DEPARTMENT OF SCIENCE, MINISTRY OF INDUSTRY

DEPARTMENT OF FISHERIES, MINISTRY OF AGRICULTURE

APPLIED SCIENTIFIC RESEARCH CORPORATION OF THAILAND

COOPERATIVE RESEARCH PROGRAMME NO. 31

IMPROVEMENTS IN THE PRODUCTION OF FISH SAUCE (NAMPLA)

RESEARCH PROJECT NO. 31/4

ACCELERATION OF NAMPLA FERMENTATION BY ARTIFICIAL MEANS

REPORT NO. 1

ACCELERATION OF NAMPLA FERMENTATION BY A BIOLOGICAL QUICK PROCESS

BY

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F O R E W O R D

Production of fish sauce (nampla) is a considerable industry in Thailand, involving almost 400 factories with a total output of some 30 million litres. The conventional method of manufacture is primitive and time-consuming, taking about one year for "fermentation" and ageing.

Past attempts to speed up production have not been successful. Several rapid methods have been evolved but they have not been adopted because the resulting product is not acceptable to consumers.

The Department of Science (Ministry of Industry) has given attention to this problem from time to time over the past two decades. Some work is also in progress in the Department of Fisheries (Ministry of Agriculture). ASRCT proposed a cooperative research programme on this topic in which each of the three agencies could participate, and the present studies are part of the resulting joint effort.

ACCELERATION OF NAMPLA FERMENTATION BY A BIOLOGICAL QUICK PROCESS

By Sman Vardhanabhuti*, Jiraporn Chouvalit*, Yuk-Hang Sombatpanit*, and Pravat Lauhasiri*

SUMMARY

A new biological quick process has been found by which good quality primary fish sauce from a Stolephorus species (Pla Kratak) can be obtained in two months. The method is based on adjusting the salt content and maintaining the fermentation at elevated temperatures ranging from 37°C to 49°C. It appears that good aroma is temperature dependent and different kinds of good aroma can be produced by changing the fermentation temperature, provided mixture contained sufficiently high salt concentration. With this new method, there is good nitrogen retention and very little ammonia in the product.

INTRODUCTION

Fish sauce (nampla) is widely used by the Thai people and the peoples of neighbouring countries as a condiment to impart a salty taste and a specific flavour and aroma to various items of food. In Thailand its manufacture is a considerable industry involving 47 large-sized factories and 348 small ones (Bung-orn Kasemsarn, personal communication) and annual production is not less than 30 million litres (Saisithi et al 1966).

The conventional method of manufacture involves the autolytic decomposition of fish in the presence of salt which takes from 8 to 12 months followed by a period of ageing in the sun for 1 to 3 months.

Research on the improvement of the production of fish sauce dates back to some fifty years when Rose' (1918) examined the production of

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fish sauce (nuoc-mam) in Vietnam. Since then further work has been published from Indochina, the Philippines, Indonesia, Japan, and the United States. In Thailand a rapid method involving acid hydrolysis was developed by the Department of Science and additional experimental work on this process was carried out by the Department of Fisheries. Unfortunately, the flavour of the product did not meet with consumer acceptance and the method has not been taken up industrially (Department of Science 1961).

The present work has been concerned with attempting to vary the conditions of the conventional process to speed up production without adversely affecting the flavour and aroma of the product.

Croston (1960) showed that the mixed enzymes of chinook salmon were inactivated in acid conditions and that they worked optimally at pH 9.0 and 49°C. Takahashi (1948) found that the enzyme prepared from pyloric caeca of the Japanese yellowtail was inactive at NaCl concentrations of less than 1 per cent if kept at 50°C, acted optimally at from 2 to 15 per cent salt (at 50°C), and decreased in activity at salt concentrations above 15 per cent. On the basis of these results it was hoped that by adjusting the concentration of salt and conducting the fermentation at a suitable temperature and pH, a reasonably good quality product could be produced in a much shorter time than the conventional method without greatly increasing the technical complexity of the process or its costs.

Conventional process of manufacture

Commonly, three species of fish are employed, namely a Stolephorus sp. (Pla Kratak), Rastrelliger neglectus (Pla Thu), and a Crossocheilus sp. (Pla Soi). The first two are marine species and the last is a fresh water species.

In the conventional method usually the freshly caught fish is mixed with salt in the proportion of 1 part of salt to 2-3 parts or more of fish depending on the freshness of fish. The salt and fish mixture is put into a big concrete tank, wooden tank, or earthen jar and kept in the shade or under the sun for a period of 8 months to a year or even

longer without stirring. Sometimes the mixture is left on a slightly sloping floor for a period upto a week to let the slime drain out and the mixture partially dry up before it is placed in the fermenting tank. In the latter case brine of certain concentration is added to make up for the liquid lost during the draining period. The fish in the fermenting tank is prevented from floating by weighting it down with bricks or hard timber placed on top of a bamboo screen. At the end of the fermentation period, the fish extract is pumped or poured out, filtered through sand or fish bones, or crude paper (กระดาษฟาง) if made in small quantity. The filtrate is then aged in the sun for a period of one to three months, during which time salt crystallizes out, and the aroma and flavour improve. This primary fish extract is either sold as a special grade fish sauce or used to flavour the lower grade products.

More brine is added to the fish residue left in the tank and fermentation is allowed to go on further until a reasonably good product is obtained. The liquid is again pumped out, filtered, blended with the primary fish extract, colouring matter, and a little sugar, and finally bottled. As many as three leachings are made and, finally, the residue is boiled to get the brine which is kept for later production, and the fish bone is sold as fertilizer.

There are many problems in the conventional method. The fermentation time is too long and necessitates a big space and a large number of tanks. Improper amounts of salt and prolonged storage usually result in the loss of valuable nitrogenous materials. The method and equipment used are not sanitary and it is usual to see maggots floating on the surface of the liquid in the tank. The profit from manufacturing fish sauce comes mainly from the sale of the leachings, not from the highly nutritious primary fish extract. Consequently the average consumer is using only flavoured brine.

MATERIALS AND METHODS

Fish, Pla Kratak (Stolephorus sp.) was obtained from Khlong Dan, a landing place for fishing boats about 50 kilometres east of Bangkok and on the coast. It was placed in plastic bags, covered with cracked ice on the outside, and brought back to the laboratory. The fish was processed right away by mixing with unrefined solar salt which was used in two proportions, i.e. 15 parts of salt to 100 parts of fish and 30 parts of salt to 100 parts of fish by weight. Mixing was done by hand. Each set of salt-and-fish mixtures, equivalent to 1.5 kilogrammes of fish, was placed in a 2-litre glass beaker and then treated by different procedures as tabulated in Table 1.

For the procedures employing two different temperatures, the mixture was incubated at the higher temperature first and then removed to the lower one.

Adjustment of pH was done by the addition of sodium hydroxide to approximately a pH of 9 before the mixture was incubated. Further additions of sodium hydroxide were made at intervals to maintain the pH at that level. The pH was brought down to 7 by the addition of dilute hydrochloric acid prior to sun exposure.

The content in the beaker was stirred once daily during processing. Each beaker was covered with a glass cover when being exposed to the sun to prevent rain water and insect from getting in. At intervals during processing and before filtering, tap water was added to replace the water lost through evaporation.

Before each preparation was exposed to the sun, a check was made to see that disintegration was complete and that at least 50 per cent liquefaction has occurred. The liquefaction was determined by centrifuging 150 millilitres of each preparation in a Martin Christ-Osterode/ Harz, type UJ 3 centrifuge at 3,000 r.p.m. for 30 minutes and liquid portion was recorded as per cent of the total volume.

After a period of sun exposure each fish-and-salt mixture was strained through cheese cloth first and then filtered through a Whatman No. 1 filter paper with the use of a Buchner funnel and a suction pump.

TABLE 1

METHODS OF PROCESSING SALT AND FISH MIXTURES

Method	Parts of salt used for each 100 parts of fish	Duration of incubation at various temperatures				pH adjustment	Period of sun exposure	Remark
		Room temperature	37°C	49°C	75°C			
A	15	3 months	-	-	-	No	1 month	
B	15	1 month	-	-	-	Yes	1 & 2 months	
C	15	-	1 month	-	-	No	1 month	
D	30	-	1 month	-	-	No	1 month	
E	15	-	-	2 weeks	-	No	1 month	
F	15	-	2 weeks	overnight	-	No	1 month	49°C first
G	15	2 weeks	-	overnight	-	No	1 month	49°C first
H	15	-	-	2 weeks	-	Yes	1 month	
I	15	-	2 weeks	overnight	-	Yes	1 month	49°C first
J	30	-	-	2 weeks	-	No	1 month	
K	30	-	2 weeks	2 nights	-	No	1 month	49°C first
L	30	2 weeks	-	2 nights	-	No	1 month	49°C first
M	15	-	-	-	2 weeks	No	2 months	
N	15	-	-	-	1 week	No	2 months	
O	15	-	-	-	2 weeks	Yes	2 months	
P	15	-	-	-	1 week	Yes	2 months	
Q	15	-	1 month	-	overnight	No	1 month	75°C first
R	15	1 month	-	-	overnight	No	1 month	75°C first

For the mixtures that yielded good quality products as judged by chemical analysis, colour, and aroma, the residue was further leached by adding corresponding salt solution (i.e. 15 per cent or 30 per cent solar salt in tap water) up to the original volume and exposed further in the sun for another period.

Chemical analysis of the products were made at the following intervals:

(1) When the fish was completely disintegrated.

(2) Before exposure to the sun.

(3) After sun exposure and filtration.

(4) At intervals during leaching.

Each sample was analysed for pH, specific gravity, total solids, sodium chloride, total nitrogen, ammonia nitrogen, and formaldehyde nitrogen contents.

A Beekman pH metre Model GS was used to measure pH.

Specific gravity was determined by filling a 10-ml volumetric flask with the material and weighing it. The weight of the fish sauce is then divided by that of an equal volume of distilled water to obtain the specific gravity.

The total solid content, the NaCl content, the total nitrogen content and the ammoniacal nitrogen content were determined by AOAC methods (Horwitz, 1965). Formaldehyde nitrogen was determined by the method of Sörensen (1909). The figure for organic nitrogen was obtained by subtracting the ammoniacal nitrogen content from the total nitrogen content, and that of amino nitrogen by subtracting the ammoniacal nitrogen content from that of the formaldehyde nitrogen.

Good quality products were sent out, each along with a sample of conventional product of good quality, to a number of persons for tasting.

RESULTS

Disintegration of fish

With samples made by mixing 15 parts of salt to 100 parts of fish, complete disintegration was obtained overnight at 49°C and 75°C when the mixture was stirred the following morning. At 37°C it took three days for complete disintegration of the fish. At 75°C the mixture smelled very much like cooked fish.

Adjusting the pH of the fish-and-salt mixture to pH 9.0 with sodium hydroxide resulted in slightly more rapid disintegration at 37°C and 49°C. There was no difference in the rate of disintegration at 75°C. Initially it took about 16 grammes of sodium hydroxide to bring the pH of 1 kilogramme of fish up to 9.0. More sodium hydroxide is needed at intervals to keep the pH of the fish and salt mixture at this level.

At room temperature it took approximately 3 months for complete disintegration to occur, and 1 month at pH 9.

With samples made by mixing 30 parts of salt to 100 parts of fish, complete disintegration occurred after 40 hours at 49°C, and about one month at 37°C. About 5 per cent of the fish disintegrated at the end of 2 months at room temperature.

The effect of pH adjustment was not studied at this salt concentration, since it was found earlier that by so doing the product smelled very much of ammonia and the aroma was not good.

Liquefaction of the fish and salt mixture

With the preparations made by mixing 15 parts of salt to each 100 parts of fish, there was slightly more than 70 per cent liquid portion with the mixture kept at 49°C and at pH 9 for 15 days. 65 per cent liquid was obtained with the preparations kept at 49°C without pH adjustment and also at 37°C at pH 9 after the same length of time. Slightly over 50 per cent liquid was obtained with the preparation kept at 37°C without pH adjustment after 15 days.

The liquid portion of the mixture kept at 75°C for two weeks and

then exposed to the sun for one month was found to be 60 per cent. The mixture kept at 75°C and at pH 9 for two weeks then neutralized with hydrochloric acid to pH 7 and exposed to the sun for one month also yielded 60 per cent liquid.

The mixture kept at room temperature for one month at pH 9 then neutralized in the same manner and exposed to the sun for one month yielded 65 per cent liquid. The mixture kept at room temperature without pH adjustment for 3 months also yielded 65 per cent liquid.

With the preparations made by mixing 30 parts of salt to each 100 parts of fish, the mixture kept at 49°C for two weeks yielded 60 per cent liquid. The one kept at 37°C for one month also yielded 60 per cent liquid. The fish in the mixture kept at room temperature did not completely disintegrate (after 3 months) at the time of writing this report. pH adjustment was not attempted with preparations using this higher salt content.

Chemical composition of various products

Table 2 summarizes the results of chemical analyses of various products kept at room temperature.

Table 3 summarizes the results of chemical analyses of various products kept at 37°C.

Table 4 summarizes the results of chemical analyses of various products treated at 49°C.

Table 5 summarizes the results of chemical analyses of various products treated at 75°C.

Table 6 gives comparative figures for chemical content of good quality products prepared by our methods and that of good quality conventional fish sauces.

At the time this report was being written, leachings were not yet completed.

Aroma and flavour

The results of tasting for aroma and flavour of good quality products are given in Tables 7 and 8.

TABLE 2

PHYSICAL AND CHEMICAL PROPERTIES OF PRODUCTS PROCESSED AT ROOM TEMPERATURE (27°- 35°C)

Method of treatment	pH	Sp.gr.	Total solid (g %)	NaCl (g %)	Total N (g %)	Organic N (g %)	Formal. N (g %)	Amino N (g %)	Ammonia N (g %)	Liquid portion from 150 ml (ml)	Aroma	Colour
Method A, before sun exposure	7.8	1.136	21.7	13.3	1.94	1.33	1.36	0.75	0.61	100	Bad	Brownish yellow
Method A, sun exposure for one month	7.7	1.116	21.6	11.6	1.70	1.16	1.20	0.66	0.54	93	Bad	Brown
Method B, neutralized and exposed to sun for one month	6.6	1.135	23.0	13.6	1.63	1.32	0.94	0.63	0.31	100	Bad	Yellow
Method B, sun exposure for two months	6.2	1.31	22.8	13.0	1.59	1.30	0.94	0.65	0.29	-	Fairly good	Brownish

TABLE 3

PHYSICAL AND CHEMICAL PROPERTIES OF PRODUCTS PROCESSED AT 37°C

Method of treatment	pH	Sp.gr.	Total solid (g %)	NaCl (g %)	Total N (g %)	Organic N (g %)	Formal. N (g %)	Amino N (g %)	Ammonia N (g %)	Liquid portion from 150 ml (ml)	Aroma	Colour
Method C, before sun exposure	7.8	1.148	26.7	13.0	2.44	1.94	1.57	1.05	0.52	80	Bad	Brownish yellow
Method C, sun exposure for one month	6.9	1.152	26.9	13.3	2.39	1.87	1.62	1.10	0.52	80	Fairly good	Brown
Method D, before sun exposure	5.6	1.225	35.8	23.4	1.93	1.77	0.98	0.82	0.16	95	Good	Reddish brown
Method D, sun exposure for one month	5.4	1.265	36.9	22.9	2.21	2.03	1.09	0.91	0.18	89	Good	Reddish brown

TABLE 4

PHYSICAL AND CHEMICAL PROPERTIES OF PRODUCTS PROCESSED AT 49°C

Method of treatment	pH	Sp.gr.	Total solid (g %)	NaCl (g %)	Total N (g %)	Organic N (g %)	Formal N (g %)	Amino N (g %)	Ammonia N (g %)	Liquid portion from 150 ml (ml)	Aroma	Colour
15 parts salt, kept at 49°C for one day without pH adjustment	6.0	1.168	30.8	13.1	2.61	2.45	0.87	0.71	0.16	-	-	-
Method E, before sun exposure	6.8	1.143	24.9	14.2	1.83	1.65	0.94	0.76	0.18	100	Fairly good	Brownish
Method E, sun exposure for one month	6.4	1.142	25.1	14.8	1.84	1.59	1.09	0.84	0.25	80	Fairly good	Brownish
Method F, before sun exposure	7.3	1.142	24.5	13.8	1.87	1.60	1.07	0.80	0.27	80	Bad	Yellow
Method F, sun exposure for one month	6.7	1.146	24.3	14.3	1.87	1.58	1.19	0.90	0.29	80	Good	Yellowish Brown
Method G, before sun exposure	5.9	1.157	28.1	14.0	2.24	2.00	1.08	0.84	0.24	75	Fairly good	Reddish yellow
Method G, sun exposure for one month	6.3	1.136	25.3	12.5	2.08	1.78	1.22	0.92	0.30	110	Bad	Brown

TABLE 4

(continued)

Method of treatment	pH	Sp.gr.	Total solid (g %)	NaCl (g %)	Total N (g %)	Organic N (g %)	Formal. N (g %)	Amino N (g %)	Ammonia N (g %)	Liquid portion from 150 ml (ml)	Aroma	Colour
Method H, before neutralization	8.8	1.159	27.1	15.2	1.86	1.77	0.81	0.72	0.09	110	Bad	Deep yellow
Method H, neutralized and exposed to sun for one month	7.0	1.154	25.7	15.2	1.75	1.62	0.85	0.72	0.13	90	Fairly good	Brown
Method I, before neutralization	9.6	1.146	25.4	14.5	1.80	1.75	0.65	0.60	0.05	100	Bad	Yellow
Method I, neutralized and exposed to sun for one month	6.6	1.148	26.1	15.2	1.72	1.55	0.95	0.78	0.17	85	Fairly good	Yellow
30 parts salt, 49°C without pH adjustment for one night	5.8	1.204	35.8	19.4	2.41	2.26	0.70	0.55	0.15	-	-	-
Method J, before sun exposure	5.8	1.221	35.1	24.1	1.94	1.75	0.88	0.69	0.19	95	Good	Reddish brown
Method J, sun exposure for one month	5.5	1.195	32.5	20.6	1.92	1.72	0.89	0.69	0.20	90	Fairly good	Brown

TABLE 4

(continued)

Method of treatment	pH	Sp.gr.	Total solid (g %)	NaCl (g %)	Total N (g %)	Organic N (g %)	Formal. N (g %)	Amino N (g %)	Ammonia N (g %)	Liquid portion from 150 ml (ml)	Aroma	Colour
Method K, before sun exposure	5.7	1.218	34.4	23.1	1.87	1.70	0.86	0.69	0.17	80	Fairly good	Pale reddish yellow
Method K, sun exposure for one month	5.6	1.190	31.7	20.0	1.88	1.70	0.89	0.71	0.18	90	Good	Brown
Method L, before sun exposure	5.7	1.210	36.7	23.1	1.79	1.62	0.81	0.64	0.17	75	Fairly good	Yellow
Method L, sun exposure for one month	5.6	1.198	32.3	20.7	1.90	1.71	0.96	0.77	0.19	89	Good	Yellowish brown

TABLE 5

PHYSICAL AND CHEMICAL PROPERTIES OF PRODUCTS PROCESSED AT 75°C

Method of treatment	pH	Sp.gr.	Total solid (g %)	NaCl (g %)	Total N (g %)	Organic N (g %)	Formal. N (g %)	Amino N (g %)	Ammonia N (g %)	Liquid portion from 150 ml (ml)	Aroma	Colour
Method M, before sun exposure	6.8	1.132	23.7	13.64	1.62	1.53	0.52	0.43	0.09	-	-	-
Method M, sun exposure for one month	6.6	1.141	24.8	14.47	1.76	1.67	0.72	0.63	0.09	90	Fairly good	Reddish brown
Method M, sun exposure for two months	6.1	1.139	24.1	14.03	1.67	1.53	0.72	0.58	0.14	-	Good	Reddish brown
Method N, sun exposure for one month	6.8	1.160	26.0	16.55	1.64	1.49	0.67	0.52	0.15	70	Good	Brown
Method N, sun exposure for two months	6.6	1.136	21.9	14.22	1.40	1.24	0.54	0.38	0.16	60	Good	Yellowish brown
Method O, sun exposure for two weeks	6.9	1.124	22.1	12.70	1.45	1.39	0.61	0.55	0.06	-	-	-
Method O, sun exposure for one month	7.0	1.133	22.8	13.30	1.52	1.42	0.61	0.51	1.10	90	Bad	Reddish brown

TABLE 5

(continued)

Method of treatment	pH	Sp.gr.	Total solid (g %)	NaCl (g %)	Total N (g %)	Organic N (g %)	Formal. N (g %)	Amino N (g %)	Ammonia N (g %)	Liquid portion from 150 ml (ml)	Aroma	Colour
Method O, sun exposure for two months	6.4	1.131	22.3	13.03	1.46	1.38	0.58	0.50	0.08	115	Fairly good	Reddish brown
Method P, sun exposure for one month	8.5	1.175	28.8	18.09	1.72	1.67	0.48	0.43	0.05	75	Fairly good	Brown
Method P, sun exposure for two months	7.7	1.138	23.0	14.41	1.37	1.34	0.38	0.35	0.03	75	Good	Yellowish brown
Method Q, before sun exposure	7.0	1.153	25.5	15.6	1.77	1.60	0.78	0.61	0.17	55	Good	Yellow
Method Q, sun exposure for one month	6.3	1.145	25.4	15.0	1.73	1.52	0.89	0.69	0.21	62	Bad	Yellowish brown
Method R, before sun exposure	7.2	1.145	24.8	15.4	1.75	1.55	0.76	0.56	0.20	65	Good	Pale yellow
Method R, sun exposure for one month	6.8	1.118	20.6	12.2	1.37	1.20	0.70	0.53	0.17	70	Good	Yellow

TABLE 6

COMPARATIVE ANALYTICAL FIGURES OF CONVENTIONAL PRODUCTS AND PRODUCTS MADE AT ASECT

Product	pH	Sp.gr.	Total solid (g %)	NaCl (g %)	Total N (g %)	Organic N (g %)	Formal. N (g %)	Amino N (g %)	Ammonia N (g %)
Conventional primary fish extract of three years standing without sun exposure	6.9	1.217	33.1	22.5	1.91	1.56	1.32	0.97	0.35
First quality Vietnamese fish sauce, figures from Fisheries Products Manual, p. 126, 1961	-	-	-	27.5	2.2	1.5	1.6	-	0.7
Product obtained by Method C	6.9	1.152	26.9	13.3	2.39	1.87	1.62	1.10	0.52
Product obtained by Method D	5.4	1.265	36.8	22.9	2.21	2.03	1.09	0.91	0.18
Product obtained by Method F	6.7	1.146	24.3	14.3	1.87	1.58	1.19	0.90	0.29
Product obtained by Method J	5.5	1.195	32.5	20.6	1.92	1.72	0.89	0.69	0.20
Product obtained by Method K	5.6	1.190	31.7	20.0	1.88	1.70	0.89	0.71	0.18
Product obtained by Method L	5.6	1.198	32.3	20.7	1.90	1.71	0.96	0.77	0.19

TABLE 7

RESULTS OF TASTING FOR AROMA AND FLAVOUR OF TWO CONVENTIONAL PRIMARY FISH SAUCES

Type of sample	No. of tasters	No. of acceptors	No. of non-acceptors	Acceptor: non-acceptor ratio
Conventional product Sample A	34	26	8	3.3 : 1
Conventional product Sample B	24	15	9	1.7 : 1

TABLE 8

RESULTS OF TASTING FOR AROMA AND FLAVOUR OF THE BETTER PRODUCTS MADE BY THE VARIOUS NEW METHODS

Type of product	No. of tasters	No. of acceptors	No. of non-acceptors	Acceptor: non-acceptor ratio
Product prepared by Method C	11	6	5	1.2 : 1
Product prepared by Method D	11	10	1	10 : 1
Product prepared by Method E F	11	6	5	1.2 : 1
Product prepared by Method I J	10	5	5	1 : 1
Product prepared by Method K	14	9	5	1.8 : 1
Product prepared by Method L	9	8	1	8 : 1

Yields

The yields of primary extracts of good quality products are given in Table 9.

TABLE 9

YIELD OF ACCEPTABLE PRIMARY FISH SAUCE
FOR EACH KG OF FISH USED

Type of product	Yield in ml
Product prepared by Method D	590
Product prepared by Method J K	604
Product prepared by Method K L	590

DISCUSSION

From the results tabulated in Table 2 it is apparent that fish sauce fermentation at room temperature using 15 parts salt for each 100 parts of fish that yielded 13.3 per cent NaCl in the fish extract took too long a time (three months) for complete disintegration and sufficient liquefaction. Moreover, there was too much ammonia formed (0.61 per cent) and great reduction in the organic nitrogen content (1.36 per cent) of the extract. The aroma of the fish extract was bad. By raising the pH to 9 the disintegration and liquefaction were accelerated, but the organic nitrogen content of the extract did not improve and the aroma remained bad. Further aging in the sun (up to two months) improved the aroma slightly.

When the fermentations were conducted at 37°C (Table 3) improvements resulted. The extract obtained from 15 parts salt mixture, Method C, at the end of one month were found to be highly nutritive, with organic nitrogen content of 1.94 per cent, amino nitrogen content of 1.05 per cent and formaldehyde nitrogen content of 1.57 per cent. It contained 0.52 per cent ammonia nitrogen. The aroma of the extracts was not good but this improved from sun exposure. The latter, at the end of one month,

caused only slight reduction of the total nitrogen content and organic nitrogen content, but the amino nitrogen content, formaldehyde nitrogen content, and ammonia nitrogen content remained practically the same. When the mixture consisting of 30 parts of salt for each 100 parts of fish was fermented at 37°C (Method D) for one month, good disintegration and liquefaction resulted. The extract was of good aroma. Sodium chloride content was 23.4 per cent. There were slight reductions in total nitrogen content, the organic nitrogen content, and amino nitrogen content when compared with the product obtained with 15 parts salt and rather marked reductions in formaldehyde nitrogen content and ammonia nitrogen content. However, the chemical content of this product compared well with that of good quality fish extract prepared by conventional method after more than three years of fermentation (see Table 6) and conformed to the regulation set up by the French Government for Vietnamese fish sauce (Gouvernement Général de l'Indochine 1943).

At 49°C (Table 4), with 15 parts salt to each 100 parts of fish, good quality fish extract was obtained in two weeks and, with additional sun exposure, the quality slightly improved but the aroma did not quite match those of the conventional product. When the fish-and-salt mixture was kept at 49°C overnight and then placed at 37°C for two weeks, the chemical contents remained good, but the aroma was not good. The aroma improved with sun exposure for one month without detrimental effects on the chemical contents. The results were the same with the mixture kept at 49°C overnight and then placed at room temperature for two weeks with and without further exposure to the sun. Increasing the pH to 9 gave better liquefaction, but this brought about more loss of total nitrogen content and caused bad aroma which improved somewhat by sun exposure.

Good quality fish extract was obtained when 30 parts salt to each 100 parts of fish mixture was kept at 49°C for two weeks (Method J). The fish took a little longer time to disintegrate completely (two nights) but good liquefaction was obtained at the end of the period.

Good quality sauces with <u>better</u> aroma were obtained when the mixture was kept at 49°C for only two nights and then either placed at 37°C (Method K) or at room temperature (Method L) for two weeks and then aged in the sun for one month. Slightly less liquefaction occurred at room

temperature than at 37°C. The chemical contents of these sauces compared well with those of the conventional product (Table 6) and also conformed with the French regulation for Vietnamese fish sauce.

Conducting the fermentation at 75°C (Table 5) with 15 parts salt to each 100 parts of fish prevented putrid odour and produced good aroma and flavour sauces, but there were much less total nitrogen, organic nitrogen, formaldehyde nitrogen, and amino nitrogen in the extract. Very little ammonia was formed, however. Liquefaction was also less. Because of too low formaldehyde nitrogen content, the products obtained at this temperature did not pass the French regulation for Vietnamese fish sauce. When the salt and fish mixture was kept at 75°C for one day and then placed either at 37°C or room temperature for one month, there were more total nitrogen, organic nitrogen, formaldehyde nitrogen, and amino nitrogen released in the extract. Liquefaction was still less and the products did not pass the French regulation because there was still too low formaldehyde nitrogen present.

From these results it is apparent that disintegration and liquefaction of fish developed best at 49°C, and fairly well at 37°C, both for products containing 15 parts salt and those with 30 parts salt. Retardation occurred at the higher salt level but this was slight. However, the proper aroma, the most essential factor for good fish sauce, was obtained at the higher salt level, and at 37°C. For the product made with 30 parts salt and kept at 49°C even for two nights and then kept at 37°C for two weeks (Method K), very faint cheese-like and somewhat penetrating odour was detected. Slight putrid odour was present when the mixture was kept at 49°C for two nights and then placed at room temperature for two weeks (Method L). It appears then that putrefaction occurs more narkedly at room temperature and less so at 37°C and higher. If the salt concentration is raised to about 23 per cent at 37°C putrefaction appears to be completely inhibited. There was still some putrefactive activity at this latter temperature if the salt concentration was about 13 per cent. The putrid odour was not detected with the product made with 15 parts salt and kept at 49°C for two weeks, but the smell of this product was highly penetrating instead. This peculiar penetrating cheese-like odour that developed at 49°C, even after two nights at this temperature, require further investigation. It could

conceivably occur as the result of oxidation of fish oil at elevated temperature or as the result of the activity of some thermophilic micro-organisms.

Tasting for the aroma and flavour of nampla is a very difficult thing to evaluate. It depends entirely on what the taster has been accustomed to or brought up with. The tastes of different members of a single family are usually the same. Even with conventional products the ratio of individuals accepting the product to those not accepting it ranges from 3.25 to 1 to 1.67 to 1 (Table 8). With this point in mind it was decided to take a product with the ratio 1.5 to 1 as the base line to accept it for good quality. Consequently, it appears that the products prepared by Methods D and L are very well accepted by most of the tasters — much better than the two conventional products (Table 8). The product obtained by Method K was also accepted. The products obtained by Methods C, E, F and J were not accepted. The authors realize that the number of individuals tasting each product is still very small. More samples will be sent out later for tasting.

Samples of products made by Methods D, K, and L were sent out to a few nampla producers and it was very interesting to find that they all prefer the more pungent products (prepared by Methods K and L) to the blander one prepared by Method D. This is explainable by the fact that the producers are accustomed to consuming the primary fish extract, which is usually not sold to the public without further blending. The average tasters tasting the various products are used to consume the blended fish sauce on sale in the market. It may be possible, therefore, that a little further blending might make the other products more acceptable still.

Our results seem to confirm Takahashi's (1948) finding that fish enzymes are more active at sodium chloride concentrations of less than 15 per cent and that they are less active at higher salt concentrations. They are more active at pH 9 and at 49°C, and less so at neutral pH and lower temperature similar to what was reported by Croston (1960). We also found that the best nitrogen retention was obtained at the lower salt concentrations and at 37°C (Method C). With 15 parts salt mixture kept at 37°C for one month, the figure for total nitrogen and organic

nitrogen were 2.44 per cent and 1.94 per cent respectively. Following one month of sun exposure, these figures became 2.39 per cent and 1.87 per cent respectively. More rapid nitrogen losses occurred at both room temperature and at 49°C. Higher salt concentrations resulted in less total nitrogen and organic nitrogen even at 37°C. However, with the product obtained by Method D (30 parts salt, 37°C for one month) total nitrogen, organic nitrogen, and formaldehyde nitrogen strangly backed up after one month of sun exposure. This could mean that more disintegration occurred during sun exposure, and requires further investigation.

It should also be noted that, with the good quality products made with 30 parts salt, the content of ammonia nitrogen remains satisfactorily low and in fact lower than that of the conventional product. The product made by Method C which contains 15 parts salt has higher ammonia nitrogen content but still lower than that of the first quality Vietnamese fish sauce (Table 6). This again indicates more putrefaction with lower salt content, since ammonia is one of the usual end products of putrefactive process.

It is unfortunate that the product obtained with 15 parts salt at 37°C has rather unpleasant odour and slightly bitter taste since this preparation proves to be highly nutritious. Work is in progress to prevent the development of this unpleasant odour, suspected by us to be the result of putrefactive bacteria on certain components of the fresh raw fish. The optimum fermentation temperature and salt concentration have yet to be determined. It will also be tested later whether further addition of salt after some initial period with lower salt content would improve the aroma and preserve better the nitrogenous contents of the finished product.

Our methods were based on increased enzymatic and microbial activity to hasten the fermentation and the development of good aroma. It will be interesting to study whether so doing would result in the increase in vitamin content of the finished products. Many of these micro-organisms are good synthesizers of vitamins.

We were able to obtain products with different aroma from different methods used. It is hoped that with this knowledge we may be able to produce fish sauce suitable for the taste of the people in neighbouring

countries. Work along this line will be attempted in the near future.

We plan also to try using larger fish, i.e. the Pla Thu and Pla Soi, which are also used for preparing conventional fish sauce, and non-edible portion of big fresh sea fish as well.

CONCLUSIONS

By adjusting the salt content and maintaining the fermentation at elevated temperatures ranging from 37°C to 49°C, good quality primary fish sauce could be produced from Pla Kratak (Stolephorus sp.) in two months. Using 15 parts salt to each 100 parts of fish, a highly nutritious product was obtained at 37°C (Method C), but this product did not have acceptable aroma and was slightly bitter when tasted plain. When the salt content was increased to 30 parts for each 100 parts of fish, acceptable products were obtained (Method D).

At this higher salt level, the temperature of 37°C (Method D) appears to favour the development of the usual aroma acceptable to the average Thai consumers, while 49°C (Method K) results in the more pungent and cheese-like aroma, and room temperature (Method L) produces mild putrid odour which is not disagreeable. Connoisseurs and producers seem to prefer the last two types to the first one.

There is good nitrogen retention and very little ammonia formation in the products made by this new biological quick process.

It is believed that good quality and sanitary fish sauce could be advantageously produced by the new method in a modern type of plant without too much technicality and added cost. Moreover it appears that different types of aroma could be produced as desired to suit the taste of each particular group of consumers.

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