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Fatty Acids and Sensory Acceptance of a Dietary Sodium–Potassium Fish Sauce

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Two varieties of fish, (1) flounder, a lean fish with low fat (1.6%), and (2) trout, a fatty fish with 9.2% fat, were used in the preparation of fish sauce. The preparation of sauce consisted of fermenting a mixture of uneviscerated ground fish and sodium and potassium salts in different fish-to-salt ratios. Desired ratios of these salts in the sauce were obtained by mixing appropriate volumes of individual salt-based sauces. Identification of fatty acids was accomplished by gas chromatography. Results showed that all samples contained volatile fatty acids from C-2 to C-6 and nonvolatile fatty acids from C-8 to C-18 in various proportions. In all the samples, isovaleric acid was in the highest concentration followed by acetic and isobutyric acids. Sensory analyses indicated that a sodium–potassium dietary fish sauce could prove to be a possible replacement for an all sodium chloride sauce provided the salt ratios do not exceed 40:60.

Fermented fish products are manufactured in vast quantities for human consumption in Southeast Asia. These foods provide an excellent means of fish preservation in areas where few canning or freezing facilities are available. The protein diet of the people in these areas is closely linked with cured and fermented fish products. Fermented fish products are commonly used to brighten monotonous rice dishes, and somewhat more than 50 different kinds of fermented fish products are utilized in Southeast Asia (Amano, 1962).

Various sauces or pastes are prepared from a wide variety of both fresh and saltwater fishes, mainly clupeids, carangids, and cyprinids. Each country has its own national fermented fish products. For example, the Japanese have "Katsuo-Shiokara" made from the visceral organs of tuna; the Cambodians have "Nuoc-mam Gauca" made from fish liver; the Indonesians have "Trassi" made from planktonic shrimp which is similar to the Filipino "Bagoong" and the "Kapi" of Thailand. However, one of the most popular fermented products which is famous among the people of the Far East and distributed throughout the entire area is Nuoc-mam or Nam-pla, a fish sauce made in Vietnam, Cambodia, the Philippines, and Thailand.

Sodium chloride is a major ingredient in making fish sauce, its concentration ranging between 25 and 32% in the finished product. In fact, NaCl is essential for human and animal growth, but the level needed by human subjects has not been established with any degree of certainty. There is adequate evidence that the daily ingestion of 250–375 mg of NaCl (100–150 mg of Na) by adults can be maintained without any apparent signs of abnormalities (Dahl, 1972; Institute of Food Technologists, 1980). However, from this low requirement of the human body for NaCl, the average daily intake has been estimated as ranging from 10 g for Americans to 27 g for inhabitants of certain areas of Japan and most of Southeast Asia. Beneficial effects have been observed following the limitation of sodium salt intake by patients with a variety of diseases. Some investigators concluded that there exists a causative relationship between a high salt intake and hypertension (Dahl, 1960; Meneely et al., 1957; Meneely and Ball, 1958).

Therapeutical salt restriction was employed in 1901 in patients with edematous heart disease (Achard and Loeper, 1901; Bennett, 1979). The treatment was subsequently extended to congestive heart failure, hypertension, renal diseases, cirrhosis of the liver, toxemia of pregnancy, and Meniere's disease (Kempner, 1948; Jones, 1979). It has been reported that one-fourth of hypertensive patients respond by a decrease in blood pressure after salt restriction.

Man's requirement for salt is so low that most patients find it a hardship to be subjected to a salt-restricted diet. For this reason, efforts have been made to develop a seasoning agent to replace sodium salt. It has been suggested that high naturally occurring dietary KCl protects against human addition of excessive NaCl (Meneely et al., 1957; Michelsen et al., 1977). The use of NaCl and KCl mixtures in diets of populations susceptible to hypertension would be the most practical way to decrease the incidence of this disease.

Essentially no research has been reported on a dietary sodium–potassium fish sauce which can be used by people who are on a sodium-restricted diet. Therefore, the present investigation was undertaken with the following objectives: (1) to develop a dietary sodium–potassium fish sauce; (2) to make a sensory evaluation of the sauce in terms of color flavor, and overall quality; (3) to isolate and identify certain key flavor components such as volatile and nonvolatile

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Table I. Formulation of Fish Sauce[a]

sample	KCl	KCl:fish	NaCl	NaCl:fish	H_2O
		Flounder			
1	600	1:2.3			0.5
2	800	1:2.5			0.5
3			600	1:2.3	0.5
4			800	1:2.5	0.5
		Trout			
1	600	1:3.3			0.5
2	800	1:2.5			0.5
3			600	1:3.3	0.5
4			800	1:2.5	0.5

[a] Sample weight: 2 kg. Storage temperature: 37 °C.

fatty acids which impart a characteristic flavor to the sauce.

MATERIALS AND METHODS

A review of literature indicated that commercially produced fish sauces in Southeast Asia are fermented at least 9 months for the sauce to develop the desirable flavor. Therefore, the present investigation is limited to the isolation, identification, and measurement of fatty acids in the test samples that were fermented for 6- and 9-month periods.

Sample Collection and Preparation of Fish Sauce. Two varieties of fish, flounder, a lean fish with low fat content (1.6%), and trout, a fatty fish with 9.2% fat, were selected for the preparation of fish sauce. These fresh fish were purchased from a trawler operator in Baton Rouge, LA. Immediately after being caught, the fish were selected for uniform size and weight (each flounder measured 8–10 in. long and 0.5–0.8 in. thick and weighed about 1.0 lb; each trout measured 10–12 in. long and 1–1.5 in. thick and weighed about 1.0 lb). They were packed in crushed ice and transferred to the L.S.U. Food Science laboratory where the uneviscerated fish were washed with clean tap water and stored in polyethylene bags in the cold room (4 °C) until further use. Elapsed time from catching until receipt at the laboratory was generally less than 15 h.

The method of preparation of fish sauce consisted of mixing uneviscerated ground fish with either NaCl or KCl in different fish-to-salt ratios (Table I). The fish were ground in a heavy duty grinder (Oster electric grinder). There was a total of eight ground fish samples (four samples of each species), each of which weighed 2 kg. The fish–salt mixtures were transferred to eight 1-gal glass jars fitted with screw caps. The fermentation was allowed to continue for 9 months at 37 °C, which is the recommended optimum temperature for commercial fish sauce production. Periodically, at 3-month intervals, clear sauce samples were drawn from the glass jar for chemical analyses. The desired ratios of sodium and potassium salts in the fermented fish sauce were obtained by mixing appropriate volumetric amounts of the sodium and potassium based sauces.

Extraction of Volatile and Nonvolatile Fatty Acids. The extraction of volatile fatty acids was achieved by using the method of Kabot and Ettre (1963). Two hundred milliliters of each sample was extracted with 500 mL of dichloromethane for 5 h at room temperature. For thorough mixing and complete extraction, the solution was continuously stirred by a magnetic stirrer. The dichloromethane solution was shaken with an equal volume of 0.1 N NaOH solution in order to transfer acidic components from the organic layer to the aqueous layer. To the separated aqueous layer was added 85% H_3PO_4 and the pH was adjusted to 8.0 after which the solution was evaporated to dryness at room temperature under reduced pressure. The white powder obtained, a mixture of sodium salts of acidic components, was acidified with a small volume of 2 N H_2SO_4, and then 10 mL of ether was added to extract fatty acids. The ether extract was dried with a small amount of anhydrous Na_2SO_4 powder. The resulting ether solution was filtered through Whatman No. 541 filter paper. The resulting filtrate was directly submitted to gas chromatography.

Nonvolatile fatty acids can be extracted either as free acids or as their methyl esters. However, extraction of nonvolatile free fatty acids is time consuming and laborious. Therefore, numerous investigators have extracted them in their methyl ester form which is the method used in the present investigation.

The methylation of fatty acids was achieved by using the method of Metcalfe and Schmitz (1961). For preparation of methyl derivatives of nonvolatile fatty acids, 5 mL of BF_3–methanol (12% w/v) was added to 0.5 g of a mixture of sodium salt of fatty acids (the white powder obtained during the extraction of volatile fatty acids) in a 15-mL conical centrifuge tube. The tube was stoppered tightly and placed in a 60 °C water bath for 30 min; 0.5 mL of chloroform was added and the tube inverted 30 times to extract the methyl esters. Brief centrifugation clarified the chloroform layer which was then removed with a Pasteur pipet to a 12 × 75 mm screw-cap culture tube. Contamination of the chloroform extract with water was avoided by first removing the aqueous layer with a pipet attached to an aspirator pump. The extract was dried with anhydrous $MgSO_4$. Both extracts were stored for 24–48 h at −20 °C before analysis by gas chromatography.

Isolation, Identification, and Quantitative Measurement of Volatile and Nonvolatile Fatty Acids. *Volatile Fatty Acids.* A Perkin-Elmer Model 990 gas chromatographic unit, employing a flame ionization detector (FID), was used. An 8 ft long coil-shaped stainless steel column of $^1/_8$-in. inside diameter was packed with 10% FFAP (a modified Carbowax 20 M treated with 2-nitroterephtalic acid) on 80–100-mesh Chromosorb W, AW. The column temperature was programmed from 60 to 150 °C at 12 °C/min. The detector temperature was 175 °C. Nitrogen was used as a carrier gas at a flow rate of 40 mL/min. The sample was injected directly into the gas chromatograph, and the fatty acids were analyzed by comparing the retention times of the peaks with those of standard samples.

Nonvolatile Fatty Acids. The procedure used to isolate, identify, and quantify the nonvolatile fatty acids by gas chromatography was identical with the one used for volatile fatty acids except for the column packing materials and detector and column temperatures. The column was packed with 20% DEGS on 60–80-mesh Chromosorb W, AW. The column temperature was 205 °C (isothermal) with a detector temperature of 250 °C.

Quantitative analysis was achieved by the Internal Normalization Method (Kabot and Ettre, 1963). In this method, the concentration of each component in the sample was calculated by weight percent composition in terms of relative percent peak area. The molar concentration of the components were determined by the method of Relative Molar Concentration (Preston and Spreckelmeyer, 1971).

Sensory Evaluation. Sensory evaluation based on flavor, color, and overall quality such as appearance and mouth feel was performed on sodium and potassium salt based fish sauce. The test samples consisted of seven mixtures of Na salt sauce and K salt sauce in different percent ratios (KCl:NaCl: 0:100, 20:80, 40:60, 50:50, 60:40,

Table II. Analysis of Variance on Acceptability of Flavor, Color, and Overall Quality of Fish Sauce Made by Replacing Various Levels of NaCl with KCl

source	df	flavor m.s.[c]	color m.s.	overall quality m.s.
judge (J)	9	0.874[b]	2.200[b]	3.557[b]
treatment (T)	6	80.157[b]	9.080[b]	73.675[b]
salt content (S)	1	6.914[b]	0.014	1.728[a]
fish variety (F)	1	0.357	0.128	2.057[b]
$T \times S$	6	0.097	0.080	0.286
$T \times F$	6	0.257	0.561[a]	0.715[a]
$S \times F$	1	0.514	0.228	0.057
$T \times S \times F$	6	0.097	0.228	0.332
residual	243	0.259	0.235	0.301
corrected total	279	2.015	0.491	2.00

[a] Significant at the 5% level. [b] Significant at the 1% level. [c] Mean square.

Table III. Treatment Means from Analysis of Variance on the Acceptability of Flavor, Color, and Overall Quality of Fish Sauce (Trout and Flounder) with Various KCl:NaCl Ratios[a]

KCl:NaCl	flavor	color	overall quality
0:100	4.87	4.85	4.80
20:80	4.45	4.87	4.62
40:60	4.02	4.67	4.27
50:50	3.60	4.75	3.90
60:40	3.17	4.32	3.30
80:20	1.57	3.97	2.00
100:0	1.15	3.65	1.25
overall means	3.26	4.44	3.45

[a] Mean scores: 1 = least acceptable; 5 = most acceptable. Each mean is the average of 40 observations.

80:20, and 100:0). The percent ratios of these mixtures were obtained by combining appropriate volumetric ration (KCl, 300 and 400 g/kg of fermented fish; NaCl, 300 and 400 g/kg of fermented fish) with similar sauces and similar salts. The sauces were fermented for 9 months (fermentation period commercially used) including 1 month of ripening. The taste panel consisted of ten individuals, two females and eight males, who were students from Southeast Asia and whose daily diets included fish sauce. The panelists, who were 20–35 years old, were presented with seven samples at a time for evaluation based on hedonic scale of 1–5.

During the test period, the panel members were not allowed to talk to one another and the fish sauce samples were served as a dip for fresh boiled shrimp.

The sensory data was submitted to an analysis of variance which examined the effects of salt levels, KCl:NaCl percent ratios, and fish varieties (flounder and trout) on the flavor, color, and overall quality. All tests of significance were made at the 0.01 and 0.05% levels of probability (Snedecor, 1959).

RESULTS AND DISCUSSION

Sensory Evaluation. Statistical analyses of the taste panel data indicated that salt content (KCl:NaCl ratios) influenced flavor ($p < 0.01$) and overall quality ($p < 0.05$) as shown in Table II. Results in Table III show that the trend toward acceptability of flavor and overall quality terminates at the 60:40 ratio of KCl and NaCl. A rejection of these attributes at higher KCl ratios could be due to the known unpleasant bitter taste of potassium salt. However, in cases of fish sauce with various KCl:NaCl ratios (Table III), the samples which contained KCl from 0 to 60% were shown to be well accepted in flavor, color, and overall quality. The least desired samples were those containing 80 and 100% potassium salt. The high salt concentration and fish variety did not significantly ($p > 0.05$) affect the acceptance because of sauce color but significantly reduced the flavor ($p < 0.01$) and overall quality ($p < 0.05$). The sauce prepared by fermenting fish at low salt concentration was more favorable. It is thought that using as low a salt content as 300 g/kg of fermented fish in making fish sauce is more desirable than the 400 g/kg of fermented fish (Table IV). The interaction of treatment on fish was found to significantly ($p < 0.05$) reduce the color acceptance and overall quality (Table II and III). This probably was due to the high content of fat in fatty fish which inhibits the sugar–amino browning reaction during fermentation (Gottschalk and Partridge, 1950).

Table IV. Taste Panel Treatment Combination Means for Flavor, Color, and Overall Quality of Fish Sauce of KCl:NaCl Mixtures at Two Levels of Salts Concentrations[a]

	low salt (300 g/kg of fermented fish)		high salt (400 g/kg of fermented fish)	
	trout	flounder	trout	flounder
flavor	3.500[b]	3.342[c]	3.100[b]	3.114[c]
color	4.400	4.500	4.442	4.428
overall quality	3.600[d]	3.457[e]	3.471[d]	3.271[e]

[a] Each mean is the average of 70 observations. [b,c] Results with similar letters were significantly different ($p < 0.01$). [d,e] Results with similar letters were significantly different ($p < 0.05$).

Table V. Volatile Fatty Acid Composition

volatile fatty acids	mol/L			
	flounder, low fat (1.6%), stored		trout, high fat (9.2%), stored	
	6 months	9 months	6 months	9 months
acetic acid	0.0619	0.0447	0.0344	0.0412
propionic acid	0.0302	0.0189	0.0206	0.0103
isobutyric acid	0.0464	0.0086	0.1186	0.0705
butyric	0.0103	0.0068	trace	trace
isovaleric acid	0.5022	0.3818	1.2900	1.1782
caproic acid	0.0022	trace	trace	trace
total	0.6533	0.4608	1.4636	1.3002

Table VI. Nonvolatile Fatty Acid Composition

nonvolatile fatty acids	μmol/L			
	flounder, low fat (1.6%), stored		trout, high fat (9.2%), stored	
	6 months	9 months	6 months	9 months
caprylic acid	0.00028	trace	0.00096	0.00115
pelargonic acid	0.00086	0.00048	0.00086	0.00028
capric acid	0.00869	0.00724	0.02125	0.01014
undecyclic acid	0.00053	0.00077	trace	trace
lauric acid	0.00072	0.00135	0.00086	0.00579
myristic acid	0.00294	0.00231	0.00067	0.00178
palmitic acid	trace	trace	0.00183	0.00019
stearic acid	0.00019	trace	0.00903	0.00028
oleic acid	trace	trace	0.00096	trace
linoleic acid			0.00086	trace
arachidic acid			trace	trace
total	0.01421	0.01215	0.03728	0.01961

It appears that the utilization of KCl to replace NaCl in making fish sauce is quite acceptable if the salt ratio of KCl:NaCl is not above 60:40. The concentration of 300 g/kg of fish fermented is more favorable. Both fatty and lean fish yielded good-quality fish sauce.

Volatile and Nonvolatile Fatty Acids. The fatty acid composition of fish sauces from trout and flounder fermented for 6- and 9-month periods are presented in Tables V and VI. The test samples consisted of mixtures of two sauces prepared at two salt levels (low, 300 g of KCl or NaCl/g of fish fermented; high, 400 g of KCl or NaCl/kg of fish fermented) in the ratio of 50:50.

Chromatograms of the samples showed that all the samples contained volatile fatty acids from C-2 to C-6 in various proportions. The flounder sample fermented for 6 months had the highest percent isovaleric acid (77.4%); the lowest was caproic acid (0.28%). The total volatile fatty acids of this sample was 0.6535 mol/L. However, a significant decrease (0.4608 mol/L) was observed in the same sample at the end of 9 months of fermentation. The highest reduction was mostly in propionic, isobutyric, butyric, and caproic acids.

The number and kind of volatile fatty acids found in trout samples fermented for 6 and 9 months were similar to those observed in flounder samples, although in different proportions (Tables V and VI). The total volatile fatty acids for trout samples at the end of 6 months of fermentation was 1.4636 mol/L corresponding to 0.6535 mol/L found in flounder. However, it was interesting to note that the total volatile fatty acids in trout samples fermented for 9 months was almost 3 times greater than that in the corresponding flounder samples (trout, 1.3002 mol/L; flounder, 0.4608 mol/L).

In all the samples, isovaleric acid was the highest in percent composition followed by acetic and isobutyric acids. Acetic, propionic, and butyric acids were the same as those found in Vietnamese fish sauce (Nguyen An Cu and Vialard Goudou, 1953; Truong Van Chom, 1963). Isobutyric, isovaleric, and caproic acids, were not reported to be presented in the Vietnamese sauce. The flavor of fish sauce is partly due to the cumulative effect of all the volatile fatty acids. Therefore, high or low concentrations of any individual volatile fatty acid cannot be assumed to contribute to poor quality in the whole fish.

Chromatographic analyses of the samples showed that all four samples (trout and flounder sauces fermented for 6 and 9 months) contained nonvolatile fatty acids from C-8 to C-18 in various proportions (Table VI). However, trout samples for both 6- and 9-month periods of fermentation exhibited small amounts of linoleic and arachidic acids. The 6-month flounder sauce samples had the highest percent capric acid (61.45%); the lowest was oleic acid (trace amount). The total nonvolatile fatty acids of this sample was 0.01421 μmol/L. However, a slight decrease (0.01215 μmol/L) in the total nonvolatile fatty acids was observed in the same sample of 9-month fermentation. Reduction of individual nonvolatile fatty acids occurred in caprylic, capric, myristic, and stearic acid concentrations. It was interesting to note that these reductions were accompanied by a slight increase in undecylic and lauric acids.

The number and kind of nonvolatile fatty acids for trout samples at the end of 6 months of fermentation was 0.03728 μmol/L, corresponding to 0.01421 μmol/L found in flounder. No appreciable change in the total nonvolatile fatty acid content was observed between trout and flounder samples when both were fermented for 9 months.

The data obtained in this investigation suggest that it is extremely difficult to indicate to what extent the individual fatty acids contribute to the characteristic flavor of fish sauce. The flavor of fish sauce can be due to the overall effects of both volatile and nonvolatile fatty acids along with other biochemical reactions that generally occur in fermentation. Compounds resulting from bacterial breakdown of protein or other nitrogenous compounds occurring in fish may play an important role in the flavor of fish sauce. Another possibility would be that this flavor is due to some breakdown oxidation products of long-chain polyunsaturated fatty acids that are common in fish lipids. This view receives support from the fact that poultry or other farm animals fed large quantities of fish oil sometimes develop a distinct characteristic fishy odor in the meat. This type of odor is not a rancid one but, rather, is described as being characteristically fishy. Flavors associated with fish sauce, on the other hand, are generally believed to result from volatile fatty acids (acetic, propionic, isobutyric, butyric, isovaleric, and hexanoic acid) which are produced by *Pediococus halophilus* and found during the fish sauce fermentation (Velankar, 1952).

Registry No. NaCl, 7647-14-5; KCl, 7447-40-7; acetic acid, 64-19-7; propionic acid, 79-09-4; isobutyric acid, 79-31-2; butyric acid, 107-92-6; isovaleric acid, 503-74-2; caproic acid, 142-62-1; caprylic acid, 124-07-2; pelargonic acid, 112-05-0; capric acid, 334-48-5; undecylic acid, 112-37-8; lauric acid, 143-07-7; myristic acid, 544-63-8; palmitic acid, 57-10-3; stearic acid, 57-11-4; oleic acid, 112-80-1; linoleic acid, 60-33-3; arachidic acid, 506-30-9.

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