CHAPTER 3
THE EFFECTS OF SKIM MILK POWDER AND WATER ADDITION ON THE GROWTH OF YOGURT STARTER CULTURES IN CORN MILK

3.1 INTRODUCTION

Thailand exported canned and frozen sweet corn products for an amount of more than 650 million Baht in 2002 (Ketnil, 2002). Sweet corn also has been processed locally further to be corn milk that either be pasteurized or heat-treated in the range of UHT treatment. The good color, aroma and appearance together with the sweetness of the corn milk were the main sensory characteristics that were looked by its consumers (Rauengpanyawatana, 1998). The milk was also have a good vitamin composition including 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 100 g of the sweet corn milk (USDA, 2004).

Yogurt is principally manufactured by the growth of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* (De Brabandere and De Baerdemaeker, 1999; Lourens-Hattingh and Viljoen, 2001; Tamime and Robinson, 1999). In general, cow's milk is used as a yogurt substrate. Nevertheless, 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 mango pulp, soymilk and buffalo milk (Kumar and Mishra, 2004).

In this study, sweet corn milk was used as a media for the growth of traditionally yogurt starter cultures. Using corn 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. Producing yogurt from the corn milk would give an advantage of a low fat content and the absence of cholesterol in the final product (USDA, 2004).

Previous research showed that the production of fermented soymilk would give a better final product if it was added with SMP. SMP was

supplemented into soymilk to provide essential nutrients, mainly lactose, for the growth of the yogurt starter cultures (Cheng *et al.*, 1990; Granata and Morr, 1996; Karleskind *et al.*, 1991; Tamime and Robinson, 1999).

The main objective of this study was to determine the effects of SMP and distilled water supplemented in corn milk on the growth of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. SMP would be a source of lactose and cow's milk protein that were expected to give a better support for the growth of yogurt starters. Addition of distilled water would mainly affect the solid content of corn milk

3.2 MATERIAL AND METHODS

3.2.1 Sweet corn milk

The sweet corn used in this study was an ATS-5 that harvested on the 23rd day after silking of the corn plant. The sweet corn was purchased from Thaweesak Sweet Corn Group, Chiang Mai province, Thailand in April-May 2004. The preparation of corn milk solution and the storage condition were followed the method in section 2.2.1.

3.2.2 Stock culture

The freeze-dried starters of *S. thermophilus* No. 894 (ATCC 19258) and *L. delbrueckii* subsp. *bulgaricus* No. 892 (ATCC 11842) (Thailand Institute of Scientific and Technological Research, Thailand) were grown for 18 h at 37°C in M17 broth (Merck, Germany) and MRS medium (Merck, Germany), respectively. The 1 loop of each growth microorganisms was transferred into 10 ml of litmus milk. The litmus milk was prepared in a test tube by mixing 16% (w/v) SMP (Mission, Thailand), 2% (w/v) of 1% (w/v) litmus concentration (BDH, England), and 0.3% (w/v) yeast extract (Difco, USA). The litmus milk was diluted with distilled water. Enough amount of calcium carbonate (Merck, Germany) was added to cover the bottom of the test tube. The mixture was sterilized for 15 min at 121°C and immediately cooled to 37°C by soaking in tap water. The inoculated culture was incubated for 18 h at 37°C and stored at 5°C until use (Sankhavadhana, (2001) with modification).

3.2.3 Mother culture

Individual mother culture was freshly prepared before conducting the experiment by inoculating 1 loop of stock culture in 100 ml of sterilized milk

medium, which contained 16% (w/v) SMP, and 0.1% (w/v) yeast extract. The inoculated culture was incubated at 37°C for 18 h and kept at 5°C until use (Sankhavadhana, (2001) with modification).

3.2.4 Corn milk yogurt

To study the growths of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in corn milk, the corn milk was added with 3 different levels of a SMP (Mission, Thailand) including 0, 7.5 and 13.5% (w/v) and 3 different levels of distilled water using ratios of corn milk to distilled water of 1:0, 1:1 and 1:2. The milks added with distilled water were preheated at 90°C for 5 min before adding SMP. The milk samples were then heated at 95°C for 5 min and cooled down immediately to 40°C. At 40°C, the milks were inoculated at a ratio of 1:1 with 2% (v/v) mixture of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. The inoculated milk was incubated at 40°C for 48 h. During the incubation time, samples were taken at 0, 2, 4, 6, 8, 10, 12, 14, 18, 24 and 48 h. The experiment was carried out in triplicates.

3.2.5 Pesticide residues

The sweet corn samples were taken from Thaweesak Sweet Corn Group, Chiang Mai, Thailand in November 2006. The sweet corn samples were evaluated for the pesticide residues in Pyrethroid group using method of Steinwandter (1985).

3.2.6 Chemical analysis

Samples of corn milk or yogurt were analyzed for total acidity, pH value and total solid. The total acidity and total solid were measured according to AOAC methods no. 947.05 and no. 990.20, respectively (AOAC, 2000). The result of total acidity was expressed as % lactic acid. For the pH measurement, a pH meter (Consort C830, CE, Belgium) was employed.

3.2.7 Microbiological analysis

The corn milk or yogurt samples were subjected to microbiological analysis for the viable numbers of yogurt starter cultures. *S. thermophilus* was enumerated using M17 agar (Merck, Germany) that was acidified to pH 6.8 by 1 M HCl (Merck, Germany) as described by IDF (1997). The samples were incubated at 37±1°C under aerobic condition for 48 h. *L. delbrueckii* subsp. *bulgaricus* was enumerated by MRS agar (Merck, Germany) that was acidified to pH 5.4 using 100% glacial acetic acid

(Merck, Germany). The samples were incubated at 37±1°C under anaerobic condition for 72 h (IDF, 1997).

3.2.8 Statistical analysis

The collected data was analyzed statistically by an Analysis of Variance using a Factorial Experiment in CRD with 2 factors. The first factor was SMP concentrations, which were 0, 7.5 and 13.5% (w/v). The second factor was the ratios of corn milk to distilled water, which were 1:0, 1:1 and 1:2. If the F value from the Analysis of Variance was significant, a Duncan's New Multiple Range test was utilized to determine differences between treatment means (Montgomery, 2001). The statistical calculation was performed using SPSS 10.0.1 software (SPSS Inc., Chicago, USA).

3.3 RESULTS AND DISCUSSION

3.3.1 Pesticide residues

Use of pesticide in pyrethroid group was reported during production of ATS-5 sweet corn plant (Kongthong, 2006). Pesticides contaminated in corn milk would inhibit or retard the growth yogurt cultures (Tamime and Robinson, 1999; Yagüe *et al.*, 2001). Accordingly, the sweet corns were determined for pesticides in pyrethroid group including bifenthrin, permethrin, l-cyhalothrin, cypermethrin, cyfluthrin, fenvalerate and deltamethrin (Appendix A-2). The results revealed that the sweet corn samples were not contained any residue of the tested pesticides. The effects on growth of yogurt cultures would, therefore, resulted from other factors, except the pesticide residue.

3.3.2 Solid content of corn milks supplemented with SMP and/or distilled water

The amount of solid in corn milk (control treatment) was equal to that in soymilk yogurt (Granata and Morr, 1996), but higher to that in cow's milk yogurt, which was 14-15% (Tamime and Robinson, 1999). As expected, the level of solid content was increased with increasing level of SMP and/or decreasing level of added water (Table 3.1).

Table 3.1 The levels of total solid content (%) of corn milks supplemented with different concentrations of SMP and/or distilled water

SMP (%,w/v)	Corn milk-to-distilled water ratio		
	1:0	1:1	1:2
0	17.65±0.16[d]	9.00±0.02[g]	5.72±0.04[h]
7.5	23.32±0.30[b]	14.86±0.30[e]	12.51±0.15[f]
13.5	27.76±0.18[a]	20.39±0.04[c]	17.67±0.08[d]

* Values in a column followed by different letters were significantly different treatments ($P<0.05$)

During 48 h fermentation at 40°C, the solid levels of different corn milk treatments were not changed (Figure 3.1). The stability of solid content was also observed in soymilk fermentation (Lee *et al.*, 1990). This result implied that the change in chemical and/or physical properties of corn milk yogurt could not be detected by a measurement of total solid.

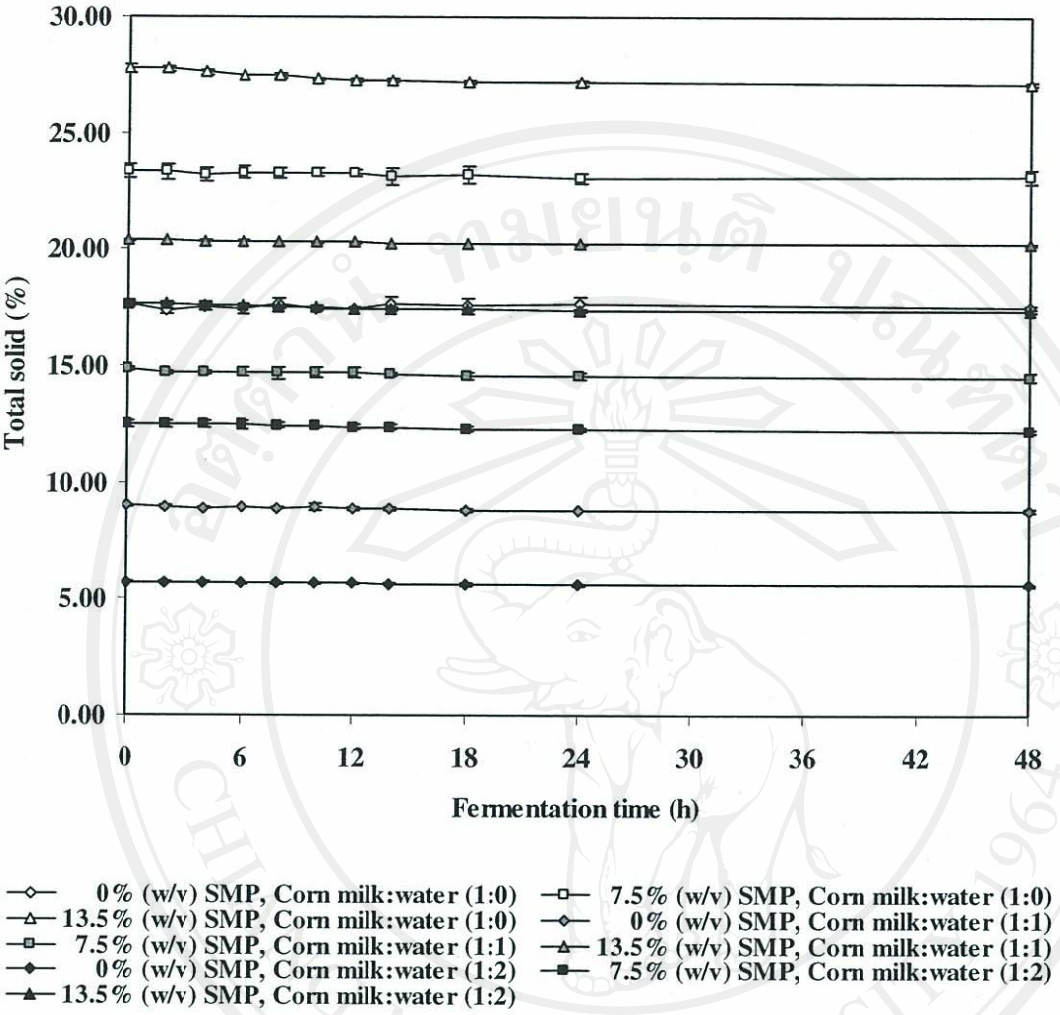

Figure 3.1 Total solid of corn milk prepared from different levels of SMP and distilled water during fermentation at 40°C.

3.3.3 Development of acidity during fermentation of corn milk yogurt

Acidity is the most important parameter that can be used to directly monitor the progress of fermentation. The development of acidity was evaluated by measuring the values of pH and total acidity (Figures 3.2 and 3.3). As shown in Figure 3.2, pH values of the corn milks dropped sharply within the first 6-8 h incubation time. Then, the pH values were stable for the extended incubation time. This finding was agreed with the study of soymilk yogurt (Wang *et al.*, 2002; Yazici *et al.* 1997). The initial pH values between different corn milk treatments were slightly differed depending on the levels of SMP and/or distilled water. The reason was because pH of SMP,

6.63±0.01 (Tamime and Robinson, 1999), was slightly lower than that of the corn milk.

Although the initial pH of different treatments was slightly differed, the progress of acid development during fermentation was noticeably affected by the level of solid content. At high level of solid content (Table 3.1), the lower reduction of pH was observed (Figure 3.2). The lower reduction of pH was possibly due to the buffering capacity of added protein as stated by Gastaldi *et al.* (1997); Granata and Morr (1996); Kristo *et al.* (2003); Tamime and Robinson (1999) and Yazici *et al.* (1997). The similar changes of acidity and pH during fermentation were observed in corn milk yogurt studied by Pangsanit (2002) and Prasertcheeva (2003).

The time taken the pH to reach 4.4-4.6 was used as an indication of fermentation time in the manufacturing of yogurt (De Brabandere and De Baerdemaeker, 1999; Puvanenthiran *et al.*, 2002; Tamime and Robinson, 1999; Wu *et al.*, 2001). As can be seen in Figure 3.2, an increasing in the amount of SMP and/or the corn milk caused the fermentation time to increase considerably. The sample contained the highest solid content, i.e. 13.5% (w/v) SMP, required more than 48 h fermentation, whereas the sample with lowest solid content, i.e. 0% (w/v) SMP required only 6 h fermentation.

Changing of acidity was corresponded to the levels of total solids in the milks. In general, higher solid content resulted in higher level of initial acidity (Figure 3.3) as SMP itself provided some acidity (Tamime and Robinson, 1999). During the fermentation, production of acid was enhanced by the increasing of solid level. The presence of nutrients from SMP would support the growth of yogurt starter cultures (Cheng *et al.*, 1990; Karleskind *et al.*, 1991), which produced lactic acid. The corn milks contained less than 10% solid would achieve the maximum level of 0.68% lactic acid after 48 h fermentation. Whereas those that contained the higher levels of total solid would generally experience a sharp increase in the content of lactic acid within the first 12 h of the fermentation.

The initial lactic acid levels of the corn milks were higher than that of the normal acidity of cow's milk, ≤0.2% (Fox and McSweeney, 1998; Tamime and Robinson, 1999) and of cow's milk yogurt, 0.9-1.4% (Duboc and Mollet, 2001; Kosikowski, 1997). However, the pH as well as acidity used as an indication of

fermentation in the manufacturing of corn milk yogurt could be used at the pH value of cow's milk fermentation of below 4.6. This was because zein protein in corn could not provide the gel structure of yogurt itself. It was the milk protein, which was supplemented into corn milk that was responsible for gel structure. Accordingly, the similar final pH could be used to monitor the fermentation of corn milk yogurt. For soymilk yogurt, its final lactic acid content varied from 0.72-1.21% (Granata and Morr, 1996; Cheng *et al.*, 1990 and Lee *et al.*, 1990) which was similar to the level of acidity in this study.

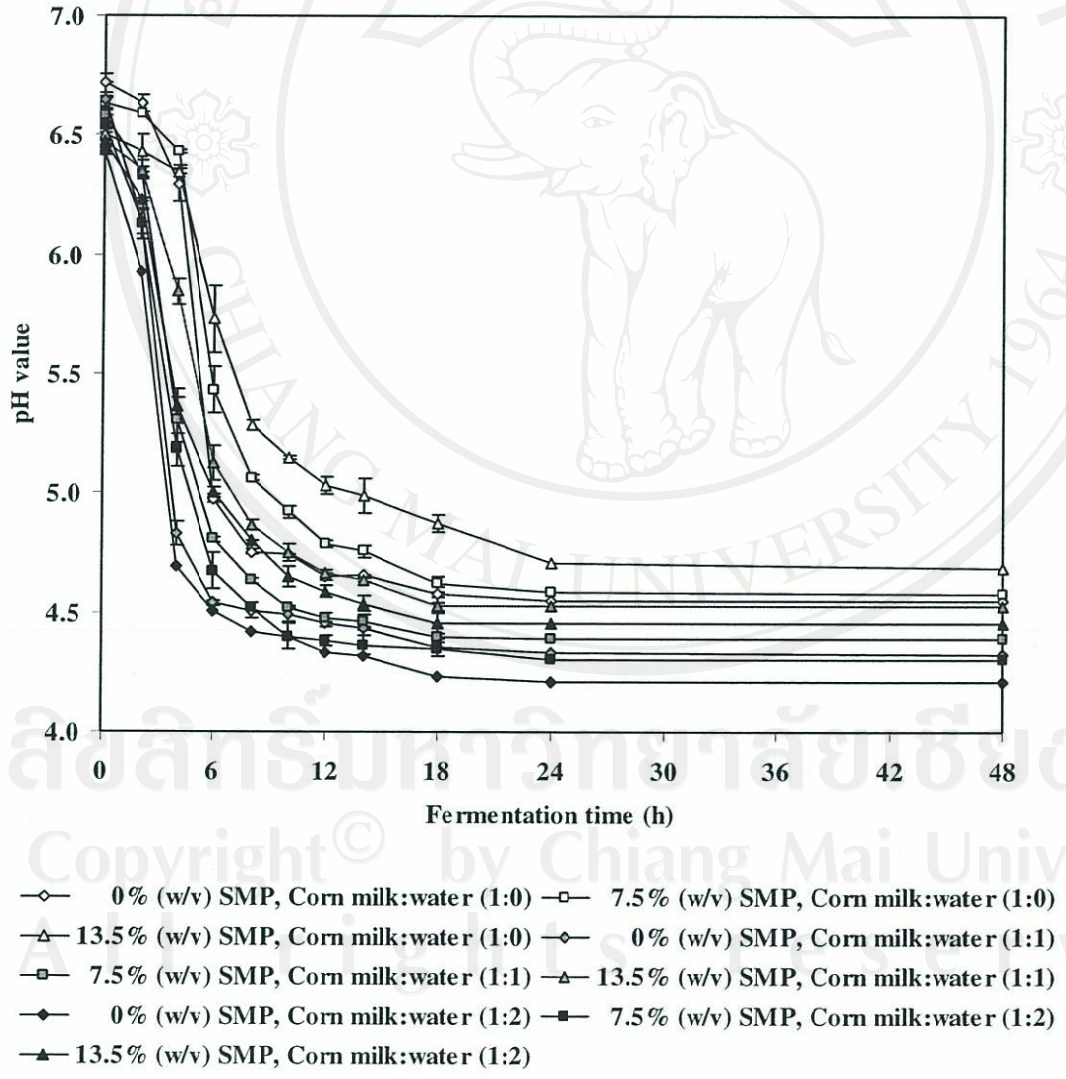

Figure 3.2 pH values of corn milk prepared from different levels of SMP and distilled water during fermentation at 40°C.

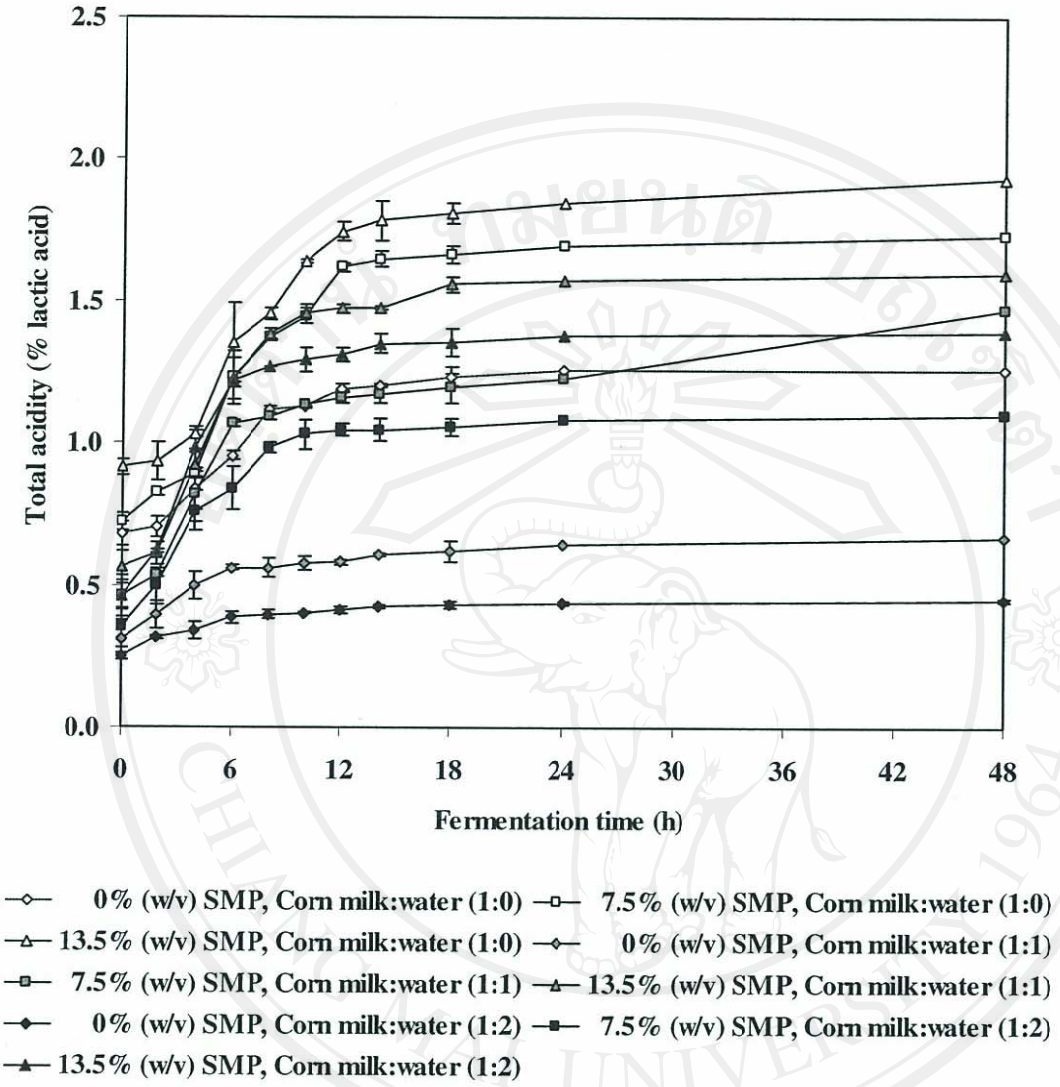

Figure 3.3 Total acidity (% lactic acid) of corn milk prepared from different levels of SMP and distilled water during fermentation at 40°C.

3.3.4 Changes of yogurt starter cultures during fermentation of corn milk yogurt

3.3.4.1 Growth of *S. thermophilus*

Growths of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were monitored during 48 h of fermentation. Figure 3.4 showed the fast growth of *S. thermophilus* in corn milk without SMP and water additions during the first 2 h of fermentation. The maximum populations of *S. thermophilus* were obtained at 6 h. When the fermentation, time was prolonged, the survival numbers of the streptococci were reduced. At the end of the fermentation, the counts were 1.47±0.39 log CFU/ml.

The presence of SMP, which contained casein and lactose, could generally provide a better support in maintaining the viability of the *S. thermophilus* than the nutrients in the corn milk alone.

The reduction of *S. thermophilus* was caused partly by high acidity. The pH values below 5.5 was reported for having a negative effect on the growth of *S. thermophilus* (Adams and Moss, 2000; Lourens-Hattingh and Viljoen, 2001). Sugar in corn milk, mainly sucrose, might not enough for maintain the viability of the organism. The similar growth pattern of *S. thermophilus* during fermentation was observed of soymilk (Wang *et al.*, 2002) and cow's milk (Dave and Shah, 1997; Krasaekoopt *et al.*, 2001; Vinderola *et al.*, 2000).

Different levels of distilled water in corn milks did not significantly effect the growth pattern of *S. thermophilus*. However, at higher levels of distilled water, the reduction in the survival number of the organism became lower. This could be due to the reduction in acidity of corn milk.

3.3.4.2. Growth of *L. delbrueckii* subsp. *bulgaricus*

Growth patterns of *L. delbrueckii* subsp. *bulgaricus* were different from those of *S. thermophilus* (Figure 3.5). The difference could possibly result from the inability of *L. delbrueckii* subsp. *bulgaricus* to utilize sucrose (Wang *et al.*, 2002), the main sugar in corn milk. The influence of SMP on the growth of *L. delbrueckii* subsp. *bulgaricus* was less than that of *S. thermophilus*. Overall, counts of *L. delbrueckii* subsp. *bulgaricus* in corn milk were continuously decreased from 8.33 log CFU/ml at the beginning to 0.50 log CFU/ml at the end, except the milks supplemented with SMP and distilled water exhibited distinct reduction after 6 and 10 h, respectively. The addition of distilled water exhibited a less reduction in the viable numbers of *L. delbrueckii* subsp. *bulgaricus*, especially after 10 h fermentation. The presence of higher levels of distilled water would produce corn milks with lower acidity and lower sucrose content that favor the growth of *L. delbrueckii* subsp. *bulgaricus.* Although, *L. delbrueckii* subsp. *bulgaricus* also could survive at low pH values itself (Lourens-Hattingh and Viljoen, 2001; Walstra *et al.*, 1999). In general, a high survival rate of *L. delbrueckii* subsp. *bulgaricus* was established in the corn milk that was supplemented with 7.5% (w/v) SMP and distilled water at a ratio of 1:2.

Supplementation of distilled water at a ratio of 1:2 was employed in the further experiments.

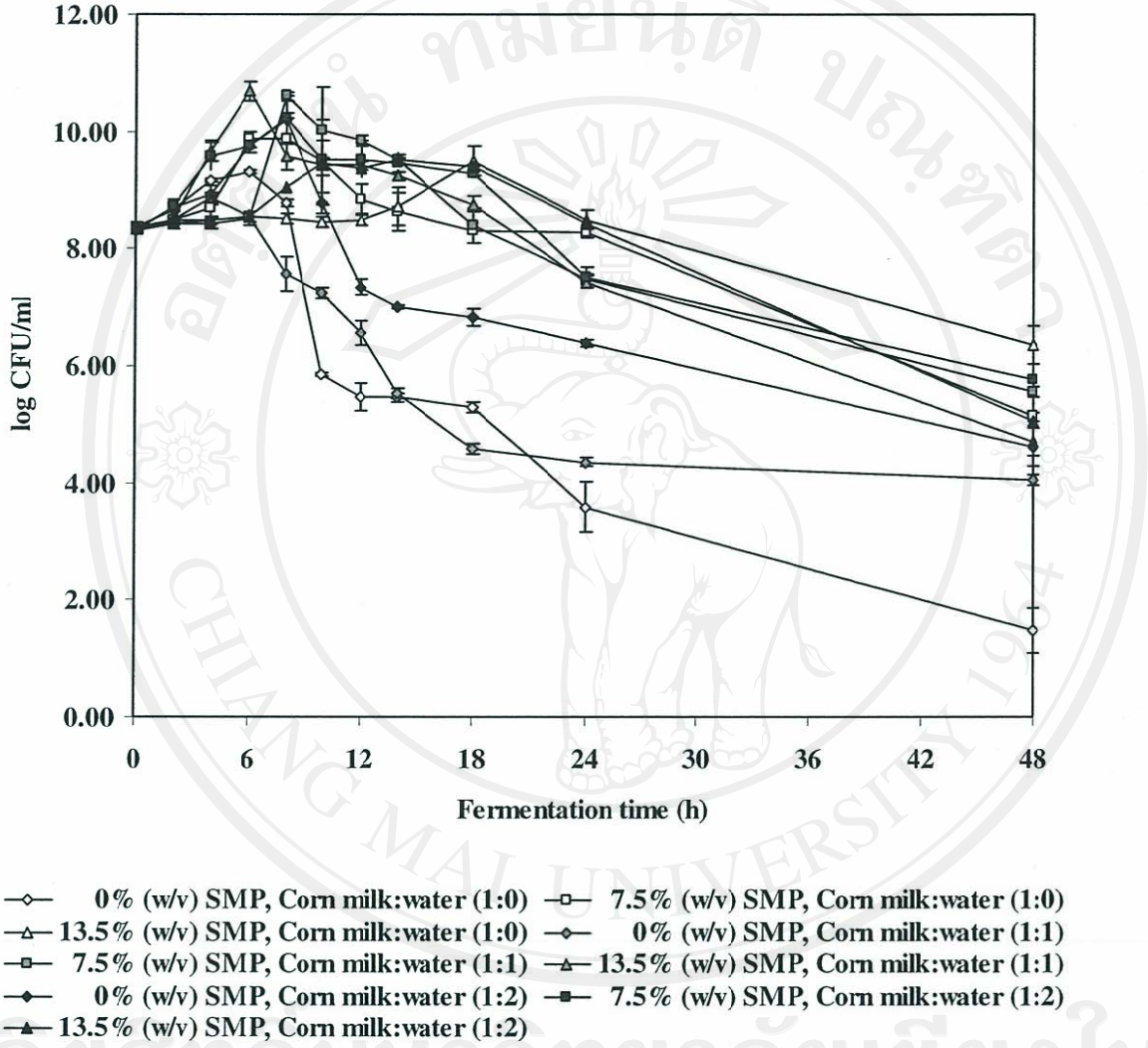

Figure 3.4 The viable number of *S. thermophilus* in corn milk prepared from different levels of SMP and distilled water during fermentation at 40°C.

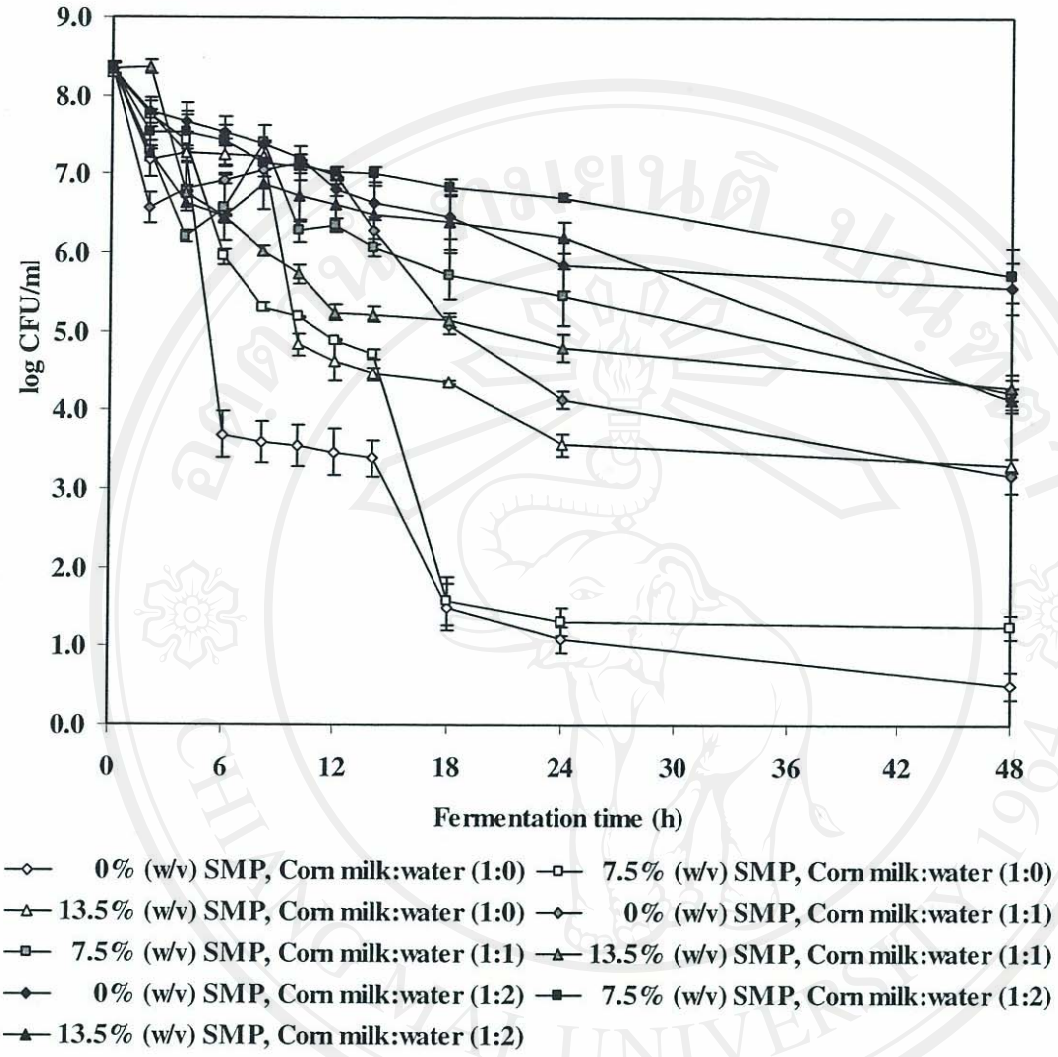

Figure 3.5 The viable number of *L. delbrueckii* subsp. *bulgaricus* in corn milk prepared from different levels of SMP and distilled water during fermentation at 40°C.

3.4 CONCLUSIONS

The development of acidity by yogurt starter cultures in corn milks was positively correlated to levels of the supplemented SMP. *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* demonstrated different growth patterns during corn milk fermentation. *S. thermophilus* could grow for more than 2 log CFU/ml during the first 6 h incubation time at 40°C, whereas the viable numbers of *L. delbrueckii* subsp. *bulgaricus* was steadily decreasing throughout 48 h fermentation. The corn milk alone

was found to be an unsuitable yogurt substrate for both microorganisms. The additions of SMP and distilled water could improve the viability of these microorganisms.

Supplementation of 7.5% (w/v) SMP and distilled water at a ratio of 1:2 was a suitable condition for corn milk yogurt production. At this condition, the corn milk yogurt had pH value below 4.6, lactic acid content fell in the range of general yogurt, and high survivals of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were obtained.