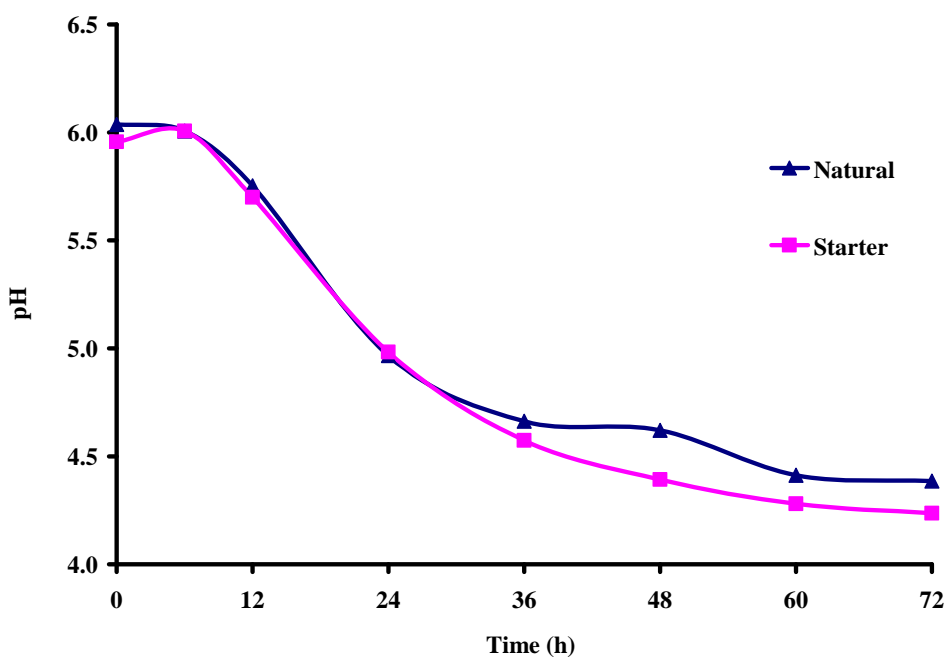


## RESULTS

### 1. Chemical profiles of Nham

#### 1.1 pH

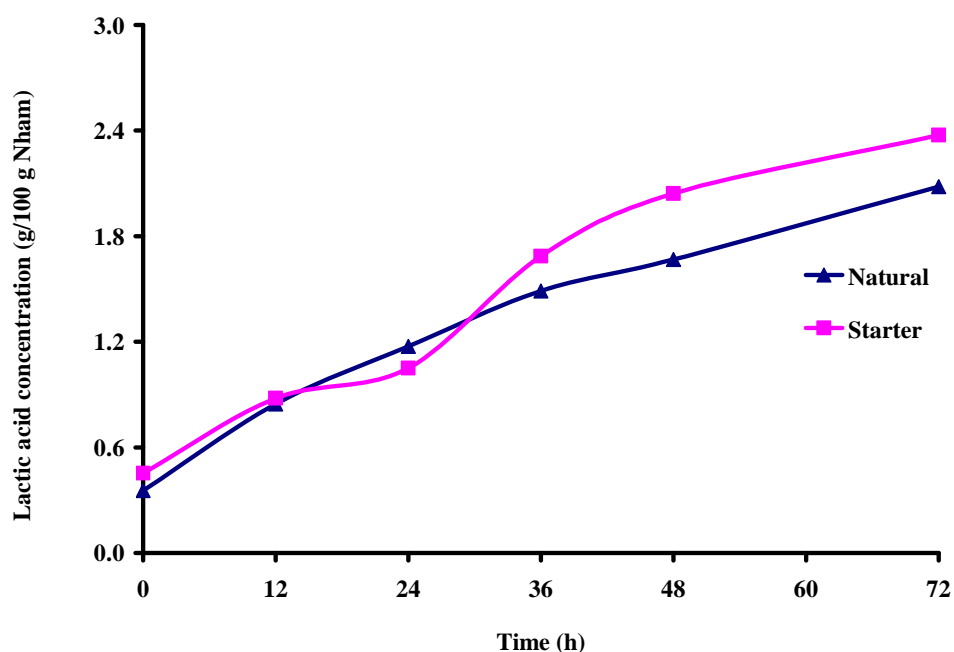
The overall pH profiles were similar in both types of Nham fermentation (Figure 7). The initial pH of all samples was approximately 6.0. In both types of fermentation, the pH was relatively stable up to 6 h of fermentation and sharply decreased after 12 h. During 24 h of fermentation, the pH value was not different between natural and starter cultured Nham fermentation. However, the pH value in starter culture fermentation was lower than that in natural fermentation after 36 h and until the end of fermentation. The pH value of natural and starter cultured Nham w decreased to 4.6 after 36 h and 48 h of fermentation, respectively. At the end of fermentation, the final pH was approximately 4.2 and 4.4 in Nham produced with and without starter culture, respectively.



**Figure 7** Changes in pH profiles during Nham fermentations.

## 1.2 Organic acid

The overall of lactic acid profiles were similar in both types of fermentation. The production of lactic acid tended to increase and became highest at the end of fermentation (Figure 8). The initial concentration of lactic acid in both types of fermentation was between 0.4-0.5 g/100 g Nham. During 24 h of fermentation, lactic acid concentration was not different between natural and starter culture Nham. Starter cultured Nham contained higher amounts of lactic acid than natural Nham after 36 h and until the end of fermentation. At the 72 h of fermentation, the final lactic acid concentration was 2.1 and 2.4 g/100 g Nham in natural and starter cultured Nham, respectively. In addition, only small amount of acetic acid and pyruvic acid were found in both type of Nham fermentation (data not shown). The changes in pH coincided with the production of lactic acid during the fermentation. The increased in lactic acid was correlated with the changes in pH. This indicates that the production of lactic acid contributed to the decrease in pH.

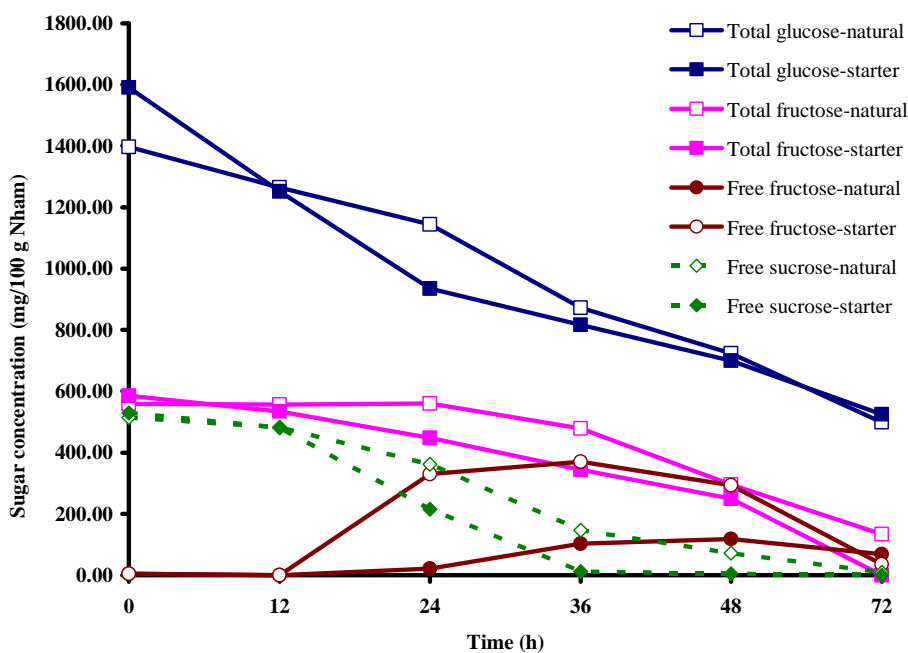


**Figure 8** Changes in lactic acid profiles during Nham fermentations.

### 1.3 Sugar concentration

The pattern of sugar concentration in both types of fermentation was similar. However, there are significantly different when comparing each type of sugar in more detail as described below (Figure 9).

The pattern of total glucose depletion was similar in both types of fermentation. From the start of fermentation, the total concentration of this sugar was continuously declined to a level of approximately 500 mg/100g Nham at 72 h of fermentation. However, total glucose depletion rate was faster in starter cultured Nham in the first 2 days.



**Figure 9** Changes in sugar profiles during Nham fermentations.

Total fructose concentration has been continuously depleted in both types of fermentation but with different rate. In natural fermentation, total fructose concentration remained constant during the first 24 h of fermentation before decreased to 133.91 mg/100g Nham at 72 h. In contrast, total fructose in starter

cultured Nham was continuously depleted with the greater rate than those observed in natural Nham. This residual sugar was not detected in starter cultured Nham at the end of the studying period.

The pattern of free fructose was similar in both types of Nham during the early phase (0-12) of fermentation but significantly different when the fermentation proceeded to 24 h and continued to differ until the end of fermentation. The starting fructose concentration in both types of fermentation was between 4.4 to 4.7 mg/ 100g Nham. In both types of Nham fermentation, fructose concentration was decline to a non-detectable level at 12 h of the fermentation. At 24 h of fermentation, the amount of free fructose abruptly increased to the high level at 330.3 mg/ 100g Nham in starter cultured Nham while only 20.9 mg/ 100g Nham was detected in natural fermentation. At the 36 h of fermentation, the concentration of fructose in starter cultured Nham was slightly increased up to the highest level at 370.1 mg/ 100g Nham. In contrast, natural Nham, the amount of fructose gradually increased to the high level at 103.1 mg/ 100g at the 48 h of fermentation. At the end of fermentation, the amount of fructose in the natural and starter cultured Nham were 68.5 and 40.0 mg/ 100g Nham, respectively.

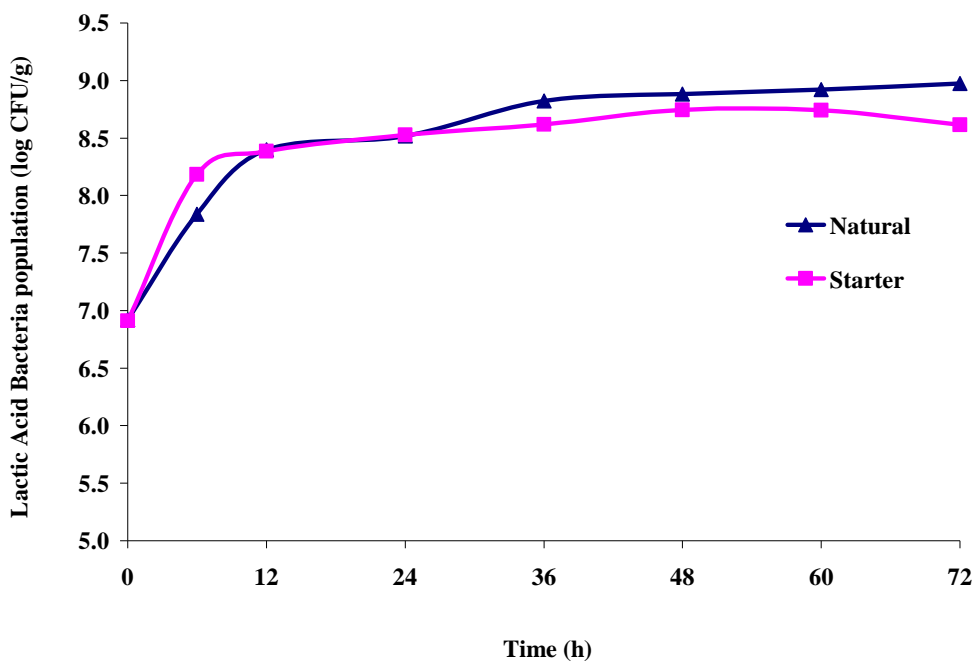
For free sucrose concentration, it was similar in both types of fermentation. There was a period of rapidly declined in sucrose level between 12-36 h followed by slightly decreased for the remaining of the fermentation. In addition, residual sucrose was not detected in starter cultured Nham after 3 days.

The free sugar availability in both types of Nham was relatively low (except sucrose) in the early part of fermentation. Thus total fructose and glucose concentration change was most likely come from bound sugar in garlic and cooked rice respectively. Thus sucrose and cooked rice were the most likely source of sugar used in both types of Nham fermentation. In starter culture inoculated Nham, there were a large increase in availability of free fructose after 24 h into the fermentation. This may be the cause of significantly lower pH of Nham with starter cultured Nham.

## 2. Microbiological characteristics of Nham

### 2.1 Lactic Acid Bacteria population (LAB)

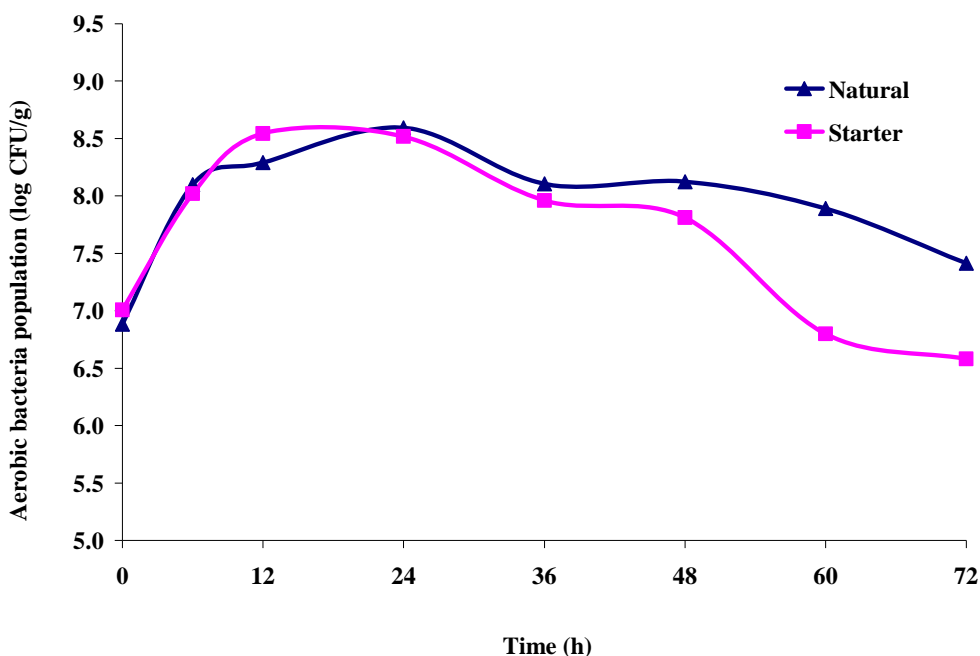
The total LAB counts were mainly similar in both types of Nham samples as shown in Figure 10. The initial population of LAB in both types of fermentation was approximately  $8.4 \times 10^6$  CFU/g. The LAB populations sharply increased at the initial stage to  $10^8$  CFU/g after 6 h and 12 h of fermentation in starter cultured and natural Nham, respectively. At the later stage of fermentation, the total number of LAB in both types of Nham was relatively stable or slightly increased up to 72 h of fermentation. At the end of fermentation, the numbers of LAB in the natural and starter cultured Nham were  $9.4 \times 10^8$  CFU/g and  $4.2 \times 10^8$  CFU/g, respectively. Starter cultured Nham has resulted in a more rapid increase of LAB cell counts than in the natural Nham at the early stage of fermentation. However, the counts of LAB in starter cultured Nham were slightly lower than in the natural Nham after 36 h of fermentation until the end of fermentation.



**Figure 10** Population of LAB during Nham fermentations.

## 2.2 Aerobic bacteria population

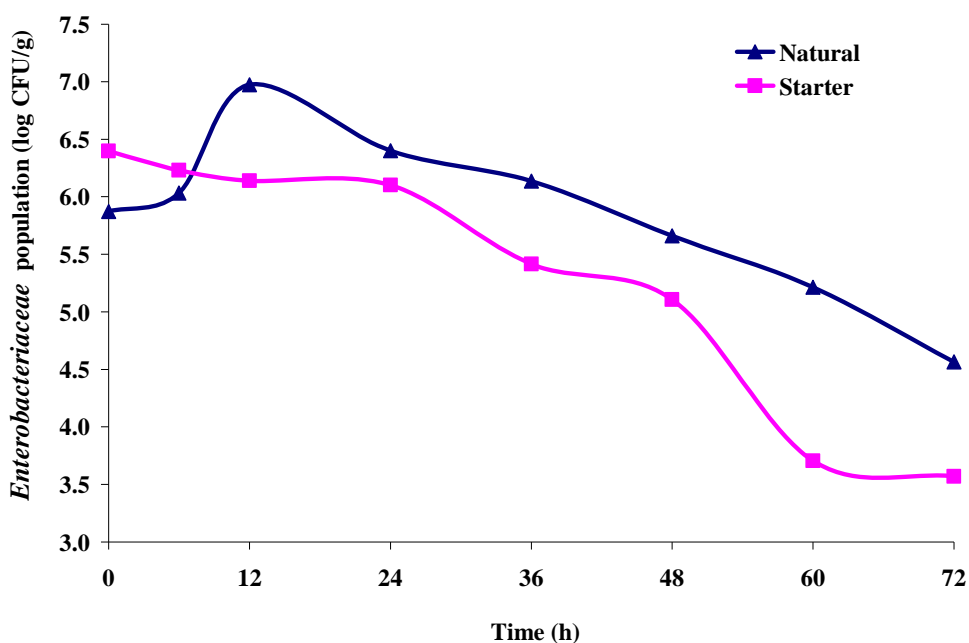
Aerobic plate count (APC) representing aerobic mesophile bacteria showed the different growth pattern from LAB population during Nham fermentation. However, the aerobic bacteria populations were mainly similar in both types of Nham fermentation. The aerobic bacteria counts were increased to the highest number at the early stage of the fermentation prior to being decreased toward the end of fermentation as shown in Figure 11. The initial population of aerobic bacteria in all was approximately  $10^7$  CFU/g. The number of aerobic bacteria increased to the highest number at 12 h and 24 h of fermentation in starter cultured Nham ( $3.5 \times 10^8$  CFU/g) and natural Nham ( $4.0 \times 10^8$  CFU/g), respectively. The total number of aerobic bacteria in both types of Nham fermentation tended to decreased toward the end of fermentation. At the end of fermentation, the counts of aerobic bacteria in starter cultured and natural Nham were  $3.8 \times 10^6$  CFU/g and  $2.7 \times 10^7$  CFU/g, respectively.



**Figure 11** Population of aerobic bacteria during Nham fermentations.

### 2.3 *Enterobacteriaceae* population

The patterns of population were different between natural and starter culture fermentation (Figure. 12). The initial number of *Enterobacteriaceae* was observed to be lower in natural Nham ( $7.5 \times 10^5$  CFU/g) than in starter cultured Nham ( $2.5 \times 10^6$ CFU/g). In natural fermentation, the *Enterobacteriaceae* population was sharply increased between 6 and 12 h of fermentation to the highest level of approximately  $9.6 \times 10^6$  CFU/g. The total number of *Enterobacteriaceae* decreased after 12 h and until the end of fermentation. On the other hand, the number of *Enterobacteriaceae* in starter cultured Nham gradually decrease toward the end of fermentation. At 72 h of fermentation, the final counts of *Enterobacteriaceae* in starter cultured and natural Nham were  $3.9 \times 10^3$  CFU/g and  $3.7 \times 10^4$  CFU/g, respectively. The counts of *Enterobacteriaceae* in starter cultured Nham were clearly lower than in natural Nham. This indicates that the suppression of *Enterobacteriaceae* was more effective using a starter culture.



**Figure 12** Population of *Enterobacteriaceae* during Nham fermentations.