CHAPTER 7

SURVIVAL OF *B. licheniformis* IN NISIN ADDDED PASTEURIZED IMITATED MILK SYSTEM AS AFFECTED BY NISIN ADDITION TIME AND STORAGE TEMPERATURES

7.1 Introduction

Nisin was produced by fermentation of *Lactococcus lactis* subsp. *lactis* (Thomas *et al*., 2000). Nisin is best added as an aqueous solution, usually mixed in the liquid part of products during the process (Nissen *et al*., 2001; Samelis *et al*., 2003). For example, nisin can be combined with the brine solution of a canned food and then mixed the brine into the whole product. In dairy desserts and milks, it can be added into a small quantity of milk and then mixed the milk into the bulk part, filled and processed. Although nisin can be added as a powder, it is essential to ensure that the protein disperses thoroughly throughout the food matrix. The best time to add nisin is at the last practice stage before heat processes (de Vuyst and Vandamme, 1994; Thomas *et al*., 2000).

In this section, IMS solutions prepared based on the results of previous sections were made and inoculated with a spore suspension of *B. licheniformis* that was heat treated prior to the inoculation. The inoculated IMS solution was then added with 100 IU/ml nisin. The addition time of nisin was varied either 0, 30, 60 or 120 min before or after a pasteurization process. The objective of this chapter was to understand whether the addition time of nisin would affect the effectiveness of nisin in inhibiting the growth of bacilli in the pasteurized IMS solutions in order to extend the shelf life of the IMS solutions or milk at low storage temperatures.

7.2 Material and methods

7.2.1 Addition before pasteurization

IMS solutions prepared according to the method in the section 4.2.1 were produced using 2% (w/v) fat and 1% (w/v) WPI based on the results in the sections 4 and 6. No addition of casein and lactose was done into the IMS solutions, since these

milk components produced a negative effect on the activity of nisin. The IMS solutions were then inoculated with 3.70±0.02 log cfu/ml *B. licheniformis* spore suspension and added with 100 IU/ml nisin. The addition time of nisin was varied at 0 (directly before pasteurization), 30, 60 and 120 min before a pasteurization process at 72ºC for 15 s. As a control, an IMS solutions without nisin addition was also produced. After the heat treatment, different IMS solutions were quickly cooled down in a running tap water and stored at 4 and 10ºC.

7.2.2 Addition after pasteurization

A similar experiment procedure as in this section 7.2.1 was carried out in this sub-section. Nisin was added at 0 (directly after pasteurization), 30, 60 and 120 min after the pasteurization process. Since the antimicrobial compound was supplemented into pasteurized IMS solutions, nisin solution prepared at a concentration of 2000 IU/ml using distilled water was filtered sterilized by 0.45 µm syringe filter (Acrodisc®, USA) prior to the supplementation. IMS solution should be final concentration of nisin at 100IU/ml. The inoculated *B. licheniformis* suspension had a microbial population of 3.59±0.12 log cfu/ml.

7.2.3 Chemical analysis

7.2.3.1 Total acidity measurement

A similar method as in the section 4.2.4.

7.2.3.2 pH measurement

A similar procedure as in the section 4.2.4.

7.2.4 Microbiological analysis

7.2.4.1 Total viable microorganisms

A similar microbiological method as in the section 4.2.5.

7.2.4.2 Spore count

A similar procedure as in the section 4.2.5.

7.2.4.3 Thermoduric bacteria.

A similar analysis method as in the section 4.2.5.

7.2.5 Nisin assay

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

7.2.6 Experimental design

The experiment results were analyzed statistically using a Factorial in Completely Randomized Design with 2 factors. The first factor was the time addition of nisin, including at 0, 30, 60 and 120 min before or after pasteurization. The second factor was storage temperatures, which were at 4 and 10°C. DMRT was then used to determine differences between treatment means. The analysis was carried on using a SPSS program (SPSS version 10.0) (SPSS Inc., Chicago, USA).

7.3 Results and discussion

7.3.1 The effect of nisin addition time before pasteurization on the effectiveness of nisin to inhibit *B. licheniformis* in the IMS solutions

Chemical composition of IMS solutions

Results from the Chapter 3 showed that supplementation of 100 IU/ml nisin in the IMS solutions was the best concentration of the antimicrobial compound to inhibit the growth of survival microorganisms in pasteurized milk, therefore this concentration of nisin continued to be applied in this section. For the composition of the IMS solution, data in the Chapter 4 suggested that the presence of 2% fat in the IMS solution produced a significant lower TVM count than the control treatment (no fat), therefore the same fat level was used in this section. The addition of carbohydrates either lactose or sucrose (Chapter 5) did not give a positive effect on the activity of nisin in inhibiting the bacilli population, so no addition of carbohydrates was done in this section. The presence of casein and WPI (Chapter 6) demonstrated different results on the effectiveness of nisin. The casein molecule, which is the major milk protein, had an adverse effect on the activity of nisin, whereas, the WPI could support the activity of nisin against the target microorganisms. Since the concentration of 1% WPI in the IMS solutions displayed a significant reduction in the TVM count compared to the control treatment (no WPI) during storage at 10ºC, this WPI level was incorporated into the IMS solutions studied

in this section. The final nisin added IMS solutions had a composition of 2.09±0.01% (w/v) fat, 1.25±0.01% (w/v) protein and 0.19±0.01% (w/v) carbohydrate. The total solid of the solution was 3.84±0.01% (w/v) with 1.82±0.04% (w/v) solid non fat (Figure 7.1).

The chemical composition of the nisin added IMS solutions was not significantly ($P>0.05$) different to the control treatment (no nisin addition). A slightly higher protein level of the IMS solutions compared to the 1% (w/v) addition level of WPI could contribute from the supplementation of UHT whipped cream as a source of milk fat that had only a fat level of 36% in the product.

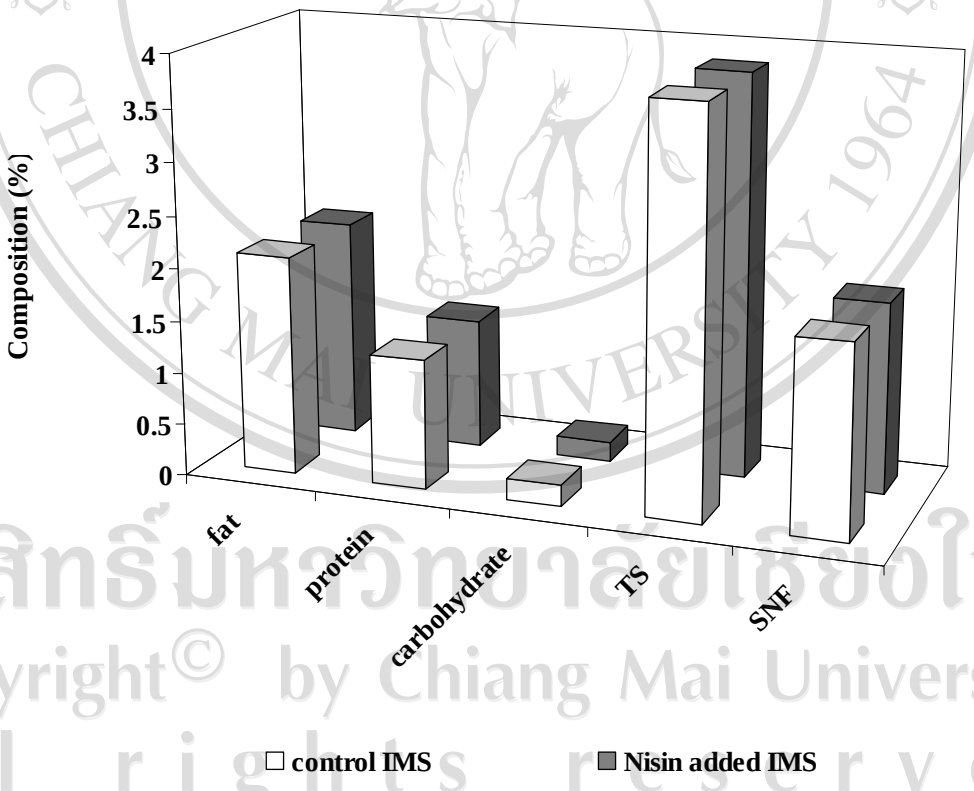

Figure 7.1 Chemical composition of IMS solutions with and without nisin addition supplemented before pasteurization.

7.3.2 The microbiological effect of nisin addition time before pasteurization on the effectiveness of nisin to inhibit *B. licheniformis* in the IMS solutions

7.3.2.1 TVM count

In this section, IMS solutions were divided into five batches. All of the IMS batches were inoculated with *B. licheniformis* spore suspension at a level of 3.70±0.02 log cfu/ml prior to the nisin addition. One of the IMS batch was not added with nisin and was used as a control. For the rest of four batches, nisin was supplemented at 0 (directly before pasteurization), 30, 60 and 120 min before pasteurization at 72ºC for 15 s. After the pasteurization treatment, different IMS samples were stored at 4 and 10ºC storage temperatures.

The presence of nisin in the IMS solution had a significant effect ($P \leq 0.05$) in enhancing the microbial destruction during the pasteurization process. When nisin was absent from the IMS solution, a reduction in the TVM count of 1.07 to 1.08 log cycle was recorded. Whereas in the presence of 100 IU/ml, a reduction in the TVM number between 1.78 and 2.00 log cfu/ml was found, which was 0.7 log cycle higher than the control treatment. Different addition time of nisin did not give any significant effect ($P > 0.05$). This finding indicated that nisin caused the vegetative cell of *B. licheniformis* to be more susceptible to the heat treatment.

During 21 days storage of 4 and 10ºC (Figure 7.2), the control IMS treatment had a significant increase ($P \leq 0.05$) in its TVM count. A significant ($P \leq 0.05$) higher TVM count of more than 3 log cycle was also found when the control treatment was stored at 10ºC at the end of the storage period. On the other hand, 100 IU/ml nisin had a significant effect ($P \leq 0.05$) in controlling the growth of the vegetative bacilli in the IMS solutions. At 4ºC storage temperature, the IMS solutions that were supplemented with nisin 30 to 120 min before the pasteurization process experienced a continued reduction in their TVM count for up to 0.61 log cycle during the first 7 days storage. This effect was not recorded in the IMS solutions that were supplemented with nisin directly before the pasteurization treatment. Although all of the nisin added IMS solutions had an increase in their TVM counts for the rest of the

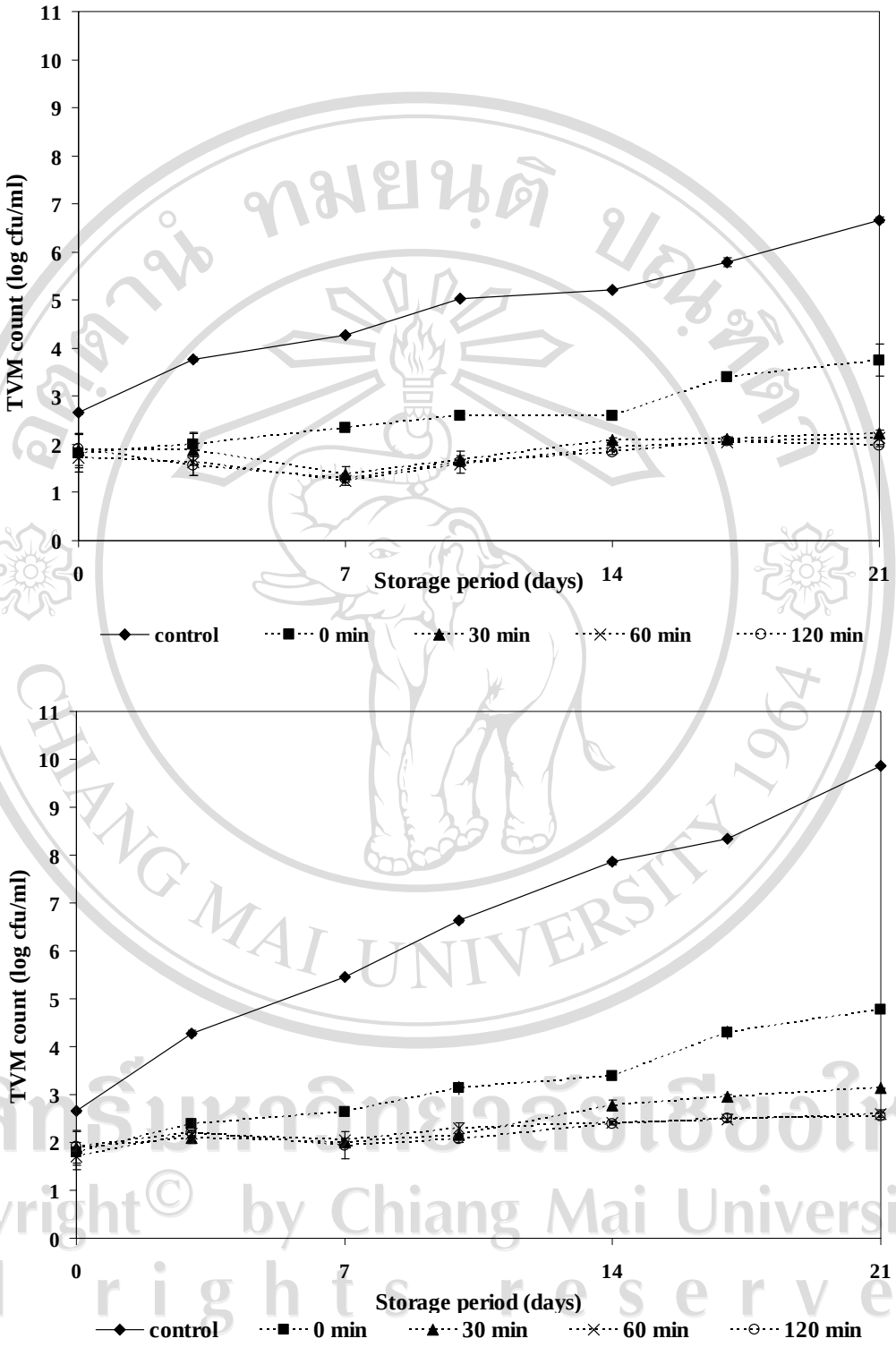

Figure 7.2 Total Viable Microorganisms of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times before pasteurization during storage at 4ºC (a) and 10ºC (b).

storage period, on the 21st day of storage, the IMS treatment added with nisin directly before pasteurization contained a significant ($P \leq 0.05$) higher TVM count compared to those of the IMS treatment that were supplemented with nisin 0.5 to 2 h before the pasteurization process (Figure 7.2a). This result showed clearly that the addition time of nisin played an important role in inhibiting the growth of bacilli population during storage. Giving some period of time for the antimicrobial compound to interact with microorganisms before the main heat treatment would produce a better control for the microbial growth during storage. Increasing the contact time from 30 to 120 min before the pasteurization process did not significantly ($P > 0.05$) increase the effectiveness of nisin, even though lower TVM number were recorded in the IMS solutions with longer nisin contact time.

When the IMS solutions were stored at 10ºC, a similar result as the finding at 4ºC was found (Figure 7.2b). However, a slight reduction of the TVM count between 0.14 and 0.25 log cycle was only occurred in the IMS solution that were supplemented with nisin 60 and 120 min before the pasteurization process during the 3rd and 7th days of storage. The TVM count of the IMS solutions was also significantly ($P \leq 0.05$) higher than those of the IMS solutions kept at lower temperature. This finding was similar to the control treatment showing a better support of higher storage temperature for the microbial growth. Interestingly, even at 10ºC, the IMS solution that had 120 min contact time with nisin before the pasteurization process had a significant ($P \leq 0.05$) lower TVM number than that of the IMS solutions that was added with nisin directly before the pasteurization and stored at 4ºC after 7 days storage. This result clearly suggested that increasing the contact time of nisin with microbial population gave a positive effect for the activity of nisin in controlling the microbial growth during storage. A minimun contact time of 30 min should be given for nisin before the main heat treatment to produce this effect.

7.3.2.2 Spore count

Directly after the pasteurization process, the spore count of the control IMS treatment was significantly ($P \leq 0.05$) higher than those of the IMS solutions supplemented with nisin (Figure 7.3). Between the nisin added IMS solutions, the IMS solutions with shorter nisin contact time had significantly ($P \leq 0.05$) lower initial spore counts. During 21 days of storage, increasing number of spore was noticeable in the control IMS treatment. Significantly ($P \leq 0.05$) higher spore counts in the IMS solution stored at higher storage temperature were also recorded and were similar to the TVM result (Figure 7.2). On the other hand, the nisin added IMS solutions had a slow reduction in their spore count in the first 10 days of storage before increase in the rest of the storage period. This changing was not significantly ($P \leq 0.05$) affected by the storage temperature and the nisin addition/contact time.

The data of the spore count presented a good control of nisin against the bacilli spore during pasteurization and subsequent storage period. The addition time of nisin was only found to significantly affect the spore number during the heat process. Storage temperatures of 4 and 10ºC were not a major issue that affected the activity of nisin. Storage time of the product would be more important in controlling the spore outgrowth, since the nisin availability reduced at longer storage time (Figure 7.7) and the effect of nisin could be sporastatic rather than sporocidal.

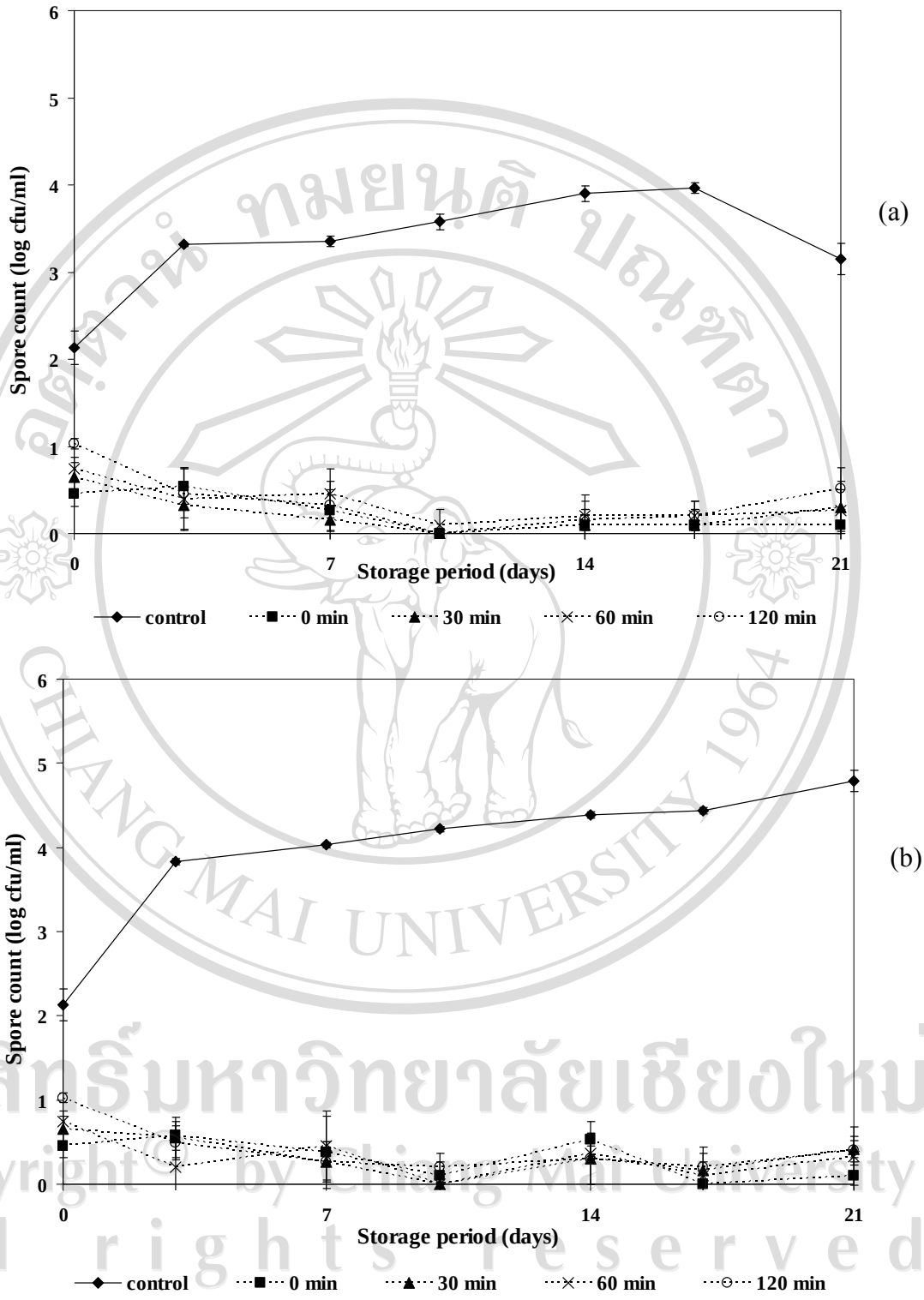

Figure 7.3 Spore count of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times before pasteurization during storage at 4ºC (a) and 10ºC (b).

7.3.2.3 Thermoduric count

The effectiveness of nisin against thermoduric bacteria in the IMS solution was not significantly ($P>0.05$) affected by the nisin addition time, but it was influenced by the storage temperature particularly at longer storage period (Figure 7.4). After the pasteurization process, the control IMS treatment had significantly ($P\leq0.05$) higher thermoduric organism compared to those of the IMS solutions supplemented with nisin. During the storage period, the number of thermoduric count in the control IMS treatment was slightly increased in the first 3 days of storage before became more stable for the rest of the storage period. Different storage temperatures did not significantly ($P>0.05$) affect the number of thermoduric bacteria in the control IMS solutions. This finding might be affected by the fact that the studied storage temperatures were not the optimum temperature for the thermoduric bacteria (Adam and Moss, 2000). For the IMS solutions supplemented with nisin, the thermoduric organisms in the samples were mainly stable during 21 days of storage. Applying a higher storage temperature of 10ºC caused the nisin treated IMS solutions to have significantly ($P \leq0.05$) higher thermoduric counts than those of the IMS samples stored at 4ºC after 17 days of storage. Data in this subsection demonstrated that a better control of thermoduric microorganisms in milk could be done by combining nisin and low storage temperatures.

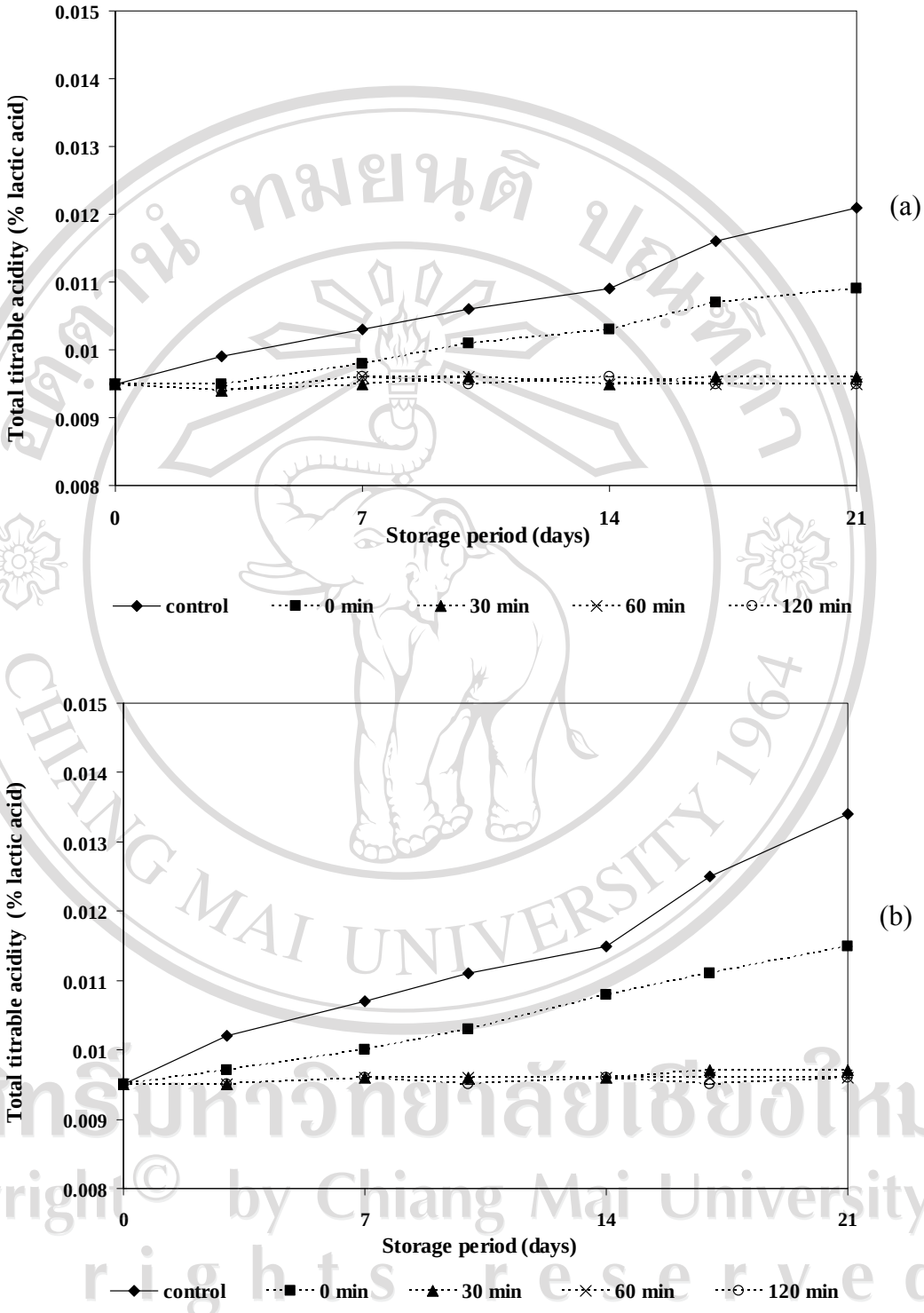

Figure 7.4 Thermoduric count of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times before pasteurization during storage at 4ºC (a) and 10ºC (b).

7.3.3 The chemical effect of nisin addition time before pasteurization on the effectiveness of nisin to inhibit *B. licheniformis* in the IMS solutions

7.3.3.1 pH value

The growth of microorganisms, particularly for the TVM (Figure 7.2), in the IMS solutions during 21 days of storage was accompanied with a significant ($P \leq 0.05$) reduction in the pH of the solution (Figure 7.5). The control IMS solutions also had a significant ($P \leq 0.05$) higher reduction rate than that of the IMS solutions supplemented with nisin and was significantly ($P \leq 0.05$) affected by the storage temperature. The pH reduction in the IMS solutions supplemented with nisin was not significantly ($P > 0.05$) affected by the nisin addition time and storage temperatures.

7.3.3.2 Acidity value

Although in general the result of the acidity measurement of different IMS treatments (Figure 7.6) corresponded to the finding of the pH (Figure 7.5), the acidity of the IMS solutions supplemented with nisin directly before pasteurization had significantly ($P \leq 0.05$) higher value than those of the IMS solutions supplemented with nisin 30 to 120 min before pasteurization after 10 days of storage. This finding directly reflected the TVM result (Figure 7.2) and demonstrated that the measurement of acidity could be more sensitive than the pH measurement in this experiment.

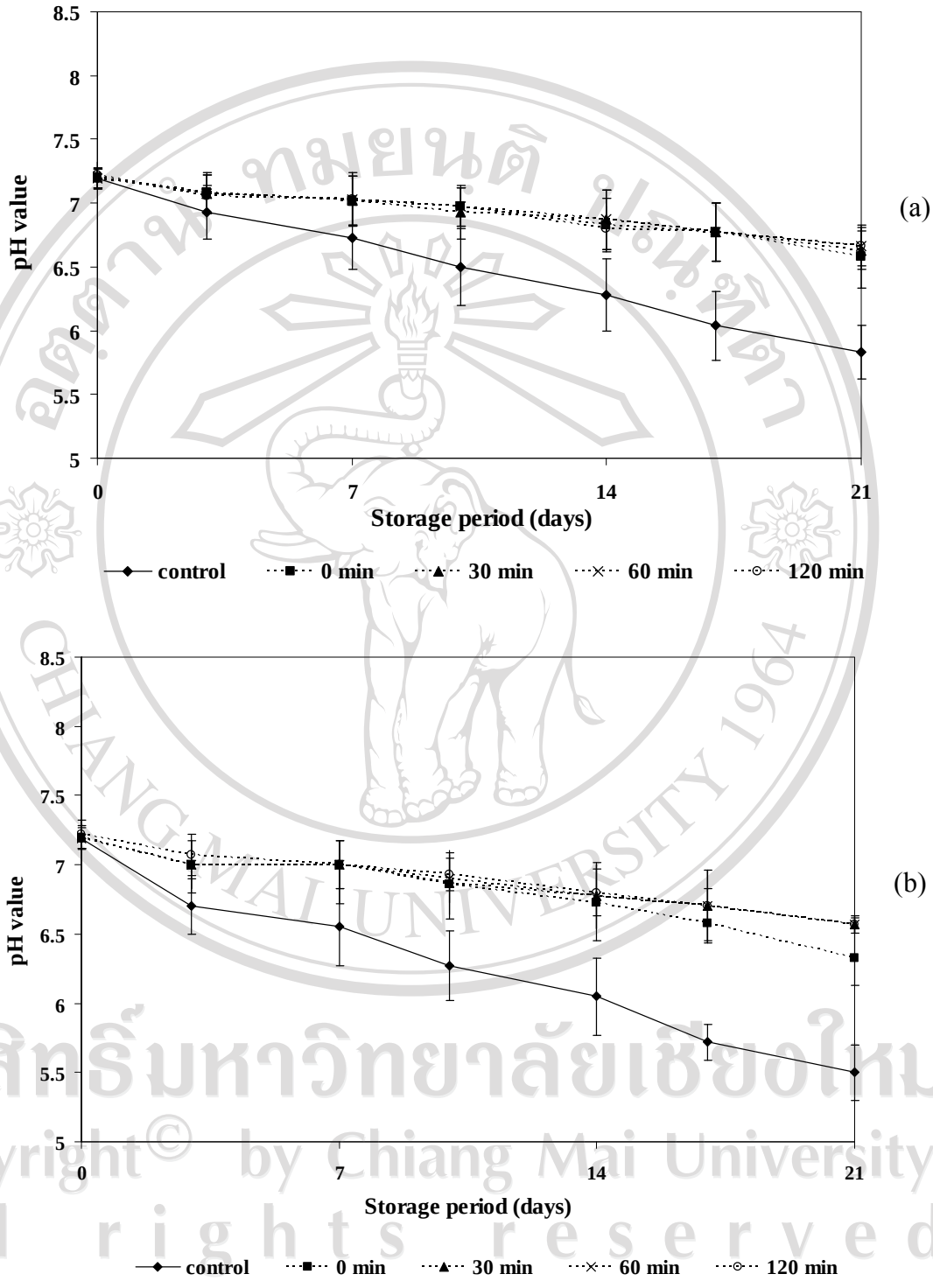

Figure 7.5 pH value of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times before pasteurization during storage at 4ºC (a) and 10ºC (b).

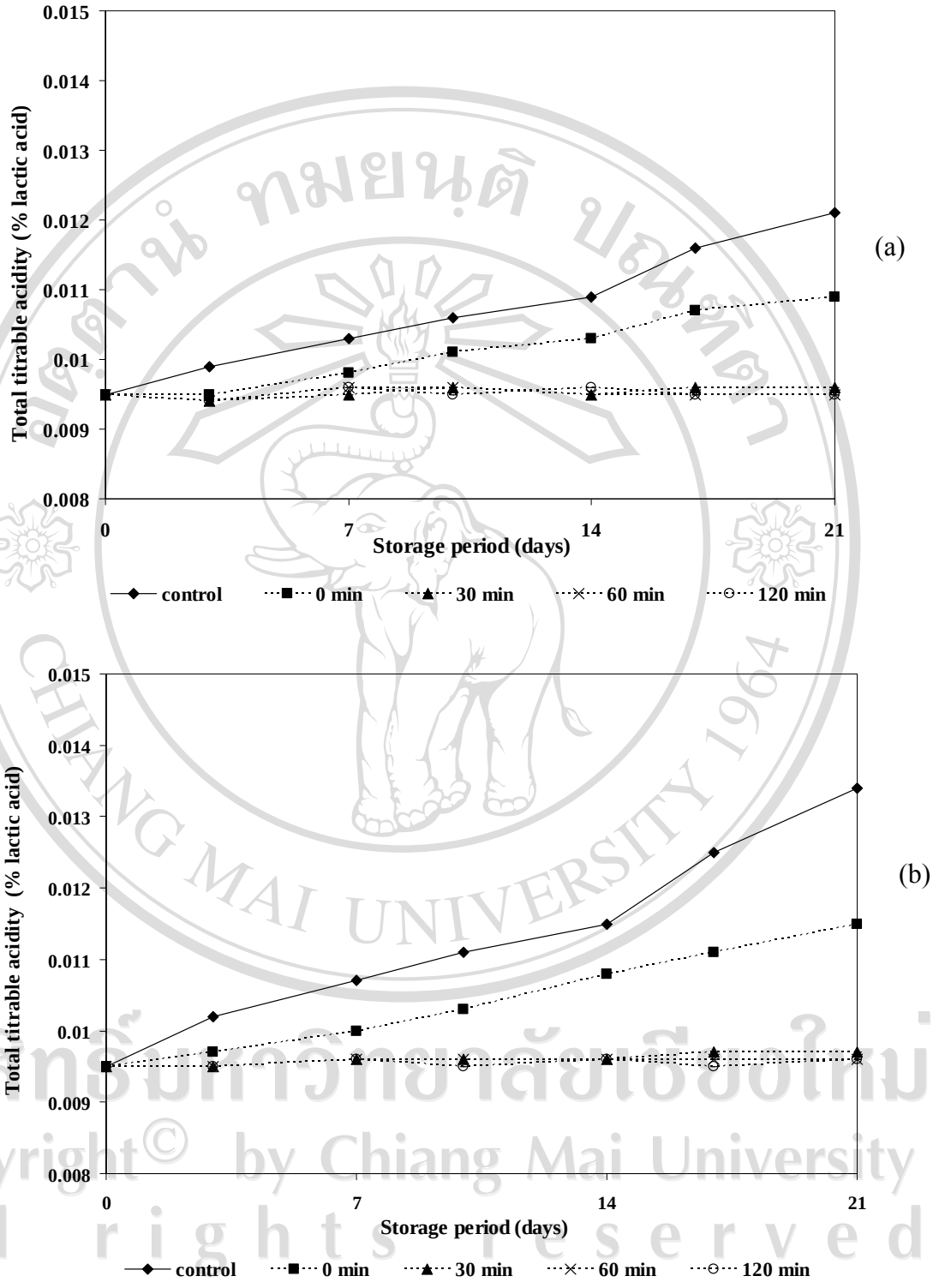

Figure 7.6 Total acidity of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times before pasteurization during storage at 4ºC (a) and 10ºC (b).

7.3.4 Nisin assay of the IMS solutions affected by the nisin addition time before pasteurization

The residual nisin activity of the IMS solutions studied in this section can be seen in Figure 7.7. Collected data demonstrated that the nisin activity was significantly ($P \leq 0.05$) affected by the storage time and the addition time of the antimicrobial compound into the IMS solutions before pasteurization, but was not affected by the storage temperature. Longer storage period produced lower nisin activity. At the same time, supplementation of nisin directly before pasteurization caused the compound to have significantly ($P \leq 0.05$) higher reduction rate than that of the supplementation which was carried out 30 to 120 min before pasteurization after 7 days of storage. This finding might explain significant higher TVM counts in the first supplementation treatment (Figure 7.2). Reduction of the nisin activity below 47.26±0.00 IU/ml after 7 days of storage might also influence the growth of TVM in the IMS solutions supplemented with nisin 30 to 120 min before pasteurization. This microbial growth could also be affected by a better adaptation of the microorganisms in the presence of nisin and a recovery from heat injured mechanisms after 7 storage days. No nisin activity was detected in the control IMS treatment.

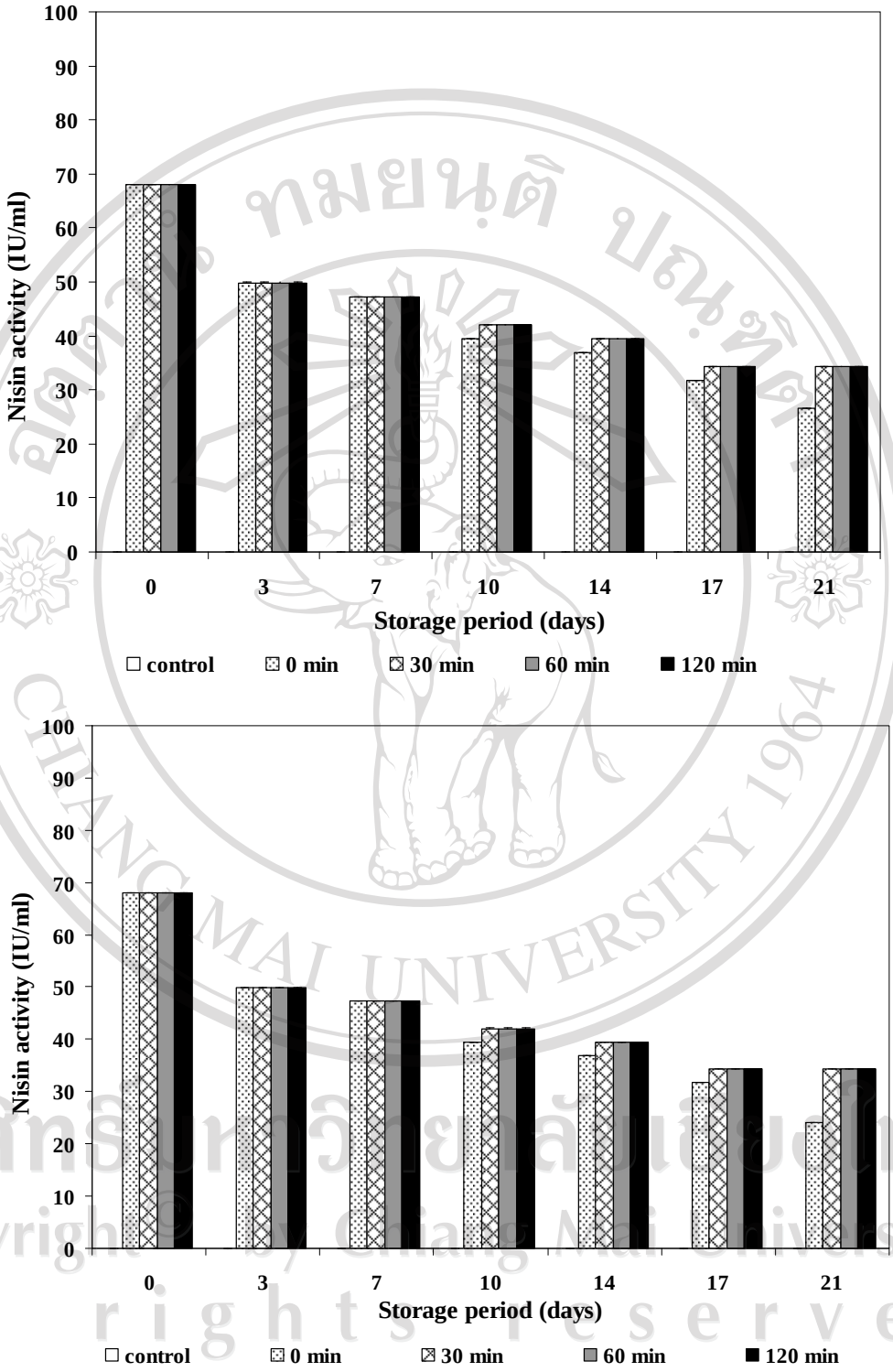

Figure 7.7 Nisin activity of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times before pasteurization during storage at 4ºC (a) and 10ºC (b).

7.3.5 The effect of nisin addition time after pasteurization on the effectiveness of nisin to inhibit *B. licheniformis* in the IMS solutions

Chemical composition of IMS solutions

The IMS solution produced in this subsection was prepared from 2% fat and 1% WPI and divided into five small batches. One of these IMS batch was used as a control treatment, whereas the other four batches were supplemented with 100 IU/ml nisin at different addition time. The nisin IMS solutions were composed of 2.08±0.01% (w/v) fat, 1.26±0.01% (w/v) protein and 0.20±0.01% (w/v) carbohydrate (Figure 7.8). These IMS solutions had 3.84±0.01% (w/v) total solid and 48.18% of this amount was solid not fat. The composition of the nisin added IMS solutions was not significantly ($P>0.05$) different to that of the control IMS treatment.

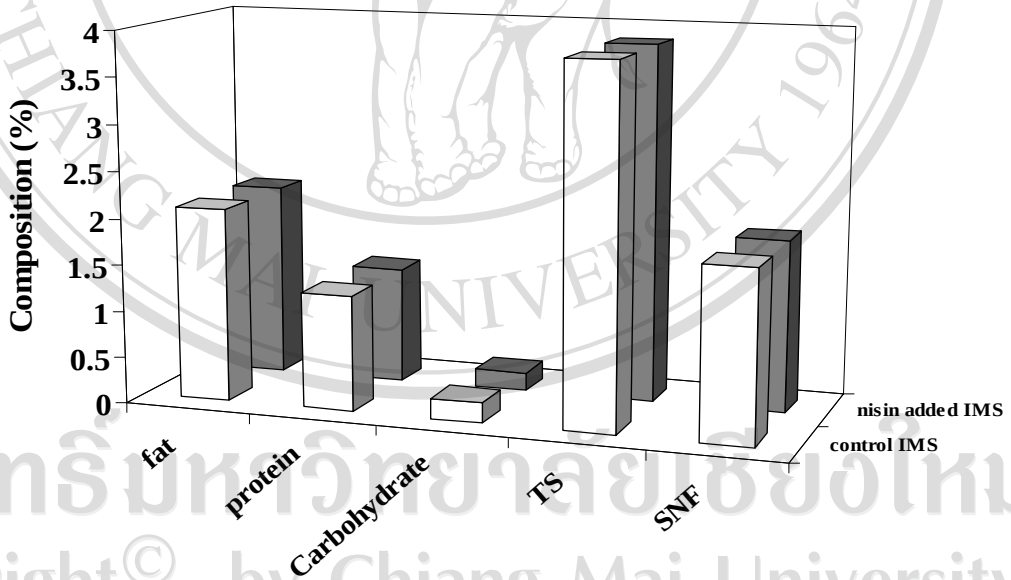

Figure 7.8 Chemical composition of IMS solutions with and without nisin addition supplemented after pasteurization.

7.3.6 The microbiological effect of nisin addition time after pasteurization on the effectiveness of nisin to inhibit *B. licheniformis* in the IMS solutions

7.3.6.1 TVM count

Using an initial *B. licheniformis* population of 3.59±0.12 log cfu/ml, a pasteurization process of 72ºC for 15 s could reduce the bacilli population for 0.93 log cycle in the control IMS treatment (Figure 7.9). Supplementation of 100 IU/ml nisin directly after the pasteurization produced a significant ($P \leq 0.05$) higher reduction rate of 2.03 log cycle compared to that of the control IMS treatment. However, adding the nisin solution 30 to 120 min after the pasteurization process gave the highest reduction rate of 2.19 to 2.20 log cycle and was significantly ($P \leq 0.05$) different to that of the IMS solutions supplemented with nisin directly after the pasteurization. This finding confirmed the result in the section 7.3.2.1 that nisin and pasteurization could work synergistically to reduce the bacilli population. In this section, it was demonstrated that heat injured bacteria were susceptible to the presence of nisin. However, there was not any simple explanation for the result that found longer nisin lag period prior to the addition produced higher reduction rate in the bacilli population. A more detail experiment might need to be carried out to explain this result.

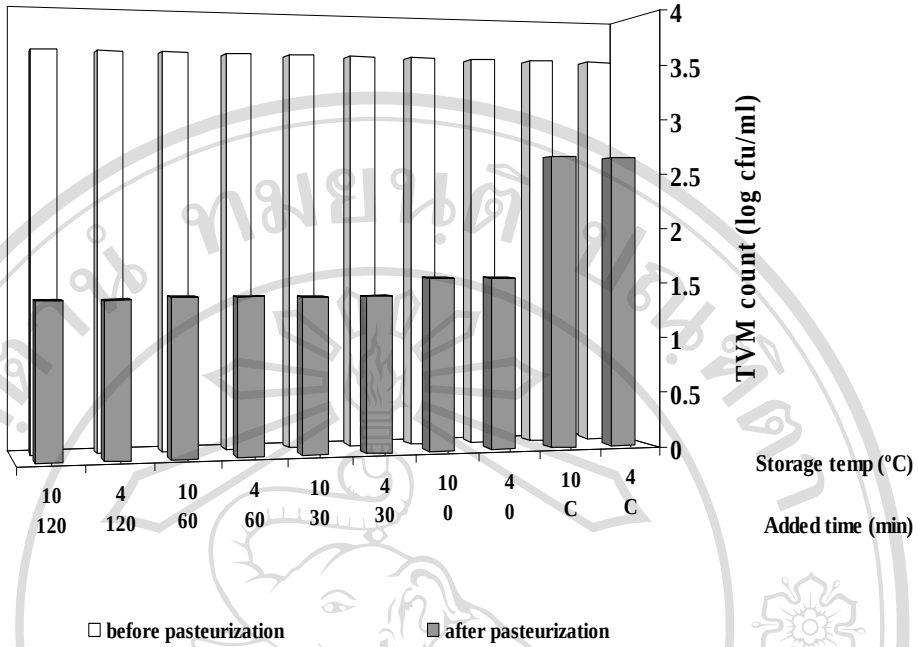

Figure 7.9 Total Viable Microorganisms of the IMS solutions with and without 100 IU/ml nisin supplemented after pasteurization and measured before pasteurization and directly after pasteurization or nisin addition.

During 21 days of storage at 4 and 10ºC, the control IMS solutions had a significant ($P \leq 0.05$) increase in their TVM count and were significantly ($P \leq 0.05$) affected by the storage temperature after 3 days storage (Figure 7.10). On the other hand, the presence of nisin played an important role in inhibiting the growth of the bacilli population and produced a slow increase in the TVM number throughout the storage period. This effectiveness of nisin caused the TVM number in the nisin added IMS solution to be significantly ($P \leq 0.05$) lower than that of the control IMS solutions. From the 7th day of storage onward, the storage temperature significantly ($P \leq 0.05$) affected the TVM number of the IMS solutions supplemented with nisin immediately after pasteurization, whereas the same effect was occurred after 17 days of storage for the IMS solutions supplemented with nisin 30 to 120 min after pasteurization. This result demonstrated a better control of nisin against TVM population at higher storage

temperature when the protein compound added at longer lag period after pasteurization. On the 14th day of storage, the IMS solutions supplemented with nisin directly after pasteurization contained significantly ($P \leq 0.05$) higher TVM numbers than those of the IMS treatments supplemented with nisin 30 to 120 min after pasteurization. Therefore at the end of the storage period, the IMS solution supplemented with nisin 120 min after pasteurization and stored at 4ºC had significantly ($P \leq 0.05$) the lowest TVM count compared to those of the other nisin added IMS treatments and the control IMS solution.

TVM data in this section demonstrated that nisin could be supplemented into food products after the main heat treatment, although an extra preparation to separately sterilize the nisin solution and a particular attention during the supplementation step needed to be carried out. The effectiveness of nisin supplemented after pasteurization against microbial population depended on the addition time (lag period) after pasteurization, the storage temperature and storage period.

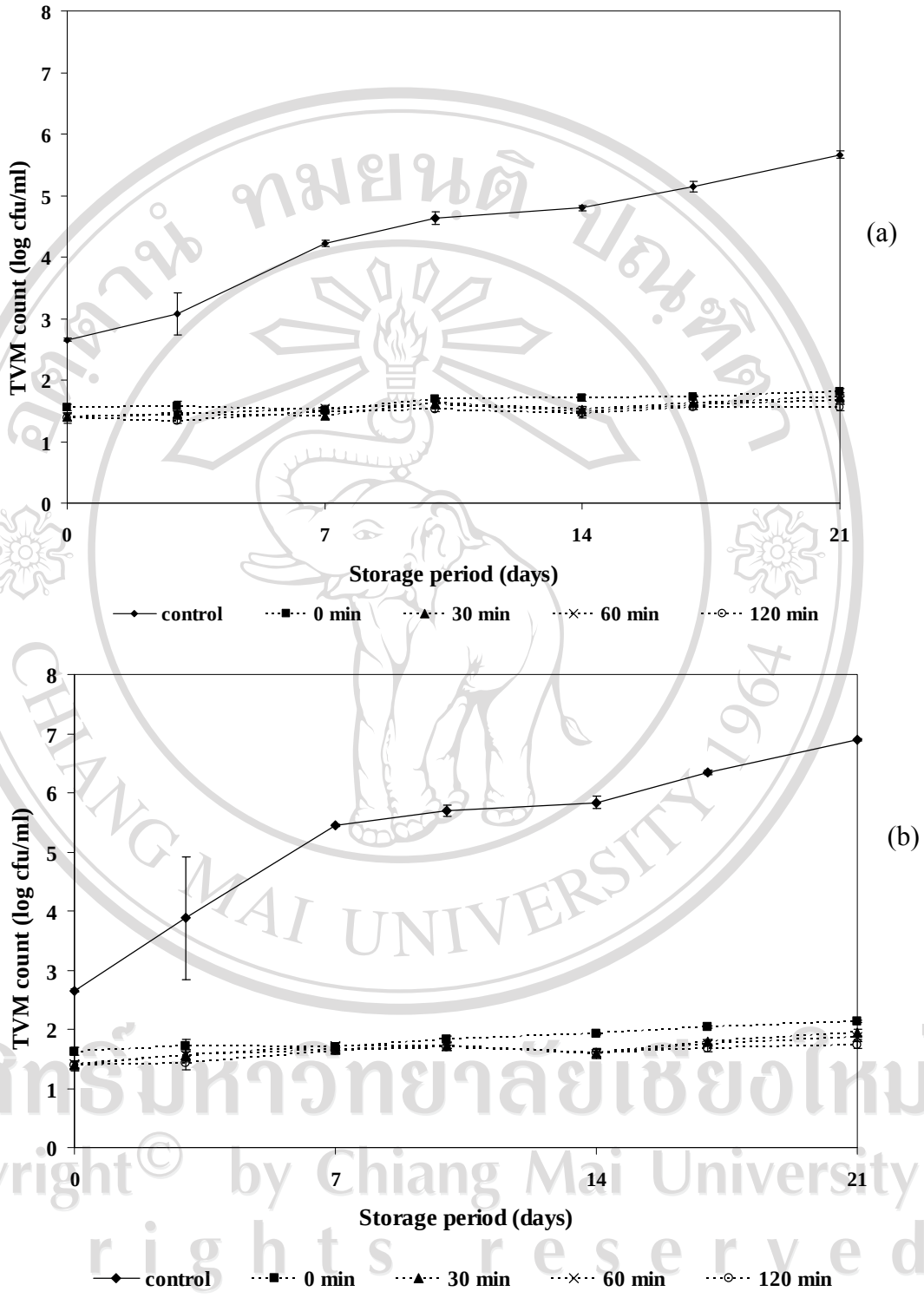

Figure 7.10 Total Viable Microorganisms of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times after pasteurization during storage at 4ºC (a) and 10ºC (b).

7.3.6.2 Spore count

In general, the result of the spore displayed a similar pattern as the finding in the TVM count (Figure 7.10). The addition time (lag phase) of nisin after pasteurization, storage temperatures and storage time affected the activity of nisin against the bacilli spore form. However these effects might not be seen clearly due to a lower number of spore after pasteurization and a slow growth of the microorganisms during the storage period.

The control IMS solutions had significantly ($P \leq 0.05$) higher spore count directly after pasteurization and during 21 days of storage compared to those of the nisin added IMS treatment (Figure 7.11). The storage temperature significantly ($P \leq 0.05$) affected the spore number in the IMS control treatment after 17 days of storage. The nisin added IMS solutions had a spore count of lower than 1.00 log cycle for most of the studied storage period, except for the spore count in the IMS solution supplemented with nisin directly after pasteurization on the last day of storage. This last treatment, especially for the IMS samples stored at 10ºC, contained higher spore counts compared to those of the other nisin added IMS solutions after 21 days of storage. On the other hand, the spore counts of the IMS solutions supplemented with nisin 120 min after pasteurization and kept at 4ºC produced the lowest spore number and was significantly lower than those of the other nisin added IMS solutions at the end of the studied storage period.

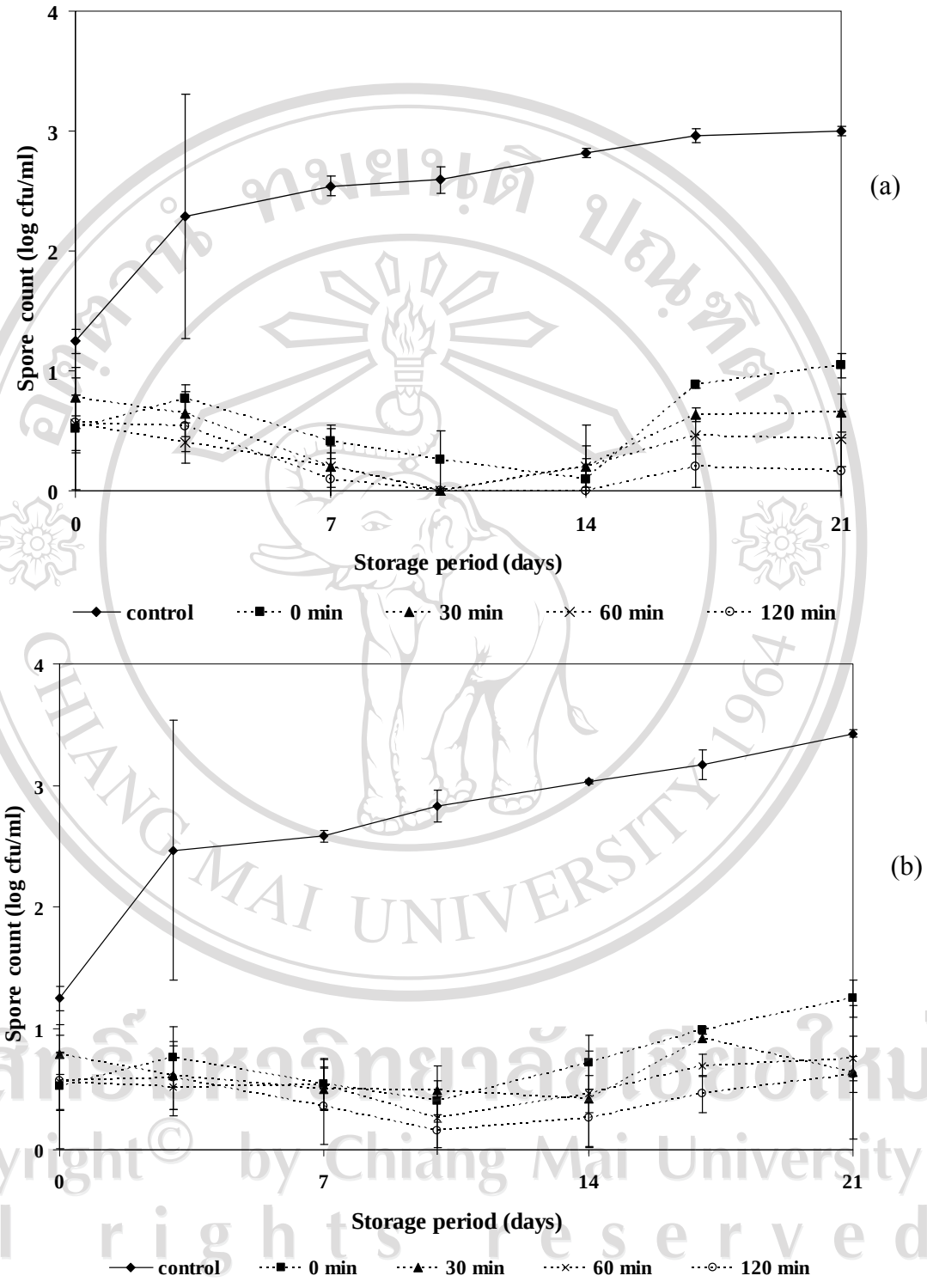

Figure 7.11 Spore count of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times after pasteurization during storage at 4ºC (a) and 10ºC (b)

7.3.6.3 Thermoduric count

The presence of nisin significantly (P≤0.05) reduced the thermoduric counts in the IMS treatments between 0.39 and 0.53 log cycle lower than that of the control IMS treatment (Figure 7.12). During storage at 4 and 10ºC for 21 days, the control IMS solution experienced significant increases in its thermoduric count for up to 2.64 and 3.87 log cycle, respectively, and were significantly (P≤0.05) affected by the storage temperature after 7 days of storage. A slow increase in the thermoduric count of the nisin added IMS solutions was recorded. At 4ºC storage temperature, the IMS solutions supplemented with nisin at 0 (directly), 30, 60 and 120 min after pasteurization had an increase in thermoduric bacilli for 0.34, 0.15, 0.12 and 0.16 log cycle, respectively, after 21 days of storage. Applying a higher storage temperature caused a higher increase in the thermoduric count of the IMS solutions of 0.63, 0.36, 0.29 and 0.32 log cfu/ml, respectively, at the end of the storage period. An increase in the thermoduric count for 2 times in the nisin added IMS solutions kept at 10ºC compared to that of the samples stored at 4ºC showed that the storage temperature affected the effectiveness of nisin against the thermoduric bacilli. However, the addition time of nisin after pasteurization did not produce a significant effect in inhibiting the growth of thermoduric bacilli within 21 days of storage.

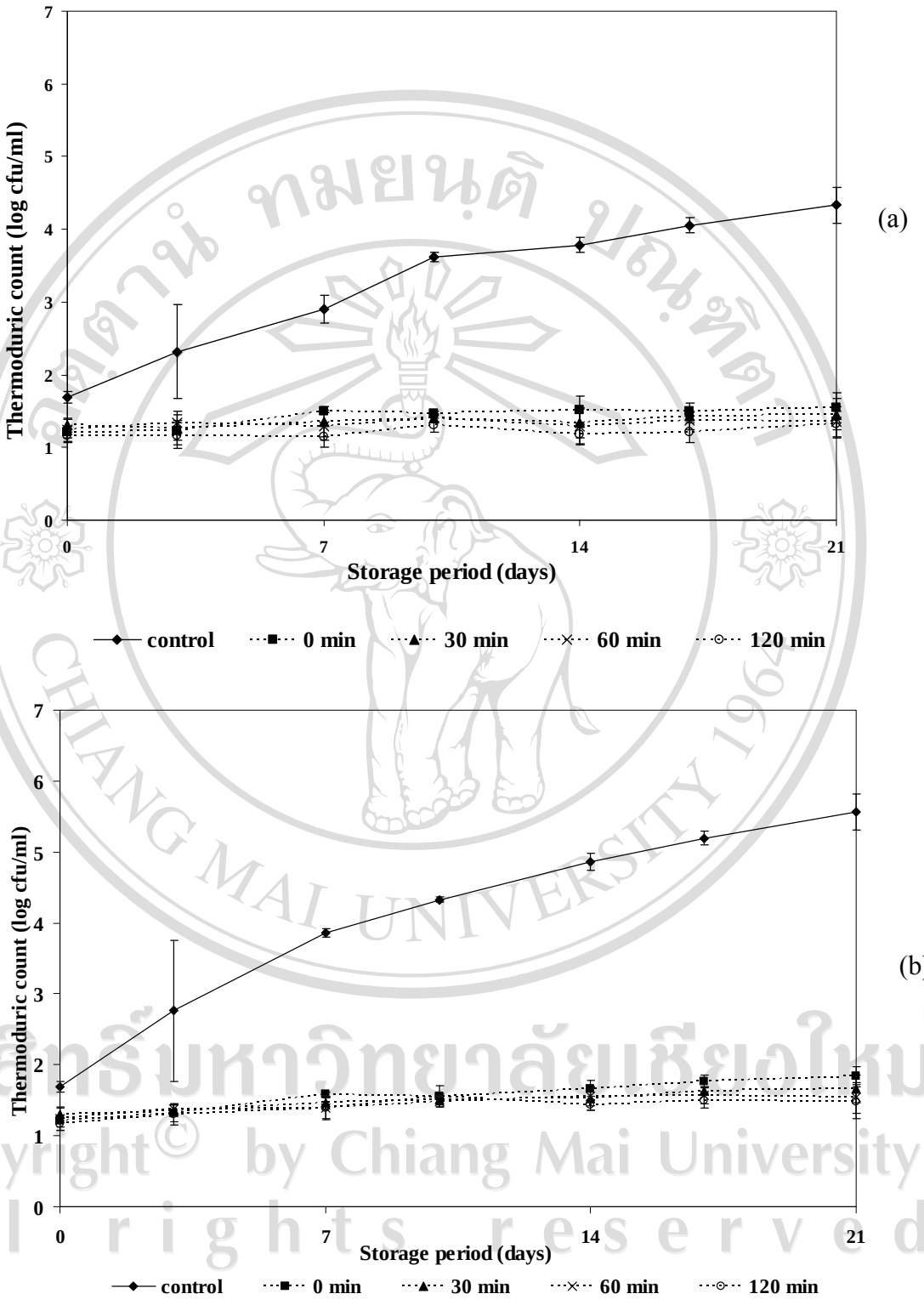

Figure 7.12 Thermoduric count of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times after pasteurization during storage at 4ºC (a) and 10ºC (b).

7.3.7 The chemical effect of nisin addition time after pasteurization on the effectiveness of nisin to inhibit *B. licheniformis* in the IMS solutions

7.3.7.1 pH value

Changing in the pH value of the IMS solutions supported the result of the microorganism growth in the IMS treatments (Figures 7.10 and 7.12). Significant ($P \leq 0.05$) reductions in the pH value of the control IMS solution were recorded during the storage period. Lower pH values of the control IMS solution stored at higher storage temperature were found throughout 21 days of storage and were significantly different ($P \leq 0.05$) at the of the storage period (Figure 7.13). The pH reduction in the nisin added IMS solutions occurred at a slower rate than that of the control IMS treatments. Although the storage temperature did not significantly ($P > 0.05$) affected the pH of the nisin added IMS solution, lower pH values in the IMS samples stored at 10ºC were noted. The nisin addition time after pasteurization also did not significantly ($P > 0.05$) affected the pH of the IMS solutions supplemented with nisin.

7.3.7.2 Acidity value

As expected, the acidity of different IMS solutions increased during storage period. The storage temperature, the presence of nisin and the nisin addition time after pasteurization influenced the increase in the acidity value (Figure 7.14). The control IMS treatment had the highest increasing rate of the acidity followed by the IMS solution supplemented with nisin directly after pasteurization and the IMS solutions supplemented with nisin 30 to 120 min after pasteurization. The IMS solutions supplemented with nisin directly after pasteurization had significantly ($P \leq 0.05$) higher acidity values than those of the other nisin added IMS solutions after 10 days of storage at both storage temperatures. This finding was similar to the result in the section 7.3.3.2 (Figure 7.5) and supported the results of the TVM measurement (Figure 7.10) that supplementation of nisin directly after pasteurization was not as effective as the supplementation of nisin at longer lag period after pasteurization in controlling the growth of vegetative bacteria.

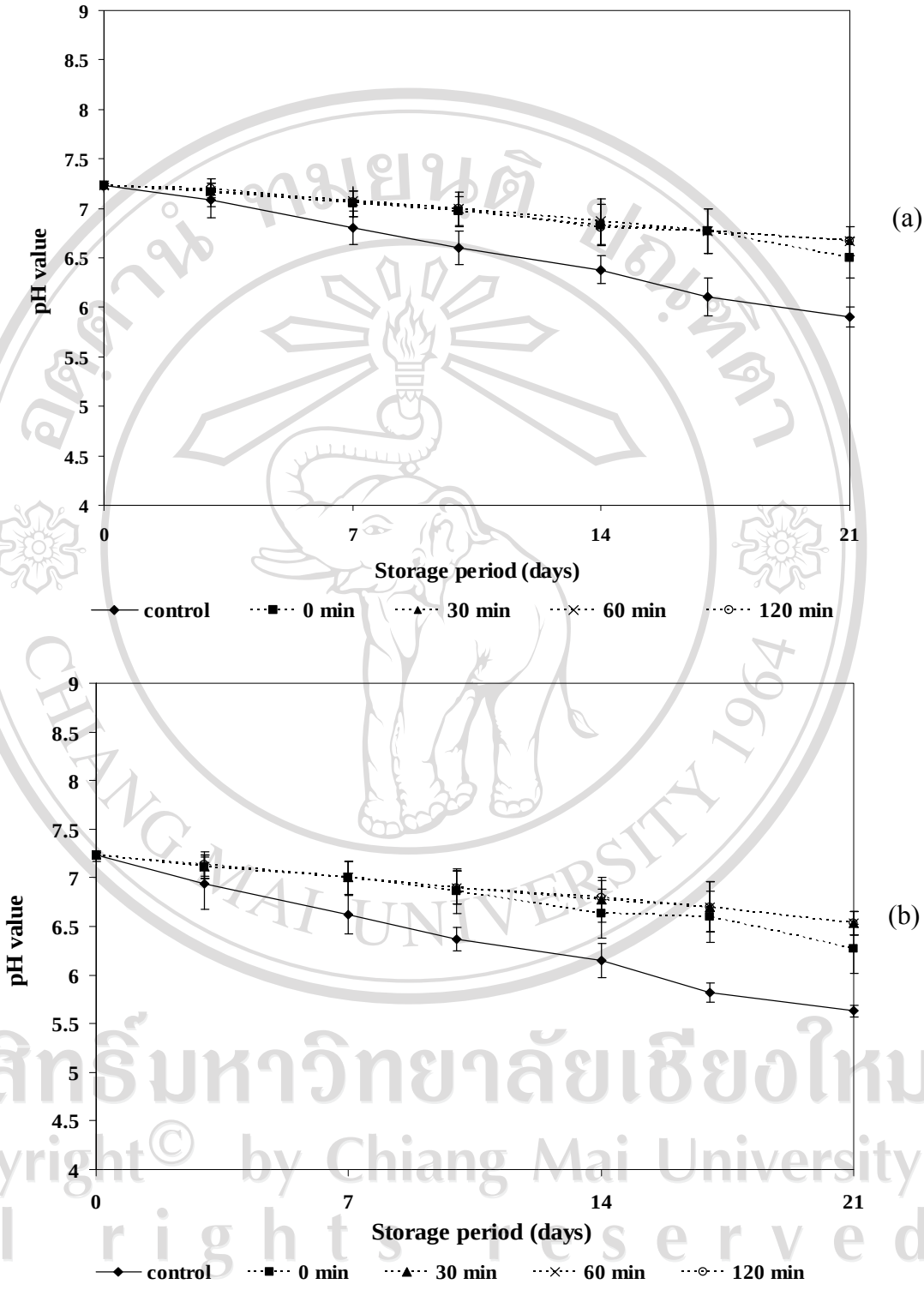

Figure 7.13 pH value of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times after pasteurization during storage at 4ºC (a) and 10ºC (b).

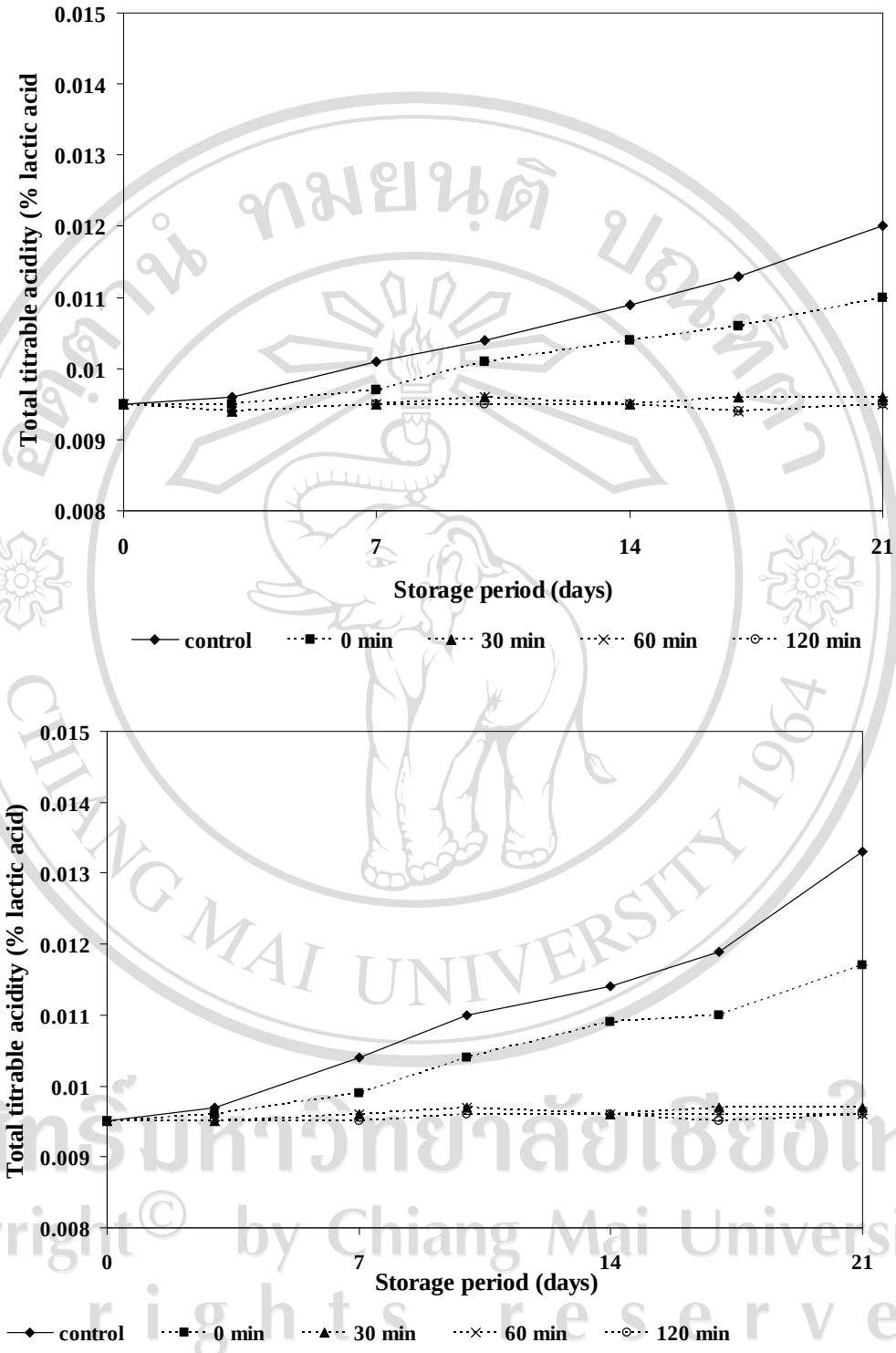

Figure 7.14 Total titrable acidity of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times after pasteurization during storage at 4ºC (a) and 10ºC (b).

7.3.8 Nisin assay of the IMS solutions affected by the nisin addition time after pasteurization

Although it has been reported that nisin was heat sensitive at higher pH value, it was not expected that the addition of nisin after pasteurization produced similar reduction as when the antimicrobial compound was supplemented before pasteurization (section 7.3.4). When nisin was incorporated before pasteurization, the concentration of nisin at the beginning of the storage period was 67.93±0.06 IU/ml (Figure 7.7), whereas the nisin concentration in this section (added after pasteurization) was 65.34±0.06 IU/ml on the first day of storage (Figure 7.15). This result might indicate that a mild heat treatment, such as pasteurization, might not be fully responsible in the reduction of nisin activity. Other factors, such as composition of food products, microbial load in food products and pH of food products could give a more significant effect in the reduction of the nisin activity in a food product that passed a pasteurization treatment.

Storage temperature and storage time affected the availability of nisin during 21 days of storage at 4 and 10ºC (Figure 7.15). However, the addition time of nisin after pasteurization did not affect the residual nisin activity in the nisin added IMS solutions. No nisin activity was detected in the control IMS treatment.

7.4 Conclusions

Data in this section clearly demonstrated that the presence of 100 IU/ml nisin had a significant ($P \leq 0.05$) effect in improving the microbial quality of the IMS solutions and reducing the chemical changing in the product. Selection of storage temperature and storage period are important factors that affect the effectiveness of nisin to inhibit microbial growth and extend the product shelf life. The supplementation of nisin can be done before or after the main heat treatment. However, an addition step of nisin with a lag period of 30 to 120 min before or after pasteurization would enhance the work of nisin against different types of microorganism in the product compared to the addition of the protein directly before or after pasteurization. This information will be important for food manufactures that use nisin in their product in designing their food production process.

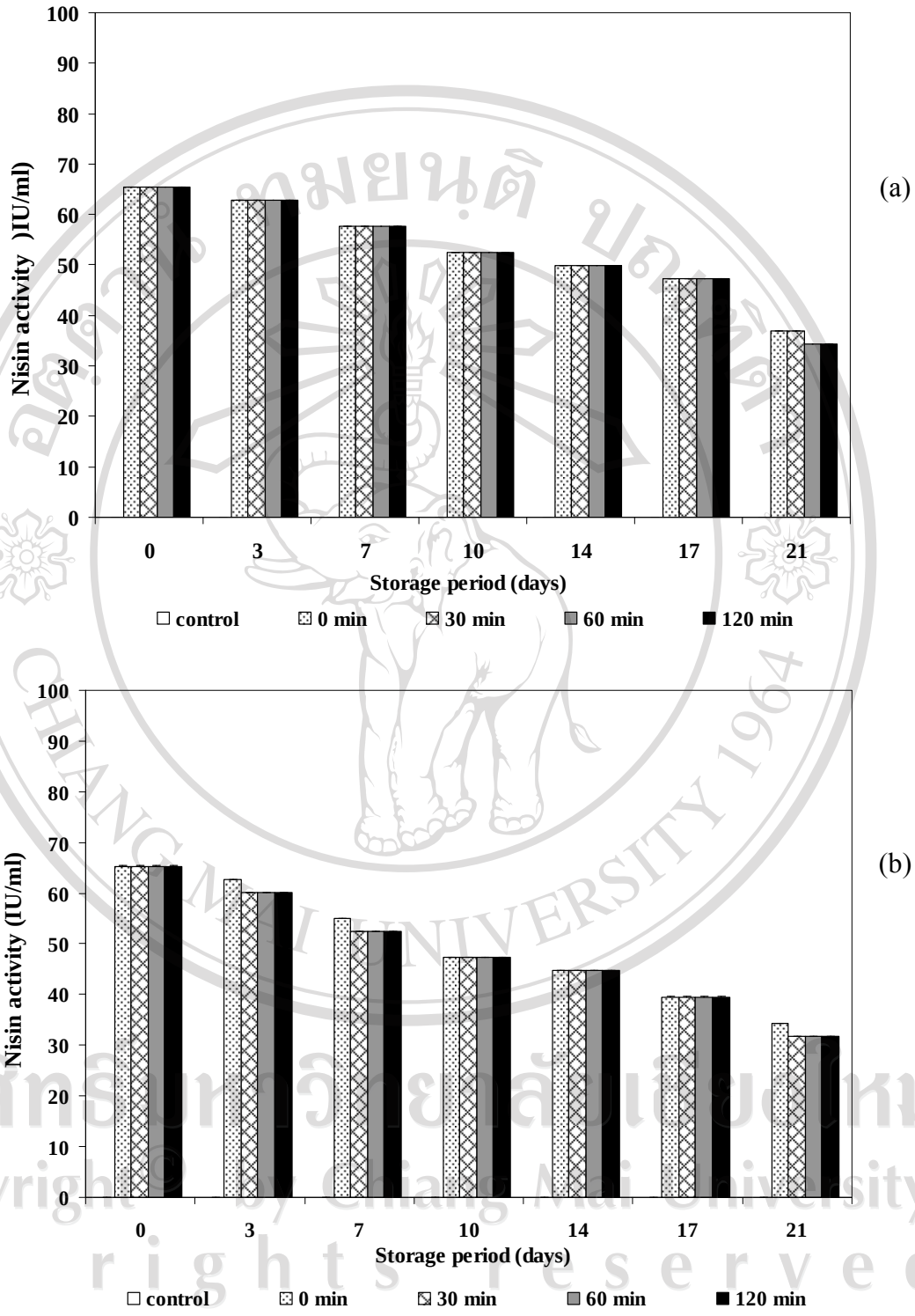

Figure 7.15 Nisin activity of IMS solutions with and without 100 IU/ml nisin supplemented at different addition times after pasteurization during storage at 4ºC (a) and 10ºC (b).