EFFECT 0F CHITOSAN, FRESH GARLIC AND PEDIOCIN PA-1 PRODUCER (*Pediococcus pentosaceus* TISTR 536) ON *Staphylococcus aureus* IN NHAM (THAI FERMENTED MEAT) MODEL BROTH

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Abstract – The study informs the beneficial effect of 5% of fresh garlic on the recovered of pediocin PA-1 producer (*Pediococcus pentosaceus* TISTR 536) as starter in the high concentration of Chitosan (CS, 5000 ppm) during Nham model broth (NMB) fermentation. Besides, the effect of CS (5000 ppm) combined with 5 % fresh garlic and *P. pentosaceus* TISTR 536 on *S. aureus* were also determined in NMB, it was found that these 3 ingredients in NMB exhibited an interactive effect on *S. aureus* and could diminish this pathogenic microorganism in NMB within 42 h. The results implied that CS and pediocin PA-1 producer can be used in Nham production in order to produce a safety of traditional Thai fermented meat product.

I. INTRODUCTION

Chitosan (CS) has been reported to be possessed as various functional properties such as intestinal lipid binding and serum cholesterol lowering effects [1, 2], water binding [3], antioxidative and preservative effects in muscle foods [4] and emulsifying capacity [5]. Chitosan has also been informed the inhibitory effect on various gram negative and gram positive bacteria including Salmonellae and *Staphylococcus aureus* [7, 8]. The main objective of our earlier study was to investigate whether CS (concentrations of 100, 500 and 1000 ppm) has antimicrobial effect on some pathogens (*Salmonella* Anatum, *Salm.* Derby and *Staphylococcus aureus*) which are associated with Nham (a popular fermented sausage in Thailand, mainly composed of lean pork, sliced cooked pork rind, cooked rice, garlic and salts) during fermentation. The results from our earlier study informed that higher concentration of chitosan (500 and 1000 ppm) in Nham model broth (NMB) exhibited higher inhibitory effect on all pathogens than NMB with 100 ppm of chitosan [8]. The study for effect of CS on some lactic acid bacteria (LAB) such as *Lactobacillus plantarum*, which mostly associated in various traditional Thai fermented meat products [9], and *Pediococcus pentosaceus* TISTR 536, which is pediocin PA-1 producing strain isolated from Nham [10] by using spot-on-lawn method and Nham Model Broth (NMB) has revealed that, between two studied LAB strains, *P. pentosaceus* TISTR 536 exhibited a higher resistance to Chitosan than *L. plantarum* ATCC 14917 [11]. Hence, this study is to further report the interactive effect among chitosan (5,000 ppm), fresh garlic (5%) and pediocin PA-1 starter cultures (10^6 cfu/ml) on *S. aureus* in Nham model broth (NMB).

II. MATERIALS AND METHODS

Microorganisms : *Pediococcus pentosaceus* TISTR 536, which is pediocin PA-1 producing strain isolated from Nham [10], was used as starter culture for Nham model broth (NMB). *Staphylococcus aureus* ATCC 12600, a food pathogenic bacterial strain, was used for this study.

Medium :
Nham model broth (NMB) : NMB without nitrite, which simulated the conditions of Nham production (a_w. 0.970, pH 6.3, micro-aerophilic condition with paraffin oil) [12], was used as a model instead of Nham product. *P. pentosaceus* TISTR 536 starter culture at a level of 10^6 cfu/ml and 5 % fresh sterilized garlic, was used for the study of their inhibitory effect on *S. aureus* ATCC 12600 at the level of 10^6 cfu/ml in NMB. The samples of each studied condition in NMB were left to ferment at 30°C for 2 days after *P. pentosaceus* TISTR 536 and *S. aureus* ATCC 12600 inoculation. The pH and percentage of lactic acid were investigated in NMB every 6 h, the growth of *P. pentosaceus* TISTR 536

and *S. aureus* ATCC 12600 in NMB were determined every 12 h [12].

MRS broth : medium modified was used as cultivation medium for *Pediococcus pentosaceus* TISTR 536. Pure cultures of *P. pentosaceus* TISTR 536 was transferred to MRS broth and incubated at 30 ^{o}C for 24 h.

Trypticase soy broth (TSB) : the medium was used as a cultivation medium for *Staphylococcus aureus* ATCC 12600. Pure cultures from trypticase soy agar (TSA) slant was transferred to TSB and incubated at 37 ^{o}C for 24 hours.

Preparation of fresh sterile garlic for NMB : Fresh sterile garlic was prepared by the method recommended by Swetwiwathana et al. [12] and contained in NMB for 5 % (w/v).

III. RESULTS AND DISCUSSION

The study of the interactive effect of CS salts (5,000 ppm sodium), 5 % fresh garlic and LAB starter cultures (*P. pentosaceus* TISTR 536 at 10^6 cfu/ml) on 10^6 cfu/ml of *S. aureus* ATCC 12600 was performed. The results (Fig. 1) showed that *S. aureus* could grow in NMB and gradually grow from 10^6 cfu/ml up to 10^8 cfu/ml after 48 h of incubation. Five percent fresh garlic could retard the growth of *S. aureus* at the early stage of NMB fermentation, while *P. pentosaceus* TISTR 536 could also retard the growth of this pathogen and reduce the cell number of *S. aureus* after 24 h of NMB fermentation due to the higher amount of lactic acid produced by *P. pentosaceus* TISTR 536 which led the pH of NMB decreased from 6.15 to 4.60 after 24 h of fermentation (Table 1). Among the 3 studied factors, NMB with 5,000 ppm CS showed the best inhibitory effect on *S. aureus.* Chitosan could gradually diminish all cells of *S. aureus* in NMB after 48 h of NMB fermentation. Additionally, CS with *P. pentosaceus* TISTR 536 as starter in NMB and CS, fresh garlic and *P. pentosaceus* TISTR 536 as starter showed the best results in diminishment of *S. aureus* cells from NMB after 42 h of NMB fermentation.

This can be explained because CS alone itself can exhibit an inhibitory effect on *S. aureus* as reported by many researchers [7, 8]. The use of CS together with *P. pentosaceus* TISTR 536 as starter culture and fresh garlic, *P. pentosaceus* TISTR 536 can grow and produce higher lactic acid during fermentation (data not shown) which led to the reduction of pH in NMB (Table 1). With the reduction of pH in NMB, CS exhibited a higher inhibitory effect on *S. aureus,* even more than the higher pH [13]. Besides, by the report of *P. pentosaceus* TISTR 536 which is known as pediocin PA-1 producing strain [10] and fresh garlic which contained allicin and exhibited an effect on *S. aureus* [14], the best inhibitory effect on *S. aureus* had been exhibited when these 3 studied factors were combined in NMB when compared to NMB with CS alone.

IV. CONCLUSION

The results from this study revealed the inhibitory effect of Chitosan (CS), fresh garlic and pediocin PA-1 producer (*P. pentosaceus* TISTR 536) as starter culture on *S. aureus* in Nham model broth (NMB) fermentation. Thus CS and pediocin PA-1 producer can be used in Nham production in order to produce a safety of traditional Thai fermented meat product.

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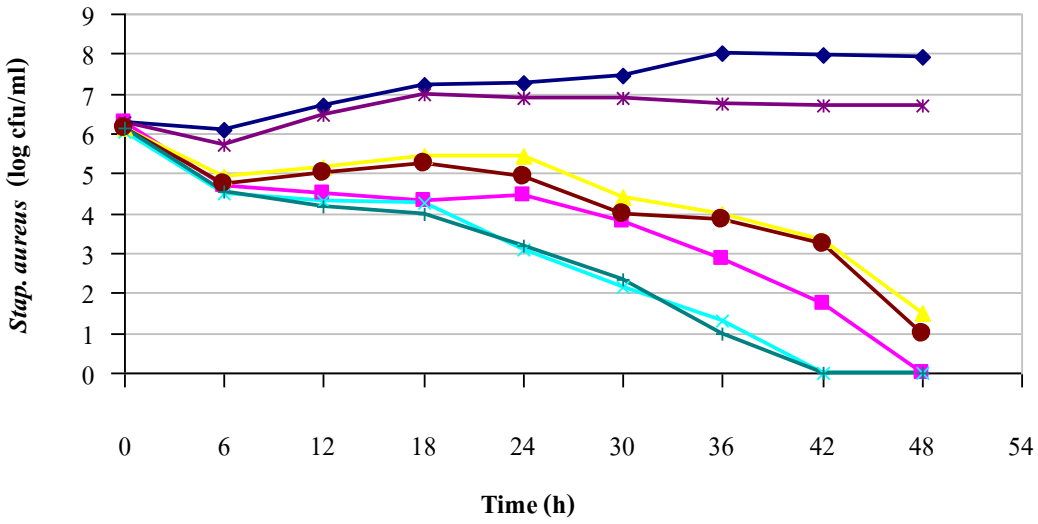

Figure 1 : Effect of chitosan (CS) 5000 ppm , *P. pentosaceus* TISTR 536 (10^6 cfu/ml) and 5% fresh garlic (w/v) on *S. aureus* (10^6 cfu/ml) in NMB : *S. aureus* ( ), *S. aureus*+ CS ( ), *S. aureus* + *P. pentosaceus* ( ), *S. aureus* + *P. pentosaceus* + CS ( ), *S. aureus*+ Garlic ( ), *S. aureus* + *P. pentosaceus* + Garlic ( ) and *S. aureus* + *P. pentosaceus* + Garlic+ CS ( )

Table 1. Effect of chitosan (CS) 5000 ppm , 5 % fresh garlic, *P. pentosaceus* TISTR 536 (10^6 cfu/ml) and *S. aureus* (10^6 cfu/ml) on the change of pH and percentage of lactic acid during 48 h of NMB fermentation.at room temperature (30 ^{o}C)

sample	pH					Lactic acid (%)				
	Incubation period (h)					Incubation period (h)				
	0	12	24	36	48	0	12	24	36	48
Pp	6.15	5.29	4.49	4.02	3.91	0.23	0.39	0.50	0.60	0.64
PpCS	6.10	6.07	5.17	4.85	4.51	0.26	0.29	0.45	0.54	0.58
PpSt	6.15	4.84	4.60	4.38	4.00	0.23	0.42	0.51	0.52	0.60
PpStCS	6.14	6.00	5.53	5.50	4.88	0.26	0.30	0.32	0.32	0.50
PpStG	6.10	4.80	4.50	4.32	3.98	0.23	0.44	0.56	0.61	0.65
PpStGCS	6.15	5.98	5.01	4.81	4.45	0.23	0.32	0.35	0.42	0.53

Pp - NMB with *P. pentosaceus* TISTR 536
PpCS - NMB with *P. pentosaceus* TISTR 536 and CS 5000 ppm
PpSt - NMB with *P. pentosaceus* TISTR 536 and *S. aureus*
PpStCS - NMB with *P. pentosaceus* TISTR 536, *S. aureus* and CS 5000 ppm
PpStG - NMB with *P. pentosaceus* TISTR 536 , *S. aureus* and 5 % fresh garlic
PpStGCS - NMB with *P. pentosaceus* TISTR 536 , *S. aureus*, 5 % fresh garlic and CS 5000 ppm