CHAPTER 4

RESULTS AND DISCUSSION

In general, samples of noni fruit / juice were subjected to eight types of microbiological examination, including total plate count (TPC), an enumeration of yeasts and moulds, Gram negative bacteria, lactic acid bacteria, coliform bacteria (by a Most Probable Number (MPN) method), proteolytic bacteria, *Bacillus* spp. and *Clostridium* spp.

The purpose of TPC analysis is to count the aerobic mesophilic microorganisms in 1 ml substance. High TPCs give on indication of low environmental sanitation, low standard of processing and contamination of raw material during a preparation of noni juices and noni products. However, high TPCs do not have any correlation with the presence of pathogenic microorganisms (Collins *et al.*, 1989).

The enumeration of yeasts and moulds has the purpose to know the storage quality of noni products. The presence of certain moulds is also harmful since they produce metabolites that may cause intoxication to the customers (Jawetz, 1987).

Coliform bacteria measured by a MPN method used to estimate the number of coliforms in 1 ml substance. The presence of coliform bacteria, especially *Escherichia coli* indicates faecal contamination of man or animals in the product or raw materials (Collins *et al.,* 1989).

Gram negative bacteria are generally recognized as the causative agents of halmful diseases because most of Gram negative bacteria are pathogenic bacteria. Therefore, the enumeration of these bacteria was also conducted in noni products (Limyati and Juniar, 1998).

Lactic acid bacteria are typically characterized as Gram positive, non-spore forming, nonmotile, catalase negative bacteria growing under microaerobic or strickly anaerobic conditions. Some species produce lactic and acetic acids when they use glucose as a carbon source. The lactic acid bacteria of *Lactobacillus* spp. are claimed to provide a number of health benefits, including antimicrobial effects against pathogenic bacteria, anti-tumor effects and protection against antibiotic-associated diarrhea or food allergy (Limyati and Juniar, 1998). *Lactobacillus* spp. is reported to be acid bile tolerant and survive in the gastro-intestinal tract.

The presence or absence of proteolytic bacteria is checked, because its presence indicates contamination from soil, raw material or low hygiene of the producers during the preparation of the product (Limyati and Juniar, 1998).

Bacillus spp. and *Clostridium* spp. are spore-forming bacteria and some species are pathogenic bacteria. They produce spores as well as vegetative cells. The spores can survive adverse conditions such as drying and pasteurization. These organisms are common in the environment and in many foods. Some strains produce enterotoxins if allowed to grow, for example *Bacillus cereus*. The emetic toxin of *B. cereus* is particularly heat resistant (126°C for 90 min) whereas the diarrheagenic toxin is inactivated by exposure to 56°C for 30 min. High levels (>10^5 CFU/g) are necessary to produce toxin to cause illness. *Bacillus* spp. can also cause food spoilage and food poisoning (Roberts *et al.*, 1995).

Clostridium spp. is commonly found in mammalian feaces and soil. The spores persist in the environment and often contaminate raw food materials. Spores may survive cooking and rapid growth if the food is not chilled prompty. If the organisms are eaten in a large amount from a food, they can sporulate in the gastrointestinal tract and produce enterotoxin, which causes diarrhea (Bell *et al.*, 1997).

4.1 microbiological composition of fresh noni fruit noni juices and fermented noni juices

The microbiological composition of fresh noni fruit can be seen in Table 4.1. The data showed that fresh noni fruit contained coliform bacteria (75 MPN/g), and lactic acid bacteria (5.00 ± 0.05 log CFU/ml). The total viable count of the fruit was 5.52 ± 0.00 log CFU/ml. The high number of TPC could be due to the present of Gram negative bacteria (5.13 ± 0.02 log CFU/ml), yeasts and moulds (5.00 ± 0.05 log CFU/ml) and *Bacillus* spp. (4.50 ± 0.01 log CFU/ml). Other studies have reported a significant antimicrobial effect on different strains of bacteria, such as

Staphylococcus aureus, Pseudomonas aeruginosa, Proteus morgaii, Bacillus subtilis, E. coli, Helicobacter pylori, Salmonella spp. and *Shigella* spp. (Bushnell *et al.,* 1950; Atkinson, 1956; Dittmar, 1993). Another study showed that an acetonitrile extract of the dried fruit inhibited the growth of *P. aeruginosa, B. subtilis, E. coli* and *Streptococcus pyrogene* (Locher *et al.,* 1995). Furthermore, they showed that the anti microbial effect is highly dependent on the stage of ripeness and on processing, being greater when the fruit is ripe, without drying. Perry and Metzger (1980) mentioned that noni juice has some antibacterial activity besides other activities such as analgesic and anthelminthic.

The microbiological composition of a commercial fermented noni juice (Lookyohthammachart) showed high numbers of TPC, lactic acid bacteria, Gram negative bacteria and a count for yeasts and moulds (Table 4.1). At the same time, the juice had a low number of proteolytic bacteria. Coliform, *Bacillus* spp. and *Clostridium* spp. were not detected in the fermented noni juice samples, which could be due to a high number of lactic acid bacteria that can inhibit food spoilage and food poisoning bacteria such as *Bacillus cereus, Clostridium botulinum, Clostridium perfringens, S. aureus* and *Listeria monocytogenes* (O'Sullivan *et al.,* 2002). Lactic acid bacteria can also produce lactic and acetic acids including other acids such as formic acid, free fatty acid and other substances such as ammonia, ethanol, hydrogen peroxide, diacetyl, acetoin, acetaldehyde, benzoate, bacteriolytic enzyme, bacteriocin and unidentified products (Abee *et al.,* 1995; Adams, 1999). These products from lactic acid bacteria help to prevent the growth of food spoilage and pathogenic bacteria. The microbiological composition of a commercial noni juice (Siam Noni) showed that the juice had lower numbers of TPC, yeasts and moulds, Gram negative bacteria, lactic acid bacteria and proteolytic bacteria compared to those of fresh noni fruit and/or a fermented noni juice (Table 4.1). Coliform bacteria, *Bacillus* spp. and *Clostridium* spp. were also not being detected in the commercial noni juice. A different microbial result in the commercial noni juice could be due to a heat treatment that was employed to the product during the production process (Suprederm, 2003) and selection of the raw material.

Table 4.1 Microbiological composition in fresh noni fruit, noni juices and fermented noni juices

No. of microorganisms	Samples		
	Fresh noni fruit	Siam Noni	Lookyohthammachart
TPC (log CFU/ml)	5.52 ± 0.00[b]	2.81 ± 0.00[a]	6.10 ± 0.07[c]
Yeasts and moulds (log CFU/ml)	5.00 ± 0.05[b]	3.61 ± 0.02[a]	5.03 ± 0.01[b]
Gram negative bacteria (log CFU/ml)	5.13 ± 0.02[b]	3.22 ± 0.02[a]	6.05 ± 0.01[c]
Lactic acid bacteria (log CFU/ml)	2.89 ± 0.00[b]	2.52 ± 0.00[a]	5.87 ± 0.01[c]
Coliform (MPN/g)	75[b]	<3[a]	<3[a]
Proteolytic bacteria (log CFU/ml)	3.64 ± 0.02[b]	2.52 ± 0.01[a]	2.50 ± 0.01[a]
Bacillus spp. (log CFU/ml)	4.50 ± 0.01	ND	ND
Clostridium spp. (log CFU/ml)	2.69 ± 0.01	ND	ND

Different letter within a row showed significantly different (p≤ 0.05)

4.2 Chemical analysis in fresh noni fruit, noni juices and fermented noni juices

For the chemical analysis, samples of noni fruit, a commercial noni juice and a commercial fermented noni juice were analyzed for their pH values and total titratable acidity (TA). The chemical analysis result for fresh noni fruit, a commercial noni juice and a commercial fermented noni juice is shown in Table 4.2. It could be seen clearly from this Table that different noni products had significantly different pH values and TA ($p \leq 0.05$). The commercial fermented noni juice (Lookyohthammachart) sample significantly contained the highest TA value and the lowest pH value compared to those of the other noni products. This finding was mainly due to the production process of the fermented noni juice (Lookyohthammachart) that contained a processing step of fermentation in which microorganisms had a time period to produce lactic acid and other organic acids.

Table 4.2 Chemical analysis in fresh noni fruit, noni juices and fermented noni juices

Samples	Chemical analysis	
	Total acidity (g/100ml)	pH
Fresh noni fruit	0.95 ± 0.01^{a}	4.65 ± 0.00^{c}
Siam Noni	1.60 ± 0.01^{b}	4.44 ± 0.02^{b}
Lookyohthammachart	2.98 ± 0.00^{c}	3.54 ± 0.02^{a}

Different letter within a column showed significantly different ($p \leq 0.05$)

4.3 Microbiological composition in fermented noni juice during fermentation

To have a better understanding about the microbial composition of the commercial fermented noni juice and knowledge for changes in the microbial composition of fermented noni juices, a production of a fermented noni juice in a laboratorium-scale was conducted for 36 weeks. During the fermentation period, samples of fermented noni juices were collected on 0, 2, 4, 6, 8, 10, 12, 24 and 36 weeks to be analyzed for their microbiological composition. The result of this monitoring is displayed in Table 4.3. The Table showed clearly that the numbers of Gram negative bacteria, coliform, proteolytic bacteria, *Bacillus* spp. and *Clostridium* spp. were decreased significantly during 36 weeks fermentation period at room temperature. Coliform was the first bacteria group that was detected at the minimum detectable level of MPN methods on the 4^{th} week of the fermentation period. *Clostridium* spp., proteolytic bacteria, *Bacillus* spp. and Gram negative negative bacteria were not detected in noni juice samples after 8, 10, 10 and 36 weeks fermentation period, respectively, although these bacterial groups were found for more than 4.19 log CFU/ml at the beginning of the fermentation time. On the other hand, the counts of TPC and lactic acid bacteria were significantly increased during the fermentation period. An increase for up to 1.78 log CFU/ml for lactic acid bacteria within the first 2 weeks of the incubation period might be responsible for the increase in the TPC count at the same fermentation period. The increase in the lactic acid bacteria population might also partly responsible for the decrease in the numbers of other microorganisms, including Gram negative bacteria, coliform, proteolytic bacteria, *Bacillus* spp., *Clostridium* spp., yeasts and moulds. From the second week of the fermentation period onwards, lactic acid bacteria were the dominant microorganism in the fermented noni juice and reached a population of 8.42 ± 0.004 log CFU/ml at the end of the fermentation period.

Bacterial pathogens such as *Salmonella* spp., *E. coli* and *S. aureus* may occasionally occur in fruit juice, where they will have been derived from the orchard soil, farm and process equipment or human sources. However, the acidity of the product can prevent their growth and such microorganisms do not survive for long in the fermented product. Bacterial spores from *Bacillus* spp. and *Clostridium* spp. can survive for long period and are frequently found in fermented noni juice but no concern in relation to either spoilage or public health because of the low pH value of the product. However their presence may be indicative of poor plant hygiene (Limyati and Juniar, 1998).

One of the primary objectives of fermentation is a production of ethanol from simple sugars. Various intermediates in the Embden-Meyerhof-Parnass pathway can also be converted to from a diverse range of other metabolites, including glycerol (up to 0.5%). Diacetyl and acetaldehyde may also occur, particularly if the fermentation occurs in the presence of flavour-producing microorganism. Other metabolic pathways will operate simultaneously with the formation of long and short chain fatty acids. If other microorganisms are also present in the fermentation (e.g. lactic acid bacteria) these can convert the fruit acid malic and quinic acid to lactic and dihydroshikimic acids, respectively, thereby reducing the acidity of fermented noni juice (Anonymous, 2001).

The presence of yeasts and mould in the fermented noni juice was also affected by lactic acid bacteria, as shown in Table 4.3. A high number of yeasts and mould at a level of 6.12 ± 0.01 log CFU/ml at the beginning of the fermentation period was reduced for up to 1.24 log CFU/ml within 2 weeks period at room temperature. Although Jay (1996) had reported that lactic acid bacteria grow rapidly at pH around 4.0 and slow growth at pH below 3.6, yeasts and moulds are also microorganisms that can survive and grow at low pH values. Yeast has an optimum growth pH of 4.5-6.0 and fungi have an optimum growth pH of 3.5-4.0. This information supported the finding in this study that eventhough the number of yeasts and mould was declined during the fermentation period, the survival number of yeasts and mould in the fermented beverages, lactic acid bacteria such as *Lactobacillus* spp. and *Leuconotoc* spp. can grow and cause souring, turbidity and sometime slime production. If wild yeasts are present, the yeasts can cause spoilage in fermented beverages by producing unfinable turbidity, excess gas and turbidity. The wild yeast spoilage would be varied depending on the yeast type that was responsible for the spoilage. Some wild yeast could also produce high levels of C_4 to C_{10} fatty acid, such as isobutyrate, isovalerate, etc. Wild yeasts were able to grow during fermentation period and in the finished fermented beverages (Galland, 2003).

Table 4.3 Microbiological composition in fermented noni juice during fermentation period

Fermentation period (week)	Type of Microorganisms (log CFU/ml)							
	TPC (log CFU/ml)	Yeasts and moulds (log CFU/ml)	Gram negative bacteria (log CFU/ml)	Lactic acid bacteria (log CFU/ml)	Coliform (MPN/g)	Proteolytic bacteria (log CFU/ml)	*Bacillus* spp. (log CFU/ml)	*Clostridium* spp. (log CFU/ml)
0	6.05 ± 0.01^{a}	6.12 ± 0.01^{g}	6.20 ± 0.00^{a}	3.27 ± 0.00^{a}	75^{a}	4.50 ± 0.01^{a}	4.87 ± 0.00^{a}	4.19 ± 0.01^{a}
2	7.12 ± 0.01^{b}	4.88 ± 0.02^{e}	5.24 ± 0.00^{b}	5.06 ± 0.02^{b}	7^{b}	3.68 ± 0.01^{b}	4.14 ± 0.08^{b}	3.35 ± 0.00^{b}
4	7.31± 0.00^{c}	4.48 ± 0.02^{c}	4.45 ± 0.01^{c}	5.86 ± 0.00^{c}	<3^{c}	3.65 ± 0.00^{c}	3.34 ± 0.00^{c}	2.70 ± 0.00^{c}
6	7.04 ± 0.00^{c}	3.40 ± 0.02^{a}	4.39 ± 0.01^{d}	6.01 ± 0.04^{d}	<3^{c}	2.81± 0.00^{d}	2.82 ± 0.01^{d}	2.47 ± 0.00^{d}
8	7.94 ± 0.00^{d}	4.11 ± 0.05^{b}	4.19 ± 0.01^{e}	7.52 ± 0.00^{e}	<3^{c}	1.36 ± 0.02^{e}	1.21 ± 0.05^{e}	ND
10	7.99 ± 0.00^{d}	5.45 ± 0.01^{f}	3.82 ± 0.16^{f}	8.00 ± 0.00^{f}	<3^{c}	ND	ND	ND
12	8.00 ± 0.02^{d}	4.88 ± 0.03^{e}	3.35 ± 0.01^{g}	8.35 ± 0.00^{g}	<3^{c}	ND	ND	ND
24	7.51 ± 0.01^{e}	4.68 ± 0.19^{d}	2.81 ± 0.05^{h}	8.42 ± 0.00^{h}	<3^{c}	ND	ND	ND
36	7.46 ± 0.02^{e}	4.63 ± 0.19^{d}	ND	8.42 ± 0.00^{h}	<3^{c}	ND	ND	ND

Different letter within a column showed significantly different ($p \leq 0.05$)

4.4 Chemical analysis in fermented noni juices during fermentation period

Changes in the chemical properties of fermented noni juices during fermentation period are shown in Table 4.4. The collected data in Table 4.4 showed clearly that the acidity of noni juices was significantly increased ($p \leq 0.05$) during the fermentation period at room temperature. The TA of noni juices had a significant increase in the first 10 weeks fermentation period to reach a value of 3.18 ± 0.04 followed by a slight decrease afterwards. On the other hand, the pH values of fermented noni juices were significantly reduced within the first 10 weeks fermentation time and reached a value of 3.45 ± 0.03. After this fermentation period, the pH values of fermented noni juices were slightly increased confirming the result of the TA measurement. Development of acidity in fermented noni juices was mainly due to the growth of lactic acid bacteria, as was shown in Table 4.3.

Table 4.4 Chemical analysis in fermented noni juices during fermentation period

Fermentation period (Week)	Chemical analysis	
	Total acidity(g/100 ml)	pH
0	0.93 ± 0.01^{a}	4.91 ± 0.00^{a}
2	1.34 ± 0.10^{b}	4.76 ± 0.02^{b}
4	1.53 ± 0.05^{c}	4.51 ± 0.02^{c}
6	1.97 ± 0.11^{d}	4.25 ± 0.02^{d}
8	2.65 ± 0.03^{e}	3.85 ± 0.02^{e}
10	3.18 ± 0.04^{f}	3.45 ± 0.03^{f}
12	3.12 ± 0.04^{f}	3.48 ± 0.03^{f}
24	3.01 ± 0.02^{g}	3.53 ± 0.03^{g}
36	2.98 ± 0.02^{h}	3.53 ± 0.01^{g}

Different letter within a column showed significantly different ($p \leq 0.05$)

Lactic acid bacteria have been reported to produce lactic and organic acids (Jay, 1996). At the same time, the bacteria would grow slowly after the pH of fermented noni juices reached a value below 3.6 as was stated by Jay (1996). Therefore, after 10 weeks fermentation period, there was not any significant acidity development in fermented noni juices. From this finding, it could be summarized that during noni juice fermentation, lactic acid bacteria could grow and produce organic acids as the result of their metabolism (Jay, 1996). The presence of lactic acid in the fermented noni juices could inhibit both Gram positive and Gram negative bacteria growth by diffusing into microbial cells and its dissociation form of hydrogen ion (H^+) affected microbial cell metabolism. The increase in the level of lactic acid might not exclusively done by lactic acid only, since there was a possibility that the presence of yeast could also carry out sugar fermentation. References from other scientific studies have also shown that beside lactic acid, acetic acid was another major fatty acid of fermatation beverages, which could include fermented noni juices (Anonymous, 2001). The levels of acetic acid present in fermented beverages vary considerably, but its presence has a strong effect on total acidity, pH value, flavor and aroma of fermented beverage.

The formation of CO_2 in fermented beverages is one of the products from heterofermentation process by lactic acid bacteria. Where CO_2 can substitute oxygen content in fermentation, it will generate anaerobic condition that is undesirable for aerobic microorganisms especially moulds. In addition, CO_2 reduced both intracellular and surrounding pH value that could affect cell membrane to be destroyed. This condition could happen in the absence of sugar and other compounds utilized by yeasts and lactic acid bacteria (Anonymous, 2001). The low pH condition in a fermented food was sufficient to prevent the growth of food spoilage and pathogenic bacteria.

4.5 The effect of different heat treatments on the microbiological quality of noni juices

It is a common practice to do a heat treatment for fruit juices in order to extend their shelf lives. The literature review in Chapter 2 also displayed that the production of Siam noni used pasteurization as one of the processing steps (Suprederm, 2003). In this section, different heat treatment conditions, including pasteurization and boiling, were assessed for their effectiveness in reducing the microbial load of fresh noni juices. The collected data for the microbial composition of different heat-treated noni juices is displayed in Table 4.5. Data from this Table indicated that the intensity of different heat treatments was significantly affected the microbial composition of the heat-treated noni juices. All of the heat-treated juices contained coliform bacteria at the minimum detection level of the MPN method, which gave an assurance that the processing conditions used in this study would be sufficient to protect the consumer health from pathogen bacteria in the coliform group, especially *E. coli* (Srimuang, 2004). For other microorganism types, the result in Table 4.5 showed that the microbial composition of boiled noni juices was lower than those of the other 3 pasteurization processes, particularly for the TPC, Gram negative bacteria, lactic acid bacteria and *Bacillus* spp. In addition, the 3 pasteurized noni juices contained proteolytic bacteria at a level between 2.69-2.79 log CFU/ml. Higher microbial populations in the 3 pasteurized noni juices could be due to the intensity of the pasteurization treatment that was not severe enough to kill spore-forming bacteria. Spores of facultative or obligate anaerobic bacteria were reported to have higher heat resistant than those of the microbial spores of obligate aerobes (Srimuang, 2004).

The primary functions of pasteurization were destroyed microorganisms and deactived pectic enzyme. Organisms associated with the fruit and capable of growing in noni juices are primarily lactic acid and acetic acid bacteria, yeasts and moulds. Lactic acid bacteria grow rapidly in fruit juice especially *Lactobacillus* and *Leuconostoc* spp. that can grow well at temperature of 20-37°C but do not grow at temperature of 54°C or above. Harvested fruits may be invaded or contaminated by

Table 4.5 The microbiological quality of noni juices affected by different heat treatments

Type of Microorganisms	Heat treatments			
	64°C 15 min	72°C 1 min	80°C 15 s	100°C 10 min
TPC (log CFU/ml)	2.83 ± 0.02^{ns}	2.72 ± 0.00^{ns}	2.90 ± 0.02^{ns}	2.26 ± 0.21^{ns}
Yeasts& Moulds (log CFU/ml)	2.51 ± 0.02^{a}	2.67 ± 0.01^{b}	2.81 ± 0.01^{c}	3.22 ± 0.03^{d}
Gram negative bacteria (log CFU/ml)	2.58 ± 0.03^{b}	3.20 ± 0.01^{d}	2.86 ± 0.01^{c}	2.48 ± 0.01^{a}
Lactic acid bacteria (log CFU/ml)	2.63 ± 0.00^{b}	3.41 ± 0.02^{d}	3.21 ± 0.01^{c}	2.47 ± 0.01^{a}
Coliform (MPN/g)	<3	<3	<3	<3
Proteolytic bacteria (log CFU/ml)	2.77 ± 0.02^{ns}	2.69 ± 0.02^{ns}	2.79 ± 0.02^{ns}	ND
Bacillus spp. (log CFU/ml)	3.36 ± 0.06^{c}	3.27 ± 0.05^{bc}	3.20 ± 0.01^{b}	2.65 ± 0.01^{a}
Clostridium spp. (log CFU/ml)	3.58 ± 0.00^{d}	3.48 ± 0.01^{c}	2.77 ± 0.01^{a}	3.36 ± 0.01^{b}

Different letter within a row showed significantly different ($p \leq 0.05$)

yeasts and moulds. Moulds are of concern only when fruit handling and juice equipment room are not kept in a sanitary condition. Effective culling out of moulds, rotten fruit helps to minimize the introduction of these organisms into the plant and into the processed juice. It is important to recognize that the optimum pasteurizing temperature used in any process is one which reduces microorganisms and deactivates detrimental enzymes without adversely affecting the taste, quality and nutritional value of the juice (Jay, 1996).

4.6 The effect of different storage conditions on the microbiological quality of noni juices

The microbiological quality of heat-treated noni juices was also monitored during storage at 4°C and room temperature for 21 days. The monitoring results for heat-treated noni juices processed at 64°C for 15 min, at 72°C for 1 min, at 80°C for 15 s and at 100°C for 10 min stored at 4°C are shown in Figures 4.1, 4.2, 4.3 and 4.4, respectively.

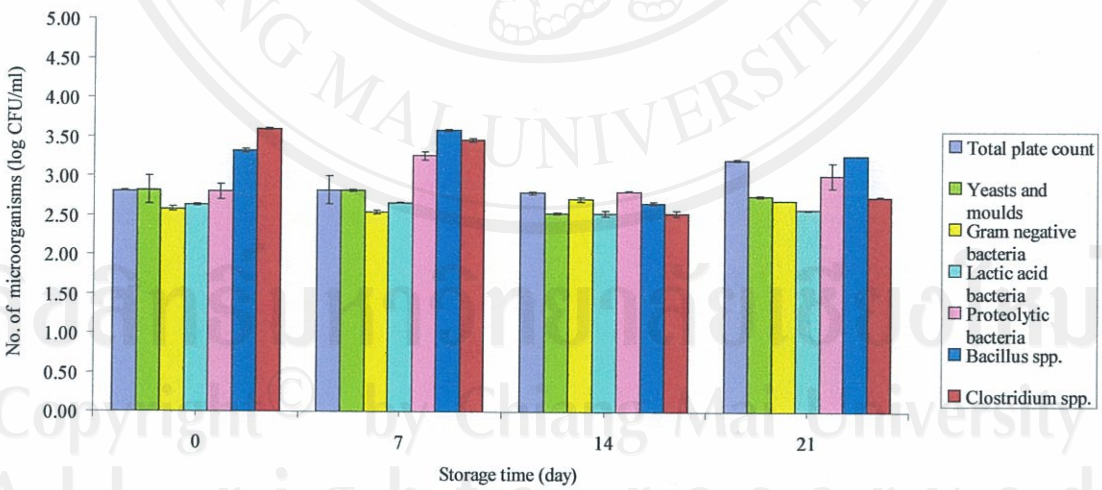

Figure 4.1 The microbiological quality of pasteurized noni juices at 64°C for 15 min and stored at 4°C for 21 days

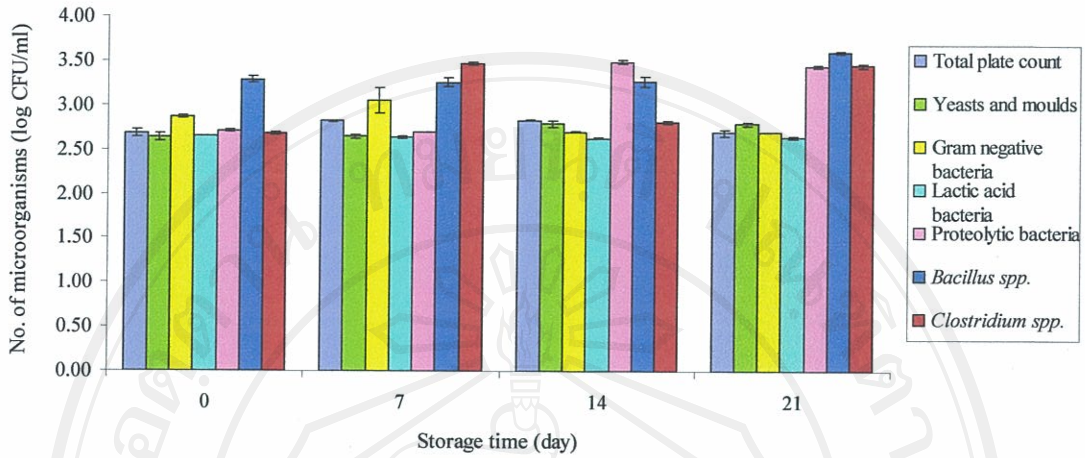

Figure 4.2 The microbiological quality of pasteurized noni juices at 72°C for 1 min and stored at 4°C for 21 days

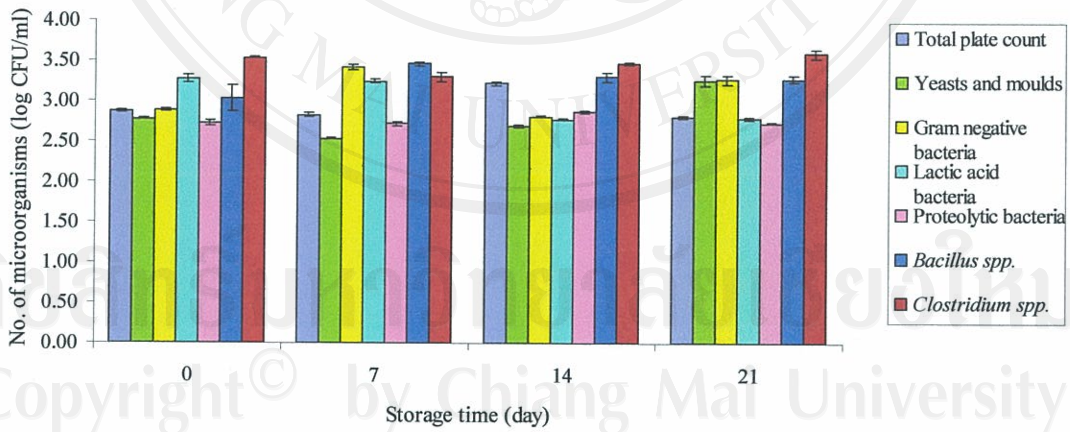

Figure 4.3 The microbiological quality of pasteurized noni juices at 80°C for 15 s and stored at 4°C for 21 days

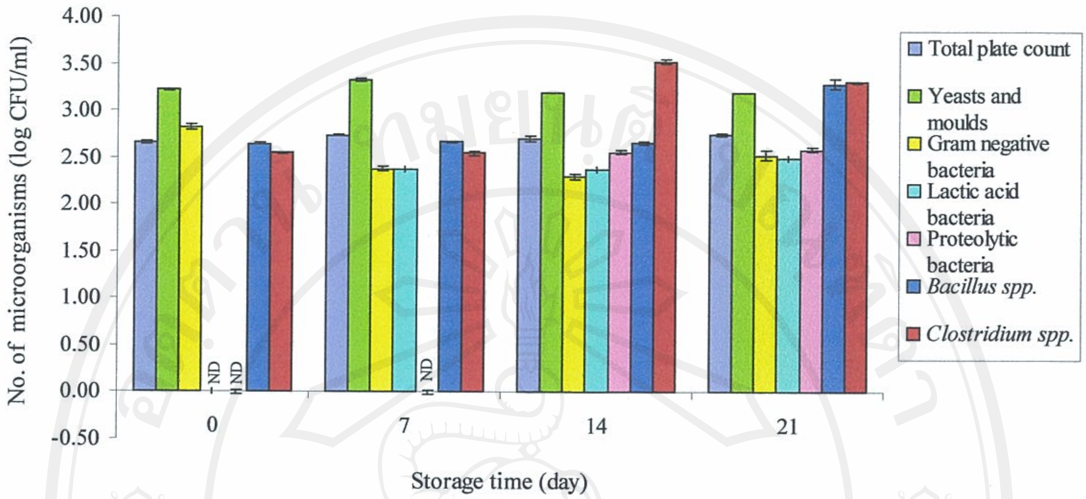

Figure 4.4 The microbiological quality of boiled noni juices at 100°C for 10 min and stored at 4°C for 21 days

In general, the microbiological composition of the heat-treated noni juices in this section was almost similar to the previous data in Table 4.5, except for the lactic acid bacteria in the noni juices heated at 100°C for 10 min. In this section, these noni juice samples did not contain any lactic acid bacteria, which might be due to different initial microbial loads of noni fruit. For coliform bacteria, the analysis result for different heat-treated noni juices showed that the bacteria was at the minimum detection level of the MPN method (< 3 MPN/g) directly after the heat treatments and throughout the storage period. This finding confirmed the safety of the heat-treated noni juices kept at 4°C that would not support the growth of coliform bacteria.

The monitoring results of heat-treated noni juices kept at 4°C generally displayed that all of the microorganism groups tested in this study could only grow at a slow rate during 21 days storage. Most of the microbial populations were only increased up to 0.50 log CFU/ml. However, proteolytic bacteria and *Clostridium* spp. in noni juices treated at 72°C for 1 min and *Bacillus* spp. and *Clostridium* spp. in noni juices heated at 100°C for 100 min could increase up to 1.0 log CFU/ml during the storage time. It is also interesting to note that eventhough lactic acid bacteria and proteolytic bacteria were not been detected in the noni juices heated at 100°C for 10 min directly after the heat treatment, both microorganism groups were grown up to 2.59 log CFU/ml during the storage period, which was a level that almost similar to the other pasteurized noni juices. This finding indicated that heat-injured bacteria might not be easily grown in microbiological media directly after a heat treatment process. A short storage period might be useful in giving a correct microbial load in a heat-treated food sample (Srimuang, 2004). Pasteurization process is primarily designed to eliminate or reduce the number of microorganisms to an acceptable level and provide conditions that limit the growth of pathogenic and spoilage microorganisms. At the same time pasteurization treatments rely on storage under refrigerated conditions for a specified maximum period. These combination treatments are intended to prolong the shelf life of fruit juices. Because of refrigeration greatly slows microbial growth and reproduction, but does not efficience to kill the spore formers. Furthermore, the spores of facultative or obligate anaerobes are more resistant to severe condition than the microbial spores of obligate aerobes. Fortunately most pathogens are mesophilic and do not grow well at temperature around 4°C. Refrigerated items may be ruined by growth of psychrophilic and psychrotrophic organisms, particularly if water is present. Thus refrigeration is a good technique only for shorter-term storage of pasteurized fruit juices (Srimuang, 2004).

Besides a storage temperature of 4°C, this section also investigated storage of heat treated noni juices at room temperature for 21 days. The monitoring results for microbial population in heat-treated noni juices stored at room temperature are exhibited in Figures 4.5-4.8 and Table 4.6. Comparing the microbial results of heat-treated noni juices stored at room temperature to those stored at 4°C, the data clearly displayed that room temperature storage significantly supported the growth of all microorganisms in heat-treated noni juices. The high numbers of TPC found in the heat-treated noni juices were mainly due to a significant increase in yeasts and moulds counts, Gram negative bacteria, lactic acid bacteria, proteolytic bacteria, *Bacillus* spp. and *Clostridium* spp., which could increase for up to 3.0 to 5.0 log CFU/ml. However, a lower increase in the lactic acid bacteria population (only up to 1.14 log CFU/ml) in the noni juices treated at 64°C for 15 min and at 80°C for 15 s should be noted. This finding indicated that the microbial population of heat-treated noni juices kept at room temperature storage would not be dominated by lactic acid bacteria. The heat-injured lactic acid bacteria might need a longer adaptation period than other microorganism groups. Causing the bacteria not to be a major bacteria in the juices. Results in this section clearly demonstrated that pasteurization is not a severe heat treatment to kill all microorganisms in fruit juices, particularly spore-forming bacteria (Srimuang, 2004). In addition, the spores of facultative or obligate anaerobes are more heat resistant than the microbial spores of obligate aerobes (Jay, 1996). Keeping pasteurized noni juices without any additional preservation method at room temperature would support the growth of all types of microorganisms in the juices, including pathogen and spoilage microorganisms.

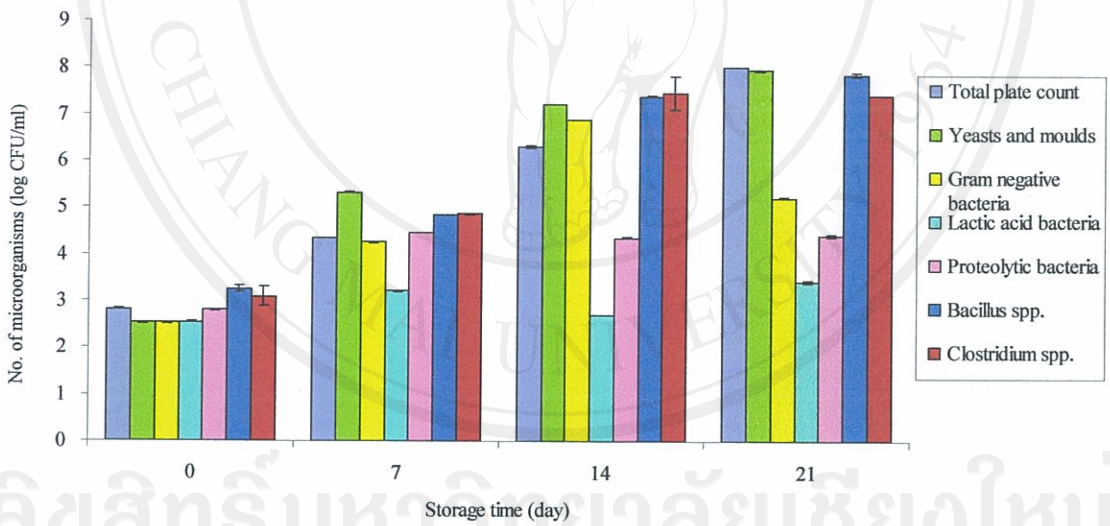

Figure 4.5 The microbiological quality of pasteurized noni juices at 64°C for 15 min and stored at room temperature for 21 days

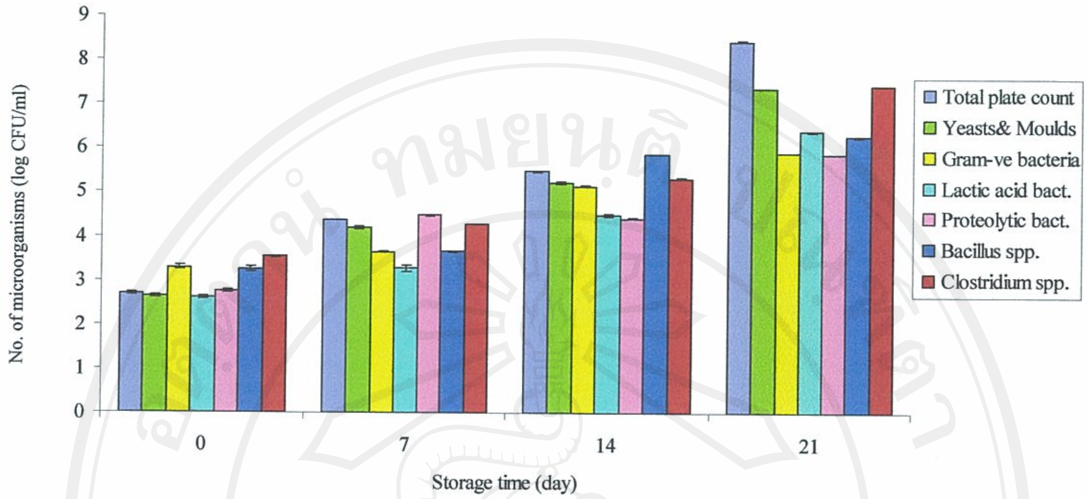

Figure 4.6 The microbiological quality of pasteurized noni juices at 72°C for 1 min and stored at room temperature for 21 days

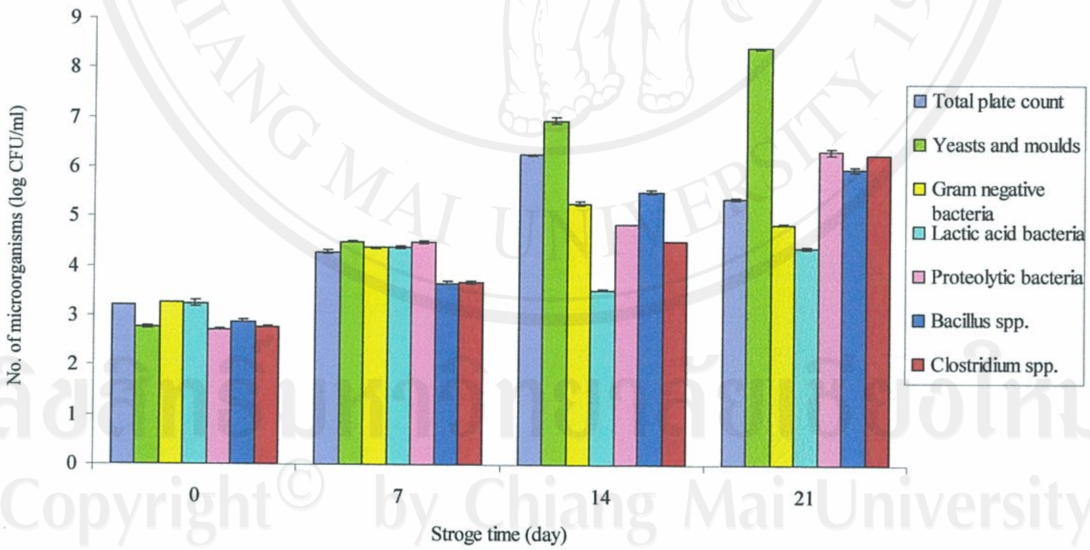

Figure 4.7 The microbiological quality of pasteurized noni juices at 80°C for 15 s and stored at room temperature for 21 days

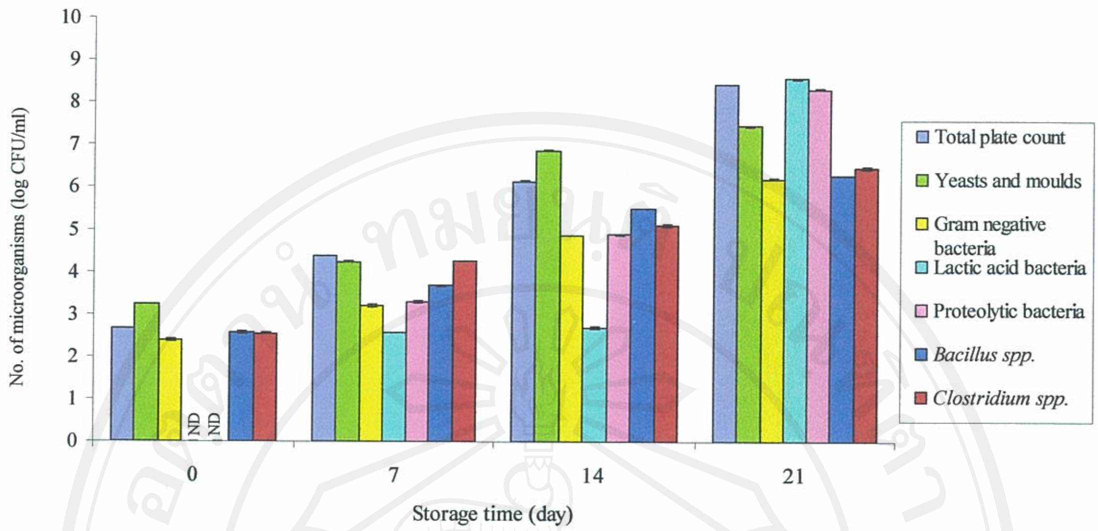

Figure 4.8 The microbiological quality of boiled noni juices at 100°C for 10 min and stored at room temperature for 21 days

Table 4.6 Coliform bacteria (MPN/g) of heat-treated noni juices stored at room temperature for 21 days

Heat treatments	Storage time (day)			
	0	7	14	21
at 64°C for 15 min	< 3[a]	14[b]	75[c]	150[d]
at 72°C for 1 min	< 3[a]	15[b]	43[c]	150[d]
at 80°C for 15 s	< 3[a]	14[b]	75[c]	210[d]
at 100°C for 10 min	< 3[a]	14[b]	75[c]	210[d]

Different letter within a row showed significantly different ($p \leq 0.05$)

It is interesting to note for the growth of lactic acid bacteria and proteolytic bacteria in the boiled noni juices kept at room temperature. Although these microorganism groups were not detected in the noni juices directly after boiling

(Figure 4.8), both microorganism groups were grown for more than 8.32 log CFU/ml at the end of the storage period. A higher increase of more than 2.19 and 1.99 log CFU/ml for lactic acid bacteria and proteolytic bacteria, respectively, in the boiled noni juices compared to those of the other pasteurized noni juices indicated that the intensity of heat treatments had a significant effect on the microorganism growth in the subsequent storage period.