CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

1.1 STATEMENT AND SIGNIFICANCE OF THE PROBLEM

Sweet corn is a special variety of corns. It has high sugar content with a good consumer acceptance due to its appealing taste and flavor. In commercial practices, the corn can be processed further for different sweet corn products, including sweet corn milk. The sweet corn milk is sweet and has a high nutritional value from the presence of vitamin A, B_1, B_2, B_6, C and niacin (USDA. 2004). The corn milk also has a low fat content with almost no saturated fatty acid and cholesterol. Thus, the corn milk can be categorized as a healthy food and it can be beneficial for people that are concerned with a healthy life-style. Beside that, the consumption of the corn milk will also be attractive for vegetarian people, who are avoiding the consumption of meat and meat products or people that have an allergenic reaction to animal's milk.

Today, sweet corn milk has been commercially manufactured in Thailand. Corn milk products, such as pasteurized and sterilized corn milks, can be obtained in local (fresh) markets and supermarkets. However, there has not been any corn milk yogurt available yet. Despite of the fact that there is plenty of information regarding soymilk yogurt, there is a little publication that studied about corn milk yogurt. In the light of this circumstance, a study about corn milk yogurt will not only sustain the development of corn milk products, but it will also give a new understanding about a different raw material in the production of fermented products.

Yogurt is the most popular fermented product that had been studied extensively. Although yogurt was initially produced using cow's milk as a substrate, nowadays different raw materials have been studied to understand their suitability as a yogurt substrate, including soymilk (Granata and Morr, 1996), coconut milk (Siripanporn *et al.*, 2000), grape juice (Öztürk and Öner, 1999) and a combination of mange pulp, soymilk and buffalo milk (Kumar and Mishra, 2004). Especially, there was an extensive research regarding reduction of beany flavor of soymilk yogurt in the last two decades. The reduction of this flavor has contributed to an increase in the consumption of soymilk products (Granata and Morr, 1996). However, the overall flavor of the soymilk still becomes a hurdle for some groups of people.

A previous report cited that the sweet corn milk had a good aroma and was accepted for its color, aroma and appearance by the sensory panelists (Rauengpanyawatana, 1998). There was a publication in Chinese language by Xinyum *et al.* (1998) that studied a production of instant corn milk powder. However, there is not any publication yet that showed a deep study about corn milk yogurt.

In this study, sweet corn milk was used as a media for the growth of traditionally yogurt starter cultures. Using this vegetable milk as a yogurt substrate was not only intended to increase the diversification of fermented products, but it was also aimed to combine the good sensory characteristics of the corn milk with the well-known yogurt flavor. Moreover, the corn milk yogurt would give an advantage of a low fat content and the absence of cholesterol in the final product. This would offer a benefit for people that are concerned with healthy food products.

1.2 RESEARCH OBJECTIVES

1.2.1 To evaluate the effect of different cultivars and different harvest stage of sweet corns on corn milk composition.

1.2.2 To determine the effect of skim milk powder (SMP) and water addition in corn milk on the growth of yogurt microorganisms.

1.2.3 To elucidate the effect of milk protein and lactose on corn milk yogurt.

1.2.4 To study the effect of gelatin on properties of corn milk yogurt.

1.2.5 To compare the effect of addition of individual and mixed starter cultures on the growth of yogurt microorganisms.

1.2.6 To compare characteristics and shelf lives of corn milk yogurt with commercial yogurt.

1.3 LITERATURE REVIEW

1.3.1 Introduction of sweet corn

Sweet corn (*Zea mays Saccharata*) is classified in a Gramineae family (Pulham, 1997). The sweet corn plant can grow well all year around in Thailand with its highest yield on January to May (Thanwiset, 2003). The sweet corn plant is cultivated extensively in Chiang Mai, Ratchaburi, Suphan Buri and Buri Rum (Ketnil, 2002).

The agriculture production of sweet corn has been mainly processed for canned and frozen products. In fact, Thailand is one of the main producer and exporter of sweet corn products in the world. Thailand generally exported canned sweet corn for an amount of 20,000 tons/year, which was equaled to more than 600 million Baht. For frozen sweet corn, the amount was about 1,000 tons/year, which was more than 50 million Baht (Ketnil, 2002). Besides these two main products, recently the fresh sweet corn in Thailand is also processed for corn milk. The milk is commercially produced as pasteurized and sterilized, mainly ultra high temperature (UHT), corn milks. The selling of fresh sweet corns in local markets is also famous after they have been steamed.

1.3.2 Varieties of sweet corn

The varieties of sweet corn in Thailand are comprised of ATS-1, ATS-2, ATS-5, Bicolor, Suwan-1, Suwan-3, Hybrid, Sweet-50 and Sweet-45 (Puhlam, 1997; The National Research Council of Thailand, 2000). The two most varieties cultivated in Chiang Mai are ATS-2 and ATS-5 because they give a high yield output and high quality products. In fact, the ATS-2 and ATS-5 were the most popular varieties in 1999 and 2003, respectively (The National Research Council of Thailand, 2000). The ATS-5 will give a higher yield production, more weight per ear and is sweeter than the ATS-2 variety (Thanwiset, 2003). For example, the weight per ear of the ATS-5 and ATS-2 are 500 g and 250 g, respectively. The total soluble solid of the ATS-5 and ATS-2 are 14-16°Brix and 15°Brix, respectively. However, the ATS-2 is reported to have a better aroma than ATS-5 (Chamornman, 2000).

The sweet corn plants flower and fertilize between the male flowers on the top, which is also called as pollen, and the female flowers to produce sweet corns. The female flowers contain silk on the top of the flowers to help the fertilization process. After the fertilization process completed, the sweet corn seeds will be developed on the corncobs. For the sweet corn varieties of ATS-2 and ATS-5, the corns will produce silk after seeding for 48-52 days (Chamornman, 2000; Department of Agriculture, 2002). Normally, these corn varieties are harvested on the 19-23 days after 50% of sweet corn plants are silking, which is also called as a milking stage (Puhlam, 1997).

1.3.3 General composition of sweet corn

Sweet corn has high sugar content from its sucrose and fructose with a total carbohydrate content of 19.02% (based on wet basis, Table 1.1) (Hall, 2003; Permoranz, 1987). The corn has a good eating quality and consumer acceptance because of its good aroma. The good aroma consist of many compounds, including ethanol, acetaldehyde, methanethiol, hydrogen sulfide and dimethyl sulfide (DMS). The present of DMS is the most important component for the good aroma of sweet corn (Azanza *et al.*, 1996; Tracy, 2001).

Sweet corn has a high nutritional value from its chemical components and it is suitable to be further processed as canned, frozen and corn milk products (Makhlouf *et al.*, 1995; Tracy, 2001). The chemical composition of sweet corns is presented in Table 1.1.

Table 1.1 The chemical composition of sweet corn

Sweet corn compounds	The percentage of compounds	
	Dried basis	Wet basis
Protein	9.90-13.31	2.18-3.20
Carbohydrate	76.00-79.12	18.45-19.02
Fat	4.91-5.20	1.18-1.26
Fiber	2.10-9.57	0.51-2.30
Vitamin C	$(2.20\text{-}3.10)\times10^{-2}$	$(5.4\text{-}7.6)\times10^{-3}$

Source: Hall (2003); Makhlouf *et al.* (1995) and Permoranz (1987).

The two main protein components of sweet corn are zein (prolamins) and corn glutelin. The zein is alcohol-soluble protein, while the glutelin is alkali-soluble protein (Cabra *et al.*, 2005; Lásztity, 1996; Pomeranz, 1987). For the sugar content of sweet corn, sucrose and fructose are the main carbohydrates that give sweetness to the corn. The fat content in the sweet corn is 1.18-1.26% (Table 1.1), which contains 83.0% of unsaturated fatty acids (Pomeranz, 1987). Within this amount of unsaturated fatty acid, the sweet corn has 57.4% linoleic acid (C18:2) (Pomeranz, 1987).

However, the nutrient content in the sweet corn can be varied from a batch to batch production, depending on their cultivars and the stage of maturity on the harvesting time (Makhlouf *et al.*, 1995).

1.3.4 Sweet corn milk

One of the sweet corn products that is interesting to be considered further is sweet corn milk. The product has been commercially produced and it has some advantages compared to cow's milk. In the first instance, a commercial sweet corn milk brand Malee I-Corn from Maleesampran Ltd., Nakhon Pathom claimed that the fat content in their sweet corn milk product is only 0.49%, which is much less than the cow's milk, that contained around 4.0-4.4% fat (Walstra *et al.*, 1999). The second benefit is the amount of saturated fatty acids and cholesterol. The similar commercial sweet corn milk brand Malee I-Corn also stated that the product does not contain saturated fatty acids and cholesterol, where the cow's milk has 1.9% saturated fatty acids and 0.01% cholesterol (Fox and McSweeney, 1998; Walstra, *et al.*, 1999). The sweet corn milk also has 24 IU of vitamin A, 0.020 mg of vitamin B_1, 0.030 mg of vitamin B_2, 0.020 mg of vitamin B_6, 3.7 mg of vitamin C and 0.520 mg of niacin in every 100 g of sweet corn milk (USDA, 2004). For these reasons, another commercial sweet corn milk brand Royal's Corn produced by Sahajaraenenterprise Ltd., Bangkok has made an advertisement that their sweet corn milk product is a healthy food.

1.3.5 Processing of yogurt

Fermented dairy products have received a lot of interests in the last few decades due to their nutritional values and a distinct taste of the products. Many countries in the world have developed their own fermented products. However, up to date yogurt is the most well known and studied all over the world (Vinderola *et al.*, 2002; Wu *et al.*, 2001).

Yogurt is a fermented dairy product produced by the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* in warm milk. After the milk is incubated at a specific time and temperature, the yogurt production is completed. The yogurt itself is characterized with a smooth, viscous gel, soft curd and delicate flavor (Joint FAO/WHO, 2000; Kosikowski, 1997; Tamime and Robinson, 1999).

There are many volatile flavor components in yogurt, including acetic acid, diacetyl and acetaldehyde. The amounts of these flavor compounds that have detected in cow's milk yogurt are 0.03-0.05 kg/m^3 (0.5-0.8 mM), 0.8-1.5 mg/kg (0.01-0.02 mM) and 10 mg/kg (0.2 mM) for acetic acid, diacetyl and acetaldehyde, respectively (Walstra *et al.*, 1999). Acetaldehyde, which is produced by *L. delbrueckii* subsp. *bulgaricus* has a significant contribution to the unique flavor of yogurt (Kosikowski, 1997).

The methods to produce yogurt from cow's milk essentially do not have any significant changes over the years. Although there have been some refinements in the procedures, especially for the method that related to lactic acid bacteria (LAB) that will carry out the fermentation process, the essential steps of the yogurt production are still the same. These steps can be seen in the following paragraph.

Before the yogurt production is started, the level of total solids in cow's milk is firstly adjusted to have a level of 12-16 g in 100 g milk (Lee *et al.*, 1990; Tamime and Robinson, 1999). For soybean yogurt, the total solid content of the raw material mixture is normally in the range of 15.8-19.5 g in 100 g soymilk (Granata and Morr, 1996). The adjusted milk will then be heated at high temperatures, typically at 85°C for 30 min or 90-95°C for 5 min (Raphaelides and Gioldasi, 2005; Tamime and Robinson, 1999). The precise heating time will be depended on the heating temperature that is selected. After the milk is heated, the milk is cooled down to the inoculation temperature at 40-45°C, when a bacterial culture at a concentration of 2% is added. The dominant organisms in this culture are *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. For the last step of the yogurt production, the inoculated milk is incubated, either in a bulk system or in retail units, under a specific time and temperature incubation that will promote the formation of a smooth viscous coagulum and a desired aromatic flavor/aroma (Lourens-Hattingh and Viljoen, 2001; Tamime and Robinson, 1999).

During the fermentation process of yogurt, acidification is the most important parameter that will show the progress of the process and determine the quality of the final product. Consequently in the commercial production of yogurt, a measurement of pH values is mainly used to monitor the whole fermentation process (De Brabandere and De Baerdemaeker, 1999). Normally, the fermentation process is allowed to be carried out until the pH values reach 4.0 to 4.7, when the whole process will then be stopped immediately by rapid cooling to 5°C (Wu *et al.*, 2001). The chilled condition at 5°C or below is continuously maintained during the subsequent processes including packaging and distribution to consumers (Tamime and Robinson, 1999).

Changes of milk constituents and other ingredients that are added during manufacturing of yogurt have a significant contribution to the fermentation process of yogurt. These changes occur due to the fermentation activities of the inoculated starter cultures, secretion of nutritional and chemical substances from the growth activities of starter cultures, metabolites activities from the presence of other microorganisms, and also associated enzymes that are produced by the starter culture and/or other microorganisms (Lourens-Hattingh and Viljoen, 2001).

In cow's milk fermentation, the addition of LAB has a main role to utilize lactose that is naturally present in the milk. The lactose is used by the bacteria as a substrate and converted into lactic acid. The presence of lactose as a free sugar is splitted by β-galactosidase to glucose and galactose molecules. The end product, lactic acid, is mainly produced from the glucose molecules rather than the galactose molecules. Therefore, the presence of galactose in fermented milk products can be accumulated. Some of these galactose molecules can be utilized later on by the microorganisms themselves, either *S. thermophilus* or *L. delbrueckii* subsp. *bulgaricus*. However, enzymes to metabolize galactose are present in a low amount (van den Bogaard *et al.*, 2004; Walstra *et al.*, 1999).

Lactic acid is an important end product during the manufacture of yogurt from cow's milk. There are two main factors that are affected by the production of lactic acid. Firstly, lactic acid assists in destabilizing casein micelles of milk protein. The lactic acid affects the casein micelles by progressively converting a colloidal calcium phosphate complex in the micelles into a soluble calcium phosphate fraction, which can be diffused into the aqueous phase of the milk. By this conversion, the calcium micelles are being gradually depleted of calcium, which affected the micelle's balance in the milk solution. If the loss of calcium is significant, the casein will be coagulated. The coagulation of casein normally occur at pH 4.6-4.7, in which at the same time the yogurt gel is also formed. For the second factor, the production of lactic acid will give the final product, yogurt, and a distinct and specific taste, which is described as sharp and acidic. The presence of lactic acid is also contributed to the nutty and/or aromatic flavor of the product (Adam *et al.*, 2004; Tamime and Robinson, 1999).

The content of amino acids in the final yogurt product is strongly correlated with the value of titratable acidity of the product itself. For the yogurt made from cow's milk, which has a content of amino acid in a range of 3.29-10.31 mg in 100 ml milk, the final amino acid content will be in a range of 18.7 to 33 mg in 100 ml yogurt. At this amount of amino acids, the acidities of the yogurt can be found in a range of 1.0-1.4 g lactic acid in 100 g yogurt. The total amino acid content in the final yogurt would reflect a balance between proteolysis and assimilation of the bacteria cultures. Some amino acids, such as glutamic acid, proline and, to a lesser degree, alanine and serine, may not be required by the yogurt organisms. Therefore, these amino acids can be found in larger quantities in yogurts compared to the other amino acids, which are utilized by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* during their growth and/or fermentation (Tamime and Robinson, 1999). However, the total amino acid content in yogurt made from cow's milk will not differ substantially from the milk. The difference is only with the content of free amino acids, which will be higher in the yogurt due to proteolytic activities of yogurt microorganisms (De Brabandere and De Baerdemaeker, 1999).

Changes of volatile fatty acids in yogurt have been reviewed by Tamime and Robinson (1999). They found that saturated fatty acids and oleic acid had a significant increase, while linoleic and linolenic acids in the glyceride fraction were decreased during the yogurt production. The general increase of free fatty acids was moderate, although stearic and oleic acids were increased significantly and the monoglyceride fraction was disappeared completely during fermentation. During the manufacture and storage of yogurt, there was an appreciable increase in the total level of volatile fatty acids in yogurt by *L. delbrueckii* subsp. *bulgaricus*. This could be due to the activities of endopeptidases and/or exopeptidases rather than lipases because the lipases were normally inactivated after a normal pasteurization time and temperature combination. Beside that, some proteolytic enzymes could exhibit esterase activities.

For their growth requirements, yogurt starter bacteria require some vitamins, including a group of vitamin B. Tamime and Robinson (1999) reported that a wide range of lactobacilli utilized vitamin B_{12} when they were grown in SMP. For most of the time, the required vitamins are available in cow's milk and used by organisms during fermentation. However, the extent of the needed quantities of these vitamins is depended on the rate of inoculation, the strains of yogurt starters and the fermentation conditions (Lourens-Hattingh and Viljoen, 2001; Tamime and Robinson, 1999). Although the yogurt starter bacteria utilize some vitamins, they also produce other vitamins, such as niacin, folic acid and to a lesser degree, vitamin B_6. The concentrations of these vitamins will be increased during the production of yogurt because the vitamins are actively synthesized by the starter cultures, especially by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Tamime and Robinson, 1999). It was recorded that a folic acid producing strain of *S. thermophilus* would increase the folic acid content of yogurt within 3.5 h of incubation. After that, the level of the acid would be decreased rapidly, which indicated that *L. delbrueckii* subsp. *bulgaricus* had started to grow and utilized the vitamin that was produced by *S. thermophilus*. In commercial yogurts, the folic acid contents would be in a range between 3.7 to 24.5 μg/ 100 g yogurt. If the mutant strains of *S. thermophilus* were present in the yogurt, the organisms would increase the folic acid content in SMP to 38.1 μg/ 100 g yogurt (Tamime and Robinson, 1999). Nergiz and Seckin (1998) reported that the losses of nutrients could be as high as 7.28% for protein, 0.77% for fat, 71.7% for lactose, 51.8% for thiamin, 60.5% for riboflavin, 70.2% for sodium, 68.2% for potassium, 65.8% for calcium and 50.2% for phosphorus.

1.3.6 Yogurt starter bacteria

LAB organisms are characterized by their high demand for essential growth factors, including peptides and amino acids (Abu-Tarboush, 1996; Shihata and Shah, 2000). The main amino acid that is important for the LAB is valine (Walstra *et al.*, 1999).

LAB is ubiquitous and the prime agents in producing fermented milk and milk products. Their activities are promoted as starters used in the manufacture of butter, cheese, yogurt and cultured products. Some strains of LAB can produce as much as 1.5% lactic acid in milk. LABs are classified as homofermentative if they produce only lactic acid and heterofermentative if they produce acetic acid, alcohol and CO_2 as well as lactic acid (Walstra and Jenness, 1984; Walstra *et al.*, 1999).

The primary role of LAB in the yogurt manufacturing is to promote aggregation of casein micelles in cow's milk by lowering the pH of the substrate. Some strains of LAB can even make a further contribution to the physical structure of yogurt by a production of extracellular polysaccharide (EPS). The presence of EPS in the final yoghurt product is perceived as giving a better mouthfeel and a more cohesive (ropy) texture. Actually, the exopolymers from LAB are polysaccharides that are composed of repeating units (branched), containing α- and β-linkages. Although, the LAB can secrete many different types of these exopolymers as was reviewed by Haque *et al.* (2001), the monomer composition of these exopolymers is seemed to be similar. The monomer units that are normally present include D-galactose, D-glucose and L-rhamnose. D-glucose and L-rhamnose present in different ratios for different types of exopolymers (De Vuyst and Degeest, 1999).

The production of yogurt from milk is made though a protocooperative action of two homofermentative LAB, which are *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Adam *et al.*, 2004; Amoroso and Manca da Nadra, 1992; De Brabandere and De Baerdemaeker, 1999). The symbiotic relationship between *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* is developed due to the proteolytic nature of the *L. delbrueckii* subsp. *bulgaricus* to produce essential amino acids. The amino acid nitrogen that is produced by the *L. delbrueckii* subsp. *bulgaricus* will be used by the *S. thermophilus*, whereas *S. thermophilus* produces formic acid and CO_2 for *L. delbrueckii* subsp. *bulgaricus*. These activities will then establish the special relationship between the two of them (Shihata and Shah, 2000).

In the traditional yogurt fermentation, the used starter bacteria, especially *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, have to tolerate the condition of the manufacturing processes, which they are undergone as the milk is prepared to produce a bioproduct. At the peak of freshness, directly after making the yogurt, plain yogurt may contain up to one billion live *L. delbrueckii* subsp. *bulgaricus* and *S. thermophillus* per ml yogurt. These two dominant bacteria can be maintained for the yogurt manufacturing as one mixed culture or separately, as two individual cultures until they are used (Kosikowski, 1997). Although the optimum ratio between the diplococci and the rods is depended on the properties of the strains, the ratio is often to be found at a combination of 1:1. This specific ratio between the two LAB is the best to be used if the inoculums percentage is 2.5% with an incubation time of 2.5 h at 45ºC (Walstra *et al.*, 1999) or 3% inoculums with an incubation time of 2.5 h at 40-45ºC (Tamime and Robinson, 1999).

In yogurt fermentation, the ratio between *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* is constantly changed. At the beginning, the streptococci can grow faster than the lactobacilli. This is because of the fermentation growth factors produced by the *L. delbrueckii* subsp. *bulgaricus* and probably also due to proteins and small peptides being added via the inoculum, especially in the manufacture of set yogurts. Afterwards, the growth of *S. thermophilus* is slowing down by the presence of acids that is produced. At the same time, the *L. delbrueckii* subsp. *bulgaricus* has started to grow faster because of the growth factors that are formed by the *S. thermophilus*, particularly CO_2 and formic acid (Amoroso and Manca de Nadra, 1992; Walstra *et al.*, 1999). Both *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* ferment lactose in milk to lactic acid and produce flavor of the yogurt. The main flavor of diacetyl and a small amount of acetaldehyde will be produced by *S. thermophilus*, while a significant amount of acetaldehyde will be mainly developed by *L. delbrueckii* subsp. *bulgaricus* (Lourens-Hattingh and Viljoen, 2001).

The characteristics of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* for the yogurt production have been reported. The *S. thermophilus* has spherical or ovoid cell shape morphology and its diameter is less than 1 μm. *S. thermophilus* is usually forming chains or occurring in pairs. The *S. thermophilus* is a Gram-positive LAB that grows anaerobically and ferments lactose using a homofermentative pathway (Leaungsakul, 1998; Tamime and Robinson, 1999). The homofermentative characteristic of the streptococci means that the organism produces almost all of its acid as lactic acid and only a small amount of formic acid, CO_2 and O_2 (Walstra *et al.*, 1999). The type of lactic acid that is produced by the streptococci is L (+) lactate. The streptococci also produce diacetyl, a small amount of acetaldehyde and EPS from lactose in milk. However, the streptococci require the presence of B vitamins and some amino acids to enhance their growth rate. If a methylene blue solution at a concentration of 0.1 g in 100 ml solution is present in a substrate or the substrate has a pH value of 9.6, the streptococci cannot grow (Tamime and Robinson, 1999).

L. delbrueckii subsp. *bulgaricus* is recognized as an obligate homofermentative lactobacillus. It is present as a rod shape with rounded ends and a typical figure of 0.5-0.8 x 2.9 μm. The lactobacilli can occur as a single cell or in short chains of several cells. *L. delbrueckii* subsp. *bulgaricus* is able to ferment fewer types of sugar compared to *S. thermophilus*. The lactic acid that is produced by *L. delbrueckii* subsp. *bulgaricus* is D (+) lactate. Beside lactic acid, the lactobacilli can also produce acetaldehyde from lactose in milk. Some specific species of the lactobacilli can also produce EPS from lactose. Generally, *L. delbrueckii* subsp. *bulgaricus* can grow at 50-55°C (Leaungsakul, 1998; Tamime and Robinson, 1999).

In the normal cow's milk, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, can easily grow and produce acid as a by-product. The acid production rate can be faster due to a stimulation growth between the two microorganisms. Nevertheless, the fermentation conditions, including incubation time and temperatures; concentration of the starter cultures and the presence of specific agents or substances in milk may have an effect on the rate of acid development and/or the growth rate of the starter cultures (Tamime and Robinson, 1999). The specific substances include the present solid, lactose and sodium caseinate in milk. Beside these factors, a factor of ratio and percentage of the starter cultures will also affect the overall fermentation process. Some of the examples for the effects of these factors can be seen as followed: the acidity of the yogurt that causes the streptococci to decrease its growth rate, which typically at pH below 5.5. The low pH will be reached earlier if higher inoculums of starter cultures are added into milk. In this situation, the number of lactobacilli will be increased, although the whole fermentation process is done at the same incubation time. In another case, when lower inoculums are added into the milk, the ratio between the two yogurt bacteria will be shifted in favor to the streptococci (Adam and Moss, 2000; Lourens-Hattingh and Viljoen, 2001; Walstra, *et al.*, 1999). For the fermentation temperatures, increasing of temperature will give a progressive increase in the rate of pH reduction and the onset of gel network formation (Haque *et al.*, 2001). Normally, an addition of 2-3 ml starter culture into 100 ml milk is recommended. If manufacturers use DVI starter cultures (Direct-to-Vat

Inoculation, such as freeze-dried cultures), an inoculums percentage between 2.5 and 7.0 g of 100 ml milk can be used (Tamime and Robinson, 1999).

For the effect of specific substrates that are present in milk on fermentation processes, previous studies have reported that the total solid in milk would affect pH values that would be an onset point for the gelation characteristic of the yogurt. A higher total solid content in milk by an addition of SMP or milk proteins were found to be responsible for an increase of lactic acid production. However, reduction of pH is decreased because the added protein has a buffer capacity. Thus, the prolonged fermention time is appeared in the yogurt fortified with milk protein. (Gastaldi *et al.*, 1997; Granata and Morr, 1996; Kristo *et al.*, 2003; Tamime and Robinson 1999; Yazici *et al.*, 1997). The higher total solid content affects the increase of both the firmness and elasticity of milk gel. When the total solid of milk is higher, the concentration of casein particles will also increase. A higher concentration of casein particles will enhance the interactions between particles by increasing the number of bonds between particles and rate of bonding (Gastaldi *et al.*, 1997). Beside the total solids of milk, lactose is another compound that also influences the performance of yogurt cultures. The presence of 15% or 20% of lactose in fermented milks has been reported to have an inhibitory effect on *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* (Vinderola *et al.*, 2002). The excess lactose will be utilized to sweeten the final yogurt product without increasing its calorific value. This effect can be achieved by hydrolyzing the lactose using β-galactosidase, which splits the lactose into glucose and galactose. If the relative sweetness between lactose and other monosaccharides is compared, the degree of sweetness for sucrose, lactose, galactose and glucose will be 1, 0.4, 0.6 and 0.7, respectively (Tamime and Robinson, 1999). Therefore, the presence of glucose and galactose from the lactose hydrolysis will increase the overall sweetness of the yogurt. However, Tamime and Robinson (1999) reviewed that only 50% of the available lactose that was necessary to be hydrolyzed to produce a final yogurt product with an acceptable sweetness.

Generally, the amount of lactose in cow's milk is in the range of 4.6-4.8% (Fox and McSweeney, 1998; Walstra *et al.*, 1999). For the production of yogurt, the total solid of cow's milk can be increased by adding 5.8% (w/v) of SMP. If the concentration of lactose in the SMP is around 52.9%, the total lactose concentration in the yogurt mixture will be in a range of 7.6-7.8% (Haque *et al.*, 2001; Tamime and Robinson, 1999).

The effects of sodium caseinate, casein hydrolyzate and whey protein hydrolyzate on the production of acid, flavor and volatile compounds in a high protein and fiber soymilk yogurt-like product were studied by Granata and Morr (1996). The researchers found that a concentration of 0.25% sodium caseinate was the most effective substrate to decrease the pH values and increase the titratable acidity of the product, while 0.5% casein hydrolyzate and 0.25% whey protein hydrolyzate were the least effective substrates. At the similar concentration of sodium caseinate or an addition of 0.1% casein hydrolyzate, the compounds also gave a high beneficial effect for the growth stimulation of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in soymilk yogurt. This was because sodium caseinate or casein hydrolyzate provided proteins, peptides and amino acids to the microorganisms more than the whey protein hydrolyzate. An addition of 0.25% whey protein hydrolyzate was found to give the lowest beneficial effect for the growth stimulation of the yogurt cultures. For the volatile compounds of soymilk yogurts, the addition of 0.25% sodium caseinate produced the highest acetaldehyde, acetone and diacetyl concentrations, which were 1,247; 85 and 80 ng/g soymilk yogurts, respectively, compared to the addition of casein or whey protein hydrolyzates. When comparing the yogurt characteristics based on a sensory evaluation between the soymilk yogurt added with 0.25% sodium caseinate or 0.1% casein hydrolyzate with cow's milk yogurt as a control, the soymilk yogurt was not significantly different compared to the control for panelist's favoritism for the flavor and texture characteristics. The soymilk yogurt added with 0.25% sodium caseinate or 0.1% casein hydrolyzate got the best overall characteristics for the sensory evaluation because the yogurt had a smooth consistency with a creamy texture, a tan to beige color, a clean and well-balanced aroma with no off-odors and a balance yogurt flavor.

Another study that fortified whey protein powders at levels between 0.6 and 4% in yogurts found that the whey protein powder affected the total solid content of cow's milk. As the amount of added whey protein powder increased, the total solid content of the milk would also increase. The increasing total solid content of the milk would affect the characteristics of the final yogurt by producing more acetaldehyde, increasing the viscosity of the yogurt, reducing the syneresis of the yogurt and improving the results of sensory evaluation. The scores of sensory evaluation were improved by making a yogurt with a better consistency, texture and creaminess (Penna *et al.*, 1997; Tamime and Robinson, 1999).

1.3.7 Changes during storage of yogurt

During storage periods of yogurts, there were several changes that could be happened, which included:

1.3.7.1 The levels of acetaldehyde, ethyl acetate, diacetyl and ethanol

The levels of acetaldehyde, ethyl acetate, diacetyl and ethanol in yogurt during storage were reviewed by Tamime and Robinson (1999). The levels of acetaldehyde, ethyl acetate and diacetyl in sheep's milk yogurt were decreased as the storage time increased. However, in the yogurts produced from cow's milk, it was only the concentration of acetaldehyde that was decreased during 10 days storage at 4 or 10°C, while the concentration of diacetyl and ethanol were increased.

1.3.7.2 Proteolysis

The rate of proteolysis increased during storage of yogurts (Tamime and Robinson, 1999).

1.3.7.3 Titratable acidity

The values of titratable acidity in yogurt increased during the first 5 days storage at 4°C. After this period, the titratable acidity would not have any significant changes until 35 days of storage (Dave and Shah, 1997).

1.3.7.4 pH values

The initial pH values of cow's milk at 6.55-6.62 decreased significantly to 4.33-4.64 at the end of yogurt fermentation. After an initial sharp decrease of the pH values of milk during the fermentation process, a more gradual decrease of the yogurt pH values could be observed during 35 days storage at 4°C. At the end of 35 days storage, the pH values of yogurt were at a range of 4.16-4.40 (Dave and Shah, 1997).

1.3.7.5 The number of dairy starter cultures

The viability of dairy starter cultures, particularly *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* during storage of yogurts could be slightly differed. After a production of yogurt for 3.5-4.0 h at 43°C, the number of *S. thermophilus* increased by 25-28% during the first 5 days of storage at 4°C. The increase on the viability of *S. thermophilus* could be due to residual activities of the organisms thoughout that particular storage period. After that, the viability of *S. thermophilus* declined continuously by approximately 9.5-61.8% for the remainder of 35 days storage. For *L. delbrueckii* subsp. *bulgaricus*, the population increased significantly by 12- to 22-fold during yogurt fermentation from an initial inoculation population of 1.20-3.00x10^7 CFU/ml milk. After a marked initial increase, the population of *L. delbrueckii* subsp. *bulgaricus* decreased for up to 50% during the first 5 days storage at 4°C. *L. delbrueckii* subsp. *bulgaricus* had a further steady decrease in the following 5 days storage and continued by a sharp decline on the next 5 days storage. For the remainder of the storage time up to 35 days, the population of *L. delbrueckii* subsp. *bulgaricus* was below 10^5 CFU/ml. Accordingly, *S. thermophilus* could maintain its viability better than *L. delbrueckii* subsp. *bulgaricus*. *L. delbrueckii* subsp. *bulgaricus* was rapidly loss its viability during storage and a reduction of more than 4 log cycles of the population had been observed (Dave and Shah, 1997).

1.3.8 Shelf life of yogurt

The shelf life of yogurts from cow's milk during storage at 4°C (40°F) or lower is between 30 to 60 days (Kosikowski and Mistry, 1997). The main quality problem of yogurt is the acidity of the product that continues to increase during the distribution and marketing of the product. This means that the yogurt may become too sour when it is consumed. Beside that, yogurt can also become bitter due to excessive proteolysis. However, the bitterness is highly depended on the type of used starters. The development of sour taste and bitterness generally determines the shelf life of yogurt itself. Although the product is kept at refrigerator temperatures, the acidification and other changes caused by enzymes activities can be carried on, albeit in a slow phase (Walstra *et al.*, 1999).

Another defect that may occur in yogurt includes problems caused by contaminated organisms, especially yeast and moulds. The off-flavors that were produced by these microorganisms were characterized as yeasty, fruity, musty, cheesy or bitter, and occasionally soapy-rancid. The off-flavor threshold can be generally reached when the count of microorganisms is about 10^4 yeast and moulds per ml yogurt. The growth of these contaminated microbes is largely determined by the amount of available oxygen. Therefore, the volume of the container headspace and the air permeability of the container packaging material should be as low as possible. A defect due to a too low of the yogurt characteristic flavor can be another problem that may happen in yogurt. This defect may be occurred because of a too low incubation temperature or an excessive growth of streptococci or lactobacilli strains that are weak as aroma producers. Insufficient acidification caused by raw milk that is contaminated by penicillin can also lead to a bland product. The off-flavors in raw milk that is used for yogurt manufacturing can naturally cause flavor defects in the final yogurt product (Harrigan, 1998; Leaungsakul, 1998; Walstra *et al.*, 1999).

1.3.9 Yogurt from non-cow's milk

Beside cow's milk yogurts, several researchers have done intensive research to study and develop soymilk yogurt. The soymilk yogurt could be produced using 2 main microorganisms of the ordinary cow's milk yogurt, which were *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus.* The yogurt microorganisms could ferment soymilk in the presence of 2-5% lactose and 3-5% whey protein concentrate or nonfat dry milk (Cheng *et al.*, 1990; Karleskind *et al.*, 1991; Lee *et al.*, 1990). After fermentation condition at 40°C for 14 h, the bacterial cultures could produce a sufficient lactic acid, lower the pH of soymilk to 4.5 and coagulate the soy proteins. However, the resulting soymilk yogurt generally lacked with a typical yogurt flavor (Karleskind *et al.*, 1991; Lee *et al.*, 1990). The soymilk yogurt also imparted a characteristic of beany, grassy off-flavor that would be a major hurdle for its acceptance, as was reviewed by Granata and Morr (1996). When Granata and Morr (1996) developed a soymilk yogurt with an addition of 0.25% sodium caseinate, the yogurt had a balance aroma with no off-odor and a good yogurt flavor.

Siripanporn *et al.* (2000) studied a production of coconut milk yogurt that was developed using coconut milk from haft-mature coconut meat. The coconut milk was extracted, heated at 75°C for 10 minutes, and chilled at 4-10°C for 2 h. After the chilling process, the fat in supernatant was removed. The coconut milk yogurt was prepared using 12% coconut milk and 15% reconstituted SMP at a ratio of 1:1.25. The combined milk was inoculated with 4% *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* at a ratio of 1:1 and incubated for 4 h at 43°C. The finished product was found to have a low fat content with smooth texture and thick consistency. The product could be stored at least 14 days at 4-10°C.

Prasertcheeva (2003) studied a production of yogurt from corn milk. The yogurt was developed using a mixed commercial freeze-dried culture of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* at a ratio of 1:1. The corn milk was produced from a sweet corn variety of Two Color and prepared by cutting the kernels of sweet corn, extracted and mixed with water at a ratio of 1:2. An optimization for the final yogurt product could be achieved when the basic material for making yogurt contained 82.40% corn milk (w/w), 7.50% SMP (w/w), 7.00% sucrose (w/w), 0.10% gelatin (w/w) and 3.00% starter cultures (w/w). When the mixtures of the basic ingredients were incubated for 10 h at 37°C, the finished yogurt contained 20.56% total solid content (w/w), 3.73% non-reducing sugar (w/w), 4.06% reducing sugar (w/w) and 7.79% total sugar as invert sugar (w/w). The analysis of the final yogurt also showed that it had 1.11% total titratable acidity (w/w) as lactic acid and a pH value of 3.97 with a number of microorganisms of 7.10×10^9 CFU/g yogurts. The viscosity of the yogurt was 17,500 centipoises when it was measured with a Brookfield Viscometer using a needle no. 4 and 2.5 rpm. For the sensory evaluation using Mean Ideal Ratio scores, panelists accepted the yogurt for its color, smoothness, whey separation, mouthfeel, viscosity, milk powder flavor, corn flavor, acidic flavor, sweetness and overall acceptability.

1.3.10 Application of gelatin as stabilizer for yogurt

Tamime and Robinson (1999) stated that the primary aim of adding stabilizers to a milk base was to enhance and maintain the desirable characteristics of yogurt, for example body and texture, viscosity/consistency, appearance and mouthfeel.

Stabilizers are sometimes referred as hydrocolloids that have two basic functions in yogurt, which are to bind water and to improve the yogurt texture (Kumar and Mishra, 2004). The molecules of a stabilizer are capable of forming a network of linkage between the milk constituents and themselves due to the presence of a negatively charged group, for example hydrogen or carboxyl radicals, or to the presence of a salt possessing a power to sequester calcium ions. A stabilizer can achieve the process of water binding into the milk base by following several stages of development. Firstly, a stabilizer binds water as the water of hydration. Stabilizer reacts with milk constituents, mainly proteins, to increase their levels of water of hydration. Finally, it stabilizes the protein molecules in a form of a network that retards the free movement of water (Tamime and Robinson, 1999).

Kumar and Mishra (2004) studied different types of stabilizers and their addition levels on physicochemical, sensory properties, textural characteristics and amount of starter culture of Mango Soy Fortified Set Yogurt (MSFY). The study used 3 types of stabilizers, which were gelatin, pectin and sodium alginate at 3 different levels (0.2%, 0.4% and 0.6% (w/w)) together with a sample without any addition of stabilizer as a control. The results of the study showed that the addition of pectin gave the highest amount of acidity in the MSFY sample, followed by the samples that were added with gelatin and sodium alginate in an equal amount. The acidities of the gelatin and sodium alginate-added samples were still higher than the control. However, the addition of gelatin gave the highest levels of total solid content and moisture content compared to the other stabilizers. When comparing different levels of stabilizers, the amounts of acidity and total solid content were increased as the levels of stabilizers increased. In contrast, the moisture content was decreased as the levels of stabilizers increased.

All types of the stabilizers affected the syneresis of the MSFY samples. The syneresis decreased as the levels of stabilizers increased. However, among different types of stabilizers, the MSFY samples added with gelatin showed the lowest amount of syneresis. For the acetaldehyde content, the amount was lower in all the MSFY samples added with stabilizers compared to the control. Between the 3 types of stabilizers, the amount of acetaldehyde was the highest in the gelatin added samples and the lowest in the sodium alginate added samples. Measurements of color of the MSFY samples showed that the lightness (L^*) and yellowness (b^*) of the samples increased when the samples added with gelatin and decreased when the samples added with pectin and sodium alginate. However for the greenness value (a^*), the value decreased as the levels of stabilizers increased (Kumar and Mishra, 2004).

The hardness, cohesiveness and adhesiveness of the MSFY samples increased as the levels of stabilizers increased to 0.4%. However, the springiness and gumminess of the samples did not show any specific trends. In the sensory evaluation results, an addition of 0.4% gelatin got better appearance, color, yogurt's body and texture, flavor and overall acceptability compared to the other types of stabilizers (Kumar and Mishra, 2004).

The effect of stabilizers on the numbers of starter cultures, mainly *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, showed that the numbers of microorganisms increased as the levels of stabilizers increased. While, different types of stabilizers did not have any significant affects on the numbers of these microorganisms in the MSFY samples (Kumar and Mishra, 2004).

The used of gelatin as a stabilizer was also reported for corn milk yogurts at a level of 0.1% (w/w) (Prasertcheeva, 2003) and for yogurt-like soybean products at 0.5% (w/w) (Cheng *et al.*, 1990).