Chemical and microbiological quality of traditional Thai fermented meat (Nham) using pediocin PA-1 producer (*Pediococcus pentosaceus* TISTR 536) as starter culture

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Abstract – The chemiacal and microbiological properties of Nham, Thai traditional fermented meat, produced by using pediocin PA-1 producing *Pediococcus pentosaceus* TISTR 536 as starter culture were investigated and compared to naturally fermented Nham without starter culture. The fermentation of both products duration 0 and 3 days were determined for lactic acid concentrations, pH, water activities, moisture content, the growth of LAB and *Salmonella* during fermentation. The results revealed that Nham fermented with starter culture showed a higher acid content after 3 days which led to decrease the pH more rapidly than Nham produced without starter, while water activity, moisture content and the growth of total lactic acid bacteria showed no difference. After 3 days of fermentation, Nham with starter culture implied to diminish all contaminated *Salmonella* which found 100% from the mixture of Nham before leading to ferment, while Nham without starter could diminish the contaminated *Salmonella* only 50 % from the 6 batches of Nham products. The classification of the salmonella grouping informed that *Salmonella* found contaminated in Nham mixtures before leading to ferment were belonged to group B, C and E. Group C was the most tolerated group and could be found in the product after 3 days of fermentation in Nham without starter culture.

Key Words – Nham, lactic acid bacteria, *Pediococcus pentosaceus, Salmonellae*

I. INTRODUCTION

Nham, a traditional Thai fermented meat, is one of the most popular traditional dishes consumed nationwide in Thailand. A numerous reports on *Staphylococcus aureus* [1] and Salmonellae contamination in this product [2, 5] are the main public health concerned. Since there were a lot of research works aimed to control the growth of salmonellae which were mostly contaminated bacterial pathogen in Nham by using lactic acid bacteria (LAB) as starter culture in Nham [2, 4, 5] including the bacteriocin-producing LAB especially pediocin PA-1 producer of *Pediococcus pentosaceus* TISTR 536 in this Thai fermented meat product [5]. But the works were investigated only in the laboratory scale. Thus, this study were aimed to provide the use of pediocin PA-1 producer strain of *P. pentosaceus* TISTR 536, that we have isolated from Nham [4], in the production of Nham in a small and medium enterprise (SME) of Thai traditional fermented meat industry. The preliminary results of chemical and microbiological properties of Nham produced by using *P. pentosaceus* TISTR 536 compared to the control batches of naturally fermented Nham without using starter culture were conducted and reported in this study.

II. MATERIALS AND METHODS

Microorganisms

Pediococcus pentosaceus TISTR 536 which isolated from Nham and confirmed to produce pediocin PA-1 (Swetwiwwathana, 2005) was used in this study.

Medium

De Man Rogosa and Sharpe (MRS) broth (Difco™, USA), MRS agar (schalau) + 0.5%$CaCO_3$ (Merck) and Glucose Yeast Extract Peptone (GYP) broth (Utarapichat, 2530)

Preparation of starter culture

LAB strains were cultivated an aerobically in Glucose Yeast Extract Peptone (GYP) broth at 30^{o}C for 24 hr.

Preparation of Nham

Nham samples with and without *P. pentosaceus* TISTR 536 as starter were produced traditionally by Suddhiluck Innofood Co.,Ltd.

Study on effect of *P. pentosaceus* TISTR 536 starter during Nham fermentation

Chemical and microbiological determination of Nham samples with and without *P. pentosaceus* TISTR 536 as starter (obtained from Suddhiluck Innofood Co.,Ltd.) at 0 and 3 days of fermentation were sampling and determined for total lactic acid bacteria on MRS agar + 0.5% $CaCO_3$ (incubated under anaerobic condition at 30°C for 48 h), concentration of lactic acid was determined by Titratable acidity method (AOAC, 1990) , determination of pH using WTW, Inolab pH Level 1, Germany , water activity (A_W) was measured using a Hygroplam Rotronin mod. HW3 (International PBI Milano, Italy) at 25°C. Determination of percentage of moisture content in Nham product was done by the method recommended by AOAC, 2000 Determination of *Salmonella* spp. in Nham fermentation period at 0 and 3 days was done using the method recommended by ISO 6579. Classification of *Salmonella* group was done by slide agglutination method with grouping antisera purchased from S&A Laboratory Ltd., Thailand.

III. RESULTS AND DISCUSSION

Study on effect of *P. pentosaceus* TISTR 536 starter during Nham fermentation

1. Growth of lactic acid bacteria (LAB) in Nham with and without starter

The study informed that Nham inoculated with about 10^6 cfu/ml of *P. pentosaceus* TISTR 536 as starter compared to Nham control without starter, the number of LAB before fermentation (0 day) in Nham with starter revealed a log cycle higher number than Nham control without starter (5.85 and 4.96 log cfu/g respectively). After the third day of fermentation at 30°C, both Nham with and without starter revealed the growth of LAB in the product up to 3 log cycles (8.39 log cfu/g) in Nham with starter and 4 log cycle (8.23 cfu/g) in Nham without starter (Table 1). The number of LAB in Nham control without starter were known to present in various materials for Nham production such as from meat, cooked rice, and fresh garlic etc. (Swetwiwathana, 2005)

Table 1 Properties of Nham fermented with and without *P. pentosaceus* TISTR 536 as starter. (Growth of LAB, lactic acid concentration, pH and moisture content)

Fermentation time (day)		Analysis				
		LAB (log cfu/g)	pH	Lactic acid content (%)	Moisture (%)	A_W
0	With TISTR536	5.85	6.73	0.2	67.55	0.9729
	Without TISTR536	4.96	6.75	0.19	67.2	0.9774
3	With TISTR536	8.39	4.58	0.4	69.21	0.9732
	Without TISTR536	8.23	4.74	0.34	67.09	0.9778

2. Determination of lactic acid concentration, pH, water activity (A_W) and moisture content of Nham product.

When Nham products with and without starter were left to ferment for 3 days, more lactic acid content was observed in Nham contented with TISTR 536 starter than Nham without starter (0.40% and 0.34%, respectively.) (Table 1). This higher percentage of acid content in Nham with TISTR 536 starter led the pH value of Nham decreased from 6.73 to 4.58 at 3 days of fermentation, lower than Nham without starter (pH decreased from 6.75 to 4.74). By the decrease of pH corresponds to the number of increases lactic acid concentrations the Athletic raising this are indicative of the safety of consumers. As a resulted of inhibiting the growth of *Salmonella* (Swetwiwathana et al., 1999). The results revealed that the water activity (A_W) of Nham product with and without starter showed no difference after 3 days of fermentation, due to the non-water permeable casing which used in stuffing this product. But the percentage of moisture content from Nham with starter after 3 day of fermentation showed a little bit higher percentage (69.21 %) when compared to the control sample without starter (67.09 %).

3. The effect of *P. pentosaceus* TISTR 536 on microbiological safety of Nham.

According to the report on *Salmonella* contamination in Nham product and the effect of *P. pentosaceus* TISTR 536 on *Salmonella* Anatum during the maturation of Nham (Swetwiwathana et al., 2007), thus, this study had also investigated the salmonella contamination during the production of Nham from the company by comparing the treatment of Nham produced by using TISTR 536 as starter with the control sample without using starter. The results informed that from the 6 batches of Nham produced by the company Salmonellae were detected 100 % in 25 gram of each studied which had not been fermented (0 day) (Table 2). After 3 days of fermentation, Salmonellae in Nham fermented with TISTR 536 from 6 batches of samples were totally diminished from the samples and could not detected in 25 gram of sample, while Salmonellae in Nham without using starter could be detected in 25 gram of sample for 50%. The results in diminishment of Salmonellae in Nham product were concurred to the results informed the reduction of *Salm.* Anatum in Nham model broth and Nham sample was concerned to the increasing of lactic acid content and led to the decreasing of pH in Nham together with the pediocin PA-1 production of TISTR 536 strain in the product during left to ferment for 3 days. of Swetwiwathana et al. (2007) which

Table 2: Effect of the growth *salmonella* of Nham contented with and without starter culture LAB, TISTR536

Fermentation time (day)	Sample	*Salmolla* spp.			
		Nham with TISTR 536		Nham without TISTR 536	
		Number	%	Number	%
0	6	6	100	6	100
3	6	0	0	3	50

When classify the group of *Salmonella* by using slide agglutination method with grouping of antisera, the results revealed that most of salmonella group which contaminated in this product before fermentation were belonged to group B, C and E (Table 3). Three days fermentation period of Nham without TISTR 536 as starter could decrease the pH of product to 4.74 (Table 1) which was not lower enough to diminish some *Salmonella* group especially group C. The results implied that when produced this Nham product with pediocin PA-1 of *P. pentosaceus* TISTR 536 as starter culture could help to rapidly decrease the pH of the product (from 6.73 to 4.58 after 3 days of fermentation) and also diminished all contaminated *Salmonella* from the product. The results of reducing salmonella by using pediocin PA-1 producing strain had already been explained by Swetwiwathana et al. (2007).

Table 3: Salmonellae Groups found in Nham produced with and without starter culture.

Fermentation Time (Day)		Sample (batch)					
		1	2	3	4	5	6
0	Nham with TISTR 536	C	C	C	C	C	C
	Nham without TISTR 536	C, E	C	C	C, E	C, B	C
3	Nham with TISTR 536	ND	ND	ND	ND	ND	ND
	Nham without TISTR 536	C	C	ND	C	ND	ND

IV. CONCLUSION

Nham product produced by using *P. pentosaceus* TISTR 536 as starter culture revealed the better chemical quality and microbiological safety of the product when compared to Nham produced and left to naturally fermentation without using starter. The microbiological safety of this Thai traditional fermented meat to get the *Salmonella*-free product concerning to the rapidly lactic acid production during fermentation which led to decrease the pH value and the synergistic effect of pediocin PA-1 producing by *P. pentosaceus* TISTR 536 as the starter culture.

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