CHAPTER 8

SURVIVAL OF *B. licheniformis* IN NISIN ADDED PASTEURIZED IMITATED MILK SYSTEM AS AFFECTED BY PASTEURIZATION CONDITIONS AND STORAGE TEMPERATURES

8.1 Introduction

Bacillus spp. have characteristics of Gram positive rods, grow aerobically and produce spores. They are widely spreaded in soil and water. Most of the species are motile, some species are strictly aerobic, while others are facultative anaerobic. A few species of *Bacillus* spp. can cause disease. For example, a previous report presented an outbreak of *Bacillus* spp. food poisoning in a sanatorium with patients developed symptoms of profuse diarrhea, stomach cramps and vomiting (Singleton and Sainsbury, 1981; Adams and Moss, 1995).

Since *B. cereus* is ubiquitous in nature, it can predominantly present in milk and dairy products. In fact, spore forming bacteria that spoil dairy products usually originate from raw milk. The defect of raw milk does not correlate with the initial numbers of spore formers. The reason for this is because storage conditions of milk products can support the organisms growth. If a milk product is stored for a long period of time, the small numbers of initial microorganisms can grow and eventually cause a defect (Frank, 1997). In raw milk, *B. cereus* was more commonly found during summer months and was not detected in some winter months (Crielly *et al*., 1994; Larsen and Jorgensen, 1997).

The other *Bacillus* spp. that is important in milk and milk products is *B. licheniformis*. Both of *B. cereus* and *B. licheniformis* are capable to form endospores that are highly resistant to heat and drying. Janstova and Lukasova (2001) also found that *B. licheniformis* was the greatest heat resistance as compared to the other *Bacillus* species. The spore of this *Bacillus* can germinate after a heat treatment at 135°C. Therefore, the presence of *B. cereus* and *B. licheniformis* in raw milk becomes an important factor for the milk industries, especially when both of them can survive pasteurization. *B. licheniformis* can be found everywhere in the farm environment. It was reported that the count of the organism was higher during the

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winter months. Beside that, *B. licheniformis* can also be found in the laboratory raw milk that has been heat-treated at 80°C for 10 min. For the growth kinetics of *B. cereus* and *B. licheniformis*, it was shown that *B. cereus* grew faster than *B. licheniformis* at ambient temperatures (Crielly *et al*., 1994).

Nisin mainly has an activity against Gram positive bacteria. Several reviews have been published recording its antimicrobial potential (Henning *et al.*, 1986; Breukink and de Kruijff, 1999; Thomas *et al.*, 2000; Cleveland *et al.*, 2001; Wirjantoro *et al.*, 2001).

In this section of the study, experimental work was carried out to determine the effect of pasteurization conditions and storage temperatures on the effectiveness of nisin against different bacteria forms of *B. licheniformis* in the IMS solutions. During a storage period at 4 and 10ºC, routine analysis for the microbial properties, including Total Viable Microorganisms, spore count and thermoduric count and chemical characteristics, mainly pH value and total acidity of the IMS solutions were done regularly.

8.2 Materials and methods

8.2.1 Holding temperature of pasteurization

IMS solution in this section was prepared using 2% (w/v) milk fat and 1% WPI and made according to the method in the section 4.2. This IMS solution was divided into 2 batches. Into one IMS batch, 100 IU/ml nisin and a spore suspension of 3.71±0.02 log cfu/ml *B. licheniformis* were aseptically added and mixed throughly. The nisin compound was incorporated into the IMS solutions 30 min before pasteurization. For the other IMS batch, the milk solution was only aseptically inoculated with *B. licheniformis* using a same spore suspension and served as a control. This control IMS solution passed a pasteurization process at 72±1ºC for 15 s, whereas the nisin added IMS solution was further divided into 5 small batches and processed at 72±1, 78±1, 80±1, 85±1 and 90±1ºC using one holding time of 15 s. All of the heat treated IMS solutions were immediately cooled down using a running cold water after the pasteurization treatment and stored at 4 and 10ºC. Each of the IMS treatment was done in triplicate.

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8.2.2 Holding time of pasteurization

A similar procedure as in the section 8.2.1 was done. However, the nisin added IMS solution was divided into 3 small batches before being pasteurized at 72±1ºC using holding times for 15, 20 and 25 s. The initial spore suspension of *B. licheniformis* used in this subsection was 3.65±0.02 log cfu/ml. Each of the IMS treatment was carried out in triplicate.

8.2.3 Microbiological analysis

8.2.3.1 Total viable microorganisms

A similar method as in the section 4.2.5.

8.2.3.2 Spore count

A similar microbiological method as in the section 4.2.5.

8.2.3.3 Thermoduric bacteria

A similar procedure as in the section 4.2.5.

8.2.4 Chemical analysis

8.2.4.1 Total acidity measurement

A similar method as in the section 4.2.4.

8.2.4.2 pH measurement

A similar procedure as in the section 4.2.4.

8.2.5 Nisin assay

The assay was carried out according to the method in the section 3.2.6.

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8.2.6 Statistical analysis

Data of this section was divided into 2 parts based on the experiments of holding temperature and holding time of pasteurization. Results of the holding temperature of pasteurization were statistically analyzed using a 6x2 Factorial in Completely Randomized Design. The first factor was pasteurization temperatures, which were control at 72ºC, nisin added IMS solutions at 72, 78, 80, 85 and 90°C. The second factor was storage temperatures, including at 4 and 10°C. DMRT was then used to determine differences between treatment means. The analysis was carried

out using a SPSS program (SPSS version 10.0) (SPSS Inc., Chicago, USA).

Results of the holding time of pasteurization were analyzed statistically using a 4x2 Factorial Experiment in Completely Randomized Design. The first factor was holding time, including control for 15 s and nisin added IMS solutions for 15, 20 and 25 s. The second factor was storage temperatures, which were at 4 and 10°C. If F value was significant, DMRT was used to determine differences between treatment means by using a SPSS program (SPSS version 10.0).

8.3 Result and discussion

8.3.1 The effect of holding temperature of pasteurization on the effectiveness of nisin to inhibit *B. licheniformis* in the IMS solutions

8.3.1.1 Chemical composition of IMS solutions used in the different holding temperatures of pasteurization experiment

The IMS solutions used in this section were made only from 2% (w/v) fat and 1% (w/v) WPI, since the presence of lactose, sucrose and casein did not produce a positive effect on the activity of nisin against bacilli population (Chapters 4-6). A 100 IU/ml nisin was also incorporated into the nisin added IMS solutions. The composition of the control IMS solution was 2.09±0.01% fat, 1.25±0.01% protein and 0.19±0.01% carbohydrate. This solution had 3.53±0.01% total solid and 1.53±0.01% solid not fat (Figure 8.1). For the chemical composition of the nisin added IMS solutions, it was found that the composition of the solutions was not significantly different than that of the control treatment suggesting that 100 IU/ml nisin did not affect the chemical composition of the IMS solutions.

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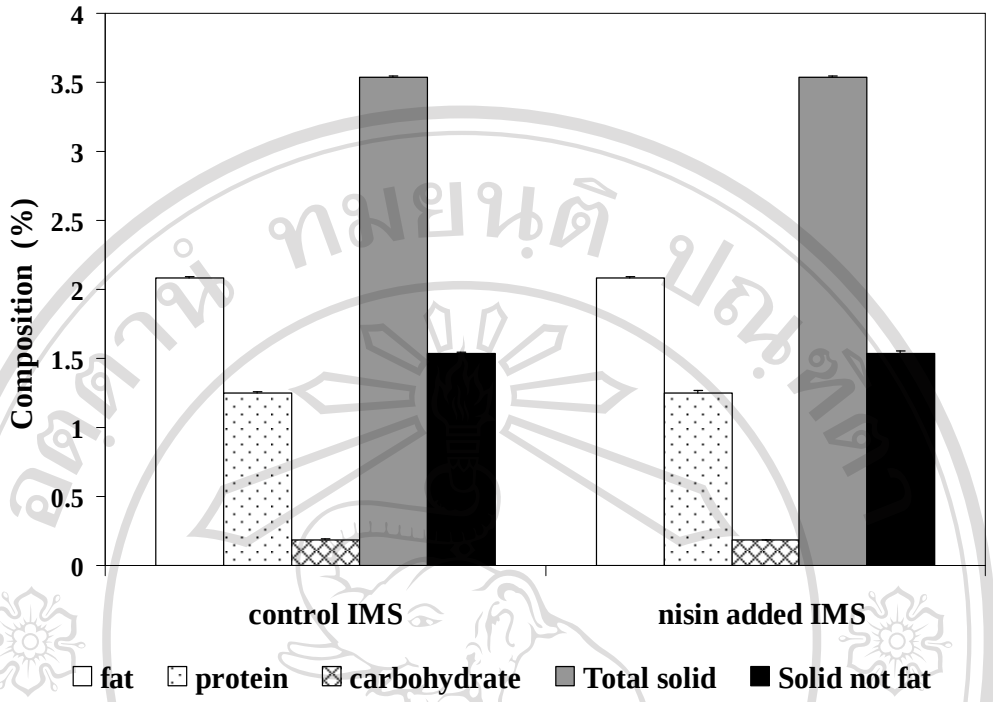

Figure 8.1 Composition of IMS solutions with and without 100 IU/ml nisin.

8.3.1.2 *TVM count*

The content of *Bacillus* in raw milk was generally lower than $5.0x10^3$ /ml (Brammley and McKinmon, 1990). However in this research, a spore suspension of *B. licheniformis* in an order of 3.0 log cycle was applied in most of the experimental work to take into an account a high number of *Bacillus* spp. in raw milk that has been reported to be affected by the seasons of the year (Criely *et al*., 1994; Larsen and Jorgensen, 1997). In this section, the studied population of *B. licheniformis* was in an order of 3.71±0.02 log cfu/ml. This microbial population was reduced to be 2.06±0.11 log cfu/ml directly after a pasteurization process at 72ºC for 15 s (Figure 8.2). The presence of nisin could reduce further the bacilli population. The reduction of the bacilli population was also higher at higher pasteurization temperatures. However, it was only the nisin added IMS samples heated at 85 and 90ºC for 15 s that contained significantly ($P \leq 0.05$) lower bacilli population compared to that of the control IMS treatment (no nisin addition) processed at a minimum pasteurization condition. Although results in this section did not find a significant reduction of the

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B. licheniformis population in the presence of nisin after the heat treatment at the normal pasteurization condition, the combination of nisin and higher pasteurization temperatures produced a synergistic effect, particularly at holding temperatures of 85 and 90ºC.

Applying storage temperatures of 4 and 10ºC brought a significant ($P \leq 0.05$) effect on the growth of TVM in the IMS solutions (Figure 8.3). A significant ($P \leq 0.05$) higher increasing number of the TVM count in the control IMS solutions compared to those of the nisin added IMS solutions was found after 3 days of storage. At the same storage time, a higher and significant ($P \leq 0.05$) increase of 0.56 log cycle of the TVM count was found in the control IMS solutions stored at 10ºC compared to that of the control IMS treatments stored at lower storage temperature. The increase in the TVM number of the control IMS solutions continued throughout the studied storage period. The bacilli population in the control IMS treatments stored at 4 and 10ºC increased for up to 3.26 and 4.17 log cycle, respectively after 21 days of storage showing the importance of storage temperature in controlling the microbial growth in pasteurized milk products.

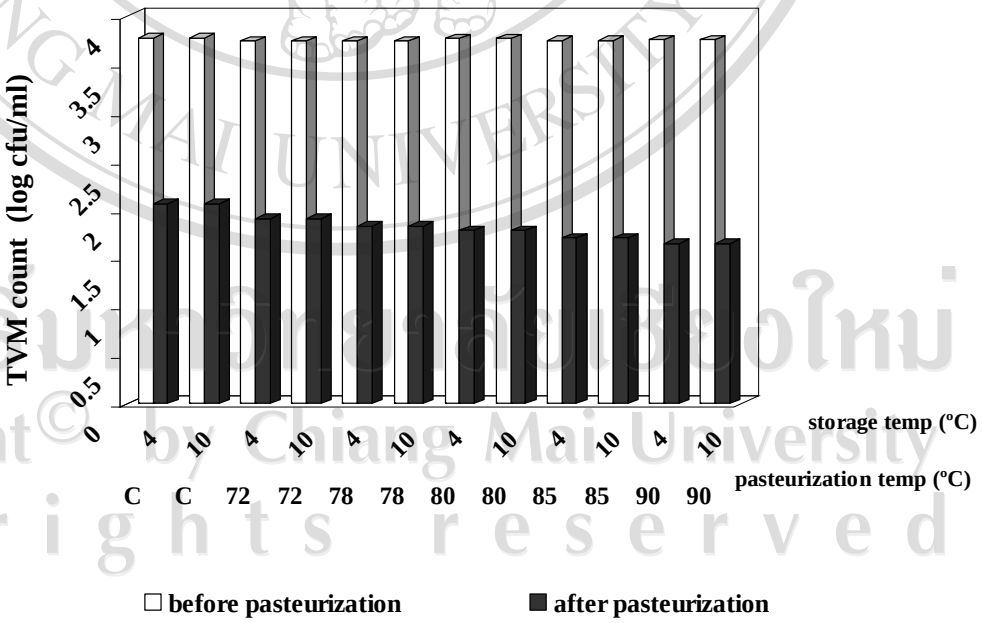

Figure 8.2 Total Viable Microorganism of the IMS solutions directly before and after pasteurization heated at different holding temperatures for 15 s.

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For the nisin added IMS solutions, the TVM count in these solutions increased at a much slower rate than that of the control IMS treatment (Figure 8.3). Increasing the bacilli population in the nisin added IMS treatment was significantly ($P \leq 0.05$) affected by the holding temperatures of pasteurization, storage temperatures and storage period. The holding temperatures of the pasteurization were mainly affected the initial number of the *B. licheniformis* population at the beginning of the storage period, whereas higher storage temperatures produced higher increasing rates of the bacilli population and was significantly different ($P \leq 0.05$) after 17 days of storage. Therefore, it was recorded that the nisin added IMS treatments processed at 72ºC for 15 s and stored at 10ºC had significantly ($P \leq 0.05$) higher number of TVM enumeration than those of the other nisin added IMS solutions from 3 days of storage onward. On the other hand, the nisin added IMS treatments heated at 90ºC for 15 s and stored at 4ºC had the lowest number of the TVM enumeration throughout the storage period.

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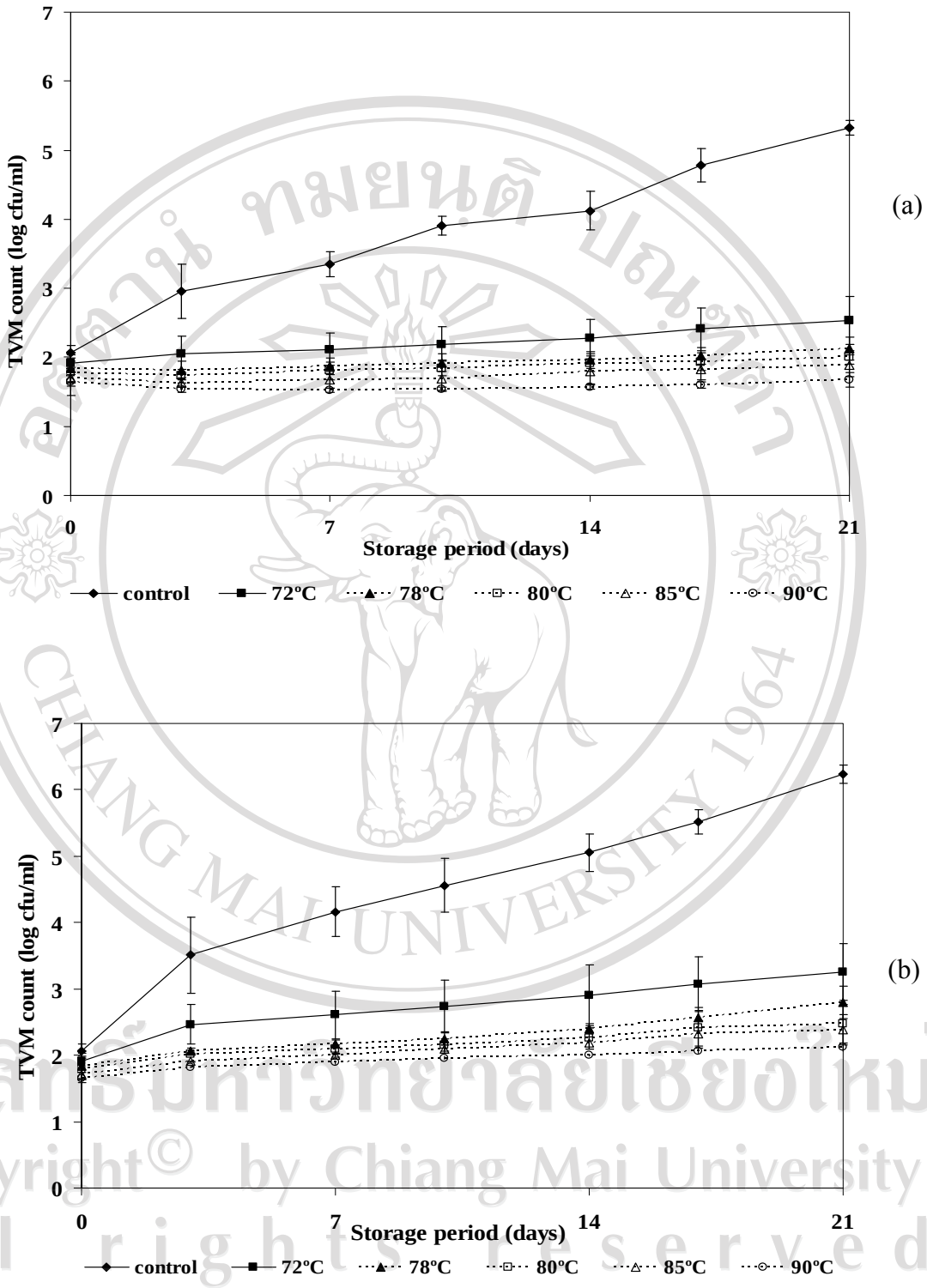

Figure 8.3 Total Viable Microorganism of IMS solutions with and without 100 IU/ml nisin and heated at different holding temperatures for 15 s during storage at 4ºC (a) and 10ºC (b).

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8.3.1.3 Spore count

Similar to the TVM finding, the result of the spore count demonstrated that nisin and holding temperatures of the pasteurization process worked synergistically in reducing the bacilli spore form in the IMS solutions. In the presence of nisin and higher holding temperatures, lower initial spore counts of the IMS treatments after the pasteurization treatment were found (Figure 8.4). The nisin added IMS solutions processed at 85 and 90ºC for 15 s had significantly ($P \leq 0.05$) lower spore counts compared to that of the control IMS treatment.

During 21 days storage, the spore count in the control IMS solutions had a continuous increase, reaching microbial populations of 2.41±0.16 and 2.94±0.48 log cfu/ml for the control IMS samples stored at 4 and 10ºC, respectively, at the end of the storage period from the initial spore count of 1.71±0.16 log cfu/ml. The storage temperature did not significantly ($P > 0.05$) affect the increase in the spore count of the control IMS solutions, even though at higher storage temperature, a double increase in the spore number was recorded.

For the nisin added IMS solutions, the spore count of the samples was significantly ($P \leq 0.05$) lower than that of the control IMS treatments after 3 days of storage. Changing in the spore count of the nisin added IMS solutions was also differed compared to that of the control IMS solutions and was more affected by the storage temperatures rather than by the holding temperatures of pasteurization. At 4ºC, the spore of the nisin added IMS solutions experienced a slow reduction throughout the storage period. Whereas at 10ºC, the spore of the nisin added IMS solutions was reduced in the first 3 to 7 days of storage before became more stable until at the end of the storage period. Different holding temperatures of pasteurization were mainly affected the initial spore population of the nisin added IMS solution at the beginning of the storage period. Finding in this section displayed that the activity of nisin against bacilli spore form was not only effective during pasteurization by producing lower initial spore count, but also during storage by maintaining low numbers of spore even reducing it when was combined with a storage temperature of 4ºC. For the last effect, it would also be affected by a constant presence of nisin in the IMS solutions (Figure 8.8).

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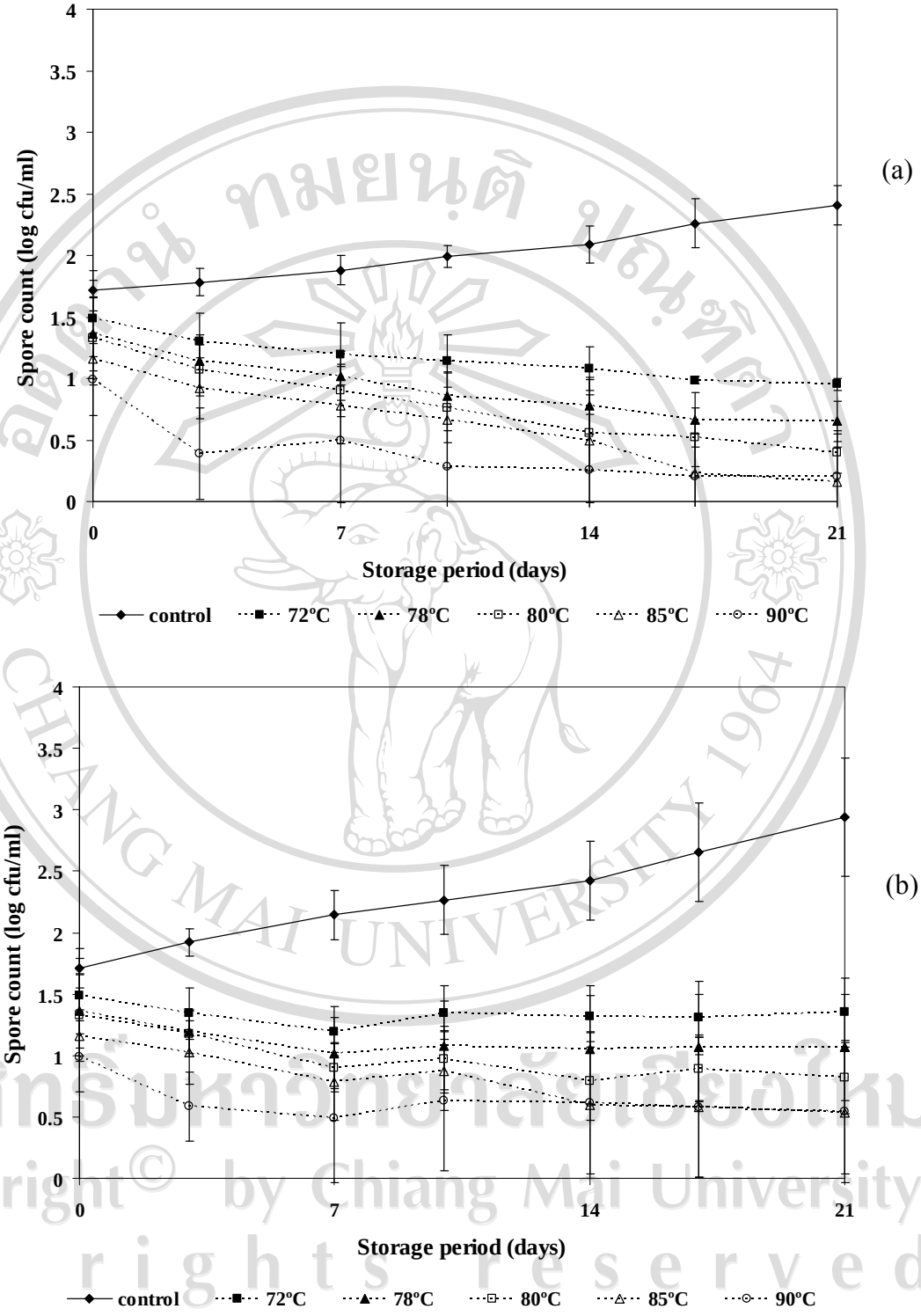

Figure 8.4 Spore count of IMS solutions with and without 100 IU/ml nisin and heated at different holding temperatures for 15 s during storage at 4ºC (a) and 10ºC (b).

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8.3.1.4 Thermoduric count

Changing in the thermoduric count of different IMS solutions during storage was in the same trend as in the TVM measurement (Figure 8.3) although occurred at lower numbers of microbial populations (Figure 8.5). The effectiveness of nisin against thermoduric bacilli in the IMS solutions was demonstrated during pasteurization at higher holding temperatures and during storage by producing a slow increase in the thermoduric population. The storage temperatures produced a significant ($P \leq 0.05$) effect on the thermoduric population of the control IMS treatment and the nisin added IMS solution processed at 72ºC for 15 s after 3 days of storage. Applying higher holding temperatures of pasteurization reduced the effect of storage temperatures on the growth of the thermoduric bacilli in the nisin added IMS treatments. Different holding temperatures of pasteurization mainly affected the initial thermoduric count in the IMS solutions after the pasteurization treatment. Finding in this section suggested that the growth of the survival thermoduric bacilli in the pasteurized IMS solutions was more influenced by the presence of nisin and storage temperatures compared to the holding temperatures of the pasteurization treatment.

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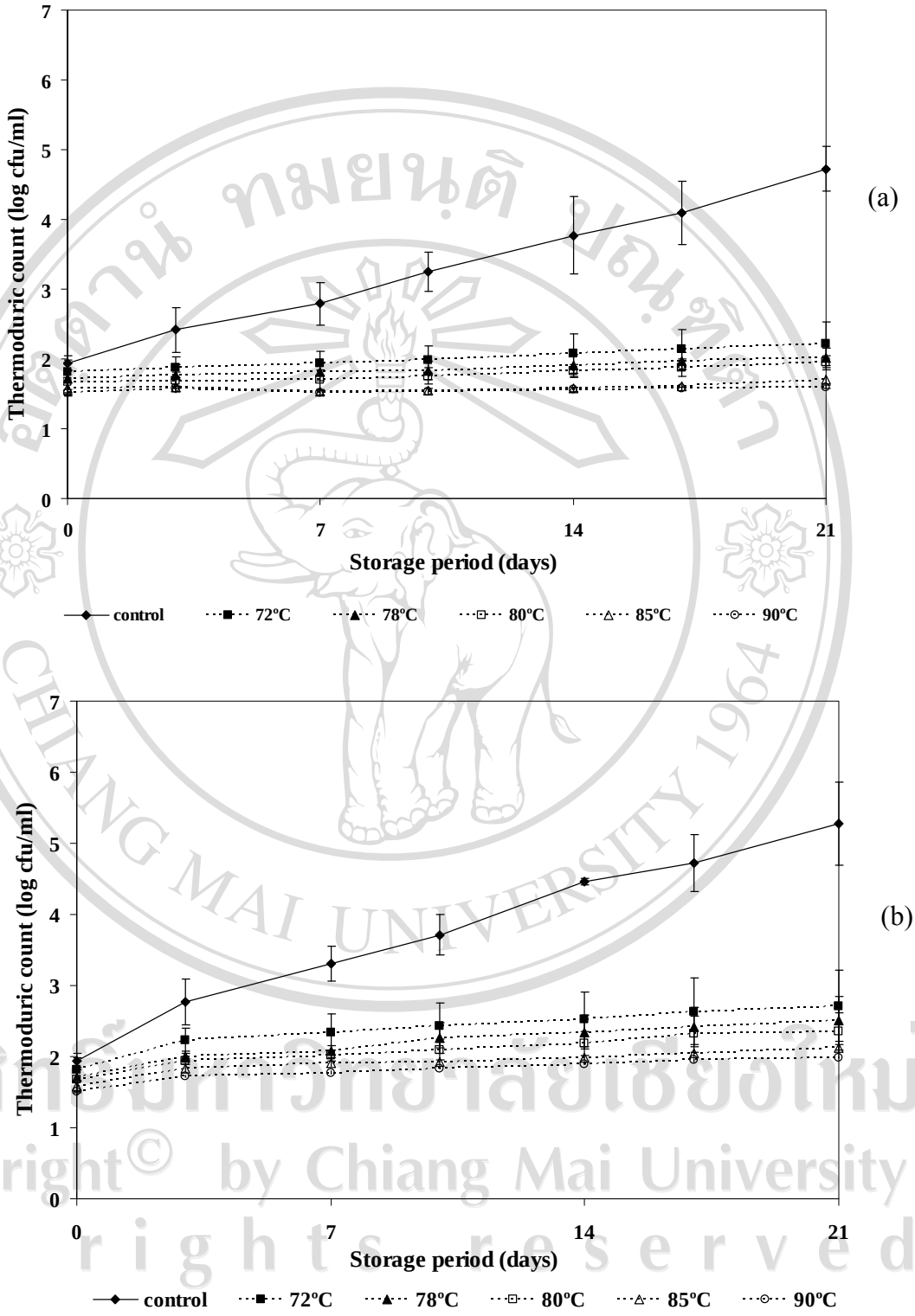

Figure 8.5 Thermoduric count of IMS solutions with and without 100 IU/ml nisin and heated at different holding temperatures for 15 s during storage at 4ºC (a) and 10ºC (b).

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8.3.1.5 pH value

Reduction in the pH of the IMS solutions occurred both for the control and the nisin added IMS treatments (Figure 8.6). A higher rate of pH reduction was significantly recorded in the control IMS solutions stored at 4ºC after 10 days of storage, whereas the same reduction was happened in the control treatments stored at higher storage temperature after 7 days of storage. Reduction of the pH of the nisin added IMS solution that was occurred at a slower rate was corresponded to lower increase in its microbial population (Figures 8.3-8.5). Lower pH values were noted for the nisin added IMS solutions stored at higher storage temperature, particularly for the nisin added IMS solutions pasteurized at 72ºC for 15 s.

8.3.1.6 Acidity value

Results of the total acidity measurement directly responded to the finding of the pH measurement. The acidity of different IMS solutions increased during the studied storage period (Figure 8.7). A higher and significant increase of the acidity of the control IMS treatment occurred after 3 days of storage and was significantly affected by the storage temperature. The same finding was also found for the nisin added IMS solution pasteurized at 72ºC for 15 s on the 10^{th} day of storage onward. This result showed that the acidity of the IMS solutions was more affected by the holding temperatures of pasteurization compared to the pH of the solutions.

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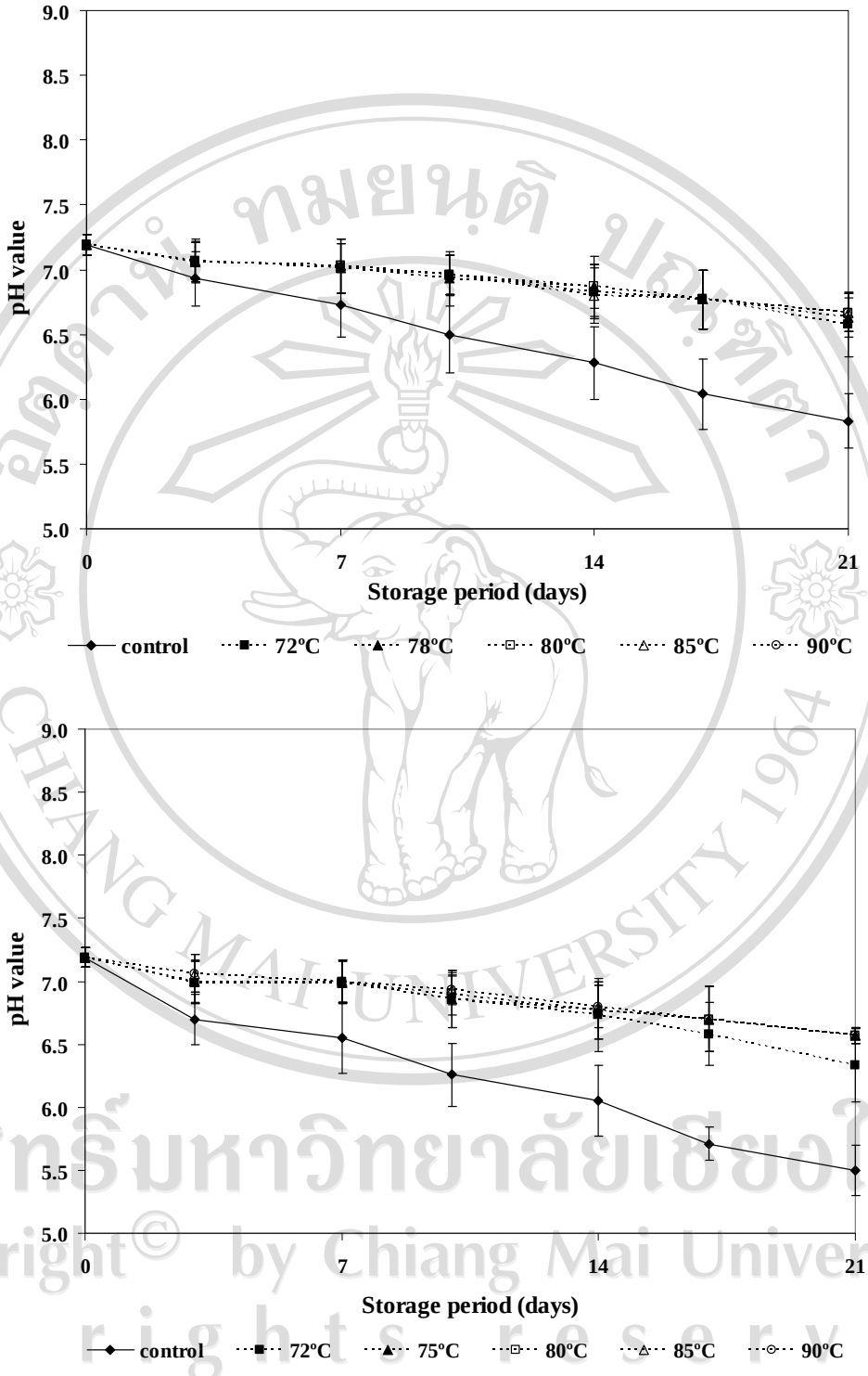

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Figure 8.6 pH value of IMS solutions with and without 100 IU/ml nisin and heated at different holding temperatures for 15 s during storage at 4ºC (a) and 10ºC (b).

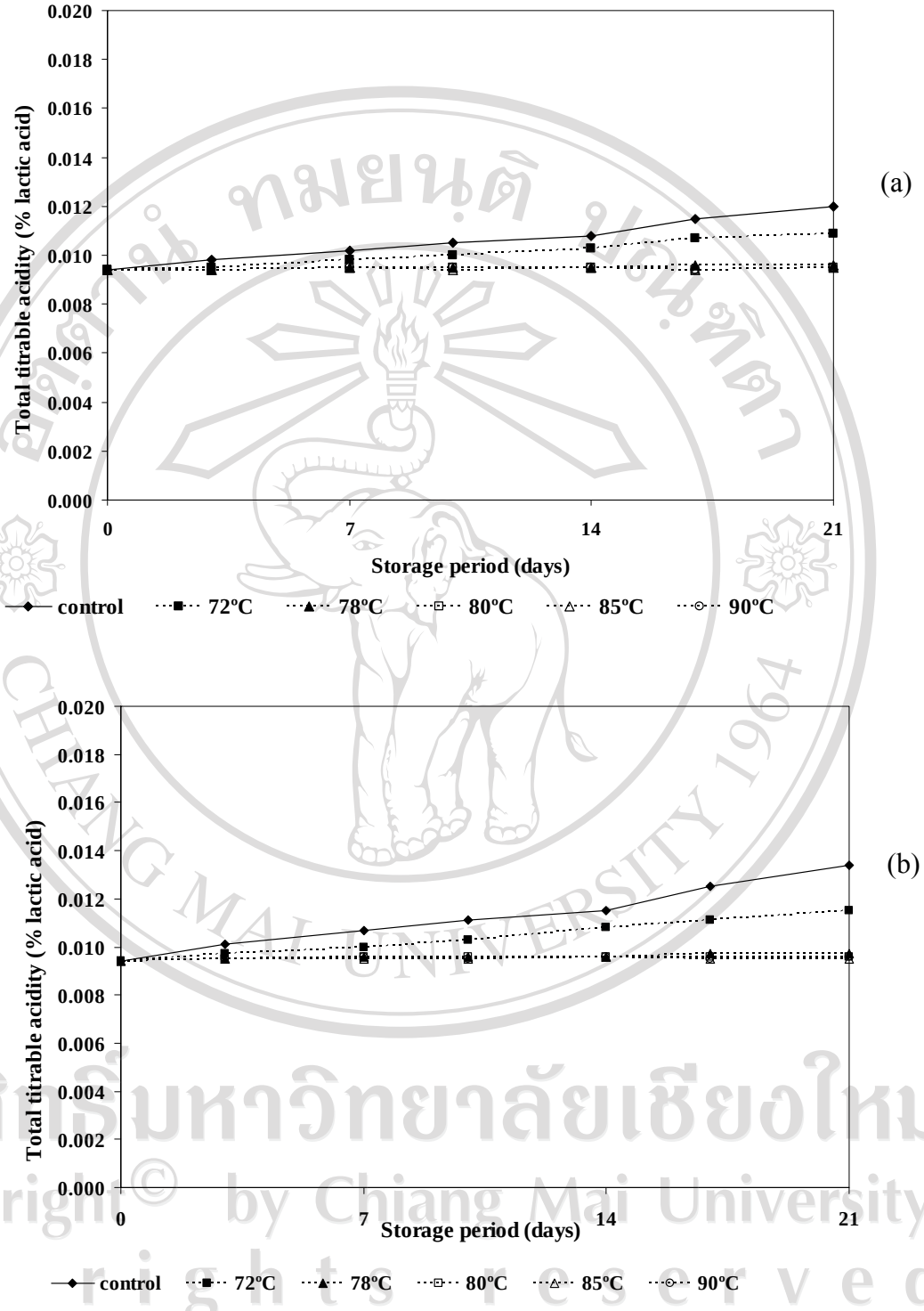

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Figure 8.7 Total acidity of IMS solutions with and without 100 IU/ml nisin and heated at different holding temperatures for 15 s during storage at 4ºC (a) and 10ºC (b).

8.3.1.7 Nisin assay

Residual nisin activity in the IMS solutions displayed that the effectiveness of the protein against microorganisms was affected by holding temperatures of pasteurization, storage temperatures and storage time (Figure 8.8). Higher holding temperatures of the pasteurization significantly reduced the residual nisin activity in the IMS solutions particulary during the storage period. The finding was similar to the report of Thomas *et al*. (2000). The effect of the storage temperature on the activity of nisin could be seen at longer storage period (after 2 weeks in this study). Whereas increasing the storage period was effectively reduced the residual nisin acitivity. No nisin activity was detected in the control IMS treatment. Results from the nisin activity measurement suggested that beside the storage temperature and storage time, holding temperature of pasteurization also needed to be calculated to ensure that the antimicrobial compound would be present in the pasteurized food products throughout the storage period.

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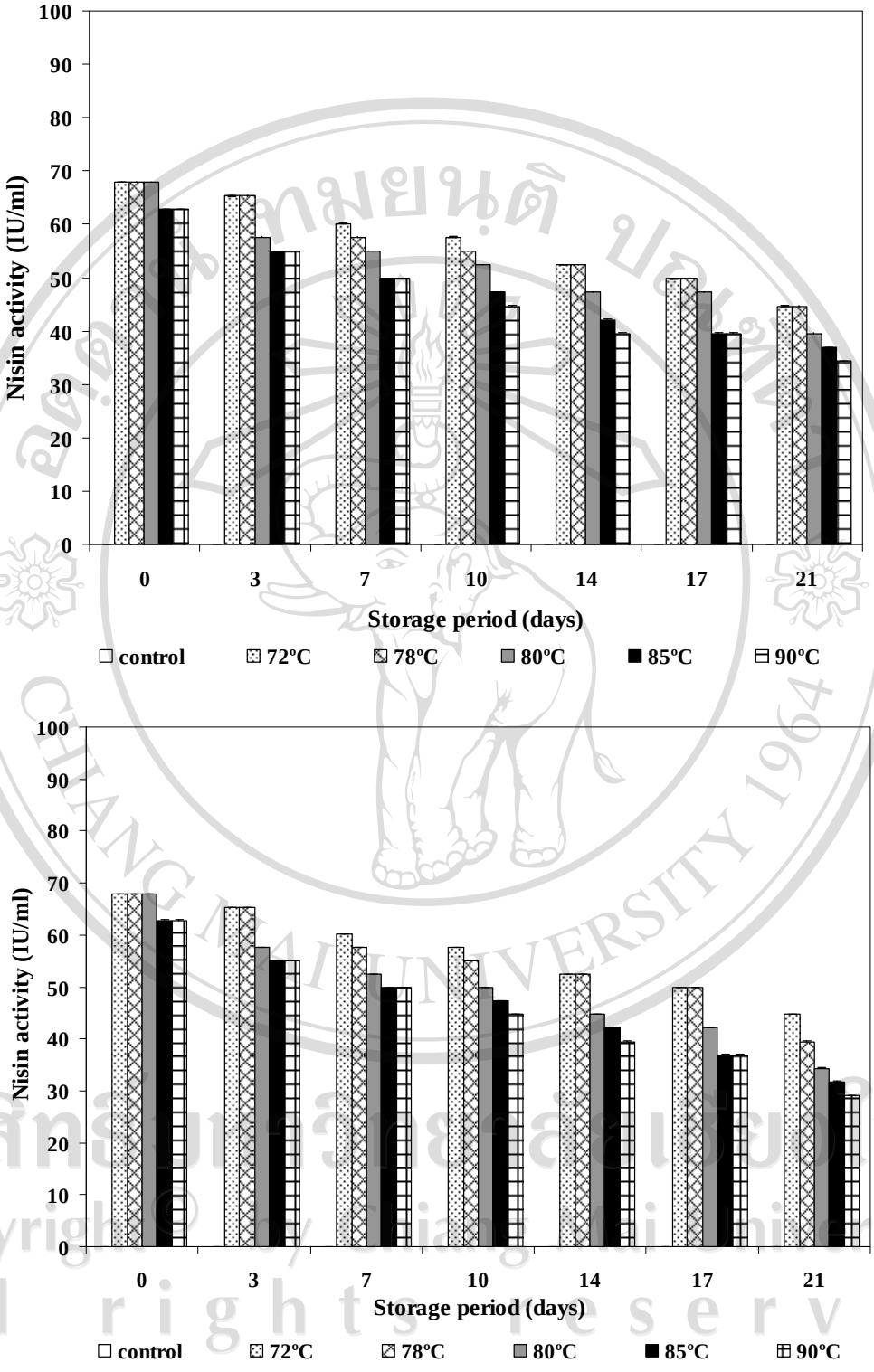

Figure 8.8 Nisin activity of IMS solutions with and without 100 IU/ml nisin and heated at different holding temperatures for 15 s during storage at 4ºC (a) and 10ºC (b).

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8.3.2 The effect of holding time of pasteurization on the effectiveness of nisin to inhibit *B. licheniformis* in the IMS solutions

8.3.2.1 Chemical of the IMS solutions used in the different holding times of pasteurization experiment

Supplementation of 2% (w/v) fat and 1% (w/v) WPI produced an IMS solution that composed of 2.07±0.01% (w/v) fat, 1.23±0.01% (w/v) protein and 0.21±0.01% (w/v) carbohydrate. The total solid of the solution was 3.71±0.01% (w/v) with 43.94% of this amount was solid non fat (Figure 8.9). After dividing this IMS solution into 2 batches, an addition of 100 IU/ml nisin into one of these batches did not significantly (P>0.05) modify the chemical composition of the nisin added IMS solutions. The composition of the IMS solutions in this section was also similar to that of the section 8.3.1.1.

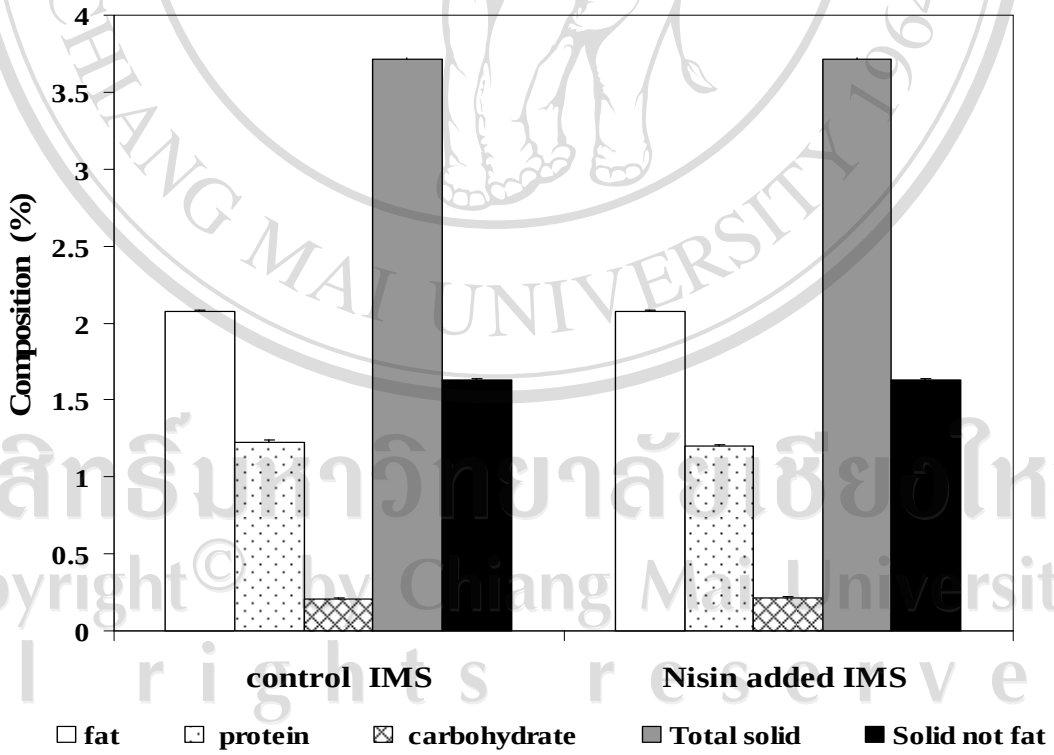

Figure 8.9 Composition of IMS solutions with and without 100 IU/ml nisin.

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8.3.2.2 *TVM count*

Using an inoculation suspension of *B. licheniformis* of 3.65±0.02 log cfu/ml, the pasteurization process at 72ºC for 15 s reduced the bacilli population for 1.71 log cycle directly after the heat treatment (Figure 8.10). The addition of nisin and applying longer holding times of pasteurization of 15 to 25 s further decreased the bacilli population, which reached for up to 1.87 log cycle. However, this additional reduction did not produce a significant effect on the initial *B. licheniformis* population in different IMS solutions at the beginning of the storage period. This finding suggested that increasing the holding temperature of pasteurization (section 8.3.1.2) would be more effective in reducing TVM population compared to extending the holding time of pasteurization.

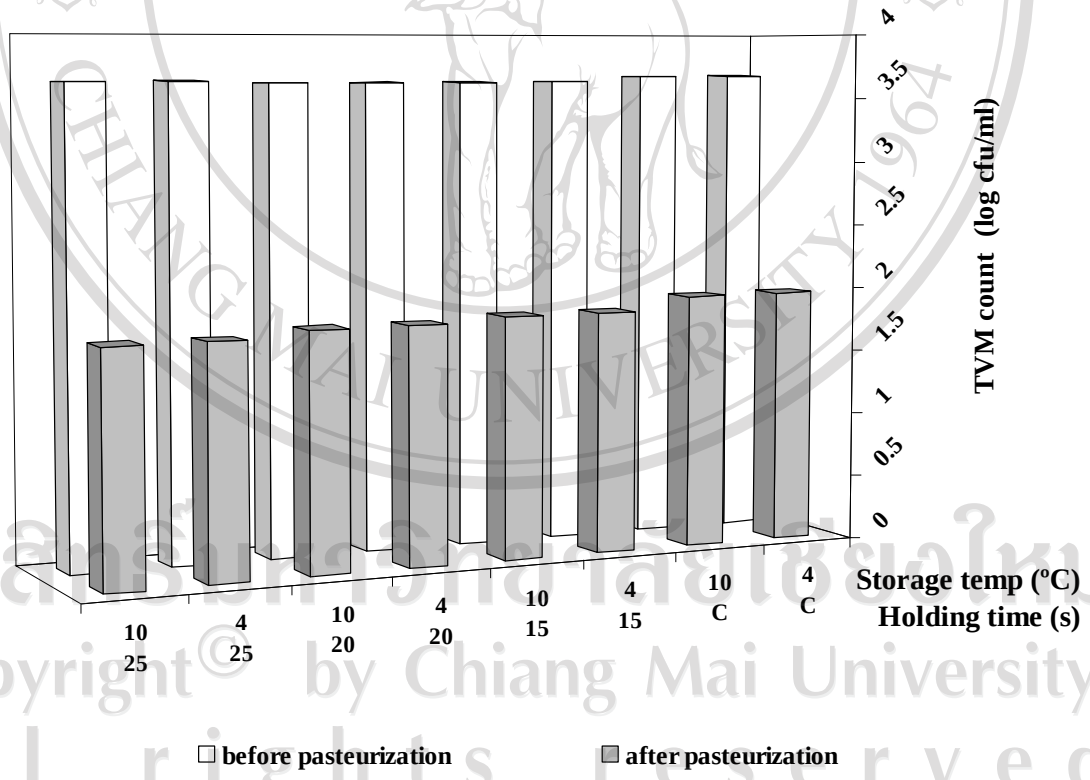

Figure 8.10 Total Viable Microorganisms of the IMS solutions directly before and after pasteurization at 72ºC using different holding times.

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The effectiveness of nisin to inhibit the vegetative cells of *B. licheniformis* was clearly displayed during storage at 4 and 10ºC. The control IMS treatment had a significant increase in its TVM population throughout the storage period (Figure 8.11). Significant ($P \leq 0.05$) and higher increasing rates of the TVM of the control IMS solutions compared to that of the nisin added IMS solution were found after 3 days storage. The storage temperature was also significantly ($P \leq 0.05$) affected the TVM number of the control IMS treatments from 3 days storage onward.

The increase of the TVM population in the nisin added IMS solution occurred at a much slower rate (Figure 8.11). After 21 days at 4 and 10ºC, the TVM population in the nisin added IMS solutions only increased for up to 0.63 and 0.98 log cycle, respectively. Similar increases of the TVM population in the control IMS treatments could be found within 3 days of storage. The higher storage temperature caused the TVM in the nisin added IMS solutions to have a higher increasing rate. However, it was only significant in the nisin added IMS solutions processed at 72ºC for 15 s after 17 days storage. Longer holding time of pasteurization decreased the effect of storage temperature on the activity of nisin to inhibit the TVM population. Different holding times of pasteurization did not significantly influence the growth of the TVM population during the studied storage period, even though the TVM population of the nisin added IMS solution heated at 72ºC for 25 s and stored at 4ºC was the lowest compared to those of the other IMS treatments throughout the storage period.

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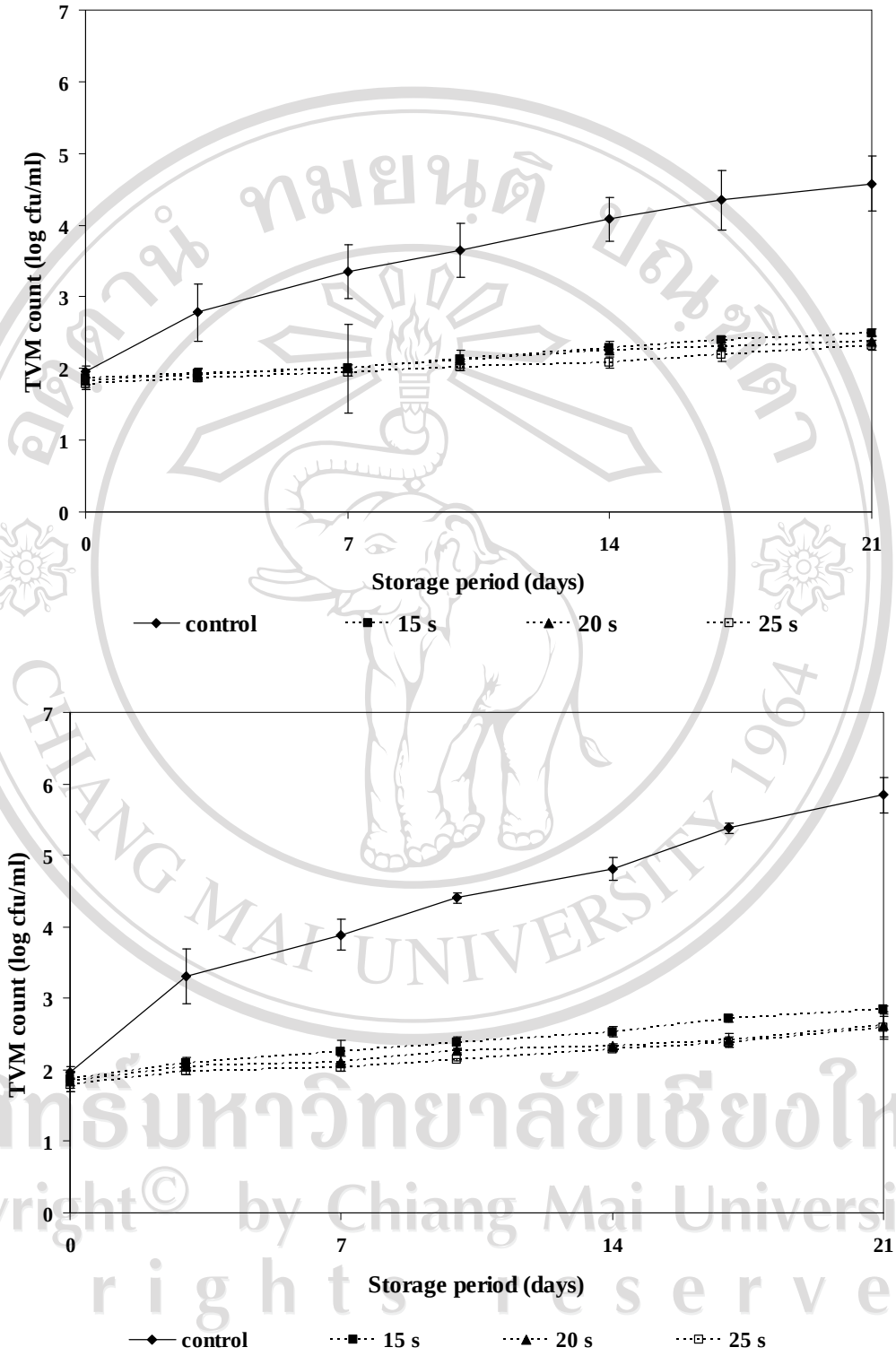

Figure 8.11 Total Viable Microorganism of IMS solutions with and without 100 IU/ml nisin and heated at 72ºC using different holding times during storage at 4ºC (a) and 10ºC (b).

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8.3.2.3 Spore count

Nisin was also found to significantly ($P \leq 0.05$) affect the outgrowth of bacilli spores in the IMS solutions. When nisin was absent in the IMS solution, the solution had a significant ($P \leq 0.05$) higher initial spore count after pasteurization and experienced increases in its spore number throughout the storage period (Figure 8.12). On the other hand, the presence of nisin could maintain and even reduced, when it was combined with a storage temperature of 4ºC, the spore population in the IMS solutions during 21 days of storage. Different storage temperatures and holding times of pasteurization did not significantly ($P > 0.05$) influence the effectiveness of nisin against the bacilli spore. Finding in this section was supported by a continuous presence of nisin in the IMS solutions during the studied storage period (Figure 8.16), since the effect of nisin to inhibit the outgrowth of spore form could be sporostatic (de Vuyst and Vandamme, 1994).

8.3.2.4 Thermoduric count

Results of the thermoduric measurement (Figure 8.13) showed similar graphs as the finding for the TVM enumeration (Figure 8.11), which indicated that the thermoduric bacilli was the major bacilli type in the IMS solutions. The initial thermoduric count in different IMS solutions was not significantly ($P > 0.05$) different (Figure 8.13). The effectiveness of nisin to inhibit thermoduric *B. licheniformis* was significantly ($P \leq 0.05$) demonstrated from 3 days storage onward. The storage temperature significantly ($P \leq 0.05$) affected the growth of the thermoduric population in the IMS solution without nisin after 7 days of storage. The same factor significantly affected the nisin added IMS solutions processed at 72ºC for 15 s after 10 days of storage. The lowest thermoduric bacilli population throughout 21 days of storage was found in the nisin added IMS solution heat treated at 72ºC for 20 and 25 s.

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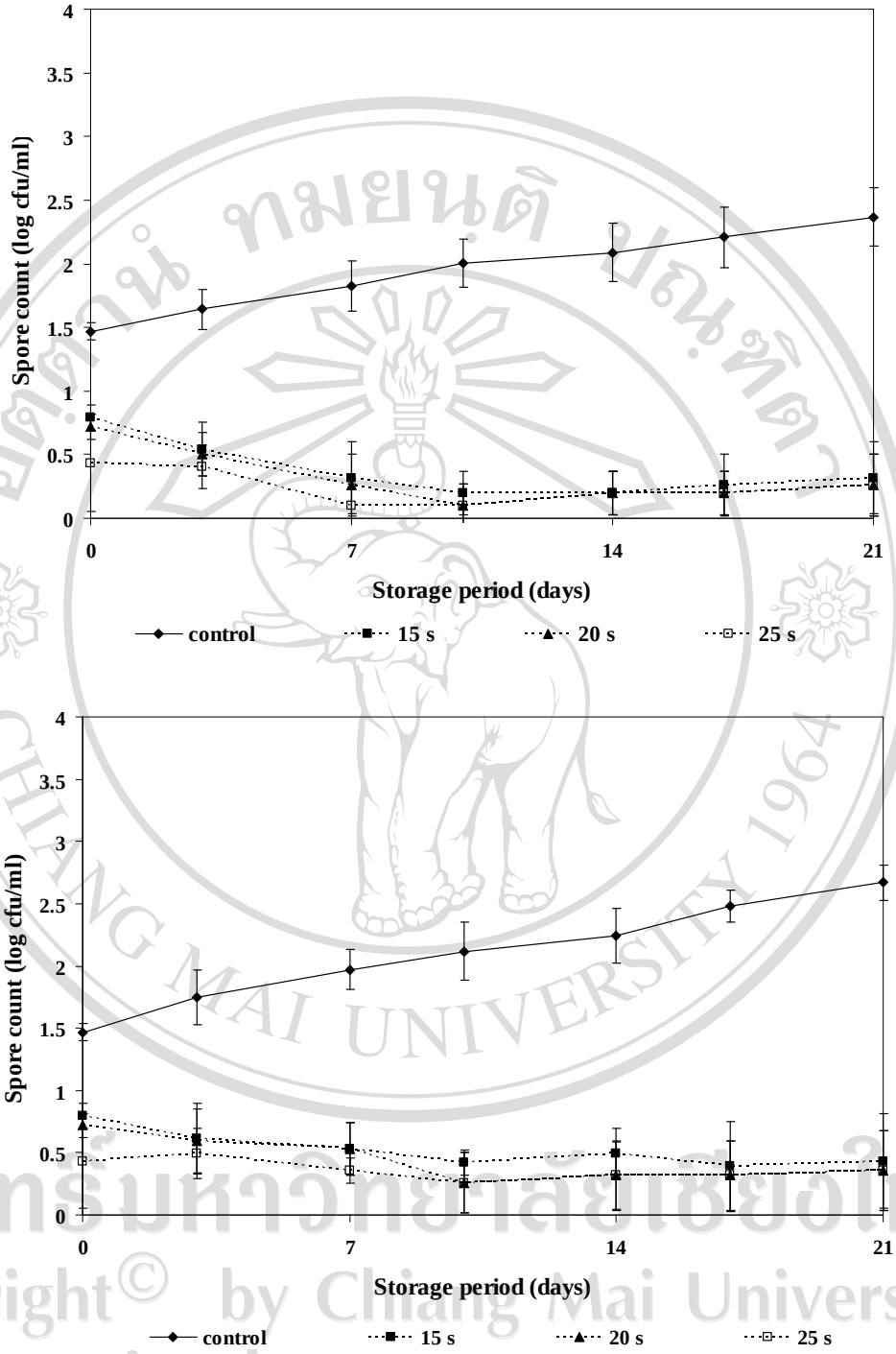

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Figure 8.12 Spore count of IMS solutions with and without 100 IU/ml nisin and heated at 72ºC using different holding times during storage at 4ºC (a) and 10ºC (b).

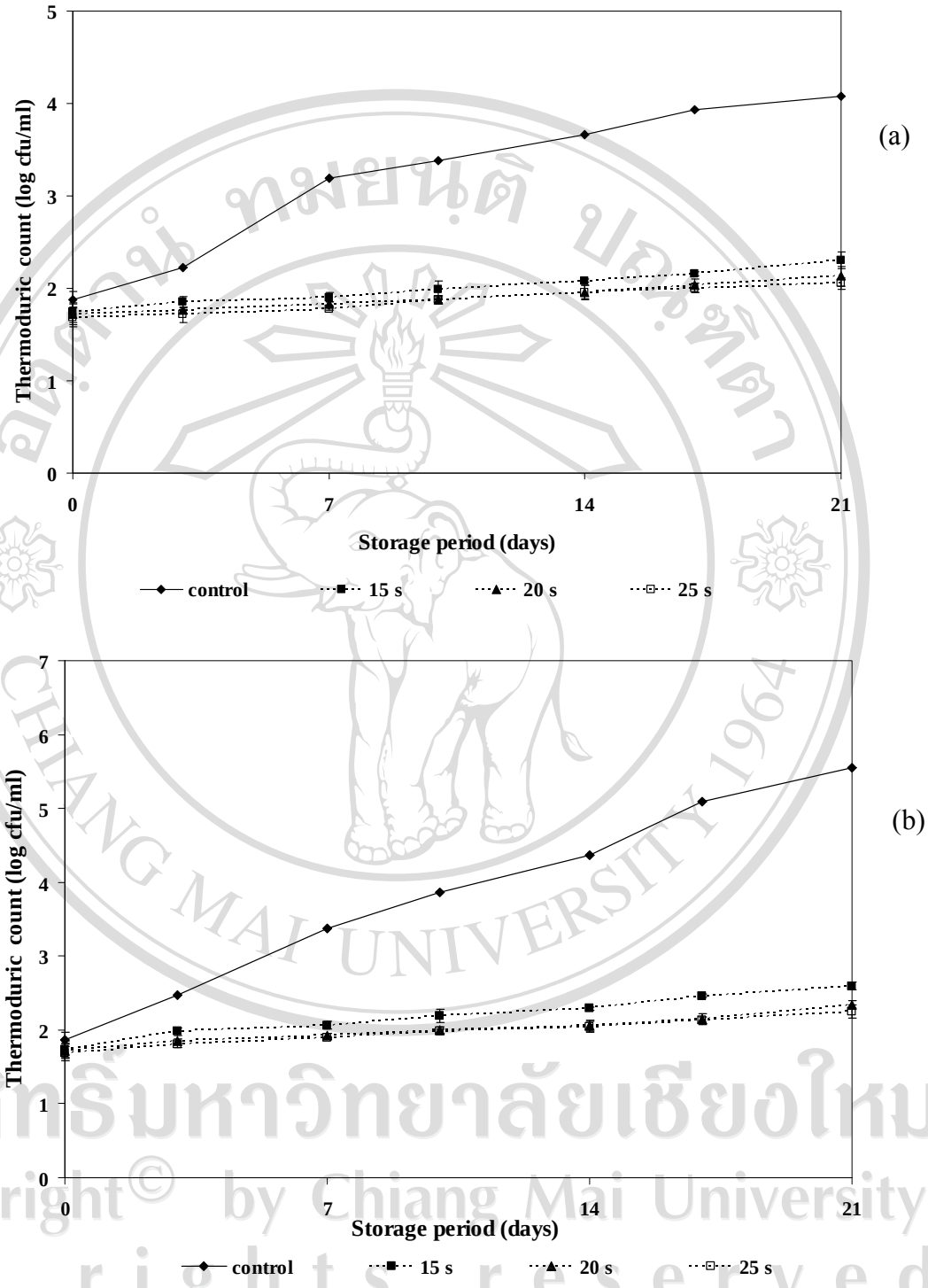

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Figure 8.13 Thermoduric count of IMS solutions with and without 100 IU/ml nisin and heated at 72ºC using different holding times during storage at 4ºC (a) and 10ºC (b).

8.3.2.5 pH value

Following the microbial growth in the IMS samples (Figures 8.11 and 8.13), the pH of the IMS solutions reduced during 21 days storage (Figure 8.14). The control IMS solutions had a significant ($P \leq 0.05$) higher reducing rate for their pH value compared to those of the nisin added IMS solutions, especially at 10ºC storage temperature. Different storage temperatures and holding times of pasteurization did not give a significant ($P > 0.05$) effect on the reduction of the pH value of the IMS solutions.

8.3.2.6 Acidity value

Responding to the decrease of the pH, the total acidity of different IMS solutions increased at different rates during the storage period (Figure 8.5). A significant and higher increasing rate of the acidity of the control IMS treatments was found since the 3rd day of storage. The nisin added IMS solutions processed at 72ºC for 15 s also had a significant and higher increasing rate for its acidity compared to those of the other IMS solutions supplemented with nisin and heated at longer holding times after 10 days of storage. The storage temperature was only significantly ($P \leq 0.05$) affected the control IMS treatments and the nisin added IMS solutions processed at 72ºC for 15 s after 3 and 14 days of storage, respectively. Although changing in the acidity of the IMS solutions occurred within 0.0084% lactic acid during 21 days of storage, this measurement was sensitive to detect the chemical variation in the IMS solutions processed at different holding times of pasteurization.

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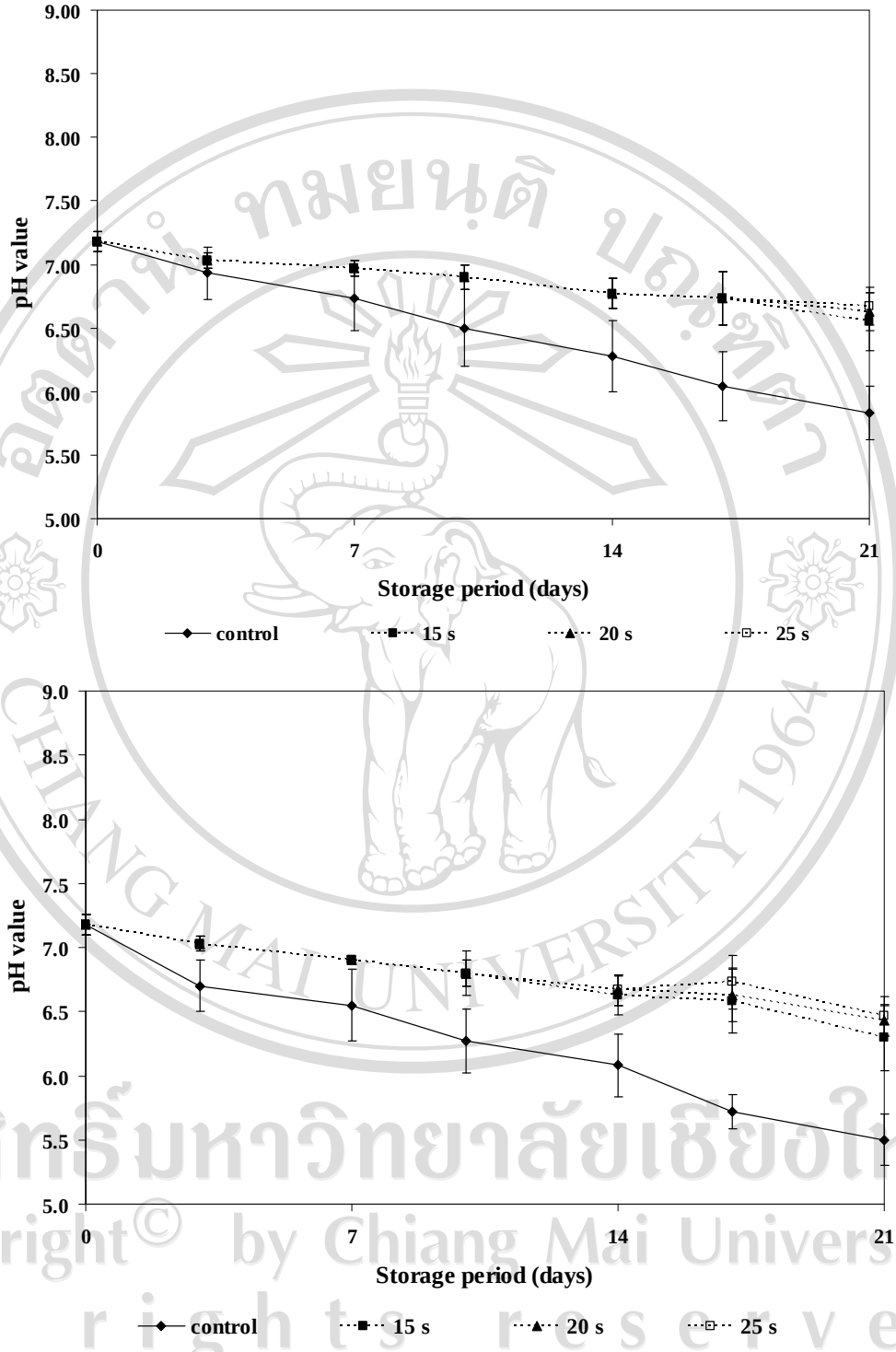

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Figure 8.14 pH value of IMS solutions with and without 100 IU/ml nisin and heated at 72ºC using different holding times during storage at 4ºC (a) and 10ºC (b).

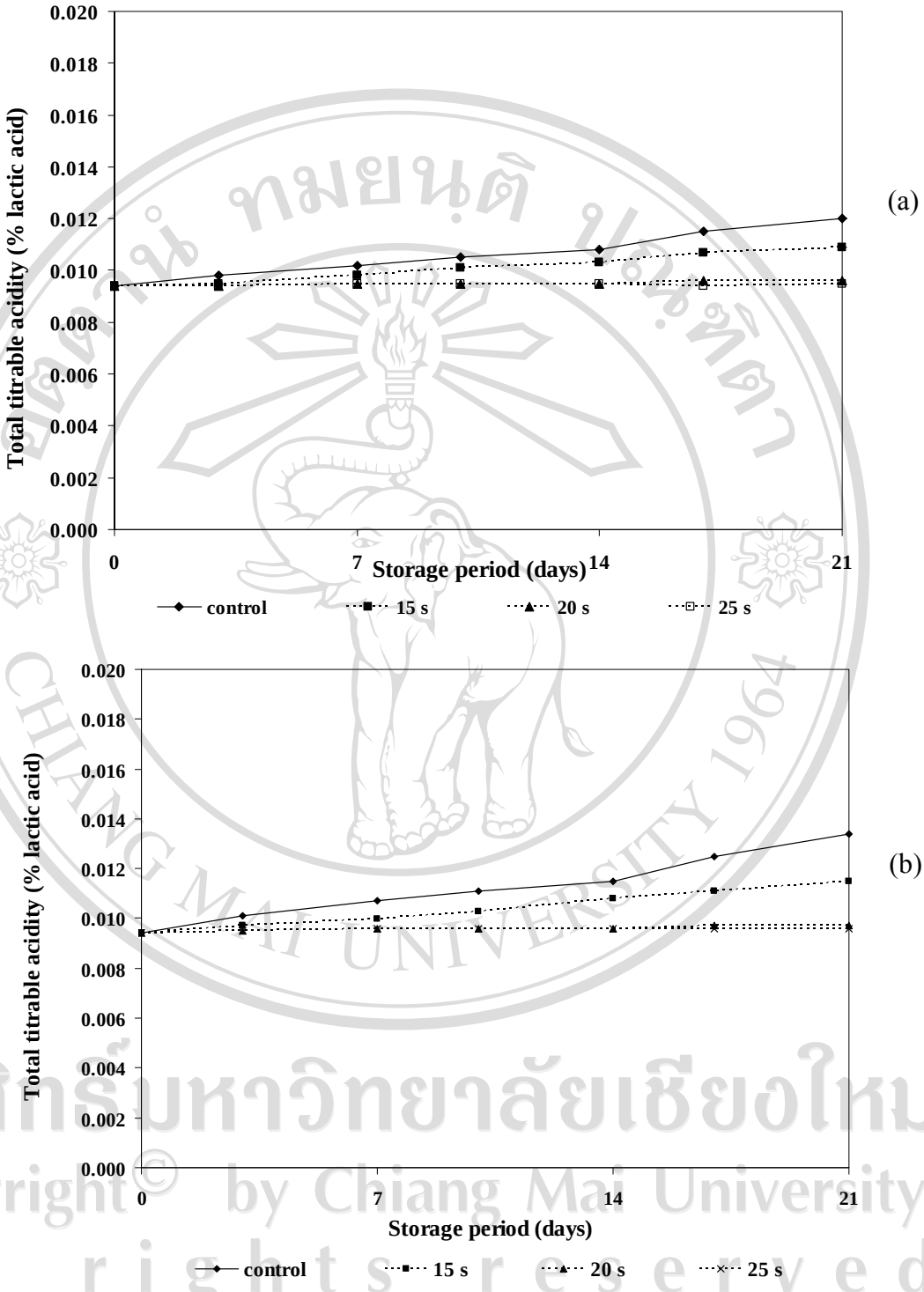

Figure 8.15 Total acidity of IMS solutions with and without 100 IU/ml nisin and heated at 72ºC using different holding times during storage at 4ºC (a) and 10ºC (b).

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8.3.2.7 Nisin assay

Although different holding times of pasteurization did not give significant (P>0.05) effects on the chemical and microbial properties of the IMS solutions supplemented with nisin during 21 days of storage, except for total acidity, this heat treatment factor did produce an effect on the residual nisin activity during the storage period, particularly after 10 days of storage (Figure 8.16). At 4ºC, the nisin added IMS solutions processed at 72ºC for 15, 20 and 25 s had reductions in their nisin activity for 31.01, 33.59 and 36.17 IU/ml, respectively. While applying a higher storage temperature produced higher reductions of 38.76, 41.34 and 43.93 IU/ml, respectively. This result showed clearly that the activity of nisin in the IMS solutions was affected by the holding times of pasteurization, storage temperature and storage time. Since longer holding times of pasteurization did not significantly reduce the microbial population of the IMS solution (Figures 8.11 – 8.13), applying a minimum holding time of 15 s for the pasteurization process would extend the availability of nisin in the solution and subsequently produce a longer shelf life. No nisin activity was detected in the control IMS solutions.

8.4 Conclusions

Data in this section emphasized that nisin was significantly affected the chemical and microbial characteristics of the IMS solutions. Improvement in the microbial quality and reducing changes in the chemical property of the IMS solutions during 21 days at 4 and 10ºC could be achieved by the presence of 100 IU/ml nisin. Increasing holding temperature of pasteurization affected the microbial growth, acidity and residual nisin activity in the IMS solutions, while applying longer holding time of pasteurization only influenced acidity and residual nisin activity of the IMS solution. This finding suggested that beside storage temperature and storage time, the pasteurization condition should also be included in the consideration of the nisin application in food products, especially dairy products.

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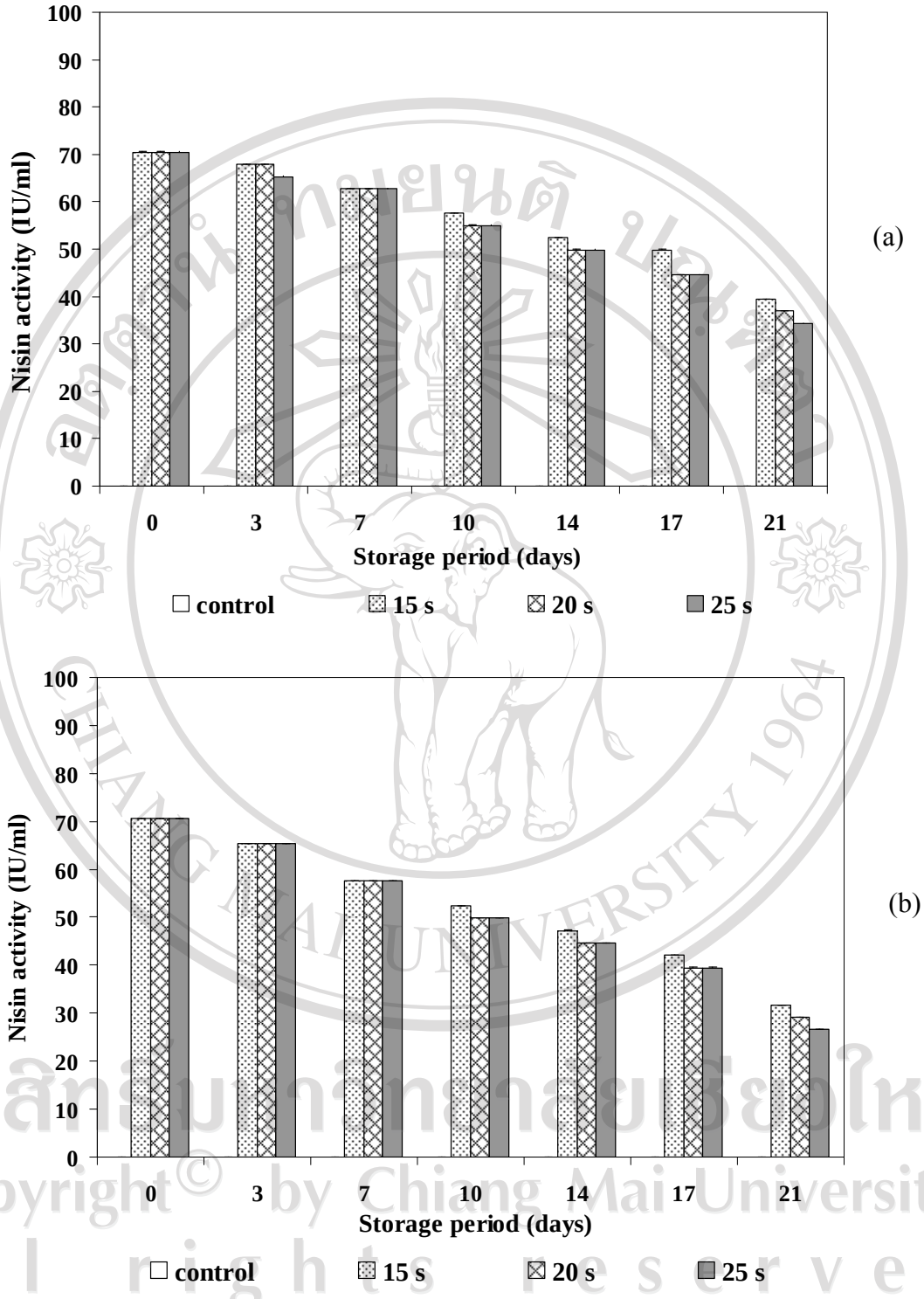

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Figure 8.16 Nisin activity of IMS solutions with and without 100 IU/ml nisin and heated at 72ºC using different holding times during storage at 4ºC (a) and 10ºC (b).