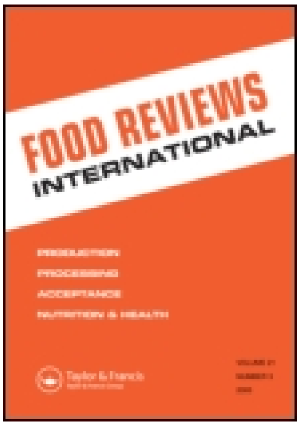

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FISH SAUCE PRODUCTS AND MANUFACTURING: A REVIEW

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FISH SAUCE PRODUCTS AND MANUFACTURING: A REVIEW

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ABSTRACT

Fish sauce, due to its characteristic flavor and taste, is a popular condiment for cooking and dipping. Biochemically, fish sauce is salt-soluble protein in the form of amino acids and peptides. It is developed microbiologically with halophilic bacteria, which are principally responsible for flavor and aroma. This review article covers the manufacturing methods of fish sauce, factors affecting the quality of fish sauce, nutritional values of fish sauce, microorganisms involved with fermentation, and flavor. In addition, rapid fermentation to reduce time and new parameters to estimate the quality of fish sauce are reviewed. Along with a new approach for estimating the quality of fish sauce, the quantitative analysis of degradation compounds from ATP and other specific protein compounds in fish sauce are discussed.

INTRODUCTION

Fish sauce is a clear brown liquid with a salty taste and mild fishy flavor. Generally, the conventional method used to produce fish sauce in Thailand, Korea, Indonesia, and other countries in Asia is to store salted whole small fish (e.g., anchovies) in underground concrete tanks or earthenware for 9 to 12 months in order to complete hydrolysis (1,2). Fish sauce is usually used as a condiment

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in cooking. Fish sauce contains all essential amino acids and is especially high in lysine. Many vitamins and minerals are also found in fish sauce. Fish sauce is a very good source of vitamin B_{12} and minerals such as sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), and phosphorus (P) (1). Even though fish sauce contains a wide range of nutrients, its nutritional value is compromised due to the high concentration of salt (3).

Fermented fishery products have been consumed since ancient times. Roman fermented fish sauce (*garum*) was originally made from the viscera and blood of mackerel (4). Mackerel blood coagulates rapidly under high salinity and is broken down slowly by halotolerant enzymes from viscera (5,6). After a 9-month fermentation period, *garum* was obtained from the clear brown liquid drained from the fermentation tank and the unhydrolyzed tissue in the fermentation tank was used to produce fish paste, which was a stronger and thicker sauce (7). *Garos*, a fish sauce produced in Greece, was made from the liver of *Scomber colias* (8). The production of *garos* was fairly rapid because of the high concentration of proteolytic enzyme in the liver. *Aimeteon* was another fish sauce made during the ancient Greek period. It was made from the blood and viscera of tunny fish. *Botargue* and *ootarides* were two types of fish sauce produced in Italy and southern Greece until the 19th century (4).

In Southeast Asia, and especially in Thailand, fish sauce production has annually extended deeper into international markets. Fish sauce is currently very popular in Southeast Asia and with Asian people in Western countries and is known by different names depending on the country. In Malaysia, fish sauce is called *budu*; in the Philippines, *patis*; in Indonesia, *ketjap-ikan*; in Burma, *ngapi*; in Cambodia and Vietnam, *nouc-mam* (or *nouc-nam*); in Thailand, *nampla*; in Japan, *ishiru* or *shottsuru* (9); in India and Pakistan, *colombo-cure*; in China, *yeesu*; and in Korea, *aekjeot* (7,10).

In Thailand, fish sauce is classified by the Thai Public Health Ministry into three types based on the production process: pure fish sauce, hydrolyzed fish sauce, and diluted fish sauce (1). Pure fish sauce is derived from fresh fish or fish residue obtained from fish fermented with salt or brine. Hydrolyzed fish sauce can be obtained from the hydrolysates of fish or other kinds of animals, which are often treated with hydrochloric acid (HCl) or other hydrolyzing processes that are approved by the Thai Public Health Ministry. Diluted fish sauce is obtained from pure fish sauce or hydrolyzed fish sauce, but is diluted using approved additives or flavoring agents.

This article will primarily review fish sauce manufacturing, factors affecting fish sauce quality, chemical and microbiological composition, flavor, rapid fermentation, and parameters estimating the quality of fish sauce.

FISH SAUCE MANUFACTURING

Fish sauce results from the physical, chemical, and microbiological changes that occur at high salt concentration and low oxygen levels. Fish and salt are the primary raw materials for fish sauce production. Generally, mixing fish and salt is the first step in making fish sauce. The ratio of fish and salt varies from 2:1 to 6:1 depending on the country (7). Other details involved in fish sauce manufacturing vary among fish sauce producing countries as well, in order to make a desirable product for the specific consumer groups.

Traditional *nouc-mam* processing has been reviewed extensively (3,7,11, 12,13). For homemade fish sauce, fish is ground, pressed by hand, and then placed into clay jars in layers with salt in an approximate ratio of 3:1 fish to salt. Shrimp can also be used instead of fish, but it is not popular (14). The jars are then almost completely buried in the ground. The containers are closed tightly and left for several months. At the initial stage of fermentation, the bloody liquid (*nuoc-boi*) is drained off the fermentation tank in about 3 days (3,7). The supernatant liquid is decanted carefully from the fermentation vessels. Today, this traditional method is still used in rural areas of Vietnam. The fermentation time for small fish is around 6 months and extends to 18 months if larger fish are used (15,16,17). The first supernatant collected from the first fermentation cycle is referred to as primary or high quality *nouc-mam*, or *nuoc-nhut* (7). Then hot brine is added into the fermentation tank to extract more *nouc-mam*. This is referred to as secondary or low quality *nouc-mam*. The *nouc-mam* extracted by boiling brine has a low shelf life due to its low salt content and high pH value. Some additives, such as caramel, molasses, roasted maize, or roasted barley, can be added to the fish before the second extracting cycle to improve the color of the product (15,16,18). Instead of using additives, high quality *nouc-mam* is commonly added to low quality *nouc-mam* to enhance its color and flavor (7). Additional fish sauce production procedures are listed in Table 1.

Thai fish sauce (*nampla*), has recently become popular among Western consumers, especially in the United States. Thailand is the leading fish sauce producer in the world. The fish sauce industry in Thailand has expanded from a domestic scale to an international leader over the last 50 years. Because of the different culture and appetite of Thai consumers, *nampla* processing is quite different from *nouc-mam* processing. *Nampla* production starts with cleaning fresh fish with cold water to remove impurities and to reduce the quantity of microorganisms in the raw materials (1). Generally, cleaned fish will be mixed with salt in a 2:1 or 3:1 ratio (fish:salt) (w/w), depending on the area of production. Then, salt-mixed fish is transferred to a fermentation tank where a bamboo mat is laid on the bottom of the tank (Fig. 1). Another layer of bamboo mat is placed on top of the fish and loaded with heavy weight to keep the fish flesh in the brine that is extracted from the fish during fermentation. Brine will reach the top of the fish flesh within the first week of fermentation. After 12–18 months of fermentation, the supernatant is first transferred from the fermentation tank to the ripening tank. After 2–12 weeks of ripening, first grade *nampla* is obtained (10).

Second grade and low quality *nampla* can be produced in the same manner as in the production of low quality *nouc-mam*. In Thailand, BX water or Mikei water is

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Table 1. Summary of Fish Sauce Processing Methods and Types of Fish Used in Various[a] Countries

Country	Name	Fish Species Commercially Used	Method Fish:Salt/ Fermentation Time
Cambodia	*Nouc-mam*	*Stolephorus spp.*	3:1–3:2/2–3 months
		Ristrelliger spp.	
		Engraulis spp.	
		Decapterus spp.	
	Nouc-mam	*Clarius spp.*	
	Gau-ca	*Ophicephalus spp.*	
France	*Pissala*	*Ahya pellucida*	4:1/2–8 weeks
		Gobius spp.	
		Engraulis spp.	
		Atherina spp.	
	Anchovy	*Engraulis encrasicholus*	2:1/6–7 weeks
Greece	*Garos*	*Scomber colias*	Liver only, 9:1/8 days
Hong Kong	*Yeesui*	*Sardinella spp.*	4:1/3–12 months
		Engraulis pupapa	
India and Pakistan	*Colombo-cure*	*Ristelliger spp.*	Gutted fish with gills removed and tamarind added
		Cybium spp.	
		Clupea spp.	6:1/12 months
Indonesia	*Ketjap-ikan*	*Stolephorus spp.*	6:1/6 months
		Clupea spp.	
		Leiagnathus	
		Osteochilus spp. (fresh water fish)	
Japan	*Shottsuru*	*Astroscopus japonicus*	5:1/6 months, malt added
		Clupea pilchardus	
Korea	*Aekjeot*	*Astroscopus japonicus*	3–4:1/12 months
		Engraulis japonica	
Malaysia	*Budu*	*Stolephorus spp.*	3–5:1/3–12 months, palm sugar/tamarind added
Philippines	*Patis*	*Stolephorus spp.*	3–4:1/3–12 months
		Clupea spp.	
		Decapterus spp.	
		Leionathus spp.	
Thailand	*Nampla*	*Stolephrous spp.*	1–5:1/5–12 months
		Ristrelliger spp.	
		Cirrhinus spp.	

[a] Adapted from (7).

applied to improve the quality of low grade or secondary *nampla* (3,19). BX-water or Meiki water is the by-product of monosodium glutamate (MSG) production and is a rich source of glutamic acid, which improves the nitrogen (N) content of low quality *nampla* in order to meet the requirements of the Thai Industrial Standard Institute. Caramel color and other additives, which are not harmful for consumers, are also added to improve color and flavor qualities of *nampla*. The production scheme of typical *nampla* is shown in Figure 2.

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Figure 1. Fish sauce fermentation tank used in *nampla* production.

In the northeastern states of Malaysia, *budu*, similar to *nouc-mam* and *nampla*, is produced (3). In Malaysia, fish sauce is not as popular as in Thailand or Vietnam. The manufacturing process, as well as the changes that occur during *budu* production, have been studied (19). Budu is usually produced from fish left over from fish drying or when the weather is not suitable for drying fish (3). Small fish are mixed with salt in a 3:2 ratio (fish:salt) (w/w). Mixed fish are loaded into circular concrete tanks (~0.9 m diameter × 1 m deep) and covered with a plastic sheet. Weights are placed to press fish in order to enhance osmotic dehydration. Due to the higher salt concentration in *budu*, the rate of fermentation and end product formation are different from *nouc-mam* and *nampla* (7). After a 3–12 month fermentation period, the salt-fermented fish is ground up at irregular intervals, mixed with tamarind

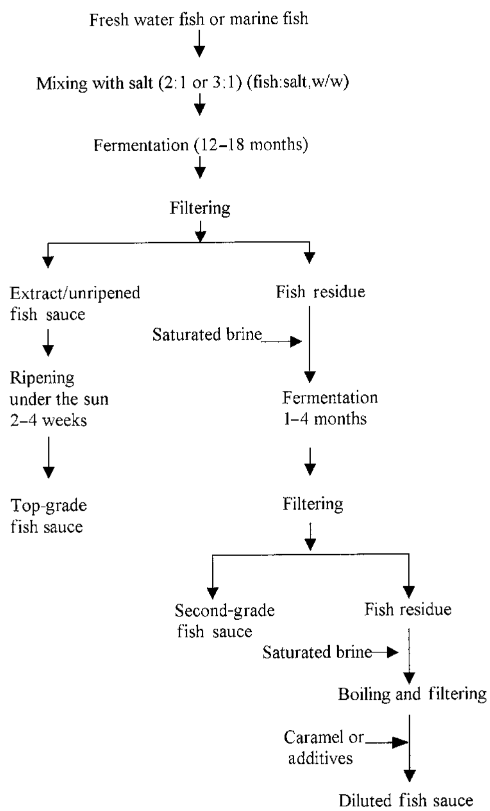

Figure 2. Traditional *nampla* production scheme. Adapted from (1).

and caramelized palm sugar, and boiled. It is then cooled and filtered before bottling. This sweetened product has a darker appearance than *nampla* and *nouc-mam* (3).

Korean fish sauce, *aekjeot* (or *jeotkuk*) is typically prepared by putting anchovies and salt (20–30%) in alternating layers. The amount of salt added is dependent on the freshness, fat content, and storage temperature of the fish. For the first few days, salt and fish are thoroughly mixed to accelerate the penetration of salt. Once the salt is mixed with the flesh, the container is sealed and left at approximately 20°C for fermentation. It is common to see the highest content of free amino acids after 90 days fermentation.

Other types of fish sauce have been produced around the Asian continent. In the Philippines, *patis* is produced by fermenting sardines, anchovies, ambassids and shrimp (3). In Japan, *shottsuru* is made from *hatahata* (*Perciformes trichodontidae*) and is popular locally in Akita prefecture (20). Ishiru is another typical fish sauce, which is made from sardine or squid. Other kinds of Japanese fish sauce are prepared from sardines, cuttlefish, herring, or fish waste materials (21). Although anchovies and sardines are most frequently used for fish sauce production, it is

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obvious that many other raw materials can be used for production of good quality fish sauce. Raksakulthai and Haard (22) have characterized fish sauce produced from the Arctic capelin (*Mallottus villosus*). Recently Lopetcharat and Park (23) evaluated the potential of manufacturing fish sauce using enzyme-laden Pacific whiting (*Merluccius productus*) by combining enzymatic and microbiological degradation. They reported that quality fish sauce could be manufactured using Pacific whiting.

FACTORS AFFECTING THE QUALITY OF FISH SAUCE

There are five major factors influencing fish sauce quality: fish species, type of salt, the ratio of fish and salt, minor ingredients, and fermentation conditions. A certain aspect of fish sauce quality is also dependent on specific consumers. For example, *budu* has a dark color and is preferred by Malaysian consumers, but not by those in Thailand.

The type of fish used in manufacturing fish sauce, which varies from country to country, affects the nutritional quality of fish sauce, especially its nitrogen content. Thus, the different total nitrogen contents of anchovies and sand lance are reflected in the different protein contents of their respective fish sauces (24). Minerals and vitamins present in fish, which contribute to the nutritive value of fish sauce, also vary. Major minerals in fish are potassium (K), phosphorus (P), sulfur (S), sodium (Na), magnesium (Mg), calcium (Ca), iron (Fe), etc. Water-soluble vitamins, such as thiamin, riboflavin, niacin, and vitamins B_6 and B_{12} are also found in fish sauce (1). The nutritional composition of some fish used in fish sauce production is listed in Table 2.

Fish species also affects the type of proteins that serve as nutrients for microorganisms and substrates for enzymes, both of which hydrolyze proteins into

Table 2. Nutritional Compositions of Three Species of Fish Used in Fish Sauce Production [a]

		Different Species of Raw Materials		
Nutrients	Unit	*Stolephorus spp.* (Anchovy)	*Ristrelliger spp.* (Mackerel)	*Clupea spp.* (Herring)
Protein	g	18.0	20.0	20.2
Fat	g	0.3	6.7	4.3
Moisture	g	80.5	72.0	74.4
Calcium	mg	218	170	4.0
Phosphorus	mg	211	60	175
Iron	mg	1.7	11.9	2.0
Vitamin A	IU	139	138	195
Vitamin B_1	mg	0.02	0.03	0.12
Vitamin B_2	mg	0.04	0.62	0.05
Niacin	mg	0.60	9.20	3.00

[a] All values in this table were based on 100 g of sample. Adapted from (1).

small peptides and amino acids. Proteins are highly complex polymers made of up to 20 amino acids (25). Most proteins in fish, except connective tissue and other stroma proteins, are hydrolyzed into small peptides and amino acids. The small peptides, free amino acids, ammonia, and trimethylamine (TMA) contribute to the specific aroma and flavor in fish sauce. The cheesy aroma in *nampla* and *nouc-mam* is caused by low molecular weight volatile fatty acids, especially ethanoic and *n*-butanoic acids (26). Every fish has a slightly different fatty acid profile. Unsaturated fatty acids constitute up to 40% of the total fatty acids (27) and decrease during fermentation (28).

In addition to the chemical composition of fish, microorganisms in fish are also important to the quality of fish sauce. Microorganisms vary depending upon season, place, transportation, species, storage, and catching methods. Microorganisms found in fish and seafood are shown in Table 3. In fresh marine fish, there are about 10^2–10^7 cells/cm^2 on the mucus on fish skin and about 10^3–10^9 cells/gram in fish intestine (1). Spoilage microorganisms, such as *Escherichia sp., Serratia sp., Pseudomonas sp.*, and *Clostridium sp.* grow effectively because fish serve as a source of amino acids and additional nutrients produced by autolysis (2).

Salt is the second main ingredient in fish sauce production. Salt controls the type of microorganisms and retards or kills some pathogenic microbes during fermentation. Sea salt is usually used by the fish sauce industry because of its easy availability. Both sea salt and rock salt are mainly composed of sodium chloride (NaCl). In Thai sea salt, however, sodium chloride is 88.26 ± 2.79%, while salt from other

Table 3. Genera of Bacteria Most Frequently Associated in Fish and Seafood[a]

Genus	Gram Reaction	Frequency
Acinetobacter	−	×[b]
Aeromonas	−	×
Alcaligenes	−	×
Bacillus	+	×
Corynebacterium	+	×
Enterobacter	−	×
Enterococcus	+	×
Escherichia	−	×
Flavobacterium	−	×
Lactobacillus	+	×
Listeria	+	×
Microbacterium	+	×
Moraxella	+	×
Psychrobacter	−	×
Shewanella	−	××[c]
Vibrio	−	×
Pseudomonas	−	××

[a] Adapted from (2).
[b] × indicates known to occur.
[c] ×× indicates most frequently reported.

countries has a high NaCl content (∼97%). Other elements in sea salt are calcium sulfate ($CaSO_4$) at 0.24%, magnesium sulfate ($MgSO_4$) at 0.17%, magnesium chloride ($MgCl_2$) at 0.3%, calcium chloride ($CaCl_2$) at 0.24%, water insoluble substance at 0.4%, and water 2.4%. Mg^{2+}, Ca^{2+}, $SO_4{}^{2-}$, and other impurities retard the diffusion of NaCl into fish flesh (1). Slow diffusion rate can accelerate spoilage. In addition, heavy metal ions contained in salt often increase the oxidation rate of fatty acids in fish oil resulting in low quality fish sauce.

The effect of salt on microorganisms has been studied (2,29,30). Microorganisms such as *Halobacterium* sp., *Halococcus* sp., and *Serratia salinaria* are often associated with sea salt. The osmotic effect of salt kills or retards microbes because of plasmolysis of the microbial cells. Lowering water activity (A_w) reduces water for all metabolic activities causing a longer lag phase (2). Sodium (Na^+) and chloride (Cl^-) interrupt transferring acyl group in some bacteria. In a very high ionic environment, enzymes are easily denatured and inactivated. Thus, metabolism in bacteria cells cannot function properly or totally stops. Some bacteria are more sensitive to carbon dioxide at high salt concentration than low salt concentration (2). Oxygen is less soluble at high salt concentrations. In fish sauce fermentation tanks this results in anaerobic conditions for microorganisms because of thick layers of salt on the top of fish.

The fish to salt ratio is another factor affecting fish sauce quality. The concentration of salt affects the function of various endogenous enzymes that play an important role in protein degradation during fermentation (31). In different countries, the ratio of fish to salt (w/w) varied greatly depending on the type of fish sauce. In Japanese fish sauce (*shottsuru*), the ratio of fish to salt is about 5:1 (7). Korean fish sauce (*aekjeot*) producers use a fish:salt ratio 3:1–4:1 (32,33). *nampla*, in contrast, is made using a 1:1 to 5:1 ratio. Mixing ratios of fish and salt, according to various countries, are summarized in Table 1.

Generally, the fish to salt ratio varies depending on the size of fish used in the production and the desired final product taste. At different salt concentrations, bacterial and enzymatic activity are changed, resulting in different flavors. The chemical composition of salt also affects the type of microbiological flora during fermentation, which in turn affects the quality of fish sauce.

Low oxygen levels in the fermentation tank have a synergistic effect on selecting microorganisms for the process. On the surface of the fermentation tank, the oxygen content is quite high; however, it is limited under the liquid surface and extremely low at the bottom of fermentation tank. Anaerobic fermentation has been shown to alter the aromatic quality of fish sauce (34). Fish sauce fermentation is therefore completed under partial aerobic and anaerobic conditions.

The aroma of fish sauce is primarily due to the functions of aerobic and anaerobic bacteria present in the fermentation tank (19). Halophilic aerobic spore formers are the predominant microorganisms of fish sauce (10). *Bacillus*-type bacteria, aerobes, were found to dominate in *nampla* and they produced a measurable amount of volatile acids. *Staphylococcus* strain 109, catalase positive, was isolated and produced twice as much volatile acid as *Bacillus spp*. *Micrococcus* and *Coryneform*

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bacteria also played a major role in aroma production in *nampla*. Additionally, *Streptococcus spp.* produced a measurable amount of volatile acids (10).

In some countries, such as Malaysia and China, dark colored fish sauce is preferred over light colored fish sauce. Some minor ingredients, such as sugar and natural acids, are used to accelerate the browning reactions. In *budu*, palm sugar and tamarind are added (7). In contrast, for the production of *shottsuru, uwo-shoyu* and *ika-shoyu* malted rice and *koji* (yeast) are used to enhance microbial fermentation.

The quality of fish sauce is often evaluated subjectively, depending on the target consumers, by its flavor and color. Even though the quality of fish sauce depends on the culture and tradition of consumers, the above-mentioned factors determine the consistency, desirability, and safety of the product.

CHEMICAL AND BIOCHEMICAL COMPOSITIONS

Fish sauce is the proteineous product obtained through natural hydrolysis by endogenous enzymes and microorganisms. Obviously, the major change during the fermentation period is the conversion of proteins to small peptides and free amino acids. Chemical compositions of fish sauce (i.e., nitrogen content, pH, and volatile acids) have been investigated broadly using various fish sauces (6,10,19,35–42). Generally, as most of the polypeptide nitrogen decreases during the fermentation period, the amino acid content increases. The pH value drops due to the release of free amino acids from proteins and large polypeptides. In addition, total lipids decrease, but fatty acid composition does not change greatly during fermentation (28). Compared to soy sauce, the chemical composition of fish sauce is very similar (Table 4). The pH and NaCl content of fish sauce, however, are significantly higher than those in soy sauce. Furthermore, acetic acid is higher in fish sauce, while lactic acid is higher in soy sauce.

The average chemical and biochemical compositions of fish sauce from various countries (Burma, China, Japan, Malaysia, Philippines, Thailand, and Vietnam)

Table 4. Chemical Compositions of Fish and Soy Sauce

	Fish Sauce[a]	Soy Sauce[b]
pH	5.3–6.7	4.7–4.9
NaCl (g/dL)	22.5–29.9	16.0–18.0
Total amino acids (g/dL)	2.9–7.7	5.5–7.8
Glutamic acid (g/dL)	0.38–1.32	0.9–1.3
Total organic acids (g/dL)	0.21–2.33	1.4–2.1
Acetic acid (g/dL)	0.0–2.0	0.1–0.3
Lactic acid (g/dL)	0.06–0.48	1.2–1.6
Succinic acid (g/dL)	0.02–0.18	0.04–0.05
Reducing sugar (g/dL)	trace	1.0–3.0
Alcohol (g/dL)	trace	0.5–2.0

[a] Adapted from (33).
[b] Adapted from (38).

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were reported (38). The average NaCl content in fish sauce was $26 \pm 3.7\%$ which is higher than that of soy sauce. The average pH value ranged between 5.3 and 6.7, and most organic acids existing in fish sauce were in salt form. No sugar or alcohol was found in the fish sauce samples.

Nouc-mam

Biochemical changes of *nouc-mam* were reviewed extensively by Beddows (7). Total nitrogen content in *nouc-mam* ranged from 1.3 to 2.3%, depending on quality (15–17,43). *Nouc-mam* contained 2.3 wt.-% nitrogen: 46% in the form of amino acids and 17% in ammonia form (15). During the 120-day fermentation period, organic nitrogen reached a maximum of approximately 2.0%, with total nitrogen content being 2.38%. Approximately 86% of the total nitrogen was organic nitrogen and 49% was free amino acid nitrogen (44). *Nouc-mam* contained 0.13% Mg^{2+} and 0.035% Ca^{2+} (43). The concentration of glutamic acid, aspartic acid, lysine, leucine, valine, and isoleucine was found to be approximately 4 g/L (45). These are complimentary to the amino acids derived from cereal (7). When the amino acid compositions of commercial fish sauce manufactured in various countries were compared (Table 5), there were some differences in the concentration of certain amino acids, especially glutamic acid. This significant difference might have been linked to the improper use of MSG or MSG by-products.

Nampla

Nampla is Thai fish sauce similar to *nouc-mam* (3,45). Total nitrogen increased from 49 mmoles/100 mL to 130 mmoles/100 mL during the 9-month fermentation period. Volatile acid (lactic) increased rapidly within the first 3 months and minimally decreased after 8 months fermentation. Trimethylamine was detected in the aromatic fraction using ion exchange (10). Acetic acid was the major volatile fatty acid in *nampla* using GC-MS technique (46). According to the Thai Industrial Standard (45), NaCl content in *nampla* must be more than 200 g/L and total nitrogen content must be more than 20 g/L. The pH value of *nampla* has to be between 5.0 and 6.0. Amino acid nitrogen content must be 40–60% of the total nitrogen. Glutamic acid content per total nitrogen should lie between 0.4 and 0.8. Histidine and proline content in *nampla* are higher than fish sauce produced in other Asian countries (1).

Budu

Beddows et al. (6,47) categorized the protein breakdown and subsequent change in nitrogen content into 3 stages during *budu* (northeastern Malaysia fish sauce) fermentation: osmosis (0–25 days), releasing proteins (80–120 days), and distribution of nitrogen compounds (140–200 days) (6). Amino-N changed from 36.3 to 66.3% within a 5-month fermentation period. On the other hand, volatile-N,

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Table 5. Amino Acid Composition (mg/100 mL) of Fish Sauces

Amino Acid	China[a]	Korea[b]	Phillipine[c]	Thailand[d]	Vietnam[e]
Taurine	124.5	207.2	211.6	102.1	169.0
Aspartic acid	362.9	28.0	415.7	609.7	430.3
Threonine	222.2	90.7	298.7	379.4	534.6
Serine	138.9	ND	274.3	260.4	393.3
Glutamic acid	823.1	1803.0	944.1	1205.1	3031.9
Proline	86.4	321.7	143.8	178.7	193.0
Glycine	186.5	591.9	323.0	268.3	232.6
Alanine	437.8	1234.0	506.9	670.8	328.9
Cysteine	115.2	287.0	ND	ND	38.1
Valine	338.0	681.1	358.7	476.1	350.1
Methionine	159.5	133.7	217.3	167.0	294.6
Isoleucine	282.5	720.2	355.7	298.4	511.4
Leucine	375.4	1217.7	466.1	343.6	895.1
Tyrosine	38.4	25.0	58.4	37.2	44.9
Phenylalanine	176.2	65.5	201.5	226.7	129.5
Histidine	99.8	341.3	222.8	269.7	307.3
Lysine	667.7	1058.8	696.4	956.5	634.0
Arginine	19.0	57.8	29.9	6.8	14.9
Total	4654.0	8864.6	5724.9	6456.5	8533.5

Adapted from (24).
[a]Fish + salt.
[b]Anchovy + salt.
[c]Fish extract + salt.
[d]Anchovy fish extract + salt.
[e]Anchovy fish extract + salt.

protein-N and polypeptide-N decreased from 10.5 to 6.6%, 1.23 to 0.56%, and 52.0 to 26.5%, respectively, within the same period. Protein conversion rate increased dramatically in the first 60 days of fermentation and then became quite constant over the period of 100–200 fermentation days. There was 1.77% of total-N (organic) and 1.17% of amino-N in *budu*. Palm sugar and tamarind did not have any effect on nitrogen conversion of *budu* production (6).

Bakasang

The pH value of *bakasang* (Indonesian fish sauce) decreased from 6.55 to 5.95. *bakasang* produced by adding glucose showed a greater reduction in pH than without glucose. Like *budu* fermentation, both total soluble nitrogen and total free amino nitrogen increased during fermentation. Alanine, isoleucine, glutamic acid, and lysine were prominent in *bakasang*. However, proline content was low. Different salt concentrations had a great effect on the contribution of amino acid in *bakasang* (37). At different salt levels, different enzymes were activated and also the type and activity of microorganism was altered. Different enzyme and microbial action resulted in different end products (31).

Patis

For *patis*, fish sauce made in the Philippines, NaCl content ranged from 22.26%–26.44% and total solids from 28.56–37.81% (48). First class *patis* had total nitrogen more than 20 g/L. Chemical and physiological compositions of *patis* were reported as follows (49): pH value 5.1, total nitrogen 15.5 g/L, NaCl 29.1%, trimethylamine 14.9 mg N/100 mL. Glutamic acid was predominant in *patis*, 831 mg/100 mL. Alanine, lysine and aspartic acid were also found as major amino acids in *patis* at 696, 677, and 533 mg/100 mL, respectively. Acetic acid was determined to be 2.03 mg/mL.

Shottsuru

Shottsuru, Japanese fish sauce, was studied extensively by Fujii and Sakai (36,50). The pH value and NaCl content of *shottsuru* were 5.0–6.0 and 27.5–34.5% respectively (36,39,40,50). Total nitrogen content ranged between 12.2 and 20.8 g-N/L (40). The amount of trimethylamine was between 8.4 and 12.4 mg/100 mL. The predominant volatile acid was acetic acid like in *patis* (36,50). However, Ren et al. (39) reported that lactic acid was predominant instead of acetic acid. Glutamic acid was a major free amino acid at a level of 721.8 mg/100 mL. Lysine was predominant at a level of 451–581 mg/100 g, which was more than the leucine content of *shottsuru* (47). In contrast, Fujii and Sakai (50) reported that lysine content was lower than leucine content. Histidine content of *shottsuru* made from squid was 145 mg/100 g, whereas in *shottsuru* made from fish, it was more than 300 mg/100 g (40).

Yeesui

The chemical and biochemical compositions of *yeesui*, Chinese fish sauce, were studied by Ren et al. (39,40). The pH value was between 5.4 and 5.8. Salt content ranged from 31 to 33% and total nitrogen content was about 1.25 %. As an amino acid profile, glutamic acid, lysine, and alanine are predominant in *yeesui*. All amino acids in *yeesui* were lower than amino acids in *shottsuru*. Glutamic acid content in *yeesui* was almost two times lower than that of *shottsuru*. Lactic acid was found in all *yeesui* samples, but acetic acid was not found in *yeesui* made in the province of Xiamen (46).

Aekjeot

Aekjeot is literally a Korean fermented fishery product in a liquid form. It is also called Jeotkuk. The pH of anchovy Aekjeot decreased from 6.0 to 5.5 during 3 months of fermentation. The maximum content of soluble nitrogen and amino acid nitrogen was obtained after 3 months fermentation. This maximum content coincides with the optimum taste (51).

MICROBIOLOGY OF FERMENTED FISH SAUCE

Fish sauce has a very high concentration of salt (25–30%). Thus microorganisms found during fish sauce production are generally classified as halophilic (52). The important roles of bacteria in fish sauce are protein degradation and flavor-aroma development. Consequently, when fish sauce is produced under aseptic conditions, typical fish sauce aroma is not developed (6). Bacteria involved in fish sauce can be classified into two major groups.

1. Bacteria that produce proteolytic enzymes. These include: *Bacillus sp., Pseudomonas sp., Micrococcus sp., Staphylococcus sp., Halococcus sp., Halobacterium salinarium, Halobacterium cutirubrum* (1,52,53). Highly concentrated NaCl (25%) does not have any effect on the proteolytic activity of enzymes from *H. salinarium and H. cutirubrum*; however, a chelating agent such as EDTA inactivates these enzymes completely. Zinc ion (Zn^{++}) and magnesium ion (Mg^{++}) can reactivate the enzyme activity slowly (53). Enzymes from halophilic bacteria can function fully in a high salt environment, but most are inactive in the absence of salt (54). The extreme halophiles adapt themselves to metabolize amino acids more efficiently than carbohydrates (53).
2. Bacteria that relate to flavor and aroma development. Ten out of 17 *Bacillus*-type isolates produced a measurable amount of volatile acids in *nampla*. *Staphylococcus strain* 109 also produced a significant amount of volatile acid in *nampla* (10).

When microbiological changes during *bakasang* processing were monitored, a variety of bacteria grew during the first 10 days of fermentation (37), however after 20 days, *Streptococcus, Pediococcus, Micrococcus* were dominant. During 40 days of fermentation, *Enterobacter, Moraxella, Pseudomonas, Lactobacillus, Staphylococcus, Micrococcus, Streptococcus*, and *Pediococcus* were isolated from *bakasang*. Total plate count, however, reached a maximum at 10 days fermentation and decreased after 20 days of fermentation.

For *nampla*, total viable count steadily decreased as fermentation time was extended, similar to *bakasang*. *Bacillus, Coryneform, Streptococcus, Micrococcus* and *Staphylococcus* were isolated from 9-month-old *nampla* (10). *Bacillus*-type bacteria produced a measurable amount of volatile acids; however, *Staphylococcus* strain 109 produced twice as much. Extremely halophilic *Archaeobacterium*, strain ORE, was also isolated and identified from *nampla* (52). Using polar liquid analysis and DNA hybridization technique, this halobacterium was identified as *Halobacterium salinarium*, which is known to produce extracellular proteases. All isolates from fish sauce and soy sauce were Gram-positive, nonmotile cocci (55). All isolates from fish sauce were facultatively anaerobic and fermented glucose (55).

The microflora found from Korean anchovy sauce in the final stage of fermentation included *Bacillus cereus* var. I and II, *B. megaterium* var. II, *B. pumilis, Clostridium setiens, Pseudomonas halophilus*, and *Serratia marcescens* (56).

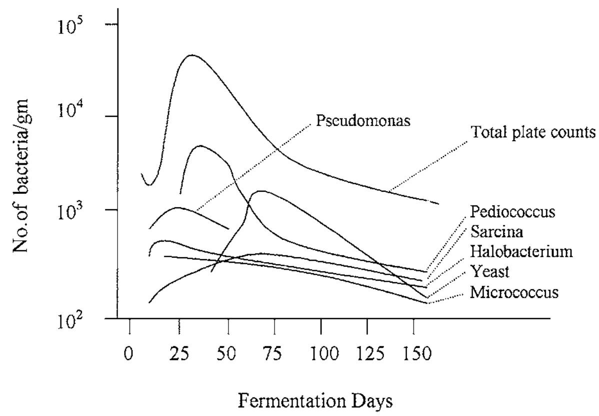

Figure 3. Changes in microflora during the fermentation of Korean anchovy fish sauce. Adapted from (57).

According to Kim and Kim (57), eleven different microorganisms were isolated during the fermentation of Korean anchovy *aekjeot*. They were all halophilic and either aerobic or anaerobic. They rapidly grew up to the maximum level and then rapidly disappeared. As indicated in Figure 3, *Pseudomonas* and Halobacterium grew well at the initial stage, whereas, total plate count, *Pediococcus*, and *Sarcina* were predominant during 40–50 days of fermentation and decreased thereafter.

FLAVOR OF FERMENTED FISH SAUCE

Flavor is the combined impressions perceived via the chemical senses from a product in the mouth resulting in aroma and taste (58). The aroma is often used as a quality index for fish sauce, but is measured somewhat subjectively by consumers (26,47). The salty taste of fish sauce is very strong and dominates other flavor constituents (47). The chemical sensory factors, especially glutamic acid, relate to umami taste and good taste imparted from the fish sauce (39,59).

Three major contributing factors in fish sauce are ammonical, cheesy, and meaty notes (26). The ammonical note has been attributed to ammonia, trimethylamine, and other basic nitrogenous compounds (10,26). In the presence of antibiotic material and rifampicin, ammonia and trimethylamine (TMA) are easily formed. Thus, these nitrogenous compounds must be derived from nonbacterial means, such as raw fish (47). Trimethylamine is linked to cheesy note in fish sauce because its threshold value is very low, 2.4 ppb in the vapor phase (60–62). In addition to imparting flavor, some volatile compounds are noted as a spoilage indicator, such as TMA, ammonia, and dimethylamine (DMA) (2,63).

Low molecular weight volatile fatty acids (VFA), in particular acetic, ethanolic, propionic, *n*-butyric and isovaleric acids have been identified as contributing to

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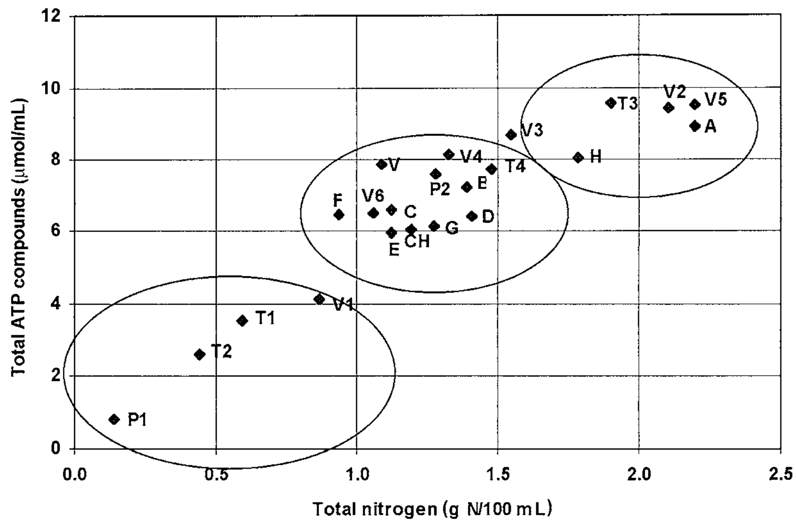

Figure 4. Relationship between total nitrogen and total compound of degraded ATP. A-H: Korean commercial fish sauce; P1-P2: Philippine commercial fish sauce; T1-T4: Thailand commercial fish sauce; V1-V7: Vietnam commercial fish sauce; CH: Chinese commercial fish sauce. Samples in the same circle are categorized as the same grade (high, medium, and low).

the cheesy note of fish sauce (10,26,48,64–66). These VFA were produced from the autoxidation of polyunsaturated acids and by bacterial action on amino acids, which are used as a carbon source (26). Butanoic acid, 3-methylbutanoic acid, pentanoic acid, and 4-methylpentanoic acid are also considered to be associated with the cheesy note in the fish sauce aroma because of their low odor threshold value, 3.89, 2.45, 4.79, and 7.10 ppb in vapor phase and quantitative values (60,61,67).

N-butanoic and *n*-pentanoic acids were derived by bacterial activity on amino acids, using (U-^{14}C)-protein hydrolysate as a substrate during fermentation, instead of glucose and oxidation of the fish lipid (47). These VFA were produced by bacteria prior to the salting process (10,47). Ethanoic and *n*-butanoic acids can be produced by the oxidation of glutamate (68). In addition, tryptophan breaks down to give ethanoic acids (69) and *Clostridium kluveri* produces *n*-butanoic acid using alcohol or ethanoic acid as substrate (70).

The maximum ratio of *n*-butyric acid to acetic acid is 1:3.3 and 1:1 in *nampla* and *nouc-mam*, respectively (71). For the highest quality *nampla* and *nouc-mam*, the ratio of *n*-butyric acid to acetic acid is 1:20. Total VFA in Hong Kong and Chinese fish sauce was only one-third of that in *nampla* (26). Volatile compounds in Taiwanese fish sauce were also identified (67).

In addition to VFA, many ketones could be responsible for the cheesy odor as well (67). Ketones did not have much effect on fish sauce flavor because of their high threshold value (61). Isopentanoic acid was the most abundant volatile acid in both *shottsuru* and *nampla*, followed by acetic, isobutanoic, *n*-butanoic and propionic acids (72). In addition to these acids, isohexanoic acid was also a major volatile acid in *nampla*. *nouc-mam* had different a volatile acid profile than *nampla* and *shottsuru*. Acetic acid was the highest volatile acid in *nouc-mam* (72). These results were different from volatile acid profiles reported previously (46,50). Acidic fraction of Patis consisted of *n*-butanoic acid at 50%, propanoic acid at 22%, isopentanoic acid at 17%, acetic acid at 4%, and isobutanoic acid at 3% (65,73).

The sensory meaty note is more complicated than the other two notes and has not been well characterized. Meaty odor was produced by the oxidation of a substance that can be extracted entirely from fish sauce with isopropanol (26). Glutamic acid also contributes to the meaty aroma in *nampla* (10), and histidine and proline may play an important role in *nampla* flavor as well (74).

Meaty aroma in *nampla* was extracted as three lactones in neutral fraction: γ-butyrolactone, γ-caprolactone, and 4-hydroxyvaleric acid lactone (26,46). Both γ-butyrolactone and γ-caprolactone have a faintly sweet and aromatic buttery aroma, while 4-hydroxyvaleric acid lactone has a pungent odor (46). *Budu* had less meaty aroma than *nampla* (47). Nitrogen-containing compounds such as pyrazines, pyridines, pyrimidines, amines and nitrile have a burnt-or amine-like odor. Together with aldehyde compounds, they may be responsible for meaty notes (61).

In addition to the major pleasant notes in fish sauce are sulfur-containing compounds, such as dimethyl disulfide and dimethyl trisulfide with threshold value of 0.427 and 1.66 ppb in vapor phase respectively, which can cause unpleasant odor in fish sauce (67). Aldehyde is also considered to have a negative effect on overall flavor in fish sauce because of their low threshold values (60,61,75).

The flavor of fish sauce is due to the cumulative effect of both the volatile fatty acids and non-volatile fatty acids along with other biochemical reactions. Compounds from both enzymatic and bacterial breakdown of protein or other nitrogenous compounds are also important to fish sauce flavor (76). Comparing flavors in *shottsuru, nampla*, and *nouc-mam* (72), *shottsuru* was a little fishy, cheesy and rancid with a sweet, but a little burnt odor. *nampla* had a more stimulating, fishy, cheesy, and rancid odor than *shottsuru*. It was a little sweet and very slightly burnt. *nouc-mam*, on the other hand, exhibited a significantly burnt smell like smoked fish products. In comparison, *patis* had a fishy, cheesy, and rancid odor and it also smelled like *tsukudani*, a traditional Japanese processed seafood (65).

The type of fish used also determines fish sauce flavor. Fish sauce from flounder, a low fat fish, had a significantly different flavor compared to fish sauce made from trout, a fatty fish, at $p < 0.05$. However, the volatile acid profile from both sauces did not relate to the amount of fat in fish. Therefore, the relative and absolute amount of VFA depends on both the type (*nampla, nouc-mam*, or *patis*) and quality of fish sauce (46).

Amino acid composition in fish sauce was also affected by different enzymes used during fermentation (77). Fish sauce supplemented with squid hepatopancreas was highly acceptable to consumers. The high content of glutamic acid was found with hepatopancreas, while the content of leucine found with pronase and the high content of alanine with trypsin and chymotrypsin. Only fish sauce developed with hepatopancreas was acceptable to consumers. This result suggested that glutamic

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acid plays an important role in fish sauce flavor and the changing enzyme system in fermentation changes the flavor of product. Histidine and lysine had been used as accelerating agents in fish sauce (64,66). Lysine did not have significant effect on aroma, but it changed the flavor of fish sauce. Inclusion of histidine shortened the fish sauce fermentation to 4 months and the product was acceptable. However, the addition of histidine did not increase the histamine content of the sauce.

RAPID FERMENTATION OF FISH

Protein hydrolysis occurs in fish sauce fermentation via autolytic activity (6,19,35). Trypsin and chymotrypsin and other digestive enzymes are principally responsible for autolysis (78,79). Trypsin-like enzyme can be recovered from fish viscera and fish sauce (80). Trypsin-like activity in *patis* fermentation increased and reached a maximum in the first month and then dramatically declined (31). The decline of trypsin-like activity in *patis* is thought to be caused by the accumulation of end products (amino acids and small peptides), inhibitors in fish blood or substances produced by bacteria.

Cathepsin activity in *patis* formation was also studied (41). Cathepsin A and D were found to be responsible for the protein hydrolysis in *patis* formation as trypsin and chymotrypsin. However, cathepsin B and D minimally effected protein degradation in *patis* (41). In contrast, when Pacific whiting and its surimi by-products, after being mixed with high salt concentrations up to the level of 25%, were subjected to autolysis at 50°C, cathepsin L-like enzymes and metalloproteases played a significant role in hydrolyzing proteins (81).

Trypsin and chymotrypsin (alkaline proteinases) are active in neutral condition and cathepsins are active in acid condition. The pH of fish sauce decreased from neutral pH (∼7) to acidic pH (∼5) during fermentation. Therefore, during the first stage of fish sauce fermentation, tyrpsin and chymotrypsin are responsible for protein hydrolysis, but cathepsins are responsible for protein degradation in fish sauce fermentation when the pH drops to the acidic region. The decrease in catheptic activity can be due to the decreased high molecular weight proteins that serve as the substrate for the enzymes (48).

In traditional fish sauce fermentation, the rate of production depends only on the activity of enzymes in the fish. Rapid fermentation has been studied by many researchers (32,33,64,82,83). Fermentation was accelerated when finely ground fish was used and when stirring was applied (32,82,83). In Patis production, increasing temperature (45°C) and reducing salt concentration can also reduce fermentation time (84). In general, the optimum temperature for fish sauce fermentation is between 35 and 45°C (18). However, Korean fish sauces were usually fermented at 20–25°C in order to maintain traditional taste and flavor.

Using natural enzymes, including bromelain, ficin, and papain, to shorten fermentation time has also been studied (17,80–82). Rate of hydrolysis of fish flesh was increased using papain (19,85). However, bromelain gave better result

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than papain and ficin. In addition, Raksakulthai et al. (76) compared the fish sauce produced using male capelin (*Mallotus villosus*) and various other enzymes. Acid hydrolysis also used to accelerate fermentation (27,86).

PARAMETERS FOR ESTIMATING QUALITY OF FISH SAUCE

The traditional method for the production of fish sauce is different from country to country. Factors such as ratio of salt to fish, fermentation temperature, fish species, and minor ingredients greatly influence the compositional and nutritional quality of fish sauce. The only quantitative parameters to determine the quality of anchovy sauce and validate the grade available in Korea and Thailand are total nitrogen content and color (Table 6). Considering the total nitrogen content can be easily fortified with other soluble proteins, and color can be adjusted using natural brown pigments, such as caramel, the use of total nitrogen content and color as a target quality parameter could mislead the market. There is a great need, therefore to develop a method to identify the quality of fish sauce without the presence of food additives.

There are several attempts being made in an effort to develop a method to assess the quality of anchovy sauce. Kim et al. (87) reported that physicochemical analysis in conjunction with sensory evaluation could be used to estimate the quality of fish sauce. The content of extractable components containing nitrogen compounds, such as free amino acids and nucleotides, to estimate the quality of fish sauce was also suggested (88,89). In addition, the aroma of fish sauce is often used as a measure of product quality (34,67,90).

Fish sauce is made as a result of almost complete hydrolysis of muscle proteins in the presence of saturated salt concentration. This hydrolytic fermentation is slowly progressed by the action of intestinal proteases and proteases generated from halophilic microorganisms. Different kinds of peptides and amino acids are produced from different biological properties of fish as affected by the muscle

Table 6. Standard Parameters for Fish Sauce in Thailand and Korea

	Thailand[a]		
Items	1st grade	2nd grade	Korea[b]
Relative density at 27	1.2	1.2	
pH	5.0–6.0	5.0–6.0	
Sodium chloride (g/L)	230	230	230
Total nitrogen (g/dL)	2.0	1.5	>1.0
Glutamic acid (g/total nitrogen)	0.4–0.65	0.4–0.6	
Amino acid (g/dL)	1.0	0.75	0.6
Moisture (%)			68

[a] Adapted from (100).
[b] Adapted from (101).

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composition of the species. As an objective index for the quality estimation of Korean anchovy sauce (Aekjeot), Choi et al. (91) investigated the biochemical properties of a specific protein that remained undigested during fermentation. An approximately 55,000-dalton protein identified on sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) was found to be in good relationship with the total nitrogen content. In addition, this protein was minimally affected during fermentation (92). Choi et al. (93) developed a quantitative method for the specific protein in anchovy sauce by liquid chromatography. Furthermore, Cho and Choi (24) reported a simple, but accurate method for measuring ATP derivatives and the ratio of hypoxanthin to uric acid to estimate the quality of fish sauce.

Histamine content can be another important target to estimate the quality of fish sauce.Histamine toxisis (Scombroid poisoning) is caused by ingesting a high level of free histidine in fish tissue that has been decarboxylated to histamine (64). Particularly, fish sauce contains a large amount of histamine when Scombroid fish are used as the raw material (94,95). Histidine in fish muscle is the primary source of free histamine in fermented products. Histamine stimulates muscles to contract or relax, particularly the heart muscle and the extravascular smooth muscle in the small intestine, and also affects the sensory and motor neurons that control gastric acid secretion (96). Many halophiles, such as *Photobacterium phosphoreum, Photobacterium histaminum sp. nov., Enterobacteriaceae, Proteus morganii (Morganella morganii), Klebsiella pneumoniae, Citrobacter freundii, Enterobacter cloacae, Hafnia alvei*, and *Escherichia coli* can produce histidine decarboxylase (49,97). Histamine formation can easily be controlled by lowering the storage temperature and implementing hygienic practices (98). Virulhakul et al. (95) analyzed 250 fish sauce samples exported from Thailand and found the histamine level was 1.16–129.46 mg/100 g. The content of histamine in 189 samples was higher than the defect action level (20 mg/100 g). The histamine content in fish sauce had no correlation with total nitrogen content (95). According to the newly proposed FDA action level for histamine (5 mg/100 g), any fish containing histamine above this level will have to be discarded or destroyed (99). This suggests that the histamine content in fish sauce is very important for safety. Development of fish sauce from lean fish appears to be a way to avoid the histamine problems associated with anchovies.

SUMMARY

Fish species and manufacturing methods used for fish sauce vary from country to country, due to culture and weather/temperature. In general, fish sauce is produced by grinding small fish, mixing with salt, and fermenting for about 12 months. During fish sauce fermentation, the proteolytic hydrolysis of fish proteins produces soluble peptides and amino acids and degrades low molecular weight compounds. Volatile compounds contribute a unique aroma and flavor and are developed during fermentation. Amino acids (glutamic acid and aspartic acid), peptides,

nucleotides, and organic acid (succinic acid) also contribute to the taste of fish sauce. In general, fish, salt, fish and salt ratio, oxygen level, and minor ingredients have tremendous effects on fish sauce quality. Three major contributory factors of fish sauce aroma are ammonical, cheesy, and meaty notes. Ammonia, amines and trimethylamine play an important role in ammonical note, which does not depend on microbial activity. Cheesy note can be contributed by low molecular weight volatile fatty acids produced by microorganisms using amino acids as a substrate. Meaty note can be produced by oxidation. *Bacillus and Staphylococcus* are found in fish sauce and produce measurable volatile fatty acids. Further investigation of accelerating the fish sauce production, fish sauce with no histamine, objective quality indices, and technology to develop low sodium fish sauce without sacrificing flavor should be conducted in the future to produce high quality fish sauce.

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