CHAPTER 2
LITERATURE REVIEWS

2.1 Soybean

The history of soybean has started since 4000 years ago, 2000 B.C, when Chinese cultivators were said to recognize the real–health value of this great yellow jewel. Chinese Emperor Shen-nong declared soybean as one of the five sacred crops. It has been an important food ingredient in China, Manchuria, Japan, Korea and Malaysia over centuries (Granata and Morr, 1996). In Thailand, the bean has mainly been cultivated in northern part of the region. The popular soybean varieties include SJ.4, SJ.5 and Chiangmai 60.

The soybean variety of Chiangmai 60

Since 1975, some Thai researchers has tried to mix two varieties of soybean, which were Willium types that had a strong stem and a high number of pod per tree with SJ (F107070) types, which would produce a high yield and had a good seed quality together with a higher resistance to rust diseases. The new mixed variety was cultivated for several generations, until the last generation that was named as "7508-50-10 types". This name was later on changed to be "Chiangmai 60 type". This new variety of soybean was approved by The Research Department of the Agriculture Committee on 30 September 1987. The new soybean variety was resistance to rust diseases more than the soybean varieties of SJ.4 and SJ.5. If the rust diseases attack these soybean plants, the Chiangmai 60 type would only lose 16% of its yield, whereas the SJ.4 and SJ.5 types would lose 29% and 30%, respectively, of their yields. Beside that, the Chiangmai 60 type has little branches, which can increase the number of tree per field and it uses less fertilizer than the SJ.5 type (Chayawat *et al.*, 2003).

The plant of Chiangmai 60 can be cultivated both in the dry and raining seasons. Other important characteristics of the Chiangmai 60 type include its green leaf, width and green stem and brown hair. In general, the Chiangmai 60 plant will grow up to a height of 61 cm (Chayawat *et al.*, 2003).

In addition to its little branches, the plant has a white flower, the color of its mature pods is dark brown and the seed color is yellow. The seed has circle shape with brown eye. The weight of 100 seeds was approximately 14.5 g. The Chiangmai 60 plant will be bloomed after 35 days of planting the seed and the soybean can be havested after 97 days of planting the seeds (Chayawat *et al.*, 2003).

Table 2.1 The nutitional value of 100 g soybean

Nutritional compound	Quality
Protein	43.2 g
Fat	10.5 g
Carbohydrate	20.9 g
Calcium	240 mg
Phosphorus	690 mg
Iron	11.5 mg
Carotene (Vitamin A)	426 mg
Vitamin B	Small amounts

From: Tamine and Robinson (1999)

The nutritional value of 100 g soybean can be seen in Table 2.1. With about 50% protein/g, soybean is 2 times richer than pulses (dal) or peanut, 3 times richer than eggs and 11 times richer than cow's milk. The full nutritional comparison between soymilk and cow's milk is displayed in Table 2.2. Due to its high protein content, soybean is used as a major source of vegetable protein for human and animal nutrition in Thailand (Liu, 1999).

Table 2.2 Comparative chemical analysis (g $100g^{-1}$) of soymilk and cow's milk

Component	Milk	
	Soy	Cow
Protein	3.6	3.3
Fat	1.9	3.9
Carbohydrate	2.8	4.7
Ash	0.4	0.7
Water	91.3	87.4

From: Tamine and Robinson (1999)

Proximate composition of soybean

In general, cultivated soybeans are approximately comprised of 8% hull, 90% cotyledons, and 2% hypocotyl axis. Cotyledons contain the highest percentage of both protein and oil, whereas the hull has the lowest values of these components (Liu, 1999).

- **Protein**

Soybean is a major source of vegetable protein for human and animal nutritions. Like proteins of other sources, soy protein provides calories, essential amino acids, and nitrogen. However, the nutritional quality of proteins is a function of many factors, including amino acid composition (Liu, 1999).

Amino acid composition of soy protein

Soy protein is low in sulfur-containing amino acids, with methionine being the most significant limiting amino acid, followed by cysteine and thereonine. However, soy protein contains sufficient lysine, which is deficient in most of the other cereal proteins (Liu, 1999).

Lipid

During processing, components extracted from soybeans by organic solvents such as hexane are classified as crude oil. Major components of crude oil are triglycerides (or triacylglycerols) (Table 2.3). Minor components include phospholipids, unsaponifiable material, free fatty acids, and trace metals. Unsaponifiable material consists of tocopherols, phytosterols, and hydrocarbons (Liu, 1999).

Triglycerides

Refined soybean oil contains more than 99% triglycerides (Table 2.3). Triglycerides are neutral lipids, each consisting of three fatty acids and one glycerol that link the three acids.

Fatty Acid Composition

The range of fatty acid composition among soybean germaplasm has been reported to be C16:0, 8-17%; C18:0, 3-30%; C18:1, 25-60%, C18:2, 25-60%; and C18:3, 2-15%. The presence of a double bond in an unsaturated fatty acid also makes it susceptible to oxidation, leading to the development of an off-flavor (Liu, 1999).

Table 2.3 Typical composition of crude and refined soybean oil

Components	Unit	Crude oil	Refined oil
Triglycerides	%	95-97	more than 99
Phosphatides	%	1.5-2.5	0.003-0.045
Free fatty acids	%	0.3-0.7	less than 0.05
Unsaponifiable matter	%	1.6	0.3
Plant sterols	%	0.33	0.13
Tocopherols	%	0.15-0.21	0.11-0.18
Hydrocarbons	%	0.014	0.01
Trace metals			
Iron	ppm	1-3	0.1-0.3
Copper	ppm	0.03-0.5	0.02-0.06

Source: Liu (1999)

Carbohydrate

The term carbohydrate, also known as saccharide, and its derivatives include simple sugars (mono- and disaccharide), oligosaccharides, and polysaccharides.

Soluble carbohydrates (Liu, 1999)

Soybeans contain trace amounts of monosaccharides, such as glucose and arabinose, and measurable amounts of di- and oligosaccharides, with sucrose in the range of 2.5-8.2%; raffinose, 0.1-0.9%; and stachyose, 1.4-4.1%. Among the soluble carbohydrates, raffinose and stachyose receive more attention, mainly because their presence has been linked to flatulence and abdominal discomfort associated with human consumption of soybean and soy products. These oligosaccharides cannot be digested in the human duodenal and small intestinal mucosa. Although the presence of the oligosaccharides in soybeans and soy products is generally considered undesirable in terms of their flatus activity, recent studies have shown some beneficial effects of the dietary oligosaccharides in humans as included :

1. Increasing population of indigenous bifidobacteria in the colon which, by their antagonistic effect, suppress the activity of putrefactive bacteria
2. Reducing toxic metabolites and detrimental enzymes
3. Preventing pathogenic and autogenous diarrhea by the same mechanisms as described in the reduction of detrimental bacteria
4. Preventing constipation due to production of high levels of short-chain fatty acids by bifidobacteria
5. Protecting liver function due to reduction of toxic metabolites
6. Reducing blood pressure
7. Having anticancer effects
8. Producing nutrients such as vitamins also due to increase activity of bifidobacteria

Minor Components

In addition to soluble carbohydrates, soybeans also contain various minor components, including minerals, vitamins, phytin, and phenolics (Liu, 1999).

2.2 Soybean yogurt

Granata and Morr (1996) studied the effects of caseinate, casein hydrolyzate and whey protein hydrolyzate on the production of acid, flavor and volatile compounds in a high protein and fiber soymilk. The soymilk was produced by blending soaked, boiled and dehulled soybeans with Swiss cheese whey ultrafiltration permeate. The blended milk was then fermented with a yogurt culture, a mixture of *S. thermophilus* and *L. bulgaricus*. The concentrations of lactic acid, key volatile compounds, i.e., acetaldehyde, acetone, and diacetyl, and the flavor and texture of the resulting soymilk based yogurt formulated with added caseinate or casein hydrolyzate were comparable to those in a cow's milk control.

Kamaly (1997) studied the growth rates and changes in pH of two strains of bifidobacteria, *Bifidobacterium longum* and *Bifidobacterium bifidum,* cultured in reconstituted skimmed milk (RSM), soy milk and modified de Man Rogosa Sharpe (MRS) broth. The growth rates of both strains were in the following order: modified MRS broth > RSM > soymilk. Both strains exhibited proteolytic activities and were more pronounced in soymilk than in RSM.

Nilanon (2000) studied a production of soybean yogurt with a mixture culture of *L. bulgaricus* and *S. thermophilus*. The work was concentrated to investigate the fermentation period and storage time that influenced the growth of lactic acid bacteria and the production rate of lactic acid (1.5%). The highest total number of lactic acid bacteria (2.2 x 10^7 CFU/ml) was achieved at 20 and 22 h with 1.2 x 10^7 and 1.25 x 10^7 CFU/ml of *L. bulgaricus* and 1.0 x 10^7 and 0.95 x 10^7 CFU/ml of *S. thermophilus,* respectively. The optimum storage time of soybean yogurt with a good keeping eating quality was in a range of 0 to 15 days at 5°C. During this storage time, the acid content and pH level were not statistically affected. If the yogurt was kept longer during the storage, the number of lactic acid bacteria found in the yogurt was reduced. The acceptance of soybean yogurt was not statistically different than a yogurt prepared from cow milk. However, the acceptance was significantly different between the soybean yogurt and a commercial yogurt.

Sankhavadhana (2001) studied the formulation and process of a yogurt-like fermented milk product developed from 15.5% full cream milk powder, 10.0% skimmed milk powder, 0.075% carageenan, 0.33% *L. acidophilus* La-5, 0.33% *Lactobacillus casei* Lc-01 and 0.33% *B. bifidum* Bb-12. The product was fermented at 37^{0}C for 14 hours and then kept either at 5 or 8^0C for 4 weeks. Storage at 5^0C showed less change in the physical, chemical and organoleptic properties than storage at 8^0C. After 2 weeks of storage, the numbers of *B. bifidum* reduced to be lower than 10^7 CFU/g both at 5 and 8^0C storage temperatures. The results indicated that the shelf life of the yogurt-like fermented milk product was 2 weeks at 5^0C.

2.3 Starter micro-organisms used in fermented dairy products

In fermented dairy products, starter cultures are added into milk, e.g. starter cultures for yogurt production. Preparation of starter cultures must be done without the presence of antibiotics and bacteriophage. The yogurt production will normally add 1–2% starter cultures into heated milk and then incubate at 42^0C for 3-4 h or at 30^0C for 16–18 h. During the incubation time, the starter cultures grow and change the milk into yogurt (Nanasombut, 1996).

Several types of microorganisms use as starter cultures in fermented dairy products are as followed:

2.3.1 *Streptococcus*

Usually *Streptococcus* spp. is used in cheese producing industries and other fermented milks. *Streptococcus* spp. likes to grow at medium temperatures (mesophilic) with an optimum growth temperature for growth at temperature less than 30^0C, for example *S. lactis.* subsp. *lactis, S. lactis* biovar. *diacetylactis* and *S. lactis* subsp. *cremoris*. However, some types of *Streptococcus* spp. like to grow at high temperatures (37–45^0C), for example *S. thermophilus* (Nanasombut, 1996).

The microorganisms can ferment sugar, i.e. glucose, and produce mainly lactic acid, therefore they are identified as homofermentative lactic acid bacteria (Nanasombut, 1996).

2.3.2 *Lactobacillus*

In 1990, Mono was the first researcher to isolate facultative anaerobic straight rods from the faeces of breast-fed infants, which he typified as *Bacillus acidophilus*, a generic name for intestinal lactobacilli. *Lactobacillus* is generally characterized as Gram-positive, non-sporeforming and non-flagellated rods or coccobacilli (Gomes and Malcata, 1999). Types of *Lactobacillus* are divided into homofermentative and heterofermentative lactic acid bacteria. The homofermentative type is the main type that is used in food industries, including *L. delbrueckii* subsp. *lactis, L. delbrueckii* subsp. *bulgaricus, L. acidophilus* and *L. helveticus*. For the heterofermentative type, it will include *L. casei* subsp. *casei* and *L. plantarum* (Nanasombut, 1996).

Lactobacillus bulgaricus

L. bulgaricus is a bacterium used for the production of yogurt, not only in Bulgaria, but also in many other countries. It is a typical organism in Bulgaria (thrives freely on the Balkan Peninsula), and is very difficult to reproduce in other parts of the world, hence the name - Bulgaricus. However, the bacterium is now used worldwide. The bacteria are fed into the milk and produce lactic acid, which helps preserve the milk. It partly breaks down lactose and is often acceptable to sufferers of lactose intolerance. One of the biggest importers of the bacteria is Japan (Nanasombut, 1996).

2.4 Probiotics

The word 'probiotic' derived from a Greek language, means 'for life' and has had many definitions in the past. Definitions such as 'substances produced by protozoa that stimulate the growth of another' or 'organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance' were used.

These general definitions were unsatisfactory because substances could include chemicals such as antibiotics (Lourens–Hattingh and Viljoen, 2001).

The definition of probiotics has been broadened to a mono- or mixed-culture of live microorganisms, which applied to man or animal (e.g. as dried cells or as a fermented product), beneficially affects the host. This definition implies that probiotic products, for example bio-yogurt, contain live microorganisms and improve the health status of the host by exerting beneficial effects in the gastrointestinal tract (Lourens–Hattingh and Viljoen, 2001).

Live cultures of probiotic bacteria, such as *L. acidophilus* and *Bifidobacterium* spp., in the diet are claimed to provide several therapeutic benefits including prevention of cancer, reduction in the level of serum cholesterol, management of normal intestinal flora and improvement in lactose utilization in lactose malabsorbers (Lourens–Hattingh and Viljoen, 2001).

For therapeutic benefits, the minimum level of probiotic bacteria in yogurt has been suggested to be 10^5 to 10^6 viable cells per ml or g of product. The probiotic culture can be added prior to fermentation simultaneously with the conventional yogurt cultures or after fermentation when the product is cooled down to 4^0C, before packaging (Lourens–Hattingh and Viljoen, 2001).

2.4.1 *Lactobacillus acidophilus*

L. acidophilus is the most commonly used probiotic, or ''friendly'' bacteria. Such healthy bacteria inhabit the intestines and vagina and protect against the entrance and proliferation of ''bad'' organisms that can cause disease. This is accomplished through a variety of mechanisms. For example, the breakdown of food by *L. acidophilus* leads to a production of lactic acid, hydrogen peroxide, and other by-products that make the environment hostile for undesirable organisms. *L. acidophilus* also produces lactase, an enzyme that breaks down milk sugar (lactose) into simple sugars. People who are lactose intolerance do not produce this enzyme. For this reason, *L. acidophilus* supplements may be beneficial for these specific individuals. The physiological and biochemical characteristeristic of *L. acidophilus* are displayed in Table 2.4 (Gomes and Malcata, 1999).

The growth condition of *L. acidophilus*

Growth of *L. acidophilus* may occur at temperature as high as 45°C, but the optimum growth occurs within 35-40°C. Its acid tolerance varies from 0.3% to 1.9% titratable acidity, with an optimum pH lying between 5.5-6.0 (Gomes and Malcata, 1999).

Table 2.4 Physiological and biochemical characteristics of *Bifidobacterium* spp. and *L. acidophilus*

Character	*Bifidobacterium* spp.	*Lactobacillus acidophilus*
Physiology	Anaerobic	Microaeropilic
Lactic acid configuration	L	DL
Sugar metabolism	Heterofermentative	Homofermentative

From: Gomes and Malcata (1999)

2.4.2 *Bifidobacterium* spp.

In 1990, Henry Tisseir was a first researcher to isolate *Bifidobacterium* from the faeces of breast–fed infants at the Pasteur Institute, Paris, France (Shah *et al.*, 1995).

Bifidobacterium spp. is Gram positive bacteria (Nanasombut, 1996) and has rod–shaped. The bacteria are non-gas–producing and anaerobic microorganisms (Table 2.4). Bifidobacteria is also generally characterized as non-spore forming, non-motile and catalase-negative anaerobe bacterium and has various shapes for example, short, curved rods, club-shaped rod and bifurcated Y-shaped rods. Presently, 30 species are included in the genus *Bifidobacterium*, 10 of which are from human sources (dental caries, faeces and vagina), 17 from animal intestinal tracts or rumen, two from wastewater and one from a fermented milk (Gomes and Malcata, 1999). *B. bifidum* is also a slow–growing bacterium, weak growth and acid production in milk, which invariably requires long fermentation times and conditions of anaerobiosis. In addition, during fermentation bifidobacteria produces acetic and lactic acids at the ratio 3:2, so excessive growth may yield products with vinegar-like taste and aroma, which may not be acceptable to consumers (Gomes and Malcata, 1999).

Table 2.5 Commercial products containing *Bifidobacterium* spp. and *Lactobacillus acidophilus*

Product	Country of origin	Microorganisms
A-38	Denmark	*L. acidophilus, B.bifidum*, mesophilic lactococci *Leuconostoc mesenteroides* subsp. cremoris
Acidophilus buttermilk	USA	*L. acidophilus, L. mesenteroides* subsp. *cremoris*, mesophilic lactococci
Progurt		*B. bifidum, L. acidophilus*, mesophilic lactococci
Acidophilus milk	Several Countries	*L. acidophilus*
Acidophilus yeast milk	Former USSR	*L. acidophilus, Saccharomyces fragilis, Saccharomyces cerevisiae.*
A-B Yoghurt	France	*B. bifidum, L. acidophilus*
Cultura	Denmark	Ibidem
Milky	Italy	Ibidem
Nu-Trish A/B milk	USA	Ibidem
Biomild	Several Countries	*Bifidobacterium* spp., *L. acidophilus*
Acidophilus yoghurt (ACO-yoghurt)	Several Countries	*L. acidophilus, L. delbrueckii* - subsp. *bulgaricus, S. thermophilus.*
8-Active	France	*Lactobacillus acidophilus, B. bifidum, L. delbrueckii subsp. bulgaricus, S. thermophilus*
Fresh BA	UK	Ibidem
Kyr	Italy	Ibidem
Yoplus	Australia	Ibidem

Table 2.5 (Continue)

Product	Country of origin	Microorganisms
Biogarde	Germany	*L. acidophilus, B. bifidum, S. thermophilus*
Ofilus	France	Ibidem
Philus	Norway	Ibidem
Bifidus milk	Several Countries	*B. bifidum, B. longum*
Bifighurt	Germany	*B. bifidum, S. thermophilus*
Biogurt	Germany	*L. acidophilus, S. thermophilus*
Biokys	Czech Republic	*L. acidophilus, B. bifidum, Pediococcus acidilactici*
Mil-Mil	Japan	*L. acidophilus, B. bifidum, Bifidobacterium breve*
Akult	Japan	*L. acidophilus, B. bifidum, B. breve, L. casei* subsp. *casei*

From: Gomes and Malcata (1999)

The growth condition of *Bifidobacterium* spp.

1. The optimum pH for bifidobacteria growth is 6-7, with virtually no growth at pH 4.5-5.0 or below or at pH 8.0-8.5 or above.

2. The optimum growth temperature is 37-41°C, with maximum growth temperatures at 43-45°C and virtually no growth at 25-28°C or below (Gomes and Malcata, 1999).

The benefit of probiotic (acidophilus and bifidus) (Kailakailasapathy and Jameschin, 2000)

Some commercial food products that contained *Bifidobacterium* spp. and *L. acidophilus* can be seen in Table 2.5. The early claims for the health benefits of yogurt have only recently been supported, in part, by scientific study. Although more research is needed, several nutritional and health benefits have been suggested for acidophilus and bifidus cultures, including:

1. Protection against diarrhea

Application of acidophilus and bifidus cultures has been found to reduce the incidence and severity of several forms of diarrhea. There are several proposed mechanisms. For example, acidophilus and bifidus cultures can compete for space in the digestive system as well as produce antibacterial substances against illness causing microorganisms. Probiotics can also increase the body's own immune response (defence mechanisms) although how is happened is yet to be understood.

2. Effect on vaginal microflora.

Eating yogurt with acidophilus has been reported to reduce vaginal thrush infections and vaginosis. At the same time, the lactose individuals will have better tolerances for the presence of lactose in milk.

3. Amount of milk

The presence of probiotic will give an opportunity for the lactose intolerance people to consume milk. This is partly due to the digestion of lactose by the bacterial culture.

4. Large bowel (colon) health

Studies show that probiotics may have a role in the treatment of constipation and irritable bowel. There is also interest in the role of probiotics in inflammatory bowel disease, such as ulcerative colitis and Crohn's disease, because of their effects on the immune response of the bowel. There may also be some protection against bowel cancer by detoxification of cancer causing substances and effects on the turnover of cells of the bowel.

2.5 Factors that affect the growth of probiotics

Compounds including lactose, galactose, glucose, protein hydrolyzates, yeast extract, proteose peptone, casitone, polypeptone and phytone that were added to enrich soy milk were shown to give appreciable stimulation for growth and acid production of *B. bifidum* (Kamaly, 1997). In the same experiment, *B. longum* was not stimulated by carbohydrate and protein hydrolyzate substrates. Among amino acids tested, a sulphur-containing amino acid, cysteine, was found to increase the populations of *B. longum* and *B. bifidum* by 5 times compared with soy milk without cysteine. A combination of amino acids, cysteine and threonine had a potential synergistic effect on the growth and acid production of bifidobacteria. A desirable yogurt-like fermented milk was prepared using reconstituted skimmed milk enriched with soy milk (20%), kappa-carrageenan, a mixture of amino acids, cysteine and threonine (0.05% each) and a single inoculum of bifidobacteria. The product was incubated for 24 h at 37^0C (Kamaly, 1997).

Sucrose

Sucrose is one of the family of sugars otherwise known as saccharides in a group called carbohydrates. Carbohydrates, as the name implies, contain carbon and hydrogen plus oxygen in the same ratio as in water. The saccharides are a large family with a general formula of $C_nH_{2n}O_n$. Sucrose, $C_{12}H_{22}O_{11}$, is a disaccharide, a condensation molecule made up of two glucose molecules (Ansari, 1999).

Sucrose can be found everywhere, including many fruits and vegetables. For economical extraction, it is only sugar in sugar beet and sugar cane that are found in sufficient quantity.

Chemical properties/reaction tendencies of sucrose (Ansari, 1999)

- Sweet taste
- Colorless crystals, crystalline masses or white crystalline powder
- Stable in air
- Finely divided sugar is hygroscopic and absorbs up to 1% moisture which is given up on heating to 90°C
- Chars and emits characteristic odor of caramel
- Sucrose does not reduce Fehling's solution, form an osazone, or show mytarotation.
- Sucrose is hydrolyzed to glucose and fructose by diluted acids and by invertase, a yeast enzyme
- Sucrose is fermentable, but resists bacterial decomposition when it is presence in high concentration
- Sucrose is a compound resulting from a chemical combination of the element carbon, which is usually a black solid when free, and the two elements hydrogen and oxygen, which are colorless gases when uncombined.

2.6 Prebiotic

Prebiotics are complex sugars that cannot be metabolised directly by humans but serve as a carbohydrate source for intestinal flora. Oligosaccharides, such as fructooligosaccharides, lactulose, raffinose, stachyose and inulin oligomers, are used as 'prebiotics' or bifidogenic factors. It is not clear at this time which type of prebiotics is the most effective one.

Prebiotic benefits

A prebiotic is a nondigestible ingredient that stimulates the growth and activity of the beneficial microbiota in the gastrointestinal tract. Numerous studies confirm the fact that the prebiotic passes through the stomach and small intestine undigested and results in an increase in the beneficial colonic microflora, such as bifidobacteria and lactobacilli. These bacteria are associated with improved health in the colon, which can influence overall well being throughout the body. In addition, studies show that as beneficial bacteria proliferate in the colon, the numbers of potentially harmful bacteria such as *Escherichia coli* and clostridia decline.

2.6.1 Fibersol-2 (Colebank, 2003)

Fibersol-2 is a unique soluble dietary fiber produced from starch by a combination of dextrinization and enzymatic treatments. The product has been well-developed containing branched chemical structures of polysaccharides that are composed not only by 1,4- and 1,6-glucosidic bonds, but also contained 1,2- and 1,3-linkages. These branched structures, except 1,4-linkages, are resistant to human digestible enzymes and they mainly pass undigested to the large intestine. In the large intestine, the fiber can be partially fermented by Fibersol-2, whereas the fractions that are not utilized are excreted. Some functional properties of the dietary fiber are arranged in Table 2.6.

Table 2.6 The functional properties of fibersol-2 in its applications

Applications	Properties
Beverages	- Improve scratchy taste and unpleasant flavors of vitamins and create smooth taste. - Reduce the bitterness of amino acids. - Reduce the bitterness of isoflavones. - Reduce the fishy flavors of omega-3 fatty acid.
Juice drinks	- Improve mouth-feel and achieve mild/smooth taste. - Reduce the bitterness of citrus flavors (lemon, grapefruit) - Reduce the acid taste of vinegar, noni fruit, lemon, etc.
Dairy drinks	Reduce the strong and bitter, and/or astringent tastes from emulsifiers and/or proteins.
Coffee & tea drinks	Reduce the strong, bitter, and astringent tastes and create mild/smooth taste.
Soy beverages	Mask soy flavors.
Alcohol drinks	Reduce the strong taste of alcohol and create a more mild taste.
Intensive sweeteners (Aspartame, Sucralose, Acesulfame K, Stevia,	Mask irritating after taste. Improve the overall sweetness profile.

From: Colebank (2003)

2.6.2 Fructo-oligosaccharides (FOS)

FOS are one of prebiotics, non-digestible food ingredients that benefit the host by stimulating the growth of beneficial microflora, or the actual beneficial bacteria, such as acidophilus and bifidum. Bifidobacteria may inhibit the growth of pathogenic bacteria, such as *Clostridium perfringens* and diarrheagenic strains of *E. coli.* FOS typically refer to short-chain oligosaccharides comprised of D-fructose and D-glucose, containing from 3 to 5 monosaccharide units. Those are called oligofructose. FOS, also called as neosugar and short-chain FOS (scFOS), are produced on a commercial scale from sucrose using a fungal fructosyltransferase enzyme. FOS are comprised of one molecule of D-glucose in the terminal position and from 2 to 4 D-fructose units. In terms of chemistry, a fructooligosaccharide is a glucose molecule bonded to multiple fructose molecules. These bonds cannot be broken down by enzymes in the human small intestine - allowing the compound to reach the large intestine intact, where it becomes a substrate for colonic bacteria. Groups of these oligosaccharides can be found in food products such as beans, blueberries, and onions (Hidaka *et al.*, 1986).

Mechanism of fructooligosaccharides action

The possible anticarcinogenic activity of FOS might be accounted for, in part, by the possible anticarcinogenic action of butyrate. Butyrate, along with other short-chain fatty acids, is produced by bacterial fermentation from FOS in the colon. Some studies suggest that butyrate may induce growth arrest and cell differentiation, and may also upregulate apoptosis, three activities that could be significant for antitumor activity (Hidaka *et al.,* 1986).

FOS may also aid in increasing the concentrations of calcium and magnesium in the colon. High concentrations of these cations in the colon may help to control the rate of cell turnover. High concentrations of calcium in the colon may also lead to the formation of insoluble bile or salts of fatty acids. This might reduce the potential damaging effects of bile or fatty acids on colonocytes (Hidaka *et al.*, 1986).

FOS may bind/sequester mineral such as calcium and magnesium in the small intestine. The short-chain fatty acids formed from the bacterial fermentation of FOS may facilitate the colonic absorption of calcium and, possibly, also magnesium ions. This could be beneficial in preventing osteoporosis and osteopenia (Hidaka *et al.*, 1986).

Pharmacokinetics of fructooligosaccharides

Little digestion of FOS occurs in the stomach and small intestine following ingestion of FOS. FOS are fermented in the colon by bifidobacteria and some other bacteria to produce the short-chain fatty acids acetate, propionate and butyrate; gases hydrogen, hydrogen sulfide, carbon dioxide and methane; and lactate, pyruvate and succinate. Some acetate, propionate and butyrate are absorbed from the colon and transported by the blood circulation to various tissues where these short-chain fatty acids undergo further metabolism. Many short-chain fatty acids are metabolized by the colonocytes. Butyrate is an important respiratory fuel for the colonocytes. Those with ileostomies may have a microbial population colonizing their ileum. In those cases, FOS could be fermented by some of the bacteria, much as they are in the colon (Jung *et al.*, 1989).

Benefit indications of fructooligosaccharides

FOS appears to be of benefit in modulating the microbial ecology of the gastrointestinal tract, boosting gastrointestinal immunity. FOS may also protect against colon cancer, and may have favorable lipid effects in some cases. In addition, FOS may also aid in calcium absorption (Jung *et al.*, 1989).

2.6.3 Tomato Juice

Tomatoes are the second most consumed vegetable in North America. Tomato juice contains 93.1% moisture, 4.89% carbohydrate, vitamins and minerals. The juice is low in protein and fat (Yoon *et al.*, 2004).

Tomatoes are excellent sources of a good number of functional components. Many of these functional components have anti-oxidative or hormonal properties. Others may promote the growth of organ tissues or biologically healthy bacteria in the intestinal tract. In addition to, the vegetables are the highest sources of the functional components lycopene and oligosaccharides (Yoon *et al.*, 2004).

Benefits of tomato consumption

Tomatoes and tomato products are rich with lycopene, a powerful anti-oxidant that picks up free radicals in the body, and can play a key role in that process. In addition, tomatoes can prevent cancer of breast, prostate, colon, cervix, lung and digestive tract, as well as cardiovascular disease and degenerative disease of the eye, osteoporosis, diabetes and other diseases (Yoon *et al.*, 2004).

2.7 Utilization of stabilizers in fermented dairy products

In the production of yogurt, stabilizers are often added to the base with the primary aim is to enhance and maintain the desirable characteristics of the final yogurt product, including body and texture, viscosity/consistency, appearance and mouthful. Stabilizers are sometimes referred to as hydrocolloids and their mode of action in yogurt includes two basic functions. The first is binding water and the second is a promotion to increase the yogurt viscosity. Different types of hydrocolloids (in the amounts of volume) used in the U.S.A can be seen in Table 2.7 (Ruttanapanon, 2002).

Table 2.7 The amounts of volume of hydrocolloids used by the U.S.A industries.

Hydrocolloids	The utilization volume (million pounds)		
	Food industries	Other industries	Total Volume
Corn starch	600.0	2,500.0	3,100.0
Gum arabic24.0	7.0	31.0	
Guar gum	20.0	45.0	65.0
CMC (Carboxyl-Methyl Cellulose)	16.0	100.0	116.0
Pectin	12.0	-	12.0
Alginate	10.0	2.0	12.0
Gum ghatti	10.0	1.0	11.0
Locust bean gum	9.0	3.0	12.0
Carrageenan	9.0	0.2	9.2
Xantane gum	3.0	9.0	12.0
MethylCellulose	2.0	53.0	55.0
Tragacanth gum	1.3	0.2	1.5
Karaya gum	1.0	7.0	0.8
Agar	0.3	0.4	0.7
Fercellarane	0.3	-	0.3

From: Ruttanapanon (2002)

2.7.1 Carrageenan

Carrageenan is a naturally occurring family of carbohydrate extracted from red seaweed. Types of carrageenan are lambda, kappa, and iota carrageenans. Lambda carrageenan is a compound with 3,6-anhydro-D-galactose units. The contents of this carrageenan is highly being sulfated, therefore the carrageenan is easily soluble under most conditions. Kappa carrageenan contains 3,6-anhydro-D-galactose as part of the repeating unit and fewer sulfate groups, therefore it is less hydrophilic and less soluble. The intermediate carrageenan is iota carrageenan. This carrageenan is more hydrophilic than kappa-carrageenan by the presence of 2-sulfate and the position of this 2-sulfate, which counteracts the less hydrophilic character of the 3,6-anhydro-D-galactose residue (Ruttanapanon, 2002).

Carrageenan exhibits the solubility characteristics normally shown by hydrophilic colloids. It is water soluble and insoluble in most organic solvents. Water miscible alcohol and ketones, while themselves non-solvents for carrageenan, are tolerated in a mixture with carrageenan solutions at levels up to 40%. More highly polar solvents, such as formamide and N,N-dimethylformamide, are tolerated in still higher proportion and each of these solvents can cause a marked swelling of the polymer. Functional carrageenans are used mainly for thickening, suspending and gelling agents (Ruttanapanon, 2002).

The solubility characteristics of carrageenan in water are influenced by a number of factors. The most important factors are (Ruttanapanon, 2002):

a. the type of carrageenan
b. the present of counter ions
c. other solutes
d. temperature
e. pH

Kappa-carrageenan

[-(1⟶3) - β-D-galactopyranose-4-sulfate- (1⟶4)-3,6-anhydro- α -D-galactopyranose- (1⟶3)-], kappa -carrageenan is produced by alkaline elimination from polysaccharides isolated mostly from the tropical seaweed *Kappaphycus alvarezii* (also known as *Eucheuma cottonii*). The experimental charge/dimer is 1.03 rather than 1.0 with 0.82 molecules of anhydrogalactose rather than one (Ruttanapanon, 2002).

The important characteristic of kappa-carrageenan (Ruttanapanon, 2002).

- It is soluble in hot water.
- The addition of potassium ions induces the formation of a durable, brittle gel; it also increases the gelling and melting temperatures.
- The compound can make strong, rigid gel, with some syneresis. It forms a helix structure with K^+ ions.
- Ca^{++} causes helices to aggregate and the gel to contract and become brittle.
- It produces a slightly opaque gel. The gel become clears with sugar.
- It contains approximately 25% ester sulfate and 34% 3,6-anhydro-D-galactose.
- It is compatible with water miscible solvents.
- It is insoluble in most organic solvents.
- The typical use levels are 0.02 to 2.0%.

2.7.2 Guar gum

Guar gum (often called guaran) is extracted from the seed of the leguminous shrub *Cyamopsis tetragonoloba*, where it acts as a food and water store. The plant is an annual leguminous plant originating from India and Pakistan, but now it also cultivated in the U.S.A (Ruttanapanon, 2002).

Structural unit

Guar gum is a galactomannan consisting of a (1→4)-linked with α-D-mannopyranose backbone with branchpoints from their 6-positions linked to α-D-galactose (i.e. 1→6-linked-α-D-galactopyranose). There are between 1.5-2 mannose residues for every galactose residue (Ruttanapanon, 2002).

Molecular structure

Guar gum is made up from non-ionic polydisperse rod-shaped polymers and it can comprise up to 10,000 residues. Higher galactose substitution also increases the stiffness (i.e. decreases the flexibility), but reduces the overall extensibility. The galactose residues prevent strong chain interactions, as few unsubstituted clear areas have the minimum number (about 6) required for the formation of junction zones. Of the different possible galactose substitution patterns, the extremes of block substitution and alternating substitution give rise to the stiffer, with greater radius of gyration and most flexible conformations respectively (random substitution being intermediate). If the galactose residues were perfectly randomized, it is unlikely that molecules would have more than one such area capable of acting as a junction zone, so disallowing gel formation. A block substitution pattern, for which there is some experimental evidence, would allow a junction zone formation if the blocks were of sufficient length (Ruttanapanon, 2002).

Functionality

Guar gum is an economical thickener and stabilizer. It hydrates fairly rapidly in cold water to give highly viscous pseudoplastic solutions of generally greater low-shear viscosity when compared with other hydrocolloids. High concentrations (~ 1%) are very thixotropic but lower concentrations (~ 0.3%) are far less so. Guar gum does not form gels but does show good stability to freeze-thaw cycles. Its shows high low-shear viscosity but it is strongly shear thinning. Being non-ionic, it is not affected by ionic strength or pH, but it will be degraded at extreme pH at high temperature (e.g. pH 3 at 50°C) (Ruttanapanon, 2002).

Guar gum is an emulsifier, thickener and stabilizer approved for use in a wide range of food, cosmetics, and pharmaceuticals. It is sold as a white to yellowish odorless powder, which is available in different viscosities and different granulometries depending on the desired viscosity. Its viscosity is a function of temperature time and concentration. One advantageous property of guar gum is that it thickens without the application of heat. Various applications of guar gum are exercised of its different properties (Ruttanapanon, 2002).

The important of guar gum properties are (Ruttanapanon, 2002):

- Easy solubility in cold and hot water
- Film forming property
- Resistance to oils, greases and solvents
- Better thickening agent
- Water binding capacity
- High viscosity
- Functioning at low temperatures.

Guar gum utilization in commercial products

In bakeries, dairies and in production of meat, dressings and sauces, guar gum is an important natural food supplement with high nutritional value for weight gain and cholesterol reduction. In cosmetics, especially shampoos and toothpastes, guar gum is used primarily as a thickening and suspending agent. In beverages, it is used as a stabilizer for preparing chocolate drinks and juices. Guar is also widely used in tobacco, leather, insecticides and pesticides, crayons, adhesives, etc. Guar gum comes in different forms - from seeds to powder. Main types of guar gum include guar seed, un-dehusked split, refined split, pulverized guar gum powder, guar protein and guar meal (Ruttanapanon, 2002).

2.7.3 Carboxymethylcellulose (CMC)

CMC is a gum of water-soluble cellulose manufactured by reacting sodium monochloroacetate with alkali cellulose to form sodium carboxymethylcellulose. It dissolves in hot or cold water and is fairly stable over a pH range of 5.0 to 10.0, but acidification below pH 5.0 will reduce the viscosity and stability except in a special acid that can produce a stable type of CMC. A variety of CMC types is available which differs in viscosity and degree of substitution (the number of sodium groups per unit). It functions as a thickener, stabilizer, binder, film former and suspending agent. It is used in a variety of foods including; dressings, ice cream, baked goods, puddings and sauces. In general, the usage range is from 0.05 to 0.5 % (Igoe and Hui, 2003).