CHAPTER 2
LITERATURE REVIEW

2.1 Green soya bean

Green soya bean or vegetable soya bean is a special variety of soya bean (*Glycine max* L. *Merr.*) with a characteristic of green or green-yellow color, soft texture and large seed size (resulting from high moisture content and specially selected varieties). Green soya beans are normally hand-picked at about 80% maturity in the green-yellow pod (Catharina *et al.*, 1999) and the seeds have expanded to fill 80-90% of the pod width. Therefore, the beans are also known as immature soya beans or green soya beans. Like other types of soya beans, e.g. field-dried soya beans, the seeds of green soya bean varieties are rich in protein and highly nutritious (Konovsky *et al.*, 1994). However, there are compositional differences between immature and mature soya beans. Green soya beans will normally contain protein in the range of 11-16% and oil in the range of 8-11%, on the wet-basis. The protein and oil contents of green soya beans on the moisture-free basis will be very close to that of mature soya beans (Liu, 1999).

If green soya beans are compared with field-dried soya beans, then the green soya beans offer three distinct features (Liu, 1999):

- Their green color and soft texture enhance their appeal as a vegetable.
- The beany or bitter flavor intensity of green soya beans is much lower than that of mature soya beans (0.54 vs. 1.6, respectively).
- The green soya bean has higher amounts of ascorbic acid and β-carotene. The amounts of the two vitamins are close to or even higher than those of germinated soya beans.

In addition, the green soya bean has lower amounts of antinutritional factors, including trypsin inhibitors, oligosaccharides and phytates. However, at present there are some constraints for wide consumption of green soya beans. Difficulty in harvesting is a major factor; when the tender green soya beans are bruised or damaged, oxidative deterioration occurs rapidly, leading to off-flavor formation and browning. Other constraints include low overall field yields and some degree of beany flavor that is hard to eliminate (Liu, 1999).

Green soya bean is one of the traditional soy foods in many Asian countries. In Japan, it is known as *edamame* (pronounced "eh-dah-mah-meh"). Green soya bean has a sweet and delicious taste and can be eaten as a snack either boiled in water or roasted. The fresh beans can also be mixed into salads, stir fried, combined with mixed vegetables, make tofu, ice creams and similar dessert items. In Asian countries such as China, Japan, Thailand and Taiwan, green soya bean pods are sold fresh on the stem with leaves and roots or stripped from the stem and packaged fresh or frozen as either pods or beans. In Thailand, production of frozen green soya bean began in the 1990s (Lin and Shanmugasundaram, 2001). In 2000, the green soya bean production reached about 2,000 ton and was exported to Japan (Mohameda and Mentreddyb, 2004). There are currently three major processors in Thailand. They process a total of 9,000 ton/year, of which 8,700 ton are exported to Japan and 300 ton to the United States and other countries.

There are a few qualities that are desirable for green soya beans to be consumed as vegetables. These include large seed size, soft texture, good flavor and high amounts of protein, free amino acids and total sugars. Factors affecting these attributes include cultivar, growing seasons, harvest time and storage conditions (Mohameda and Mentreddyb, 2004).

2.1.1 Chemical compositions

During seed development and maturation, young soya beans undergo many compositional changes before reaching maturity. During soya bean maturation, weight and color change and dry matter increases from 16 to about 90%. The average fresh weight of most green soya bean varieties, with the exception of a few black colored varieties such as Tambagura, expressed as mg/seed, increases from 300 to a peak at 568 and then decreases to about 209 at maturity (Mohameda and Mentreddyb, 2004).

1. Moisture content: The moisture content of fresh green seeds ranged from 53.9-56.1%. Differences between genotypes were not significant. Seed moisture content is a critical factor that affects time of harvest since it is an integral part of organoleptic characteristics of green soya bean (Mohameda and Mentreddyb, 2004).

2. Protein and oil: During maturation, soya beans undergo mass synthesis of storage proteins and lipids. The lipids are stored in oil bodies, mainly in the form of triglycerides, while the proteins are reserved in another organelle known as protein bodies. Since green soya beans are normally harvested between 50-60 days after flowering, they contain 11-16% protein and 8-11% oil on a fresh weight basis. In a study conducted in Georgia in the United States, the mean protein content of 11 Japanese green soya bean genotypes was 36% on a dry weight basis. This is about 86% of the total protein of mature dry bean (Mohameda and Mentreddyb, 2004).

3. Fatty acid compositions: Rubel *et al.* (1972) found that between 24-40 days after flowering, the amounts of all fatty acids increase. During the remaining stage of seed development, relative percentage of fatty acids remains essentially constant. Different varieties of soya bean seeds affected the percentage of individual fatty acids.

4. Amino Acid Compositions: In general, there is a decrease in total free amino acids during seed development, which may contribute to a better taste in green soya beans compared to mature ones (Mohameda and Mentreddyb, 2004).

5. Carbohydrates: Sugars detected in soya bean seeds include glucose, fructose, galactose, sucrose, raffinose and stachyose. Sucrose appeared early in the seed development, followed by raffinose and stachyose, which were not detected until 40-50 days after flowering. Dimethyl sulfoxide soluble starch reaches a maximum value at 30-40 days after flowering and then declines sharply to almost nonexistent at the mature stage. Green soya beans contain higher amounts of simple sugars and much less in amount of oligosaccharides compared with mature types. This is consistent with a common impression that flatulence is infrequent after ingestion of green soya beans. (Mohameda and Mentreddyb, 2004).

Oligosaccharides of soya beans have been generally considered undesirable, because raffinose and stachyose are factors responsible for the flatulence and abdominal discomfort often experienced after ingestion of soya beans. However, these oligosaccharides have been reported to support the growth of bifidobacteria and to play an important role in health benefits from soya bean (Mohameda and Mentreddyb, 2004).

6. Vitamins: Some vitamins that were detected in green soya bean included tocopherol (δ, γ and α), ascorbic acid and β-carotene. Ascorbic acid in green soya beans could be as high as 40 mg/100 g on a fresh weight basis. It decreases to 2 mg/100 g for soaked weight at full maturity. Similarly, green soya beans contain as much as 0.46 mg/100 g of β-carotene on a fresh weight basis and can be as low as 0.12 mg/100 g for soaked weight when beans are fully matured (Mohameda and Mentreddyb, 2004).

2.1.2 Biologically active compounds

1. Trypsin Inhibitor: Trypsin Inhibitor (TI) levels increased during soya bean maturation. Cultivar has a great influence on TI activities during soya bean seed development, but the green soya bean generally has lower levels of TIs than mature seeds. Furthermore, TIs in green soya beans are more susceptible to heat destruction than those in mature seeds. For green soya beans, boiling in water or steaming for 20 min completely eliminated their TI activities. However, for mature soya beans, 100% destruction could only be achieved by soaking plus boiling. However, a heating process such as blanching eliminates most of the activities of these inhibitors. One-third of the activity of TI remains in green soya bean seed even after boiling for 5 min (Mohameda and Mentreddyb, 2004).

2. Phytate: a calcium-magnesium-potassium salt of inositol hexa-phosphoric acid, commonly known as phytic acid, occurs in certain cereal and legume seeds including soya bean. Phytate is the main source of phosphorus in soya bean seed and is known to form complexes with phosphorus, proteins and minerals such as Ca, Mg, Zn, and Fe. This reduces the bioavailability of these minerals, affects seed germination and seedling growth and causes deficiencies in non-ruminant animals. Green soya beans also contain a smaller amount of phytic acid, which is widely believed to interfere with mineral absorption in our bodies. The mean phytate content was found to be 1.26% (dry matter basis), with a range of 1.08-1.39% in several green soya bean genotypes (Mohameda and Mentreddyb, 2004).

3. Isoflavones: Several isoflavones, including malonylgenistin, malonyldaidzin and malonylglycitin have been detected in soya bean. Isoflavones have been shown to exert many health benefits including cancer prevention and control. However, their presence is partially responsible for objectionable taste of soy products. Low amounts of isoflavones are consistent with the fact that green soya beans taste less bitter and less astringent than mature types. Isoflavones cause a sour or bitter flavor. Current research indicates that these are important phytochemicals associated with health benefits to humans from soya bean (Mohameda and Mentreddyb, 2004).

4. Saponins: There is significant variation in saponin content and pattern of accumulation in soya bean. The variation in saponin composition in soya bean seeds is explained by different combinations of 5 genes controlling the use of soya-sapogenol glycosides as substrate. Phenotypes of more than 1,000 soya beans were classified into 8 saponin types, and the frequency of phenotypes was different between the cultivated and the wild soya bean (Mohameda and Mentreddyb, 2004).

The group A saponins are responsible for an undesirable bitter and astringent taste. At the same time, however, 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-py-4-one (DDMP)-conjugated saponins and their degradation products, or subgroups B and E saponins, have health benefits such as inhibition of the infectivity of the acquired immunodeficiency syndrome (AIDS) virus (human immunodeficiency virus or HIV) and inhibition of the activation of the Epstein-Barr virus early antigen. The reduction, by genetic means, of saponins possessing undesirable characteristics, together with an increase of the other saponins with health benefits, is important. In this regard, the content of group A saponins is reported to depend more closely on genetic characteristics than on environmental effects. Therefore, the identification of a group of mutants deficient in group A saponins would contribute to the improvement of soya bean based foods (Mohameda and Mentreddyb, 2004).

5. Phytosterols: Mean value for β-sitosterol level was found to be 234.8 μg/g, which was the highest in tested green soya bean genotypes, whereas mean levels of campesterol and stigmasterol were significantly lower (45.6 and 44.6 μg/g, respectively). The concentration of δ-tocopherol in soya bean seeds was found to be the highest during the early pod development phase under field conditions, but decreased during the later stages. At the same time α- and γ-tocopherols increased (Mohameda and Mentreddyb, 2004).

Given that phytate, tocopherols, phytosterols, and isoflavones have significant health benefits through a reduction in blood serum cholesterol levels, reduction in the risk of cardiac diseases, cancer and so on, a higher amount of these compounds in green soya bean is desirable despite their undesirable effects on organoleptic characteristics (Mohameda and Mentreddyb, 2004).

2.1.3 Nutritional quality

When compared with corn, green peas or green beans, green soya beans have four times more fiber and much higher contents of iron, calcium, vitamin C and protein. The nutritional value and quality of green soya bean is superior to that of certain selected soy products such as natto and tofu as well (Table 2.1). Of greater importance is the fact that green soya beans contain higher levels of isoflavones than many other non-soy food products (Mohameda and Mentreddyb, 2004).

Low contents of anti-nutritional factors and soft texture of green soya beans should improve protein digestibility. In one study, green soya beans were shown to have higher values of protein efficiency ratio (PER) than mature ones when fed to rats. In another study, the net protein use and PER of green soya beans were found to be comparable to those of casein and lean beef (Mohameda and Mentreddyb, 2004).

Table 2.1 Nutritional content of some green soya bean and pea products

Composition	Units	Natto	Momen Tofu	Vegetable Soybean	Pea	Green Pea
Energy	Kcal/100g	200.0	77.0	582.0	30.0	96.0
Water	g/100g	59.5	86.8	71.1	90.3	75.7
Protein	g/100g	16.5	6.8	11.4	2.9	7.3
Lipid	g/100g	10.0	5.0	6.6	0.1	0.2
Nonfibrous Carbohydrates	g/100g	9.8	0.8	7.4	5.4	13.0
Fiber	g/100g	2.3	0.0	1.9	0.8	2.9
Dietary fiber	g/100g	-	-	15.6	-	6.3
Ash	g/100g	1.9	0.6	1.6	0.5	0.9
Calcium	mg/100g	90.0	120.0	70.0	55.0	28.0
Phosphorus	mg/100g	190.0	85.0	140.0	60.0	70.0
Iron	mg/100g	3.3	1.4	1.7	0.8	1.9
Sodium	mg/100g	2.0	3.0	1.0	1.0	3.0
Potassium	mg/100g	660.0	85.0	140.0	60.0	70.0
Carotene	mg/100g	0.0	0.0	100.0	620.0	360.0
Vitamin B_1	mg/100g	0.07	0.07	0.27	0.12	0.25
Vitamin B_2	mg/100g	0.56	0.03	0.14	0.10	0.12
Niacin	mg/100g	1.1	0.1	1.0	0.6	1.9
Ascorbic acid	mg/100g	0.0	0.0	27.0	34.0	18.0

Source: Mohameda and Mentreddyb (2004)

2.1.4 Organoleptic features

Besides nutritional advantages, green soya beans have several organoleptic features that are superior to mature ones. These include green color, larger seed size, softer texture, sweeter and better taste, and lower beany flavors. Large-seed size results from two factors: high moisture content and genotypic selection for large-seed trait. The age of the seed tissue and genotypic selection account for the soft texture of green soya beans. The sweet and somehow delicious taste of green soya beans is attributed to their high content amounts of simple sugars and free amino acids and low levels of isoflavones. Quality characteristics and associated chemical compounds are shown in Table 2.2.

Table 2.2 Quality characteristics and associated chemical compounds of green soya beans

Characteristic	Associated Chemical Compounds	
Taste	Ascorbic acid, sucrose, glutamic acid and alanine make green pods and seeds tasty.	
Flavor	Cis-jasmone and (Z)-3-hexenyl-acetate.	
Nutritional factors	Protein, lipid, fiber, sucrose, ascorbic acid, essential amino acids, Vitamins and minerals.	
Antinutritional factors	Phytate	1. Phosphorus, proteins and minerals. 2. Reduce bioavailability and cause deficiencies of minerals. 3. Significant varietal difference.
	Trypsin inhibitor	Binds proteolytic enzymes and reduces protein efficiency ratio.
	Saponins, isoflavones and phenolic acids	Sour/bitter and astringent flavor; but associated with health benefits to humans.
	Stachyose and raffinose	Cause flatulence, which leads to abdominal discomfort.

Source: Mohameda and Mentreddyb (2004)

1. Pod and seed appearance: Although green soya bean is sought for its health benefits, morphophysiolocal traits determine its marketability and profitability. Pod size, its color and number of seeds per pod are important morphological traits that determine marketability and price of green soya bean. Generally, pods with more than two seeds in each secure higher prices than those with fewer seeds. The pod color is important and bright green is most desirable. Yellowing of the pods reflects declining freshness and degradation of ascorbic acid. Quality properties such as color, texture and seed size of green soya bean are a function of development time (Mohameda and Mentreddyb, 2004).

The special grade of green soya bean should have 90% or more pods containing 2 or 3 seeds. The pods should be perfectly shaped, completely green, no injuries and no spots. The grade B green soya bean should have 90% or more pods with 2 or 3 seeds, but it can be a lighter green, slightly spotted, injured or malformed and have short pods or small seeds. The grade A is the intermediate between special grade and grade B. In these three grades, pods must not be overly mature, diseased, insect damaged, one-seeded, malformed, yellowed, split, spotted or unripe (Mohameda and Mentreddyb, 2004).

The color of seeds changes from green to light green, yellow-green, yellow and then to buff-brown. The best time to pick green soya beans for direct consumption is when the seed color changes from green to light green. At this stage, the seeds are at about 80% maturity, sucrose levels are at their peak and many other desirable seed quality traits are also at their peak levels (Mohameda and Mentreddyb, 2004).

2. Texture: Texture also contributes to green soya bean quality. The soya beans with hard seeds receive low scores. Until the middle pod-filling stage, the seeds tend to have a soft seed coat, which then becomes harder with advancement toward maturity. (Mohameda and Mentreddyb, 2004).

There is no standard available on the desired texture for green soya bean. There are many factors that might contribute to the hardness of green soya bean seeds.

Thehardness of green soya bean seeds harvested at different maturity stages (Tsou and Hong, 1990).

Pods after prolonged cooking are generally softer and therefore the desired hardness can be obtained through the control of cooking time. Fresh pods are better blanched than boiled to preserve the bright green color and flavor. Pods and seed for the frozen green soya bean market are blanched by placing the pods and seeds in boiling water at 100°C for 2-3 minutes, then immersed in cold water at 0°C followed by freezing at -40°C. The blanched pods and seed are stored at -18°C. However, extended cooking time may cause the breaking of pods or degradation of pod color (Mohameda and Mentreddyb, 2004).

3. Flavor and taste: It was reported that beany characteristics did not have any significant trends with maturation, but there was a significant increase in the intensity value of bitter flavor in mature soya bean. The lower flavor in green soya beans was partially due to lower lipoxygenase activity (Mohameda and Mentreddyb, 2004).

Saponins and isoflavones are responsible for off-flavors and their thresholds are organoleptically low. The higher content of total saponins is observed in the seed hypocotyl fraction than in other seed fractions and ranges from 0.62-6.16%. The content of saponins in soya bean seed varied with the maturity of seed and was more dependent on the variety than on the cultivation year (Mohameda and Mentreddyb, 2004).

Flavor and texture of boiled green soya bean are also highly correlated to their sensory scores. The boiled or blanched soya bean contains a characteristic sweet flower-like and beany flavor. A combination of ascorbic acid, sucrose, glutamic acid and alanine make pods and seeds tasty. Whereas cis-jasmone and (Z)-3-hexenyl-acetate have been reported to confer desirable flavor (Mohameda and Mentreddyb, 2004).

There are many taste-related substances in soya bean seed, such as sugars, amino acids, organic acids, inorganic salts, flavonoids and saponins. Tsou and Hong (1990) indicated that sucrose, which is the predominant sugar in green soya bean, is responsible for its sweetness. Therefore, analysis of sucrose content is most important in the evaluation of the sweetness of green soya bean (Mohameda and Mentreddyb, 2004).

Volatile flavor of the boiled green soya bean is highly correlated with quality. Characteristic flower-like flavor components of boiled green soya bean are cis-jasmone, (Z)-3-hexenyl-acetate, linalool and acetophenone. Major components, l-octen-3-ol, 1-hexanol, hexanal, 1-pentanol, (E)-3-hexen-l-ol, 2-hepta-none and 2-pentylfuran, the beany flavor, are also detected in green soya bean. Boiling gives seeds their characteristic flavor because of heat-induced substances such as furans and ketones, and easy evaporation of volatiles due to rupture of tissue and cells. Cell rupture accompanied by freezing gives undesirable flavor because of lipid peroxides. The flavor components might be cyclo N-O substances (Mohameda and Mentreddyb, 2004).

2.1.5 Factors affecting quality attributes

Harvest time affects green soya bean quality mainly because of compositional changes during maturation. In one report with three green soya bean cultivars, ascorbic acid, sugars and free amino acids decreased with seed maturation. Seed maturity, growth environment and cultural practices affect the quality of soya bean seeds at harvest. (Mohameda and Mentreddyb, 2004).

1. Genotypes: Green soya bean cultivars with traits desirable for fresh consumption have been developed through conventional breeding. These varieties, once referred to as a "garden type of soya beans", are now known as green soya bean. The growth habit of these genotypes is similar to conventional grain soya bean bred for oil, but they are generally larger in seed size, tender in texture, lower in beany flavor, higher in protein and lower in oil and yield. The Japanese varieties tend to have larger seeds with greater flavor than the American genotypes. Significant variations in isoflavones among green soya bean genotypes have been documented (Mohameda and Mentreddyb, 2004).

2. Growing location and season: The photothermal characteristics of a location determine variety selection. Soya bean varieties are classified into maturity groups depending upon their temperature and day-length requirements. Location, climatic patterns and biotic stress cycles are major considerations in the selection of cultivars. Green soya bean yields are generally higher under cooler conditions where temperatures do not exceed 27°C during the pod-filling and seed development phases. Higher temperatures during the seed development phase result in poor quality and shriveled and fewer seeds/pod (Mohameda and Mentreddyb, 2004).

Seasonal differences influence seed quality and phytochemical contents such as isoflavones, tocopherols, phytosterols and saponins. The location and crop season characterized by the differences in environmental characters influence the seed weight and germination rate. Similar changes were also reported for seed composition. Large-seeded green soya bean varieties were reported to have poor germination. For different seed sizes within a variety, small seeds had better germination than larger seeds (Mohameda and Mentreddyb, 2004).

Akazawa and Fukushima (1991) reported both genotypic and year-to-year variations in free amino acids, total sugars, proteins and starch contents of green soya bean. The free amino acids were generally higher in green soya bean cultivar than in conventional grain soya bean cultivar whereas the year-to-year variation depended on solar radiation from flowering to harvest. Seasonal differences are manifest in variation in solar radiation, temperatures, day length and precipitation. Tocopherol metabolism appears to be influenced by environmental stresses such as drought, indicating that phytonutrients such as vitamin E may be influenced by weather (Mohameda and Mentreddyb, 2004).

3. Pre-harvest: To produce high-grade green soya bean, good crop management practices must be applied. Problems with insect and cyst nematode should be closely monitored (Mohameda and Mentreddyb, 2004).

4. Period of harvest: The optimum time for harvesting fresh green soya bean to combine the best product quality with maximum yield is rather complex and it is often a compromise depending upon the consumer, the market and the end-product requirements. Because the quality is mainly evaluated by the appearance, the superiority or inferiority of production districts is decided by propriety of harvest period and by post-harvest processing. To determine the most suitable period for harvesting, the relationships of days after flowering, pod expansion, seed components and pod color should be taken into consideration (Mohameda and Mentreddyb, 2004).

The length and width of pods can be known relatively early during the growth period, and thereafter seeds rapidly expand. The thickness and weight of pods increase after the pod expansion. Taste of the green soya bean is highly correlated to the sucrose content or glutamic acid of seed. Therefore, the sugar and free amino acid contents provide a good estimate of the tastiness of green soya bean. The taste is known to deteriorate in the latter stages of development, mainly due to the decrease in content of sugars and free amino acids (Mohameda and Mentreddyb, 2004).

Pod color is important for evaluation of the grades. Harvested pods are graded into four classes; A, B, C and D, with A being the best pods and D being the pods with the most undesirable traits. Green soya bean is harvested at about 33–38 days after flowering (DAF) depending on pod color and thickness. The pods at harvest are generally bright green in color and lose their brightness after harvest. Good qualities of green soya bean are good taste, deep green color of pods, full expansion of pods and uniform pods without infections or injuries. To obtain uniform pods, it is important to protect plants against diseases and insects. Using pod color as a guide, it is suitable to harvest before 40 DAF (Mohameda and Mentreddyb, 2004).

The sensory scores of the boiled green soya bean, harvested at different times of the day, showed no significant differences in sweetness, texture and overall scores except for flavor. Both harvest time in terms of number of days after planting and harvesting hour in the day affect the quality of green soya bean. Data showed that after harvest, the shorter the time before blanching and cooling, the better the quality (Mohameda and Mentreddyb, 2004).

5. Harvesting: Most green soya beans are harvested by hand. When the green soya beans are sold in markets still attached to the stems, the plants are hand cut or pulled out by the roots, and unacceptable pods and lower leaves are culled, and the branches tied together in small bundles. For the sale of pods alone, plants are cut and the pods are stripped off. After sorting, 300-500 g of pods is put into a polyethylene net bag and 10 or 20 bags are packaged in a corrugated cardboard box (Mohameda and Mentreddyb, 2004).

6. Post-harvest handling: Those pods having only one seed or those injured or diseased are removed by hand. Since, the market value of green soya beans is mainly determined by their appearance, the sorting process is extremely important and a producing area that excels at processing and sorting is given a superior rating by consumers (Mohameda and Mentreddyb, 2004).

Research concerning quality degradation is limited. Green soya beans belong to the vegetable group with a high rate of respiration. After harvest, sugar content decreases rapidly at higher temperatures. Free amino acids also decrease in a short period; content of alanine and glutamic acid was reduced to two-thirds and one-half of the harvest, respectively, when the pods were placed under room temperature (26 ± 2°C) and 66% humidity for 24 h. In this case, a decrease in sweetness and taste could be recognized after 10 h (Mohameda and Mentreddyb, 2004).

The changes in the quality of pods attached to the stem with leaves and roots or of the stripped pods were studied after harvest. Iwata *et al.* (1982) reported that pods on the stem possessed better quality than stripped pods, whereas Osodo (1978) reported that the pods maintained bright green color when they were wrapped with a low-density polyethylene film. The pod color deterioration is accelerated under low humidity conditions, whereas deterioration is prevented under high relative humidity. The fresh green seeds packed and sealed in airtight plastic bags could be stored for about a year when placed in controlled environment chambers set at 15-20°C temperature and 50% relative humidity. The pods stripped by machine often turned brown after two or three days, because the browning substances such as phenol oxidases are enzymatically synthesized within the injured cells (Mohameda and Mentreddyb, 2004).

Handling soya beans under cool conditions is important to maintain their high quality. Most vegetables are pre-cooled in two ways: (a) air-cooling and (b) vacuum-cooling. For green soya beans, vacuum-cooling is effective in maintaining their good quality, because the temperature can be reduced quickly. It is important for quality maintenance to save time in harvesting and sorting to the start of pre-cooling. Minamide and Hata (1990) reported that after harvest, ascorbic acid and free amino acids in green soya beans decreased rapidly but total sugar content remained almost unchanged during seven-day storage at 20°C. Increases in protein and starch contents with storage were also reported (Mohameda and Mentreddyb, 2004).

Green soya beans are packed in net bags and then put into corrugated cardboard boxes. The following procedures should help maintain the quality of soya beans in high humidity by (a) spreading moisture absorbing sheets in a box or (b) preventing transpiration by wrapping the soybeans with polypropylene film instead of the net bag. The high humidity seems effective in preventing wilting and maintenance of the deep green pod color (Mohameda and Mentreddyb, 2004).

Some reports indicate that green soya bean qualities might change during cold storage, for example loss of moisture, vitamin C, sugar, amino acid and chlorophyll degradation. Proper storage conditions are essential for green soya bean to maintain its quality. Pre-cooling was effective in maintaining better quality green soya beans during storage. Polyethylene (PE) or Polypropylene (PP) bags with 0.32% pores also can maintain good quality of green soya beans. The PE bag-packed samples retained more vitamin C, remained greener and suffered less weight loss than that packed in net bags. The hardness of all samples increased during storage (Mohameda and Mentreddyb, 2004).

Data indicated that vitamin C content decreased during storage. Green soya bean stored at 0°C had the lowest changes in color index (Mohameda and Mentreddyb, 2004).

7. Effect of processing: The effect of cooking retail green soya beans (without pods) or "green soya peas" by boiling or microwave radiation was described as followed. Cooking in a microwave resulted in a lower loss of isoflavones than cooking in boiling water. Thus, microwave-heating green soya bean in the pods, or for the shelled beans rather than boiling in water, allows for a greater retention of isoflavones. Also, cooking the green pods allows greater retention of isoflavones compared to shelled beans (Mohameda and Mentreddyb, 2004).

Murphy (2001) and Anderson and Wolf (1995) measured the group B saponins found in soya beans. The saponin levels in the raw green soya bean beans are higher than in mature soya beans. Cooking by boiling and microwave heating did not result in any statistical differences in saponin levels in shelled beans or green pods (Mohameda and Mentreddyb, 2004).

During thermal processing, trypsin inhibitors decreased at a much faster rate in green soya beans than mature beans when both types of beans were not presoaked, presumably due to high initial moisture content. There was also a decrease in oligosaccharide upon heating, but phytate showed little change (Mohameda and Mentreddyb, 2004).

2.2 Yoghurt

Yoghurt is one of the most popular fermented milk products worldwide and has widespread consumer acceptance as a healthy food. It provides an array of nutrients in significant amounts, in relation to its energy and fat content, making it a nutrient-dense food. In particular, yoghurt can provide the body with significant amounts of calcium in a bioavailable form. Furthermore, yoghurt has many health benefits beyond the basic nutrition it provides, such as improved lactose tolerance, a possible role in body weight and fat loss and a variety of health attributes associated with probiotic bacteria (Mckinley, 2005).

Yoghurt has a history of thousands of years. They have come to be enjoyed everywhere in the world for their characteristic refreshing acid taste (Fellows, 1998). Yoghurt is manufactured from pasteurized milk having the desired percentage of butterfat, which may range from 0-3.2%. If the product contains butterfat, it normally is homogenized before being cultured to ensure that cream does not rise to the surface during the fermentation process. In most instances, the yoghurt mix is fortified with nonfat dried milk solids before pasteurization to improve the body and texture of the product. In some instances, stabilizers are added to enhance the water-holding capacity of the product so that a firmer body and texture may be achieved (Walstra *et al.*, 1999).

The pasteurization time and temperatures used for the yoghurt mix normally are higher than those used for fluid milk. Yoghurt mix normally is pasteurized at a temperature of 85-91°C for 30 min. This helps ensure interaction of the whey protein with casein to increase water-holding capacity, thereby improving the custard like consistency of the gel in the finished yoghurt. It also results in less wheying off of the product, thus improving the appearance (Walstra *et al.*, 1999).

The typical or traditional starter culture used to manufacture yoghurt is a mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Figure 2.1). Some yoghurt makers think that the best starter culture for the process is one containing a 1:1 ratio of rods and cocci; however, the optimal ratio is likely to depend on the characteristics of the individual strain of each species involved in the starter. However, excess numbers of *L. bulgaricus* can result in a shaper flavor of the yoghurt (Walstra *et al.*, 1999).

The two species of bacteria in the starter culture work together to produce the necessary acidity and flavor for yoghurt (Walstra *et al.*, 1999). Initially, *S. thermophilus* grows rapidly to produce diacetyl and lactic, acetic and formic acids, while *L. bulgaricus* grows slowly but possesses do not weak protease activity which releases peptides from the milk proteins. The production of peptides by *L. bulgaricus* stimulates the growth of the *S. thermophilus* and the production of formic acids by *S. thermophilus* promotes *L. bulgaricus* (Figure 2.2). *L. bulgaricus* produces most of the lactic acid and also acetaldehyde, which together with diacetyl, give the characteristic flavor and aroma of yoghurt (Fellows, 1998).

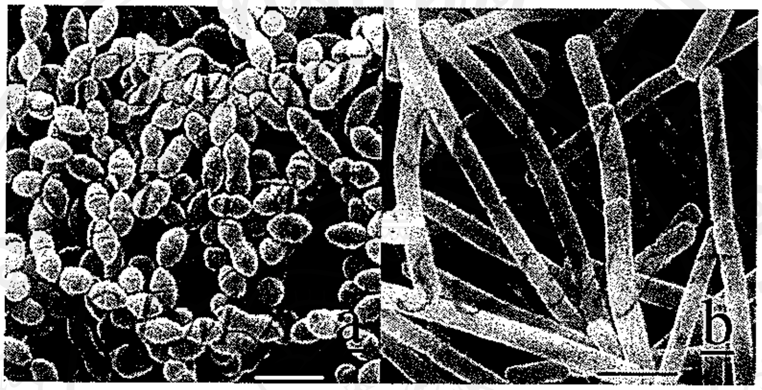

Figure 2.1 Morphologies of (a) *Streptococcus thermophilus* and (b) *Lactobacillus bulgaricus* under an electron microscope

Source: http//www.distans.livstek.lth.se:2080/S_thermo.htm and http//www.anka.livstek.lth.se:2080/L_bulg.htm [15 March, 2006]

S. thermophilus and *L. bulgaricus* exhibit a symbiotic relationship during the processing of yoghurt, with the ratio between the two species changing constantly. During fermentation, *S. thermophilus* grows quickly at first, utilizing essential amino acids produced by *L. bulgaricus.* In return, *S. thermophilus* produces lactic acid, which reduces the pH of the medium to an optimal level for the growth of *L. bulgaricus*. The production of lactic acid and to a lesser amount the formation of formic acid stimulate the growth of *L. bulgaricus*. The streptococci are inhibited at pH values of 4.2-4.4, whereas the lactobacilli tolerate pH values in the range of 3.5-3.8. After approximately 3 h of fermentation, the numbers of the two organisms should be equal. With longer fermentation, the growth rate of *S. thermophilus* declines, while *L. bulgaricus* continues to reduce the pH by producing excessive amounts of lactic acid. The pH of commercial yoghurt is usually in the range of 3.7-4.3. Although *S. thermophilus* forms acetaldehyde as a product of metabolism, the pathway is less active at normal fermentation temperatures compared to *L. bulgaricus* that produces acetaldehyde responsible for the characteristic of sharp flavour (Lourens-Hattingh and Viljoen, 2001).

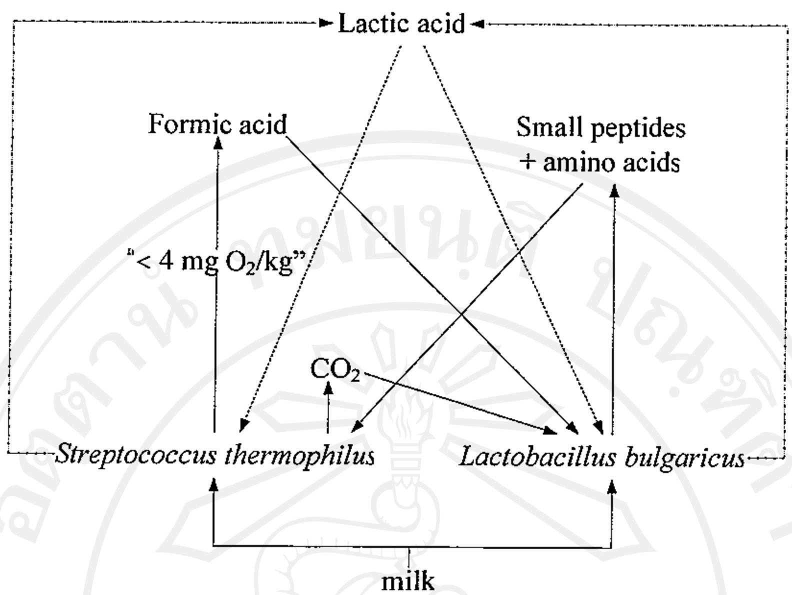

Figure 2.2 An outline for the stimulation and the inhibition of the growth of yoghurt bacteria in milk. -·-·-·-, formation of lactic acid; ········, formation of growth factors; ——, stimulation; -------, inhibition

Source: Walstra *et al.* (1999)

The consistency, flavor and aroma of yoghurt can be varied from one area to another. In some areas, yoghurt is produced in the form of a highly viscous liquid, whereas in other countries, it is in the form of a softer gel. Yoghurt is also produced in a frozen form as a dessert, or as a drink. The flavor and aroma of yoghurt are differed from those of other acidified products and the volatile aromatic substances include small quantities of acetic acid and acetaldehyde make the product has special characteristics (Bylund, 1995).

Yoghurt is typically classified as followed (Figure 2.3) (Bylund, 1995):

- Set type yoghurt, which is incubated and cooled in the package.
- Stirred type yoghurt, which is incubated in tanks and cooled before packing.
- Drinking type yoghurt is similar to the stirred type, but the coagulum is "broken down" to a liquid form before being packed.
- Frozen type yoghurt, which is incubated in tanks and frozen like ice cream.
- Concentrated yoghurt, that is incubated in tanks, concentrated and cooled before being packed. This type is also called strained yoghurt.

Yoghurt is an excellent source of protein, calcium, phosphorus, riboflavin (vitamin B2), thiamin (vitamin B1) and vitamin B12 and a valuable source of folate, niasin, magnesium and zinc. The protein that it provides is of high biological value (i.e. it contains all the amino acids essential to health), and the vitamins and minerals found in milk and dairy foods are bioavailable (i.e. available for absorption and use by the body) (Mckinley, 2005).

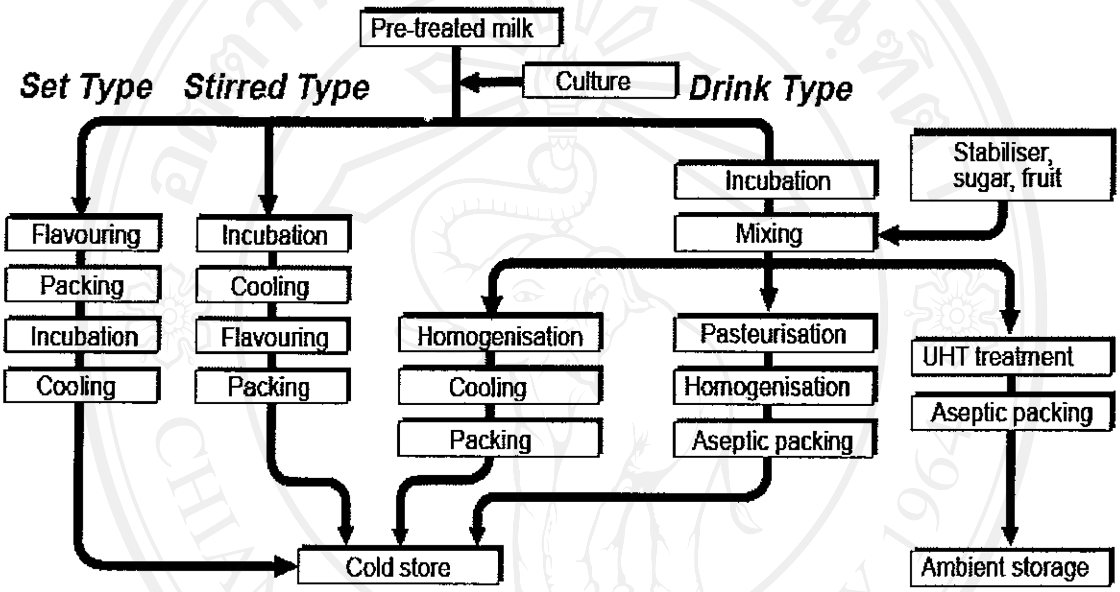

Figure 2.3 Production steps of set, stirred and drinking yoghurt
Source: Bylund (1995)

Frozen yoghurt

Frozen yogurt is a refreshing, tangy dessert that combines the flavors and textures of ice cream and sherbet. Frozen yogurt is a relative new-comer in the dessert market. Frozen yogurt is made in much the same way as ice cream and, with the exception of yogurt culture, they are made from similar ingredients. Two of the most important elements in frozen yogurt are water and air. Air is incorporated into the mixture to add volume. Water exists in the liquid elements of the mix and is considered the continuous phase, which goes from liquid to a partially solid state. Frozen yogurt is never completely frozen; it simply contains ice crystals (Tamime and Robinson, 1999).

Frozen yoghurt is classified into three main categories, soft, hard or mousse (Table 2.3). These products resemble ice cream in their physical states and they are characterised simply as having the sharp, acidic taste of yoghurt combined with the coldness of ice cream. In addition, these products contain high levels of sugar and stabilisers/emulsifiers compared with yoghurt, since these compounds are required during the freezing process to maintain the air-bubble structure (Tamime and Robinson, 1999).

Table 2.3 The composition of different frozen yoghurt.

Ingredients (%)	**Type of frozen yoghurt**		
	Soft	**Hard**	**Mousse**
Fat	2-6	2-6	2
MSNF	5-10	5-14	12
Sugar	8-20	8-16	8
Stabiliser, emulsifier	0.2-1.0	0.2-1.0	2.4
% Overrun	50-60	70-80	90

Source: Tamime and Robinson (1999)

The production of all types of frozen yoghurt is started from stirred yoghurt (Figure 2.4). Soft frozen yoghurt is made by adding 20% fruit syrup, stabilisers and emulsifiers to 80% of a cold fermented milk base, and then filling them into containers with a 50-60% overrun using a normal ice cream freezer and kept at 0 to -6°C (Nakazawa and Hosnono, 1988).

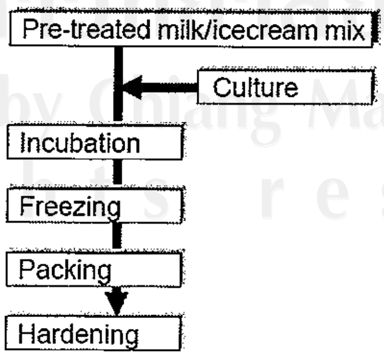

Figure 2.4 Production steps of frozen yoghurt
Source: Bylund (1995)

The hard type of frozen yoghurt, on the other hand, is more like an ice cream. It is usually contained more fruit juice (35%). The overrun is higher (70-80%) and the storage temperature is lower (-25°C). Mousse yoghurt is made by mixing a fermented milk base with a warm mousse base (a homogenized mixture of skim milk, sugar, stabilisers and emulsifiers) and freezing the mixture in a normal ice cream freezer after initial cooling. It has a higher overrun than the other two types of frozen yoghurt (90%) and is kept at a relatively high temperature, below 0°C. Frozen yoghurt sold in Japan also includes products which are relied on the foaming and emulsifying properties of milk components, with no stabilisers or emulsifiers being used (Nakazawa and Hosnono, 1988).

Frozen yoghurt is the most popular dessert made from fermented milk by *S. thermophilus* and *L. bulgaricus.* Another popular dietary adjunct that may be added to frozen desserts are *Lactobacillus acidophillus* and *Bifidobacterium* species (Marth and Steele, 1998). The addition of *L. acidophillus* and *Bifidobacterium* species produces new cultured yoghurt products. These products provide consumers with a source of microorganisms thought to have health and nutritional benefits through their growth and action in the intestinal tract. The traditional yoghurt starter cultures (i.e. *S. thermophilus* and *L. bulgaricus*) do not grow in the intestinal tract. They may provide some benefits, however, particularly in improving the utilization of lactose in persons who are lactose maldigestors (Walstra *et al.*, 1999). In addition, there is an attempt to combine a mixture of organisms, those able to colonize the gut such as, *L. acidophillus* and *Bifidobacterium* species with *S. thermophilus* to provide the characteristic yoghurt flavor (Adams and Moss, 2002).

The product characteristics of frozen yoghurt include the fact that its shelf life is relatively longer because it is distributed in a frozen condition. It should be noted that this means that counts of lactic acid bacteria must be maintained at $\geq 10^7$ CFU/g during the distribution period. The ways in which counts of lactic acid bacteria in frozen fermented milks are affected by changes in contents of milk components, sugars, etc. have been studied. Bacterial survival was increased when sucrose concentration was increased by 10%. However, it was decreased when sucrose was replaced by glucose. A slight increase in the bacterial survival was recorded when the milk solids content (dried skim milk) was increased. The study was carried out with *L. bulgaricus* and *S. thermophilus,* and the overall results indicated that *L. bulgaricus* had a high sensitivity for changes in the various yoghurt components (Nakazawa and Hosnono, 1988).

In general, manufacturers attempt to limit the amount of acetaldehyde flavor in the frozen yoghurt, believing that most customers do not prefer that flavor which characterizes plain yoghurt. Yoghurt definitely has some acidic flavor as compared with a low fat ice cream containing the same amount of fat (Marshall and Arbuckle, 1996).

The apparent reason that frozen yoghurt has been preferred over a similarly comprised and prepared ice milk product is that the yoghurt bacteria are thought by many people to assist in digestion of lactose and to have other health-promoting properties (Marshall and Arbuckle, 1996).

Frozen yoghurt products are all relatively low in fat content. In America, the label of this class of products varies with the fat content, with the same names applying as in ice cream products of the same fat content: <0.5 g total fat per 4 fl oz (113.4 g) serving is nonfat frozen yoghurt; 0.5-3.0 g per serving is low fat frozen yoghurt; and above 3 g per serving is labeled as frozen yoghurt. It is not expected that the industry will market reduced fat frozen yoghurt, because the highest fat content of frozen yoghurt is in the vicinity of 4%. Thus, it is obvious that a reduced fat product would contain about 3% fat. If the finished product weights 70 g per 4 fl oz (113.4 g) serving, at 3% fat a serving, the product would contain only 2.1 g of fat and would qualify for the low fat label (Marshall and Arbuckle, 1996).

2.3 Ice cream

Ice cream is a frozen mixture of a combination of milk components, sweeteners, stabilizers, emulsifiers and flavoring. Other ingredients such as egg products, colorings and starch hydrolysates may be added also. This mixture, called a mix, is pasteurized and homogenized before freezing. Freezing involves rapid removal of heat while agitating vigorously to incorporate air, thus imparting the desirable smoothness and softness of the frozen product (Marshall and Arbuckle, 1996).

The broad term frozen desserts refers to ice cream and related products. Specific products include ice cream and its lower fat varities, frozen custard, mellorine (vegetable fat frozen dessert), sherbet, water ice and frozen confections (Table 2.4). Some of these desserts are served in either soft frozen or hard frozen form (Marshall and Arbuckle, 1996).

2.3.1 Compositions and properties

The composition of ice cream varies widely depending on the intended market. Typical composition of a product labeled vanilla ice cream is 11% milk fat, 11% nonfat milk solids (NMS), 12% sugar, 5% corn syrup solids and 0.3% stabilizer/emulsifier. This makes the total solids approximately 39%. Products having the words flavored or artificially flavored along with the name of the flavoring on the label tend to contain the minimal amounts of solids from milk and to contain higher amounts of whey solids and corn syrup solids than do product having the name of flavoring only. The range in composition may be 0-20% fat, 8-15% NMS, 13-20% sweetener, 0-1.0% stabilizer-emulsifier and 32-43% total solids (Marshall and Arbuckle, 1996).

The physical structure of ice cream is a complicated physicochemical system. The three phrases of the system are liquid, solid and gas. Air cells and ice crystals are dispersed in a continuous liquid phase. The liquid phase also contains solidified fat, colloidal milk proteins, insoluble milk salts and lactose crystals in some cases, colloidal stabilizers and sugars and soluble salts in solution (Marshall and Arbuckle, 1996).

Table 2.4 Typical ice cream formulas.

Types of Ice cream	Fat % wt	MSNF % wt	Sugar % wt	E/S % wt	Water % wt	Overrun % vol
Dessert ice	15	10	15	0.3	59.7	110
Ice cream	10	11	14	0.4	64.6	100
Milk ice	4	12	13	0.6	70.4	85
Sherbet	2	4	22	0.4	71.6	50
Water ice	0	0	22	0.2	77.6	0

Fat:	Milk, cream, butter or vegetable fat
Water:	May include flavouring or colouring matter
MSNF:	Milk solids-non-fat (protein, salts, lactose)
Sugar:	Liquid or solid sucrose (10% of sugar may be glucose or non-sugar sweetener)
E/S:	Emulsifier and stabilizer, e.g. monoglycerides, gelatin, alginate
Overrun:	Amount of air in product
Other ingredients:	Egg, fruit and chocolate pieces may be added during processing.

Source: Bylund (1995)

2.3.2 Ingredients

There are several ingredients that are used to produce an ice cream. Advantages and limitations of these ingredients can be seen in Table 2.5. Details of each ingredient will be as followed:

1. Fat: Fat, which makes up 12% of the volume of dairy ice cream, may be either milk fat or vegetable fat. In the former case it may take the form of whole milk, cream, butter or butter oil. Some or all of the milk fat in ice cream may be replaced by vegetable fat in the form of rapeseed oil, cotton seed oil, coconut seed oil or palm kernel oil. Use of vegetable fat in ice cream is however, prohibited in some countries (Teknisk, 2002).

2. Solids-non-fat: Solids-non-fat consists of proteins, lactose and mineral salts. They are added in the form of milk powder and condensed skim milk. For best results, the quantity of solids-non-fat should always be in a certain proportion to the quantity of fat. Thus for the manufacture of ice cream with a fat content of 12%, the amount of solids-non-fat should be about 11-11.5%. Solids-non-fat has high nutritional value and an ability to improve the texture of the ice cream by binding and replacing water. They also significantly affect the correct distribution of air in the ice cream during the freezing process (Teknisk, 2002).

3. Sugar: Sugar is added to adjust the solids content in the ice cream and to give it the sweetness which customer prefer. The ice cream mix normally contains between 10 and 18% sugar. Many factors influence the sweetening effect and product quality, and many different types of sugar can be used, for example cane and beet sugar, glucose, lactose and invert sugar (a mixture of glucose and fructose). Sorbitol is used in the manufacture of ice cream for diabetics. Sweetened condensed milk is sometimes used, contributing to both the sweetening effect and the solids-non-fat content (Teknisk, 2002).

4. Emulsifiers: Emulsifiers are substances which assist emulsification by reducing the surface tension of liquid products. They also help to stabilize the emulsion once formed. Yolk of egg is a well-known emulsifier, but is expensive and less effective than the most commonly used types, which are mainly non-ionic derivatives of natural fats which have been esterified to give them one or more water-soluble (hydrophilic) radicals bonded to one or more fat-soluble (lipophilic) radicals. The emulsifiers used in ice cream manufacture can be divided into four groups: glycerine esters, sorbitol esters, sugar esters and esters of other origin. The amount added is usually of the order of 0.3-0.5% of the volume of the ice cream mix (Teknisk, 2002).

5. Stabilizers: A stabilizer is a substance which when dispersed in a liquid phase (water) binds a large number of water molecules. This is called hydration and means that the stabilizer forms a network which prevents the water molecules from moving freely. Stabilizers are of two kinds: protein and carbohydrate stabilizers. The protein group includes gelatin, casein, albumin and globulin. The carbohydrate group includes marine colloids, hemicelluloses and modified cellulose compounds. The stabilizer dosage usually amounts to 0.2-0.4% volume of the ice cream mix (Teknisk, 2002).

6. Flavoring additives: Flavoring additives are very important to the customer' choice of ice cream. The most commonly used flavors are vanilla, nougat, chocolate, strawberry and nut. These can be added at the mixing stage. If the flavoring takes the form of larger pieces such as nougat, nuts, fruit or jam, it is added after the mix has been frozen. Cocoa is widely used to give ice cream bars, cones and bricks a coating of chocolate. For this purpose the cocoa is mixed with fat, cocoa fat for example, to give the chocolate coating the correct viscosity, elasticity and consistency (Teknisk, 2002).

7. Coloring additives: Coloring additive is added to the mix to give the ice cream an attractive appearance and to enhance the color of fruit flavoring additives. The coloring additive is added in the form of powder or paste, which is mixed with water and boiled. Sodium benzoate is added as a sterilant. The coloring solution is usually added in a dosage of 10-20 ml per 100 l of mix. Only approved dyes and sterilants may be used (Teknisk, 2002).

8. Water and air: Water and air are important constituents of ice cream. Water is the continuous phase. It is present as a liquid, a solid and as a mixture of the two physical states. The air is dispersed through the fat-in-serum emulsion. The interface between the water and air is stabilized by a thin film of unfrozen material and by partially churned fat globules. The interfaces of the fat are covered by a layer of fat-emulsifying agent. Water in the ice cream mix comes from fluid dairy products and syrup or from added water. In the manufacture of ice cream, an overrun, or the increase in volume of ice cream over the volume of mix used, is produced by incorporation of air. The amount of air in ice cream is important because it influences quality and profits and is involved in meeting legal standards. Maintaining a uniform amount of air is essential in controlling both quality and quantity (Marshall and Arbuckle, 1996).

Table 2.5 Advantages and limitations of selected ice cream constituents.

Constituents	Advantages	Limitations
Milk fat	Increases richness of flavor and smoothness of texture, lubricates and insulates the mouth, adds body.	Relatively high cost. Hinders whipping. May limit consumption due to high calories and satiating effect.
Nonfat milk solids (NMS)	Improves texture, builds body, allows higher overrun.	High amount may cause sandy texture and cooked or salty flavor.
Sugar	Lowers freezing point. Displaces water. Improves flavor/texture. Low hardening temperature.	Excess sweetness possible. Lowers whippability. Increases freezing time.
Corn syrup solids	Low cost solids. Low relative sweetness. Bind and replace water.	Impart off-flavor when overused.
Stabilizer	Make texture smooth. Provide body.	Excess firmness may occur. High melt resistance.
Egg yolk solids	Improve whippability. Impart custard flavor.	Foamy melted product.
Total solids (TS)	Smoother texture. Firmer body. Higher nutrient content. Lessen excess coldness.	Heavy, soggy or sticky body. Reduce coldness.
Flavoring	Increases acceptability.	Intensities and harshness may be unacceptable.
Coloring	Improves attractiveness. Aids flavor identification.	Allergic reactions of some people to yellow no.5 or 6.

Source: Marshall and Arbuckle (1996)

2.3.3 The balance mix

A balance mix is one in which the proportions of ice cream ingredients will produce a satisfactory finished product (Marshall and Arbuckle, 1996).

Defects such as rancid flavor, feed flavor or uneven color cannot be corrected by changing the concentration of ice cream constituents. Therefore, they do not indicate a poorly balanced mix. However, other defects, such as (1) lack of flavor, insufficient concentration of flavoring, (2) lack of richness, insufficient concentration of fat, (3) sandiness, too high concentration of lactose or (4) weak body, low total solids or low stabilizer, may be corrected by changing the composition of the mix. These defects do, therefore, indicate that the mix is incorrectly balanced (Marshall and Arbuckle, 1996). The processing steps to make an ice cream are displayed in Figure 2.5.

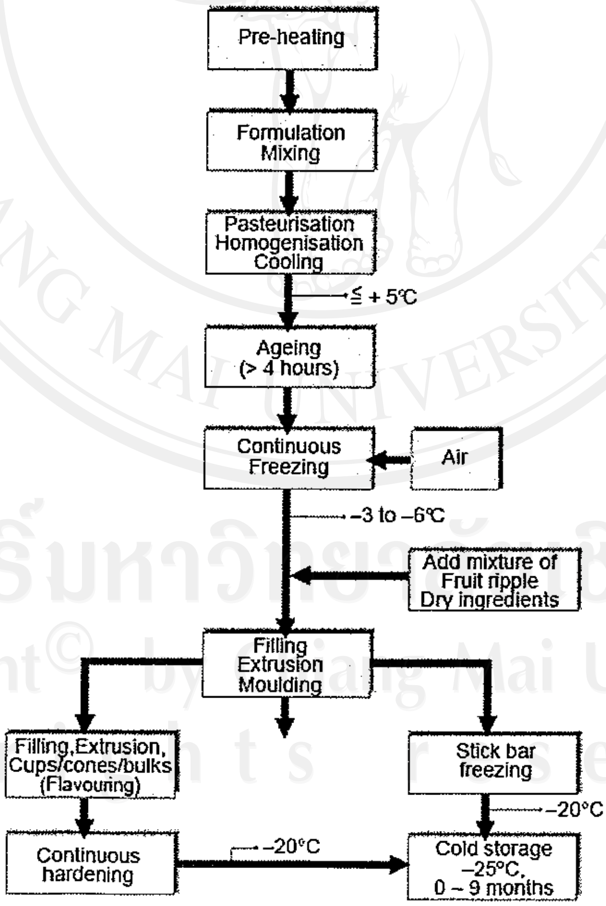

Figure 2.5 An ice cream process

Source: Bylund (1995)

Ice cream is a palatable, nutritious, healthful and relatively inexpensive food. One serving of ice cream with an average composition of 4 fluid ounces (113.4 g) and weights about 70 g supplies about 130 calories, 3 g protein, 100 mg calcium, 70 mg phosphorus, 250 international units (IU) vitamin A, 120 μg riboflavin and 30 μg thiamine (Marshall and Arbuckle, 1996).

2.4 Probiotic bacteria

Probiotic bacteria are live microbial strains that, when applied in adequate doses, beneficially affect the host animal by improving its intestinal microbial balance. Probiotic foods are food products that contain a living probiotic ingredient in an adequate matrix and in sufficient concentration, so that after their ingestion, the postulated effect is obtained, and is beyond that of usual nutrient suppliers. The probiotic strains used in dairy products most commonly belong to *Lactobacillus* and *Bifidobacterium* genera (Table 2.6) (Saxelin and Korpela, 2003).

Probiotic bacteria have been increasingly included in yoghurts and fermented milks during the last two decades. Most commonly probiotics are included lactobacilli such as *Lactobacillus acidophillus*, and bifidobacteria such as *Bifidobacterium bifidum.* A major development in functional foods pertains to food containing probiotics and prebiotics which enhance health promoting microbial flora in the intestine. There is growing scientific evidence to support the concept that a maintenance of healthy intestinal microflora may provide protection againt gastrointestinal disorders including gastrointestinal infections, inflammatory, bowel diseases and even cancer. The use of probiotic bacterial cultures stimulates the growth of preferred microorganisms, crowds out potentially harmful bacteria (Figure 2.6) and reinforces the body' natural defence mechanisms. Today, plenty of evidence exists on the positive effects of probiotics in human health (Table 2.8). However, this has usually been demonstrated in diseased human populations only. Thus there is a need for evidence for probiotic health benefits in average (generally healthy) populations (Saarela *et al.*, 2000).

Table 2.6 List of species (by alphabetical order) of the genera *Lactobacillus* and *Bifidobacterium.*

Lactobacillus		***Bifidobacterium***
L. acetotolerans	*L. jensenii*[a]	*B. adolescentis*[a]
L. acidophilus[a]	*L. Johnsonii*	*B. angulatum*[a]
L. agilis	*L. kandleri*	*B. animalis*
L. alimentarius	*L. kefir*	*B. asteroides*
L. amylophilus	*L. ke.ranofaciens*	*B. bifidum*[a]
L. amylovorus	*L. malefermentans*	*B. boum*
L. avarius	*L. mali*	*B. breva*[a]
L. bifermentans	*L. minor*	*B. catenulatum*[a]
L. brevis[a]	*L. murinus*	*B. choerinum*
L. buchneri[a]	*L. oris*[a]	*B. coryneforme*
L. casei subsp. casei[a]	*L. parabuchneri*[a]	*B. cuniculi*
L. collinoides	*L. paracasei*[a]	*B. dentium*[a]
L. confusus	*L. pentosus*	*B. gallicum*
L. coryniformis	*L. pontis*	*B. gallinarum*
L. crispatus[a]	*L. plantarum*[a]	*B. globosum*[a]
L. curvatus	*L. reuteri*[a]	*B. indicum*
L. delbrueckii	*L. rhamnosus*[a]	*B. infantis*[a]
L. farciminis	*L. ruminis*	*B. lactis*
L. fermentum[a]	*L. sake*	*B. longum*[a]
L. fructivorans	*L. salivarius*[a]	*B. magnum*
L. fructosus	*L. sanfrancisco*	*B. merycicum*
L. gallinarum	*L. sharpeae*	*B. minimum*
L. gasseri[a]	*L. suebicus*	*B. pseudocatenulatum*[a]
L. graminis	*L. vaccinostercus*	*B. psudolongum*
L. halotolerans	*L. vaginalis*[a]	*B. pullorum*
L. hamsteri	*L. viridescens*	*B. ruminantium*
L. helveticus		*B. saeculare*
L. hilgardii		*B. subtile*
L. homohiochii		*B. suis*
L. intestinalis		*B. thermophilum*

[a] Species isolated from human sources

source: Gomes and Xavier (1999)

2.4.1 Selection criteria for probiotics

The examples of criteria for selection of probiotics were reported by Gibson and Fuller (2000). They included some properties as shown in Table 2.7.

Table 2.7 Criteria for selection of probiotics.

Criteria	Details
Stain origin	Those isolated from the same species as the hosts to enhanced chance of survival.
Safety	Probiotics should be generally recognized as safe (GRAS) with minimal possibilities for the transfer of antibiotic resistance.
Survivability	Strains that have improved resistance to acid, bile salts and able to attach to the gut epithelium would have better survival characteristics both in product and after ingestion.
Production characteristics	Able to be grown in bulk culture and without genetic variation.
Processing	Strong enough to withstand the tolerant condition in production process.
Sensory properties	When added to foods, the quality should not be reduced.
Microbiological properties	Required to survive in the gastrointestinal microbial ecosystem.
Effect on the consumer	No adverse side effects such as bloating and effects on gut transit should occur.
Adherence	To enhance survival in the gut.
Effect on the pathogens	Many probiotics are able to inhibit pathogens by the production of acid, bacteriocins or competitive exclusion.
Modulation of metabolic activities	Such as the inactivation of procarcinogens.

Source: Gibson and Fuller (2000)

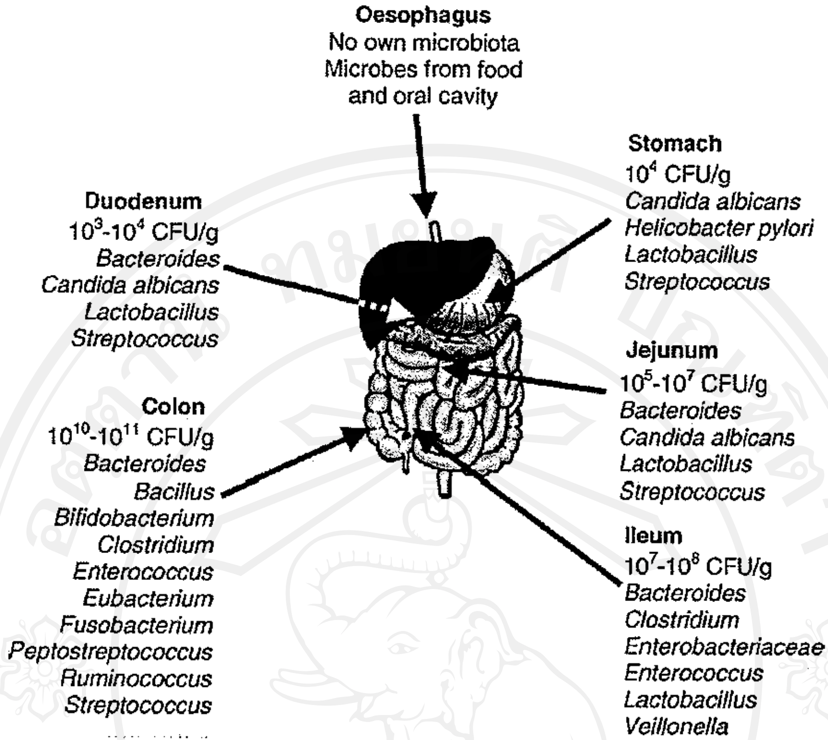

Figure 2.6 The numerically dominant microbial genera in the adult human gastrointestinal tract

Source: Isolauri (2004)

Cultures of *L. acidophillus* and *Bifidobacterium* spp. make good probiotics because they are of human origin, surviving stomach acid, intestinal bile and capable of colonizing the colon. However, milk products fermented with these organisms could not readily expand into the market place because human strains grow slowly in milk, lose viability upon storage and do not yield products with favorable organoleptic qualities. Better growth could be achieved by supplementing milk with yeast extract or whey protein and by adding threonine to promote acetaldehyde production and enhance flavor. Most *Bifidobacterium* strains cannot ferment milk by themselves because they require low redox potentials and peptides generated from the breakdown of casein. Moreover, when co-cultured with lactobacilli, they quickly become inhibited as the pH drops (Scheinbach, 1998).

Table 2.8 Potential health and nutritional benefits of functional foods prepared with probiotic bacteria.

Beneficial effect	Possible causes and mechanisms
Improved digestibility	Partial breakdown of proteins, fats and carbohydrates
Improved nutritional value	Higher levels of B vitamins and certain free amino acids, methionine, lysine and tryptophan
Improved lactose utilization	Reduced lactose in product and further availability of lactase
Antagonistic action Towards enteric pathogens	Disorders, such as functional diarrhoea, mucous colitis, ulcerated colitis, diverticulitis and enteric pathogens antibiotic colitis controlled by acidity, microbial inhibitors and prevention of pathogen adhesion or pathogen activation
Colonisation in gut	Survival in gastric acid, resistance to lysozyme and low surface tension of intestine, adherence to intestinal mucosa, multiplication in the intestinal tract, immune modulation
Anticarcinogenic effect	Conversion of potential pre-carcinogens into less harmful compounds Inhibitory action towards some types of cancer, in particular cancers of the gastrointestinal tract by degradation of pre-carcinogens, reduction of carcinogen-promoting enzymes and stimulation of the immune system
Hypocholesterolemic action	Production of inhibitors of cholesterol synthesis. Use of cholesterol by assimilation and precipitation with deconjugated bile salts
Immune modulation	Enhancement of macrophage formation, stimulation of production of suppressor cells and γ-interferon
Vaccine vehicle	Naturally occurring or rDNA vaccinal epitopes

source: Gomes and Xavier (1999)

2.4.2 *Lactobacillus acidophilus*

L. acidophilus, which is a resident species in the human digestive tract, has been used in many fermented milks. Lactic drinks and preparations of lactic acid bacteria have been produced since 1922. Advances in scientific techniques in recent years, besides clarifying various physiological effects of *L. acidophilus*, have given a more prominence prospect to products using *L. acidophilus* (Nakazawa and Hosnono, 1988).

2.4.2.1 Characteristics of *L. acidophilus*

Lactobacilli (*Lactobacillus* spp.) was first isolated from kefir and named by the Russian Kern in 1881. At present, *L. acidophilus*, *Lactobacillus salivarius*, *Lactobacillus leichmanii* and *Lactobacillus fermentum* are recognized as possible resident species in the human digestive tract (Figure 2.6); these are called resident species to distinguish them from transient species, which can only pass live through the digestive tract. The transient strains will include strains of *Lactobacillus brevis*, *Lactobacillus plantarum* and *Lactobacillus casei* (Nakazawa and Hosnono, 1988).

L. acidophilus is found to be a resident from the lower small intestine and throughout the large intestine and is detected particularly frequent in the lower small intestine. Bifidobacteria (*Bifidobacterium* spp.) on the other hand is present mainly in the large intestine (Figure 2.6) (Nakazawa and Hosnono, 1988).

L. acidophilus (Figure 2.7) shows the following characteristics (Nakazawa and Hosnono, 1988):

- It does not grow at 15°C, and does not ferment ribose.
- The optimum temperature for growth is at 35-38°C.
- The optimum pH values are in the range of 5.5-6.0.
- In cow's milk, it produces 0.3–1.9% DL lactic acid; acid producing capability are differed among strains.
- Generally, it has strict nutritional requirements. It requires acetates (or mevalonic acid), riboflavin, pantothenic acid, calcium, niacin and folic acid.

- It is resistant to bile acids.
- It produces threonine aldolase and alcohol dehydrogenase, which influence aroma.

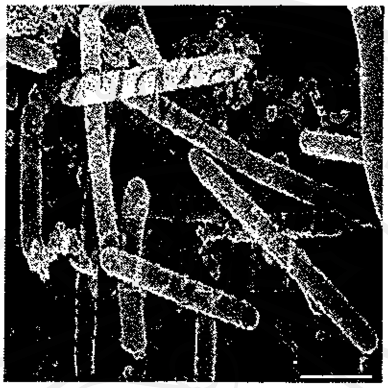

Figure 2.7 Morphology of *Lactobacillus acidophillus* under an electron microscope.
Source: http//www.anka.livstek.lth.se:2080/acidophilus.htm [15 March, 2006]

2.4.2.2 The antibacterial action of *L. acidophilus*

L. acidophilus produces organic acids, hydrogen peroxide and antibiotics. These products have been shown to be able to suppress the multiplication of pathogenic or putrefactive bacteria and they show a stronger antibacterial properties against Gram positive bacteria (e.g. *Staphylococcus aureus* and *Clostridium perfringens*) than against Gram negative bacteria (e.g. *Salmonella typhimurium* and *Escherichia coli*). It has also been claimed that the antibacterial action of *L. acidophilus* is particularly strong in inhibiting pathogenic or potentially pathogenic bacteria (Nakazawa and Hosnono, 1988).

A decrease in *Bifidobacterium* spp. in the human intestinal microflora in old age and an increase in *C. perfringens* are seen as a problem in maintaining a healthy intestinal microflora, therefore the antibacterial activity of *L. acidophilus* suggests that a consumption of *L. acidophilus* will have a beneficial effect (Nakazawa and Hosnono, 1988).

The antibiotics produced by *L. acidophilus* show the following characteristics (Nakazawa and Hosnono, 1988);

1. Lactocidin is a non-volatile non-dialysable substance soluble in ether, with a broad antibiotic spectrum incorporating Gram negative and Gram positive bacteria.

2. Acidophilin is a low-molecular-weight peptide and heat stable under acid conditions. It is known to show an antibacterial action against pathogenic bacteria *in vitro*.

3. Acidolin has an absorption peak at 255 nm and a molecular weight of 200. It is extremely heat stable and solutions from the compound are strongly acidic. It shows a broad antibacterial spectrum, particularly against spore-forming bacteria.

4. Lactosin B shows a peak absorption at 211 nm, has a molecular weight of 6000-6500, and is thought to have a peptide structure. Its antibacterial action is restricted to *Lactobacilaceae*.

5. Ammonium sulphate precipitates of filtrates from culture of *L. acidophilus* strain AC_1 and *L. acidophilus* strain AR_1 was purified on Sephades G100, and produced protein-type antibacterial substances with molecular weight of 5400 and 5200. Those substances showed antibacterial activities against *Bacillus subtilis, S. aureus, E. coli* and *Salmonella* spp., etc. The first-mentioned substance lost its activity when heated at 50°C for 20 min, but the latter was stable when heated for 80 min at 50°C under acid conditions. Both showed an optimum pH of 4.0-5.5 and were inactivated by trypsin treatment.

6. A peptide with a molecular weight of 3500 from bacterial cell extracts of *L. acidophilus* ATCC 3205 showed an antibacterial activity against *E. coli* over a wide range of pH.

2.4.2.3 Beneficial effects of *L. acidophilus* (Nakazawa and Hosnono, 1988).

1. Improving the intestinal microflora: In the intestinal tract, *L. acidophilus* is thought to suppress the multiplication of pathogenic and putrefactive bacteria which cause intestinal problems, diarrhoea and digestive upsets, etc. and contribute to maintenance of health.

2. Growth promoting effects: A significant increase in body weight was noted in mice after they were given acidophilus milk rather than untreated milk for 4 weeks. It was possible that the reasons for this were the efficiency of utilization of nutrients promoted by lactic acid fermentation and a beneficial effect of *L. acidophilus.*

The efficiencies of utilization of protein, calcium, iron and phosphorus, etc. are higher with fermented milks than with the original cows' milk. *L. acidophilus* produces organic acids, such as lactic acid, hydrogen peroxide and antibiotics and breaks down bile acids to improve the intestinal microflora and create an environment for an efficient utilization of nutrients.

3. Intensifying actions on β-galactosidase activity: There are people who find it unpleasant to drink milk because of a decrease in the secretion of β-galactosidase in the digestive tract when they become adults. The administrations of acidophilus milk and of cows' milk containing *L. acidophilus* to such people have shown a beneficial effect.

These beneficial effects of *L. acidophilus* are thought to be due to a suppression of gas-producing bacteria in the intestinal tract by *L. acidophilus* as well as the action of *L. acidophilus* β-galactosidase when there is a high proportion of *L. acidophilus* present.

4. Cholesterol-lowering action: The existence of cholesterol-utilizing *L. acidophilus* has been reported for humans. Besides anti-cholesterol factors contained in milk components, such as lactose, calcium, orotic acid, whey protein, etc., anti-cholesterol factors such as cell components of lactic acid bacteria and extrabacterial metabolites are also thought to be present in fermented milks.

5. Cancer-controlling action: Among the intestinal bacteria, there are some which act on food components and digestive secretions like bile acids to produce harmful putrefactive products such as ammonia, amines, phenols, indole and hydrogen sulphide or other carcinogenic substances. If these harmful metabolites are produced in large quantities, they promote a decrease in liver function and a development of cancer.

It is known from epidemiological investigations that eating western foods, which are high in fat, high in protein and low in vegetable fibre, is conducive to cancer of the large intestine. It has been reported that the activities of bacterial enzymes in the intestine such as β-galactosidase, nitroreductase, azoreductase and α-dehydrogenase were significantly higher in people eating a western diet than in vegetarians. Since in the large intestine these bacterial enzymes can convert chemical carcinogen precursors into carcinogens, the intensity of the activity of these enzymes can be used to monitor a development of cancer in the large intestine. In the light of this matter, the beneficial effect of administering *L. acidophilus* will have a positive effect for human health, since it has been noted to decrease the activity of the enzymes which contribute to the production of carcinogenic substances (Nakazawa and Hosnono, 1988).

When *L. acidophilus* was given to rats on a meat diet, faecal β-galactosidase, nitroreductase and azoreductase were significantly decreased. In an experiment on the development of cancer of the large intestine of rats that were given a chemical carcinogen dimethylhydrazine, the development of cancer was also retarded by giving *L. acidophilus* (Nakazawa and Hosnono, 1988).

It has been reported that in man, the administration of *L. acidophilus* or milk containing *L. acidophilus* increased counts of lactobacilli in faeces and decreased the activities of β-galactosidase and nitroreductase (Nakazawa and Hosnono, 1988).

Intestinal lactobacilli also break down nitrosamines, which have been shown to be connected with a development of stomach cancer and cancer of the small intestine. (Nakazawa and Hosnono, 1988).

2.4.3 *Bifidobacterium spp.*

Bifidobacteria was first discovered in faeces of breast-fed infants in 1899 by Tissier at the Pasteur Institute. The fact that bifidobacteria is particularly dominant as resident bacteria in the intestinal tract of breast-fed infants has been considered to be important for the prevention of infections in infants via nutritional physiology. More recently it has become clear that they are widely distributed from adulthood to old age, and the significance of the presence of bifidobacteria throughout life has attracted attention (Nakazawa and Hosnono, 1988).

2.4.3.1 Characteristics of Bifidobacteria

Bifidobacteria has been isolated from the intestinal tract and faeces of animals and from sewage, as well as from the human intestinal tract, faeces, vagina and oral cavity (Nakazawa and Hosnono, 1988).

The main characteristics shown by bifidobacteria (Figure 2.8) are as followed (Nakazawa and Hosnono, 1988; Marks, 2004):

- They are Gram positive, anaerobic, nonmotile and non-spore-forming bacteria.
- Depending on culture conditions they show Y-shapes, V-shapes, club-shaped rods, curves rods or pallisade shapes.
- They present at a length of 2-8 μm.
- They dye irregularly with methylene blue, and form clumps of 2 or more.
- The optimum temperature for growth is at 37-41°C and the full growth range extending 20-45°C
- The optimum pH is 6.5-7.0, outside the range of 4.5-8.5.
- There is species variation in the sensitivity to oxygen levels.
- The organisms ferment 1 mol glucose with fructose-6-phosphate kinase to produce 1.5 mol acetic acid and 1 mol lactic acid (Figure 2.9). They do not produce CO_2, butyric acid, propionic acid, etc.
- They do not produce catalase or indole and do not reduce nitrates.

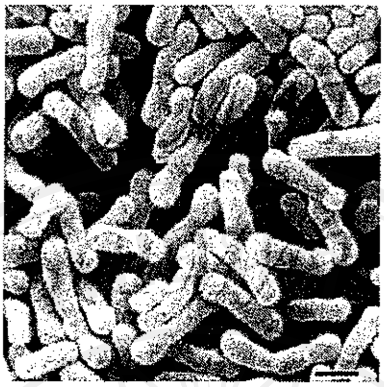

Figure 2.8 Morphology of *Bifidobacterium bifidum* under an electron microscope.

Source: http//www.anka.livstek.lth.se:2080/bifidum.htm [15 March, 2006]

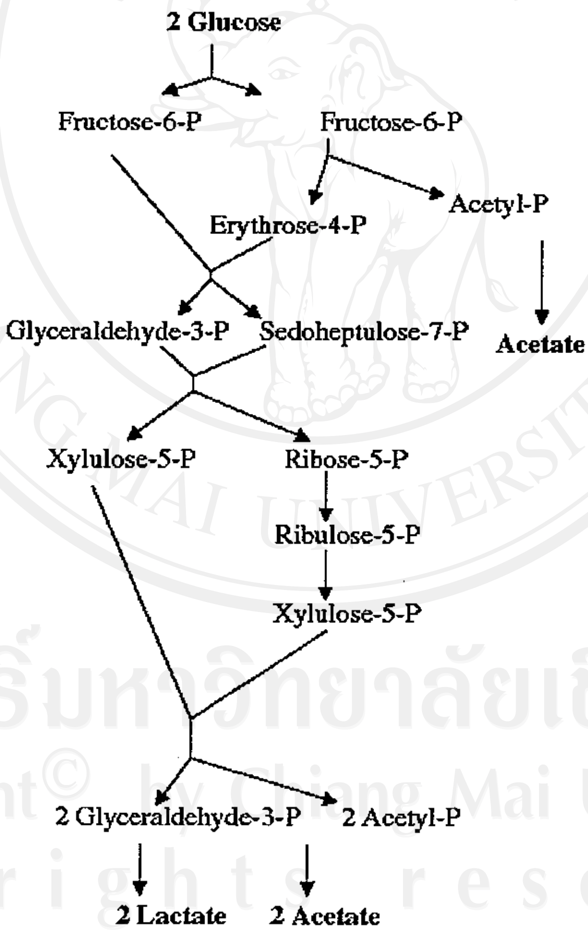

Figure 2.9 Formation of 2 molecules of lactate and 3 molecules of acetate from 2 molecules of glucose by bifidobacteria

Source: Marks (2004)

2.4.3.2 Bifidobacteria in the human intestinal tract

Bacteria inhabiting the intestinal tract of man or animals are known as intestinal bacteria, and together with other microflora they make up the intestinal microflora. There are over 100 microflora species and over 10 bacteria in the human intestinal tract, including both beneficial and harmful bacteria. The bifidobacteria, which is one of the beneficial bacteria, is present in particularly large numbers in the large intestine (Nakazawa and Hosnono, 1988).

In man, bifidobacteria first begins to appear 3-4 days after birth, and by day 5, the bifidobacteria can already be seen to be the predominant intestinal microflora. Numbers of bifidobacteria in faeces reach 10^9-11^{11}/g. The predominance of bifidobacteria in the intestinal microflora is one indication of a good health condition in babies (Nakazawa and Hosnono, 1988).

By the beginning of the 1960s, it had been shown clearly that bifidobacteria is a predominant group in the intestinal microflora in healthy people, not only in babies but also in children, adults and elderly people as well (Nakazawa and Hosnono, 1988).

The intestinal microflora is quite stable in healthy people, but varies with host physiology, food, medication, stress, age, etc. Genera which are easily affected include bifidobacteria (*Bifidobacterium*), *Enterobacteriaceae, Streptococcus, Clostridium* and *Lactobacillus* (Nakazawa and Hosnono, 1988).

People with achlorhydria, malignant gastrointestinal tumours, cancer of the large intestine, constipation or diarrhoea, etc., have low counts of bifidobacteria in their intestinal tracts. The elderly people also have lower counts of intestinal bifidobacteria due to a decrease in physiological functions such as gastric secretion, and this tendency appears to be particularly marked in senility (Nakazawa and Hosnono, 1988).

The absence of bifidobacteria in the intestinal tract implies abnormalities in health. The effects of harmful substances produced by a multiplication of harmful bacteria such as *Cl. perfringens*, etc., which take the place of bifidobacteria make it increasingly difficult to maintain a healthy state (Nakazawa and Hosnono, 1988).

2.4.3.3 Beneficial effects of Bifidobacteria (Nakazawa and Hosnono, 1988).

1. Improved protein metabolism: Bifidobacteria has a phosphoprotein phosphatase activity and can break down the α-casein in human milk. This is thought to contribute to a satisfactory absorption of human milk protein.

Nitrogen retention is good in infants with a bifidus microflora. Bifidobacteria is thought to promote the metabolism of amino acids, but the mechanism for this is unclear.

One of the roles which bifidobacteria fulfils in the intestinal tract is to suppress the multiplication of putrefactive bacteria. Accordingly, the formation of a bifidus microflora suppresses the production of putrefactive products from amino acids by putrefactive bacteria, thereby stopping losses of nutrients.

2. Improved vitamin metabolism: Bifidobacteria is predominant in the intestinal microflora of healthy people irrespective of age. They produce vitamins. Values reported for vitamins produced by bifidobacteria include vitamin B_1 7.5 µg and B_2 25 µg/g dry weight for intracellular bacterial vitamins, and B_1 25-250 µg, B_2 10 µg, B_6 100 µg, B_{12} 0.06 µg, nicotinic acid 400 µg and folic acid 25 µg/l of medium for vitamins produced outside the bacterial cells.

It is known that in adults, bacteria inhabiting the intestinal tract such as *Bacilus thiaminolyticus* can break down vitamin B_1 and cause vitamin B_1 deficiency. In such cases oral administration of vitamin B_1 is not effective; it is important first of all to improve the intestinal microflora so as to suppress the thiaminolytis bacteria, and a preparation of bifidobacteria has been suggested as an effective mean of doing this. With a presence of bifidus microflora it would also enable to have a beneficial utilization of the extracellular vitamin B_1 produced by the bifidobacteria.

3. Antibacterial actions (suppression of putrefactive and pathogenic bacteria): *In vitro*, bifidobacteria has been noted to have an antibacterial activity against pathogenic *E. coli, S. aureus, Shigella dysenteriae, Salmonella typhi, Proteus spp., Candida albicans,* etc. The main contribution to the antibacterial action shown by bifidobacteria is from the organic acids which they produce. Bifidobacteria makes 1 mol lactic acid and 1.5 mol acetic acid from 1 mol glucose. They also make small amounts of formic acid.

An antibiotic named bifidin has been isolated from *Bifidobacterium bifidum* 1452. Bifidin is stable to a heating at 100°C for 30 min; it gives a positive ninhydrin reaction, and its main components are phenylalanine and glutamic acid. It shows an antibacterial activity against *Micrococcus flavus* and *S. aureus*, being active at pH 4.8-5.5.

4. Prevention of constipation: Constipation is affected by factors such as the quality of food, regularity in taking food and the motility of the digestive tract. The organic acids produced by bifidobacteria as metabolites as they multiply are thought to stimulate the intestinal peristalsis and help with normal bowel movement. Bifidus preparations for constipation sufferers and people with irregular bowel movement have been on sale for many years.

5. Treatment of liver damage: Disturbances to liver functions cause metabolic abnormalities with ill effects from ammonia and amines, products of putrefactive bacteria. A scientific research in proving bifidus milk over a long period for patients with chronic hepatitis or cirrhosis of the liver, blood ammonia, phenol and urinary indican showed that the effect of illnesses were significantly decreased as bifidobacteria in faeces increased and the patients showed an increase in appetite and gained weight.

Beneficial effects cited for bifidobacteria include: uptake of nitrogen compounds by the bifidobacteria themselves, suppression of the putrefactive bacteria which produce ammonia and amines and depression of intestinal pH by the organic acids produced by bifidobacteria to give ionic conditions in which ammonia is not readily absorbed.

6. Immunoactivation effects: Germ-free animals have a very weak resistance to infection because they lack a capacity to produce antibodies. One significant aspect of a normal intestinal microflora is that it gives a high level of immunity.

Administration of bifidobacteria, etc., is known to raise the production of antibodies in animal experiments. Oral administration of peptidoglycan (muramyl dipeptide) from the digestion of the cell wall of bifidobacteria with lysozyme increased the immune response shown by chickens, mice and pigs (Nakazawa and Hosnono, 1988).

2.4.4 Freeze-dried cultures

When transportation and storage of cultures at -40°C is not possible, freeze-dried cultures are a good alternative to frozen concentrates. Current technology can provide highly active freeze-dried cultures that, like some frozen concentrated cultures, can be added directly to milk raw materials of a fermented product. The major disadvantage of using freeze-dried preparations in this manner is the longer lag phase they exhibit, adding an additional 30-60 min to the time normally required to make some cheeses. Preparation of freeze-dried cultures is initially similar to preparation of frozen concentrates. After freezing, the culture concentrate is placed under high vacuum to dehydrate by sublimation. The dry cells are then packaged under aseptic conditions, preferably in the absence of oxygen. Exposure to oxygen rapidly damages the cells (Marth and Steele, 1998).

2.5 Stabiliser

The word stabiliser was originally designated to a group of substances a time were known as holders, colloids, binders and fillers. The primary purpose of using a stabiliser in yoghurt ice cream is to produce smoothness in body and texture, retard or reduce ice crystal growth during storage, provide uniformity of product and increase resistance to melting. Stabiliser function through their abilities to either form gel structures in water or to combine with water as water hydration, thus increasing viscosity because they interact with other macromolecules in frozen products. As ice freezed out from a solution in a frozen dessert, stabiliser concentration, which may be only 0.25-1.0% in the unfrozen mix, increases many-fold in the frozen mixture (Marshall and Arbuckle, 1996).

2.5.1 Carrageenan

A gum that is a seaweed extract obtained from red seaweed *Chondrus crispus* or *Gigartina mammillosa* is called carrageenan (Igoe and Hui, 1995). Carrageenans are anionic polymers (they contain half-ester sulfate groups). The free acid is unstable, and carrageenans are commonly sold as mixtures of sodium, potassium and calcium salts. Commercial carrageenans are usually sold in κ, ι or λ forms. In fact, each form contains varying amounts of the other two types. For the purpose of viscosity production, λ-carrageenan is used, whereas for gelling applications, mixed calcium and potassium salts of the κ and ι types are used (Nussinovitch, 1997).

The kappa (Figure 2.10) and iota types require hot water (above 71°C) for complete solubility and can form thermally reversible gels in the presence of potassium and calcium cations, respectively. The kappa gels are brittle with syneresis while the iota gels are more elastic without syneresis. The lambda type is cold-water soluble and does not form gels. Kappa and iota carrageenans stabilize milk protein at 0.01-0.05% and to form water gels at 0.5-1.0%. Their uses include dairy products, water gel desserts and low-calorie jellies. A typical use level in water systems is 0.2-1.0% and in milk systems is 0.01-0.25% (Igoe and Hui, 1995).

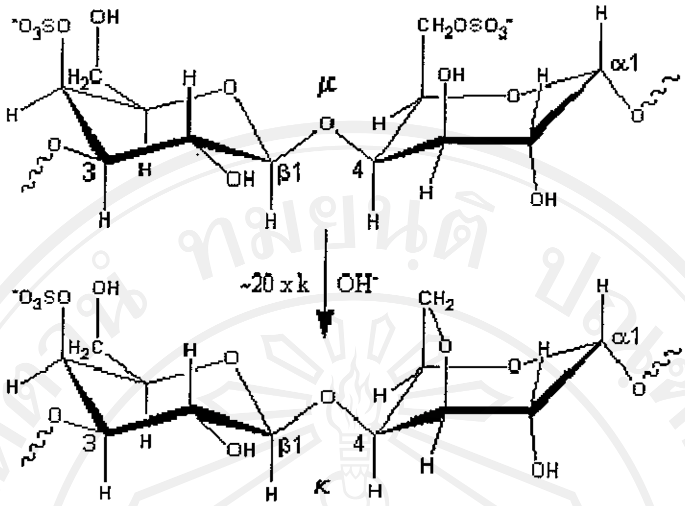

Figure 2.10 Structure of κ-carrageenan

Sourse: http://www.lsbu.ac.uk/water/hycar.html [23 August, 2006]

Carrageenans are used as binders and gelling, thickening and stabilizing agents. Typical dairy applications of carrageenans are in milk gels, puddings and pie fillings, whipped products, cold-prepared milks, acidified milks, frozen desserts, pasteurized and sterilized milk products and infant formulations. These gums are available as part of ready-made preparations, or as part of a powder to be added to milk. Brittle gels or creamier products can be achieved, depending upon the combination of κ- and ι-carrageenans and their combinations with polyphosphates. In chocolate milks, 0.025-0.035% carrageenan is added prior to pasteurization and cooling, when the interactions produce a delicate structure that keeps the cocoa particles suspended; the drink is richer and has a better oral quality. In sherbets and ice creams, carrageenans are used as stabilisers to prevent the separation of fat or other solids within the products. The use of cellulose gum and carrageenan, either alone or in combination, is essential for lowering fat and calories in confections. Cellulose gel in chocolate coatings, carrageenan in gummy confections and combined ingredients in fat-free and reduced-fat caramels and heat-stable marshmallow products can be helpful in meeting consumer demand (Marshall and Arbuckle, 1996).

2.5.2 Carboxymethylcellulose (CMC)

A gum that is manufactured by reacting sodium monochloroacetate with alkali cellulose to form carboxymethylcellulose is universally known as CMC (Figure 2.11). The compound is a water-soluble, anionic and linear polymer (Nussinovitch, 1997). 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 distability, except in a special acid-stable type of CMC. A variety of types are available which differ in viscosity and degree of substitution (the number of sodium groups per unit). It functions as a thickener, stabiliser, binder, film former and suspending agent (Igoe and Hui, 1995).

CMC is sold as a white to buff-colored, tasteless, odorless, free-flowing powder. The gum is used in a more varied range of applications than any other known water-soluble polymer (Nussinovitch, 1997). It is easily dissolved in a mixture; has a high water-holding capacity; forms weak gels by itself but gels well in a combination with carrageenan, locust bean gum or guar gum; acts also as an emulsifier; especially functional in sherbets and ices (Marshall and Arbuckle, 1996).

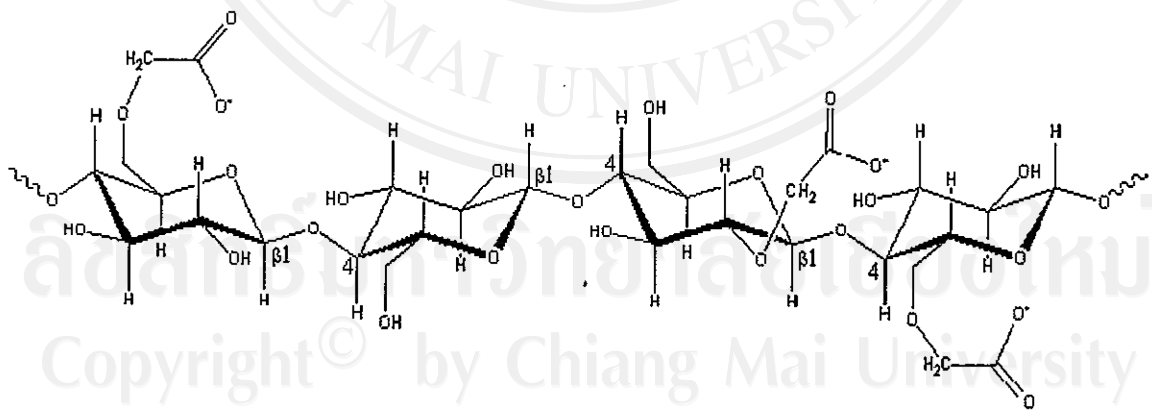

Figure 2.11 Structure of carboxymethylcellulose (CMC)

Sourse: http://www.lsbu.ac.uk/water/hycmc.html [9 June, 2006]

The use of CMC is growing in food applications, especially in developed countries where the demand for convenience has been growing rapidly. Many, or at least one, functions (binding, thickening, stabilization, moisture retention, etc.) can be achieved by the inclusion of cellulose derivatives in foods at levels of 0.1-0.5%, generally less than 1%. Texturization is beneficial in frozen desserts, other desserts and low-calorie products; thickening in bakery batters, pet foods, gravies, low-calorie foods, soups, sauces, snack foods, instant beverages and desserts; bodying in beverages, desserts and syrups; mouthfeel in products such as beverages, frozen desserts, desserts, syrups, low-calorie products and instant beverages; suspension in low-calorie products, beverages, fruit drinks, protein beverages, instant beverages and desserts; and binding properties in extruded foods, pet foods, snacks and speciality foods (Nussinovitch, 1997).

2.5.3 Guar gum

A gum that is a plant hydrocolloid extracted from two leguminous (guar) plants, *Cyamopsis tetragonolobus* and *Cyamopsis psoraloides,* is known as guar gum (Figure 2.12). Manufacturing includes separation of the endosperm from the germ and the testa, and grinding it to compose the gum. The gum is available in a wide range of sizes, which differ in their solubilization rates. Thermally degraded gums with reduced viscosities are commercially available. Steamed powders exhibit an enhanced dissolution rate and a reduction in their typical tastes. Two major grades of guar gum are marketed, food and industrial. For industrial purposes, ground endosperm is used, including small amounts of hull and germ resulting from imperfect purification. The industrial grades are manufactured with chemical additives, such as carboxymethyl, hydroxyalkyl and quaternary amine derivatives, to manipulate functional properties such as viscosity and solubility (Nussinovitch, 1997).

Guar gum is readily soluble in cold mixtures, therefore favored for High Temperature Short Time-pasteurized mixes. It may help produce excessive viscosity even at low concentrations. Different types are available to develop maximal viscosities in products from 2 to 20 h. The viscosity varies inversely with temperature, nonionic nature and straight chain structure, which enhances hydrogen bonding to other hydrophobic molecules. The polysaccharide polymer is composed of repeating mannose units with single galactose attached to alternate mannose units (Marshall and Arbuckle, 1996).

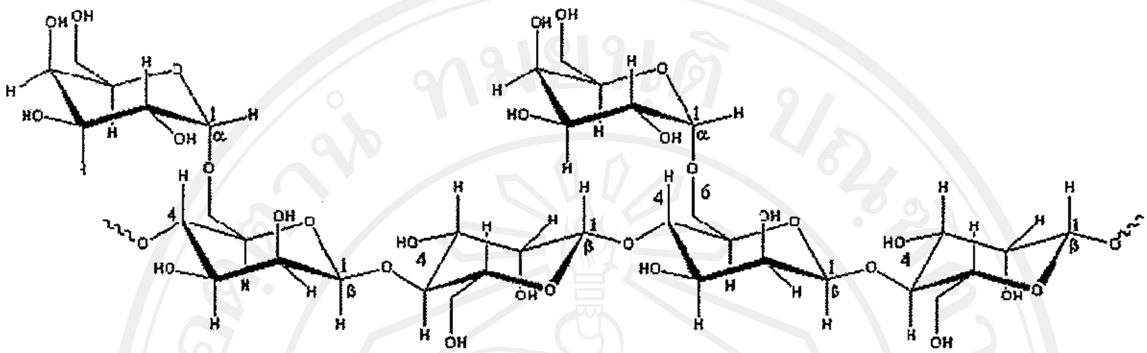

Figure 2.12 Structure of guar gum

Sourse: http://www.lsbu.ac.uk/water/hygua.html [1 September, 2006]

Guar gum is dispersible in cold water to form viscous solutions which upon heating will develop additional viscosity. A 1% solution has a viscosity range of 2,000 to 3,500 centipoises. It is a versatile thickener and stabiliser used in ice cream, baked goods, sauces and beverages at use levels ranging from 0.1-1.0% (Igoe and Hui, 1995). The maximum allowable concentration of guar in food is 2% by weight, as found in vegetable products and in fats and oils. The gum's presence in food must be listed on the label. Guar gum solutions are sometimes used as a model for non-Newtonian liquid food (Nussinovitch, 1997).