EFFECT 0F CHITOSAN ON ASSOCIATED BACTERIAL PATHOGENS IN NHAM (traditional Thai fermented meat) MODEL BROTH

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Abstract

The effect of concentrations of chitosan (100, 500, 1000, 2000 and 3000 ppm) on Nham (a traditional Thai fermented meat product) associated pathogens (*Salmonella* Anatum, *Salmonella* Derby and *Staphylococcus aureus*) was prior investigated by using spot-on-lawn technique on Mueller Hinton Agar (MHA). The results revealed that each studied chitosan concentration exhibited antimicrobial effect on all studied pathogens. When chitosan at concentration of 100 500 and 1000 ppm was confirmed for their inhibitory effect on all studied pathogens in Nham Model Broth (NMB) which left to incubate at room temperature (30-32 °C) for 48 h, the results informed that higher concentration of chitosan (500 and 1000 ppm) in NMB exhibited higher inhibitory effect on all pathogens than NMB with 100 ppm of chitosan. This study implies the possibility of using chitosan as one of Nham production ingredients in order to enhance the microbiological quality and safety of this traditional Thai fermented meat production.

Index Terms **:** chitosan, *Salmonella* Anatum, *Salmonella* Derby, *Staphylococcus aureus,* Nham**,** traditional Thai fermented meat

I. INTRODUCTION

Chitosan (CS) has been of a non-toxic and interest in the food industry, besides its antimicrobial effect, it possesses other functional properties including intestinal lipid binding and serum cholesterol lowering effects (Maezaki et al., 1993; Razdan & Pettersson, 1994), water binding (Knorr, 1982), antioxidative and preservative effects in muscle foods (Darmadji & Izumimoto, 1994) and emulsifying capacity (Lee, 1996). Chitosan, a polysaccharide comprising copolymers of glucosamine and N-acetyl-glucosamine, is obtained after alkaline deacetylation of the chitin derived from the exoskeletons of crustaceans and arthropods (Li, Dunn, Grandmaison, & Goosen, 1997). The paper report that CS disrupted bacterial cell membranes, with the release of cellular contents (Hui, Yumin, Xiaohui & Liping Sun, 2003).

Nham, a popular fermented sausage in Thailand, is mainly composed of lean pork, sliced cooked pork rind, cooked rice, garlicand salts. As a consequence of natural fermentation, the quality and consistency of Nham cannot be controlled. Since there are many reports informed the inhibitory effect of CS on various gram negative and gram positive bacteria including Salmonellae and *Staphylococcus aureus* (No et al., 2002; Fujimoto et al., 2006), thus, the main objective of our study was to investigate whether CS has antimicrobial effect on some pathogens which might be associated in Nham during fermentation. The study of CS effect on all studied microorganisms was prior conducted using spot-on-lawn method and Nham Model Broth (NMB).

II. MATERIALS AND METHODS

Preparation of chitosan solution : Concentrations of CS (85% Degree of deacetylation, A.N. LAB Thailand) at 0, 100, 500, 1000, 2000 and 3000 ppm in 1% (v/v) lactic acid were prepared and adjusted to pH 6.0 with 1N NaOH. Each concentration of CS was then sterilized at 121 °C 15 min (Jongsareejit et al., 2004).

Preparation of bacteria : To obtain pathogens inocula for the examination, *Salmonella* Anatum, *Salm.* Derby (obtained from National Institute of Health, Department of Medical Science, Bangkok, Thailand) and *Staphylococcus aureus* ATCC12600 from TSA stock cultures were 2 times cultured in Trypticase soy broth (TSB) and incubated for 18-20 h at 37 ^{o}C.

Determination of CS concentrations on pathogens : Evaluation of the effect from each CS concentration on each pathogen was studied using spot-on-lawn method (Swetwiwathana, 2005). 10 µl

of overnight cultured of each pathogenic indicator was transferred in 5 ml of melting Mueller Hinton Agar (MHA) (Concentration of each bacterial load was 10^6 cfu/ml) and seeded on MHA plate. Antimicrobial of each CS concentration was examined by dropping 10 µl of each studied CS concentration on bacterial seeded. The antibacterial activity was accessed after 24 hours incubation for zone of inhibition.

Determination of the CS concentrations on pathogens in NMB: Concentrations of CS at 0, 100, 500 and 1000 ppm were prepared in NMB (Swetwiwathana et al., 1999) and adjust to 6.0 pH. After sterilized at 121°C 15 min, overnight cultured of each studied pathogenic indicator were inoculated with an initial load about 10^6 cfu/ml. All NMB trials were left to incubate at room temperature (30-32 °C) under anaerobic condition (sterile parafin oil was poured over NMB) for 48 h. The survival of pathogen indicators in NMB was determined every 6 h (0, 6, 12, 18, 24, 30, 36, 42 and 48 h) by spread plate and pour plate techniques using trypticase soy agar (TSA) after 24 h incubation at 37°C.

III. RESULTS AND DISCUSSION

Concentrations of CS on pathogens : Effect of CS on each studied pathogens on MHA was prior examined at concentrations 100, 500, 1000, 2000 and 3000 ppm. The results (Fig. 1) revealed that all studied CS concentrations exhibited antimicrobial effect on both gram negative pathogens of *Salm.* Anatum (a) and *Salm.* Derby (b), while only 100 ppm of CS showed a little effect on gram positive *Staph. aures* (c). The study also implied that the higher CS concentrations exhibited the wider inhibition zone on MHA plate. This higher antibacterial activity of chitosan on both gram positive and gram negative was also reported by several researchers (No et al., 2002; Fujimoto et al., 2006). Thus, the lower CS concentrations from 100 – 1000 ppm were further used for our study on the effect of various CS concentrations on all pathogens which associated during Nham fermentation.

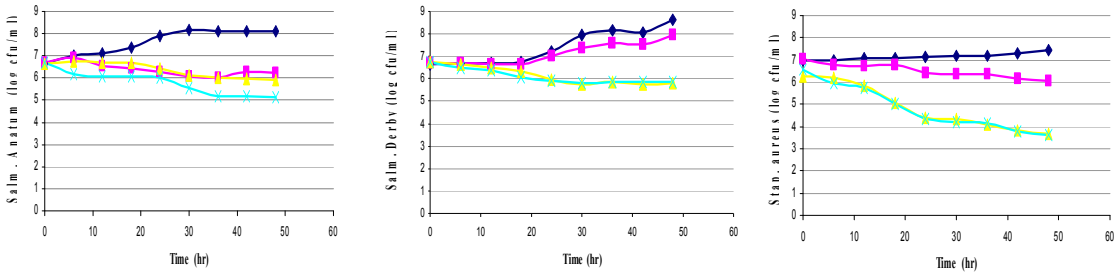
(a) (b) (c)

Figure 1. Effect of CS concentrations on bacterial pathogens : (a) *Salmonella* Anatum, (b) *Salmonella* Derby and (c) *Staphylococcus aureus.*

Effect of CS concentrations on pathogens in NMB : In order to confirm the results of CS on pathogens reduction, the study of each CS concentrations of 100, 500 and 1000 ppm was assessed for its antibacterial activity on 10^6 cfu/ml of gram negative *Salm.* Anatum (Fig. 2a) and *Salm.* Derby (Fig. 2b), and gram positive *Staph. aureus* (Fig. 2c) in NMB compared to NMB without CS (0 ppm). The results revealed that all studied pathogens could grow in this simulated model of Nham product. When compared to control NMB without CS, NMB with 100 ppm CS could only inhibit the growth of all studied pathogens during the early stage of NMB incubation. But the higher inhibition was shown in NMB with CS 500 and 1000 ppm. The results revealed that CS 500 and 1000 ppm showed the greater effect on the reduction of both salmonellae serovar in NMB for 1-2 log cfu/ml after incubated at room temperature for 48 h, while the cell number of *Staph. aureus* were reduced to 3 log cfu/ml by these same concentration. These results were concurred to the report of No et al. (2002) which reported that chitosan generally showed stronger bactericidal effects with gram-positive bacteria than gram-negative bacteria.

IV. CONCLUSION

The results from this work imply the possibility of using chitosan as one of Nham production ingredients in order to enhance the microbiological quality and safety during this traditional Thai fermented meat product. The synergistic effects between studied concentrations of chitosan and other ingredients for Nham production on all strains of Nham associated pathogens are under investigation.



(a) (b) (c)

Figure 2. Effect of concentrations of chitosan, control without chitosan (-◆-), 100 ppm (-■-), 500 ppm (-▲-) and 1000 ppm (-×-) on reducing of (a) *Salmonella* Anatum, (b) *Salmonella* Derby and (c) *Staphylococcus aureus*.

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REFERENCES

Darmadji, P., & M. Izumimoto. (1994). Effect of chitosan in meat preservation. *Meat Science*, 38, 243–254.

Fujimoto, T., Tsuchiya, Y., Terao, M., Nakamura, K., & Yamamoto, M. (2006). Antibacterial effect of chitosan solution against *Legionella pneumophila, Escherichia coli, and Staphylococcus aureus*. *Int. J. of Food Microbiol*, 112, 96-101.

Jongsareejit, B., Nakplaipan, P., & Jirachanchai, S. (2004). Chitosan : study of antimicrobial activity in food. *Thai. J. of Science*, 58, 88-95.

Knorr, D. (1982). Functional properties of chitin and chitosan. *Journal of Food Science,* 47, 593–595.

Lee, S. H. (1996). Effect of chitosan on emulsifying capacity of egg yolk. Journal of Korean Society of Food Nutrition (Taiwan), 23, 608–616.

Maezaki, Y., Tsuji, K., Nakagawa, Y., Kawai, Y., Akimoto, M., & Tsugita, T. (1993). Hypocholesterolemic effect of chitosan in adult males. *Bioscience, Biotechnology and Biochemistry*, 57, 1439–1444.

No, H.K., Park, N.Y., Lee, S.H., & Meyers, S.P. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int. J. of Food Microbiol*, 74 , 65-67.

Razdan, A., & Pettersson, D. (1994). Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentrations in broiler chickens. *British Journal of Nutrition*, 72, 277–288.

Shahidi, F., Arachchi, J. K. V., & Jeon, Y. J. (1999). Food application of chitin and chitosans. Trends in Food Science and Technology, 10, 37–51.

Swetwiwathana, A., U.Leutz., N. Lotong., & Fischer A. (1999). Controlling the growth of *Salmonella anatum* in nham. Effect of meat starter culture, nitrate, nitrite and garlic. *Fleischwirtschaft,* 79,124-128.