NHAM (THAI FERMENTED PORK) MAKING WITH STARTER CULTURES

Aran H-Kittikun, Pairote Wiriyacharee and Lakkana Ruchanagraigarn, Department of Food Science and Technology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50002, Thailand

SUMMARY

The changes in microbial counts of two famous brands of Nham (A&B) sold in Chiang Mai were monitored. It was found that *Lactobacillus plantarum* was predominated throughout the storage period of Nham A. In Nham B *Pediococcus* sp. was the major lactic acid bacteria at the beginning while the amount of homofermentative lactobacilli increased significantly from day 2 to day 10. The starter cultures used for Nham preparation were *L.plantarum* showing some inhibitory effect on *E.coli* and *Salmonella*. The sensory evaluation of starter cultures treated Nham seemed to be not better than the control. However, it needs further testing because there are many variables factors that could effect the product quality.

INTRODUCTION

Nham is traditional fermented pork of Northern Thailand. The methods of Nham making involve mixing of minced pork and sliced pork skin with salt,nitrate, rice and spices and packing the mixture tightly in banana leaves or plastic film. After 3-4 days of fermentation consumers may eat Nham directly without cooking. Traditional fermentation of Nham depends upon bacteria present on the raw materials, equipment and processing conditions. Hence, large variations in the nature and type of bacteria occur. Early microbiological investigation of Nham established that lactic acid bacteria (lactics) are the predominant microbial flora (Comenuanta 1966; Techapinyawat 1975). The coliforms counts of Nham at the beginning and the early stage of storage were very high but the counts dropped significantly after 5 days of storage (Somathiti 1982).

The use of starter cultures in meat industry helps to improve product safety,achieve consistent product, reduce process time and extend shelf-life of the product (Bacus and Brown 1981; Robinson 1984). Therefore, the objectives of the present study were (1) to determine the microbial changes during natural fermentation of Nham and select the lactics to use as starter cultures and (2) to make Nham with these cultures and evaluate the products.

MATERIALS AND METHODS

Isolation of lactic acid bacteria

Two famous brands of Nham (A and B) sold in Chiang Mai were directly obtained from the manufacturers at the day of preparation and incubated at 25°C until sampling. Fifty gram Nham samples were taken for analysis under aseptic condition by homogenizing and diluting in 0.1% peptone water. The total viable count was done on Tryptone Glucose Yeast Extract (TGY) agar and the lactics count was detected on MRS agar incubated in candled jar. Fifteen colonies were isolated from one section of the plate containing 30-300 colonies. The pure cultures were gram strained and tested for catalase reaction after 18 h on TGY agar and later identified according to Sharpe and Fryer (1966). Those lactics provided high growth rate and high acidity in MRS broth were selected to use as starter cultures.

Preparation of Nham

The Nham used for investigation of starter cultures effects was prepared in the laboratory by mixing minced pork 1 kg and sliced pork skin 1 kg thoroughly with salt 30 g, potassium nitrate 0.2 g, steamed glutinous rice 70 g, garlic 30 g, pepper 0.5 g and polyphosphates 3 g. Addition of starter cultures was done during mixing with the inoculum of 10^8 CFU/g. The mixture was tightly packed in polyvinyl chloride casing 150 g/pack and incubated at 25°C.

Microbiological analysis of Nham

Before and during storage Nham samples were taken for total viable count and lactics count, enumerated for *Staphylococcus aureus* (Braid-Parker Medium) and *Salmonella* (Trypticase Soy broth -- Selenite Cystine broth -- Bismuth Sulfite agar) and detected MPN of *Escherichia coli* (Brilliant Green Bile Broth (BGBB)-- Eosin Methylene Blue agar -- BGBB and Tryptone water 44.5 C -- IMVic test) and MPN of *Clostridium perfringens* (Thioglycollate medium -- Tryptose Sulfite Cycloserine agar).

Chemical analysis of Nham

The proximate analysis of Nham after one day of preparation was determined according to standard methods. Initial pH and pH changes during storage were done on 1 g samples blended with 90 ml distilled water. The acidity of samples filtrate was detected by titration with 0.1 N NaOH and expressed as lactic acid.

Table 1. The microbial changes of naturally fermented Nham

Sample	storage (day)	Total count ($\times 10^8$ CFU/g)	Lactics count ($\times 10^8$ CFU/g)	% of the lactics: rod	% of the lactics: coccus	pH
Nham A	0	2.3	4.6	100	0	6.35
	2	6.0	1.1	100	0	4.63
	4	8.5	1.4	100	0	4.05
	6	1.2	2.1	100	0	4.00
	10	1.6	2.2	100	0	3.90
Nham B	0	0.5	0.70	15	85	6.10
	2	1.0	1.3	70	30	4.30
	4	6.5	7.0	85	15	4.00
	6	6.0	6.5	90	10	3.96
	10	5.9	6.0	86	14	3.95

Table 2 The microbiological changes of naturally fermented and starter cultures treated Nham

Sample	Storage (day)	Total count ($\times 10^8$ CFU/g)	Lactics count ($\times 10^8$ CFU/g)	E.coli (MPN/g)	Salmonella sp. (in 25 g)	pH
	0	12.3	16.5	29	ND*	5.91
Cultures	2	8.25	5.65	<3	ND	4.65
50408+	4	7.30	5.60	<3	ND	4.52
51006	6	4.10	4.40	<3	ND	4.42
(T1)	10	4.03	4.25	<3	ND	4.10
	0	5.30	6.35	3.6	ND	5.65
Cultures	2	9.65	1.29	<3	ND	4.52
60412+	4	6.05	5.70	<3	ND	4.50
61004	6	3.95	4.65	<3	ND	4.40
(T2)	10	2.45	1.90	<3	ND	4.20
	0	0.90	0.3	>11000	S.derby	6.31
Natural	2	6.15	7.0	11000	S.derby	4.66
fermenta-	4	4.95	5.70	930	ND	4.26
tion	6	4.25	4.00	<3	ND	4.20
(C)	10	3.96	3.75	<3	ND	4.10

* not detected

Table 3 Mean panel scores for sensory attributes of Nham

Treatments	Sensory attributes				
	colour	flavor	taste	texture	overall
Natural fermentation	4.24a	3.68a	3.48a	4.16a	4.12a
Cultures 50408+51006	2.88b	3.56a	3.52a	3.24b	3.28b
Cultures 60412+61004	2.48c	3.16b	3.20a	2.60c	2.72c

* N = 25, Range of scores : 1=low and 5=high. Means within column differ significantly ($P<0.05$) if the letter differ

Sensory evaluation and statistical analysis

After 4 days of fermentation all Nham was sliced into 0.5 cm thick and 3 slices of each treatment were put on the same plate with some peanut, ginger, chilli and shallot. Twenty untrained panelists who had an experience of eating Nham were instructed to evaluate sensory attributes of colour, flavour, taste, texture and overall acceptability of Nham samples by scoring method. The data were analysed by analysis of variance.

RESULTS AND DISCUSSION

Microbiology of natural fermented Nham

The changes in microbial counts of two famous brands of Nham (A&B) sold in Chiang Mai were shown in table 1. Before incubation at 25°C the total count and the lactics count of Nham A were more than one log cycle higher than those of Nham B but at day 10 Nham B had higher counts than Nham A. It was noticed that throughout the fermentation period Nham A contained 100% homofermentative lactobacilli which were predominated by *Lactobacillus plantarum*. In Nham B *Pediococcus* sp. was the major lactics composed of 85% at the beginning while the amount of homofermentative lactobacilli increased significantly during storage. Only a few number of *Streptococcus*, *Leuconostoc* and heterofermentative lactobacilli were found during fermentation. The results indicated that the predominant lactics in Nham were the homofermentative lactobacilli.

This work tried to use pure inocula from the lactics associated with Nham. The desired characteristics of starter cultures were fast growing and producing high amount of lactic acid since consumers preferred Nham with sour taste within 3-4 days of fermentation. When all isolates were tested it was found that *L. plantarum* 50408 and 51006 from Nham A and *L.plantarum* 60412 and 61004 from Nham B were better than other lactics. Therefore, these cultures were used as starter cultures.

Chemical compositions and microbiological changes of starter cultures treated Nham.

The proximate analysis showed that both treatments (T1&T2) and the control as well as commercially prepared Nham (A&B) contained almost the same food compositions (70-74% moisture, 18-20% protein, 3.1-3.9% fat, 2.6-3.5% ash and 1.1-4.0% carbohydrate). The naturally fermented Nham had pH of 6.1-6.35 at the beginning and after storage for 10 days the pH dropped to 4.10-3.90. Both treatments had lower pH and higher

acid production than the control from the beginning. The food compositions,pH and acidity may have some effects on the microbiology and quality of Nham.

The changes in the microbial counts and types of indicator microorganisms of these Nham were shown in table 2. Since the starter cultures were used in Nham T1 and T2 therefore, at the beginning the total count and the lactics count of both treatments were more than one log cycles higher than those of the control. All Nham samples had *Cl.perfringens* and *S.aureus* counts less than 3 MPN/g and 100 CFU/g respectively. The control Nham showed high count of *E.coli* and was positive test for *Salmonella* at the beginning. On the contrary, the treatments showed much less count of *E.coli* and no *Salmonella* was detected. The results indicated that the starter cultures had an inhibitory effect on the growth of *E.coli* and *Salmonella*.

Sensory evaluation and analysis of starter cultures treated Nham.

The results of sensory evaluation of Nham were shown in table 3. The colour of the control Nham was pink while the colour of the treatments were pale brown. The texture of the control was firm and crunchy but the texture of both treatments was soft. From the sensory evaluation the application of starter cultures into Nham seemed to be not better than the natural fermentation. However, there are many factors that should be taken for consideration such as interaction of starter cultures with other Nham components and the type and amount of starter cultures. The type of panelists also has an influence on the sensory analysis which should be carefully considered as well.

CONCLUSIONS

The results of this study indicated that the natural fermentation of Nham occurred by homofermentative lactobacilli. The use of *L.plantarum* associated with Nham as starter cultures had an inhibitory effect on the enteric bacteria in Nham.

Addition of starter cultures in the form of liquid concentrates would mask the colour of Nham and add more water into Nham which resulted in Nham with inferior quality. From the sensory evaluation the application of starter cultures into Nham seemed to be not better than the natural fermentation. However, it needs further testing because there are many variable factors that could affect the quality of Nham.

ACKNOWLEDGEMENTS

The researchers are grateful to the National Research Office Committee for financial support and to the department of Medical Science, Ministry of Health for determinations of *E.coli, Cl.perfringens, S.aureus* and *Salmonella* in Nham Samples.

REFERENCES

Bacus, J.N. and Brown, W.L. (1981). *Food Technology* **35**:74.

Comenuanta, J. (1966). Bachelor Thesis in Agriculture, Kasetsart University, Bangkok (in Thai).

Robinson, R.K. (1982). Biotechnology vol.3 edited by H.J. Rhem and G. Reed, Verlag Chemie p.200.

Sharpe, M. E. and Fryer, T.F. (1966). Identification Methods for Microbiologists part A. eds. B.M. Gibbs and F.A. Skinner, Academic Press p.65.

Somathiti,S. (1982). Master Thesis in Microbiology, Kasetsart University, Bangkok (in Thai).

Techapinyawat, S. (1975). Master Thesis in Biology, Kasetsart University, Bangkok (in Thai).