Characteristics of Fish Sauce Made from Pacific Whiting and Surimi By-products During Fermentation Stage

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ABSTRACT: The fermentation condition for producing Pacific whiting fish sauce was static atmospheric fermentation with 25% salt at 50 °C. The effective enzymes in fermentation were heat stable and salt tolerant. Fermentation at 50 °C gave higher yields than at 35 °C. Total nitrogen content of whole fish fermented at 50 °C reached the equivalent level of commercial fish sauce before 15 d, supporting the strong degradation effects of Pacific whiting enzymes at earlier stages. Soluble solid and relative gravity also reached commercial level at 60 d. However, color value of unripened fish sauce was far from commercial fish sauce, indicating that ripening may be necessary to develop proper color. *Staphylococcus*, *Bacillus*, and *Micrococcus* were found as predominant microorganisms during fermentation.

Key words: fish sauce, Pacific whiting, by-products, amino acids, fermentation

Introduction

FISH SAUCE IS A CLEAR BROWN LIQUID HYDROLYSATE FROM salted fish (Amano 1962; Beddows 1985). It is widely used as a condiment and seasoning in Southeast Asia. Fish sauce is marketed using various names by different countries: nampla in Thailand, nouc-mam in Vietnam, patis in the Philippines, shottsuru in Japan, and aek-jeot in Korea. Different countries have different recipes for making fish sauce. Nampla (Thai fish sauce), which is the most dominant in the world market, is mainly produced from anchovies (*Stolephorus spp.*), mackerel (*Ristrelliger spp.*), and herring (*Clupea spp.*) (Wilaipan 1990). There are 2 major ingredients in fish sauce production, fish and salt. The ratio between salt and fish is very different, depending on the country, ranging from 1:6 to 1:2 (w/w). Traditionally, nampla is produced by mixing 1 part salt with 2 or 3 parts fish and fermenting under static atmospheric conditions in an underground concrete tank at ambient temperature (30 °C to 40 °C) up to 18 mo. The supernatant from the fermentation tank is filtered and ripened under the sun for 2 to 4 wk (Wilaipan 1990).

Microorganisms generally increase during the early fermentation stage and then decrease gradually as fermentation time is extended (Saisithi and others 1966; Ijong and Ohta 1996). Beddows and others (1979) reported that budu (Malaysian fish sauce) produced in the presence of rifampicin does not have the unique aroma of budu. Microorganisms such as *Bacillus* and *Staphylococcus*, which were isolated from nampla, bakasang (Indonesian fish sauce), and patis (Philippine fish sauce), produced a significant amount of volatile acids (Saisithi and others 1966; Ijong and Ohta 1996).

Pacific whiting (*Merluccius productus*) is the cheapest white fish in the world and abundant in the Pacific Northwest. However, it is also a problematic fish because it has strong proteolytic enzymes. This kept it from being commercially utilized until 1991, when a surimi process was developed for Pacific whiting. In 1997, 327,729 metric tons of Pacific whiting were harvested (Shapiro and others 1998). Most of the catch is commercially processed into either surimi or fillets. These processes utilize $< 30\%$ of the fish and leave $> 70\%$ of solid waste. The waste is either processed into a very cheap fishmeal or discarded. The presence of proteolytic enzymes, which cause texture softening, makes the value of Pacific whiting very low. In an attempt to combine proteolysis with fermentation, fish sauce production was thought to be a unique alternative to utilize enzyme-laden Pacific whiting and its surimi by-products.

The objectives of our study were to characterize physicochemical and microbiological changes of fish and to determine the potential for making high-quality fish sauce from Pacific whiting and its by-products. Fermentation conditions and the physicochemical transformations during fermentation were also investigated.

Materials and Methods

Materials

Pacific whiting (*Merluccius productus*) and its by-products (head, frame, guts, skin) were obtained from a local surimi processing plant and transported on ice to the OSU Seafood Laboratory. After taking a small portion of fresh samples (whole fish, fillets, and by-products) to measure autolytic activity, the remaining portions were frozen at −20 °C until fish sauce preparation.

Ten commercial anchovy fish sauce products were purchased at Asian grocery stores (Portland, Ore., U.S.A.) and analyzed for their physicochemical properties.

Chemicals

Food grade salt (NaCl) was purchased from a local grocery store. Trichloroacetic acid (TCA), bovine serum albumin (BSA), and ammonium sulfate were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Nutrient agar, anaerobic agar, and tryptic soy agar (TSA) were purchased from Difco Laboratories (Detroit, Mich., U.S.A.). TSA with 5% de-fibrinated sheep blood was obtained from Hardy Diagnostic (Santa Maria, Calif., U.S.A.).

Autolytic activities at various conditions

As a preliminary study, temperature profiles of autolytic activity were measured using fresh whole fish, by-product, and fillet. The samples were incubated at various temperatures (0, 25, 35, 45, 50, 55, 60, 65 and 70 °C) for 30 min with different salt concentrations (0, 5, 15, and 25%) at physiological pH (about 7.2 for whole fish and fillet, and about 6.8 for by-products). The autolytic reaction was stopped using 10% TCA. The TCA-sample mixtures were homogenized and centrifuged at 8000 rpm for 3 min. The autolytic activity was measured as tyrosine (Tyr) equivalency according to the Lowry assay (Lowry and others 1951). One unit of activity was defined as 1 nmole of Tyr released per min.

Fish sauce preparation

Frozen whole fish and by-products were ground with a heavy-duty grinder (model 601HP 5HP, Atutio, Astoria, Ore., U.S.A.) using 5-mm plate. Samples (210 g) of each raw material were mixed with salt (70 g). Samples were prepared by putting 6 layers of ground meat and 7 layers of salt 1 by 1 in HDPE containers. The containers were covered with snapped HDPE lids to limit evaporation, but to allow oxygen during incubation at 35 °C and 50 °C for 60 d. Incubation was terminated at 60 d when the total nitrogen content of whole fish reached a level of 22.2 to 26.9 g N/L, which was significantly higher than that of commercial anchovy fish sauce (16.3 g N/L). Static atmospheric condition was chosen because fish sauce produced under absolute anaerobic condition had a bitter taste and undesirable flavor (Sanceda and others 1992). Samples were collected from containers at Day 0, 5, 10, 15, 20, 30, 40, and 60. Additional measurements were also made irregularly after 60 d for the purpose of verification. The samples were prepared in triplicate for physicochemical determination and duplicate for microbiological analysis.

Collection of liquid

All available liquid was filtered from the whole container using 4 layers of cheesecloth and the residue was squeezed using a laboratory hydraulic press (Fred S. Carver, Inc., New York, N.Y., U.S.A.) at 1500 psi until no liquid was released. The liquid was then filtered through a filter paper #40 followed by #1 (Whatman Int'l, Maidstone, U.K.). The filtered liquid obtained was referred to as unripened fish sauce.

Physicochemical analysis

The pH of unripened fish sauce was measured by directly inserting a pH probe into the extracted liquid (Corning pH meter 240; Corning, N.Y., U.S.A.). Soluble solid was measured using a hand refractometer (model N1, Atago, Tokyo, Japan). Liquid yield was recorded. Moisture content was measured using a dry oven method (AOAC 1995).

Color characteristics of the samples were measured using a ColorQuest Hunter colorimeter (Hunter Laboratories Inc., Reston, Va., U.S.A.). Unripened fish sauce samples were placed in a 3-mm-pathlength optical glass cell (Hellma GmbH & Co., Mullheim, Germany) and reflectance measurements were obtained from the average of 4 readings for each sample, and CIE L^*, a^*, b^* values were measured using the transmission mode. The degree of brown color was separately measured using a spectrophotometer (model DU 640; Beckman, Fullerton, Calif., U.S.A.) at 420 nm.

Total nitrogen content of unripened fish sauce samples was measured using the micro-Kjeldahl method (AOAC 1995). Salt content and ammonia nitrogen were measured using a conductometric method (OAKTON™ TDS Testr2™ conductivity tester; Whatman, Hillsboro, Ore., U.S.A.) and ammonia electrode (model 95-12; Orion Research Inc., Boston, Mass., U.S.A.).

Microbiological analysis

Samples (10 g) were taken aseptically from whole container and homogenized in 90 mL of 0.9% NaCl solution. Serial dilutions of homogenates were made. Aerobic plate count (APC) and anaerobic plate count (ANPC) were determined using pour plate method on nutrient agar (Difco, Detroit, Mich., U.S.A.) fortified with 7% NaCl. Typical colonies from all plates and homogenized samples, without any dilution, were subcultured for purification and identification using tryptic soy agar (TSA) and TSA with 5% de-fibrinated sheep blood for testing β-hemolytic activity. All plates were stored at 4 °C until required. Microbiological analysis was conducted at 0, 10, 15, 20, 40, and 60 d.

Identification of microorganisms was done on the basis of morphological and biochemical properties of microorganisms using colonies obtained at 0 and 40 d according to Bergey's Manual (Holt and others, 1994). Biochemical properties were determined using the Vitek-Junior identification system (BioMerleux Vitek, Hazelwood, Mo., U.S.A.).

Statistical analysis

Data were analyzed for degree of variation and significance of difference using an analysis of variance (ANOVA) (Ramsey and Schafer 1997). All statistical analyses were performed using STATISTICA® Version/w 5.0 (StatSoft, Inc., Tulsa, Okla., U.S.A.).

Results and Discussion

Autolytic activities at various conditions

Fermentation conditions for Pacific whiting and its by-products were studied. The highest autolytic activity of ground whole fish was found with 25% salt, particularly at 50 to 60 °C (Figure 1). The effects of salt on by-products were similar, exhibiting the highest autolytic activity at 55 to 65 °C (Figure 2). In contrast, the autolytic activity in fillet was much lower than the other 2 samples, and the activity was extremely suppressed by 25% salt at 55 to 70 °C (Figure 3). These results suggest that the enzymes in Pacific whiting (whole fish and surimi by-product) are functioning at 25% salt and high temperature. Overall, autolytic activity with 25% salt was maximized at 50 °C for whole fish (Figure 1),

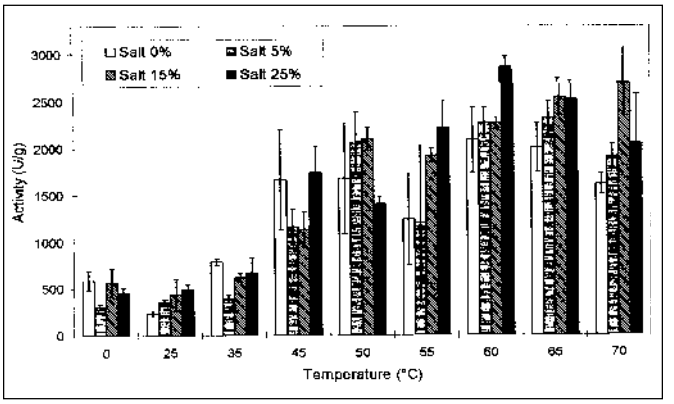

Figure 1—Temperature profiles of autolytic activity of whole Pacific whiting at various salt concentrations

and 60 °C for fillet and by-products (Figure 2 and 3). However, the 2 temperature treatments selected for subsequent experiments were at 35 °C, as a control condition used in typical fish sauce production in Thailand, and 50 °C, as experimental condition. Fifty degree Celsius was selected due to high activity of the enzymes, as reported, and tolerable environment for the mesophiles, which are believed to play an important role in fish sauce production (Wilaipan 1990; Jay 1996). Noda and others (1982) reported that 1 alkaline proteinase and 1 acid proteinase were stable in the presence of 15% to 20% NaCl.

Physicochemical Properties

Liquid yield. More liquid was extracted at Day 0 than Day 5, except with the by-products at 50 °C and liquid yield increased rapidly at Day 10. In all cases, liquid yield increased promptly from Day 5 to Day 10 of fermentation (Table 1). After Day 10, the increase was slow. Reduction of liquid yields at Day 5 was possibly caused by the migration of salt and re-equilibration of soluble components. The osmotic period of our samples was much shorter than 0 to 25 d for whole fish used in budu production (Beddows 1985). This was probably due to a difference in sample conditions, ground fish against whole fish. Grinding, through mechanical disruption of cells and expanding surface area, probably accelerated the osmosis of fish juice in the presence of salt. More liquid was obtained at 50 °C than 35 °C, and from by-products than from whole fish. Beddows (1985) obtained a maximum liquid yield (about 70%) from whole anchovies at 30 °C after 140 d. However, increased fermentation temperature (to 50 °C) and grinding helped to get more liquid yield in less time. Solid residues from each treatment were inversely related to the liquid yield (not reported).

Moisture. Moisture content at Day 0 was about 72.5% for whole fish and about 73% for by-products. Their values rapidly decreased for the first 10 d and continued to decrease up to 60 d of fermentation. Final moisture at 60 d was about 69% for by-products and about 68% for whole fish (Table 1). This was within the moisture range (60 to 75%) of commercial fish sauce (Table 2). Bersamin and Napugan (1961) reported that the moisture content of patis ranged from 62 to 74%.

Total nitrogen. The extraction of total nitrogen from Pacific whiting fish sauce fermentation was faster than that from nampla fermentation. The release of water-soluble proteins from cells by osmotic pressure resulted in an increased total nitrogen content (15.7 g N/L for 50 °C fermented whole fish) during the first 10 d (Table 1). Total nitrogen content in nampla changed from 7 to 18 g N/L during the 9 mo fermentation period (Saisithi and others 1966). Total nitrogen content is the only objective index used to classify the quality of nampla (Wilaipan 1990). High-quality nampla and patis must have a total nitrogen content of > 16.3 g N/L based on the Kjeldahl method (Wilaipan 1990; Bersamin and

Table 1—Physicochemical characteristics of unripened fish sauce (I)

		Whole Fish		By-product	
Characteristics	Day	50°C	35°C	50°C	35°C
Liquid yield (%)					
	0	45.5(0.0)	44.8(0.0)	44.0(0.0)	43.3(0.0)
	5	42.0(0.0)	32.4(0.0)	47.6(0.0)	41.0(0.0)
	10	64.3(0.0)	50.5(0.0)	64.8(0.0)	66.2(0.0)
	15	69.5(0.0)	52.4(0.0)	73.8(0.0)	71.4(0.0)
	20	70.5(0.0)	55.0(0.0)	77.1(0.0)	71.4(0.0)
	30	71.9(0.0)	54.3(0.0)	76.2(0.0)	75.7(0.0)
	40	72.8(0.0)	57.1(0.0)	73.3(0.0)	72.8(0.0)
	60	78.1(0.0)	59.5(0.0)	80.7(0.0)	77.1(0.0)
Moisture content (%)					
	0	72.4(0.1)	72.4(0.1)	73.1(0.4)	73.1(0.4)
	5	69.6(0.1)	70.4(0.1)	69.7(1.0)	71.1(0.0)
	10	68.6(0.5)	69.5(0.6)	68.6(1.2)	70.5(1.4)
	15	68.4(0.1)	69.8(0.3)	69.8(0.0)	70.1(0.0)
	20	68.7(0.0)	69.6(0.0)	69.8(0.1)	69.8(0.0)
	30	68.3(0.1)	69.2(0.0)	69.6(0.0)	69.8(0.1)
	40	68.4(0.0)	69.0(0.0)	69.6(0.0)	69.6(0.0)
	60	68.0(0.0)	67.7(0.3)	69.1(0.1)	68.9(0.0)
Total nitrogen (g-N/L)					
	0	6.4(0.1)	5.8(0.0)	10.3(0.1)	4.21(0.3)
	5	10.1(0.3)	7.5(0.1)	10.3(0.0)	7.8(1.2)
	10	15.7(0.5)	8.0(0.2)	11.5(0.2)	9.6(0.3)
	15	16.8(0.2)	8.6(0.5)	12.6(0.5)	10.2(0.2)
	20	17.1(0.3)	13.0(0.3)	11.6(0.4)	11.9(0.2)
	30	17.0(0.4)	14.6(0.1)	12.0(0.6)	11.6(1.1)
	40	17.7(0.1)	13.9(0.5)	11.0(0.1)	12.1(0.1)
	60	26.9(0.3)	22.2(0.3)	10.5(0.2)	11.7(0.6)
Ammonia Nitrogen (Mole/L)					
	0	0.02(0.00)	0.02(0.00)	0.02(0.00)	0.02(0.00)
	5	0.04(0.00)	0.03(0.00)	0.02(0.00)	0.02(0.00)
	10	0.06(0.00)	0.04(0.00)	0.03(0.00)	0.03(0.00)
	15	0.05(0.00)	0.03(0.00)	0.03(0.00)	0.03(0.00)
	20	0.04(0.00)	0.03(0.00)	0.02(0.00)	0.02(0.00)
	30	0.01(0.00)	0.01(0.00)	0.01(0.00)	0.01(0.00)
	40	0.02(0.00)	0.02(0.00)	0.01(0.00)	0.01(0.00)
	60	0.01(0.00)	0.01(0.00)	0.01(0.00)	0.01(0.00)

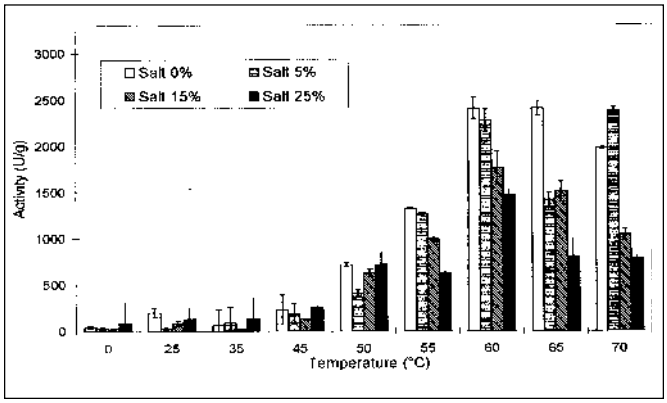

Figure 2—Temperature profiles of autolytic activity of Pacific whiting by-products at various salt concentrations

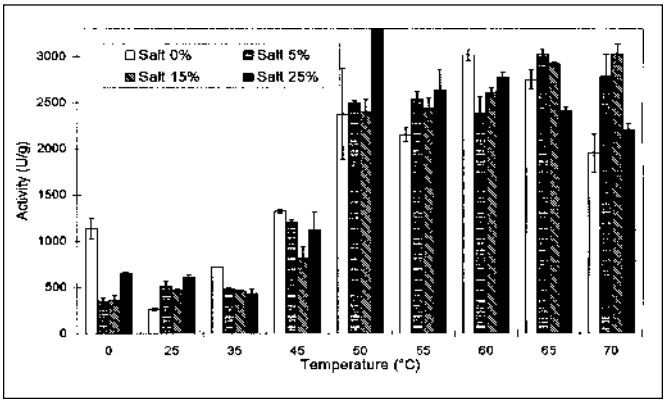

Figure 3—Temperature profiles of autolytic activity of Pacific whiting fillets at various salt concentrations

Napugan 1961). A total nitrogen content, equivalent to or higher than 16.3 g N/L, was obtained from whole fish at 15 d (50 °C) and 60 d (35 °C), supporting the strong degradation effects of Pacific whiting enzymes in the presence of salt as illustrated in Figure 1. However, the fish sauce from by-products reached this level after 112 d (not reported). The results suggest that fermentation for fish sauce from whole Pacific whiting can be completed in 60 d. Based on total nitrogen content (16.3 g N/L), 12 mo of fermentation is reportedly enough for nampla (Saisithi and others 1966) and 154 d for budu (Beddows and others 1979). Faster fermentation for fish sauce made from Pacific whiting whole fish and by-products was most likely due to combined effects of high enzymatic activity and grinding.

Ammonia nitrogen. The ammonia nitrogen reached a maximum level at Day 10 and then decreased to a minimum at Day 30 (Table 1). The ammonia level did not change for the next 30 d. After 112 d, no ammonia was detected (data not reported). The increased ammonia nitrogen content during the first 15 d could be due to fish enzymes that were active during early days of fermentation (Beddows and others 1980). However, the diminishing of ammonia in the sample might have been caused by several factors. First, the production system was an open system so that the ammonia could slowly dissipate into the air. This process is possible because the pH in all treatments was higher than the pK_b of ammonia (4.74) (Table 2). Another tentative explanation is the formation of Schiff base in the reaction of amine with aldehyde or ketone group (Wade, 1991). The further reaction from Schiff base between amine and aldehyde or ketone, which is well known as the Maillard reaction, is believed to play an important role in color and flavor development of fish sauce during the ripening step. Ammonia was suggested as one of the key components of volatile bases giving ammonical notes (Dougan and Howard 1975). However, there is no evidence that ammonia is the aroma-active component for ammonical notes in fish sauce. Only instrumental analyses and informal sensory descriptions were made in many studies (Dougan and Howard, 1975; Sanceda and others, 1992). Therefore, the role of ammonia on the sensory characteristics will be further studied and properly compared to the instrumental analysis (Lopetcharat and Park 2001).

Salt. Salt content in the fish sauce increased at Day 5. Thereafter, it remained constant at about 250 to 300 g/L during 60 d of fermentation (Table 2). It was slightly higher than in most nampla, 250 g/L (Wilaipan 1990), but slightly lower than the average values of 10 commercial fish sauces (Table 3).

Relative gravity. Relative specific gravity of Pacific whiting fish sauce reached the level (1.2 g/mL) of commercial fish sauce (Table 3) after 30 d of fermentation (Table 2). Fish sauce produced at 50 °C reached the commercial level faster than that made at 35 °C. The increased concentration of solute dissolved in the liquid phase was responsible for the development of specific gravity. Increased relative specific gravity was due to greater solubility of amino acids and small peptides at higher temperature.

Soluble solid. The average soluble solids content in commercial fish sauce was between 40 to 56 °Brix (Table 3). Soluble solids content of unripened Pacific whiting fish sauce had a soluble solids content of about 40 °Brix after 60 d of fermentation (Table 2). Measurements of soluble solids by a refractometer could be used to estimate the degree of protein hydrolysis during fermentation. The refractive index of the solution is dependent on the amount of free amino acid and small peptides released through protein degradation.

Table 2—Physicochemical characteristics of unripened fish sauce (II)

		Whole Fish		By-product	
Characteristics	Day	50°C	35°C	50°C	35°C
Salt content (g/L)					
	0	287(0)	290(10)	253(0)	253(0)
	5	319(5)	389(5)	470(9)	415(5)
	10	296(0)	296(0)	305(0)	305(0)
	15	366(0)	348(0)	331(0)	337(101)
	20	343(10)	331(0)	319(10)	319(10)
	30	308(10)	302(10)	296(0)	296(0)
	40	279(0)	314(0)	314(0)	308(20)
	60	290(5)	258(5)	290(5)	290(5)
Relative gravity (g/mL)	0	1.17(0.01)	1.17(0.01)	1.17(0.01)	1.17(0.01)
	5	1.18(0.01)	1.17(0.01)	1.17(0.01)	1.170.03)
	10	1.18(0.0)	1.16(0.00)	1.17(0.02)	1.18(0.02)
	15	1.19(0.01)	1.16(0.00)	1.20(0.02)	1.18(0.03)
	20	1.21(0.01)	1.19(0.01)	1.19(0.02)	1.20(0.02)
	30	1.22(0.01)	1.20(0.02)	1.21(0.02)	1.20(0.02)
	40	1.21(0.00)	1.19(0.02)	1.22(0.01)	1.22(0.02)
	60	1.21(0.01)	1.24(0.02)	1.22(0.01)	1.23(0.02)
Soluble solid 7 (° Brix)	0	32.8(0.2)	33.2(0.4)	31.2(0.4)	29.2(0.4)
	5	35.4(0.2)	35.6(0.2)	33.2(0.0)	32.6(0.7)
	10	32.2(0.2)	33.8(0.8)	31.2(0.4)	34.0(0.4)
	15	36.3(0.5)	34.0(0.2)	33.6(0.8)	34.4(0.4)
	20	36.5(0.1)	35.6(0.4)	34.6(0.6)	34.5(0.3)
	30	36.6(0.2)	36.0(0.4)	33.5(0.1)	34.4(0.2)
	40	37.7(0.1)	37.2(0.4)	36.8(0.4)	37.2(0.4)
	60	39.5(0.5)	39.2(0.6)	38.4(0.4)	39.0(0.6)
pH					
	0	6.58(0.00)	6.61(0.00)	6.71(0.00)	6.75(0.00)
	5	6.39(0.00)	6.43(0.00)	6.12(0.00)	6.19(0.00)
	10	6.45(0.00)	6.40(0.00)	6.11(0.00)	6.26(0.00)
	15	6.32(0.00)	6.29(0.00)	6.17(0.00)	6.11(0.00)
	20	6.37(0.00)	6.40(0.00)	6.21(0.00)	6.20(0.00)
	30	6.34(0.00)	6.32(0.00)	6.20(0.00)	6.17(0.00)
	40	6.27(0.00)	6.26(0.00)	6.10(0.00)	6.09(0.00)
	60	6.27(0.00)	6.24(0.00)	6.20(0.00)	6.13(0.00)

pH. The pH of the unripened fish sauce produced for this study was between 6.1 and 6.3 after 40 d of fermentation (Table 3). The pH of raw materials also dropped from 7.0 to 7.2 to 6.6 to 6.8 d after salt was added. This was probably due to the dissociation of amino acids and small peptides in the presence of salt. Ijong and Ohta (1996) reported the pH of bakasang ranged from 5.95 to 6.5. Mizutani and others (1992) evaluated the pH values from 15 commercial fish sauce samples and reported pH values between 5.3 and 6.7. However, our evaluation of commercial fish sauce indicated the average pH was 5.48 (Table 3). Most nampla is seasoned during ripening with food grade additives such as citric acid and sorbic acid to lower pH and adjust color (Mabesa and others 1972).

Color. Unripened fish sauce produced from Pacific whiting had different color properties compared to the commercial anchovy fish sauce based on a* and b* values (Table 3 and 4). Yellow hue (+ b*) was the dominant component in both commercial fish sauce and fish sauce made with Pacific whiting. Regarding a* values, the commercial fish sauce exhibited more red hue (20.17) (Table 3), while unripened Pacific whiting fish sauce possessed a slight green hue (−1.49 to −2.43) (Table 4). Transformation of hue from green to red is expected to occur during the ripening process. Color values of fish sauce are often determined subjectively. The most common objective method to determine the color of fish

Table 3—Physicochemical characteristics of commercial fish sauce

Characteristics	Mean (Standard deviation)
Relative gravity (mg/mL)	1.21 (0.05)
Moisture content (%)	64.51 (5.03)
pH	5.48 (0.32)
Salt content (g/L)	302.64 (23.96)
Brown color (A_{420})	2.79 (0.88)
Soluble solid (° Brix)	48.00 (7.64)
Total nitrogen (g-N/L)	16.26 (5.94)
L*	58.24 (7.49)
a*	20.17 (5.12)
b*	71.80 (6.52)

Mean values represent 10 commercial fish sauces made from anchovies

Table 4—Changes in color characteristics in unripened fish sauce

Color		Whole Fish		By-product	
Characteristics	Day	50°C	35°C	50°C	35°C
L*	0	93.5(0.0)	94.6(0.0)	93.8(0.0)	93.3(0.0)
	5	96.1(0.0)	96.0(0.0)	96.1(0.0)	96.4(0.0)
	10	96.0(0.0)	96.3(0.0)	96.2(0.0)	96.3(0.0)
	20	95.8(0.0)	96.2(0.0)	96.2(0.0)	96.2(0.0)
	40	95.4(0.0)	98.7(0.0)	96.0(0.0)	96.0(0.0)
	60	ND	ND	ND	ND
a*	0	-0.57(0.02)	-0.62(0.02)	-0.61(0.01)	-1.01(0.01)
	5	-0.94(0.01)	-0.83(0.01)	-0.81(0.01)	-0.74(0.01)
	10	-1.39(0.01)	-0.94(0.00)	-1.08(0.00)	-1.00(0.00)
	20	-1.70(0.01)	-1.33(0.01)	-1.07(0.01)	-1.28(0.01)
	40	-2.43(0.01)	-1.79(0.01)	-1.51(0.01)	-1.49(0.01)
	60	ND	ND	ND	ND
b*	0	7.04(0.01)	7.08(0.04)	9.21(0.02)	7.62(0.04)
	5	3.58(0.06)	2.96(0.01)	2.96(0.01)	2.07(0.00)
	10	5.10(0.03)	2.97(0.01)	3.71(0.02)	3.12(0.03)
	20	6.42(0.00)	4.37(0.01)	3.86(0.03)	4.20(0.01)
	40	9.83(0.01)	6.42(0.03)	5.77(0.01)	5.28(0.01)
	60	ND	ND	ND	ND
Brown Color (A_{420})	0	1.52(0.01)	1.70(0.00)	3.96(0.02)	2.28(0.00)
	5	0.35(0.0)	0.52(0.00)	0.37(0.02)	0.25(0.00)
	10	0.28(0.0)	0.23(0.01)	0.23(0.02)	0.25(0.01)
	20	0.39(0.0)	0.32(0.00)	0.26(0.01)	0.27(0.00)
	40	0.62(0.01)	0.57(0.01)	0.37(0.01)	0.38(0.00)
	60	0.81(0.01)	0.54(0.00)	0.51(0.01)	0.46(0.00)

ND: not determined

sauce is to measure absorbance at 420 nm. The brown color decreased rapidly from Day 0 to Day 5, and the minimum value was observed at Day 10. Thereafter, the absorbance increased gradually (Table 4). Low absorbance for the first 5 to 10 d was probably due to dispersed protein particles in the liquid phase. Brown color for whole fish at 50 °C showed the highest value (0.81) at 60 d. After 112 d of fermentation, brown color increased in absorbance to between 0.58 and 1.33 (data not reported). The brown color in fish sauce was caused by nonenzymatic browning reactions (Wilaipan 1990). Absorbance at 420 nm of commercial fish sauce, which was ripened with additional processes for color and flavor development, varied from 1.1 to 4.2 with an average value of 2.8 (Table 3). Due to the higher protein content in whole fish, unripened fish sauce produced from whole fish was darker and brown color development was faster than in fish sauce produced from by-products, especially at 50 °C. Most of the nitrogenous compounds in fish sauce are free amino acids and small peptides, which contribute to brown color development. Even though reducing sugar content in fish is low, carbohydrate derivatives, such as glucose-6-phosphate and other substances present in the metabolic pathways, can also act as reactants to initiate the Maillard reaction (Kawashima and Yamanaka 1996).

Table 5—Changes of aerobic and anaerobic plate counts during fish sauce fermentation

		Whole Fish		By-products	
Characteristics	Day	50 °C	35 °C	50 °C	35 °C
Aerobic plate counts (cfu/g)	0	280	280	280	380
	10	1000	0	1950	5700
	15	380	320	1060	0
	20 to 60	0	0	0	0
Anaerobic plate counts (cfu/g)	0	250	112	250	250
	10	7000	0	7000	730
	15	800	200	800	0
	20 to 60	0	0	0	0

Table 6—Major microorganisms identified from Pacific whiting during fermentation

Fermentation days	Major Microorganisms
0	*Staphylococcus saprophyticus* *Staphylococcus simulans* *Staphylococcus xylosus*
40	*Micrococcus kritinae* *Staphylococcus xylosus* *Staphylococcus equorum* *Bacillus*

Microbiological Characteristics

The patterns of microbial growth were similar for both aerobic and anaerobic plate counts (Table 5). The initial microorganism load was low (about 10^2 CFU/g), which was comparable to previous reports on anchovy fish sauce (Saisithi and others 1966; Wilaipan 1990; Ijong and Ohta 1996). The lower number of microorganisms was probably due to the use of frozen fish samples (resulting in freezing injury) and the salt effect. A significant increase in the number of halophilic microorganisms was observed at 10 d during Pacific whiting fish sauce fermentation, except for whole fish incubated at 35 °C, and then decreased rapidly to an undetectable level at 20 d. However, the increase in the number of microorganisms at Day 10 for 35 °C seemed to be an experimental error. The decreased microorganisms during fermentation are caused by high concentrations of salt and reduced pH (Jay 1996). Between 20 to 60 d of fermentation, no microorganism count was observed (Table 5), except the presence of 1 to 2 colonies at 40 d. These colonies were used for further identification.

Using colonies obtained at 0 and 40 d, more than 100 different strains were isolated. Three significant microorganism genera were identified in all samples from Pacific whiting: *Staphylococcus*, Bacillus, and *Micrococcus* (Table 6). *Bacillus*-type bacteria and *Staphylococcus* strain 109 were isolated from nampla and produced a measurable amount of volatile acids (Saisithi and others, 1966). Furthermore, *Micrococcus*, *Coryneform*, and *Streptococcus* are commonly found in anchovy fish sauce (Saisithi and others 1966; Sands and Crisan 1974; Ijong and Ohta 1996).

In addition to flavor development, *Staphylococcus*, *Bacillus*, and *Micrococcus* also produced proteolytic enzymes that are active in the presence of high salt concentration

(Norberg and Hofsten 1968; Wilaipan 1990 and Thongthai

and others 1992). However, changes of species of *Bacillus* in nampla during fermentation were also observed (Sands and Crisan 1974). The results from this study and others indicate that microorganisms should play an important role in the later stage of fermentation (> 60 d) and the ripening stage. Protein degradation by these microorganisms leads to the production of volatile compounds from amino acids and small peptides as raw materials.

Studies on the role of microorganisms need to be further investigated because there is no conclusive evidence for the role of microorganisms. Several researchers, including Beddow and others (1979) and this study, attempted to use sterilized conditions to investigate the effect of nonmicrobiological degradation. However, these studies did not address alterations such as the effect of antibacterial substances on enzymatic activity and the interactions of those chemicals with amine. When radiation was used in our study, it activated the enzyme activity in fish (data not reported).

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