of epithelial tight junctions Modification of intestinal permeability Modulation of immune response Production of antimicrobial products Decomposition of pathogenic antigens
Stimulation of immune system	Recognition by toll-like receptors—induction of innate and adaptive immunity: - downregulation of pro-inflammatory cytokines and chemokines - upregulation of phagocytic activity - regulation of helper T cells balance

2.4 Soybean as a source of bioactive molecules

2.4.1 Soybean

Soybean or soya bean (*Glycine max* (L.) Merr., *Leguminosae*) has provided nutrition and healing power for human. In the name of health, North Americans are increasingly developing a taste for this Old World staple, in both the traditional and newer forms. The Chinese cultivators have regarded the real-health value of the soybean since 2,000 B.C ago. Soybeans have been well known for their nutrition properties, rich protein contents, high quality of amino acids, high nutrition value, and low cost of production. Recently, functional effects for health of soybean and soybean food products have received much attention. Soybean had been world wide into traditional soy foods, which are usually divided into two groups: (1) fermented products such as miso, soy sauce, tempeh, and natto, and (2) non-fermented products such as tofu and soymilks. In the soybean fermentation process, finished products such as miso, tempeh, natto, and soy or tamari sauce are produced by a host of beneficial yeast, mould and bacteria. Whole-food, fermented soy powders, milks, and yogurts are also cultured with multiple species of beneficial bacteria (Faraj and Vasanthan, 2004; Pierce, 2004).

2.4.2 Nutrition of soybeans

Soybeans have been well known for their health effects, nutrition properties, rich protein contents, high quality of amino acids, high nutrition value, and low cost of production. Nutrition facts of raw mature soybean were exhibited in Table 2.3, referred from United States Department of Agriculture (USDA), National Nutrient Database for Standard Reference. Soybeans contain about 40% protein and up to 20% fat. Whole soybeans are good sources of macronutrients such as proteins, fats and carbohydrates, and minerals.

Table 2.3 Nutrition values of raw mature soybean (Anonymous, 2006)

Nutrient	**Per 100 grams soybean**
Proximates	
Water	8.54 g
Energy	416 kcal
Energy	1742 kj
Protein	36.49 g
Total lipid (fat)	19.94 g
Ash	4.87 g
Carbohydrate, by difference	30.16 g
Fiber, total dietary	9.3 g
Minerals	
Calcium, Ca	277 mg
Iron, Fe	15.70 mg
Magnesium, mg	280 mg
Phosphorus, P	704 mg
Potassium, K	1797 mg
Sodium, Na	2 mg
Zinc, Zn	4.89 mg
Copper, Cu	1.658 mg
Manganese, Mn	2.517 mg
Selenium, Se	17.8 mcg

Table 2.3 (Continued)

Nutrient	**Per 100 grams soybean**
Vitamins	
Vitamin C, total ascorbic acid	6.0 mg
Thiamin	0.874 mg
Riboflavin	0.870 mg
Niacin	1.623 mg
Pantothenic acid	0.793 mg
Vitamin B-6	0.377 mg
Folate, total	375 mcg
Folic acid	0 mcg
Folate, food	375 mcg
Folate, DFE	375 mcg_DFE
Vitamin B-12	0.00 mcg
Vitamin A, IU	0 IU
Vitamin A, RAE	0 mcg_RAE
Retinol	0 mcg
Vitamin E (alpha-tocopherol)	0.85 mg
Vitamin K (phylloquinone)	47.0 mcg

Table 2.3 (Continued)

Nutrient	**Per 100 grams soybean**
Lipids	
Fatty acids, total saturated	2.884 g
14:0	0.055 g
16:0	2.116 g
18:0	0.712 g
Fatty acids, total monounsaturated	4.404 g
16:1 undifferentiated	0.055 g
18:1 undifferentiated	4.348 g
Fatty acids, total polyunsaturated	11.255 g
18:2 undifferentiated	9.925 g
18:3 undifferentiated	1.330 g
Cholesterol	0 mg
Phytosterols	161 mg

Table 2.3 (Continued)

Nutrient	**Per 100 grams soybean**
Amino acids	
Tryptophan	0.530 g
Threonine	1.585 g
Isoleucine	1.770 g
Leucine	2.972 g
Lysine	2.429 g
Methionine	0.492 g
Cystine	0.588 g
Phenylalanine	1.905 g
Tyrosine	1.380 g
Valine	1.821 g
Arginine	2.831 g
Histidine	0.984 g
Alanine	1.719 g
Aspartic acid	4.589 g
Glutamic acid	7.068 g
Glycine	1.687 g
Proline	2.135 g
Serine	2.115 g

2.4.3 Varieties of soybean in Thailand

In Thailand, breeding of soybean began in 1960 by selection of lines from Japan and Taiwan. Nowadays, there has been continuous release of novel varieties including SJ 4, SJ 5, Nakhon Sawan 1 (SN 1), Chiangmai 1 (CM 1), CM 2, CM 3, CM 4, CM 60, Sukhothai 1 (ST 1), ST 2, ST 3, Chakkrabhandhu 1 (CB 1) and Khonkaen University 35 (KKU 35) (Tantasawat et al., 2011). In Thailand, soybean was majorly cultivated in the northern area, including varieties CM 1, CM 60, SJ 2, SJ 4 and SJ 5. CM 60 is popular variety in northern Thailand due to it can adapt to wide range of environment and provides high yield (Department of Agriculture, 1996). The variety CM 60 was approved by The Research Department of the Agriculture Committee on 30 September 1987. The variety of CM 60 was resistance to rust diseases more than the varieties of SJ 4 and SJ 5. The variety of CM 60 type has little branches, therefore, this variety can increase the number of tree per field and it uses less fertilizer the SJ 5 type (Chayawat et al, 2003). Furthermore, the CM 60 can be cultivated both in dry and raining seasons.

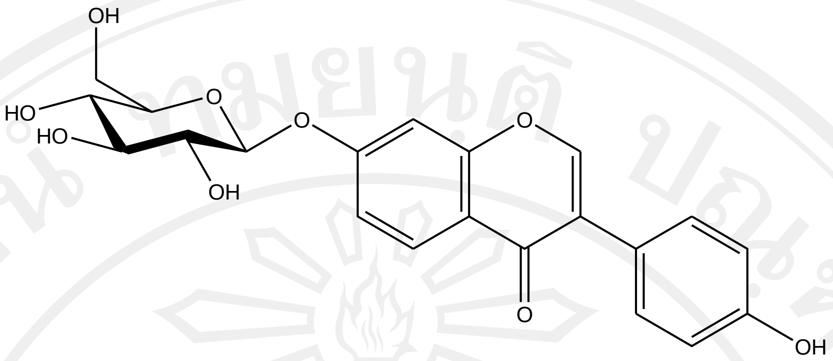
The proximate compositions and isoflavone contents of soybeans of 6 varieties commonly grown in Thailand are shown in Tables 2.4 and 2.5, respectively. The chemical structures of isoflavones are shown in Figure 2.2.

Table 2.4 Proximate compositions of whole seeds of 6 soybean varieties commonly grown in Thailand (Chaiyasut et al., 2005)

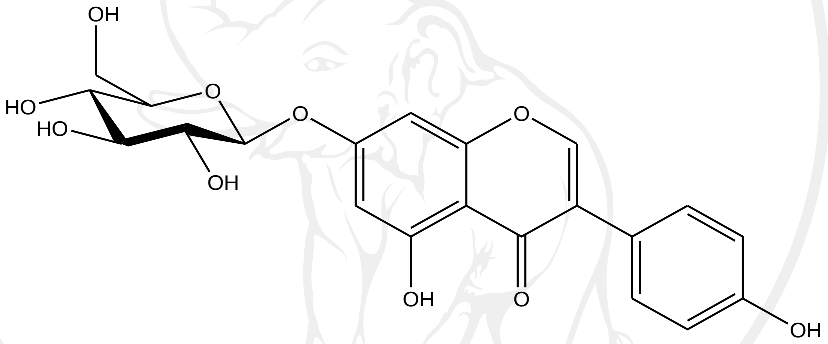
Variety	Content (%, dry wt basis)				
	Protein	Lipid	Carbohydrates	Crude fiber	Ash
Rajamangala	37.61 ± 0.36	14.71 ± 0.10	42.24 ± 0.17	6.11 ± 0.40	5.44 ± 0.10
Chiangmai 1	37.59 ± 0.30	20.82 ± 0.28	36.28 ± 0.56	4.90 ± 0.21	5.31 ± 0.01
Chaingmai 60	37.62 ± 0.04	18.00 ± 0.62	38.90 ± 0.77	8.02 ± 0.44	5.49 ± 0.09
Sor Jor 2	39.45 ± 0.18	19.19 ± 0.19	35.91 ± 0.25	5.09 ± 0.50	5.46 ± 0.01
Sor Jor 4	38.54 ± 0.35	20.34 ± 0.06	35.79 ± 0.41	5.91 ± 0.61	5.34 ± 0.09
Sor Jor 5	39.49 ± 0.33	18.54 ± 0.12	36.48 ± 0.46	5.32 ± 0.18	5.49 ± 0.12

Table 2.5 Isoflavones of whole seeds of 6 soybean varieties commonly grown in Thailand harvested during April and May (Chaiyasut et al., 2005)

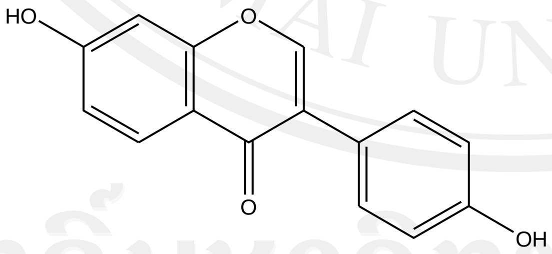
Variety	Isoflavones (mg/kg) ± S.D. (n=4)			
	Daidzin	Genistin	Daidzein	Genistein
Rajamangala	927.1 ± 63.5	949.2 ± 31.5	3.1 ± 0.9	7.1 ± 1.0
Chiangmai 1	446.3 ± 104.7	360.2 ± 25.7	2.8 ± 0.6	10.0 ± 2.8
Chaingmai 60	588.5 ± 68.4	825.6 ± 8.9	3.8 ± 0.7	26.0 ± 2.1
Sor Jor 2	948.6 ± 19.0	834.5 ± 14.0	5.2 ± 1.2	27.5 ± 4.5
Sor Jor 4	408.4 ± 65.3	610.3 ± 95.0	3.9 ± 0.6	18.9 ± 2.2
Sor Jor 5	303.2 ± 18.0	281.1 ± 11.0	2.9 ± 0.9	9.4 ± 3.5

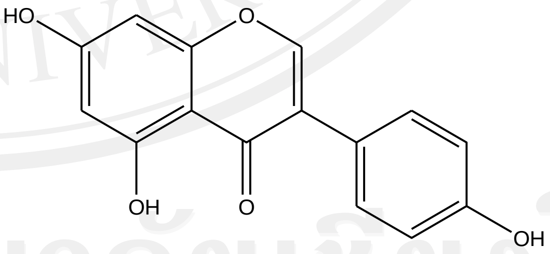


Daidzin



Genistin





Daidzein **Genistein**

Figure 2.2 Chemical structures of isoflavones

2.4.4 Bioactive compounds and health effects of soybean and soy foods

Functional effects of soybean and soybean food products for the prevention and treatment of diseases have received world wide attention. Soybean and soy foods are recognized as rich in protein, minerals, both soluble and insoluble dietary fiber components, which offer many health benefits (Rinaldi et al., 2000). The importance of soybean oligosaccharides as prebiotics and their role in colon function is being widely recognized (Lan et al., 2007). The isoflavones genistein and daidzein are unique to soybeans and have numerous functional effects on health (Otieno et al., 2007). The major non-nutrients molecules called phytochemicals (plant-derived compounds) found in soybeans that have potential long-term health benefits, including polyphenols (phenolic acids, isoflavones, tannins, and saponins), phytic acid, protease inhibitors, and lectins (Singh et al., 2008).

2.4.5 Soybean isoflavones

The three soybean isoflavones are genistein, daidzein, and glycitein. The isoflavones from soybeans have estrogenlike properties in human cells (Pham and Shah, 2007). They are classified as a flavonoid group of phytoestrogens due to their ability to interact with estrogen receptors in cells. The nonsteroidal phytoestrogenic isoflavones in soybean have focused on the potential roles in prevention against several major chronic diseases, including cardiovascular diseases, cancers, and osteoporosis (Anderson and Garner, 2000). In soybean, there are two groups of isoflavones: aglycones and aglycone-glycoside conjugates. Aglycones are the only group that possesses the estrogenic activity (Pham and Shah, 2007).

The majority of isoflavones in soy and soy foods exist as glycosides or glycones that have a glucose molecule attached to them. The single glucose molecule will be removed and the aglycone remain when glucosidase enzyme attack the glycosides (Anderson and Garner, 2000). The isoflavones predominantly exist as biologically inactive glycoside conjugates ranged 83.90%-98.37% in nature or nonfermented soy foods (Pham and Shah, 2007). The isoflavone aglycones are absorbed faster and greater amounts than their glycosides counterpart by the small intestine as part of micelles created by the action of bile. Some aglycone molecules are absorbed directly through the gut wall and a small percentage of digested glycones complete their absorption by entering the lymphatic drainage as part of chylomicrons. On the other hand, isoflavones glycosides are very poorly absorbed from the gut because their hydrophillicity and larger molecular weight than aglycones (Anderson and Garner, 2000; Pham and Shah, 2007). In addition, aglycones have been demonstrated to be more stable than isoflavone glycosides during the storage at different temperatures (Otieno et al., 2006b). The circulation of isoflavones in blood is complex, due to the lipid solubility of these molecules and weak attractive forces of binding with proteins in the circulating blood (Pham and Shah, 2007). Pham and Shah (2007) reported that the aglycones genistein and daidzein had almost equal bioavailability for absorption. Genistein was found to be highly bioavailability in rats and was shown to participate in the enterohepatic cycle.

In the last few years, several groups of probiotic lactobacilli which can exhibit β-glucosidase activities, are able to hydrolyze isoflavone glycosides to aglycones (Otieno et al, 2007). Thus the administration of β-glucosidase producing lactobacilli in soybean food products may lead to an incorporation of functional health benefits as well as that from transformation of isoflavone glucosides to bioactive isoflavone aglycones.

2.4.6 Cholesterol-lowering properties of soybean

Coronary heart disease (CHD) is a leading cause of mortality and morbidity in world populaions with over several million deaths each year. Elevates blood cholesterol is an important modifiable risk factor in the development of CHD (WHO, 2002). It has been shown that reduce blood cholesterol may therefore reduce the risk of heart disease. Beneficial effects of soybean consumption on blood cholesterol are the most consistently reported findings. Several reports have shown that the daily consumption of 30 g to 60 g of soy protein provides to decrease in total and LDL cholesterol (bad cholesterol) of between 10% and 20% in serum cholesterol. The consumption of soybean protein in place of animal protein reduces level of blood cholesterol (Cho et al., 2007).

The potential mechanisms of soybean responsible for the effects on serum cholesterol concentrations or the risk of coronary heart disease are elicited various hypotheses. These include the amino acid composition of soybean protein, an interruption of the intestinal absorption of bile acids and dietary cholesterol, direct effects on the hepatic metabolism of cholesterol, alteration of the hormone concentration involved in cholesterol metabolism, and the effects of active compounds such as isoflavones, fiber and saponins in soybeans (Anderson et al., 1999; Greaves et al., 1999).

Cho et al. (2007) indicated that the peptides in soybean protein hydrolysate (SPH) with bacterial proteases have a hypocholesterolemic effect via the mechanisms three approaches including (1) blockage of bile acid and/or cholesterol absorption, (2) inhibition of cholesterol synthesis, and (3) stimulation of low density lipoprotein receptor (LDL-R) transcription, in which soy peptides can effectively stimulate LDL-R transcription in human liver cell line and reduce blood cholesterol (Cho et al., 2007).

Moreover, there were a meta-analysis published in 1995 reported that ingestion of 47 g soy protein/day reduced total serum cholesterol, LDL cholesterol and triacylglycerol by 9.3%, 12.9% and 10.5%, respectively. On contrary, soybean protein increased high density lipoprotein (HDL) cholesterol (good cholesterol) by 2.4% (Taku et al., 2007).

Ali et al. (2004) indicated that dietary soybean isoflavones significantly with the quantity 0.1% mixed with feed diet lowered total and LDL cholesterol in obese SHR/N-cp rats. Lien et al. (2009) also reported that diet supplemented that soy aglycones of isoflavone significantly lowered LDL cholesterol levels and increased HDL cholesterol in ovariectomized (OVX) Spraque-Dawley rats. The proposed mechanisms for cholesterol-lowering effect of phytoestrogens are the up-regulation of LDL receptors and/or the inhibition of endogenous cholesterol synthesis. Phytoestrogens in soy protein may stimulate the clearance of cholesterol, probably by up-regulating LDL receptors, and thereby increasing LDL receptor activity (Tham et al., 1998).

The blood cholesterol can be decreased by soybean nutrients and by soybean isoflavones. Soybean isoflavones lowered serum total and LDL cholesterol but did not change HDL cholesterol and triacylglycerol. Soy protein with or without isoflavones also improved lipid profiles. Furthermore, the reduction in LDL cholesterol was larger in hypocholesterolemic subjects than in normocholesterolemic subjects. Soy protein and soy isoflavones would have synergistic or additive effects on cholesterol-lowering property (Taku et al., 2007).

2.4.7 Anticarcinogenic effects of soybean

A number of different bioactive compounds in soybeans may be responsible for several kinds of anticarcinogenic activity. These bioactive compounds include a protease inhibitor (the Bowmam-Birk inhibitor), a trypsin inhibitor, isoflavones (genistein and diadzein), inositol hexaphosphate (phytic acid), saponins and sterol (Venter, 1999). Venter (1999) also reviewed that Bowman-Birk inhibitor (BBI) which is contained 20% to 25% of the total protease inhibitor content of soybean protein has shown the greatest suppression of carcinogenesis in animal carcinogenesis assays. Soybean isoflavones also had been suggested that responsible for tumor inhibition and preventing a wide range of cancers.

Hormone-related cancers of the breast, ovary, endometrium, and prostate have been indicated to vary by as much as 5- to 20-fold between populations. There is a little evidence to demonstrate that any potential weak estrogenic effects of dietary isoflavones have a clinically relevant impact on breast cancer risk in women. There had been reported a significant reduction in breast cancer risk among both premenopausal and postmenopausal women who consume soy phytoestrogens (Tham et al., 1998). Tyrosine kinase inhibitors (TKI) have potential as anticancer agents in both prevention and therapeutic protocols. Soybean aglycone (genistein) is a specific inhibitor of tyrosine protein kinases, topoisomerase II, and protein histidine kinase (Thiagarajan et al., 1998). In addition, genistein inhibits DNA topoisomerase II and ribosomal S6 kinase, both of which may lead to protein-linked DNA strand breaks in cancerous cells, arrest of tumor cell growth, and induction of differentiation of several malignant cell lines into lines that may be benign (Yamashita et al., 1990; Tham et al., 1998).

2.4.8 Effect of soybean on oxidative stress and inflammatory markers

There was reported soybean phytoestrogens, fiber and polyunsaturated fat, which are associated with reduce oxidative stress, levels of inflammatory marker and improved endothelial function (Azadbakht et al., 2008). The mechanisms through which soybean effects oxidative stress, inflammatory state and endothelial function may be related to the effects of soybean phytoestrogens, specific fatty acids or fibers. Soy isoflavones (genistein and daidzein) are recently recommended hormone replacement therapy (HRT) in restoring immune physiology, since they may have a better ratio of anti-oxidant/estrogen potencies which may protect against immune senescence, in addition to complementation of hormone levels. Moreover, soy phytoestrogens can enhance nitric oxide release and bioavailability, reduce cell adhesion molecules and inflammatory markers (Azadbakht et al., 2008). In case of elderly humans also have poorly maintained cellular redox during ageing affects immune (NF-kB) and hormone receptor (ER) functions as well, besides hormonal imbalances. Furthermore, the age-related changes in the activity of proinflammatory cytokines (e.g. IL6) are beginning to elicit that may have a significant impact on the function of all these body parts. Soy phytoestrogen is claimed hormonal activity through integration of hormonal ligand activities and interference with signaling cascades that relief hormonal ailment, decrease oxidative damage, prevent cancer and/or reduce NF-kB-related inflammatory disorders as well (Dijsselbloem et al., 2004). Soy phytoestrogens elicited less reactive of oxidative effect may be due to donate hydrogen atom to free radicals and/or increase antioxidant enzyme concentrations (Kulling et al., 2001; Azadbakht et al., 2008).

2.5 Fermentation

2.5.1 Fermentation and lactic acid bacteria

The term fermentation means an energy-generation process in which organic compounds act as both electron donors and terminal electron acceptors, which is applied to describe both aerobic and anaerobic carbohydrate breakdown processes (Stanbury et al., 1999). The original and primary purpose for food fermentation was to achieve a preservation effect. However, with the development of the many effective alternative preservation technologies which are now commonly available, particularly in the western world, this is no longer the most pressing requirement and many of these foods are manufactured because their unique flavor, aroma and texture attributes, functional properties are much appreciated by the consumer. The conditions generated by the fermentation are essential in ensuring the shelf-life and microbiological safety of the products (Caplice and Fitzgerald, 1999).

The characterization of the microorganisms responsible for the fermentation towards the end of the 19th century led to the isolation of starter cultures which could be produced on a large scale to supply factories involved in the manufacture of dairy products. This significant development had a major impact on the processes used and contributed to ensuring consistency of product and reliability of fermentation (Caplice and Fitzgerald, 1999). The conventional method of fermenting plant used industrially is natural fermentation brought about by the indigenous drawbacks that limit process yields and affect final product quality, at the some time making it difficult to anticipate the course of fermentation, since the process is dependent on many different factors, some of which are hard to control. The microbial load of fresh fruits can be highly variable and unquestionably exerts a decisive influence on the proper course of fermentation (Ballesteros et al., 1999).

Lactic acid-producing fermentation is an old invention. Many different cultures in various parts of the world have used it to improve the storage qualities, palatability, and nutritive value of perishable foods such as milk, vegetables, meat, fish, legumes, and cereals. The organisms that produce this type of fermentation, lactic acid bacteria, have had an important role in preserving foods, preventing food poisoning, and indirectly feeding the hungry on every continent (Salminen and Wright, 1998). Lactic acid fermentation of cabbage to produce sauerkraut has been widely studied for many years. With the popularity and success of sauerkraut, many other vegetables have been fermented, such as cucumbers, beets, turnips, cauliflower, green beans, green tomatoes, Brussels sprouts, and mixed vegetables (*Kimchi*), celery and radishes, and carrots (Roberts and Kidd, 2005) and many other plants have been fermented. Spontaneous fermentation thus leads to variations in the sensory properties of the products. It was shown that the use of starter culture helps to standardize the fermentation by controlling the microbial flora, particularly the aerobic flora (Gardner et al., 2001).

2.5.2 Fermented soybeans (Faraj and Vasanthan, 2004; Pierce, 2004)

Fermentation leads to improved nutrition value and/or bioavailability, which are sometimes not present in the original substrate. Furthermore, food fermentation containing soybeans are destroyed undesirable beany flavours in many fermentations. Several benefits of soybean fermentation were indicated that include following:

(1) Improved digestibility. Unfermented soybeans are difficult to digest, partly due to the high amount of protein enzyme inhibitors and hard-to-digest sugar structures. During the fermentation process, the enzymes produced by the beneficial bacteria and other microbes break down, or predigest, the specific complex carbohydrates (sugars) found in soy and most other legumes. This process also renders the proteins more digestible and easier to assimilate than those in the whole soybean. For those with a compromised digestive system or difficulty digesting protein, this is especially helpful.

(2) Enhanced nutrition. Soy fermentation converts minerals such as calcium, iron, magnesium, potassium, selenium, copper, and zinc into more soluble forms and can also increase vitamin levels in the final product. Some beneficial yeasts, such as *Saccharomyces cerevisiae*, are able to concentrate large quantities of thiamin, nicotinic acid, and biotin, thus forming an enriched product.

(3) Medicinal benefits. Substances in fermented soy foods have been found to alleviate the severity of hot flushes, to have a protective effect against the development of cancer, to cause a reduction in cholesterol, and to inhibit the progression of atherosclerosis. The probiotic bacteria produced during soy fermentation are known to enhance healthy intestinal flora and correct digestive tract imbalances.

(4) Increased bioavailability of isoflavones. Isoflavones (phytoestrogens naturally occurring in soy) are converted by the bacteria into their "free" or aglycone forms for improved absorption and more effective usage within the body.

2.5.3 Fermented food containing plant using probiotic lactic acid bacteria

Although suppliers of lactic cultures have numerous starter cultures for the diary and meat sectors, there are very few cultures designed for plant and medicinal plant fermentations. Thus, studies on the evaluation and development of effective microbial inoculants to rapidly initiate fermentation of vegetables, fruits, cereal and other plants are warranted.

Lactic acid fermentation has been extensively studied in legumes for the production of acid foods with longer shelf-life and improved nutritive characteristics, cultures of lactobacilli have the ability to utilize flatulence-producing oligosaccharides, increase in essential amino acids, and improve the vitamin content. The effects of lactobacilli upon oligosaccharide, phytate and alkaloid contents, as well as on the nutritive value of lupine (*Lupinus albus* cv. Multulopa) lead to a final fermented lupine with nutritional advantages, obtaining a lupine beverage low in oligosaccharides and phytates, as well as with as improved content of riboflavin (Camacho et al., 1991).

The popularity of vegetable juices produced by lactic fermentation is growing because they represent a new type of drink and because of their high nutritive value, with a high content of vitamins and minerals. Vegetable juices (mixture of cabbage and carrot juice) produced by lactic acid fermentation by using *Lactobacillus plantarum* as a starting culture, do not have salt or spices added, so they are suitable for dietetic use. *L. plantarum* produced the best sensoric values of fermented vegetable juices because the concentration of acetic acid produced gave a very good taste (Karovičová et al., 1994).

Lactic acid bacteria (LAB) are mainly responsible for the fermentation of vegetables such as cabbage, carrots and beet-based vegetable products, but the indigenous LAB flora varies as a function of the quality of the raw material, temperature and harvesting conditions. There are no commercial cultures available that are specifically designed for the fermentation of carrots and beets, so the most widely available cultures for plant fermentations were tested, which silage were inoculated. The control products (uninoculated) were darker in color than the starter inoculated products. With respect to taste, all three inoculated products were judged to be better tasting than the controls (Gardner et al., 2001).

The fermentation product of herbs (consisting of Lavender, Lemon balm, Mugwort and Loquat) by lactic acid bacteria (FHL), in which *Enterococcus faecalis* TH10 predominated, was assayed for antifungal activity against tinea (dermatophyte that usually infects keratin tissues). The antifungal activity of FHL was as high as that of a synthetic fungicide, nonproteinaceous compounds or organic acids in FHL could inhibit the growth of the dermatophyte tinea under low-pH conditions, and that malonic acid and acetic acid could have especially high antifungal activity against tinea (Kuwaki et al., 2002).

Lactococcus lactis strain (LL3) isolated from mothers' milk was used to produce fermented soymilk. The strain survived at levels of over 7 log cfu/ml for 3 weeks in the fermented soymilk. A consumer survey was carried out to compare the acceptability of the fermented product with a similar product made with *L. lactis* ATCC11545 originally isolated from cow's milk. Blind samples produced by fermentation with the two strains were rated equally attractive, whereas information on the origin of the strains significantly enhanced the pleasantness of the fermented soymilk (Beasley et al., 2003).

Production of Spanish-style green olives, like other natural vegetable fermentations, is a spontaneous, traditional lactic acid fermentation based on an empirical process which relies upon microorganisms present in the raw material and processing environment. In the traditional process, olives are handled in order to favor the growth of *Lactobacillus plantarum,* which is thought to be essential to provide the amount of lactic acid needed for preservation as well as for its characteristic flavor. In this regard, the use of suitable *L. plantarum* starter cultures has the potential to improve the microbiological control of the process, increase the lactic acid yield and provide the production of Spanish-style fermented green olives of consistently high quality (Leal-Sánchez et al., 2003).

Two strains of lactic acid bacteria, *Lactobacillus acidophilus* CCRC 14079 and *Streptococcus thermophilus* CCRC 14085 were used in single culture and in combination with either *Bifidobacteria infantis* CCRC 14633 or *Bifidobacteria longum* B6 for the production of fermented soymilk. Results revealed that *L. acidophilus* and *S. thermophilus* were capable of metabolizing stachyose and raffinose in soymilk. In addition, *S. thermophilus* exploited these substrates more efficiently than *L. acidophilus*. During the 24-32 h of fermentation with single culture of either *L. acidophilus* or *S. thermophilus*, content of raffinose, stachyose, sucrose and pH in soymilk decreased, while content of fructose and glucose plus galactose increased (Wang et al., 2003).

Fermented plant juice is non- alcoholic beverages, produced from different kind of plants (Kantachote et al., 2004). FPJ composed of 3 main compositions; plant, water and sugar informs of sucrose or molasses. The FPJs are believed that not only promoting health beverage but also could cure some diseases. Fermented medicinal plant juice (FMPJ) containing *M. citrifolia* Linn. and *Lactobacillus* spp. starter culture were shorten the fermentation process and reduce the risk of fermentation failure. They were found at maximum values of all kinetic parameters, might be due to detect the contaminated microorganisms at low amount. FMPJ also had antimicrobial activities to *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Candida albicans* (Chaiyasut, 2004; Sirilun, 2005).

Four strains of lactic acid bacteria starter are *Lactobacillus plantarum* KFRI 00144, *Lactobacillus delbrueckii* subsp. *lactis* KFRI 01181, *Bifidobacterium breve* K-

101 and *Bifidobacterium thermophilum* KFRI 00748 tested for β-glucosidase enzyme activity. It was found that four β-glucosidase-producing lactic acid bacteria have great potential for the enrichment of bioactive isoflavones in soymilk fermentation, with an average 7.1-fold increase of aglycones (daidzein and genistein) was observed (Pyo et al., 2005).

Soymilk fermented with 3 selected *Lactobacillus acidophilus* strains were stored at various temperatures (−80°C, 4°C, 25°C and 37°C) for 8 weeks and the concentration of isoflavones determined weekly using RP-HPLC. Isoflavone aglycones as well as isoflavone glucosides largely appeared to be stable during storage (Pb0.01). Interestingly, the aglycone forms showed much smaller degradation as compared to glucoside forms at all the storage temperatures studied. Of the isoflavone aglycones, daidzein was found to be the most stable followed by genistein, while glycitein was least stable. Isoflavone aglycones such as glycitein, daidzein and genistein showed smaller degradation constants in fermented soymilk at lower storage temperatures (−80°C and 4°C) and higher degradation constants at higher storage temperatures (25°C and 37°C) with each strain. In contrast, glucosides glycitin and daidzin showed higher degradation at lower storage temperatures (−80°C and 4°C) and lower degradation at higher storage temperatures (25°C and 37°C). Storage temperature was therefore found to be very important in regulating the rate of degradation soy isoflavones in fermented soymilk (Otieno et al., 2007).

Chien et al. (2006) reported that fermented soy milk containing individual and mixed starter LAB (*S. thermophilus* BCRC 14085 or *L. acidophilus* BCRC 14079 or *B. infantis* BCRC 14633 or *B. longum* B6) exhibits a lower total isoflavone content (81.94-86.61 μg/ml) than non-fermented soymilk (87.61 μg/ml). They found that isoflavone reduction relate to the increasing of aglycone during fermentation coincides with the increase of β-glucosidase activity of starter organisms. The increased aglycone content in fermented soy milk would enhance the health benefits on human host.

Soymilk is fermented with lactic acid bacteria (*Lactobacillus acidophilus* CCRC 14079 or *Streptococcus thermophilus* CCRC 14085) and bifidobacteria (*Bifidobacterium infantis* CCRC 14633 or *Bifidobacterium longum* B6) individually, and in conjunction. It was found that in fermented soymilk both the inhibition of ascorbate autoxidation, and the reducing activity and scavenging effect of superoxide anion radicals varied with the starters used, but nevertheless are significantly higher than those found in unfermented soymilk. In general, antioxidative activity in soymilk fermented with lactic acid bacteria and bifidobacteria simultaneously is significantly higher ($P<0.05$) than that fermented with either individually. Moreover, antioxidative activity increases as the fermentation period is extended (Wang et al., 2006).