Chapter 4

Results and discussion

The result of this present work evaluated the effect of LAB starter cultures including *Pediococcus pentosaceous*, *Pediococcus acidilactici*, *Weissella cibaria*, *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus sakei* on the quality characteristics of Thai fermented sausage. The experimental results with the starter cultures (inoculated batches) were compared with naturally fermented sausage (control batch). Fermented sausages were characterized by a rapid decrease in pH during fermentation accompanied by the development of microbiology, cured meat pigment, glucose, organic acids and flavour. For flavour development, the sausage volatile compounds, 3-methyl-butanal, 3-methyl-butanoic acid and 3-methylbutanol derived from leucine were investigated by GC. In addition, this chapter stated the proteolytic effect of starter cultures on Thai fermented sausage. The hydrolysis of sarcoplasmic and myofibrillar proteins was evaluated. Non-protein nitrogen, peptides and free amino acids released were determined. The prepared Thai fermented sausages were submitted to sensory evaluation to assess the differences between control sample and samples inoculated with starter cultures. Finally, the mathematical modelling of fermented sausage was developed and verified with experimental results.

4.1 Microbiological analysis

The trajectories of microbial counts in the Thai fermented sausage during processing are presented in Figure 4.1. The growth pattern of total viable count (TVC) was enumerated on nutrient agar, which is not selective agar. Besides lactic acid bacteria (LAB), all microorganisms can grow on this agar as illustrated in Figure 4.1a. It was observed that the TVC count was in accordance with the expected growth of LAB in all processes. The population of TVC was higher than that of LAB due to growth of other microorganisms at 0 h. Thus, fermentation of Thai sausage was dominated by LAB. Table 4.1 presents statistic of amount of microbiological analysis

at the end of fermentation. Sausages inoculated with *P. acidilactici*, *P. pentosaceus*, *L. pentosus* and *L. sakei* showed significantly higher amount of TVC ($p < 0.05$) than control (uninoculated sausage). The amount of TVC obtained from sausage inoculated with *L. plantarum* was insignificantly higher ($p > 0.05$) than control. In *W. cibaria* batch, the amount of TVC was significantly lower ($p < 0.05$) than that of control. Sausages inoculated with *P. acidilactici*, *L. pentosus* and *L. sakei* were not significantly different ($p > 0.05$), but they showed the significant difference from *P. pentosaceus*, *W. cibaria* and *L. plantarum* as shown in Table 4.1.

LAB counts increased during the fermentation of both the control and inoculated sausages as shown in Figure 4.1b. Initial LAB counts in the sausages with starters added were higher than in the control due to the introduction of starters. An inoculation concentration of 2×10^7 CFU/g was attempted, but the population of LAB observed in inoculated sausages was variable at 0 h due to the contamination of raw pork with LAB house flora prior to the addition of external starter cultures. However, the statistical test was performed. The LAB count at 0 h was not significant difference ($p>0.05$) among inoculated sausages. The LAB count of the inoculated batches at 60 h showed significant higher ($p < 0.05$) than the control as shown in Table 4.1. The number of LAB in inoculated sausages increased and reached maximum levels of ~9.7-10.3 log cfu g^{-1} sausage at the end of fermentation, whereas the control attained a maximum of 9.1 log cfu g^{-1} sausage. Inoculation with of LAB showed significant difference among *P. pentosaceus*, *W. cibaria*, *L. plantarum*, and *L. sakei* while *P. acidilactici* and *L. pentosus* showed no significant difference ($p > 0.05$) as shown in Table 4.1. The results were consistent with previous studies showing that LAB are commonly found in naturally fermented sausages. For example, Ammor et al. (2005) reported that *L. sakei* strains were isolated from traditional dry sausage. Bonomo et al. (2008) characterized LAB from traditional fermented sausages of the Basilicata region. The results showed that *L.sakei* was the predominant species (67%) followed by *P. pentosaceus* (16%), *Leuconostoc carnosum* (8%), *L. plantarum* (4%), *L. brevis* (2%) and *Leuconostoc pseudomesenteroides* (2%). Malti and Amarouch, 2008 found that the LAB constituted the major microflora of the naturally fermented sausage, because the cell numbers of the total viable count and MRS count were comparable after day 3 of the fermentation. In addition, Tanasupawat and Komagata (1995)

described that homofermentative strains of *L. pentosus*, *L. plantarum*, *L. sakei*, *P. acidilactici*, *P. pentosaceus* and other Lactobacillus spp. occurred in a variety of fermented Thai food such as Nham, Pla-som and Sai-krog-prieo.

The counts of staphylococci/micrococci increased in the inoculated batches to a level of 6-6.5 log cfu g^{-1} sausage at 24 h, thereafter, the counts decreased to 4.4-5 log cfu g^{-1} sausage depending upon the starter. The sausage inoculated with *P. pentosaceus* showed the significant lowest ($p < 0.05$) staphylococci/micrococci count, while the staphylococci/micrococci counts in control samples slowly decreased to 5.4 log cfu g^{-1} sausage as showed in Figure 4.1c. At the end of fermentation, the count of staphylococci/micrococci in *L. sakei* batch was significantly different from other inoculation batches ($p < 0.05$). Moreover, there was no significant difference ($p > 0.05$) among *P. acidilactici*, *W. cibaria* and *L. pentosus* batches. Inoculation with *L. plantarum* was not significantly different ($p > 0.05$) from *L. pentosus* and *P. acidilactici* as shown in Table 4.1.

Yeast and mold counts of about 5.86 log cfu g^{-1} were found in sausages before fermentation. A reduction of yeast and mold counts was observed at 24 h of fermentation in all batches, as shown in Fig 4.1d. After 60 h of fermentation, the inoculation sausages with LAB were significantly lower than control ($p < 0.05$) as shown in Table 4.1. The population of yeast and mold was decreased to 4.3-4.6 log cfu g^{-1} sausage in sausage inoculated with starters and to 5.2 log cfu g^{-1} sausage in control sample. Yeast and mold counts of *P. pentsaceus*, *W. cibaria* and *L. plantarum* batches were not significantly different ($p > 0.05$). Yeast and mold counts of *P. acidilactici* batch were significantly different ($p < 0.05$) from *L. pentosus* and *L. sakei* batches. There was not significantly different ($p > 0.05$) between *L. pentosus* and *L. sakei* batches. The results are consistent with the observations of Annalisa et al. (2007), Visessanguan et al. (2006) and Yongjin et al. (2006) who considered acidification to be the main cause of staphylococci/micrococci, yeast and mold inhibition in dry fermented sausages. Moreover, the pH drop below 4.5, which was well above the tolerance level of many yeast and mold. Thus, acidification alone cannot account for the reduction of yeast and mold. Additionally, certain antimicrobials such as bacteriocins are antibacterial proteins which are produced by

LAB. The bacteriocins can kill or inhibit the growth of other bacteria (Cleveland, et al., 2001; Leroy and de Vuyst, 1999; Malti and Amarouch 2008).

The production of fermented sausages was based on spontaneous fermentation due to the development of the natural microflora presented in raw meat. LAB dominated the fermentation process. Inoculation of the sausage mixture with a starter culture composing of selected LAB, i.e., homofermentative lactobacilli and pediococci improved the quality and safety of the final product (Leroy et al., 2006). They caused rapid acidification of meat through the production of organic acid, mainly lactic acid. Metabolic products of LAB, such as organic acids and bacteriocins, are known as antimicrobial agents (Bromberg et al., 2004). The dominance of LAB could inhibit the growth of pathogens and avoid spoilage which sometimes caused food-borne diseases (Cleveland, et al., 2001; Ammor et al., 2006). This is an important characteristic related to probiotic effects of LAB. Starters LAB have been demonstrated to reduce pathogenic bacteria in meat fermentation. Bartholomew and Blumer (2006) studied the inhibition of Staphylococci by lactic acid bacteria in country-style hams. *L. plantarum* and *P. cerevisiae* inoculated in model meat system inhibited *S. epidermidis*. The staphylococci would gradually die off as the lactic acid microorganisms produced lactic acid. Similar to the study of Antara et al. (2004), the use of *L. plantarum* and *P. acidilactici* as starter cultures could suppress micrococcus growth. Moreover, Prachyakij et al. (2008) reported that yeast contamination in the fermented plant beverages was inhibited by *L. plantarum*.

Figure 4.1

Microbiological count profiles in Thai fermented sausages with or without starter cultures during fermentation: (a) Total viable count (TVC), (b) Lactic acid bacteria (LAB) count, (c) Staphylococci/Micrococci count, (d) Yeast and mold.

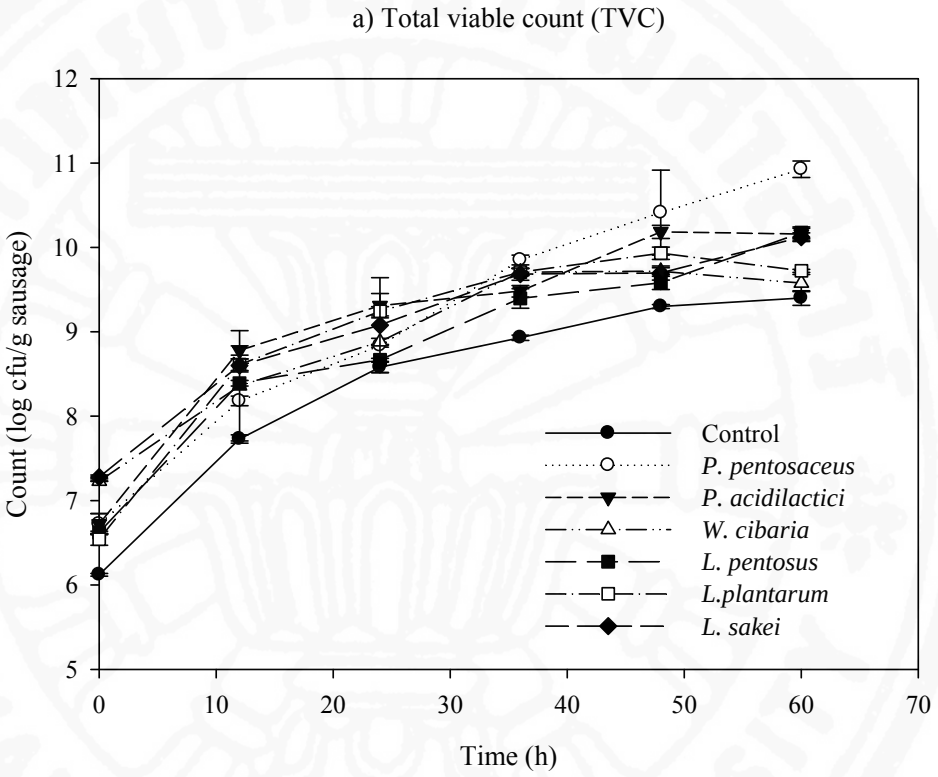
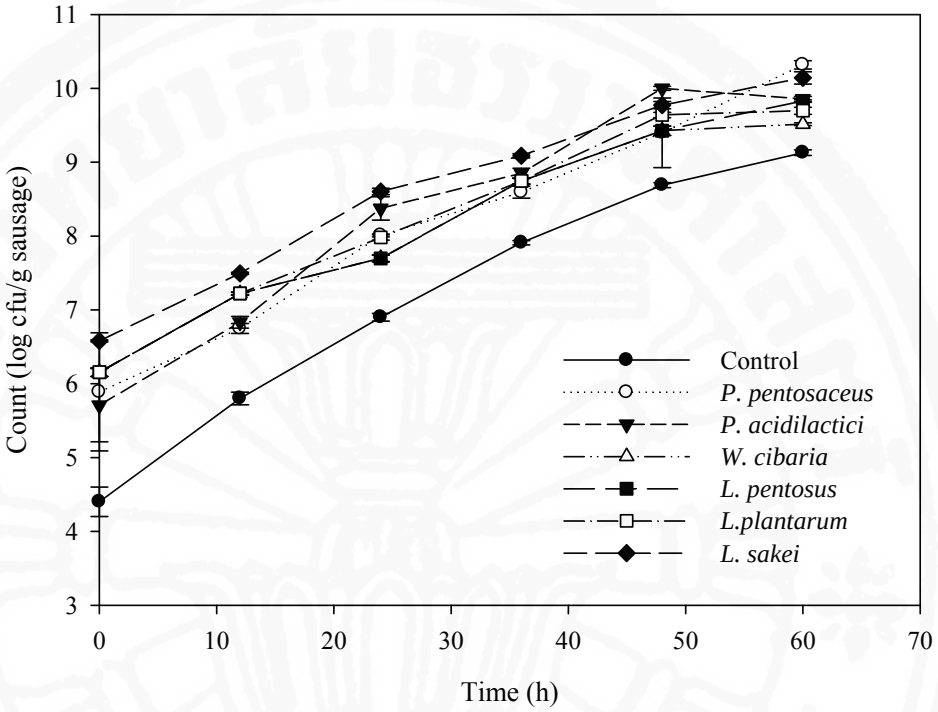


Figure 4.1 (Continued)

b) Lactic acid bacteria (LAB)



c) Staphylococcus/micrococcus

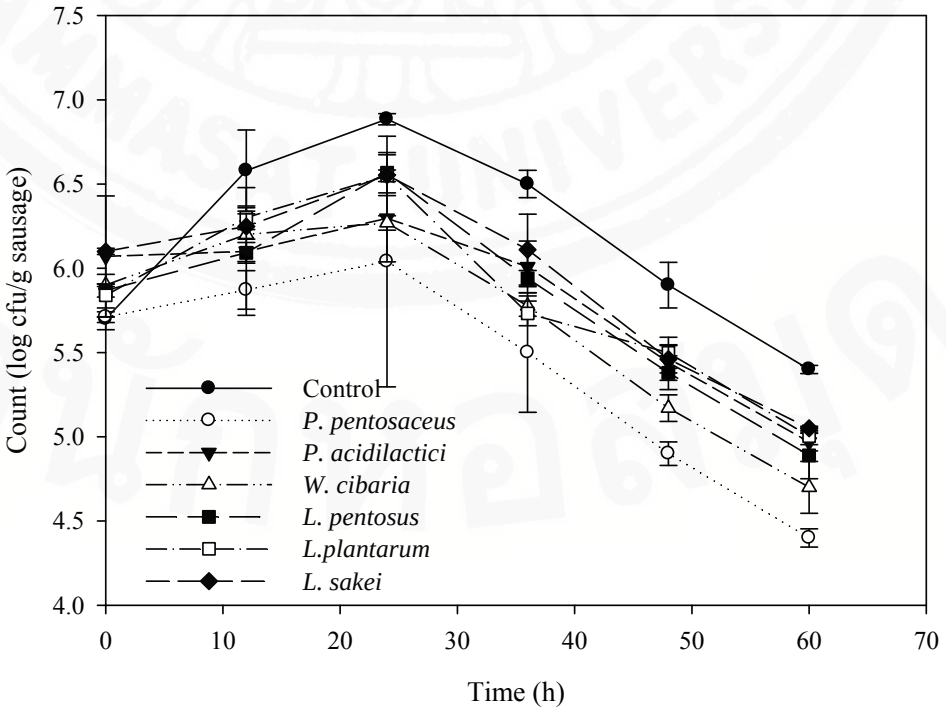


Figure 4.1 (Continued)

d) Yeasts and molds

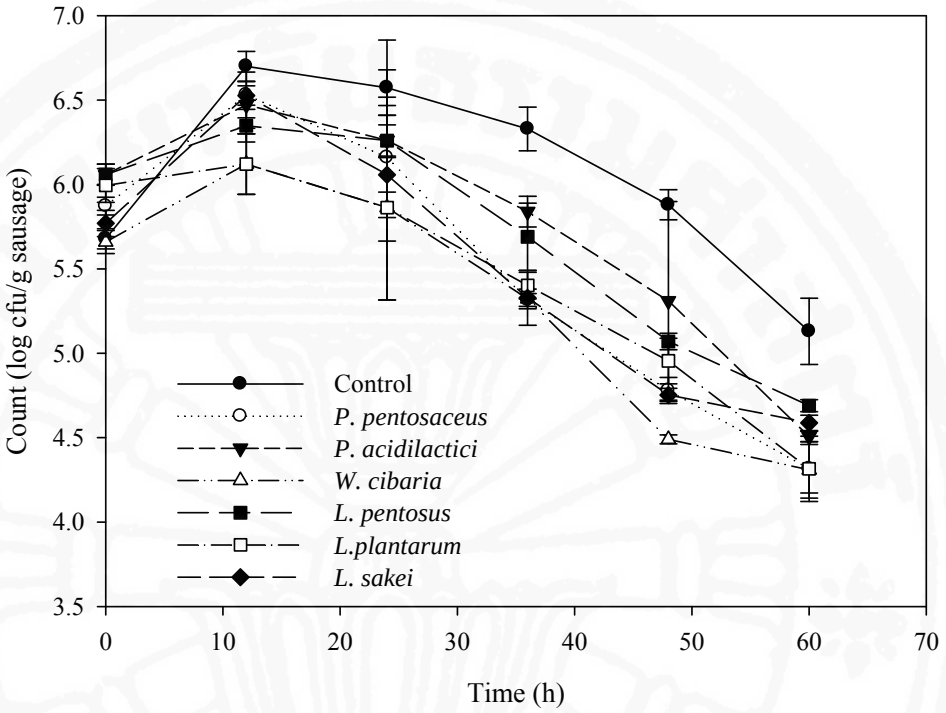


Table 4.1

Microbial population of Thai fermented sausage inoculated with/without starter cultures at 60 h of fermentation

Sausage samples	Microbial population (log CFU/g)			
	TVC	LAB	Staphylococci/ micrococci	Yeast and mold
Control	9.74±0.09^{c}	9.25±0.11^{a}	5.40±0.024^{a}	5.13±0.20^{a}
P. pentosaceus	10.92±0.10^{a}	10.31±0.05^{b}	4.40±0.054^{b}	4.32±0.14^{d}
P. acidilactici	10.15±0.07^{d}	9.86±0.04^{d}	4.97±0.053^{de}	4.51±0.31^{b}
W. cibaria	9.57±0.1^{b}	9.52±0.01^{c}	4.70±0.15^{d}	4.31±0.17^{d}
L. pentosus	10.17±0.07^{d}	9.84±0.09^{d}	4.89±0.05^{de}	4.69±0.10^{c}
L. plantarum	9.72±0.02^{c}	9.71±0.02^{e}	5.00±0.049^{e}	4.32±0.19^{d}
L. sakei	10.12±0.05^{d}	10.11±0.08^{f}	5.05±0.14^{c}	4.59±0.045^{bc}

$^{a-f}$Values with different superscript letters in the same column are significantly different ($p < 0.05$)

4.2 Chemical analysis

4.2.1 pH

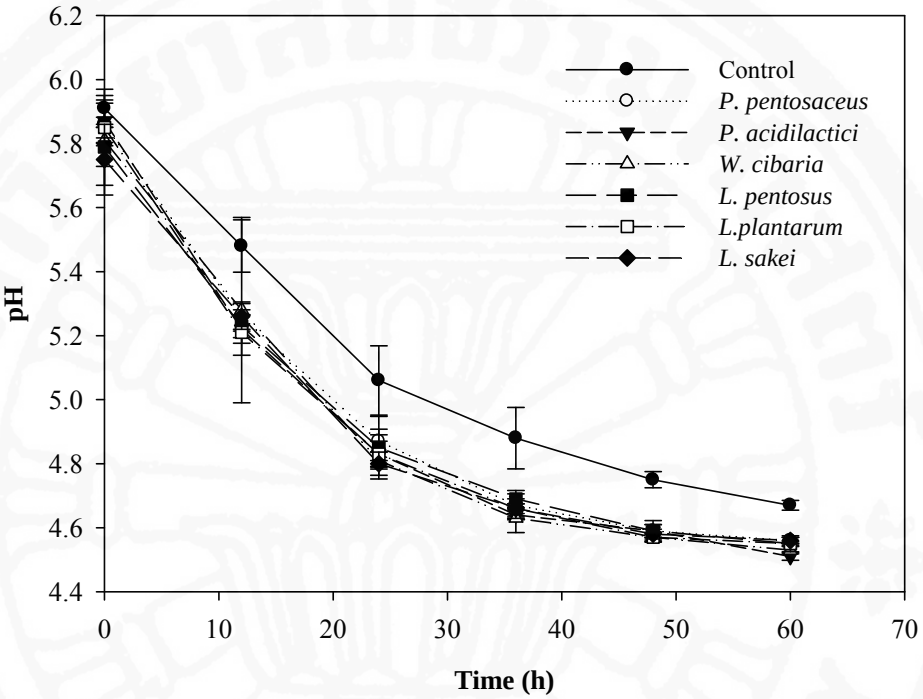
The change in pH of the Thai fermented sausages is shown in Figure 4.2. The initial pH of all batches was about 5.87. The pH was drastically dropped during the growth phase between 12 h and 48 h of fermentation and then slowly decreased after 48 h. The lowest pH value was attained at 60 h of fermentation. As fermentation time increased, sausages inoculated with LAB starters exhibited significant lower pH than the control ($p < 0.05$). At the end of fermentation, pH of sausages inoculated with starter *P. acidilactici*, *P. pentosaceous*, *W. cibaria*, *L. plantarum*, *L. pentosus* and *L. sakei* were 4.56, 4.51, 4.53, 4.55, 4.55 and 4.56, respectively, while that of control

sample was 4.67. Sausage inoculated with *P. pentosaceus* was significantly different ($p < 0.05$) in comparison with other inoculation batches except *L. plantarum*. No significant difference ($p > 0.05$) found among *P. acidilactici*, *W. cibaria*, *L. pentosus* and *L. sakei* batches as illustrated in Table A.8, Appendix A. This verifies the effectiveness of LAB starter cultures for sausage acidification. The decrease in pH values of the fermented sausages corresponded to the production of organic acids such as lactic acid and acetic acid by LAB (Erkkilä et al. 2001; Komprda et al. 2004).

Generally, measuring pH is sufficient as an indicator of the progress of the fermentation phase. During fermentation, the increase of acid production was observed along with the concomitant decrease in pH due to the transformation of sugar to acids by LAB. The rapid growth of LAB at the initial stage of fermentation was beneficial in decreasing the pH of sausages, which was responsible for reducing or eliminating undesirable bacteria. At the beginning of fermentation, the pH of sausages was regulated by the production of lactic acid. Besides after 48 h, the pH may not be influenced by lactic acid production alone. As reported by Demeyer et al. (1979), the change in pH of fermented sausages was also caused by nitrogen, acetate, and fatty acid concentration observed. The product pH can be affected by many factors, including the “buffering capacity” (the resistance to change in pH) of the meat mix. The low pH (4.3-4.5) of Thai fermented sausages produced using starter cultures might have inhibited pathogens and avoided spoilage. Therefore, fermented sausages inoculated with starter cultures are considered to be a safe food product.

Figure 4.2

Profiles of pH in Thai fermented sausages with/without starter cultures during fermentation.



4.2.2 Cured meat pigment

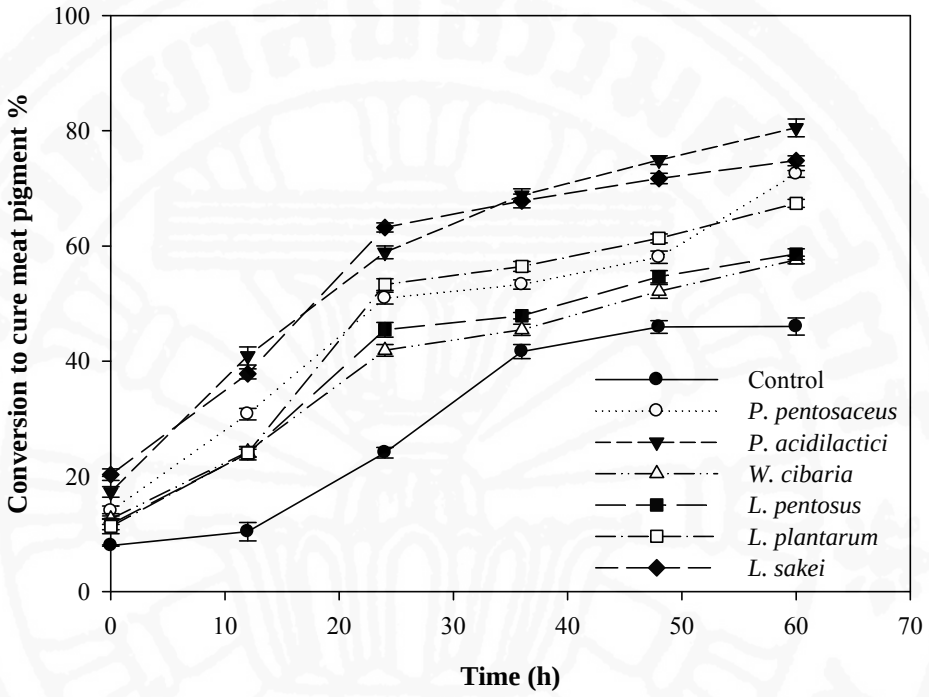
The percentage of total heme pigments existing as cured heme pigments in fermented sausages is shown in Figure 4.3. The percentage conversion to cured pigments increased during fermentation and particularly pronounced increments were found in sausages fermented with starters. In control experiments the percentage conversion to cured pigments slightly increased in the first 12 h followed by a modest increase. Similar results were also reported by Vural (1998) and Moller et al. (2003). The sausage inoculated with *P. acidilactici* showed the best conversion to cured meat pigment. At the end of fermentation, the sausage inoculated with *P. acidilactici* presented the highest conversion at 80.5% and followed by *L. sakei* (74.8%), *P. pentosaceus* (72.5%), *L. plantarum* (67.4%), *L. pentosus* (58.6%) and *W. cibaria* (57.6%). On the other hand, control sausages without starter cultures showed a

significant least conversion ($p < 0.05$) to cured meat pigments during processing. Except sausage inoculated with *W. cibaria* and *L. pentosus*, all inoculated batches showed significant difference in the percentage of cured pigments among each other ($p < 0.05$) as illustrated in Table A.9 , Appendix A. Further increases in the percentage of cured pigments, which were obtained from LAB inoculated sausages were occurred upon a rapid reduction in the pH at ambient temperature (30-35 °C) after 24 h. Vural (1998) reported that these increases in cured pigment conversion during fermentation were caused by the decrease in sausage pH and temperature.

The pigment responsible for the characteristic cured meat colour is a myoglobin derivative, nitrosylmyoglobin, MbFe(II)NO. Several lactic acid bacteria proved to be capable of converting MbFe(III) (grey/brown) to the bright red cured meat pigment MbFe(II)NO. In addition, the microorganisms were capable of generating nitric oxide (NO) from oxidation of the guanidium group in L-arginine rather than from nitrate or nitrite by bacterial nitric oxide synthase (Arihara et al. 1993; Gündoğdu et al. 2006; Morita et al. 1997). The NO reacts with myoglobin, which is coordinated to central Fe (II) in heme. Gündoğdu et al. (2006) isolated LAB from various fermented food products for their ability to convert metmyoglobin to bright red derivatives. They reported that *L. plantarum*, *Leuconostoc mesenteroides* and *P. acidilactici* were found to be NO producing bacterial strains. As in previous study by Møller et al. (2003), inoculation with *L. fermenum* and *P. pentosaceus* gave the highest potential for color formation, as they exhibited high activity for generating MbFe(II)NO. In addition, Kawahara et al. (2006) summarized that the use of *L. sakei* as starter culture without adding nitrite in a cured and smoked pork loin product could form the red colour. They also observed the reduction of free-arginine in their sample. Therefore, LAB, especially, *P. acidilactici*. could be used as meat starter culture which capablely formed cured colour without addition of nitrite or nitrate.

Figure 4.3

Profiles of percentage of total heme pigment conversion to cured heme pigment for Thai fermented sausages with/without starter cultures during fermentation.



4.2.3 Glucose

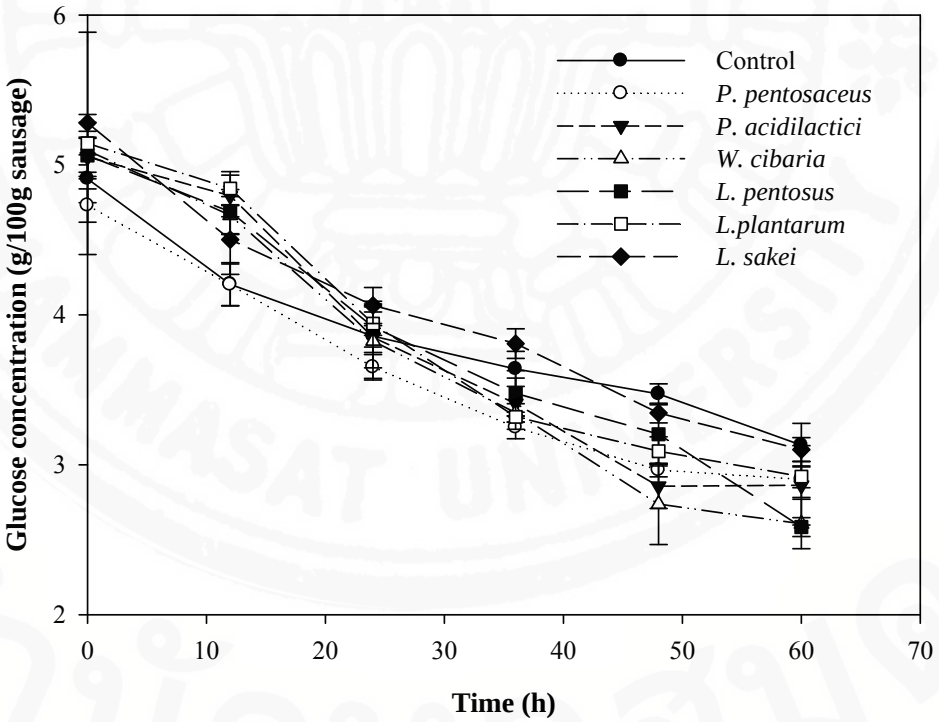
Glucose concentration profiles are presented in Figure 4.4 and Table 4.2. The initial glucose concentrations of all batches were about 12-13 mg/mL. After 12 h of fermentation, the amount of glucose decreased to 6.4-7.8 mg/mL until the end of fermentation for all batches. Initial glucose consumption rate is shown in Table 4.1. The result indicated that sausage inoculated with *W. cibaria* had the highest initial glucose consumption rate at 5.30×10^{-2} g/100g·h whereas the control showed the lowest at 4.26×10^{-2} g/100g·h. Nevertheless, there was no significant difference ($p > 0.05$) between inoculated sausages and control batch (Table 4.2).

One of the important criteria for lactic acid bacteria used in meat fermentation is the initial conversion of the added carbohydrate to lactic acid, the primary acid responsible for decreasing the pH in fermented sausage (Lee et al. 2006; Hammes and

Knauf 1994). Furthermore, carbohydrates are a primary source of energy for microorganism. According to Adamberg et al. (2006), LAB could produce their major energy requirements by fermenting amino acids and thus utilizing carbohydrates mainly for biomass synthesis. In this study, Thai fermented sausage was used cooked rice as carbon source. Thus, LAB had to initially break down all starch into glucose. As expected, glucose concentration decreased (see Figure 4.4) and accompanied with the increase of LAB population (see Figure 4.1a) and lactic acid concentration during fermentation time (see Figure 4.5a).

Figure 4.4

Profiles of glucose concentration in Thai fermented sausages inoculated with lactic acid bacteria and naturally fermentation (control).



4.2.4 Organic acids

The results of organic acid production such as lactic acid, acetic acid and formic acid are presented in Figure 4.5. The decrease of pH was attributed to organic acids, mainly lactic acid production by LAB. Lactic acid production was higher than acetic acid and formic acid production. The increase of lactic acid concentration was observed in all cases as shown in Figure 4.5a. However, the increase of lactic acid concentration ranged from 1.89 to 2.11 g/100g in inoculated sausage at the end of fermentation compared to 1.57 g/100g sausage in the control. *P. acidilactici* showed the best significant acidification performance ($p < 0.05$) as indicated by the highest lactic acid titre (Table 4.2). The result is consistent with other studies for similar products using *L. curvatus*, *L. plantarum* and *P. acidilactici* as starters (Visessanguan *et al.* 2006; Lee *et al.* 2006; Vural 1998). In Table 4.2, the lactic acid production rate of control was significantly lower ($p < 0.05$) than sausages inoculated with *P. acidilactici*, *W. cibaria*, *L. pentosus*, *L. plantarum* and *L. sakei*. For the inoculation batches, there was significant difference ($p < 0.05$) among *P. pentosaceus*, *P. acidilactici*, *W. cibaria* and lactobacilli group. No significant differences ($p > 0.05$) observed in comparison with *L. pentosus*, *L. plantarum* and *L. sakei*. The lactic acid yields are shown in Table 4.2. Accordingly, lactic acid yields observed in sausages inoculated with LAB were significantly higher ($p < 0.05$) than that of control sample. The significant maximum lactic acid yield ($p < 0.05$) was achieved in sausage inoculated with *P. acidilactici* at 0.89 g lactic acid/g glucose. With the exception in *P. pentosaceus* batch, no significant differences of lactic acid yield ($p > 0.05$) were observed among the inoculation batches.

The evolution of the acetic acid concentration during processing exhibited different behavior depending on the particular batch as shown in Figure 4.5b. Control and sausages inoculated with *P. pentosaceus*, *W. cibaria* and *L. pentosus* showed the increase in acetic concentration for the first 24 hours as shown in Figure 4.5b. Afterwards, the acetic concentration decreased until the 60^{th} hour. However, at the end of fermentation, the acetic acid concentration in the control at 0.029 g/100g sausage was significantly higher ($p < 0.05$) than in other inoculated batches. In sausages inoculated with *P. acidilactici*, *L. plantarum* and *L. sakei*, the acetic acid

concentrations slightly increased and were lower than in the other batches ($p < 0.05$). The concentration of acetic acid decreased due to acetate assimilation. Acetate is a central intermediate in the overall carbon cycle. The exclusive utilization of acetate enters the central metabolism at the level of acetyl-coenzyme A (CoA) (Meister et al., 2005). As in case of acetic acid, formic acid increased to a maximum value within the first 24 hours for *W. cibaria* and *L. sakei*, and at 36 hours for the control, *P. pentosaceus*, *P. acidilactici*, *L. plantarum* and *L. pentosus* batches as shown in Figure 4.5c. At the end of fermentation, the formic acid concentration in the control was 0.036 g/100g sausage which was significantly higher than ($p < 0.05$) that in the inoculated batches with *P. pentosaceus* and *L. sakei*. However, Durá et al. (2004) and Visessanguan et al. (2006) reported that the fermented sausages inoculated with starter cultures showed higher acetic acid concentration than the control batch. Variety strains of lactic acid bacteria can produce different organic acids, i.e. lactic acid, acetic acid and formic acid (Yun et al., 2003). The control sausage produced higher concentration of acetic acid and formic acid than the sausages inoculated with LAB due to the presence of various strains of lactic acid bacteria. Lactic acid was the major end product of sausage fermentation. The use of LAB starter cultures in Thai fermented sausages showed similar fermentation characteristics which was the yield of lactic acid to total organic acids higher than 97% (Table A.14, Appendix A). It can be concluded that the sausage fermentation by *P. pentosaceous*, *P. acidilactici*, *W. cibaria*, *L. plantarum*, *L. pentosus* and *L. sakei* was homofermentation.

Fermentation results in an increase in organic acids along with a concomitant decrease in pH. LAB such as *Lactobacillus*, *Pediococcus*, and *Weissela* fermented carbohydrates via different pathways resulting in homo-, hetero-, or mixed acid fermentation (Stiles and Holzapfel, 1997). The homofermentation routes produce more than 85% lactic acid as a major end product of glucose catabolism through glycolysis pathway, while the hetero- or mixed acid fermentation routes give not only lactic acid (50%), but also formic and acetic acids as byproducts. The presence of acetic acid and formic acid can be attributed to a shift from homo- to hetero-fermentative metabolism of the selected starter cultures under conditions of environment stress such as limitation of oxygen, nutrient and salt concentrations and low pH level. Bobillo and Marshall (1992) determined lactate and acetate production

by *L. plantarum* under acidic conditions. In continuous culture, under glucose-limited aerated conditions, maximum acetate production was observed at pH 5.0, whereas pH values of 4.5 resulted in lower levels of acetate. A probable attribution is that the enzymes involved in the anaerobic pyruvate formate-lyase have an alkaline optimum pH (Lindmark et al. 1969). Bobillo and Marshall (1992) studied the effect of salt and oxygen availability on growth and acid production by *L. plantarum*. Their results have shown that the presence of salt in the culture medium delayed growth, reduced the cell density, retarded the consumption of glucose and citrate, and significantly reduced the ratio of acetate to lactate production. The main acid product of the aerobic metabolism by *L. plantarum* was acetate, whereas the main acid of the anaerobic metabolism was lactate. Yun et al. (2003) studied the various carbon sources and glucose concentrations influencing lactic acid fermentation by *Enterococcus faecalis* RKY1. Among the various carbohydrates tested, galactose was converted into formic and acetic acids as major products, whereas xylose, glycerol, whey, and starch were poorly utilized by *E. faecalis* RKY1. In batch lactic acid fermentations, glucose, fructose, and maltose were efficient for homofermentative production of lactic acid. Erkkilä et al. (2001) reported that lactic acid was the main flavour component, but the presence of low acetic acid concentration was actually essential for sausage flavour. Therefore, changes in environmental pH, oxygen availability and carbon sources could influence the fermentation end-product spectrum of LAB cultures by affecting the regulatory mechanisms involved in acid production.

Figure 4.5

Profiles of major organic acid concentrations in Thai fermented sausages inoculated with or without starter cultures during fermentation:
(a) Lactic acid, (b) Acetic acid, (c) Formic acid.

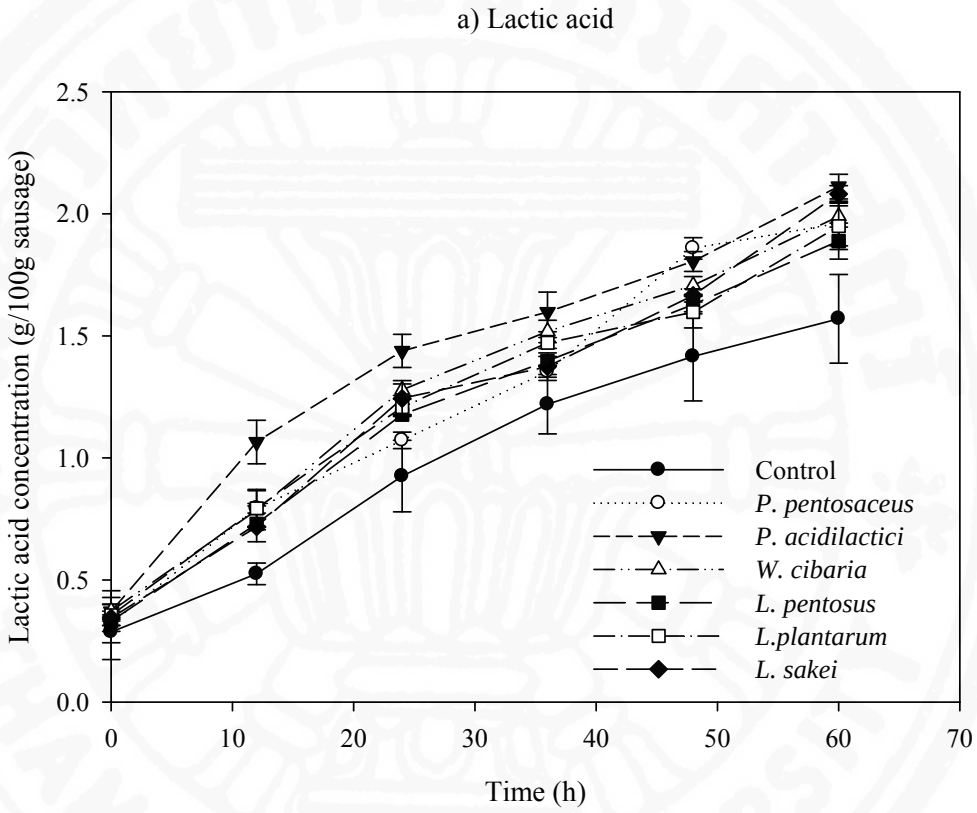
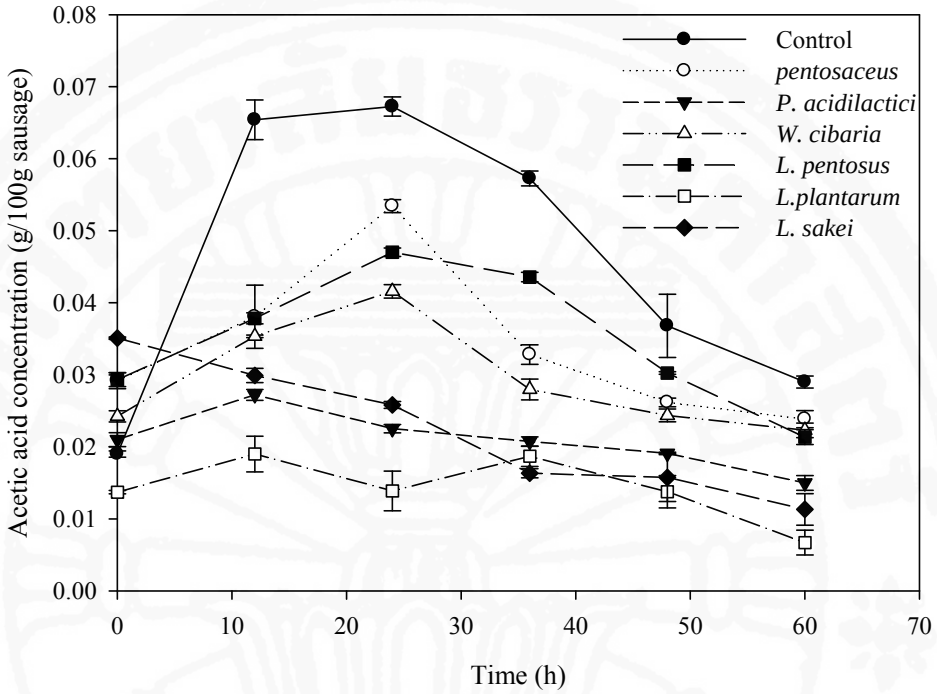


Figure 4.5 (Continued)

b) Acetic acid



c) Formic acid

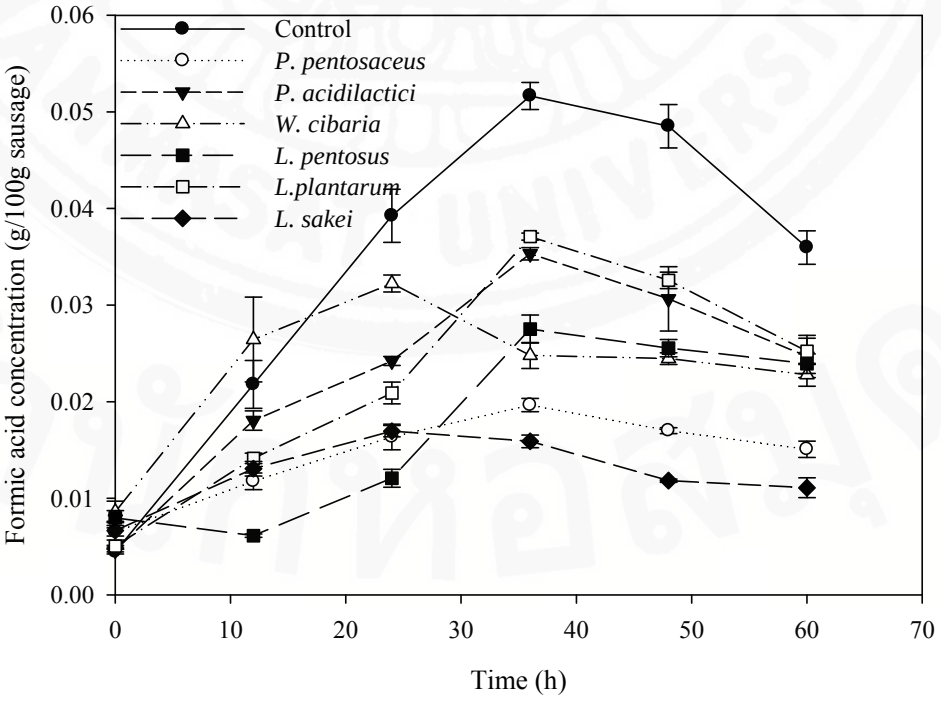


Table 4.2

Estimation of the glucose consumption rates, lactic acid production rates and lactic acid yields during fermentation of Thai fermented sausages

Fermentation process	Initial glucose consumption rate (g/100g·h)	Initial lactic acid production rate (g/100g·h)	Lactic acid yield $Y_{p/s}$ (g lactic acid/g glucose)
Control	4.26×10^{-2a}	2.66×10^{-2a}	0.63^{a}
P. pentosaceus	4.48×10^{-2a}	3.12×10^{-2a}	0.71^{a}
P. acidilactici	5.02×10^{-2a}	4.45×10^{-2b}	0.89^{b}
W. cibaria	5.30×10^{-2a}	3.78×10^{-2c}	0.75^{b}
L. pentosus	4.77×10^{-2a}	3.53×10^{-2d}	0.74^{b}
L. plantarum	5.00×10^{-2a}	3.55×10^{-2d}	0.72^{b}
L. sakei	5.07×10^{-2a}	3.75×10^{-2cd}	0.74^{b}

[a-b] Values in a low followed by the same letter are not significantly different (p<0.05).

4.3 Proteolysis

Overall proteolysis in fermented sausages was quantified by determination of protein, non-protein nitrogen contents and amino nitrogen. Free amino acid and non-protein nitrogen, formed by the synergistic action of peptidase (EC 3.4) upon myofibrillar and sarcoplasmic proteins, have been used as indicators of protein fragmentation during the fermentation of meat product. To assess the protein degradation during fermentation, sarcoplasmic and myofibrillar protein fractions extracted from inoculated sausages and control sausage were subjected to SDS-PAGE.

4.3.1 Change in protein compositions

Trends of myofibrillar, sarcoplasmic proteins and non-protein nitrogen (NPN) during the fermentation of sausages inoculated with LAB starter cultures and uninoculated sausage (control) expressed as relative nitrogen content (%) are shown in Figure 4.6. As fermentation proceeded, changes in the protein fraction of Thai fermented sausage were illustrated by the decrease in the myofibrillar and sarcoplasmic proteins, accompanied by the increase in NPN fraction. At the end of fermentation, Thai fermented sausages inoculated with *L. plantarum*, *L. pentosus* and *L. sakei* showed the fastest and largest decrease in both sarcoplasmic and myofibrillar proteins (see Figures 4.6a and 4.6b). A slight decrease in these proteins was observed in fermented sausages inoculated with *P. acidilactici*, *P. pentosaceus* and *W. cibaria*. The control sample exhibited the lowest decrease in myofibrillar and sarcoplasmic proteins. At 60 h of fermentation, Thai fermented sausages inoculated with LAB showed significant decrease in sarcoplasmic and myofibrillar protein fractions ($p < 0.05$). Similar to the study of Visessanguan et al. (2006), Nham inoculated with starter culture LAB, *L. curvatus* showed a progressive decrease in the sarcoplasmic and myofibrillar protein fractions and higher amount of NPN values in fermented sausage. NPN is an indicator for the degree of proteolysis in fermented sausage. NPN rapidly increased during the first 36 h of fermentation and gradually increased to 181-244% within 60 h as shown in Figure 4.6c. Inoculation with *L. plantarum*, *L. pentosus* and *L. sakei* generally resulted in higher increase in NPN (223, 228 and 244%, respectively). The increase in NPN was highest for *L. sakei* inoculated sausage and the lowest (181%) for the control sample. At the end of fermentation, a concentration of NPN observed from LAB inoculated sausages was significantly higher than control ($p < 0.05$). Moreover, Visessanguan et al., 2006 reported the increase in NPN content during pork sausage fermentation with inoculation of *L. cuvatus*. In contrast, Klement et al. (1973) did not observe changes in NPN content in a fermented sausage, probably due to the use of pediococci as the starter culture. According to the study of Geisen et al. (1992), pediococci did not have any significant proteolytic activity.

Figure 4.6

Profiles of protein compositions in Thai fermented sausages during fermentation with different starter cultures: a) myofibrillar protein fraction, b) sarcoplasmic protein fraction and c) non-protein nitrogen fraction

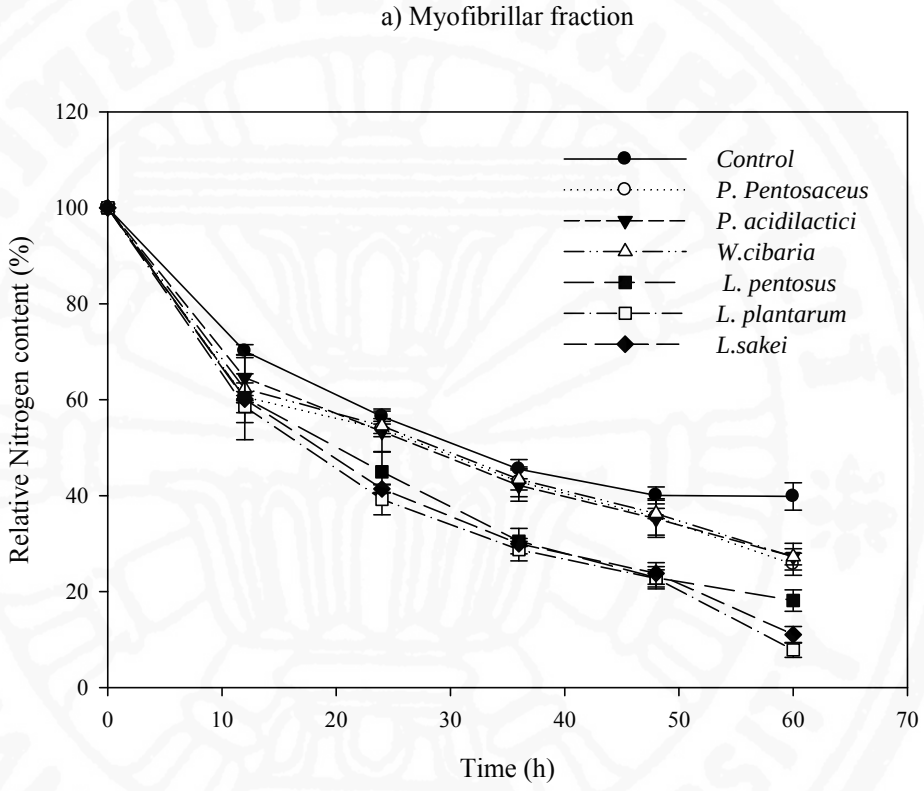
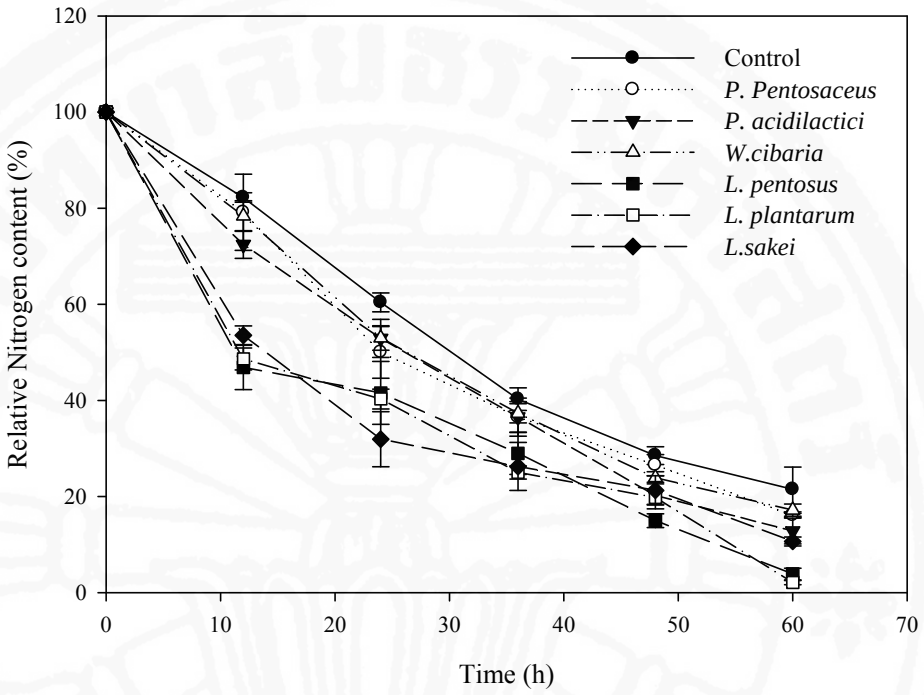
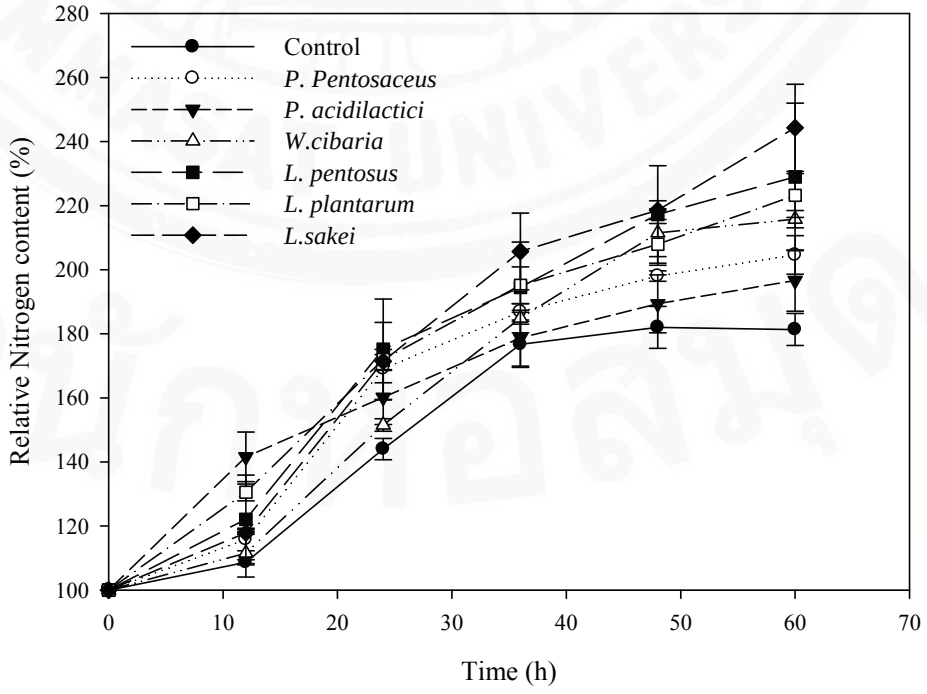


Figure 4.6 (Continued)

b) Sarcoplasmic fraction



c) Non-protein nitrogen



4.3.2 Protein degradation

The effect of starter cultures on the sarcoplasmic and myofibrillar protein degradation was assessed by SDS-PAGE. The electrophoretic profiles of sarcoplasmic proteins extracted during fermentation of sausages are shown in Figure 4.7 and Table 4.3. The hydrolysis of sarcoplasmic proteins in the seven batches was comparable. Bands corresponding to sarcoplasmic proteins with molecular weights of ~29, 42 and 97 kDa decreased and disappeared within the first 12 h of fermentation in each batch due to proteolysis. Polypeptide band with molecular weights of 26 kDa was produced at 36 h in all sausage samples. Furthermore, the intensity of additional 36 and 63 kDa polypeptide bands progressively increased throughout fermentation. Similar to the study of Hughes et al. (2002), polypeptides with molecular weights of 36 kDa increased throughout the fermentation. The pattern of proteolysis observed on SDS-PAGE was similar to those reported by Durá et al. (2004) and Casaburi et al. (2007). The pattern of proteolysis observed on SDS-PAGE was similar to those reported by Hughes et al. (2002) and Durá et al. (2004). Hierro et al. (1999) and Molly et al. (1997) demonstrated that the initial degradation of sarcoplasmic proteins may be due to the action of endogenous muscle enzymes. The endoproteolytic activity responsible for the initial breakdown of protein in dry sausages has been attributed mainly to endogenous muscle protease, cathepsin, activated by the drop in pH (Verplaetse, 1994). However, Casaburi et al. (2007) found that the electrophoretic profiles of the sarcoplasmic proteins extracted from sausage inoculated with *L. curvatus* AVL3 was different from uninoculated sausage (control). Their SDS-PAGE profiles of the control sample did not show any changes during ripening. These results described that the weak activity of the endogenous protease in uninoculated sausage may be explained by the high pH of the control sausage at 6.18 that could have affected endogenous protease activity. Moreover, Fadda et al. (1998 and 1999a) studied the hydrolysis effects of whole cells and cell extracts of *L. curvatus*, *L. sakei* and *L. plantarum* on pork muscle sarcoplasmic proteins. Their SDS-PAGE results showed that the inoculation of whole cells caused the decrease and disappearance of protein bands at approximately 97, 43 and 29 kDa. No proteolytic changes were observed when the cell extracts was used. In contrast, the mixture of whole cells and cell

extracts caused a severe degradation of bands of about 97 kDa, while other bands were partially hydrolyzed. Therefore, sarcoplasmic proteins were hydrolyzed by proteolytic lactobacilli.

Figure 4.7

SDS-PAGE of sarcoplasmic proteins throughout the fermentation time of Thai fermented sausages: a) control (uninoculated sausage) 0-60 h (lanes 1-6), sausages inoculated with *P. acidilactici* 0-60 h (lanes 7-12); b) *P. pentosaceus* 0-60 h (lanes 1-6), sausages inoculated with *W. cibaria* 0-60 h (lanes 7-12); c) *L.sakei* 0-60 h (lanes 1-6), sausages inoculated with *L. pentosus* 0-60 h (lanes 7-12), sausages inoculated with *L. plantarum* 0-60 h (lanes 13-18) and lanes and M = Standard marker.

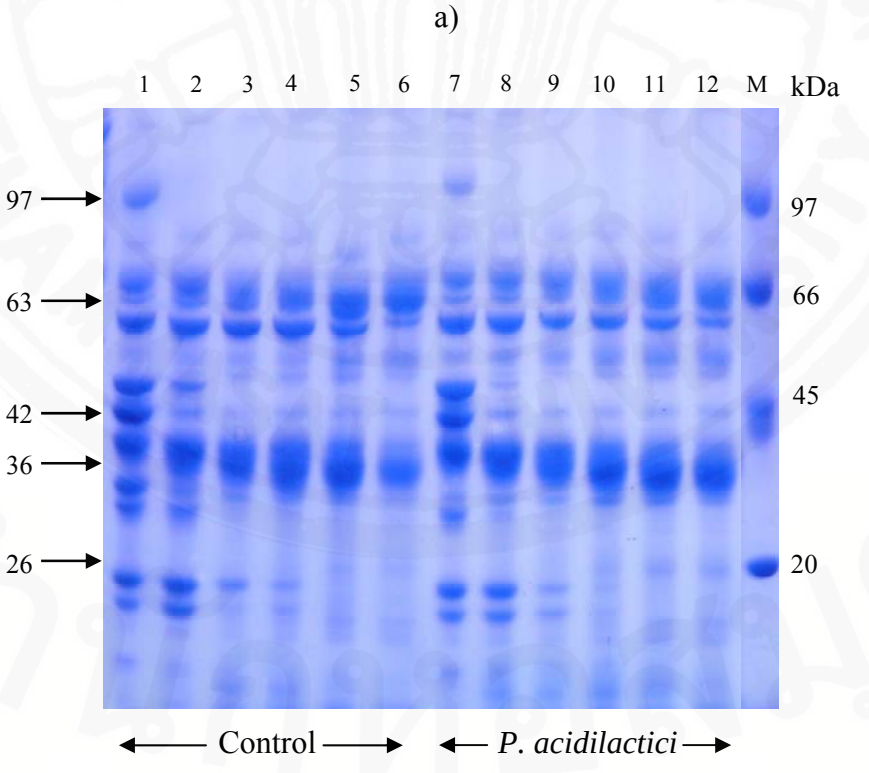


Figure 4.7 (Continued)

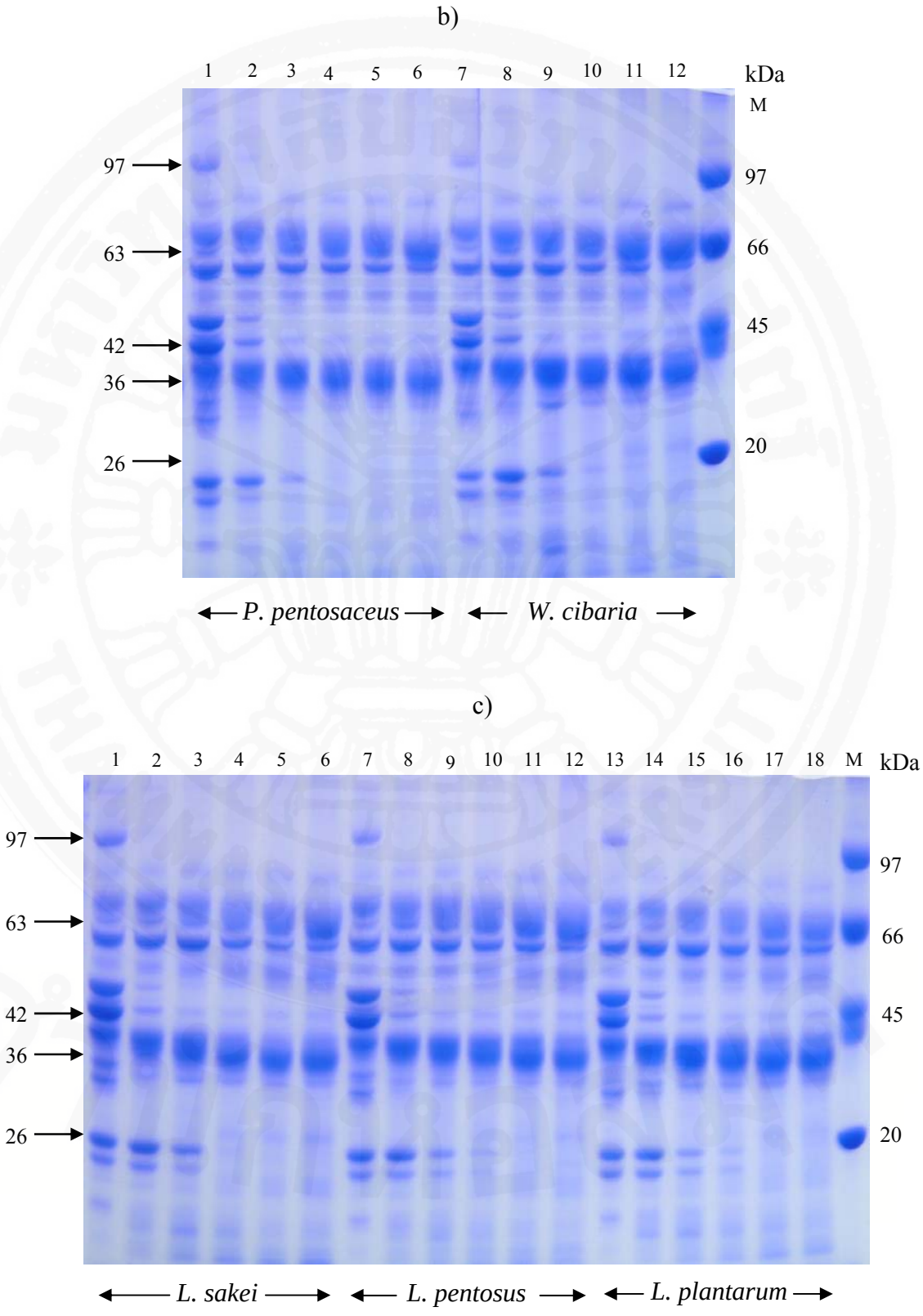


Table 4.3

Relative density (%) of the electrophoretic bands obtained for sarcoplasmic proteins extracted from fermented sausages inoculated with or without starter cultures

Batch	Molecular Weight (kDa)				
	97	63	42	36	26
Control					
0	11.78	1.42	10.56	13.38	ND
12	ND	15.49	8.32	21.51	ND
24	ND	20.87	ND	33.54	ND
36	ND	22.09	ND	32.97	0.95
48	ND	23.77	ND	36.01	5.14
60	ND	30.60	ND	42.78	5.41
L. plantarum					
0	7.56	2.62	11.25	16.03	ND
12	3.67	3.35	5.61	26.41	ND
24	ND	5.68	ND	36.15	ND
36	ND	22.09	ND	32.97	0.95
48	ND	24.40	ND	35.02	4.57
60	ND	22.63	ND	33.80	6.98
L. pentosus					
0	7.19	3.50	9.97	14.59	ND
12	ND	5.89	3.74	29.09	ND
24	ND	9.37	ND	32.68	ND
36	ND	24.29	ND	30.34	ND
48	ND	25.69	ND	31.15	5.49
60	ND	25.87	ND	30.44	5.73
L. sakei					
0	9.59	2.85	10.99	12.24	ND
12	ND	3.80	4.28	28.05	ND
24	ND	4.26	ND	32.44	ND
36	ND	20.96	ND	31.52	4.82
48	ND	21.99	ND	31.54	5.27
60	ND	23.32	ND	30.91	6.68
P. acidilactici					
0	8.40	3.00	10.72	15.44	ND
12	0.86	9.17	5.69	28.66	ND
24	ND	10.47	ND	36.73	ND
36	ND	21.97	ND	33.76	3.02
48	ND	23.24	ND	35.81	6.25
60	ND	23.78	ND	35.57	7.66

ND = not detected

Table 4.3 (Continued)

Batch	Molecular Weight (kDa)				
	97	63	42	36	26
P. pentosaceus					
0	7.48	1.97	9.51	14.64	ND
12	4.82	4.17	1.60	25.36	ND
24	ND	10.47	ND	36.73	ND
36	ND	26.47	ND	34.38	1.73
48	ND	25.57	ND	34.50	1.85
60	ND	27.48	ND	35.47	2.21
W. cibaria					
0	6.99	2.03	5.46	18.43	ND
12	1.18	3.32	5.65	29.54	ND
24	ND	6.31	ND	35.50	ND
36	ND	23.34	ND	33.33	2.41
48	ND	24.65	ND	31.07	8.65
60	ND	27.12	ND	30.05	8.29

ND = not detected

The electrophoretic profiles of myofibrillar proteins extracted during fermentation of sausages are shown in Figure 4.8 and Table 4.4. The 200 kDa band corresponding to myosin heavy chain (MHC), a 140 kDa band and a 45 kDa band of actin (Hughes et al. , 2002) were observed to decrease in all samples as shown in Table 4.4. However, the degradation was faster in samples inoculated with starter cultures: *L. plantarum*, *L. pentosus*, *L. sakei, P. acidilactici*, *P. pentosaceus and W. cibaria*. The band corresponding to myosin heavy chain (MHC) decreased during fermentation as observed by lower intensity and disappearance completely after 24 h of fermentation in the samples inoculated with *L. plantarum*, *L. pentosus*, *L. sakei* and *P. acidilactici* and after 48 h of fermentation in the samples inoculated with *P. pentosaceus* and *W. cibaria*. As in MHC, the degradation of a band with molecular weight of 45 kDa occurred rapidly within the first 12 h in samples inoculated with *L. plantarum*, *L. pentosus*, *L. sakei* and *W. cibaria* and within 24 h in samples inoculated with *P. acidilactici* and *P. pentosaceus*. In contrast to the control sample, MHC and actin decreased over the entire fermentation period (see Figure 4.8 and Table 4.4). Consequently, the intensity of polypeptide bands with molecular weights of 26, 32, 60

and 75 kDa, believed to be degradation products, increased in all sausages. Similar results were obtained from sausage model system inoculated with *L. sakei* (Sanz et al., 1999). Furthermore, Hughes et al. (2002), Durá et al., (2004), Visessanguan et al., (2006), Yungjin et al., (2006) and Casaburi et al., (2007 and 2008) described that the degradation of MHC during the ripening of fermented sausages with starter cultures addition was faster than uninoculated sausages. Fadda et al. (1999b) studied proteinase and peptidase activities of whole cells, cell extracts and the combination of both whole cells and cell extracts from *L. plantarum* on muscle myofibrillar proteins. In control samples, the activity of endogenous proteinase was responsible for the degradation of protein bands of 200 kDa (myosin), 66 kDa and 45 kDa (actin). When whole cells were inoculated, myosin and actin were partially hydrolyzed, while other bands of intermediate molecular mass (50 to 35 kDa) appeared. The same results were obtained when cell extracts were used. The protein pattern obtained when only cell extract were incorporated was identical to that obtained from control sample. The result of myofibrillar protein degradation suggests that both endogenous muscle enzymes and bacterial proteinases contributed to the initial degradation of those proteins.

Figure 4.8

SDS-PAGE of myofibrillar proteins throughout the fermentation time of Thai fermented sausages: a) control (uninoculated sausage) 0-60 h (lanes 1-6), sausages inoculated with *P. acidilactici* 0-60 h (lanes 7-12); b) *P. pentosaceus* 0-60 h (lanes 1-6), sausages inoculated with *W. cibaria* 0-60 h (lanes 7-12); c) *L.sakei* 0-60 h (lanes 1-6), sausages inoculated with *L. pentosus* 0-60 h (lanes 7-12), sausages inoculated with *L. plantarum* 0-60 h (lanes 13-18) and lanes and M = Standard marker.

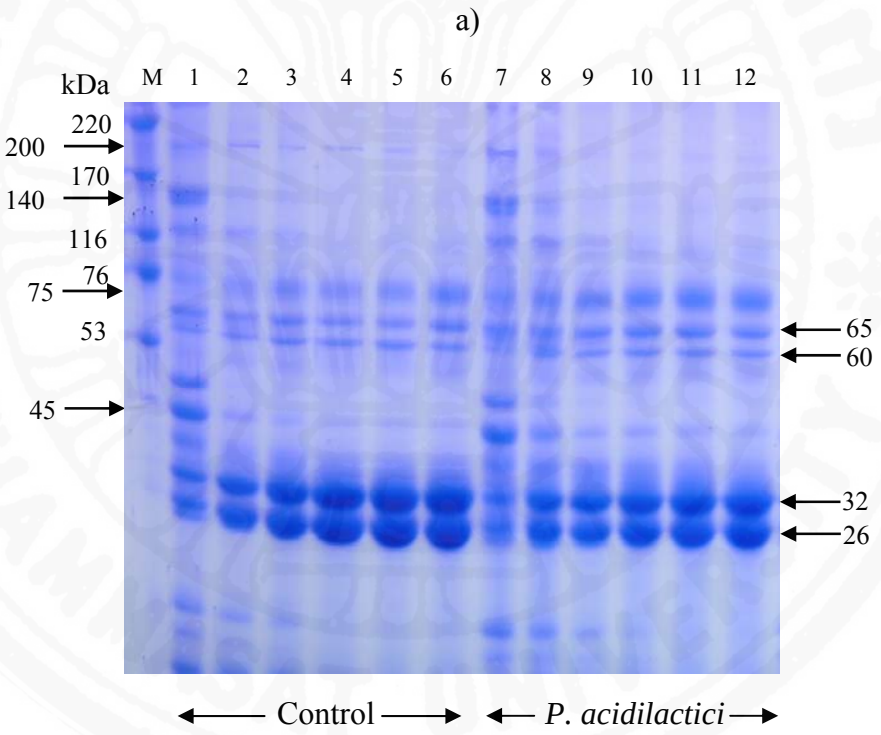


Figure 4.8 (Continued)

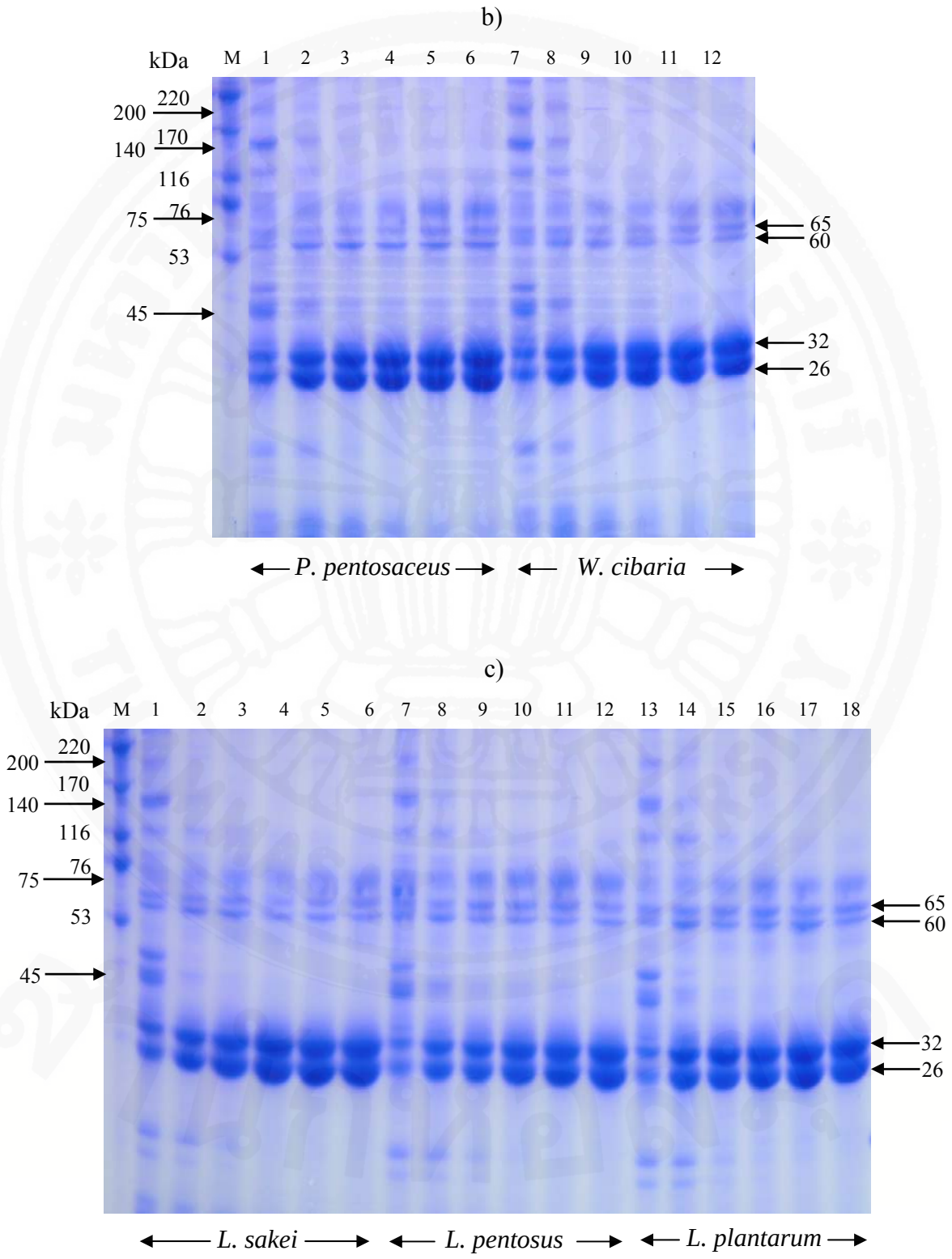


Table 4.4

Relative density (%) of the electrophoretic bands obtained for myofibrillar proteins extracted from fermented sausages inoculated with or without starter cultures

Batch	Molecular Weight (kDa)							
	200	140	75	65	60	45	32	26
Control								
0	7.13	10.34	8.08	5.90	2.84	8.11	12.08	13.47
12	5.87	6.00	9.12	7.39	4.94	4.26	18.65	18.91
24	4.38	1.86	9.83	8.33	6.15	3.20	32.25	21.43
36	3.95	1.05	13.12	8.59	6.70	2.41	33.54	21.09
48	3.85	ND	12.41	9.45	7.00	2.39	37.07	21.77
60	2.28	ND	12.51	9.31	8.33	1.63	38.25	22.86
L. plantarum								
0	6.63	11.41	5.94	6.21	5.02	9.41	9.86	12.47
12	3.92	3.48	9.45	6.83	4.26	1.64	28.74	19.68
24	1.98	2.82	10.76	6.88	5.22	0.29	33.13	19.97
36	ND	0.31	13.48	8.42	6.56	ND	40.24	25.76
48	ND	ND	12.63	8.36	6.97	ND	41.49	25.98
60	ND	ND	14.11	8.70	7.35	ND	40.34	25.26
L. pentosus								
0	7.58	8.59	12.09	3.61	4.71	6.83	10.47	13.22
12	5.58	6.64	11.18	4.56	4.80	4.83	22.05	14.22
24	2.10	5.14	16.37	7.14	6.72	ND	31.07	19.86
36	ND	ND	18.84	8.71	7.65	ND	39.62	24.34
48	ND	ND	19.16	8.81	7.86	ND	38.71	23.84
60	ND	ND	17.83	8.96	7.64	ND	37.13	25.53
L. sakei								
0	6.31	9.89	5.48	4.34	4.48	7.97	18.24	12.86
12	4.19	4.85	5.18	4.28	5.93	0.69	26.77	18.30
24	1.06	3.49	11.19	5.74	6.82	ND	34.79	23.45
36	ND	ND	12.28	6.46	8.22	ND	42.66	30.99
48	ND	ND	15.82	5.23	8.08	ND	41.04	27.33
60	ND	ND	16.33	5.81	8.30	ND	40.54	27.21
P. acidilactici								
0	5.08	9.07	6.78	5.15	4.92	7.45	19.38	13.39
12	3.24	6.06	7.17	5.14	5.97	3.21	26.51	18.64
24	2.29	ND	9.51	5.74	7.39	0.33	32.64	22.18
36	ND	ND	7.05	8.42	7.21	ND	40.23	27.76
48	ND	ND	6.20	8.12	6.86	ND	40.78	28.56
60	ND	ND	14.14	7.87	6.17	ND	39.80	28.23

ND = not detected

Table 4.4 (Continued)

Batch	Molecular Weight (kDa)							
	200	140	75	65	60	45	32	26
P. pentosaceus								
0	5.85	8.89	7.19	4.65	3.56	6.22	18.69	14.12
12	5.57	5.14	8.99	4.31	6.60	2.11	26.95	17.22
24	4.27	2.78	10.45	4.48	7.32	2.10	30.88	21.97
36	3.37	2.92	12.68	5.03	8.16	ND	34.60	24.63
48	2.23	ND	16.32	6.60	8.77	ND	35.92	25.71
60	ND	ND	15.66	6.62	8.42	ND	38.42	27.09
W. cibaria								
0	7.48	12.62	7.33	4.54	4.70	7.25	10.67	13.49
12	4.66	5.90	8.05	4.45	6.54	3.54	23.88	17.34
24	2.92	4.72	11.39	4.92	7.66	ND	33.27	20.69
36	2.40	3.78	12.95	4.98	8.68	ND	36.62	23.22
48	1.91	3.21	13.29	5.89	8.82	ND	36.98	23.44
60	ND	2.80	13.94	6.06	8.97	ND	37.61	24.28

ND = not detected

4.3.3 Free amino acid generation

The free amino acids (FAA) were determined during fermentation to evaluate the effect of starter cultures on proteolysis. The results of free amino acid (FAA) generation of the control (uninoculated sausage) and LAB inoculated sausages are shown in Table 4.5. The principle of amino acids presented in the initial mixture was Glu, Ala, Gly and Arg with the concentration higher than 25 mg/100g sausage. Several authors have reported that the amino acids predominant in the initial meat mixture were Glu, Ala, Cys and Gln (Beriain et al., 2000; Casaburi et al., 2007 and 2008). Total FAA increased throughout the fermentation time. At the end of fermentation, a significant greater rise in total FAA was detected in sausages inoculated with LAB compared to control (1.03-1.24 fold) as also reported by Hierro et al. (1999), Bruna et al. (2000a and 2000b), Bolumar et al. (2001) and Hughes et al. (2002). In Table 4.5, the inoculation of sausages with *P. acidilactici* and *L. plantarum* exhibited the highest increase in the total FAA content which was 1.24 and 1.23 times

higher than control, respectively. In addition, the ratio of the final and the initial concentration of amino acids in each batch mainly released during processing were 2.3-3.46 fold of Trp, 3.08-14.45 fold of Phe, 2.41-4.33 fold of Ile, 2.27-13.03 fold of Lys, 1.12-2.6 fold of Arg and 1.41-2.43 fold of Val. Similar result observed in this study is in the agreement with previous reports (Hughes et al., 2002; Durá et al., 2004; Casaburi et al., 2007; Casaburi et al., 2008) describing an increase of Val, Leu, Ile, Phe and Met during the fermentation of sausages prepared with or without starter culture addition. Fadda et al. (1999b) reported that the Lys, Arg, and Leu levels which were main amino acids found in myofibrillar protein increased due to the proteolytic activity. In addition, these amino acids also constitute some of the major components of pork myosin (Bandman, 1987). Moreover, the reduction in the concentration of some amino acids such as Ser, Gln and Glu (Figure A.2, A.4, Appendix A) in all batches was due to microorganism metabolism as suggested by Hughes et al. (2002), Durá et al. (2004) and Ordóñez et al. (1999). Adamberg et al. (2006) found that *L. plantarum* also used Ser as an energy source under carbohydrate-limited condition. Figure 4.9 shows the pyruvate, an intermediate of carbohydrate, metabolism in LAB. Pyruvate may be produced from Ser by dehydratase activity which is active in anaerobic environment. The consumption of free amino acids upon inoculation of LAB into the sausages was due to the requirements for amino acids for the optimal growth of LAB. LAB not only use amino acids to synthesize proteins, but also as an energy source, to obtain the necessary level of internal pH in an acidic environment and to generate co-substrate for biosynthesis (Ardö, 2006).

Table 4.5

Changes in free amino acid (FAA) in Thai fermented sausages during fermentation with and without starter cultures addition

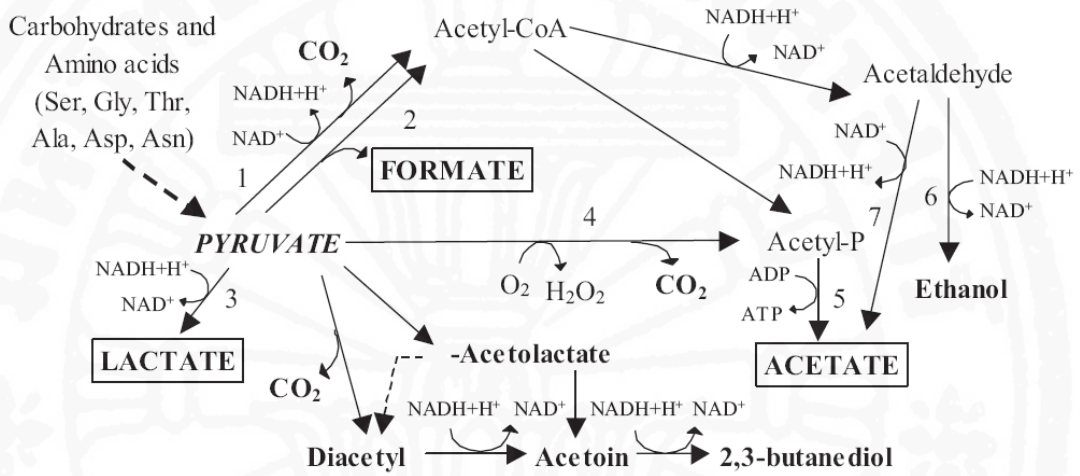
Batch*	Amino acid (mg/ 100 g sausage)																			
	Non essential FAA										Essential FAA									
	Asp	Glu	Ser	Asn	Gly	Gln	Ala	Arg	Pro	Tyr	His	Thr	Val	Met	Ile	Leu	Phe	Trp	Lys	Total
0 h																				
C	8.7	32.0	6.8	7.0	39.8	16.0	24.5	29.0	7.9	11.2	22.9	6.2	10.7	11.3	5.6	9.0	5.9	4.9	0.9	260.3
S1	9.1	31.7	6.6	9.5	33.8	17.6	22.6	39.6	10.7	8.4	17.0	4.7	11.7	8.5	6.7	8.0	4.0	5.5	1.2	256.9
S2	11.3	41.0	8.8	10.3	52.7	21.8	31.8	48.0	14.2	13.5	19.7	11.3	12.0	21.8	15.2	13.1	10.3	6.5	1.7	364.9
S3	13.4	47.1	9.8	14.5	55.2	29.8	40.1	57.2	6.3	13.8	18.1	10.9	10.0	9.0	10.6	17.5	9.5	6.1	1.2	379.8
S4	12.0	36.5	8.2	11.3	47.4	22.2	30.1	57.7	12.0	11.5	20.7	9.1	13.6	11.8	13.5	11.3	5.2	7.8	0.9	342.8
S5	11.7	51.8	10.5	13.4	60.9	27.7	35.0	51.4	11.4	17.3	17.7	10.4	14.1	18.2	12.4	14.7	7.0	8.3	1.3	395.0
S6	13.8	44.2	8.1	4.1	54.4	19.2	33.4	43.2	7.5	13.2	22.9	9.9	14.0	14.3	14.4	10.2	5.4	9.7	1.9	343.8
36 h																				
C	12.4^{a}	41.1^{a}	7.4^{a}	9.1^{a}	78.0^{bd}	22.2^{c}	52.0^{c}	63.7^{a}	7.6^{b}	16.1^{b}	32.2^{a}	9.3^{ac}	18.6^{c}	25.0^{a}	23.7^{cd}	5.9^{a}	18.9^{ab}	13.7^{bf}	2.7^{a}	459.5^{a}
S1	12.4^{a}	65.7^{b}	5.4^{b}	11.8^{b}	62.7^{a}	8.6^{a}	49.1^{a}	42.4^{b}	7.9^{b}	14.1^{b}	25.5^{bc}	10.2^{c}	14.3^{a}	13.0^{b}	20.4^{d}	10.1^{b}	26.7^{b}	12.7^{b}	2.9^{a}	415.9^{a}
S2	14.8^{bc}	86.2^{c}	5.1^{b}	14.8^{c}	90.6^{c}	21.0^{cd}	59.8^{d}	33.7^{c}	24.0^{a}	21.7^{c}	27.6^{c}	15.0^{b}	19.8^{cd}	31.4^{c}	34.4^{a}	19.0^{c}	89.7^{c}	16.0^{aeb}	15.2^{b}	639.6^{b}
S3	16.0^{c}	83.9^{c}	5.9^{b}	15.6^{c}	84.9^{cd}	17.0^{e}	60.6^{d}	48.4^{bd}	13.2^{c}	21.6^{ac}	22.0^{b}	10.1^{c}	22.1^{bde}	20.8^{a}	27.6^{bc}	9.2^{b}	52.8^{d}	13.9^{bcde}	6.9^{c}	552.5^{c}
S4	15.2^{bc}	71.7^{d}	7.2^{a}	20.0^{d}	72.3^{b}	18.6^{def}	52.6^{c}	51.6^{d}	13.5^{c}	16.9^{b}	27.9^{c}	9.9^{c}	12.4^{a}	12.9^{b}	19.9^{d}	9.7^{b}	55.4^{d}	16.5^{adf}	8.0^{c}	512.2^{c}
S5	14.5^{bc}	92.2^{e}	5.3^{b}	14.9^{c}	91.6^{c}	20.9^{cf}	58.4^{d}	33.6^{c}	13.6^{c}	20.5^{c}	23.4^{b}	14.6^{b}	24.4^{b}	24.0^{a}	28.7^{b}	19.4^{c}	85.4^{c}	18.4^{a}	12.5^{d}	616.2^{b}
S6	14.3^{b}	38.4^{a}	2.3^{c}	3.0^{e}	73.3^{b}	3.8^{b}	45.5^{b}	72.4^{e}	8.2^{b}	25.1^{a}	32.9^{a}	7.1^{a}	19.6^{ce}	22.9^{a}	19.3^{d}	7.4^{ab}	15.7^{a}	16.0^{acf}	3.0^{a}	430.2^{a}
60 h																				
C	11.9^{a}	28.5^{a}	3.6^{a}	8.7^{a}	60.6^{a}	14.5^{a}	46.8^{a}	58.5^{f}	8.4^{a}	15.8^{c}	30.1^{b}	8.5^{a}	19.4^{a}	25.3^{a}	24.3^{a}	7.7^{a}	18.1^{a}	15.3^{a}	2.7^{a}	408.6^{a}
S1	16.0^{b}	68.6^{b}	3.3^{a}	17.2^{b}	54.9^{a}	7.1^{b}	57.5^{b}	50.7^{e}	16.3^{b}	17.0^{c}	23.7^{cd}	12.2^{bd}	18.7^{a}	20.1^{b}	27.3^{a}	9.7^{ad}	36.1^{d}	14.8^{a}	6.1^{b}	477.6^{b}
S2	18.1^{bc}	96.7^{c}	4.1^{b}	17.3^{b}	91.8^{b}	18.2^{c}	66.6^{c}	43.0^{a}	29.8^{c}	23.7^{ad}	26.8^{bd}	16.0^{c}	18.8^{a}	32.7^{c}	40.0^{b}	27.3^{b}	98.0^{c}	19.8^{bc}	15.2^{c}	704.0^{df}
S3	18.6^{c}	82.4^{d}	5.4^{c}	17.1^{b}	94.6^{b}	16.0^{a}	67.9^{c}	67.1^{b}	13.5^{d}	23.9^{c}	22.2^{c}	13.2^{b}	24.3^{b}	22.3^{ab}	31.8^{c}	16.2^{c}	61.0^{b}	21.0^{bef}	8.4^{b}	626.7^{c}
S4	19.4^{c}	81.0^{d}	5.4^{c}	39.8^{c}	77.7^{c}	14.9^{a}	61.9^{bc}	82.9^{d}	20.0^{e}	19.5^{ac}	27.6^{bd}	12.4^{bd}	19.8^{a}	23.5^{ab}	32.5^{c}	17.1^{c}	75.5^{e}	23.8^{bf}	11.3^{d}	666.0^{cf}
S5	18.9^{c}	113.7^{e}	5.9^{d}	17.6^{b}	105.5^{d}	21.7^{d}	75.3^{d}	57.7^{ef}	13.3^{d}	26.6^{bd}	25.1^{cd}	18.3^{e}	19.9^{a}	32.8^{c}	33.8^{c}	25.6^{b}	80.0^{e}	19.0^{ade}	11.1^{d}	721.6^{d}
S6	19.8^{c}	35.9^{a}	2.4^{e}	4.2^{d}	91.2^{b}	2.2^{e}	58.3^{b}	112.2^{c}	12.0^{d}	24.1^{ab}	36.2^{a}	11.0^{d}	24.4^{b}	22.4^{ab}	34.7^{c}	11.2^{d}	29.7^{d}	22.3^{df}	4.3^{ab}	558.4^{e}

* C = control (uninoculated), S1 = *P. pentosaceus*, S2 = *P. acidilactici*, S3 = *W.cibaria*, S4 = *L. pentosus*, S5 = *L. plantarum*, S6 = *L. sakei*

[a-f]Values with different superscript letters in the same column are significantly different ($p < 0.05$)

Figure 4.9

Pyruvate metabolism in lactic acid bacteria (data from KEGG). 1-pyruvate dehydrogenase, 2-pyruvate-formate-lyase, 3-lactate dehydrogenase, 4-pyruvate oxidase, 5-acetate kinase, 6-alcohol dehydrogenase, 7-aldehyde dehydrogenase (Redrawn from Adamberg et al., 2006).



The effect of starter cultures on the concentration of total protein nitrogen, non-protein nitrogen and total amino acid in Thai fermented sausages are shown in Table 4.6. The proteolytic effect of LAB starter cultures on fermented sausages was evidenced by significant lower amount of total protein in the inoculated batches ($p < 0.05$) than in the control sample after 60 h of fermentation. Sausage inoculated with *L. plantarum* was significantly different ($p < 0.05$) from other inoculated sausages. There was not significantly different ($p > 0.05$) among *P. pentosaceus*, *P. acidilactici* and *W. cibaria*. *L. pentosus* batch was not significantly different ($p > 0.05$) from *L. sakei* batch (see Table 4.6). Furthermore, the hydrolytic effect of LAB was indicated by significant higher accumulation of NPN and total amino acid in inoculated samples than control sample ($p < 0.05$) as shown in Table 4.6. NPN is an indicator for the degree of proteolysis in fermented sausage. The amount of NPN was significant highest ($p < 0.05$) in *L. sakei* inoculated sausage and significant lowest ($p < 0.05$) in the control sample. Sausages inoculated with *P. acidilactici*, *W. cibaria* and *L. plantarum* were not significantly different ($p > 0.05$) in amount of NPN, but they were

significantly different ($p < 0.05$) between *P. pentosaceus* and *L. pentosus*. Moreover, a significant greater increase ($p < 0.05$) in total FAA was also detected in sausages inoculated with LAB compared to the control (Table 4.6). Sausages inoculated with *P. pentosaceus* and *L. sakei* were significantly different ($p < 0.05$) from other inoculated sausages. There were no significant differences ($p > 0.05$) observed in total FAA when sausages inoculated with *P. acidilactici* and *L. plantarum*, *W. cibaria* and *L. pentosus*. Hierro et al. (1999), Bruna et al. (2000a and 2000b), Bolumar et al. (2001) and Hughes et al. (2002) reported similar results. Fadda et al. (1998) indicated that *L. plantarum* CRL 681 had more active proteolytic system, showing the highest amino acid release in the medium after 72 h of incubation while *L. casei* showed a continued hydrolytic activity with a lower amino acids concentration along the studied period. The hydrolysis of meat proteins generates polypeptides that can be further degraded to smaller peptides and FAA by various microbial and endogenous muscle enzymes (DeMasi et al., 1990; Durá et al., 2004; Hughes et al., 2002). Molly et al., (1997) explained that protein was hydrolyzed to peptides by muscle enzymes. Consequently, the peptides initially presented in meat mixture could be taken up by the cells and also hydrolyzed by the set of bacterial enzymes (see Figure 4.10). Demeyer et al. (2000) and Sanz and Toldrá (2001 and 2002) implicated exopeptidases from *L. sakei* together with muscle aminopeptidase as being responsible for the generation of FAA from the N-amino terminal of muscle proteins and peptides. The typical microflora of sausage (lactic acid bacteria) would then have a high amount of substrate for deamination, decarboxylation and transamination reactions, and amino acids could be transformed into volatile compounds, resulting in an enhancement of flavour of dry fermented sausage.

Table 4.6

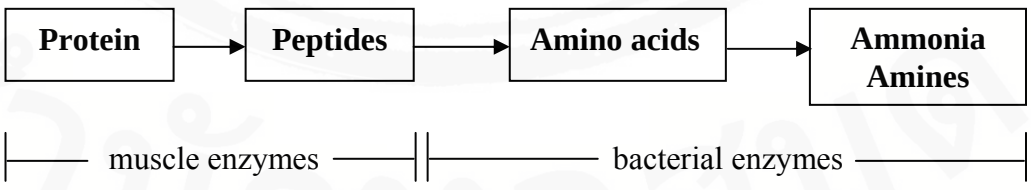
Effect of LAB starter cultures on changes in total protein nitrogen, non-protein nitrogen and total amino acid of Thai fermented sausages at 60 h of fermentation

Sausage samples	Total protein nitrogen (g/100g sausage)	Non-protein nitrogen (g/100g sausage)	Total amino acid (g/100g sausage)
Control	0.204±0.011[a]	0.192±0.008[a]	0.409±0.011[a]
P. pentosaceus	0.109±0.039[d]	0.227±0.004[b]	0.478±0.039[b]
P. acidilactici	0.118±0.021[d]	0.254±0.004[d]	0.707±0.021[ef]
W. cibaria	0.115±0.031[d]	0.256±0.005[d]	0.619±0.031[c]
L. pentosus	0.069±0.031[c]	0.239±0.009[b]	0.666±0.031[cf]
L. plantarum	0.025±0.036[b]	0.262±0.008[d]	0.722±0.036[e]
L. sakei	0.060±0.028[c]	0.275±0.007[c]	0.558±0.028[d]

[a-f] Values with different superscript letters in the same column are significantly different ($p < 0.05$).

Figure 4.10

Schematic of the hydrolysis of protein by muscle enzymes and bacterial enzymes (Molly et al., 1997)



4.4 Flavour formation

4.4.1 Change in leucine concentration

The degradation of leucine, branched chained amino acids (BCAA), generates volatile compounds involved in the flavour of dry fermented sausage. Therefore, the concentration of Leu in Thai fermented sausages inoculated with/without LAB starter cultures was assayed as shown in Figure 4.11. The control sample and sausages inoculated with *W. cibaria*, *L. pentosus* and *L. sakei* showed a decrease of Leu concentration until 24 h and followed by a slight increase. In sausages inoculated with *L. plantarum* and *P. acidilactici*, the concentration of Leu remained unchanged until 24 h and then progressively increased. The Leu concentration observed in sausage inoculated with *P. pentosaceus* increased until 12 h and then slightly decreased. At the end of fermentation, the concentration of Leu observed in sausages inoculated with LAB starter cultures was higher than in the control. The appearance may be due to microbial enzymes presence in the starter culture. Herranz et al. (2005) improved the flavor formation of dry-fermented sausages by the addition of free amino acids, especially, Leu, branched-chain amino acid. Their results showed that the starter could have used this amino acid to generate volatile compounds. Several authors, such as Berdagué et al. (1993), Larrouture et al. (2000), Montel et al. (1996) and Stahnke et al. (2002) mentioned that Leu was converted to volatile compounds; 3-methyl-butanal, 3-methyl-butanoic acid and 3-methyl-butanol by LAB as shown in Figure 4.12. The branched-chain amino acid aminotransferase encoded by *ilvE* catalyzes the conversion of methyl-branched amino acid, leucine into methyl-branched keto acid, α-ketoisocaproic acid. The keto acid is subsequently converted into the corresponding aldehyde, 3-methyl-butanal, alcohol, 3-methyl-butanol, and 3-methyl-butanoic acid.

Figure 4.11

Profiles of leucine concentration in Thai fermented sausages with/without starter cultures during fermentation.

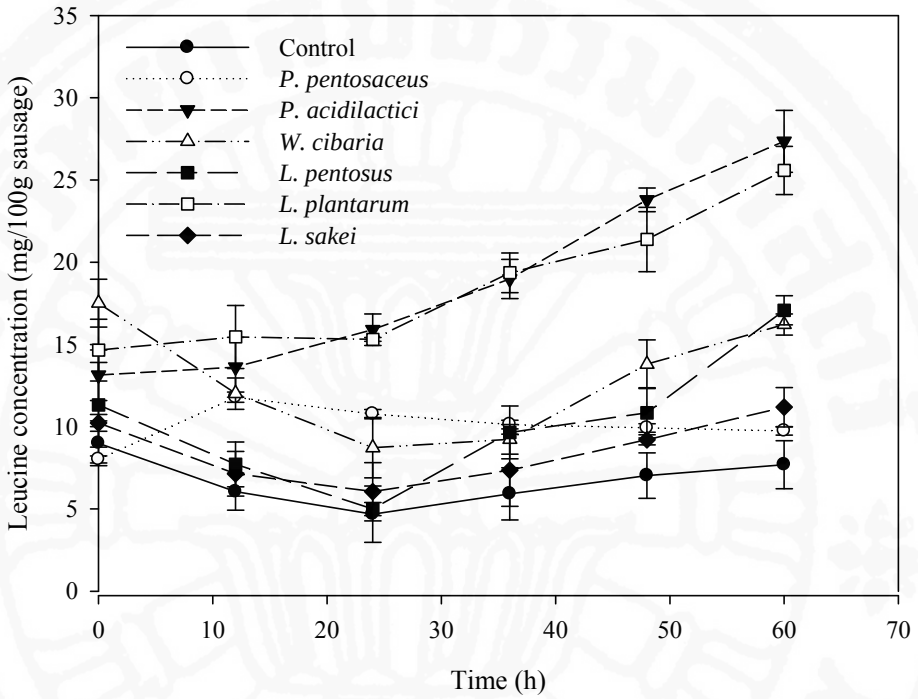
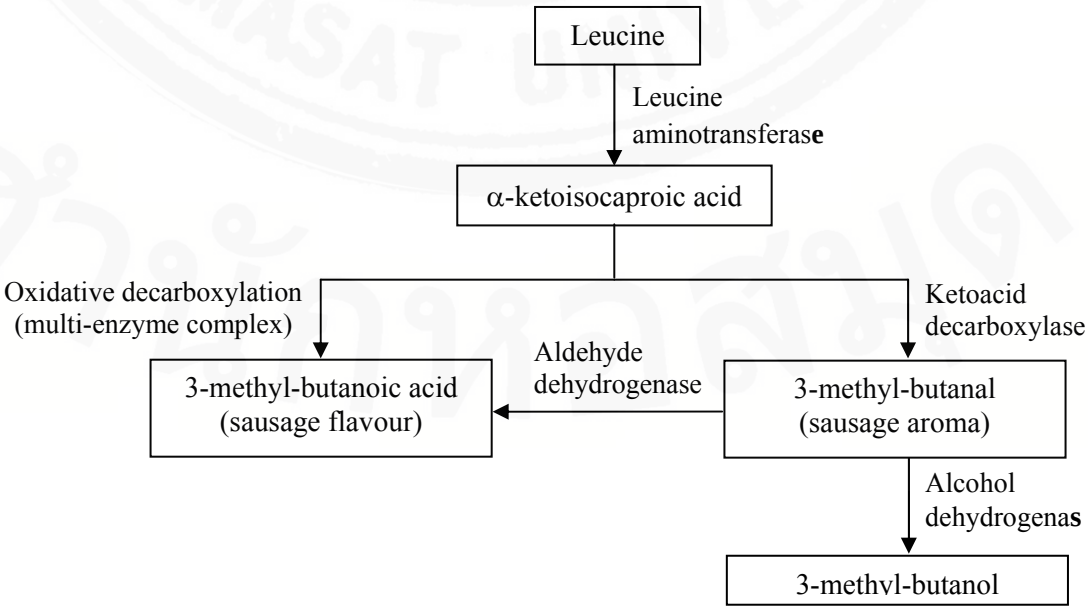


Figure 4.12

Schematic of the conversion of leucine into volatile compounds (Leroy et al., 2006).



4.4.2 Volatile compound analysis

The evolutions of the volatile compounds such as 3-methyl-butanal, 3-methyl-butanoic acid and 3-methyl-butanol derived from Leu were variable throughout the fermentation (see Figure 4.13). The production of 3-methyl-butanal was observed from sausages inoculated with *P. pentosaceus*, *P. acidilactici*, *L. pentosus* and the control. The concentration of 3-methyl-butanal obtained from the batch inoculated with *P. pentosaceus* was achieved its highest value at 24 h. As shown in Figure 4.13a, *P. acidilactici* and the control batches showed an increase in 3-methyl-butanal at 12 h and then its decrease until the end of fermentation in two batches, probably due to its reduction to 3-methyl-butanol. The sausage inoculated with *L. pentosus* yielded the lowest concentration of 3-methyl-butanal. All sausage samples showed an increase in 3-methyl-butanoic acid concentration (see Figure 4.13b). Thai fermented sausage inoculated with LAB starter cultures showed higher concentrations of 3-methyl-butanoic acid than the control. The results indicated that the production of 3-methyl-butanoic acid derived from Leu was observed in fermented sausages inoculated with LAB. Demeyer et al. (2000) and Larrouture et al. (2000) also reported that all strains of LAB were able to catabolize leucine to 3-methyl-butanoic acid. In addition, the 3-methyl-butanol, branched alcohol, derived from the 3-methyl-butanal, was observed in sausages inoculated with *P. pentosaceus*, *P. acidilactici*, *W. cibaria*, *L. plantarum* and *L. sakei* and control sample (see Figure 4.13c). The concentration of 3-methyl-butanol increased throughout the fermentation. At the end of fermentation, control sample showed the highest concentration of 3-methyl-butanol. Stahnke (1995), Montel et al. (1996), Larrouture et al. (2000) and Leroy et al. (2006) described that the compounds 3-methyl-butanal and 3-methyl-butanoic acid, derived from Leu by keto acid decarboxylation and oxidative decarboxylation, respectively, have been linked with sausage aroma (see Figure 4.12). The production of 3-methyl-butanoic acid might result from two possible catabolic pathways. The first catabolic pathway could be a decarboxylation of α-ketoisocaproic acid into 3-methyl-butanal which could be immediately dehydrogenized into 3-methyl-butanoic acid by an aldehyde dehydrogenase as shown in Figure 4.12. The second pathway could be a dehydrogenation of α-ketoisocaproic acid by a multi-enzymatic complex leading to

acyl-CoA which could be hydrolysed into 3-methyl-butanoic acid. The 3-methyl-butanal compound could be reduced to 3-methyl-butanol by alcohol dehydrogenase. It is experimentally evident that the LAB starter could accelerate the degradation of Leu to 3-methyl-butanoic acid rather than to 3-methyl-butanal. Moreover, sausages inoculated with LAB produced a lower concentration of 3-methyl-butanol than the control. Volatile compounds, 3-methyl-butanal and 3-methyl-butanoic acid have a strong effect on the sensorial qualities of fermented sausages (Larrouture et al., 2000; Stahnke, 1995; Schmidt and Berger, 1998).

Figure 4.13

Volatile compound profiles of Thai fermented sausages inoculated with/without LAB starter cultures (a) 3-methyl-butanal, (b) 3-methyl-butanoic acid, and (c) 3-methyl-butaol.

a) 3-Methyl-butanal

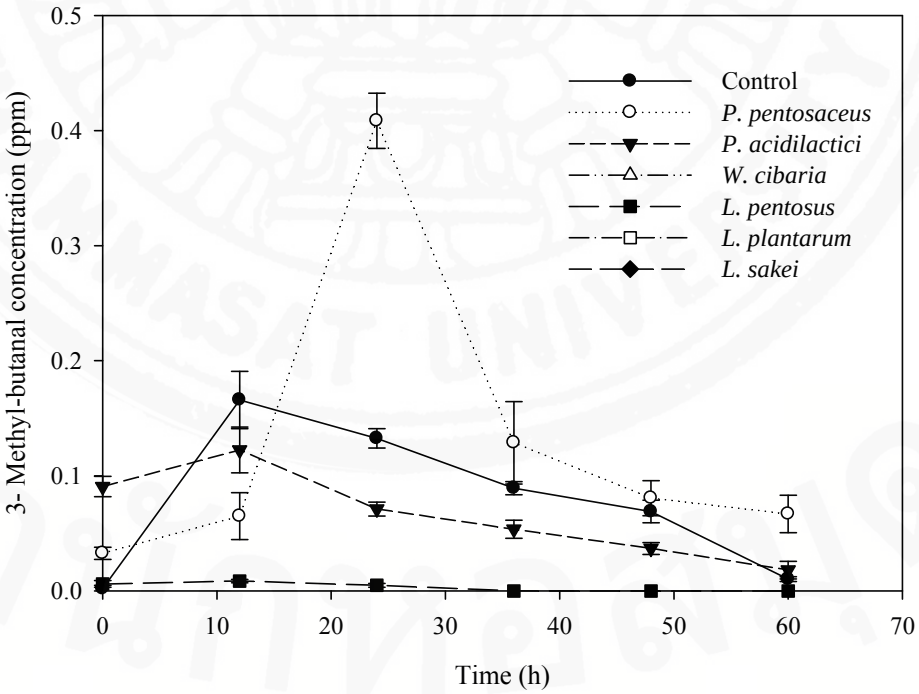
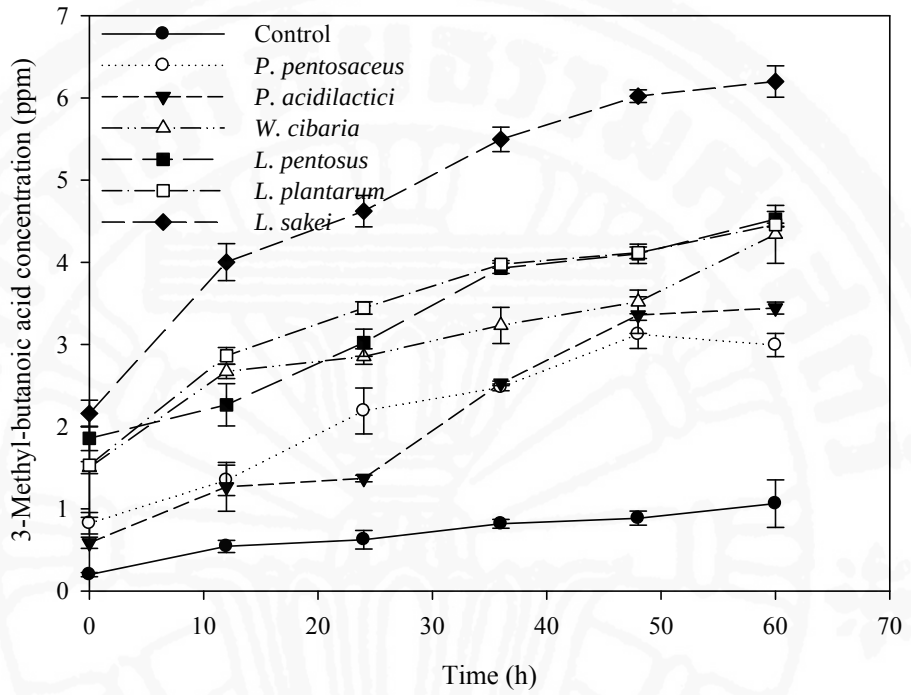
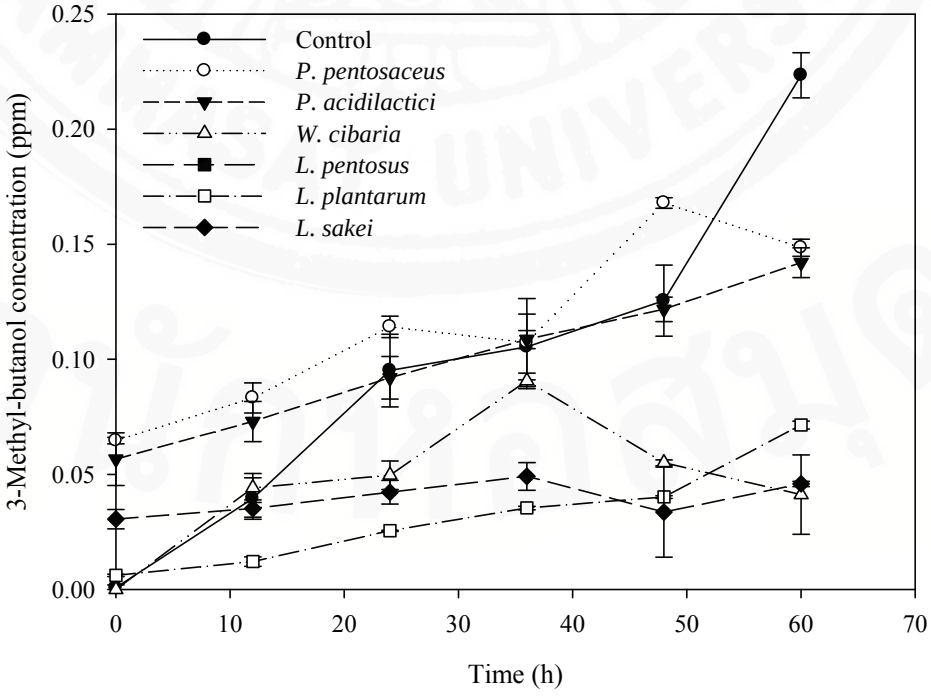


Figure 4.13 (Continued)

b) 3-Methyl-butanoic acid



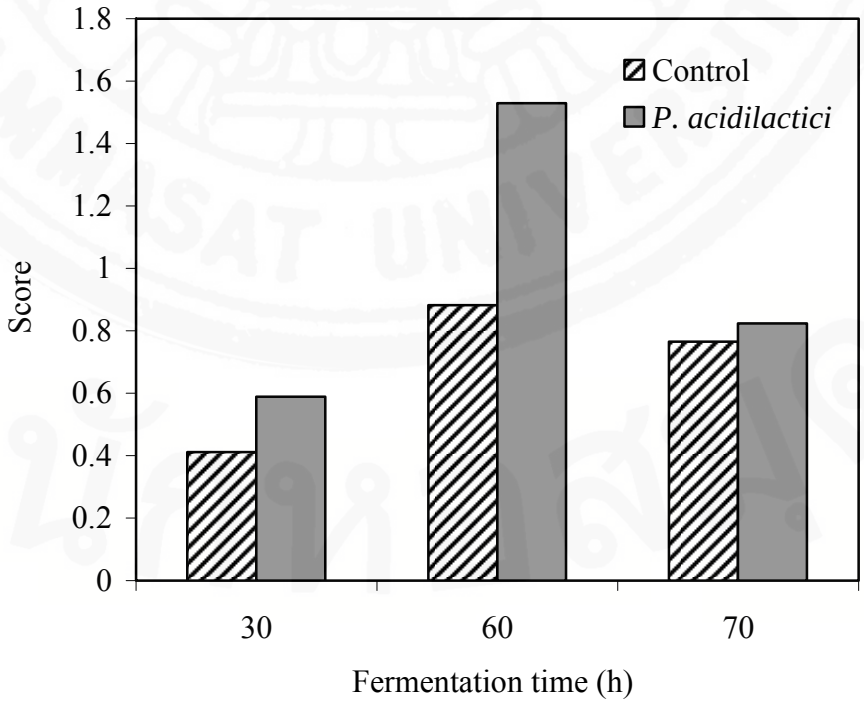
c) 3-Methyl-butanol



4.5 Sensory evaluation

The inoculation of Thai sausages with lactic acid bacteria caused the rapid the decrease of pH, and the inhibition of growth of contaminant pathogenic microorganisms. In addition, it yielded a better end-product such as cured meat pigment and lactic acid production. First, the fermentation time period of Thai fermented sausage production was investigated at 30, 60, and 70 h. The sensory evaluation was assessed between control sample and sausage inoculated with *P. acidilactici*. The result of fermentation time is presented in Figure 4.14. At 60 h of fermentation time, the sausage inoculated with *P. acidilactici* and control sample received higher overall preference score than other fermentation times, i.e., 30 and 70 h. Therefore, the fermentation time period of 60 h was applied in this study.

Figure 4.14
Sensory results of overall preference of fermented Thai sausages inoculated with *P. acidilactici* compare with control sample.



Thai fermentation sausages inoculated with LAB starter culture and the control sample were then tested for the acceptance in flavour, taste, texture and overall preference. Thai fermented sausages were presented to untrained 30-member panels for acceptability testing. The result of the test is presented in Table 4.7. The control sample received significant lower values than *P. acidilactici* inoculated sausage for all characterization ($p < 0.05$). The highest score ($p < 0.05$) for overall preference was given for the sausage inoculated with *P. acidilactici* at a score of 5.12 out of 7. Although, the scores for flavour, taste, texture, sourness, and saltiness were no significant differences ($p > 0.05$) among sausages inoculated with *W. cibaria* and *L. sakei* and control, the control had higher rating on overall preference than *W. cibaria* and *L. sakei*.

The significant differences between the mean values of sensory evaluation of Thai fermented sausages were determined by ANOVA as shown in Table 4.7. For colour score, the control batch was significantly lower ($p < 0.05$) than *L. pentosus*, *P. pentosaceus* and *P. acidilactici*. The difference was not statistically significant among inoculated sausages ($p > 0.05$). Sausage inoculation with *P. acidilactici* obtained significantly higher flavour score than control ($p < 0.05$). Sausages inoculated with other LAB starter cultures had better flavour score than control, probably related to the higher concentration of volatile compounds derived from Leu, but there were not significant differences among all batches. Sausages inoculated with *P. acidilactici* and *W. cibaria* showed significant higher score of texture ($p < 0.05$) than control while *P. pentosaceus*, *L. pentosus*, *L. plantarum* and *L. sakei* showed insignificant differences from control ($p > 0.05$). The score of texture obtained from *W. cibaria* was insignificantly different from other inoculated batches. *L. pentosus*, *P. pentosaceus* and *L. sakei* were significantly different from *P. acidilactici* ($p < 0.05$). For taste score (see Table 4.7), *P. acidilactici* and *L. plantarum* were significantly higher than control. Taste scores obtained from sausages inoculated with *P. pentosaceus* and *L. plantarum* were insignificantly different from ($p > 0.05$) other inoculated batches. Taste scores of sausages inoculated with *L. sakei*, *L. pentosus* and *W. cibaria* were not significantly different from *P. pentosaceus* and *L. plantarum*. Sausage inoculated with *P. acidilactici* showed significantly higher score ($p < 0.05$) of sourness than control. This might be due to the highest lactic acid concentration in sausage inoculated with *P.*

acidilactici. However, there were no significant different scores of sourness among inoculated batches ($p > 0.05$). No significant differences ($p > 0.05$) were found for saltiness. Finally, in overall preference, *P. pentosaceus*, *P. acidilactici* and *L. plantarum* batches obtained significantly higher score ($p < 0.05$) than control. The score of overall preference of sausage inoculated with *L.sakei* was significantly different ($p < 0.05$) from *L. plantarum*, *P. acidilactici* and *P. pentosaceus*. Overall preference score of sausage inoculated with *W. cibaria* was only significantly different ($p < 0.05$) from *P. acidilactici*. Sausage inoculated with *L. plantarum* was not significantly different ($p > 0.05$) from *L. pentosus*, *P. acidilactici*, *P. pentosaceus* and *W. cibaria*. By comparing overall preference, sausage inoculated with *L. pentosus* showed insignificantly different ($p > 0.05$) from other inoculated batches.

The result of sensory evaluation was compared with the chemical analysis. The scores for colour, flavour, taste, texture and sourness of sausage inoculated with *P. acidilactici* were higher than control sample. Agreement with chemical analysis, the sausage inoculated with *P. acidilactici* yielded better end-products such as cured meat pigment, 3-methyl-butanoic acid, 3-methyl-butanol, NPN and lactic acid.

Table 4.7

Sensory results of Thai fermented sausages inoculated with various starter cultures compare with control sausage at the end of fermentation.

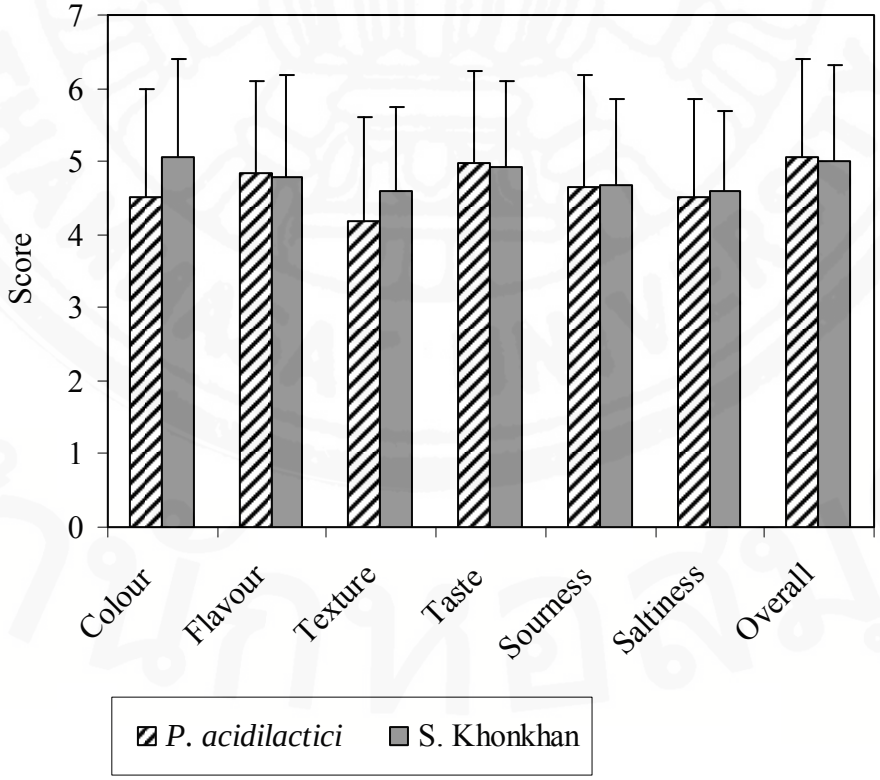
Batch	Characteristics						
	Colour	Flavour	Taste	Texture	Sourness	Saltiness	Overall preference
Control	4.56 ± 1.52^{a}	4.50 ± 1.22^{a}	4.15 ± 1.56^{b}	4.65 ± 1.41^{b}	4.26 ± 1.48^{b}	4.21 ± 1.11^{a}	4.38 ± 1.48^{b}
P. pentosaceus	5.00 ± 1.31^{b}	5.00 ± 1.20^{ab}	4.00 ± 1.56^{ab}	4.94 ± 1.48^{bd}	4.62 ± 1.43^{ab}	4.44 ± 1.43^{a}	4.67 ± 1.63^{ac}
P. acidilactici	5.26 ± 1.57^{b}	5.35 ± 1.26^{b}	4.94 ± 1.41^{a}	5.18 ± 1.45^{a}	4.97 ± 1.57^{a}	4.62 ± 1.28^{a}	5.12 ± 1.59^{a}
W. cibaria	5.09 ± 1.32^{ab}	5.12 ± 1.26^{ab}	4.56 ± 1.46^{bd}	4.79 ± 1.28^{acd}	4.56 ± 1.35^{ab}	4.44 ± 1.13^{a}	4.06 ± 1.14^{bcd}
L. pentosus	5.21 ± 1.34^{b}	5.18 ± 1.39^{ab}	4.03 ± 1.67^{be}	4.85 ± 1.36^{bd}	4.41 ± 1.55^{ab}	4.41 ± 1.26^{a}	4.68 ± 1.53^{ab}
L. plantarum	4.94 ± 1.18^{ab}	5.18 ± 1.22^{ab}	4.59 ± 1.47^{acde}	4.88 ± 1.33^{ab}	4.68 ± 1.55^{ab}	4.59 ± 1.25^{a}	4.85 ± 1.49^{ad}
L. sakei	4.71 ± 1.26^{ab}	4.79 ± 1.33^{ab}	4.15 ± 1.45^{bc}	4.50 ± 1.25^{bc}	4.32 ± 1.33^{ab}	4.15 ± 1.17^{a}	4.06 ± 1.32^{b}

[a-e] Values in a low followed by the same letter are not significantly different ($p < 0.05$)

As previously observed, *P. acidilactici* showed significant acceptance ($p < 0.05$). Thus, the next experiment was to test sensory evaluation between Thai fermented sausage inoculated with *P. acidilactici* and commercial Thai fermented sausage, under the brand name of S. Khonkhan. Thai fermented sausages were presented to untrained 40-member panels for acceptability testing. The result of acceptance in flavour, taste, texture and overall preference is presented in Figure 4.15. Sausage inoculated with *P. acidilactici* obtained lower score for colour and texture than commercial sausage, S. Khonkhan. The acceptability of flavour, taste, sourness, saltiness and overall preference of sausage inoculated with *P. acidilactici* was compared to S. Khonkhan. Unfortunately, no significant differences were found between these two batches.

Figure 4.15

Sensory results of fermented Thai sausages inoculated with *P. acidilactici* compare with commercial sausage, S. Khonkhan.



4.6 Mathematical modelling in fermented sausage

4.6.1 Model equations

The assumptions of the proposed kinetic model of Thai fermented sausage were the following: (1) the carbon and nitrogen composition in lactic acid bacteria are assumed to be identical to their composition in *Bacillus subtilis* estimated by Skolpap et al. (2004), since both lactic acid bacteria and *B. subtilis* are gram positive (2) morphology of lactic acid bacteria, verified by the experiment, is doublets (Phalakornkule and Tanasupawat 2006). Thus, the conversion factor of cell concentration of LAB is $2x10^{-12}$ g/CFU (Scharer, 2009); (3) morphological feature of *P.acidilactici*, verified by the experiment, is the mixture of doublets and tetrads (Phalakornkule and Tanasupawat 2006). Thus, the conversion factor of cell concentration is $3x10^{-12}$ g/CFU (Scharer, 2009); (4) total protein is degraded to production of biomass and non-protein nitrogen; and (5) glucose consumption mainly yields biomass and lactic acid production. The set of differential equations were solved by using the orthogonal collocation numerical coded in Matlab (MathWorks, Natick, MA) by Constantinides and Mostoufi (1999).

In all experiments of Thai fermented sausage production, the microbial growth prefers a total protein-limiting to a carbon-limiting condition. Therefore, the biomass production rate is expressed as the function of total protein concentration as:

LAB concentration (y_1):

$$\frac{dy_1}{dt} = \left(1-e^{-k_3 t}\right)\left(\frac{k_1 y_2}{k_2+y_2}\right) y_1 \left(1-\frac{y_1}{y_{1,max}}\right) - k_{12} y_1 \qquad (4.1)$$

where k_1 = maximum specific growth rate (μ_{max})

k_2 = total protein saturation constant

k_3 = time lag coefficient

k_{12} = specific rate of biomass lysis

$y_{1,max}$ = maximum LAB concentration produced

y_2 = total protein concentration.

The initial lag phase in Eq. (4.1) is expressed in terms of exponential decay function. A Monod model (Monod, 1949) accounts for the effect of the total protein concentration on the specific microbial growth rate. The term $1-\frac{y_1}{y_{1,\max}}$, the inhibition function, was suggested by the original logistic model (Fujikawa et al., 2003). The value of a monotonic inhibition function is approximately between one and zero.

The total protein concentration containing in Thai fermented sausage ingredient was utilized in biomass production and degraded into non-protein nitrogen. The total protein differential equation is expressed as follows:

Total protein concentration (y_2):

$$\frac{dy_2}{dt} = -cor_{bio}\left(\frac{N_{bio}AW_N}{MW_{bio}}\right)\frac{dy_1}{dt} + cor_{NPN}\left(\frac{N_{NPN}AW_N}{MW_{NPN}}\right)\frac{dy_3}{dt} - cor_X \qquad (4.2)$$

where AW_N = atomic weight of nitrogen

cor_{bio} = correction term for total protein consumption of biomass

cor_{NPN} = correction term for total protein degradation into non-protein nitrogen

cor_X = correction term for total protein loss due to conversion factor error of LAB concentration from CFU to g/100g sausage

MW_{bio} = formula weight of biomass

MW_{NPN} = formula weight of non-protein nitrogen

N_{bio} = mole fraction of nitrogen in biomass

N_{NPN} = mole fraction of nitrogen in non-protein nitrogen

y_1 = LAB concentration

y_3 = non-protein nitrogen concentration.

In Eq. (4.2) and all subsequent equations, the term $\frac{dy_i}{dt}$ represents the reaction rate for variable y_i. The cor_{bio} term expresses estimating uncertainty of total protein in biomass empirical formula. cor_{NPN} accounts for analytical measurement discrepancy of total protein degradation.

The production rate of non-protein nitrogen was modeled by the Luedeking-Piret equation comprising growth associated and non-growth associated terms.

Non-protein nitrogen concentration (y_3):

$$\frac{dy_3}{dt} = -k_4\frac{dy_2}{dt} + k_5 y_2 \qquad (4.3)$$

where k_4 = growth associated non-protein nitrogen formation constant

k_5 = non-growth associated non-protein nitrogen formation constant.

The model for lactic acid synthesis can be expressed as follows:

Lactic acid concentration (y_4):

$$\frac{dy_4}{dt} = k_6\frac{dy_1}{dt} + k_7\left(1 - \frac{y_4}{y_{4,\max}}\right) \qquad (4.4)$$

where k_6 = growth associated lactic acid formation constant

k_7 = non-growth associated lactic acid formation constant

y_4 = lactic acid concentration

$y_{4,\max}$ = maximum lactic acid concentration produced.

The constant term, $y_{4,\max}$, in Eq.(4.4) accounts for the maximum lactic acid concentration. The formation of lactic acid was limited by its inhibitory effect which is introduced by the term $1 - \frac{y_4}{y_{4,\max}}$.

Similarly to lactic acid model, the model for formic acid formation can be written as:

Formic acid concentration (y_5):

$$\frac{dy_5}{dt} = k_8\frac{dy_1}{dt} + k_9\left(1 - \frac{y_5}{y_{5,\max}}\right) \qquad (4.5)$$

where k_8 = growth associated constant for formic acid

k_9 = non-growth associated constant for formic acid

y_5 = formic acid concentration

$y_{5,\max}$ = maximum formic acid concentration produced.

Glucose was mainly consumed for cell growth and lactic acid synthesis. The glucose utilization was inhibited by the maximum concentration of lactic acid produced. Therefore, the glucose model equation can be expressed as:

Glucose concentration (y_6):

$$\frac{dy_6}{dt} = -k_{10}\frac{dy_1}{dt} - k_{11}\left(1 - \frac{y_4}{y_{4,max}}\right) \quad (4.6)$$

where $k_{10} = \frac{1}{Y_{X/S}} + \frac{1}{Y_{L/S}}k_6$

$k_{11} = \frac{1}{Y_{L/S}}k_7$

$Y_{X/S}$ = biomass yield

$Y_{L/S}$ = lactic acid yield

y_6 = glucose concentration.

4.6.2 Model parameter estimation

The set of differential equations were solved simultaneously by using orthogonal collocation. Initially, however, a number of parameters were not precisely known. Two experimental data sets, i.e., control and *P. acidilactici* inoculated experiments, were applied to estimate parameter values and to calibrate the model using the Gibbs parameter sampling approach (Min, 1998; Gilks et al., 1998). Each parameter to be estimated was assigned an equilateral triangular distribution comprising the maximum, the mean (most likely), and the minimum possible values.

The mean value was established by using preliminary, trial and error estimates of the parameters and comparing the predictions with the experimental results. The parameter distribution space was sampled by a Monte Carlo draw. The sampled value was applied in calculation of the values of dependent variables by integrating the differential equations Eqs. (4.1) to (4.6). The predicted values were then compared with the observed values by computing the normalized sum of squares between the observations and predictions.

The parameter sampling procedure was repeatedly carried out for a number of times by random draw using the Monte Carlo technique. The most likely set of parameters was obtained after numerous (at least 10,000) trials. In other words, each

trial yielded a set of parameter estimates. The most likely set was identified by statistical analysis of the sampled parameter vector space that met the acceptance criterion of Gilks et al. (1998).

The goodness of fit was evaluated non-parametrically by estimating the χ^2 statistic between observed and predicted concentrations. Six state variables were tested including the LAB concentration (y_1), total protein concentration (y_2), non-protein nitrogen concentration (y_3), lactic acid concentration (y_4), formic acid concentration (y_5), and glucose concentration (y_6). The observed results were comparable with the predicted results at 95% confidence level (in Appendix C). The results of modeling are illustrated in Figure 4.16 to Figure 4.21. The model parameter values of control and *P. acidilactici* inoculated experiments are listed in Table 4.8.

Table 4.8

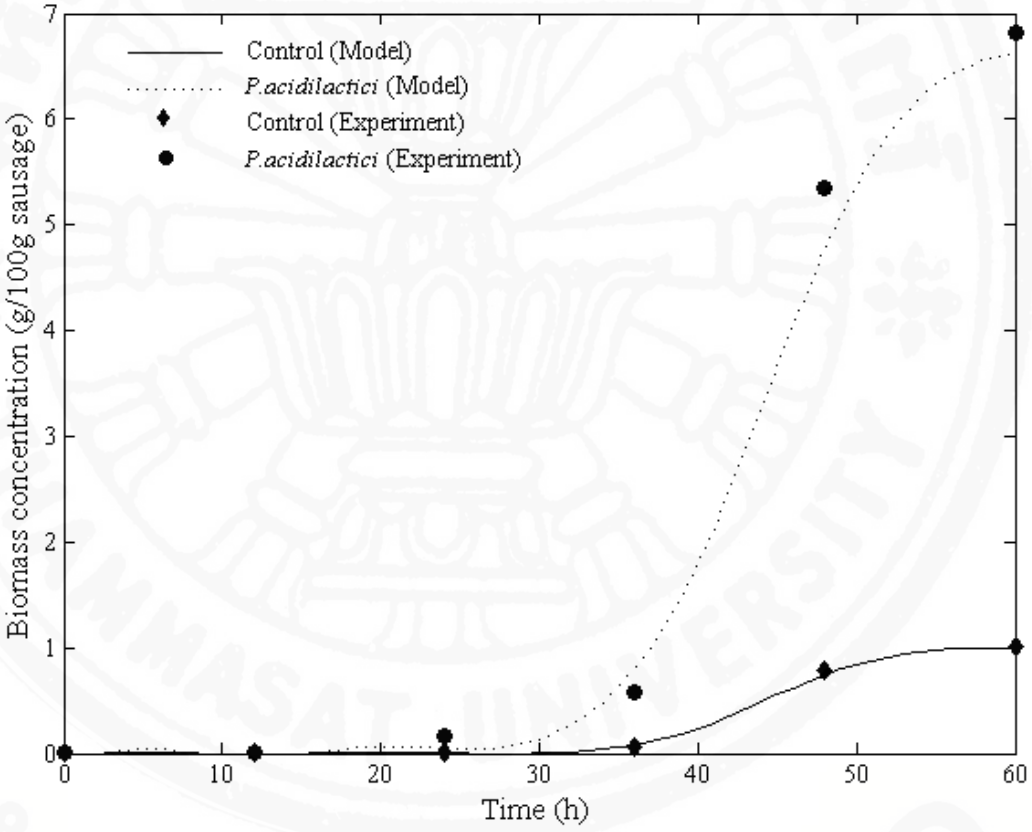
Estimated values of model parameters

Model parameter	Value for control experiment	Value for *P. acidilactici* inoculated experiment
k_1	0.330	0.244
k_2	0.061	0.088
k_3	0.142	0.141
k_4	$3.7\text{x}10^{-5}$	$4.64\text{x}10^{-4}$
k_5	$1.517\text{x}10^{-5}$	$1.275\text{x}10^{-5}$
k_6	0.414	$1.112\text{x}10^{-3}$
k_7	0.064	0.095
k_8	0.013	$2.623\text{x}10^{-4}$
k_9	$1.458\text{x}10^{-3}$	$1.7498\text{x}10^{-3}$
k_{10}	$9.855\text{x}10^{-3}$	$6.674\text{x}10^{-3}$
k_{11}	0.101	1.280
k_{12}	$1.7\text{x}10^{-3}$	$1.9\text{x}10^{-3}$
cor_{bio}	1.118	1.094
cor_{NPN}	0.832	0.723
cor_X	0.025	0.046

The modeled results of LAB concentration of all experiments showed a good agreement with the observed results as shown in Figure 4.16. For sausage inoculated with *P. acidilactici*, the calculated χ^2 values were higher than those of the control batch (see Table C.5 and C.10). However, all of estimated χ^2 values were within the listed 95% probability limit. The lower χ^2 estimates indicated that an agreement between predicted and observed cell concentration was better.

Figure 4.16

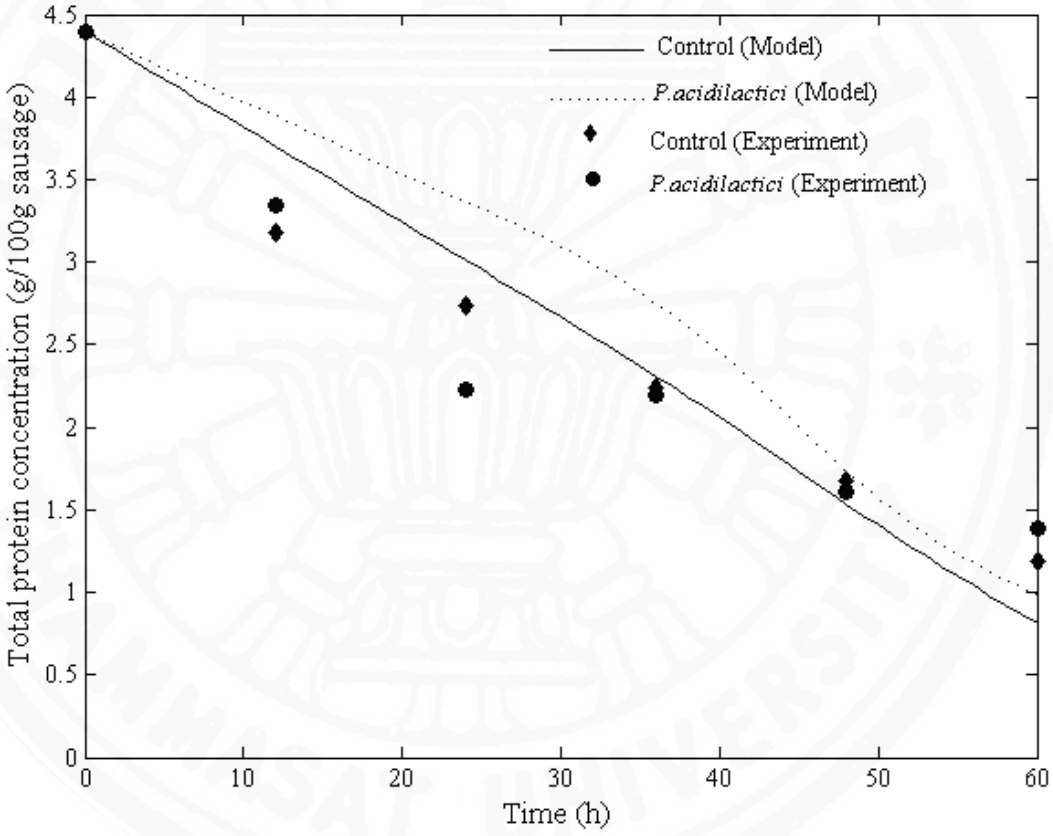
Profiles of biomass concentration



The predicted dissolved total protein concentration was compared with observation as presented in Figure 4.17. The estimated χ^2 probability of total protein concentration of sausage inoculated with *P. acidilactici* was relatively higher than that of control batch (see Table C.5 and C.10).

Figure 4.17

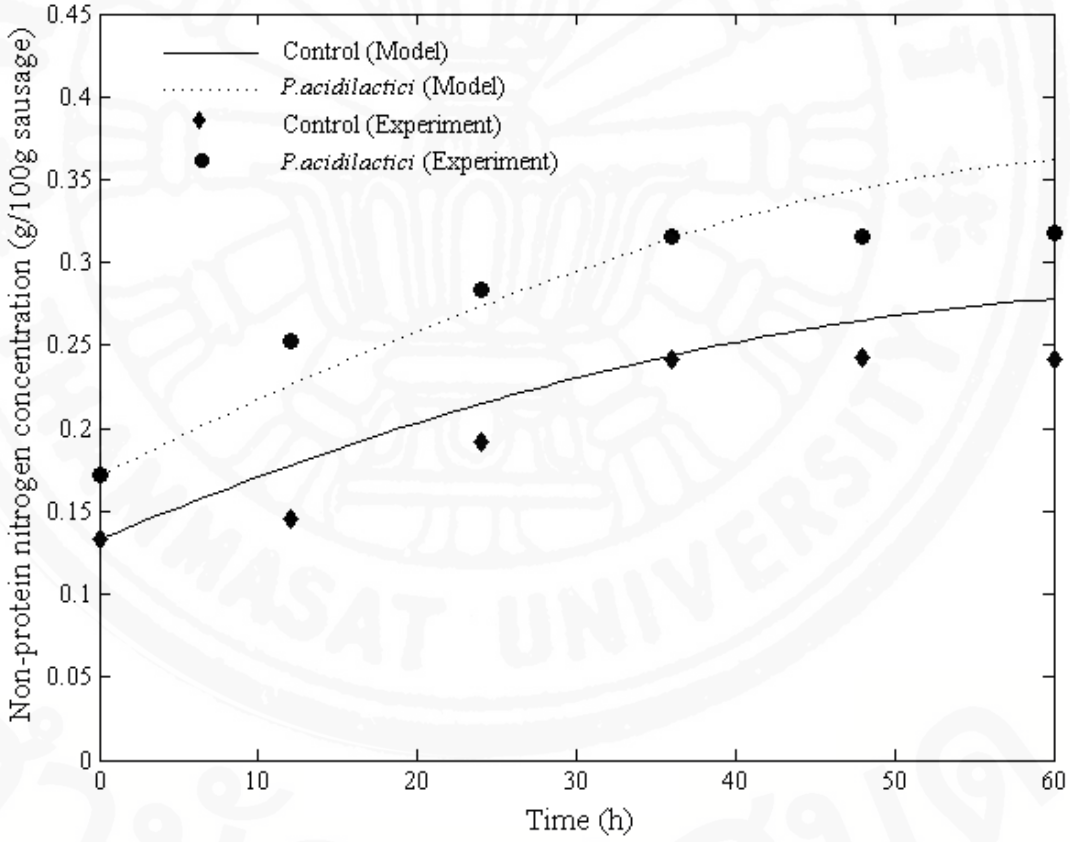
Profiles of total protein concentration



The predicted dissolved non-protein nitrogen concentration was compared with observation as presented in Figure 4.18. For the initial non-protein nitrogen production, the modeled results showed a good fit with the experimental results. The estimated χ^2 probability of non-protein nitrogen concentration of sausage inoculated with *P. acidilactici* was relatively higher than that of the control batch (see Table C.5 and C.10).

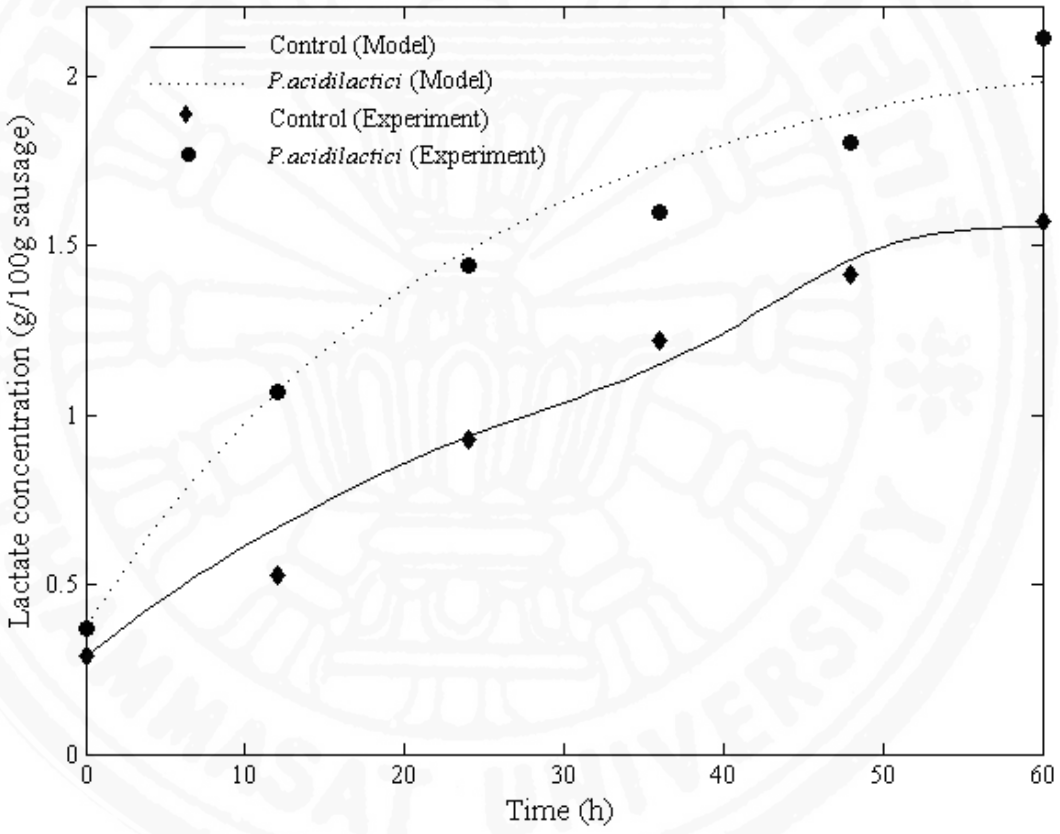
Figure 4.18

Profiles of non-protein nitrogen



The modeled results of lactic acid concentration were in good agreement with the observed results as shown in Figure 4.19. The estimated χ^2 probability of lactic acid concentration was small in all experiments (see Table C.5 and C.10).

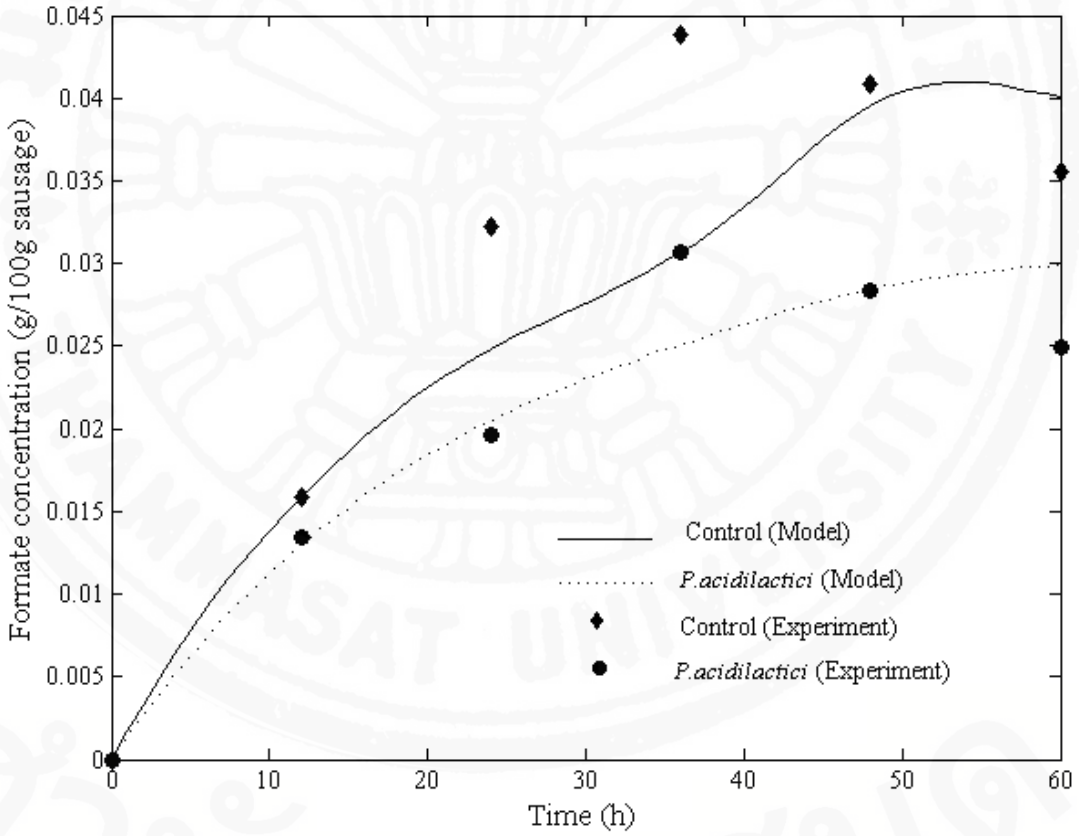
Figure 4.19
Profiles of lactic acid concentration



In contrast to control batch, the modeled results of formic acid concentrations obtained from sausage inoculated with *P. acidilactici* fitted well with the experimental results as shown in Figure 4.20. Moreover, the estimated χ^2 probability of formic acid concentration of control batch was relatively higher than that of sausage inoculated with *P. acidilactici* (see Table C.5 and C.10).

Figure 4.20

Profiles of formic acid concentration



The calculated χ^2 probability of glucose concentration was relatively small in control batch (see Table C.5). Therefore, the modeled results (see figure 4.21) showed a good agreement with the observed result. The calculated χ^2 value of sausage inoculated with *P. acidilactici* was higher than those of the control batch (see Table C.5 and C.10). However, the estimated χ^2 values were within the listed 95% probability limit.

Figure 4.21

Profiles of glucose concentration