ATTEMPTS TO IMPROVE THE TECHNOLOGY FOR FISH SAUCE FERMENTATION

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Fishery Technological Development Division, Department of Fisheries,
Kaset Klang, Chatuchak Bangkok 10900
Thailand

Running title: fish sauce fermentation

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Department of Fisheries

* Present address: Inspection Division, FDA, Ministry of Public Health,
Bangkok 10200, Thailand

** Present address: Department of Fishery Microbiology, College of Fisheries,
Mangalore-575002, India
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ABSTRACT

Fish sauce is an important fermented fishery product in Southeast Asia contributing to the nutrition and economy of the people in the region. The biochemical and microbiological changes taking place during fermentation were studied at different salt concentrations and at ambient temperature and at 40° C. Fish sauce prepare using 15% salt concentration had ammonial odour and a salt concentration of 20-25% was found to be optimal for the development of right odour and colour. At 40° C, protein solubilisation was faster but continued incubation at this temperature during the entire course of fermentation resulted with a product which was darker in colour and stronger in odour. For faster production of fish sauce, it is recommended that after an initial incubation at 40° C for 60 days, the fermentation be carried out at ambient temperature.

INTRODUCTION

Fish sauce is a fermented fishery product which is popular in many parts of Southeast and is an important part of the diet of over 250 million people in the region (Van Veen, 1965). Fish sauces are given different names in different countries eg. "Nam-pla" in Thailand, "Patis" in the Philippines, "Nuoc-mam" in Vietnam, "Budu" in

Key words: fermentation, fish sauce, anchovy, salt, temperature

present address: Inspection Div., FDA, Minist. of Public Health, Bkk., Thailand

present address: Dept. of Micro., College of Fisheries Mangalore-575002 India
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Malaysia, etc. In Thailand, “Nam-pla” is used at the rate of about 1 million bottles of 750 ml per day. Generally, marine fish such as anchovie (Stolephorus spp.), sardines (Sardinella spp.) and chub mackerel (Rastrelliger spp.) are used for making nam-pla though less commonly, freshwater fish such as silver carp (Cirrhinus jullieni), barbs (Puntius spp.), guaramy (Trichogaster trichopterus) may be used. In the traditional process, whole fish is mixed with salt in the ratio of 2-3:1, packed tightly in concrete tanks or earthen jars and allowed to ferment for 9-12 months. The supernatant liquid is pumped into open tanks and left to mature for 1-2 months. The final product is amber/brown clear liquid with salty taste and cheese like aroma (Adams et al., 1985). First grade fish sauce has high nutrional value, being rich in essential amino acids but the daily per capita consumption is limited by high salt content (Sikorski et al., 1985). Therefore, fish sauce with lower salt concentration would have better nutritional value. However, lowering the salt concentration may increase the health risk due to pathogenic microorganisms and may alter the course of fermentation. In fact, the biochemical and microbiological changes occurring during fish sauce fermentation is not clearly understood and the objective of this study was to monitor the biochemical and microbiological changes occurring during the fermentation at different salt concentrations. The possibility of accelerating the fermentation process by incubation at higher temperatures has also been investigated.

MATERIALS AND METHODS

For each experiment, about 150 kg of fresh anchovy (within 6h of harvesting) was used. solar salt was added at 15%, 20% and 25% the mixture was packed tightly in 3.5 kg quantities in glass jars. One Fish sauce fermentation set of jars were kept at room temperature (28 ± 3°C) and another set at 40 ± 3°C. At 15,30,60,90 days, samples were drawn for biochemical and microbiological analysis. Samples for organoleptic analysis were drawn at 150 and 360 days. The biochemical parameters studied were pH, total nitrogen, aminoacid nitrogen, ammonical nitrogen, formaldehyde nitrogen and total volatile base nitrogen. pH was measured using pH meter and all other biochemical parameters were analysed as described in AOAC Manual (AOAC, 1980). Total viable count of bacteria was performed by spread plate method using Tryptic
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Soy Agar (Difco). The plates were incubated at ambient temperature (28 ± 3°C) for 2-3 days. Organoleptic analysis was performed by an experienced taste panel. Amino acid content of fish sauce was estimated using amino acid analyser.

RESULTS AND DISCUSSION

The biochemical and microbiological changes observed during fish sauce fermentation at ambient temperature is illustrated in Fig 1. At all the three salt concentrations tested, the pH dropped by about 0.3 units during the first fifteen days. After this phase, the pH started rising and stabilised at about 6.2 in batches containing 20% and 25% salt. At 15% salt concentration, the pH was still rising after 30 dyas and was 7.19 on day 60 (Fig 1g). The rise in pH in the batch with 15% salt concentration is perhaps due to the formation of basic amines by microbial activity. This suggestion is supported by data on total viable bacterial count (Fig 1). At 15% salt concentration, the total viable count (TVC) was always above 5.7 log units up to 90 days whereas at 20% salt, TVC dropped to 2.15 log units on day 15 and rose marginally to 3.56 log units on day 30 and stabilised there. At 25% salt concentration, the TVC dropped steadily to 2.95 log units on day 30. The level of Total Base Nitrogen (TVB-N) was the highest at 15% salt concentration (285 mg% on day 90) and least at 25% salt concentration (141.19 mg% on day 90). The trend is illustrated in Fig 1a. Even the ammonical nitrogen (Am-N) level (Fig 1c) was highest at Fish sauce fermentation 15% salt concentration (4.56 mg% on day 90) and least at 25% salt concentration (2.51 mg% on day 90). Similarly, the levels of total nitrogen (Fig 1e), amino acid nitrogen (Fig. 1b) and formaldehyde nitrogen (Fig 1b) were higher at 15% salt and least at 25% salt. Organolpically, fish sauce prepared with 15% salt had strong ammonical odour while the batch with 25% salt had the desirable odour and colour.

Doughan and Howard (1975) noted that the flavour and aroma of fish sauce has three major components ammonical due to ammonia and trimethylamine, a cheesy component due to volatile fatty acids and a complex meaty aroma. The strong ammonical odour of fish sauce prepared with 15% salt could be due to higher microbial activity in this batch. The role of microorganisms in fish sauce fermentation
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is not clearly understood. Saisithi et al. (1966) noted that TVC decreased steadily from 3-5 log units at 15 days to 2 log units at 12 months. Though this suggests that the role of microorganisms in fish sauce fermentation is limited, Saisithi (1967) proposed that fatty acid degradation by Pediococcus halophilus would be important for development of aroma. The results of this study show that a salt concentration of 25% is important for the development of right aroma and flavour. A salt concentration of 15% would be adequate to prevent the growth or toxin production by pathogenic microorganisms (Owens and Mendoza, 1985). However, high microbial count observed during fermentation (Fig II) show that this salt concentration is not sufficient to suppress the activity of moderately halophilic bacteria which may act upon aminoacids to produce ammonia and may reduce trimethylamine oxide (TMAO) to trimethylamine (TMA). This view is supported by the observation (Fig la, lc) that levels of TVB-N and Am-N were high at 15% salt concentration. Perhaps, a salt concentration of 25% is required to suppress such moderate halophiles and to permit the dominance of halophiles such as Pediococcus halophilus which are required for the development of right odour. Thongthai and Siri Wongpairat (1989) noted that traditional fish sauce, which had a high population of Halobacterium species had the right aroma and matured faster suggesting a role for this group of bacteria in fish sauce fermentation.

Results in Fig 2 (2a-2g) show that the kinetics of fish sauce fermentation at 40°C was essentially similar to that at 28°C. Even at 40°C the fish sauce with 15% salt showed high bacterial count (5.14 log units on day 90), high TVB-N levels (285 mg% on day 90) and high ammonia levels (5.14 mg% on day 90) compared to 20% and 25% salt concentrations. Nevertheless, at low salt concentration, if results of fermentation at ambient temperature and 40°C are compared, formation of TVB-N and Am-N was slower at 40°C (TVB-N level of 219 mg% on day 60 at 40°C compared to 280 mg% at ambient temperature). This can be explained because bacterial activity, which brings about a rise in ammonia and TVB-N levels would be slowed down at 40°C. However, the fermentation appeared to be rapid at this temperature. For example, at 25% salt the total nitrogen, Am-N, TVB-N and amino acid nitrogen levels of 60 day fermentation at 40°C and 90 days fermentation at ambient temperature were
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comparable (Fig 1 and 2) showing that the process of fermentation can be hastened by increasing the temperature to 40º C. Total nitrogen and amino acid nitrogen in the batch incubated at 40º C were higher on day 90 compared to the batch left at room temperature. This indicates faster protein solubilisation at 40º C. Results in Table 1 show the aminoacid composition of 90 day old fish sauce from two commercial factories and the experimental batch at ambient temperature and at 40º C. The levels of all the amino acids with the exception of methionine was found in higher level in the sauce fermenting at 40º C compared to the batch at ambient temperature or from commercial sources. High lysine content of all batches of fish sauce noted in Table 1 is very significant because this indicates that fish sauce is a good source of this amino acid which is deficient in rice which forms the staple diet of people in Southeast Asia. Mabesa et al. (1989) noted the benefit of raising the temperature of fermentation but their protocol involved heating and agitation whereas in this study, fermentation was carried out without agitation. At ambient temperature, the fish sauce attained right colour, flavour and odour at 12 months, while at 40º C the fish sauce fermentation was complete at 6 months. However, the colour of the batch at 40º C was darker and odour stronger. Dougan and Howard (1975) noted that the three notes of odour in fish sauce, viz ammonica., meaty and cheesy could by due to different agents such as bacterial enzymes, fish enzymes and atmospheric oxidation. All these three processes would be affected by temperature and this could explain stronger odour in the batch fermented at 40º C. In the batch incubated at ambient temperature, development of cheesy odour could be noticed after 90 days. Riaz et al., (1986) noted that during fish sauce fermentation using sardines, the development of cheesy odour occurs after 80 days. To take advantage of faster protein solubilisation at 40º C and to develop the desired colour and odour, it can be recommended that the fermentation be carried out at 40º C for 60 days and then restored to ambient temperature.

The results of this study show that for production of fish sauce with desirable flavour and aroma, 25% salt concentration is essential but the process of fermentation can be quickened by incubation at 40º C for 60 days.
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ACKNOWLEDGEMENTS

This work was supported by grants from European Union Commission (STD-project) and the Royal Thai Government.

REFERENCES


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Table Amino acid composition of Fish sauce factory compared to 3 month/fish sauce at 25 ± 3° C and 40 ± 3° C

<table>
<thead>
<tr>
<th></th>
<th>Ripening Fish sauce (Anchovy)</th>
<th>3 months - Anchovy fish sauce incubated at 25 ± 3° C</th>
<th>40 ± 3° C</th>
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<tbody>
<tr>
<td></td>
<td>Factory I (mg%)</td>
<td>Factory II (mg%)</td>
<td>(mg%)</td>
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<tr>
<td>Protein</td>
<td>14.3</td>
<td>14.3</td>
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<td>Tryptophan</td>
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Figure 1. To complete the composition of fish sauce (anchovy) with 15, 20, and 25% salt then stored at room temperature (25 ± 3° C) for 90 days.
Figure 2. To complete the composition of fish sauce (anchovy) with 15, 20 and 25% salt then stored at \(40 \pm 2{}^\circ\)C for 90 days.