#### CHAPTER 7

### GROWTHS OF INDIVIDUAL OR MIXED CULTURES OF Streptococcus

thermophilus AND Lactobacillus delbrueckii subsp. bulgaricus

#### IN CORN MILK YOGURT

#### 7.1 INTRODUCTION

Yogurt is made from milk by the symbiotic relationship of two homofermentative bacteria, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Amoroso and Manca de Nadra, 1992; De Brabandere and De Baerdemaeker, 1999; Granata and Morr, 1996; Shihata and Shah, 2000; Tamime and Robinson, 1999). The optimum ratio of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* is often approximately 1:1 (Granata and Morr, 1996; Kosikowski, 1997; Walstra *et al.*, 1999). *L. delbrueckii* subsp. *bulgaricus* stimulates the growth of *S. thermophilus* by liberating amino acids and peptides from milk proteins. As a result, *S. thermophilus* can grow faster in the early stage of incubation. *S. thermophilus* in turn produces formic acid and reduces oxygen levels, which stimulates the growth of *L. delbrueckii* subsp. *bulgaricus* (Kosikowski, 1997; Shihata and Shah, 2002; Van de Water, 2003; Walstra *et al.*, 1999).

In this research, corn milk extracted from sweet corn was used as a material for yogurt making. The main objective of this study was to compare the growths of individual and mixed cultures of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* during fermentation of corn milk yogurt.

## 7.2 MATERIALS AND METHODS

#### 7.2.1 Preparation of sweet corn milk

The sweet corn used in this study was an ATS-5 that harvested on the 23<sup>rd</sup> day after silking of the corn plant. The sweet corn was purchased from the same place as section 3.2.1 in September-November 2004. The preparation of corn milk solution and storage condition were followed the method in section 2.2.1.

## 7.2.2 Starter cultures preparation

S. thermophilus and L. delbrueckii subsp. bulgaricus were prepared by the methods described in section 3.2.2 and 3.2.3.

## 7.2.3 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, 2% (v/v) yogurt starter cultures which composed of individual culture of *S. thermophilus* or *L. delbrueckii* subsp. *bulgaricus* and mixed cultures of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* at a ratio of 1:1. The inoculum was incubated at 40°C for 12 h. During the incubation time, corn milk yogurts were taken for enumeration at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0 and 12.0 h. The corn milk yogurts were prepared in triplicates for each treatment.

## 7.2.4 Microbiological analysis

The corn milk yogurts were subjected to microbiological analysis for the viable numbers of yogurt starter cultures in the samples. *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were enumerated according to section 3.2.7.

#### 7.2.5 Chemical analysis

Samples of corn milk yogurt were analyzed for total acidities, pH values and total solid content. The total acidity was measured according to AOAC methods no. 947.05 (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.

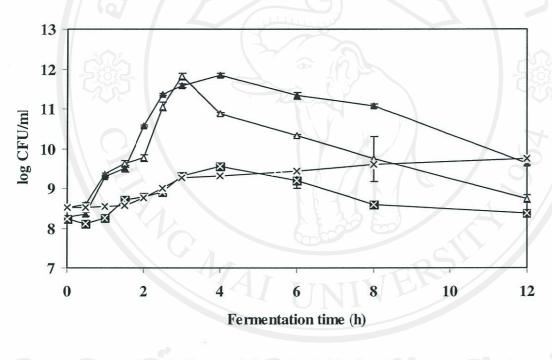
### 7.3 RESULTS AND DISCUSSION

## 7.3.1 Changes in the amount of starter cultures during fermentation

Figure 7.1 showed that the similar growths of *S. thermophilus* in mixed cultures and individual *S. thermophilus* during the first 3 fermentation. After that, the individual *S. thermophilus* decreased at the higher rate. In case of *L. delbrueckii* subsp. *bulgaricus*, the individual culture exhibited the steady increase through out the studied time, while the growth in mixed cultures was fast at the first 4 h, and then decreased continuously. According to the maximum growths of *S. thermophilus* and *L.* 

delbrueckii subsp. bulgaricus, the fermentation time would be 4 h. At 4 h fermentation, using the mixed cultures was a better choice for making corn milk yogurt.

Mixed culture showed the better growth because *L. delbrueckii* subsp. bulgaricus produced free amino acids for stimulating the growth of *S. thermophilus* while *S. thermophilus* obtained CO<sub>2</sub> and formic acid for the growth of *L. delbrueckii* subsp. bulgaricus (Beshkova et al., 1998; Shihata and Shah, 2002; Van de Water, 2003). The symbiotic action of *S. thermophilus* and *L. delbrueckii* subsp. bulgaricus relationship was reported by Amoroso and Manca de Nadra (1992).



— S. thermophilus in individual culture

▲ S. thermophilus in mixed cultures

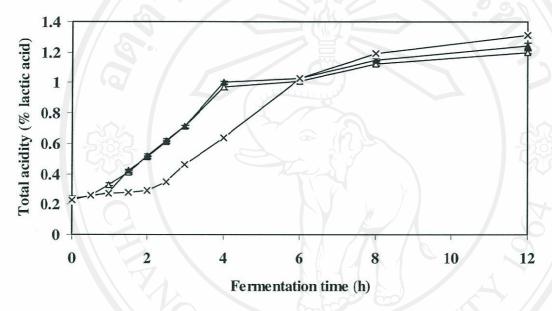
× L. delbrueckii subsp. bulgaricus in individual culture

L. bulgaricus in mixed cultures

Figure 7.1 The growth of individual and mixed culture of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in corn milk yogurt at 40°C.

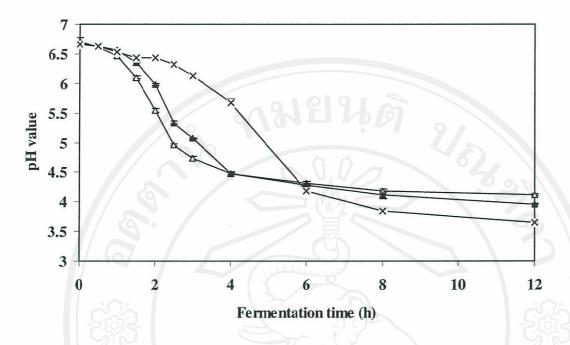
# 7.3.2 Changes in total acidity and pH values of corn milk yogurt during fermentation

The changes in total acidity (Figure 7.2) and pH values (Figure 7.3) of corn milk yogurt during fermentation by individual and mixed cultures of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were related to the growths of cultures (Figure 7.1).



- → Individual culture of S. thermophilus
- **★** Individual culture of *L. bulgaricus*
- Mixed cultures of S. thermophilus and L. delbrueckii subsp. bulgaricus

Figure 7.2 Acid production of individual and mixed cultures of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in corn milk yogurt at 40°C.



- $\triangle$  Individual culture of S. thermophilus
- × Individual culture of *L. bulgaricus*
- ▲ Mixed cultures of S. thermophilus and L. delbrueckii subsp. bulgaricus

Figure 7.3 pH changes of corn milk yogurt inoculated with individual and mixed cultures of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* at 40°C.

## 7.4 CONCLUSIONS

The fermentation of corn milk using the mixed cultures provided higher counts of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* than using the individual culture of *S. thermophilus* or *L. delbrueckii* subsp. *bulgaricus*. The optimum fermentation time of the mixed cultures system was 4 h. The changes in total acidity and pH values of corn milk yogurt during fermentation by individual and mixed cultures of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were related to the growths of cultures.