CHAPTER 8
EFFECT OF INOCULATION QUANTITY ON THE GROWTHS OF STARTER CULTURES IN CORN MILK YOGURT

8.1 INTRODUCTION

Yogurt is the result of milk fermentation by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Yogurt may be precultured once or more in a milk media, then added to the milk base with an inoculation level varying from 1 to 5% (Kosilowski, 1997; Sodini *et al.*, 2004; Torriani *et al.*, 1996). It is then kept at the optimum temperature for 3 to 6 h until pH of 4.4-4.6 (0.9 to 1.4% total acidity) is obtained (De Brabandere and De Baerdemaeker, 1999; Duboc and Mollet, 2001; Kosikowski, 1997). However, the inoculum percentage can be modulated when other factors have to be changed for practical production (Torriani *et al.*, 1996). In general, cow's milk has been used as a raw material of yogurt production, but corn milk would be used for raw material in this research. Therefore, it was important to evaluate the influence of inoculation quantity on the growth of starter culture in the corn milk yogurt.

8.2 MATERIALS AND METHODS

8.2.1 Preparation of 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 the same place and duration time as section 7.2.1. The preparation of corn milk solution and storage condition were followed the method in section 2.2.1.

8.2.2 Starter cultures preparation

S. thermophilus and *L. delbrueckii* subsp. *bulgaricus* were prepared by following the previous method as described in section 3.2.2 and 3.2.3.

8.2.3 Corn milk yogurt preparation

Distilled water was added into the corn milk in a ratio of 1:2, corn milk to distilled water, and then preheated at 90°C prior to fortification with 2% (w/v) lactose, 4% (w/v) sodium caseinate, and 0.4% (w/v) gelatin. The mixture was stirred for 5 min, following by heating at 95°C for 5 min (Raphaelides and Gioldasi, 2005) then cooled to 40°C. Consequently, yogurt starter cultures which composed of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* at a ratio of 1:1 was inoculated either at 2, 4, 6 or 8% (v/v). The inoculum was incubated at 40°C for 12 h. During the incubation time, corn milk yogurts were taken at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0 and 12.0 h for microbiological and chemical analysis. The corn milk yogurts were prepared in triplicates for each treatment.

8.2.4 Microbiological analysis

The corn milk yogurts were subjected to microbiological analysis for the viable numbers of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. The enumeration of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were accomplished by using the method that described in section 3.2.7.

8.2.5 Chemical analysis

Samples of corn milk yogurt were analyzed for total acidities, pH values and total solid content. 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 the total acidity was expressed as % lactic acid. For the pH measurement, a pH meter Consort C830 (CE, Belgium) was employed.

8.3 RESULTS AND DISCUSSION

8.3.1 Changes in the amount of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* during fermentation

The growths of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in corn milk yogurt during fermentation varied with the percentages of inoculation as shown in Figures 8.1 and 8.2, respectively. The growth profiles of *S. thermophilus* in the corn milk yogurt could be briefly divided into two groups. The first group included the samples added with 2 and 4% (v/v) starter cultures, while the second group included the samples added with 6 and 8% (v/v) starter cultures. The maximum

numbers of *S. thermophilus* in all tested sample were similar, but the amounts of *S. thermophilus* in the first group were maximum at 4 h of fermentation time, whereas the maximum numbers of the second group were obtained at 3 h. A significant reduction in the numbers of *S. thermophilus* occurred when the fermentation time was extended from 6 to 12 h, except for that of the 2% (v/v) inoculation. The similar change in growth pattern of *S. thermophilus* being found in glucose was reported by Thomas and Crow (1983).

During fermentation, the growths of *L. delbrueckii* subsp. *bulgaricus* inoculated with different percentages were comparable. The maximum amounts were attained at 4 h of fermentation time, and then the large reduction occurred. Wang *et al.* (2002) suggested that the principle factor causing the reduction of cell numbers was the acidity of the yogurt. The correlation between the reduction of cell numbers and the acidity will be examined in the followed experiment. It was observed that the numbers in all samples were greater for *S. thermophilus* than *L. delbrueckii* subsp. *bulgaricus* through out the fermentation time. This result was expected because *S. thermophilus* was able to utilize sucrose, the main sugar in corn milk, to support the growth, whereas *L. delbrueckii* subsp. *bulgaricus* could not utilize sucrose (Wang *et al.*, 2002). The better growth of *S. thermophilus* was also reported by Amoroso and Manca de Nadra (1992). Especially, the amount of *L. delbrueckii* subsp. *bulgaricus* decreased during the first 0.5 h, and then started to grow. The decreasing of *L. delbrueckii* subsp. *bulgaricus* might be because the *L. delbrueckii* subsp. *bulgaricus* was still in the lag phase, and waited for some nutrients from *S. thermophilus* (Tamime and Robinson, 1999; Walstra *et al.*, 1999). Evidently, corn milk yogurt should be used 2% (v/v) inoculation because this concentration was the lowest concentration that gave the high amounts of survival cultures at the optimum and at the end of fermentation times.

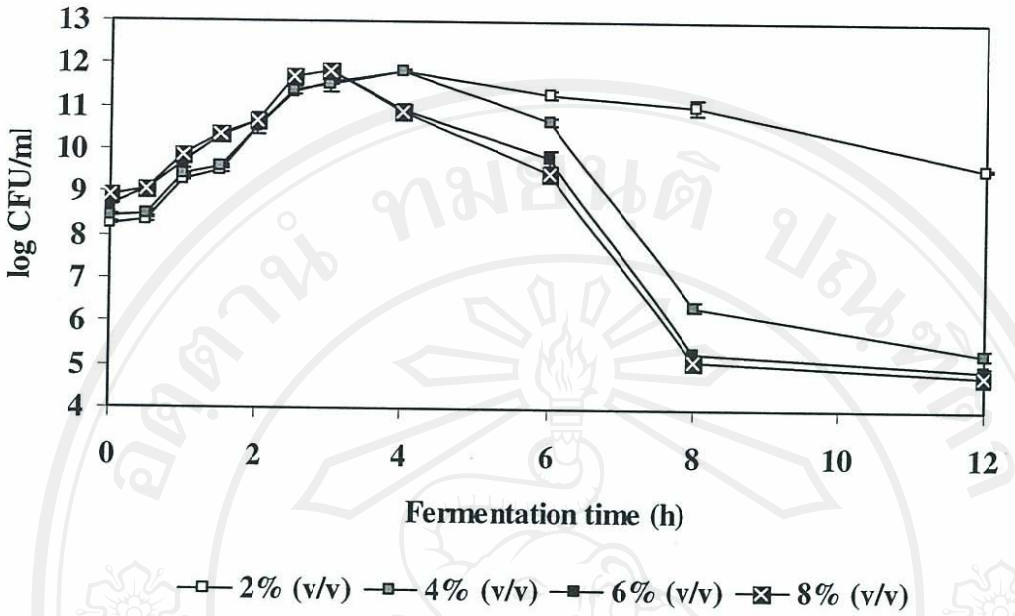

Figure 8.1 The growth of *S. thermophilus* in corn milk yogurt inoculated with different percentage of starter cultures during fermentation at 40°C.

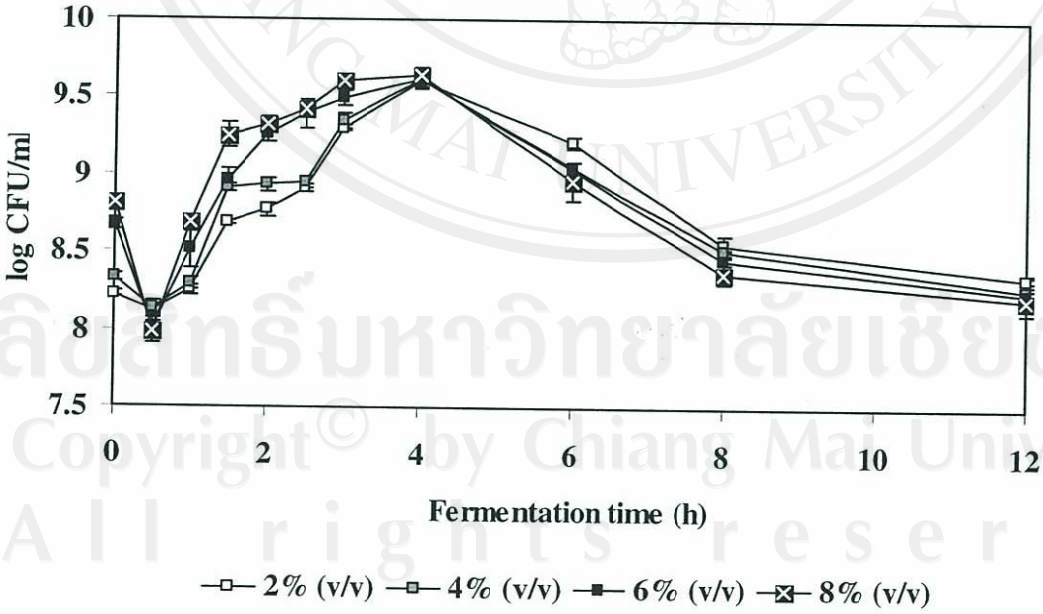

Figure 8.2 The growth of *L. delbrueckii* subsp. *bulgaricus* in corn milk yogurt inoculated with different percentage of starter cultures during fermentation at 40°C.

8.3.2 Changes in the chemical properties during fermentation

The changes of total acidity (Figure 8.3) and pH value (Figure 8.4) of corn milk yogurts inoculated with different quantities of starter cultures during fermentation were, in general, correlated to the changes of cell numbers. This finding agreed with the study of Wang *et al.* (2002). They discovered the correlation of the reduction of cell numbers of LAB and bifidobacteria, pH and the acidity in soymilk drinks.

Acid production and pH reduction were greater when inoculum percentage was higher. A similar trend was reported by Walstra *et al.* (1999). They revealed that the increasing the inoculum percentage would increase the rate of acid production. As shown in Figures 8.1 and 8.2, marked increasing in acidity and decreasing of pH were observed during the first 4 h of fermentation. Then, the changes of acidity and pH were apparently slow.

In yogurt manufacturing, fermentation is stopped at a pH below 4.6. (De Brabandere and De Baerdemaeker, 1999; Kosikowski, 1997). Thus, the optimum fermentation time for corn milk yogurts inoculated with 2 or 4% (v/v) starter cultures would be 4 h whereas those of 6 or 8% (v/v) starter cultures would be 3 h. At the optimum fermentation time, total acidity was in range of 0.95-1.03%, which was similar to the final acidity of cow's milk yogurt (0.9-1.4%) (Duboc and Mollet, 2001; Kosikowski, 1997).

The percentage of inoculum influenced on total solid content of the corn milk yogurt (Figure 8.5). The total solid content increased with the increasing of inoculation percentage. However, the total solid content of each inoculation percentage was not changed during fermentation. It might be due to solid compounds of corn milk yogurt had a little change during fermentation, as a result, the change of total solid could not be detected.

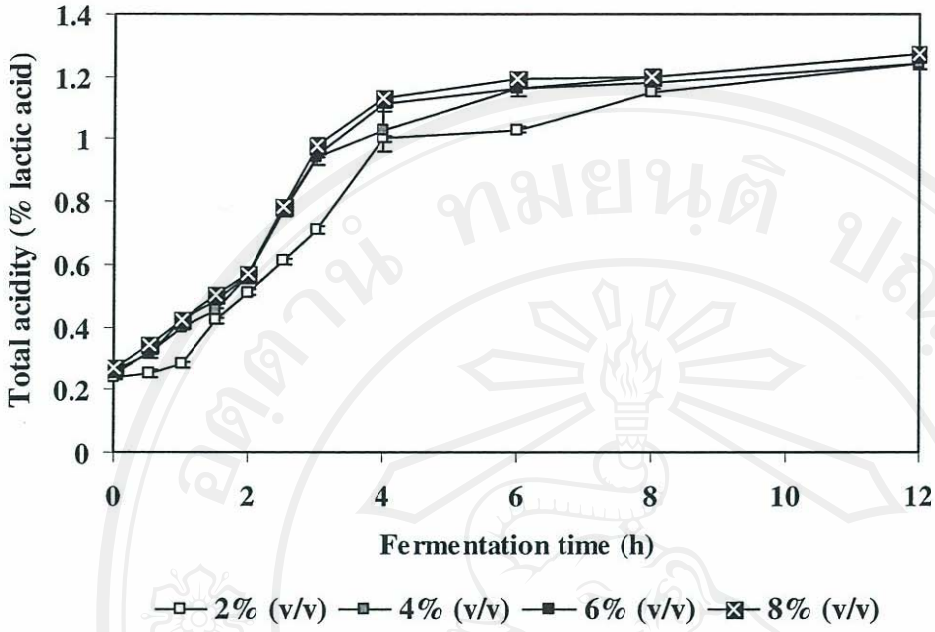

Figure 8.3 Change of total acidity of corn milk yogurt inoculated with different percentage of starter cultures during fermentation at 40°C.

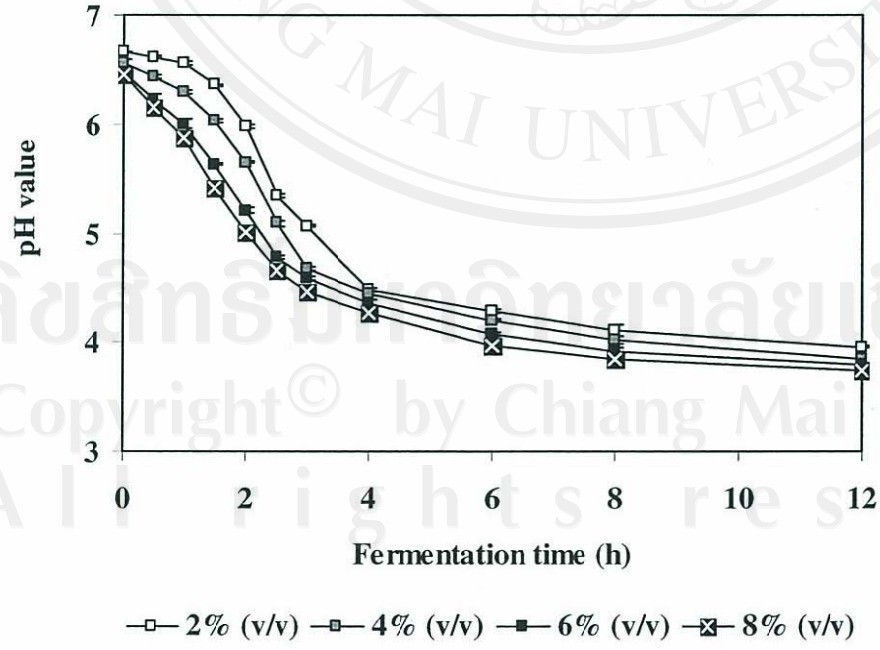

Figure 8.4 Change of pH value of corn milk yogurt inoculated with different percentage of starter cultures during fermentation at 40°C.

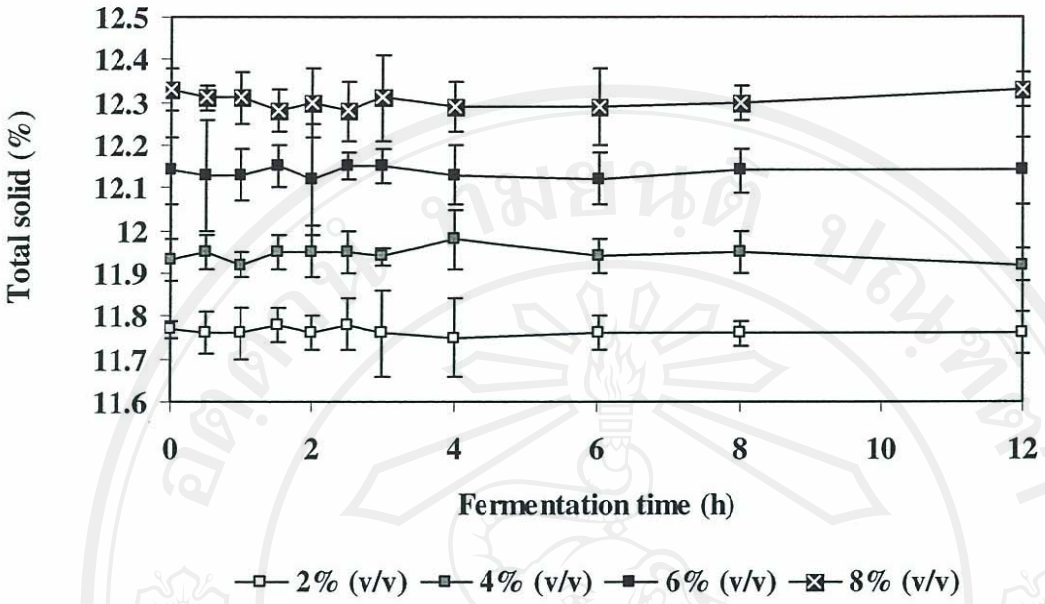

Figure 8.5 Change of total solid of corn milk yogurt inoculated with different percentage of starter cultures during fermentation at 40°C.

8.4 CONCLUSIONS

The inoculation quantity affected the growth of starter cultures in the corn milk yogurt. The maximum growth of starter cultures attained at the optimum fermentation time. The optimum fermentation time for corn milk yogurts inoculated with 2 or 4% (v/v) starter cultures was 4 h, whereas those of 6 or 8% (v/v) starter cultures was 3 h. The optimum inoculation percentage of starter culture for corn milk yogurt was 2% (v/v). The changes of total acidity and pH value of corn milk yogurts inoculated with different quantities of starter cultures during fermentation were correlated to the changes of cell numbers. Total acidity at the optimum fermentation time was 0.95-1.03%.