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# Flavor of Fermented Fish Sauce

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Forty-three previously unidentified compounds have been found in Nam-pla fermented fish sauce. This included 8 acids, 10 alcohols, 6 amines, 7 other nitrogen-containing compounds, 4 lactones, 3 carbonyls, and 5 sulfur-containing compounds. The fish sauce was solvent extracted in order to obtain a flavor isolate. This isolate was fractionated into acidic, neutral, and basic fractions via pH adjustment and liquid–liquid extraction in order to facilitate gas chromatography and mass spectrometry. All gas chromatography was done by using fused silica Carbowax 20M capillary columns.

Fish sauces constitute an important part of the diet of more than 250 million people in Southeast Asia (Van Veen, 1965). Fish sauce provides a substantial part of the protein requirements of these people.

Fermented fish sauces are made by mixing fish with salt and allowing natural fermentation and leaching to occur. For Nam-pla, a Thai sauce, small whole fish (*Stolephorus* or *Sardinella* species) are mixed with marine salt in the ratio of 3 parts of fish to 1 or 2 parts of salt. Normally 24–48 h elapse between catching the fish and the salting step during which the fish are not refrigerated. This actually initiates the fermentation process. The salt–fish mixture is transferred to large concrete tanks and left to ferment. The tanks are usually built into the ground, which maintains the temperature in the range of 35–40 °C. After 6–12 months, the brown liquid is decanted and filtered. The filtrate may be sun-ripened in earthenware for up to 3 months or bottled directly for consumption. The residue is covered with fresh brine and held for about 3 months to produce a lower quality sauce that may be blended with the first-run filtrate. Alternatively, the residue from the first fermentation may be ground and sold as paste (Saisithi et al., 1966).

During fermentation, proteolysis of fish proteins results in increasing the soluble protein. For Budu, a sauce from Malaysia, approximately 56% of the total fish protein is converted to soluble protein (Beddows et al., 1976). The yield of liquid is about 75 mL/100 g of fish. Analysis of various sauces finds total organic nitrogen ranging from 1.7% to 2.3% of which 50–60% is in the form of free amino acids (Uyenco et al., 1952; Truong-van-Chom, 1958; Saisithi et al., 1966; Beddows et al., 1979). Volatile nitrogen (mostly ammonia) comprises 7–12% of the total organic nitrogen. The pH of fish sauce is approximately 5.6 and the salt content is about 270 g/L.

Earlier investigators have found the aroma of fish sauce to be composed of a blend of ammonical, meaty, and cheesy notes (Dougan and Howard, 1975; Beddows et al., 1976). The ammonical note has been attributed to ammonia, a variety of amines, and other basic nitrogenous compounds (Saisithi et al., 1966; Dougan and Howard, 1975). Low molecular weight volatile fatty acids (VFA), in particular formic, acetic, propionic, *n*-butyric, isobutyric, *n*-valeric, and isovaleric acids, have been identified as contributing to the cheesy notes (Truong-van-Chom, 1958; Saisithi et al., 1966; Dougan and Howard, 1975; Beddows et al., 1976). The compounds responsible for the meaty aroma have not been identified.

A variety of rapid methods have been investigated in an attempt to shorten the time required to produce fish sauce. Processes involving of higher fermentation temperatures (Amano, 1962), addition of proteolytic enzymes, or use of acids (Amano, 1962; Beddows et al., 1976; Beddows and Ardeshir, 1979a), at ambient temperatures (Beddows and

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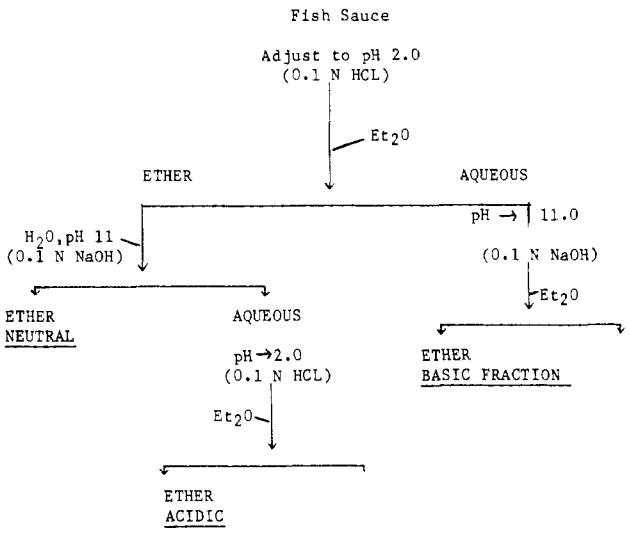


**Figure 1.** Extraction procedure used for the isolation and fractionation of volatile flavors from Nam-pla fermented fish sauce.

Ardeshir, 1979b), have been tried. The hydrolysates from the enzyme and acid treatments are similar to traditional fish sauce in soluble nitrogenous compounds and may be added to naturally produced sauce to increase production volume.

Microbiological studies of fish sauces have found *Bacillus* species to predominate (Saisithi et al., 1966; Crisan and Sands, 1975). Beddows et al. (1980) isolated a *Clostridium* species as the sole organism in Budu. The isolated species were classified as halophilic or halotolerant by the different investigators. Saisithi et al. (1966) observed that total visible counts decreased steadily from $10^3$–$10^5$/mL at 0.5 month to $10^2$/mL at 12 months during the manufacturer of Nam-pla. This suggests that the role of microorganisms in developing flavor after salting may be limited. The current study was undertaken to identify additional voltaile flavor compounds in the commercial Southeast Asian fish sauce by utilizing gas chromatography–mass spectrometry.

## MATERIALS AND METHODS

Nam-pla fish sauce was obtained locally in 750-mL plastic bottles. The fish sauce was extracted as shown in Figure 1. This extraction procedure resulted in a fractionation of the flavor components into neutral, acidic, and basic extracts. The extracts were dried by adding anhydrous $MgSO_4$ to remove water, and then each was evaporated to about 0.1 mL under $N_2$ in order to concentrate the sample for gas chromatography.

Gas chromatography was accomplished by using a Hewlett-Packard Model 5880 GC. Separation of the flavor compounds was done on a 30 m by 0.232 mm fused silica Carbowax 20M column (J & W Scientific, Rancho Cordova, CA). The column was temperature programmed from 60 to 185 °C at 5 °C/min and then held at 185 °C for 35 min. A flame ionization detector (FID) was used; the basic fraction was also chromatographed with a nitrogen–phosphorus detector (NPD).

Mass spectrometry was done by using an LKB 9000 combined GC–mass spectrometer. A glass column (3 m × 2 mm i.d.) packed with Carbowax 20M (10% on 100–120-mesh Gas-Chrom A) was used for separation of the flavor compounds. Mass spectra were compared to published spectra for identification (Cornu and Massot, 1966). The NIH-EPA Chemical Information System (National Data Corp., Interactive Sciences Division, Baintree, MA) was also used for compound identification.

Authentic samples of compounds identified by mass spectrometry were used to check retention times on the fused silica capillary column with both FID and NPD.

Table I. Major Components of the Acidic Fraction of Nam-pla Fish Sauce

| compound | % of fraction[a] |
|---|---|
| acetic acid | 29 |
| propionic acid | 14 |
| isobutyric acid | 3 |
| 4-hydroxyvaleric acid lactone | 12 |
| *n*-butyric acid | 17 |
| isovaleric acid | 6 |
| levulinic acid | 10 |
| phenylacetic acid | 3 |
| 3-phenylpropionic acid | |

[a] Percent of fraction values are calculated on an area percent basis from the GC.

## RESULTS AND DISCUSSION

The major compounds identified in the acidic fraction are listed in Table I. Collectively, these nine compounds accounted for 96% of the acidic fraction as determined by area percent measurements. The minor components, each present at less than 1% of the fraction, are included in the total list presented in Table II. The relative and absolute amounts of the short-chain volatile fatty acids (VFA) have been found to be variable, depending not only on the type of sauce (Nam-pla, Budu, Patis, etc.) but also on the quality of the product. The ratio of acetic:*n*-butyric acids was found to be approximately 1:1 in Vietnamese sauces, whereas for best quality Nam-pla this ratio may be as high as 20:1 (Dougan and Howard, 1975). The acetic acid content of Nam-pla was at levels of 3–14 g/L (Dougan and Howard, 1975) and up to 21 g/L in Budu (Beddows et al., 1979). In sauces from Hong Kong and China the total volatile acidity was as low as one-third that of Nam-pla (Dougan and Howard, 1975). These products with less VFA were described as less cheesy and more ammonical than those with greater amounts of VFA.

A mixture of the VFA listed in Table I in the same relative amounts resulted in a sharp, cheesy aroma similar to that of the acidic isolate but with a more pronounced acetic acid component. The other compounds identified had only a minor impact on the overall aroma of the fraction.

Dougan and Howard (1975) suggested the VFA were formed by atmospheric oxidation of fish lipids. However, as noted by Beddows et al. (1980), the quantity of lipid present in the fish was insufficient to account for the amount of VFA produced. It was found that when fresh fish and salt were mixed and fermented (no "spoilage period" prior to salting), very little VFA was formed (Beddows et al., 1979). Inclusion of broad spectrum antibiotics also prevented the formation of VFA, suggesting microbial involvement in the production of VFA (Beddows et al., 1979). Propionic and *n*-butyric acid levels increased prior to salting and then remained constant throughout the fermentation, while the acetic acid concentration increased as fermentation proceeded. Saisithi et al. (1966) and Beddows et al. (1980) isolated bacterial species that were able to produce VFA when inoculated on hydrolyzed rockfish (*Sebastodes* species or *Stolephorus* species). Using U-$^{14}$C-labeled protein hydrolysates, Beddows et al. (1980) observed that propionic, *n*-butanoic, and *n*-pentanoic acids appeared to be derived from amino acids via bacterial action. Other acids identified in fish sauce may also have amino acids as precursors, e.g., phenylacetic acid

Table II. Compounds Identified in Fermented Fish Sauces

| compounds | fraction[f] | ref |
|---|---|---|
| acids | | |
| formic | A | *a, b* |
| acetic | A | *a, b, c* |
| propionic | A | *a, b, c* |
| *n*-butyric | A | *a, b, c* |
| isobutyric | A | *a* |
| valeric | A | NPR |
| isovaleric | A | *a, c* |
| caproic | A | NPR |
| isocaproic | A | NPR |
| heptanoic | A | NPR |
| levulinic | A | NPR |
| benzoic | A | NPR |
| phenylacetic | A | NPR |
| 3-phenylpropionic | A | NPR |
| alcohols | | |
| ethanol | N | NPR |
| butanol | N | NPR |
| 2-methyl-1-propanol | N | NPR |
| pentanol | N | NPR |
| 3-methyl-1-butanol | N | NPR |
| 1,2-propanediol | N | NPR |
| 2,3-butanediol | N, B | NPR |
| glycerol | N, B | NPR |
| 2-furanmethanol | N | NPR |
| 4-methylcyclohexanol (t) | N | NPR |
| amines | | |
| dimethylamine | B | NPR |
| trimethylamine | B | *c, d* |
| histamine | B | *d* |
| tryptamine | B | NPR |
| tyramine | B | NPR |
| dopamine | B | NPR |
| octopamine | B | NPR |
| phenylethylamine | B | NPR |
| other N containing | | |
| ammonia | B | *b, c, d* |
| amino acids | | *d, e* |
| 2-ethylimidazole | N | NPR |
| indole | N, B | NPR |
| 3-methylindole | N, B | NPR |
| 2-methylpyrazine | N | NPR |
| 2,5-dimethylpyrazine | N | NPR |
| 2-piperidinone | A | NPR |
| 2-pyrrolidinone (t) | A | NPR |
| lactones | | |
| γ-butyrolactone | N | NPR |
| γ-caprolactone | N | NPR |
| 4-hydroxyvaleric acid lactone | A, N | NPR |
| 2-methyl-4-hydroxyvaleric acid lactone (t) | A, N | NPR |
| carbonyls | | |
| benzaldehyde | N | NPR |
| 3-hydoxy-2-butanone | N | NPR |
| 5-methyl-2-furanone | N | NPR |
| S containing | | |
| methyl mercaptan | N | NPR |
| 3-(methylthio)-1-propanol | B | NPR |
| 3-(methylthio)propionic acid (t) | N | NPR |
| 2-(4-methyl-5-thiazolidyl)-ethanol | N | NPR |
| 2-(methylthio)pentane (t) | N | NPR |

[a] Dougan and Howard (1975). [b] Truong-van-Chom (1957). [c] Beddows et al. (1979). [d] Saisithi et al. (1966). [e] Beddows et al. (1976). [f] A = acidic fraction; B = basic fraction; N = neutral fraction; NPR = not previously reported; t = tentative identification.

and 3-phenylpropionic acid from phenylalanine. The presence of caproic and heptanoic acids suggests that lipid oxidation may be responsible for some of the acids in the sauce.

Of the other major components of the acidic fraction, levulinic acid (4-oxovaleric acid) has been shown to form the breakdown of 5-(hydroxymethyl)furfural, a product of nonenzymatic browning (Noller, 1966). The γ-lactone 4-hydroxyvaleric acid lactone results spontaneously from 4-hydroxyvaleric acid.

The neutral fraction, characterized by a meaty aroma, contained five compounds present at greater than 10% of the fraction. Three of these were lactones. The γ-butyrolactone and γ-caprolactone were both faintly sweet, aromatic buttery in aroma while 4-hydroxyvaleric acid lactone had a pungent odor of weak intensity. The 3-(methylthio)propanol possessed a strong sulfury odor. The other major component, 2,3-butanediol, was also present in significant quantities in the basic fraction. This compound and glycerol are end products of fermentation by *Bacillus cereus*, *Bacillus lichenformis*, and *Bacillus subtilis* ("Bergey's Manual of Determinative Bacteriology", 1974). These three species were isolated from Nam-pla by Crisan and Sands (1975).

Among the minor constituents of the neutral fraction were a number of alcohols, mostly short chain ($C_2$ through $C_5$). Collectively, these alcohols accounted for less than 1% of the fraction. Other compounds identified (Table II) were three S compounds, two furans, two pyrazines, two indoles, an imidazole, and a single aldehyde. Benzaldehyde was the only aldehyde identified in fish sauce. The absence of this class of compounds has been noted by other investigators (Saisithi et al., 1966; Dougand and Howard, 1975). The latter workers suggested that any aldehydes formed would have been consumed in the nonenzymatic browning sequence of reactions.

On a sensory basis, the aroma of the basic fraction was dominated by ammonia and trimethylamine. These two, and to a lesser extent dimethylamine and 2,3-butanediol, were quantitatively the major constituents of this fraction. The presence of numerous other nitrogen-containing compounds was indicated by the GC profile obtained by using an NPD.

Ammonia and trimethylamine were produced in the initial weeks of fermentation and do not require bacterial involvement (Beddows et al., 1979). Metabolic pathways starting with amino acids have been elucidated for the formation of histamine (from histidine), tryptamine (from tryptophan), and tyramine, dopamine, and octapamine (from tyrosine) (Metzler, 1977).

In summary, 43 previously unidentified compounds have been identified in fermented fish sauce. The newly identified compounds included 8 acids, 10 alcohols, 6 amines, 7 other nitrogen-containing compounds, 4 lactones, 3 carbonyls and 5 sulfur-containing compounds.

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End of Symposium

# ARTICLES

## Fate of Kepone and Mirex in the Aquatic Environment

James N. Huckins,* David L. Stalling, Jimmie D. Petty, Denny R. Buckler, and B. Thomas Johnson

The degradation of Kepone and mirex were examined in fish and hydrosoils. Fathead minnows were continuously exposed to three concentrations of [$^{14}$C]Kepone and [$^{14}$C]mirex in flow-through dilutor systems and then placed in fresh water for elimination phases. After 56 days [$^{14}$C]Kepone residues were concentrated 16 600 times by fathead minnows. However, only 1–5% or 0.1–0.23 $\mu$g/g of these residues was [$^{14}$C]Kepone. Several observations suggested that some [$^{14}$C]Kepone residues present in fathead minnows were chemically bound to biogenic compounds. Similar exposures of fathead minnows to [$^{14}$C]mirex resulted in bioconcentration factors as high as 51 400 times. The half-life of [$^{14}$C]mirex was greater than 28 days in fathead minnows, and no degradation products were detected in whole body samples. No evidence of [$^{14}$C]Kepone or [$^{14}$C]mirex degradation was observed in anaerobic and aerobic hydrosoil exposures.

Kepone ($C_{10}Cl_{10}O$) and mirex ($C_{10}Cl_{12}$) are closely related insecticides differing only by the presence of an oxygen atom substituted for two chlorine atoms. Although Kepone is used in about 55 commercial products, Harless et al. (1978) pointed out that most of these products are exported to other countries for use on bananas and potatoes. However, the compound has had limited use in the United States as an ant and roach bait. Production of Kepone by Life Sciences Products, Hopewell, VA, was discontinued in 1975 because of clinical evidence of Kepone intoxication in workers. Since then, interest in Kepone has continued because extensive residues were detected in the James River below Richmond, VA, extending to the mouth of the Chesapeake Bay. Stafford et al. (1978) found Kepone residues of 0.1–20 $\mu$g/g in fish and a corresponding bioconcentration factor of 20 000 times. Skalsky et al. (1979) reported that Kepone was bound to high-density lipoproteins in human plasma and suggested that lipoprotein binding may play a role in the toxicity of Kepone.

Large quantities of mirex have been used in the last decade for the control of fire ant infestation in nine southeastern states. Residues of mirex are widespread and were detected by Hawthorne et al. (1974) in fish and other aquatic organisms. A mirex committee (Van Middelem, 1972) reported that the amount of mirex sold from 1960 to 1970 as a flame retardant was over 4 times that sold for pesticide usage. Detection of mirex in fish from Lake Ontario (Kaiser, 1974) suggested that the compound had become a major environment contaminant. Jones and Hodges (1976) and Mehendale et al. (1972) reported that soil microorganisms and rats failed to degrade mirex in laboratory experiments. However, the discovery of Kepone as a degradation product of mirex in the environment (Carlson et al., 1976) has prompted considerable concern

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