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Interactive effect of meat starter cultures, curing salts and garlic on the growth of *Salmonella anatum* in Nham

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Introduction : According to our early report on „Effect of meat starter cultures and some ingredients on the growth of *Salmonella anatum* in thai fermented meat (Nham) [an in-vitro study]" which revealed the advantage of using meat lactic acid bacterial starter cultures, nitrite and garlic in controlling the growth of studied pathogen in the created model broth [1]. Thus, this study was conducted to determine the interactive effect of the same amount of meat starter cultures, nitrite and garlic as early report on the growth of *S. anatum* during the fermentation of both NMB and Nham product. Besides, the same amount of nitrate used in the product was also studied for its interactive with meat starter cultures and garlic, and compared to the use of nitrite.

Materials and Methods

Starter cultures : Three strains, *L. curvatus, L. sakei* and *P. acidilactici*, of commercially available starter cultures (from Gewürz-müller GmbH, Stuttgart, Federal Republic of Germany) and the strain of *S. anatum* (LTH 4545, from Genera Food Technology and Microbiology, University of Hohenheim, Stuttgart) were used for this study.

Liquid medium : MRS broth - medium modified [2] was used as cultivation medium for the study LAB.

: Trypticase soy broth (TSB)- the medium [3] was used as cultivation medium for *S. anatum*. Pure cultures from trypticase soy agar (TSA) slant was transferred to TSB and incubated at 37° C for 24 h.

: The Nham model broth (NMB)- the medium simulated the conditions of Nham production (aw 0.970, pH 6.3 , microaerophilic condition with paraffin oil) [1], was used as a model instead of Nham product. The combination of using nitrite (125 ppm) and nitrate (500 ppm) as food additives by filter-sterilization [11], together with various studied starter cultures at a level of 10^6 cfu/ml and 5 % fresh sterilized garlic, were used for the study of their inhibitory effect on *S. anatum* at the level of 10^4 cfu/ml in NMB. The NMB without glucose and curing salts was used for study the effect of LAB on the growth of *S. anatum*.

The samples of each studied condition in NMB were left to ferment at 30° C for 2-3 days after LAB and *S. anatum* inoculation. Duplicate samples were determined on pH, LAB growth for every 12 h and *S. anatum* growth for every 6 h.

Preparation of fresh sterilized garlic : Local unpeeled garlic bulbs were used to prepare fresh sterilized garlic. The cloves were gently peeled, washed thoroughly with sterile distilled water, soaked in 70% ethanol for 30 minutes and washed thoroughly with sterile distilled water. Five percent of sterilized garlic was aseptically cut in small pieces and transfered into the sterilized NMB.

Preparation of Nham : The recipe of Nham (650 g minced meat, 350 g shredded cooked pig skin, 60 g cooked rice, 50 g shredded fresh garlic, 25 g salt, 3 g sodium tripolyphosphate, 0.5 g sodium ascorbate, 0.125 g sodium nitrite or 0.500 g sodium nitrate) was carried out in 1 kg batches per each strain of LAB starter culture for comparison of using nitrite (125 ppm) and nitrate (500 ppm) as food additives and their inhibitory effects on *S. anatum* in Nham. The samples without starter culture of both nitrite and nitrate added samples were also prepared for naturally fermentation (control batch). The ingredients of samples, except the control sample, were inoculated with each strain of commercial LAB at a level of about 10^6 cells/g and well mixed. Approximately 25 g of both Nham control batch mixture without starter cultures and Nham with starter cultures was stuffed into 10 x 14 cm. plastic bags. All studied samples were divided into two parts. The first part was conducted without inoculating *S. anatum* and used for determination of LAB growth and pH during Nham fermentation. *S. anatum* was added in the second part of samples to provide final concentration ranging from 10^1 to 10^3 cfu/g. The samples were thoroughly mixed from outside of the bag. The mixture was squeezed to the closed bag end and tightly wrapped with a rubber band. All samples were fermented at 30° C incubator for 6 days. Two bags per sample were examined daily after 3 days of fermentation for determining the LAB growth, pH and existence of *S. anatum*.

Microbiological and chemical analysis : Two bags per sample of Nham were examined daily after 3 days of fermentation for determining the LAB growth, existence of *S. anatum* [4] and pH [5].

Results and Discussion

Interactive effect of curing salts, fresh garlic and LAB starter on the growth of *S. anatum* in NMB

Interactive effect of using curing salts, fresh disrupted garlic and starter cultures in NMB (Figure 1) revealed a better result in rapid decreasing the number of *S. anatum* than using each of aforementioned additives, starter cultures or disrupted garlic alone. The best inhibitory effect on *S. anatum* was more achieved by using 125 ppm sodium nitrite in combination with LAB starter cultures and 5 % fresh disrupted garlic in the model broth than the broth using 500 ppm sodium nitrate. Among the use of the three studied LAB culture strains in combination with curing salts and fresh garlic in NMB, *L. sakei* was found to be most inhibitory than *P. acidilactici* and *L. curvatus*. This can be explained that *L. sakei* could rapidly produce numerous of lactic acid during fermentation in this model broth [1] which led to a rapid decrease in pH and could inhibit the growth of *S. anatum* most efficiency.

Interactive effect of curing salts, fresh garlic and LAB starter on the growth of *S. anatum* in Nham

Study on an interactive effect of curing salts, 5 % fresh garlic and LAB starter *cultures* with various amount of preinoculated *S. anatum* was performed and compared to those of natural fermented samples (without starter cultures). 25 g portion of each treatment after 3-6 days of fermentation was detected for *S. anatum* as recommended in food standard specify for salmonellae [4, 5]. The results (Table 1) showed the advantage of using *L. sakei* as a starter culture and combination with 125 ppm sodium nitrite and 5 % fresh garlic in the rapid production of salmonellae free Nham. Besides, we found that load of *S. anatum* contamination in the product also gave an effect in production of salmonellae free Nham. The results of using 10^6 cells/g of *L. sakei* in combination with 125 ppm sodium nitrite

and 5 % fresh garlic could eradicate the lowest amount of preinoculated *S. anatum* (5-10 cells/g) in Nham within 4 days of fermentation, while the higher load of preinoculated *S. anatum* (50-100 and 500-1,000 cells/g) spent more days for eradication (5 and 6 days respectively). When compared to the previous study on diminishment of *S. anatum* in NMB, we found that Nham samples revealed the longer period of fermentation in eliminating of *S. anatum* from the products than those of in-vitro model broth did. This might be explained that the inhibitory activity of the added nitrite in Nham samples might be more rapidly diminished as the result of chemical reactions with some ingredients in the samples than in NMB. It could be referred to the presence of ascorbate in the product, which could rapidly convert nitrous acid to nitric oxide and react with myoglobin to form nitric oxide myoglobin [6]. The decreasing of nitrite in Nham samples, hence, might be deficient the inhibitory effect on the presence pathogen and supported the growth pf *S. anatum* during the early stage of Nham fermentation. Then an inhibitory effect of this pathogen was occurred after the lactic acid from LAB starter was produced. Another factor which concerned to the change in pH and diminishment of *S. anatum* in Nham was that the reduction of pH in all treatment of Nham samples was more slowly decreased than in those of NMB did (Table 1 and 2). This slow reduction of pH in Nham might be the reason in delaying the production of salmonellae free Nham. An inhomogeneous and non-sterile in the production of this thai typical Nham product was, however, also a factor in slowly reduction of pH and deficient an inhibitory effect of curing salts, garlic and LAB starter cultures on *S. anatum* in the product.

Conclusions : The study revealed the advantage of using LAB meat starter cultures, curing salts and fresh disrupted garlic in controlling the growth of *S. anatum*. Use of 125 ppm sodium nitrite as a curing salt in combination with LAB and 5 % garlic exhibited more possibility in rapid production of salmonellae free Nham than use of 500 ppm sodium nitrate as a curing salt. Among these three strains of studied LAB, use of *L. sakei* as starter culture for Nham production exerted the best results in order to obtain a salmonella-free Nham product.

References : **1.** SWETWIWATHANA, A., U. LEUTZ and A. FISCHER. 1998. Wirkung von Knoblauch auf das Wachstum und die Milchsäureproduktion von Starterkulturen (Role of Garlic on Growth and Lactic Acid Production of Starter Cultures). Fleischwirtschaft. 78(4) : 294-298, 344. **2.** DE MAN, J.C., ROGASA, M. and SHARP, M.E. (1960) : A Medium for the Cultivation of Lactobacilli. J. Appl. Bacteriol. 23, 130-135. **3.** VANDERZANT, C. and D. F. SPLITTSTOESSER. 1992. Compendium Methods for the Microbiological Examination of Foods. 3 rd. Edition. Compiled by American Public Health Association (APHA) Technical Committee on Microbiological Methods for Foods. p. 1174. **4.** AOAC. 1996. Official methods of analysis 16 th ed. Association of Official Analytical Chemists, Arlington, Virginia. **5.** LOTONG, N. and A. SVETVIVADHANA. 1990. Production of Salmonella Free Nham. Annual Report, ASEAN Food Technology and Research Development Project. 1990. **6.** SILIKER, J. H., R. P. ELLIOTT, A. C. BAIRDPARKER, F. L. BRYAN, J. H. B. CHRISTIAN, D. S. CLARK, J. C. OLSON, Jr., and T. A. ROBERTS. 1980. Microbial Ecology of Foods. Volume 1 : Factors Affecting Life and Death of Microorganisms. The International Commission on Microbiological Specifications for Foods (ICMSF). Academic Press, Inc. New York.

Table 1 : pH of Nham and inhibitory effect of LAB starter cultures, nitrate (500 ppm), nitrite (125 ppm) and fresh garlic (5 %) on the inoculated *S. anatum* during Nham fermentation

sample	pH (days)				*S. anatum* recovered (days)											
					5-10 cfu/g				50-100 cfu/g				500-1000 cfu/g			
	3	4	5	6	3	4	5	6	3	4	5	6	3	4	5	6
$C+NO_2$	4.77	4.65	4.57	4.52	+	+	+	+	+	+	+	+	+	+	+	+
$C+NO_3$	4.84	4.72	4.63	4.56	+	+	+	+	+	+	+	+	+	+	+	+
$P+NO_2$	4.73	4.59	4.52	4.49	+	+	+	+	+	+	+	+	+	+	+	+
$P+NO_3$	4.77	4.62	4.55	4.52	+	+	+	+	+	+	+	+	+	+	+	+
$LC+NO_2$	4.64	4.55	4.50	4.46	+	+	+	-	+	+	+	+	+	+	+	+
$LC+NO_3$	4.68	4.58	4.52	4.50	+	+	+	+	+	+	+	+	+	+	+	+
$LS+NO_2$	4.57	4.49	4.43	4.40	+	-	-	-	+	+	-	-	+	+	+	-
$LS+NO_3$	4.61	4.52	4.48	4.46	+	+	-	-	+	+	+	-	+	+	+	+

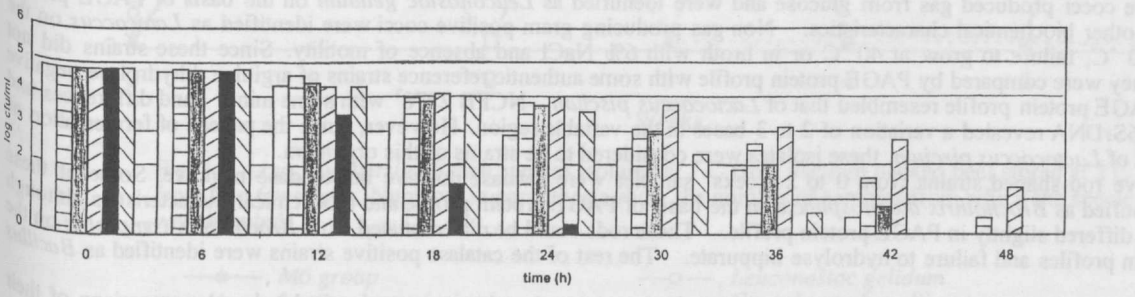
C = Nham control without starter cultures (natural fermented), P = Nham with 10^6 cells/g of *P. acidilactici* as starter culture, LC = Nham with 10^6 cells/g of *L. curvatus* as starter culture, LS = Nham with 10^6 cells/g of *L. sake* as starter culture
$+NO_2$ = Nham produced with 125 ppm sodium nitrite
$+NO_3$ = Nham produced with 500 ppm sodium nitrate

Table 2 : Growth of LAB starter cultures and pH change during fermentation in NMB with nitrate (500 ppm), nitrite (125 ppm) and garlic (5 %)

starter cultures	cfu/ml (day)				pH (day)			
	0	1	2	3	0	1	2	3
$P+NO_3+G$	$2.0x10^6$	$4.0x10^8$	$2.1x10^8$	$1.1x10^8$	6.10	4.85	4.46	4.28
$P+NO_2+G$	$2.0x10^6$	$3.0x10^8$	$1.0x10^8$	$9.8x10^7$	6.10	4.85	4.52	4.33
$LC+NO_3+G$	$4.0x10^6$	$9.7x10^7$	$6.5x10^8$	$4.6x10^7$	6.10	4.82	4.34	4.25
$LC+NO_2+G$	$4.0x10^6$	$7.3x10^7$	$5.7x10^7$	$3.6x10^7$	6.10	4.83	4.40	4.29
$LS+NO_3+G$	$5.0x10^6$	$2.1x10^8$	$8.4x10^7$	$6.8x10^7$	6.10	4.66	4.26	4.18
$LS+NO_2+G$	$5.0x10^6$	$1.2x10^8$	$9.8x10^7$	$7.6x10^7$	6.10	4.68	4.29	4.20

P = *P. acidilactici*, LC = *L. curvatus*, LS = *L. sake* ,
+G = with 5 % steriled fresh garlic, $+NO_3$ = with 500 ppm sodium nitrate, $+NO_2$ = with 125 ppm sodium nitrite

Figure 1: Effect of LAB starter culture, nitrate (500 ppm), nitrite (125 ppm) and garlic on the growth of *S. anatum* in NMB at 30° C



NM = Nham model broth, +G = with 5 % steriled fresh garlic, NO_2 = with 125 ppm sodium nitrite, NO_3 = with 500 ppm sodium nitrate
+P = with 10^6 cells/ml of *P. acidilactici*, +LC = with 10^6 cells/ml of *L. curvatus*, +LS = with 10^6 cells/ml of *L. sake*