Chapter 11
Thai Fish Sauce: A Traditional Fermented Sauce

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Contents

11.1 Introduction

Thai fermented fish product—fish sauce is known locally as "Nam Pla." Fish fermentation is widely practiced in Thailand and fish sauce is widely consumed in the country and is also the most important exported fish fermented product.

The process of fermentation for fish sauce starts when the raw material and salt are left to stand. During the fermentation process of fish sauce, some organic substances break down into smaller molecules which contribute to the typical odor, flavor, and color to the products. Similar products are also processed and consumed in other countries in the Southeast Asia, i.e., Malaysia, Laos, Vietnam, Cambodia, Myanmar, Indonesia, and the Philippines.

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11.2 Fish Sauce

Fish sauce or "Nam Pla" in Thai is the clear aqueous product of prolonged salting fish fermentation. It is made from either freshwater or saltwater fish. Anchovies (small saltwater fish) are traditionally used in good quality fish sauce; however, fish and parts of fish can be used in fish sauce fermentation (Phithakpol et al. 1995; Lopetcharat 1999) Natural fish sauce requires 9–12 months fermentation. Fish sauce is used as a flavoring ingredient in Thai cooking as well as other Southeast Asian cooking; it is called differently such as Nuoc-nam (Vietnam), Gua-ca (Myanmar), Kecap ikan (Indonesia), Patis (Philippines), and Nam-pa (Laos). Good quality fish sauce imparts good aroma and flavor, contains essential amino acids, is rich in vitamin B especially vitamin B_{12}, and also supplies minerals that include calcium, phosphorous, iodine, and iron (Areekul et al. 1974; Garby and Areekul 1974; Suwanik 1977). Thai fish sauces (Fig. 11.1) are produced for local consumption and for export to nearby countries and others such as the European Union, the USA, Canada, Japan, Australia, and New Zealand.

In Thailand, fish sauce is classified as "standardized food" where quality standards are defined by regulation authorized by Food and Drug Administration, under the Ministry of Public Health (FDA 2000). Genuine fish sauce shall be clear and free of sediment, has color, odor, and flavor inherent of that specific characteristics of genuine fish sauce, if only sodium chloride salt is used it shall be not less than 200 g in 1 L of fish sauce, total nitrogen not less than 9 g/L of fish sauce, amino acid nitrogen not less than 40 % and not more than 60 % of total nitrogen, and ratio of glutamic acid to total nitrogen not less than 0.4 and not more than 0.6 (Ministry of Public Health 2000). Histamine, a toxic biogenic amine derived from enzymatic decarboxylation of amino acid histidine, has a maximum permitted level of 400 mg/kg food (Codex Std 302-2011). Using spectrofluorometry technique, Muangthai and Nakthong (2014) analyzed ten fish sauces bought from supermarkets in Bangkok and found histamine content in the range of 7.5–15.11 mg/kg fish sauce. The low level of histamine indicates the good quality of fish raw material used in fish sauce manufacturing.

Fig. 11.1 Example of the Thai commercial fish sauce

11.3 Manufacturing Process

Natural fish sauce is made from the very fresh whole fish mostly small fish, often salting on board after catch. Fish are mixed with salt at a variable ratio from 4:1 to 1:1 (w/w); the ratio of fish to salt depends on size of fish and traditional recipe of each manufacturer. Either solar (sea) salt or rock salt can be used in the process. The fish and salt mixture is placed in large earthen or concrete containers lined with a layer of salt on the bottom, and topped with a layer of salt. Figure 11.2a shows the fermentation of fish sauce in concrete containers exposed to sunshine during the day. Figure 11.2b, c showed the fermentation in glass tanks that permitted observation of the changes of the mixture during fermentation. During the first few months, a woven bamboo mat with heavy rocks is placed on the top layer to prevent the fish in the mixture from floating during fermentation. Approximately after a period of 6 months, the light brown colored liquid is removed from the fish mixture through an outlet at the bottom of the container that facilitates separation of the sediment from the liquid. This liquid is then allowed to ferment in the sun until good aroma and flavors are developed. This top grade fish sauce is corrected to meet quality standards and is then ready for bottling. The high quality fish sauce requires about 9–12 months fermentation; therefore, it is quite challenging for researchers to find means of reducing fermentation time and still produce good aroma and flavors fish sauce. The remaining sediment from the fermentation process is mixed with brine solution, and retained for a period of 2–3 months to extract fish flavors; it is then filtered and bottled as second- and third-grade fish sauce. In commercial fish sauce, sugar, caramel color, and monosodium glutamate may be added to adjust color and flavor.

11.4 Research and Development of Thai Fish Sauce

Thai fish sauce has been traditionally processed in households and small-scale industries where research and development have been carried out by processors and kept secret within the families. Over the past 40 years, many scientific studies had been carried on Thai fish sauce and published for public information. The topics of these studies involved microbiology and chemistry of fish sauce, also methods to improve fish sauce processing and quality.

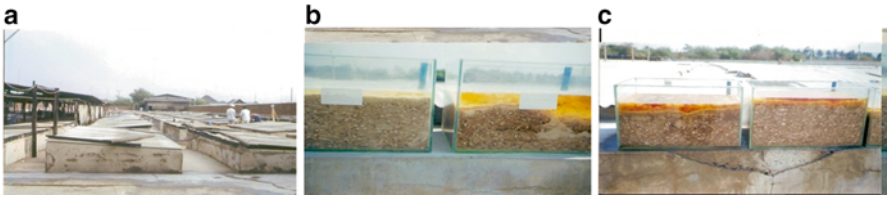

Fig. 11.2 Fish sauce fermentation, (**a**) fermentation tank, (**b**, **c**) the changes of fish and salt mixture during fermentation

11.4.1 Microbiology and Chemistry of Fish Sauce

During the 1–2 month of fish sauce fermentation, the concentration of soluble nitrogen compounds in fish and salts mixture increases which involve endogenous enzyme in fish muscle and viscera. The activity of trypsin-like proteinase has been reported by Gildberg and Shi (1994) and Sirighan et al. (2006), also cathepsin (Lopetcharat and Park 2002) during the first period of fermentation. However, high salt concentration (15–20 %) and low pH (5.5) condition do not favor these proteinase activities.

The changes of microorganism during the beginning of fish sauce fermentation were studied by Thongthai and Siriwongpairat (1978) who demonstrated that after the first month of fermentation the number of aerobic bacteria that tolerate 5–10 % salt that present in a small number will decrease and the aerobic halo-tolerant bacteria will increase from 10^7 to 7×10^8 cells/mL after 3 weeks of fermentation. More specific bacteria that involved in the first stage of fermentation have been identified by Saengjindawong and Winitnuntarat (1984) who found microorganisms to be *Micrococcus roseus, M.varians, Pediococcus cerevisiae, P. halophilus, Bacillus pumilus, B. megaterium, B. firmus, B. alvei, and B. laterosporus*. Halophilic bacteria that showed high proteinase activity are also found during fish sauce fermentation (Thongthai et al. 1992; Chaiyanan et al. 1999; Hiraga et al. 2005). In the late stage of fermentation, many groups of bacteria have been isolated by researchers. Saisithi et al. (1966) reported that *Staphylococcus* sp., *Bacillus* sp., coryneforms, *Streptococcus* sp., and *Micrococcus* sp., isolated from Thai fish sauce after 9 months fermentation were responsible for producing volatile acids that impart good odor in Thai fish sauce. Among these isolates, *Staphylococcus* sp. produced the highest volume of volatile acids. Saisithi (1967) pointed out that five volatile organic acids namely formic, acetic, propionic, isobutyric, and one unknown acid provide Thai fish sauce aroma, and water soluble nitrogen compounds, histidine, proline, and glutamic acid provide fish sauce flavor. There were 20–22 amino acids identified in the first few months of fish sauce fermentation and reduced to 13 amino acids in the final stage of fermentation. This is due to the involvement of amino acids in nonenzymatic browning reactions which resulted in an amber color development in fish sauce (Saisithi 1967, 1968; Raksakulthai and Haard 1992). Most studies (Saisithi et al. 1966; Liptasiri 1975; Suntinanalert 1979; Chaiyanan and Chaiyanan 1983; Thongthai et al. 1992; Chaiyanan 2000) showed that halophilic and halo-tolerant bacteria were involved through the whole process of fish sauce fermentation. Liptasiri (1975) isolated seven halo-tolerant bacteria namely *Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp., coryneforms, *Streptococcus* sp., *Lactobacillus* sp., and *Sarcina* sp. from Thai fish sauce. She pointed out that the first four species provide protease enzyme during fermentation where the product of enzyme reaction may involve in fish sauce flavors and color development. These groups of bacteria were similar to the reports of Saisithi et al. (1966) and Suntinanalert (1979). Parts of bacteria come from salts used in fermentation, as Suntinanalert (1979) found *Halobacterium* sp., and *Halococcus* sp., in most solar salt samples sampling from different suppliers in Thailand; however, *Bacillus* sp., *Micrococcus* sp., and

Staphylococcus sp. were found in some of the sea salt samples. She also investigated bacteria in rock salt samples in Thailand and found *Micrococcus* sp., *Bacillus* sp., and coryneforms. The high salt content (more than 20 %) in fish and salt mixture during fish sauce fermentation help eliminating spoilage bacteria and promoting growth of halophilic and halo-tolerant bacteria. *Pediococcus halophilus* is important in providing good odor in Thai fish sauce (Chaiyanan and Chaiyanan 1983). Saisithi (1987) recommended that the optimal salt concentration for fish sauce fermentation is 20 % by weight. Samittasiri (1986) isolated halophilic bacteria from fish sauce and found that *Halobacterium salinarium* is the bacteria that provide good odor of fish sauce. Later, Klomklang (1995) isolated bacteria that can produce acid, enzyme protease, lipase and amylase from fish sauce collected from different fermentation time; among these isolates she identified and reported that *Staphylococcus saprophyticus* 0113 can hydrolyze lipids and provide acid; *Bacillus pantothenticus* 0118 can hydrolyze starch and protein; *Halobacterium salinarium* 0509 was able to grow in the presence of 25–30 % sodium chloride; and an unknown Gram-negative rod-shaped bacteria 0406 can hydrolyze starch. Proteolytic bacteria, which can grow well in a medium containing high salt, have been isolated from fish sauce (Chaiyanan and Chaychotcharoen 1987; Chaiyanan 2000). Saisithi (1994) concluded in his book that excess salt addition is costly and will slow down the fermentation rate. Salt could retard protein digestion if its concentration is higher than 15 %. Most traditional fermentation took 9–12 months or more; the long fermentation time may be the result of high salt concentration which retards fish muscle digestion at the beginning of fermentation and also slows down bacterial activities.

Although halophilic bacteria that involved in Thai fish sauce fermentation can be isolated, commercially provided pure culture that can help reducing fermentation time and providing good fish sauce odor and flavor has not been successfully accomplished.

11.4.2 Methods to Improve Fish Sauce Processing and Its Quality

Fish sauce is the product of fish hydrolysis where the solid fish is liquefied; this involves the activities of bacteria and fish enzymes under high salt and micro-aerobic conditions. Among the different methods attempting to reduce fish sauce fermentation time that have been investigated by Thai researchers, the addition of enzymes, either crude or pure, obtained from plants, animals, and bacteria, to fish and salts mixture has been favored (Suwunnasart 1966; Mittranond 1983; Poosaran 1986b; Raksakulthai et al. 1986). Suwunnasart (1966) compared the effect of commercial protease obtained from microorganism (*B. amyloliquefaciens*) and papain. Crude enzyme at concentration of 0.4 % of fish weight was added to minced fish and salt liquid mixture and left to ferment at 37 °C for 68 days. The quality of the enzyme-added fish sauce was reported to be better than the non-enzyme-added

sample, where papain-added fish sauce showed the highest amino acid nitrogen content. Gongtip (1965) added bromelain during fermentation and also obtained a good quality fish sauce with shorter fermentation time. Mittranond (1983) produced fish sauce by adding proteinase T (commercial enzyme) and crude pyloric enzyme (prepared from saltwater fish) to hydrolyze fish protein during fermentation. The resulting fish sauce exhibited unsatisfied quality due to the lack of good fish sauce odor. However, the addition of enzyme plus *Halobacterium* (isolated from fish sauce sample) gave good odor liquid fish sauce (Mittranond 1983). It has been shown that addition of crude or pure enzyme can help accelerate fermentation rates; however, extra process time would be needed to help promote the production of volatile compounds that are responsible for good fish sauce odor.

The reduction of fermentation time is also carried by means of the addition of koji (the common name of the fungus *Aspergillus oryzae*). Jongsereejit (1990) screened 12 strains of *Aspergillus oryzae* isolated from the soy sauce koji and sake of Japan, the soy sauce koji of Thailand, and the fish sauce koji of Philippines for protease and amylase activities. Only *Aspergillus oryzae* F and *Aspergillus oryzae* W 215 have the highest protease and amylase activities. In this study, 12.5 % koji from selected strains and 5 % salt were added to anchovy fish and left to ferment for 1 day at 50 °C then added salt to 25 % and incubated at 40 °C for 7 days. The resultant hydrolysate had a good odor, flavor, and reddish brown color fish sauce with 28.93 % sodium chloride and amino acid nitrogen of 11.84 mg/mL. The incorporation of koji appeared to accelerate protein hydrolysis, color development as well as flavor and aroma development. It should also be emphasize that protease activity is inhibited by the increase in salt concentration. In 1995, Klomklang studied the fermentation of fish sauce by using halophilic bacteria with koji. In this study, 12.5 % koji from *Aspergillus oryzae* W 215 plus *Halobacterium salinarium*, added to fish and 25 % salt mixture, then left to ferment for 42 days, showed good odor and flavor fish sauce. The result also indicated that *Halobacterium salinarium* was responsible for good color, flavor, and odor fish sauce. It should be noted that these studies did not compare the experimented fish sauce with commercial fish sauce.

The production of fish sauce by means of acid hydrolysis has been studied by Department of Science (1961) and Poosaran (1986a). The hydrolysate was obtained within 7 days with high degree of protein hydrolysis. However, the color and odor of fish sauce is not good when compared to commercial fish sauce.

Boonpan (2002) produced crude and purified ribonuclease from a halo-tolerant *Pseudomonas* sp. No. 3241 isolated from Thai fish sauce. This enzyme requires an optimum salt concentration of 18.0 %, and shows ability to digest RNA into 5′-GMP (an important flavor enhancer). Application of 0.5 % (w/w) crude enzyme during fermentation enhances good flavor and odor production in fish sauce. Bovornreungroj (2005) produce halophilic protease (HP) from *Halobacterium salinarum* PB407 isolated from fish sauce, then apply to fish sauce fermentation. Ribonuclease from a halo-tolerant *Pseudomonas* sp. No. 3241 (HR) was also applied during fermentation. The conclusion was that fish sauce with added 1.5 % HP or 0.5 % HR at the first

period of fermentation showed a potential to compete with conventional fish sauce as it took only 6 months to ferment and showed good sensory scores.

The reviewed studies showed the potential of the addition of selected enzyme as well as koji to accelerate fish protein digestion in the first period of fish sauce fermentation, then halo-tolerant bacteria promote good odor and flavor formation in the last period; this will reduce the fermentation time compared to the conventional fermentation. However, all of the experiments were carried on laboratory scale. No report has been published as for a commercial trial.

11.5 Nutritional Values of Thai Fish Sauce

Thai fish sauce Nutrition Facts per serving size of 15 mL obtained from two different commercial brands are shown in Table 11.1. The sauce contained of 2 g protein, no fat and low carbohydrate (indicated as sugar), high sodium content (1620–1190 mg), and small amount of calcium and iron. Small amounts of vitamins, i.e., niacin, vitamin B_6, vitamin B_{12}, and folic acid are declared in some fish sauce nutrition fact tables. Vitamin B_{12} is actually produced by microorganisms during fish fermentation. Although fish sauce contains high protein, it is not promoted as a good protein source because it contains a high salt content which is not considered to be desirable in healthy diets.

Table 11.1 Nutrition Facts for Thai fish sauce (15 mL amount per serving)

Nutrition facts			Nutrition facts		
Brand A fish sauce			Brand B fish sauce		
Serving size 15 mL			Serving size 15 mL		
Amount per serving			Amount per serving		
Calories 10		Calories from fat 0	**Calories** 10		Calories from fat 0
% Daily value[a]			**% Daily value**[a]		
Total fat 0 g		**0 %**	**Total fat** 0 g		**0 %**
Saturated fat 0 g		**0 %**	Saturated fat 0 g		**0 %**
Cholesterol 0 mg		**0 %**	**Cholesterol** 0 mg		**0 %**
Sodium 1620 mg		**68 %**	**Sodium** 1190 mg		**50 %**
Carbohydrate as sugar 1 g		**1 %**	**Carbohydrate as sugar** 1 g		**1 %**
Dietary fiber 0 g		**0 %**	Dietary fiber 0 g		**0 %**
Protein 2 g		**4 %**	**Protein** 2 g		**4 %**
Vitamin A 0 %	•	Vitamin C 0 %	Vitamin A 0 %	•	Vitamin C 0 %
Calcium 0 %	•	Iron 2 %	Calcium 1 %	•	Iron 2 %

[a]Percent daily values are based on a 2000 cal diet. Your daily values may be higher or lower depending on your calorie needs

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