CHAPTER 2

LITERATURE REVIEW

Nowadays, consumers are aware of the link among lifestyle, diet and good health which explains the emerging demand for products that are able to enhance health beyond providing basic nutrition. The list of health benefits accredited to functional food continues to increase and the probiotics are one of the fastest growing categories within food for which scientific researchers have demonstrated therapeutic evidence (Soccol et al., 2010). The nation that food could serve as medicine was first conceived thousands of years ago by the Greek philosopher and father of medicine, Hippocrates, who once wrote: “Let food be your medicine and medicine be your food” (Chow, 2002). Functional foods are defined as: foods that contain some health-promoting components beyond traditional nutrients. In general, the term refers to a food that has been modified in some way to become functional. One way in which foods can be modified to become functional is by the addition of probiotics. Health-benefits derive by the consumption of foods containing probiotic bacteria are well documented and probiotic products are available worldwide.

2.1 Probiotics

2.1.1 Definition of probiotics

The term probiotic is a relatively new word meaning “for life” was originally proposed in 1965 by Lilley and Stillwell (Schmid et al., 2006). It is derived from the Greek language and it is currently used to name bacteria associated with beneficial effects for humans and animals (Fooks et al., 1999). The original observation of the positive role played by some selected bacteria is attributed to Eli Metchnikoff, who suggested, “the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes” (Metchnikoff, 1908). Lilly and Stillwell (1965) describe the word probiotic as “substance secreted by one microorganism which stimulates the growth of another.” While, Sperti (1971 cited in Itsaranuwat, 2003) was described the term of probiotic to “tissue extracts that stimulate microbial growth.” Parker (1974) was the first to use the term probiotic in the sense that is used today. He gave the definition of probiotics as “organisms and substances which contribute to intestinal microbial balance”, the definition also including antibiotics. However, Fuller (1989) defined probiotics as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.” This definition stressed the requirement of viability for probiotics and introduced the aspect of a beneficial effect on the host (Itsaranuwat, 2003). The definition of probiotics is continuously argued between microbiologists. According to Salminen (1996 cited in Itsaranuwat, 2003), a probiotic is “a live microbial culture or cultured dairy product which beneficially influences the health and nutrition of the host”, whereas in the same year, Schaafsma (1996 cited in Itsaranuwat, 2003) suggested the oral probiotics are “living microorganisms which upon ingestion in certain numbers, exert health effects beyond inherent basic nutrition.” At present, the most generally used definition is that of Fuller (Salminen et al., 1998). However, Diplock et al. (1999 cited in Lee, 2009) puts it as “ probiotic food is functional if they have been satisfactorily demonstrated to beneficially affect one or more target functions in the body beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction in the risk of diseases.” Whereas, Naidu et al. in (1999 cited in Lee, 2009) said “A microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract.” next, Tannock in 2000 (Lee, 2009) observed that long-term consumption of probiotics was not associated with any drastic change in the intestinal microbiodata composition, and thus proposed an alternative definition: Microbial cells which transit the GI tract and which, in doing so, benefit the health consumer.”

However, recently, Schrezemeir and de Vrese (2001) defined the term of probiotic as “a product containing viable, defined micro-organisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a complement of the host and by exert beneficial health effects in this host.” In addition, probiotics are defined as live microorganisms when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001). The consumption of probiotic products is growing very fast all over the world and probiotics are generally considered generally regarded as safe (GRAS). Since the word probiotics refers to microorganisms that are able to confer health benefits on humans and that have been industrially prepared for nutritional and pharmaceutical use.

Probiotics have come to Thailand as dairy products such as yogurt, drinking yogurt, and powder milk since 2001 (Lee et al. 2012). To provide better information relating to its health claim to ensure standards and protect consumers, Thai FDA, Ministry of Public Health Thailand started regulating probiotic usage for food product since 2008 (FDA 403/2551). To assess the properties of probiotics, it is suggested that the following guidelines are needed. For food application, probiotic microorganisms should be able to survive passage through the digestive tract and to proliferate in the gut.

2.1.2 The selection criteria for potential probiotics

A general agreement among scientist has been reached, at least in general terms, on the properties that a strain must have in order to be further tested for human probiotic use (Ouwehund et al., 1999):

(1) Human origin, if intended for human use

(2) Safe for food and clinical use

(3) Survive during gastric transit

(4) Acid and bile stability

(5) Adhesion behavior to gut epithelial tissue

(6) Clinically validated and document health effects

(7) (Good technology properties

The strains generally present into the intestinal flora of the host will be targeted by researchers, who assume that these bacteria have a better chance of out-competing normalflora bacteria and of establishing at a numerically significant level in their new host (Morelli, 2000). Probiotics was consumed orally, in case acid and bile resistance are preferable traits since it is desirable for a probiotic to survive gastrointestinal transit. The adhesion to mucosal surfaces by probiotic organisms is an important ability for the colonization of the human gastrointestinal tract, prevents their elimination by peristalsis and provides a competitive advantage over pathogens (Kos et al., 2003). The adhesion process can be divided into two steps: 1) reversible adhesion due to long-range forces and 2) subsequent interaction mediating a direct contact between microorganisms and supports surfaces such as the hydrophobic interaction of microorganism and support (Wang and Han, 2007).

2.1.3 The selection of a health-promoting probiotics

The probiotic strains must possess the ability to overcome the extremely low pH and the detergent effect of bile salts, and arrive at the site of action in a viable physiological state. They should be capable of co-aggregation, resistant to gastro intestinal fluid and adhere to the intestinal mucosa. However, besides the various essential characteristics, the organisms should exhibit health benefits with functional properties. The organisms have developed various functional characteristics. Clinically proven, various health effects have been reported for lactobacilli, such as cholesterol reduction, diarrhea prevention, enhancement of lactose intolerance symptoms, anticancer effects, synthesis and enhancing the bioavailability of nutrients and immune-modulatory effects, all of which are considered functional aspects of probiotic criteria. In order to exert their beneficial effect, probiotics must survive in the gastrointestinal (GI) tract, persist in the host, and provide safety for the consumer (De-Vries et al., 2006).

A number of benefits derived from probiotic-containg have been reported (Schaafsma et al., 1998; Fooks et al., 1999) and include the following:

(1) Enhanced lactose digestion

(2) Prevention/treatment of acute rotavirus and antibiotic-induced diarrheas'

(3) Improvement of the balance between microbial populations in the gut

(4) Enhancing the bioavailability of nutrients

(5) Suppression of cancers

(6) Reduction of serum cholesterol/ prevention the risk of coronary heart disease

(7) Detoxificant/binding mycotoxin

The beneficial effects of probiotics likely result from several complexes, interacting mechanisms that will differ for different strains and sites of action. These mechanisms may include competition for binding sites to the intestinal wall, competition for essential nutrients, production of antimicrobial substances, stimulation of *mucin* production, stabilization of the intestinal barrier, improvement of gut transit, metabolism of nutrients to volatile fatty acids, and immune modulation (immune stimulation and immune regulation). Some of these mechanisms have been demonstrated only through laboratory experiments or animal models and are not substantiated in humans (Fooks et al., 1999).

2.1.4 Mechanisms of probiotic actions

The effects of probiotics maybe classified in three modes of action (Oelschaeger, 2010).

(1) Probiotics might be able to modulate the host's defenses including the innate as well as the acquired immune system. This mode of action is most likely important for the prevention and therapy of infectious diseases but also for the treatment of (chronic) inflammation of the digestive tract or parts thereof. In addition, this probiotic action could be important for the eradication of neoplastic host cells.

(2) Probiotics can also have a direct effect on other microorganisms, commensal and/or pathogenic ones. This principle is in many cases of importance for the prevention and therapy of infections and restoration of the microbial equilibrium in the gut.

(3) Finally, probiotic effects may be based on actions affecting microbial products like toxins, host products e.g. bile salts and food ingredients. Such actions may result in inactivation of toxins and detoxification of host and food components in the gut.

All three modes of probiotic action are in all likelihood involved in infection defense, prevention of cancer and in stabilizing or reconstituting the physiological balance between the intestinal microbiota and its host. However, it has to be stressed that there seems not to be one probiotic exhibiting all three principles, at least not to that extent that it could be a remedy for prevention or therapy of all mentioned kinds of disease. It depends on the metabolic properties, the kind of surface molecules expressed and components to be secreted which probiotic actions a certain probiotic strain might show.

2.1.5 Beneficial health effects of probiotics

The probiotic foods should be safe and must contain the appropriate probiotic organisms in sufficient numbers at the time of consumption. Therefore, the probiotic strains selected should be suitable for large-scale industrial production with the ability to survive and retain their functionality during production and storage as frozen or dried cultures. It must survive during the food processing operations, and also in the food products into which they are finally formulated (Figure 2.1).

Probiotics provide a number of health benefits mainly through maintenance of normal intestinal microflora, protection against gastrointestinal pathogens, enhancement of the immune system (Gilliland, 1990), reduction of serum cholesterol level and blood pressure (Rasic, 2003), anti-carcinogenic activity (Rasic, 2003), improved utilisation of nutrients and improved nutritional value of food as illustrated in Figure 2.2. Therapeutic applications of probiotics include prevention of infantile diarrhea, urinogenital diseases, osteoporosis, food allergy and atopic diseases; reduction of antibody-induced diarrhea; alleviation of constipation and hypercholesterolemia; control of inflammatory bowel diseases; and protection against colon and bladder cancer (Tripathi and Giri, 2014).

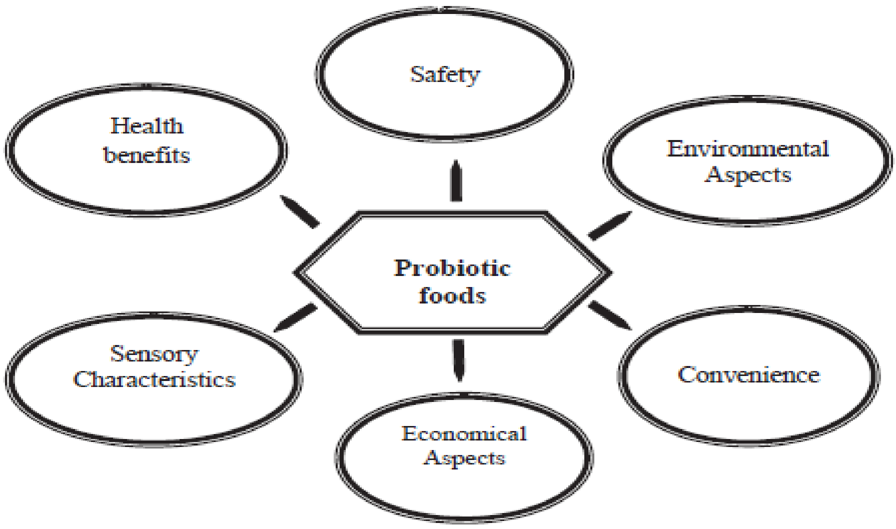

Figure 2.1 Qualitative aspects of probiotic food products

Source: Tripathi and Giri (2014)

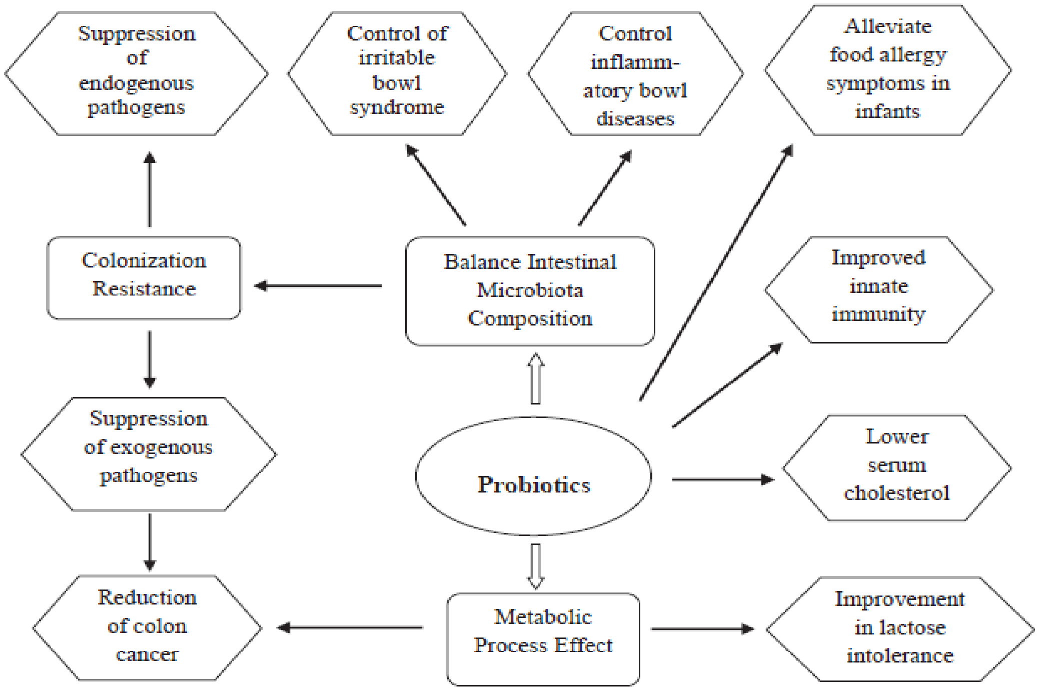

Figure 2.2 Probiotics consumption and health benefits

Source: Parves et al. (2006)

There are several evidences supporting potential clinical applications of probiotics in the prevention and treatment of gastrointestinal, urinogenital tracts and respiratory diseases (Gardiner et al., 2002). Mann and Spoerry (1974) discovered that blood serum cholesterol levels reduced significantly by drinking yogurt fermented with wild strains of *Lactobacillus sp*. Harrison et al. (1975) reported that serum cholesterol decreased by consuming infant formula added with cells of *Lb. acidophilus*. Similarly, Gilliland (1990); Gill and Guarner (2004) showed control of serum cholesterol levels in adult human experiments. It is hypothesized that these benefits may result from the growth and action of the probiotics during the manufacturing of cultured foods, while some may result from the growth and action of certain species of probiotics in the intestinal tract (Rasic, 2003 cited in Tripathi and Giri, 2014).

2.2 Lactic acid bacteria (LAB)

LAB is regarded as a major group of probiotic bacteria (Metchnikoff, 1908; Schrezenmeir and de Verse, 2001) and it constitutes an integral part of the healthy gastrointestinal (GI) microecology and is involved in the host metabolism. Fermentation has been specified as a mechanism of probiotics (Gibson and Fuller, 2000; Metchnikoff, 1908). LAB along with other gut microbiota ferment various substrates like lactose, biogenic amines and allergenic compounds into SCFA and other organic acids and gases (Gibson and Fuller, 2000; Jay, 2000). In addition, it can synthesizes enzymes, vitamins, antioxidants and bacteriocins. With these properties, intestinal LAB constitutes an important mechanism for the metabolism and detoxification of foreign substances entering the body (Salminen, 1990).

2.2.1 Metabolism of LAB fermentation

Two main hexose fermentation pathways are used to classify LAB genera. Under conditions of excess glucose and limited oxygen, homolactic LAB catabolism one mole of glucose in the Embden-Meyerhof-Parnas (EMP) pathway to yield two moles of pyruvate. Intracellular redox balance is maintained through the oxidation of NADH, concomitant with pyruvate reduction to lactic acid. This process yields two moles ATP per glucose consumed. Representative Homolactic LAB genera include *Lactococcus, Enterococcus, Streptococcus, Pediococcus,* and group I lactobacilli. Heterofermentative LAB use the pentose phosphate pathway, alternatively referred to as the pentose phosphoketolase pathway. One mole of glucose-6-phosphate is initially dehydrogenated to 6-phosphogluconate and subsequently decarboxylated to yield one mole of CO_2. The resulting pentose-5-phosphate is cleaved into one mole glyceraldehyde phosphate (GAP) and one mole acetyl phosphate. GAP is further metabolized to lactate as in homofermentation, with the acetyl phosphate reduced to ethanol via acetyl-CoA and acetaldehyde intermediates. In theory, end-products (including ATP) are produced in equimolar quantities from the catabolism of one mole of glucose. Obligate heterofermentative LAB include *Leuconostoc, Oenococcus, Weissella*, and group III lactobacilli.

2.2.2 Carbohydate fermentation patterns

2.2.2.1 Homo-and Heterolactic fermentation

Because LAB do not possess a functional respiratory system, they have to obtain their energy by substrate-level phosphorylation. With hexoses there are two basic fermentation pathways homofermentative pathway based on glycolysis or EMP and producing virtually only lactic acid and heterofermentative or heterolactic fermentation (also known as pentose phosphoketolase pathway, hexose monophosphate shunt, or 6-phosphogluconate pathway) producing in addition to lactic acid, significant amounts of CO_2 and ethanol or acetate. Theoretically, homolactic fermentation produces 2 moles of ATP per mole of glucose consumed. In heterolactic fermentation the corresponding yield is only 1 mole of ATP if acetyl phosphate formed as an intermediate is reduced to ethanol. However, if acetyl phosphate is converted to acetic acid in the presence of alternative electron acceptors, an extra ATP is formed (Wright and Axelsson, 2012).

Hexoses other than glucose (mannose, galactose, fructose) enter the major pathways above after different isomerization and phosphorylation steps as either glucose-6-phosphate. For galactose there are two different pathways, depending on whether it enters the cell as galactose-6-phosphate or as free galactose imported by a specific permeate. List of lactobacilli by their different fermentation patterns as were shown in Table 2.1 (Hammes and Vogel, 1995).

The fermentation type is an important taxonomic criterion. The division of lactobacilli in three patterns:

(1) Group I: Obligate homofermentative lactobacilli

(2) Group II lactobacilli are facultatively heterofermentative

(3) Group III lactobacilli are obligate heterofermentative

2.2.2.2 Fermentation of disaccharide

As a case of lactose in milk, lactose is cleaved to glucose and galactose by ß-galactosidase, and both these monosaccharide can subsequently enter the major fermentation pathways. In the case of lactose-specific PEP: PTS system another enzyme, phosphor- ß-D-galactosidase, is needed to split lactose phosphate to glucose and galactose-6-6phosphate. Glucose is then processed by the glycolytic pathway, while galactose-6-phosphate enters the tagarose-6-phosphate pathway (Wright and Axelsson, 2012).

Table 2.1 List of lactobacilli separated by their different fermentation patterns

Fermentation type	Species
obligately homofermentative	*Lb. acidophilus*, *Lb. amylophilus, Lb.amylovorus, Lb. crispatus, Lb. debrueckii* subp. *bulgaricus, Lb. debrueckii* subp. *delbrueckii, Lb. debrueckii* subp. *lactis, Lb. gallinarum, Lb. gasseri, Lb. helverticus, Lb. jensenii, Lb. johnsonii, Lb. kefiranofaciens, Lb. aviaries* subsp. *araffinosus*, *Lb. aviarius* subsp *aviarius, Lb. farciminis,Lb. salivarius* subsp. *salicinus*, *Lb. salivarius* subsp. *salivarius*, *Lb. mali, Lb. ruminis, Lb. sharpeae*
facultatively heterofermentative	*Lb. acetotolerans, Lb. hamster, Lb. alimentarius, Lb. bifermentans, Lb. casei, Lb. coryniformis* subsp. *coryniformis, Lb. coryniformis* subsp. *torquens, Lb.curvatus, Lb. graminis, Lb. homohiochii, Lb. intestinalis, Lb. murinus, Lb. paracasei* subsp. *paracasei, Lb. paracasei* subsp. *tolerans, Lb. rhamnosus, Lb. sake, Lb. agilis, Lb. pentosus, Lb. plantarum*
obligately heterofermentative	*Lb. brevis, Lb. buchneri, Lb. collinoider, Lb. fermentum, Lb. fructivorans, Lb. hilgardii, Lb. kefir, Lb. malefermentans, Lb. oris, Lb. parabuchneri, Lb. reiteri, Lb. pontis, Lb. vaginalis, Lb. suebicus, Lb. vaccinostercus, Lb. sanfrancisco, Lb. connfusus, Lb. confuses, Lb. fructosus, Lb. halotolerans, Lb. viridescens, Lb. kandleri, Lb. kandleri, Lb. minor*

Source: modified from Hammes and Vogel (1995)

2.2.3 Microbial growth

Bacteria grow or multiply in numbers when exposed to a favorable environment such as food. Growth important to isolate an unknown bacteria strain involved in food bioprocessing and studies its physiological, biochemical in order to design methods to controls its growth in food.

2.2.3.1 Growth curve (Roy, 2003)

The growth rate and growth characteristics of bacteria population under a given condition can be graphically represented by counting cell numbers, enumerating CFUs, or measuring OD in a spectrophotometer at a given wavelength (above 300 nm, usually at 600 nm) of a cell suspension. If the CFU values are enumerated at different times of growth and a growth curve is plotted using log10 CFU vs time (log10 CFU is used because of high cell numbers), a plot similar to the one illustrated in Figure 2.3. The plot has several features that represent the conditions of the cells at different times.

Initially, the population does not change (lag phase). During this time, the cells assimilate nutrients and increase in size. Although the population remains unchanged because of change in size, both cell mass and OD show some increase. Following this, the cell number starts increasing, first slowly and then very rapidly. The cells in the population differ initially in metabolic rate and some multiply, and then almost all cells multiply. This is the exponential phase (also called logarithmic phase). Growth rate at the exponential phase follow first-order reaction kinetics and can be use to determine generation time.

Following this, the growth rate slows down and finally the population enters the stationary phase. At this stage, because of nutrient shortage and accumulation of waste products, a few cells die and a few cells multiply, keeping the living population stable. However, if one counts the cells under the microscope or measures cell mass, both may show an increase, as dead cells may remain intact. After the stationary phase, the population enters the death phase, in which the rate of cells death is higher than the rate of cell multiplication. Depending on the strain and conditions of the environment, after a long period of time some cells may still remain viable. This information is important to determine some microorganism's criteria in food, especially controlling spoilage and pathogenic microorganisms in food.

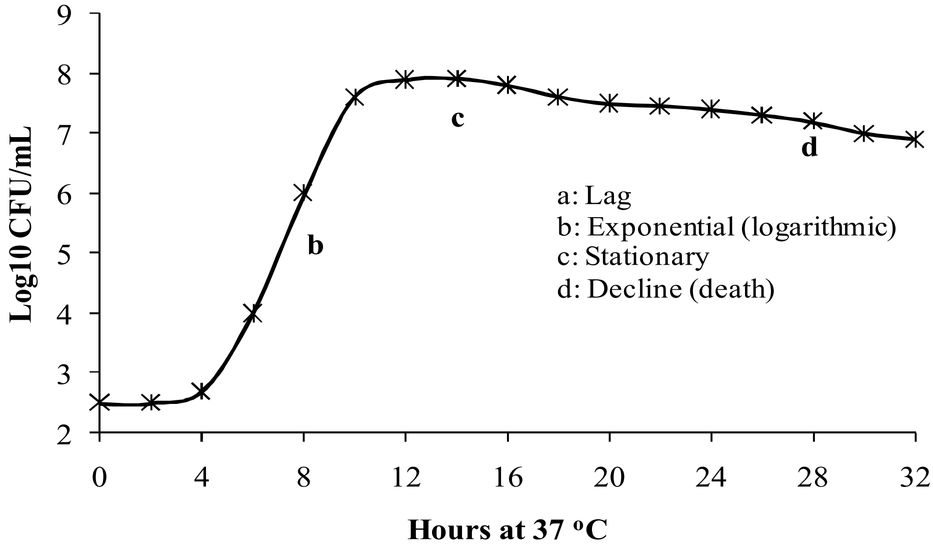

Figure 2.3 Bacterial growth curve showing changes in cell numbers of *Pediococcus acidilactici* H during 32 h incubation at 37°C in a broth.
Source: Ray (2003)

2.2.3.2 Carbohydrates and Growth

Major carbohydrates present in different foods, either naturally or added as ingredients, can be grouped on the basis of chemical nature as follows:

(1) Monosaccharides

Hexoses: glucose, fructose, mannose, galactose

Pentose: xylose, arabinose, ribose, riburose, xylulose

(2) Disaccharides

Lactose (galactose + glucose)

Sucrose (fructose + glucose)

Maltose (glucose + glucose)

(3) Oligosaccharides

Raffinose (glucose + fructose + galactose)

Starchyose (glucose + fructose + galactose + galactose)

(4) Polysaccharides

Starch (glucose units)

Glycogen (glucose units)

Cellulose (fructose units)

Hemicellulose (xylose, galactose, mannose units)

Dextrans (α-1,6 glucose polymer)

Pectins

Gums and mucilages

Lactose is found only in milk and thus can be present in foods made from or with milk and milk products. Pentose, most oligosaccharides, and polysaccharides are naturally present in foods of plant original.

All microorganisms normally found in food metabolize glucose, but their ability to utilize other carbohydrates differs considerably. This is because of the inability of some microorganisms to transport the specific monosaccharides and disaccharides inside the cells and inability to hydrolyze polysaccharides outside the cells.

Food carbohydrates are metabolized by microorganisms principally to supply energy through several metabolic pathways. Some of metabolic products can be used to synthesize cellular components of microorganisms (e.g. to produce amino acids by amination of some keto acids). Microorganisms also produce metabolic by products associated with food spoilage (CO_2 to cause gas defect) or food bioprocessing (lactic acid in fermented foods). Some are also metabolized to produce organic acids, such as lactic, acetic, propionic, and butyric acids, which have an antagonistic effect on the growth and survival of many bacteria (Roy, 2003).

2.2.4 Bacterial structure

The bacterial cell wall provides structural integrity to the cell, but differs from that of all other organisms due to the presence of peptidoglycan (poly-N acetylglucosamine and N-acetylmuramic acid), which is located immediately outside of the cytoplasmic membrane. Peptidoglycan is responsible for the rigidity of the bacterial cell wall, and determines the cell shape. It is also relatively porous and considered as an impermeability barrier to small substrates. The cell walls of all bacteria are not identical. In fact, the cell wall composition is one of the most important factors in the analysis and differentiation of bacterial species. Accordingly, two general types of bacteria exist, of which Gram-positive bacteria (Figure 2.4-2.5) are comprised of a thick peptidoglycan layer connected by amino acid bridges. Imbedded in the Gram-positive cell wall are polyalcohols, known as teichoic acids, some of which are lipid linked to form lipoteichoic acids. Due to lipoteichoic acids are covalently linked to lipids within the cytoplasmic membrane, they are responsible for linking peptidoglycan to the cytoplasmic membrane. The cross-linked peptidoglycan molecules form a network, which covers the cell like a grid. Teichoic acids give the Gram-positive cell wall an overall negative charge, due to the presence of phosphodiester bonds between the teichoic acid monomers. In general, 90% of the Gram-positive cell wall is comprised of peptidoglycan.

On the contrary, the cell wall of Gram-negative bacteria (Figure 2.4-2.5) is much thinner, and composed of only 10–20% peptidoglycan. In addition, the cell wall contains an additional outer membrane composed of phospholipids and lipopolysaccharides. The highly charged nature of lipopolysaccharides confers an overall negative charge on the Gram-negative cell wall. Sherbert (1978) showed that the anionic functional groups present in the peptidoglycan, teichoic acids and teichuronic acids of Gram-positive bacteria, and the peptidoglycan, phospholipids, and lipopolysaccharides of Gram-negative bacteria were the components primarily responsible for the anionic character and binding capability of the cell wall (Vijayaraghavan and Yun, 2008).

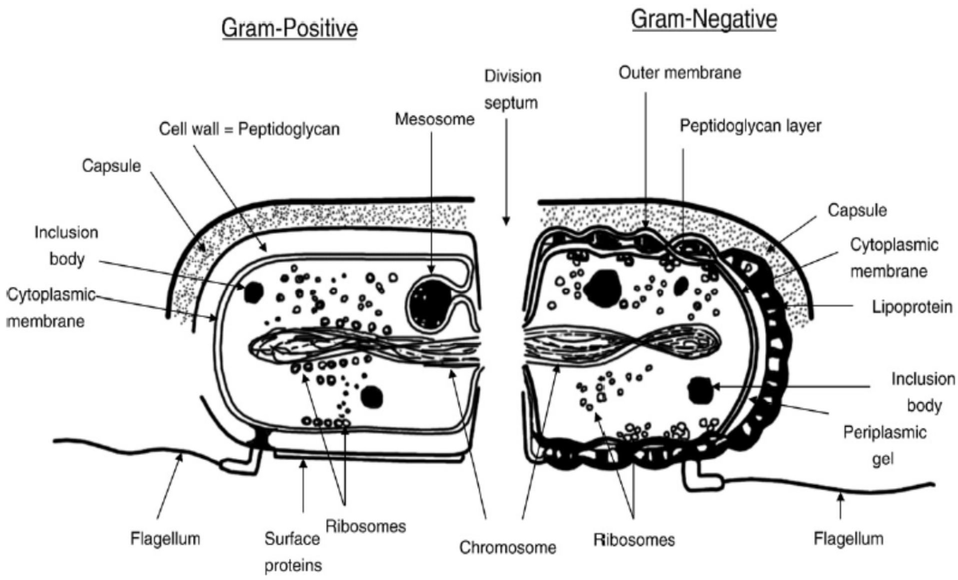

Figure 2.4 Structure of Gram-positive and negative bacteria

Source: Vijayaraghavan and Yun (2008)

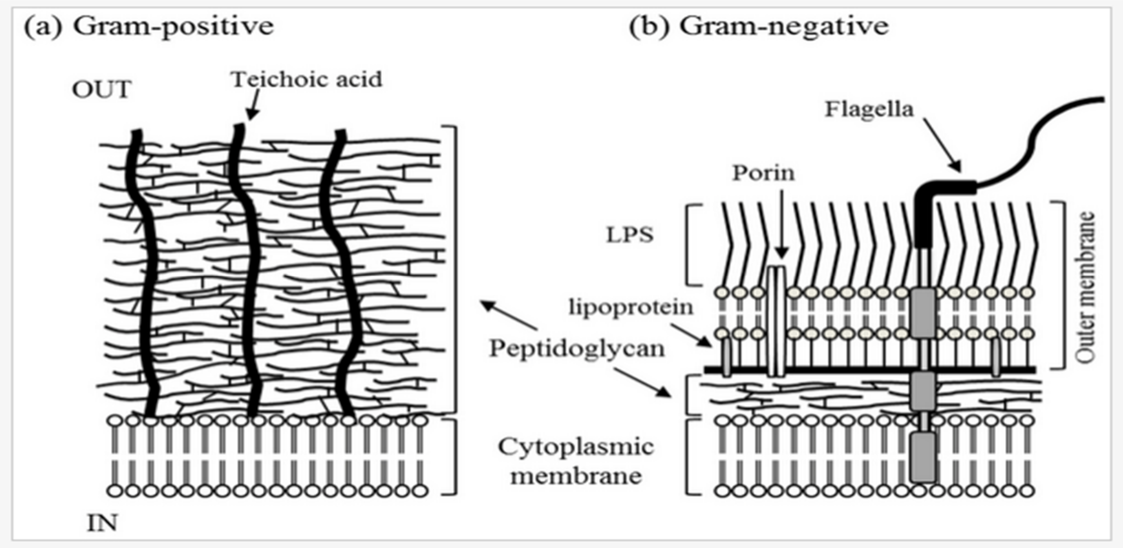

Figure 2.5 Cell wall characteristic of Gram-positive via Gram-negative bacteria. (a) Gram- positive bacteria have a thick wall composed of peptidogycans; and (b) Gram-negative bacteria have an outer membrane and a thin wall composed of peptidoglycans.
Source: Nakano et al. (2013)

2.2.5 *Lactobacillus* sp.

Lactobacillus is a Gram-positive facultative bacterium. They are a major part of LAB group having the ability to convert lactose and other monosaccharides to lactic acid. Intestinal lactic acid bacteria for humans are closely associated with the host's health because they act as an important biodefense in preventing colonization and subsequent proliferation of pathogenic bacteria in the intestine. Some species of *Lactobacillus* and *Bifidobacterium* that have been claimed as probiotics included *Lb. acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. casei*, *Lb. fermentum*, *L.b plantarum*, *Lb. reuteri*, *B. infantis*, *B. breve*, *B. animalis*, *B. adolescentis* and *B. longum*. The gastrointestinal tract of a healthy human is a harsh environment and poses a significant threat to probiotic strains. In addition, low surface tension and immune response also affect the survival of probiotic strains (Gilliland, 1979).

Lactobacillus have several scientifically established and/or clinically proved health effects, such as reduction and prevention of diarrhoea, improvement of the intestinal microbial balance by antimicrobial activity, alleviation of lactose intolerance symptoms, prevention of food allergy, enhancement of immune potency, and antitumourigenic activities(Liong, 2006). Since milk fermented with lactobacilli was first demonstrated to exhibit hypocholesterolemic effects in humans, various studies have shown that some lactobacilli exhibit cholesterol-reducing ability in human (Liong and Shah, 2005a).

2.2.6 *Lactobacillus pentosus*

Lactobacillus pentosus (*Lb. pentosus*) has since been identified as playing a traditional role in the preparation of many common foods. Also, including raw and soured milks (Kim et al., 2006), fermented cereals and vegetables (Tamminen et al., 2004), fermented meats and fish (Tanasupawat et al., 1998), and fermented beverages such as sake, tea and Scotch malt whiskey (Tanasupawat and Komagata. 1994). *Lb. pentosus* is consumed as live viable colonies in uncooked foods (e.g. soured milks and cheeses) and as dead nonviable cells in fermented foods having a later cooked stage (e.g. sourdough breads); the intentional use and natural presence of *Lb. pentosus* in foods has been international in scope, encompassing countries throughout Europe, Africa and Asia. Similar to other *Lb. pentosus* strains, strain b240 was originally isolated from Mieng, a non-salted fermented tea traditional to northern Thailand (Tanasupawat et al., 2007). Initially identified as a strain of *Lb. plantarum* (a phenotypically similar species), the bacterium was later reclassified as *Lb. pentosus* by genetic analysis using recA (Szabo et al., 2011)

Lb. pentosus is facultatively heterofermentative, as well as the so-called Group II lactobacilli. Gram-positive bacteria, rod shape with rounded ends 1-1.2 × 12-5.0 micron. The characteristics of *Lb. pentosus* are shown in table 2.2.

Table 2.2 Key characteristics of *Lb. pentosus* and *Lb. plantarum* strain

Characteristics	*Lb. pentosus*	*Lb. plantarum*
peptidoglycan type	DAP	DAP
G+C content (mol (%))	46-47.2	44-46
lactic acid isomer (s)	racemic-DL	racemic-DL
growth 15/45 (^{o}C)	+/-	+/-
carbohydrates fermented	amygdalin, L-arabinose, arbutin, galactose, cellobiose, D-fructose, β-gentiobiose, glucanate, D-glucose, glycerol, *N*-acetyl glucosamine, lactose, D-mannose, mannitol, maltose, melibiose, raffinose, ribose, salicin, sorbitol, sucrose, trehalose and D-xylose	amygdalin, L-arabinose, cellobiose, esculin, mannitol, melezitose, melibiose, raffinose, ribose, sorbitol, sucrose,

Symsbols: +, 90% or more of strains are positive; -, 90% or more of strains are negative, DAP, diaminopimelic acid. Parenthesized isomer indicate < 15% of total lactic acid. Source: modified from Zanoni *et al*. (1987); Hammes and Vogel (1995)

2.2.7 Benefits of LAB in food fermentation

Food fermentation is one of the oldest food processing and preservation methods in Asia. Fermentation adds safety, nutritional value and different flavors to what could have otherwise been a bland diet. Foods that are fermented have been subjected to the actions of microorganisms or enzymes, so that desirable biochemical changes have occurred. Moreover, fermentation is a relatively cost-effective, low energy preservation process, which is essential in ensuring the shelf-life and microbiological safety of the product (Liu et al, 2011).

Although the primary purpose was to achieve food safety, fermentation plays at least five roles:

(1) Enrichment of the diet through development of a diversity of flavors, aromas, and textures in food substrates;

(2) Preservation of food through lactic acid, alcoholic, acetic acid, and alkaline fermentations;

(3) Biological enrichment of food substrates with proteins, essential amino acids, essential fatty acids and vitamins;

(4) Detoxification during food fermentation processing; and

(5) Decrease in cooking times and fuel requirements (Steinkraus, 1996).

Fermented dairy products represent about 20% of the total economic value of fermented foods produced world-wide and the market share of such products continues to grow dairy industry is a prime consumer of various LAB strains such as *Lactobacillus, Lactococcus,* and *Leuconostoc*. For this reason, LAB used to be called "milk-souring organisms" (Liu et al, 2011).

2.3 Short chain fatty acids (SCFAs)

Short chain fatty acids (SCFAs) are synthesized by the gastrointestinal microflora. The end products from carbohydrates as substrate, are mainly SCFAs; acetic, propionic and butyric acid together with gases; CO_2, H_2, and methane. SCFAs are carboxylic acids with 1 to 5 carbon atoms that include different other functional groups, such as hydroxyl or dicarboxyl. SCFAs with different carbon chain lengths (acetate (C2), propionate (C3), butyrate (C4), valerate (C5)) are produced in varying amounts depending on the diet and the composition of the intestinal microbiota.Most microorganisms in the colon prefer to ferment carbohydrates and switch to protein fermentation when fermentable carbohydrates are depleted. While carbohydrate fermentation generally leads to health-promoting SCFAs production, protein fermentation yields branched-chain fatty acids and potentially toxic metabolites (e.g. ammonia, amines, phenols, indoles and thiols). In humans, SCFAs such as acetate, propionate and butyrate are produced as the major end products of anaerobic fermentation in large intestine resulting in significant health benefits such as anti-cancerous effect. Also SCFAs are known to fulfill 60–70% of the energy requirement of the colonocytes (Sreenivas and Lele, 2013). SCFAs arise from bacterial fermentation of carbohydrates, proteins, peptides and glycoprotein precursors. Approximately 80-90% of SCFAs, which are produced from the breakdown of dietary food, are absorbed in colon while the rest are excreted in faces (Huda-Faujan, 2010). SCFAs are rapidly absorbed and have shown to have distinct bioactivity depending on their chain length. With regard to maintenance of colonic health and barrier function. In addition, their important role as fuel for intestinal epithelial cells, butyrate has drawn most attention, as this fatty acid is the major energy source for the colonocytes. However, butyric, acetic, and propionic acids have mainly been emphasized. In particular, butyric acid was addressed to be more beneficial for promoting colonic health and more effective for stimulating the proliferation of intestinal mucosal cells than acetic and propionic acids. Butyric acid is also the main energy substrate for the colonocytes and it has been suggested to play an important role in the prevention and treatment of distal UC, Crohn's disease (CD), and cancer (Huda-Faujan, 2010). Furthermore, butyrate has been shown to have anti-inflammatory properties and to have anti-carcinogenic effects. SCFAs content in faces could be used as a biomarker for the physiological processes in the organisms as well as for the effect of nutritional interventions.

According to Scheppach (1994) about effects of SCFAs on colonic morphology and function (facts and hypotheses) in figure 2.6. Their production during bacterial carbohydrate (starch, fibre) fermentation is well established. SCFAs are the preferred energy substrates of colonocytes, especially in the distal large bowel. Probably linked to this property, they affect a range of mucosal events (absorptive processes, blood flow, mucus release, cellular differentiation and proliferation). These effects of SCFAs are possibly clinically important (adaptation to postoperative conditions, prevention of colitis).

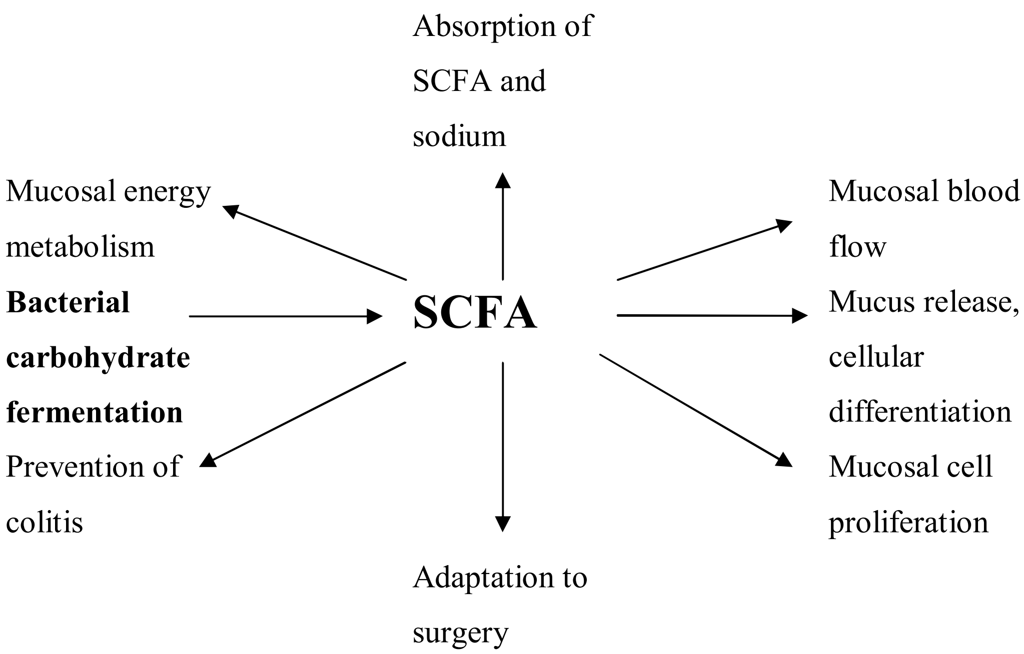

Figure 2.6 Effects of short chain fatty acids (SCFAs) on colonic morphology and function (facts and hypotheses).
Souce: Scheppach (1994)

2.3.1 Implication of substrate

The bacterial species present in the colon use different fermentation pathways, leading to differences in the SCFAs pattern generated. Indigestible carbohydrates that reach the colon are mainly non-starch polysaccharides (NSPs), resistant starches (RS) and certain oligosaccharides. In particular, NSPs are important energy substrates for large intestinal microbial fermentation, and the amount as well as the chemical and structural composition of the carbohydrate is important factors for the microbial activity in the gastrointestinal tract. Most mono- and disaccharides are rapidly absorbed in the upper intestinal tract and provide a readily available source of energy. An exception is lactose, a P-linked disaccharide, which is poorly absorbed by most of the adult population in the world due to low levels of lactase in the small intestine.

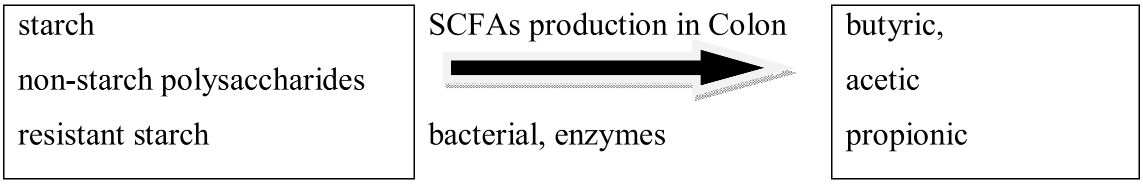

Figure 2.7 The carbohydrates as substrate fermented in the distalileum and colon
Source: Floch (2010)

When lactose is malabsorbed in the small intestine, it reached the colon for fermentation. Resistant peptides may also enter the colon and amino acid fermentation yields branched SCFAs. Starch and NSPs are fermented in the distalileum and colon. Fiber, NSPs (soluble fiber from fruits and vegetables), and prebiotics are fermented primarily by bacterial enzymes as shown in figure 2.7. Humans have essentially no enzymes that permit their production, whereas bacteria extensively ferment and produce them in the molecular ratio of approximately 60:20:20 for acetic, butyric, and propionic. The amount produced varies with different organisms and the availability of different substrates (Floch, 2010).

2.3.2 Nutritional and Health benefits of SCFAs

The SCFAs absorbed from the colon can be utilized as an energy source by the host but they contribute only to a small part (5-10%) of total energy. The colonic mucosa obtains its energy by oxidizing mainly SCFAs in the order of butyric > propionic > acetic acid. The SCFAs that escape metabolism in the colon enter the hepatic portal blood as illustrate in figure 2.8. The liver utilizes acetic acid where it is transferred into Acetyl-CoA, which can act as a precursor for lipogenesis, but also stimulates gluconeogenesis. Acetic acid in low level of concentrations can also be detected in venous blood in peripheral tissues. Propionic acid is mainly metabolized in the liver and has been proposed that may lower plasma cholesterol concentrations by inhibiting hepatic cholesterogenesis. Butyric acid is the main energy for the colonocytes. The colon epithelial cells use butyric acid before they use glucose as a nutrient (Henningsson, 2001).

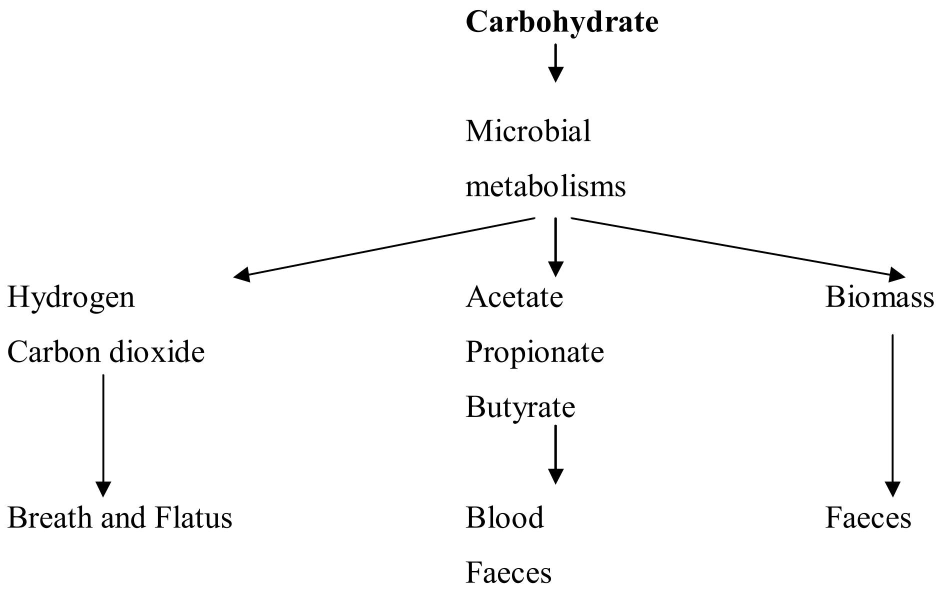

Figure 2.8 Carbohydrate fermentation in the human colon
Source: Henningsson et al. (2001)

Acetic acid is the building block for cholesterol. The functions of propionic acid are not well understood. In humans, the synthesis of cholesterol from acetic acid decreased when propionate was infused rectal. However, they seem to affect the rate of cholesterol metabolism. It is suggested that substrates that can decrease the acetic acid and propionic acid ratio may actually improve lipid risk factors. Furthermore, as much as 5% to 10% of absorbed energy can come from SCFA metabolism. Butyric acid is considered beneficial for gut health, because of it serves the main energy substrate for the colonocytes and metabolized by the cells in preference to glucose or glutamine, accounting for 70% of the total energy demand of the colonic mucosa. It has been reported to be important in the prevention and treatment of diseases of the colonic mucosa, such as distal ulcerative colitis and cancer (Henningsson, 2001). Furthermore, the energy derived from the SCFAs can be beneficial to patients with a short bowel when carbohydrates are metabolized by the microflora to produce SCFAs and then absorbed. In addition, increased SCFAs levels may also increase the solubility of certain minerals, such as calcium, and enhance the absorption and expression of calcium-binding proteins. The entire subject of microorganisms producing substances such as

SCFAs that are beneficial to the host requires further experimentation to see the therapeutic and beneficial effects (Scheppach et al.1995).

2.4 Cholesterol reducing activity

Probiotics have been considered to have potential health-promoting benefits as biotherapeutic agents (Begley et al., 2006). One of the health-promoting benefits of probiotics is their ability to reduce cholesterol (Silirun et al., 2010). Probiotic strains, especially LAB have a major role to play in the cholesterol lowering mechanism. As the cholesterol level keeps increasing in the serum, it leads to cardiac diseases. From many reports, several actions to reduce cholesterol associated with *Lactobacillus* spp. have been described as cholesterol assimilation by the bacteria, cholesterol binding to the bacterial cell wall, and BSH deconjugating of bile salt (Pereira and Gibson, 2002; Kim et al., 2008).

2.4.1 Mechanisms of cholesterol reducing effects

Several mechanisms have been suggested for cholesterol reducing activity of probiotics include deconjugating bile acids through bile salt hydrolase catalysis (Homayouni et al, 2012). Cholesterol is the precursor for the systhesis of new bile acids, the use of cholesterol to synthesize new bile would lead to a decreased concentration of cholesterol in blood. Bacteria tend to take up and assimilate cholesterol for stabilization of their cell membrane and binding cholesterol to cell walls of probiotics in intestine. Conversion of cholesterol into coprostanol (Lye et al., 2010) and SCFA such as propionate produced by probiotic bacteria may also inhibit hepatic cholesterol synthesis and/or redistribution of cholesterol from plasma to the liver (Pereira and Gibson, 2002b) hypocholesterolemic effect via altering the pathways of cholesteryl esters and lipoprotein transporters as described in Figure 2.9 (Ooi and Liong, 2010).

The mechanisms of probiotic activity to reduce cholesterol that have been proposed to three ways (assimilating, binding or by degradation).

(1) assimilate the cholesterol for their own metabolism

(2) cholesterol adherence to the bacterial cell wall or its incorporation into cells and could be bound to the cholesterol molecule,

(3) (probiotics are capable of degrading cholesterol to its catabolic products (Mahrous, 2011).

In addition, the physiological action of the end-products of SCFAs by fermentation, destabilization and co-precipitation of the cholesterol micelles, bile salt hydrolase activity of the lactobacilli, cholesterol oxidize activity, and finally production of some functional peptides.

Bile is a water-soluble end product of cholesterol in the liver, is stored and concentrated in the gallbladder, and released into the duodenum upon ingestion of foods. It consists of cholesterol, phospholipids, conjugated bile acids, bile pigments and electrolytes. Once deconjugating, bile acids are less soluble and absorbed by the intestines, leading to their elimination in the feces. Cholesterol is used to synthesize new bile acids in a homeostatic response, resulting in lowering of serum cholesterol as described in Figure 2.10 (Beglay et al., 2006). Bile salt hydrolase (BSH) is the enzyme responsible for bile salt deconjugation in the enterohepatic circulation. It has been detected in probiotics indigenous to the gastrointestinal tract and able to hydrolyze conjugated glycodeoxycholic acid and taurodeoxycholic acid, leading to the deconjugation of glyco- and tauro-bile acids.

The hypocholesterolemic effect of the probiotics has also been attributed to their ability to bind cholesterol in the small intestines. Usman (1999) reported that strains of *Lb. gasseri* could remove cholesterol from laboratory media via binding onto cellular surfaces. The ability to bind cholesterol appeared to be growth and strain specific. Kimoto *et al.* (2002) evaluated the cholesterol removal by probiotics cells during different growth conditions. Living and growing cells were compared with nongrowing (live but suspended in phosphate buffer) and dead cells by heat-killed. The authors found that although growing cells removed more cholesterol than dead cells, the heat-killed cells could still remove cholesterol from media, indicating that some cholesterol was bound to the cellular surface (Ooi and Liong, 2010).

Reduction of cholesterol, in the added cholesterol media is considered as an indication for the selection of probiotic strains with cholesterol assimilation property

(Gilliland and Walker, 1990; Lin and Chen, 2000). Some researchers have carried out the cholesterol reducing in the MRS media before and after the complete growth of *Lactobacillus* strains as a typical approach (Gilliland and Walker, 1990; Lin and Chen, 2000; Liong and Shah, 2005a). The researchers have been used a colorimetric method base on cholesterol-O-phthalaldehyde reaction, constitute the principles of Rudel and Morris (1973) for determined the cholesterol removal (Mirlohi et al., 2012). Since cholesterol reducing is a health-promoting characteristic, the idea of selection of microbial strains with cholesterol reducing effect has been developed as a tool to introduce new probiotic microorganisms (Madani, 2013).

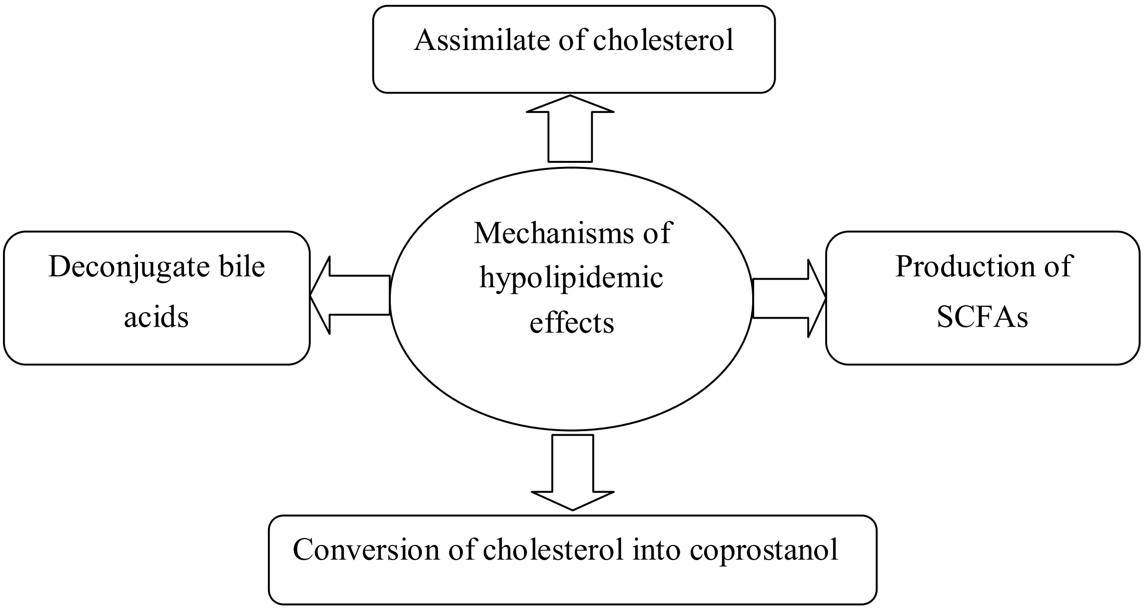

Figure 2.9 Mechanisms of hypocholesterolemic effect
Source: Mahrous (2011)

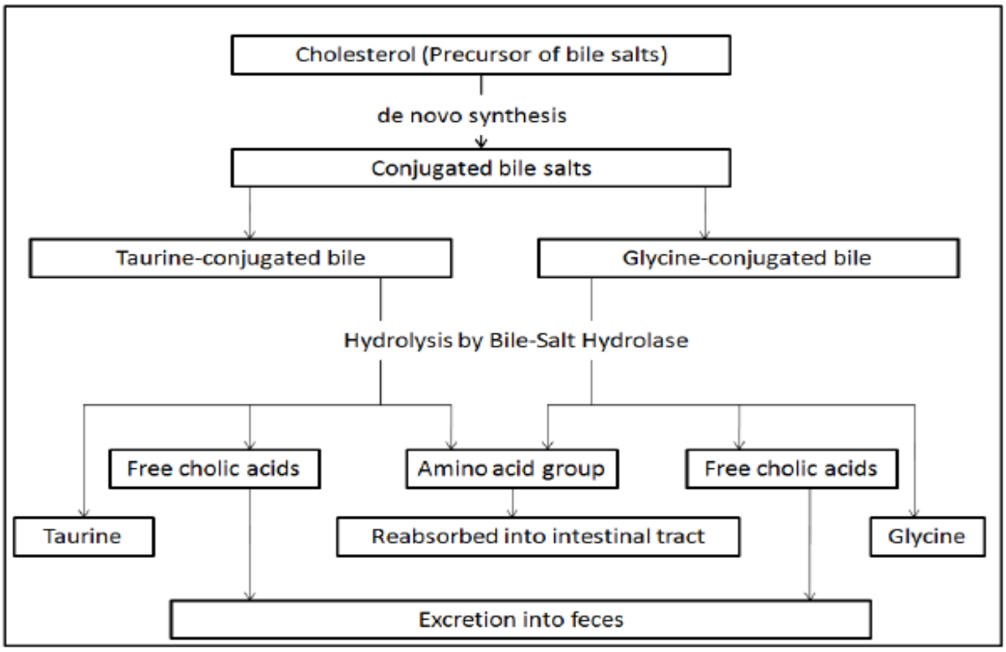

Figure 2.10 Cholesterol as the precursor for the synthesis of new bile acids and the role of bile salt hydrolase for hypocholesterolemic
Source: Ooi and Liong (2010)

2.5 Mycotoxins

Mycotoxins are the secondary metabolites produced from toxigenic fungi recognized as major food and feed contaminants. They are a source of grave concern in food contamination, resulting in mycotoxicosis in humans and animals. Typically, toxin production is influenced by moisture, time, temperature, and food or feed substrates. Contamination can occur throughout the food chain from the field, during harvesting, processing, storage, transportation, and consumption (Ezekiel et al, 2008; Anukul, 2013). The mycotoxins may be cause carcinogenic, mutagenic, teratogenic, estrogenic, neurotoxic, and immunotoxic for animals or humans (Joannis-Cassan et al., 2011). Many of the developed countries have regulations for mycotoxins in food grains and its products. However, the risk of mycotoxin exposures continues in the developing countries due to lack of food security, poverty and malnutrition (Shetty and Jespersen, 2006).

2.5.1 Zearalenone

Zearalenone (ZEA) is an estrogenic mycotoxin that can be produced by several field fungi including *Fusarium* graminearum (Gibberella zeae), *F.* culmorum, *F.* cerealis, *F.* equiseti, *F.* semitectum and *F. crookwellense*. Fungi of the genus *Fusarium* infect cereals pre-harvest in the field during blooming, but growth and toxin production may also occur post-harvest under poor storage conditions. The toxin is common in maize, but because the spores of *Fusarium* are ubiquitous, cereal crops such as barley, oats, wheat (Oliveira et al. 2014; Avantaggiato et al., 2003), rice, sorghum and soybeans are also susceptible to contamination with ZEA, both in the temperate and warmer climate zones (Golinski et al., 2010). Many countries have gathered data on occurrence of ZEA in (mainly grain-based) foods (EFSA, 2004 and Anukul et al , 2013). Depending on climatic, harvest and storage conditions, the concentrations of ZEA found in cereals and cereal products range from less than 1 to over 300 mg/kg, but rarely exceed 10 mg/kg (IARC, 1993).

ZEA not only leads to economic loss by contaminating food and feed, but it causes serious health problems in livestock and humans as well. ZEA causes alterations in the reproductive tract of laboratory animals (mice, rats, guinea pigs) and farm animals. Decreased fertility, increased number of resorptions, reduced litter size, changed weight of adrenal, thyroid and pituitary glands and change in serum levels of progesterone and oestradiol have been observed, but no teratogenic effects were found. Occurrence in mixed feeds associated with hyperoestrogenism has been reported in farm animals, particularly in pigs (Kuiper-Goodman et al. 1987). It can cause estrogenic effects, inducing reproductive toxicological effects in domestic animals and may act as a key factor in certain pregnancy disorders in humans (Zinedine et al., 2007; Lu et al. 2011).

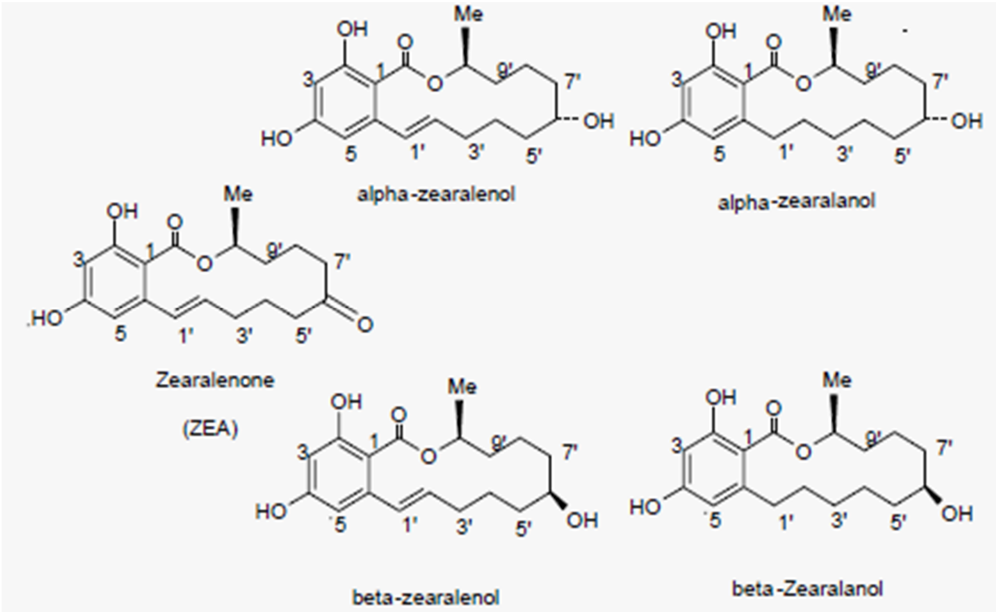

Figure 2.11. Chemical structure of ZEA and its major metabolites
Source: EFSA (2004)

ZEA (formerly denoted F2-toxin) is a resorcyclic acid lactone chemically described as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcyclic acid lactone ($C_{18}H_{22}O_5$, MW: 318.36) (Figure 2.11). The structure of ZEA allows its binding to mammalian oestrogen receptors (EFSA (2004). Estrogenic syndrome is the major toxic effect of ZEA as its structure resembles that of 17-beta-estradiol, an estrogen hormone. Hyperestrogen, vulvovaginitis, and estrogenic responses from ZEA are observed in estrogenic target cells. An amount of 1 mg/kg in feed can cause estrus in swine. maximum tolerable. ZEA was evaluated by the IARC base on Cancer. Based on inadequate evidence in humans and limited evidence in experimental animals, zearalenone was placed, together with other *Fusarium* toxins, in Group 3 (not classifiable as to their carcinogenicity to humans) (IARC, 1993). The provisional maximum daily intake established by JECFA was 0.5 µg/kg body weight (EFSA, 2004).

2.5.2 ZEA Contaminated in foods product and feeds

From Biomin's mycotoxin survey report 2011, ZEA contamination of was found highest in Asia (53%) of positive samples compared in Europe (35%), South America (28%), Oceania (26%), North America (14%), and Africa (8%), Middle East

(0%), respectively. Never the less, in Asia, the data showed highest by North Asia (63%), Southeast Asia (37%), and South Asia (4%). Average amount of ZEA contamination in Asia was 129 mg/kg, which was still within the range of the maximum limits regulated in Asian countries (Anukul et al, 2013). The regulation limits for ZEA established in various Asian countries are relatively diverse and different from those set by EU regulations. The maximum limit of ZEA in food and feed commodities in Thailand was 30-1000 ppb (all foods), Japan 1000 ppb (compound feeds) whereas, South Korea 200 ppb (grains and processed), 50 ppb (confectionaries), 20 ppb (baby foods). However, the occurrence of this toxin in processed food was largely lower than that found in the EU and in each country's regulation limits except in Indonesia, which showed a slightly higher level (Anukul, 2013).

Zinedine et al. (2007) reported that several studied found ZEA contaminate in cereals and food products in Asia. The contamination of cereals (barley and wheat) with ZEA was found in Japan (Yoshizawa and Jin, 1995; Yoshizawa, 1997) and barley, barley-based foods, corn and corn-based foods (Park et al., 2002) and rice (Park et al., 2005) were found in Korea. In addition, cereals including maize, wheat and rice were reported to contain ZEA in India, (Phillips et al., 1996; Janardhana et al., 1999). Co-contamination of maize with ZEA, NIV, Fumonisins and aflatoxins is an emerging issue in Philippines and Thailand (Yamashita et al., 1995). The contamination of maize-based food and poultry feeds with ZEA was reported in Indonesia (Nuryono et al., 2005). Throughout the globe, ZEA has been detected in a number of cereal crops such as maize, barley, oats, wheat, rice, sorghum, and rye (Zinedine et al., 2007). Depending on climatic and storage conditions, the contents of ZEA vary within the range of 0.001-8.04 mg/kg (wheat), 0.016-0.095 mg/kg (oat), and 0.004-15 mg/kg (barley) (Placinta et al., 1999).

Soybean is an important legume plant cultivated in many parts of the world for its oil and proteins, which extensively used in the manufacture of human foods and animal feedstuffs. Soybean often attacked by fungal infections and cause of mycotoxin contamination during cultivation, post-harvest or process. There is an increasing world consumer demand for high quality and innocuous food and drink products with the lowest possible level of contaminants such as mycotoxins. As a result, the food industry

in the developed world demands raw ingredients of the best quality and that conform to statutory limits where these have been set for mycotoxins (Barros et al, 2011).
Several approaches have been developed for decontamination of mycotoxins in foods. Though many approaches are available for mycotoxin decontamination, most of them are not widely available due to high cost or practical difficulties involved in detoxification process (Reddy et al., 2010). However, the methods also affected the taste and the quality of food and the toxin was not in an acceptable level (Yaowapa, 2013). In the present, LAB was investigated and further developed to use as a biosorption for reduce toxins contamination in food industry.

2.5.3 ZEA Binding by LAB

LAB is widely used in foods fermented products and is part of the intestinal microflora. Several investigations indicated that LAB has beneficial health effects in humans (Ouwehand et al., 2002). LAB as biopreservative organisms have been the focus of numerous studies. Generally, LAB is accepted as safe for use in food by the Food and Agricultural Organization of the United States (FAO) and by the European Food Safety Authority (EFSA) who have granted many species with Generally Regarded as Safe (GRAS) and Qualified Presumption of Safety (QPS) status, respectively (Franz et al., 2010). LAB is known to deliver desired technological properties and bioprotection in several different food matrices, concurrently enhancing organoleptic and textural qualities of the final product (Oliveira et al. 2014). One of the effects identified is the protection against toxins contained in foods such as heterocyclic aromatic amines, polycyclic aromatic hydrocarbons, reactive oxygen, and mycotoxins (Fuchs et al., 2008). An increasing interest has been generated by the possibility of using microorganisms to reduce mycotoxins. The ability of a mixed culture of bacteria to degrade completely ZEA from culture media was also reported by Megharaj et al. (1997). Also with other fungal toxins such as ZEA, trichothecenes and fumonisins, binding effects have been observed in chemical analytical investigations (Shetty and Jespersen, 2006). It has been shown earlier by El-Nezami et al. (2002) demonstrated that LAB detoxify aflatoxin B1 (AFB1), which is the most potent known human carcinogen and contributes to the high prevalence of liver cancer in regions such as Central Africa and China. Recently, Mokoena et al. (2005) reported that LAB fermentation could significantly reduce the concentration of ZEA in maize by 68–75% in fourth days of fermentation

2.5.4 Mechanism of mycotoxin binding by LAB

Cell wall peptidoglycan and polysaccharide are the two most important elements responsible for the binding of mutagens to LAB (Hirayama and Rafter, 2006). Both of these components are expected to be affected by heating and acids. Heating may cause protein denaturation or the formation of Maillard reaction products between polysaccharides and peptides or proteins, whereas under acidic conditions, the glycosidic linkages in polysaccharides break down releasing monomers that may be further fragmented into aldehydes. Acids may also break the amide linkages in peptides and proteins, producing peptides and the component amino acids. The peptidoglycan structure of the cell wall is usually quite thick in LAB but its thickness may be reduced and/or its pore size may be increased via heat and acid treatments. (El-Nezami et al., 2002).

Several LAB have been found to be able to bind mycotoxins *in vitro*/*in vivo* with efficiency depending on the bacterial strain. Mathematical model suggests the attachment of toxins molecules to the surface of the organism and takes two processes into consideration: binding (adsorption) and release (desorption) of toxin to and from the binding site on the surface of the microorganisms (Bueno et al., 2006). The cell walls of some LAB have been reported to be able to bind some mutagenic compounds such as amino acid pyrolysates and heterocyclic amino acids produced during cooking (Dalie et al., 2010). On the basis of the chemical moieties and interactions involved in ZEA and α-zearalenol binding by LAB, it is likely that carbohydrates and proteins were the bacterial cell components involved in the process (El-Nezami et al., 2002).

2.6 Soybean

Soybeans have become an increasingly important agricultural and worldwide annual production in the world. It is widely believed that the soybean originated in China, probably in the north and central regions, 4000-5000 years ago. From China, soybean cultivation spread into Japan, Korea, and throughout Southeast Asia (Liu, 1997). The consumption of soybean in Asian countries has increasing worldwide every year mainly due to their acclaimed health benefits. In these, soybeans and soy-products are found several phytochemicals and they appear to be the active compounds causing many beneficial health effects. Soybean milk is a widely ingested beverage in East and Southeast Asian countries. In China, annual consumption is reported to be 15-20 kg per person in all forms. While in Thailand, soybean production is not sufficient to meet human and animal needs. In 2010, about 1.8 million tons of soybeans were imported. However, soybean is a widely cultivated crop, most of it is used as the raw material for feedstuff, food protein and widely used in industrial. Several years of rigorous scientific and clinical research has established that most of the components of soybean have beneficial health effects as characterized by its preventive potential for the so-called life-style-related diseases (Dixit et al., 2011).

2.6.1 Soybean seed characteristics

The soybean belongs to the family *Leguminosae*, subfamily *Papilionoideae*, and the genus *Glycine,* L. The cultivated form, named *Glycine max* (L.) Merrill, grows annually. Most mature seeds are made of three basic parts: the seed coat, the embryo, and one or more food storage structures. The embryo contains two pieces of cotyledons that function of food reserve structure (Figure 2.12). The seed coat protects the embryo from fungi and bacteria infection before and after planting. Beside cotyledons, the embryo has three other parts: radical, hypocotyls, and epicotyls. The radical and hypocotyls, together know as hypocotyls radical or germ, are located under the seed coat. During germination, the radical becomes the primary root, whereas the hypocotyl lifts the cotyledons above the soil surface. The epicotyl is the main stem and growing point (Liu, 1997).

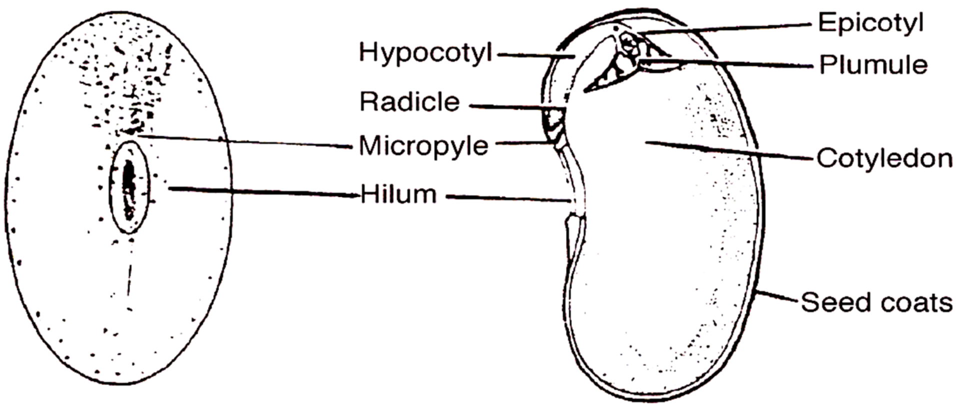

Figure 2.12 Structure of a soybean seed

Source: Liu (1997)

2.6.2 Soybean products

Soybean has been extensively used as important source of dietary protein and oil throughout the world. There are distinct differences in how the East ant the West use soybeans. In the Far East, traditionally, soybeans are made into various foods for human consumption, including tofu, soymilk, soy sprouts, miso, natto, and tempeh, whereas in the West, most soybean are crushed into oil and defatted meal. Although soybean oil is almost all human consumption, soy meal is mainly used as animal feed. Only a small portion is processed into soy protein ingredients including soy flour, concentrated, isolates, and textured soy proteins. These ingredients have functional and nutritional applications in various types of bakery, dairy, and meat products, infant formulas, and the so-called new generation soy foods. Figure 2.13 shows a general outline of soybean food utilisation base on oil beans and food beans (Liu, 1997).

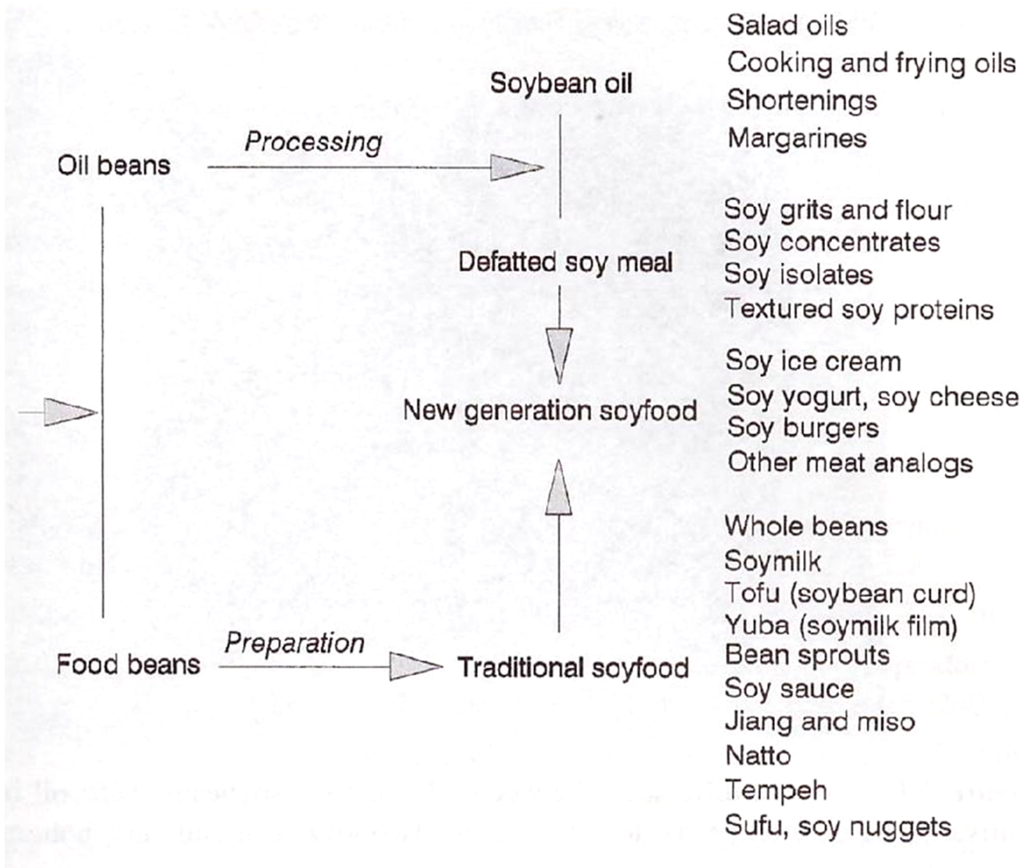

Figure 2.13 A general outline of soybean food use based on classification of oil and food beans

Source: Liu (1997)

2.6.3 Soybean nutritional properties

The soybean is one of the most economical and valuable agricultural commodities because of its unique chemical composition. Proteins and lipids, some vitamins and minerals, are major nutritionally important components of soybeans; carbohydrates are major constituents quantitatively. On average, dry soybean contains roughly 40% protein, 20% oil, 35% soluble (sucrose, raffinose, stachyose, etc.) and insoluble (dietary fiber) carbohydrate and 5% minerals and several other components including vitamins (Liu, 1997; Jooyandeh, 2011). Furthermore, soybeans contain many minor substances, some of which, such as phytase, oligosaccharide, and isoflavones.

2.6.3.1 Proteins

Soybean is good sources of protein, can be good substitutes for animal protein because it offers a complete protein profile. The protein content of soybean has rich amino acids with a good balance, which is comparable of animal proteins sources like milk and beef. Soybean contains all the essential amino acids (except methionine), which must be supplied in the diet because they cannot be synthesized by the human body. The high sufficient lysine content of soy protein makes it a good complement to cereal proteins, which are low in lysine (Jooyandeh, 2011). Soybean contains 35–40% protein on a dry-weight basis, of which, 90% consists of β-conglycinin (7*S* globulin) and glycinin (11*S* globulin) (Liu, 1997). The amino acid profile and structure of these two main proteins concern much with the physicochemical functions, including emulsification, foaming, gelation and fat binding abilities. These proteins contain all amino acids essential to human nutrition, which makes soy products almost equivalent to animal sources in protein quality but with less saturated fat and no cholesterol. Moreover, soybean is not only high quality protein, but it is now thought to play preventive and therapeutic roles for several diseases (Dixit et al., 2011).

2.6.3.2 Carbohydrates

The carbohydrates of soybeans, containing little starch and hexose, are largely polysaccharides with some oligosaccharides. Carbohydrates make up approximately 35% of the soybeans. Approximately 50% of soy carbohydrates are nonstructural in nature and include: low molecular weight sugars, oligosaccharides and small amounts of starch. The other half comprises polysaccharides that include considerable amounts of pectic polysaccharides. The small amounts of free galactose, glucose, fructose and sucrose make up the low molecular weight sugars. Galacto-oligosaccharides (raffinose, stachyose and verbascose) comprise approximately 5% of the soybeans dry matter, while starch represents less than 1%. Stachyose is a tetraose with a galactose-galactose-glucose-fructose structure, while raffinose is a triose with a structure of galactose-glucose-fructose.

Soybean oligosaccharides; SBO are a group of soluble low molecular weight oligosaccharides in soybean seeds, which include sucrose, stachyose and raffinose. Soybean oligosaccharides are defined as non-digestible oligosaccharides or non-digestible sugars except sucrose since human gastrointestinal tract does not possess α- galactosidase enzyme essential for hydrolysis of the α-1, 6 galactosyl linkages.

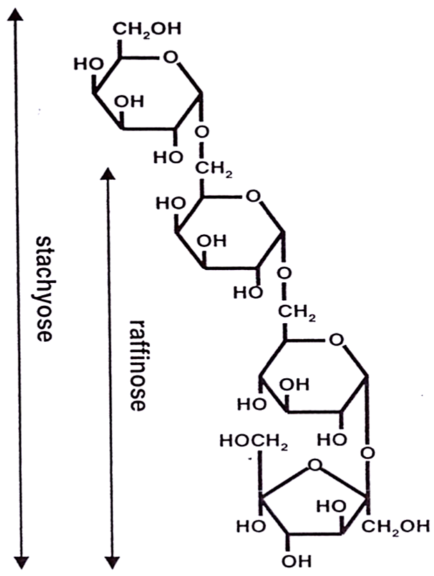

Figure 2.14 Soybean oligosaccharides are extracted directly from soybean whey. The trisaccharide raffinose and the tratasaccharide stachyose are the major oligosaccharide structures
Source: Cristendall (2006).

Therefore, oligosaccharides are supposedly involved in flatulence. The presence of these oligosaccharides impedes the full utilisation of the soybean products. With the progress of oligosaccharides researches, it was found that soybean oligosaccharides are not the direct causes of flatulence. Modern safety tests have proven that oligosaccharides are safe for human consumption. Synthesis SBO: consist mainly of the trisaccharide raffinose and the tratasaccharide stachyose. They are extracted directly from soybean whey rather than being commercially synthesized using enzymatic processes shown in Figure 2.14. Both raffinose and stachyose are resistant to digestion and are readily fermented by bifidobacteria *in vitro* (Cristendall, 2006).

2.6.3.3 Lipids and micronutrient profiles

Soybean oil provides calories, low in saturated fat, rich in the essential fatty acids and is an excellent source of vitamin A and E but contributes insignificant amounts of vitamins D and K. In other word, besides to providing omega-6 fatty acids, soybeans are among the few plant foods that provide omega-3 fat á-linolenic acids (Jooyandeh H. 2011). Linoleic acid in soybean oil is an essential fatty acid belonging to the omega -6 family of polyunsaturated, which exerts important nutritional and physiological functions. Even the α-linolenic acid is also an essential fatty acid belonging to omega-3 fatty acid family, and plays an important role in the regulation of a number of metabolic pathways (Dixit et al., 2011).

2.6.3.4 Vitamins and minerals

The water-soluble vitamins of soybean mainly include thiamine, riboflavin, niacin, pantothenic acid and folic acid. The main oil soluble vitamins include A (retinol) and E (tocopherol). The Vitamin D and K content is negligible. Vitamin A exists as the pro-vitamin â carotene. The tocopherol content of soybean varies with variety, tocopherol that is excellent natural antioxidants. The major forms of minerals in soybean are sulphates, phosphates and carbonates. Potassium is found in the soybean in the highest concentration, followed by phosphorus, magnesium, sulphur, calcium, chloride and sodium in that order.

2.6.3.5 Isoflavones

Isoflavones is a sub-group of heterocyclic plant phenolic category called flavonoids. The soybean is most abundant source of isoflavones in the nature. Soybean contains three types of isoflavone aglycone; daidzein, genistein and glycitein; each of them present in three glycosidic forms in addition to their aglycone form. Daidzein, genistein and their glycosides contribute to >90% of total isoflavone; whereas glycetein and its glycoside are present as minor component (<10%), only. Isoflavones are structurally similar to mammalian estradiol and can bind to both α and β isoforms of estrogen receptor (ER), thus called phytoestrogens. However, the isoflavones are not essential nutrients that are required to support life, still they exert many beneficial health effects, therefore, are of immense help for maintaining healthy life.

2.6.4 Soybean and health benefits

The health effects of soy components have been extensively studied through human clinical trials, experimental animal studies, and *in vitro* cell culture studies. That is, consuming soy foods as part of the normal diet would be a wise nutritional practice because soybean provides several nutrients and helps prevent cancer, osteoporosis and cardiovascular diseases. Explanations of how soy protein lowers blood cholesterol include:

(1) decrease in cholesterol absorption and increase in bile acid excretion

(2) increase in liver low density lipoprotein (LDL) receptors and faster clearance of LDL from the blood

(3) decrease in hepatic cholesterol synthesis

(4) increase in blood thyroxin and thyroid stimulating levels.

Thus soy helps to prevent osteoporosis by two ways-by daidzein affecting bone resorption and formation directly and by genistein acting as a weak estrogen. Soybeans are also free of milk sugar, that is, lactose. Therefore soy foods provide a wonderful selection of alternatives to dairy foods for people who are lactose intolerant. Moreover, soybean sugars are so effective in promoting the growth of probiotic which play a very important role in promoting health of the colon when these oligosaccharides are replacing common table sugar.

2.7 Honey

Honey was an important food as the available natural sweetener for human along time ago. In present the annual world honey production is about 1.2 million tons, which is less than 1% of the total sugar production. Honey consumption is higher in developed countries, where the home production does not always cover the market demand. Different surveys on nutritional and health aspects of honey have been compiled (Bogdanov et al. 2008).

Key points of honey:

(1) About 95% of the honey dry matter is composed of carbohydrates, mainly fructose and glucose. 5-10 % of the total carbohydrates are oligosaccharides, in total about 25 different di- and trisaccharides.

(2) Besides, honey contains small amounts of proteins, enzymes, amino acids, minerals, trace elements, vitamins, aroma compounds and polyphenols.

(3) Honey has been shown to possess antimicrobial, antiviral, antiparasitory, antiinflammatory, antioxidant, antimutagenic and antitumor effects.

(4) Due to its high carbohydrate content and functional properties honey is an excellent source of energy for athletes.

(5) Most of the health-promoting properties of honey are only achieved by application of rather high doses of honey such as 50 to 80 g per intake.

Application of honey

The application of honey as a food additive is based on its manifold properties. The antibacterial effect of honey counteracts microbial spoilage of food. The antioxidant effect of honey prevents oxidation of food during storage. Other physical and sensory properties make honey a good candidate for an additive to a wide variety of food in term of arising good sensory and rheological properties (Păucean et al. (2011). As a prebiotic, honey contains carbohydrates called oligosaccharides, which may improve gastrointestinal health by stimulating the growth of good bacteria in the colon. Honey has been shown to enhance growth, activity of *Bifidobacteria* in fermented dairy food. (Amiri, 2010)