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N-Nitrosodimethylamine in Cold-Smoked Sablefish

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Sablefish was treated with nitrite levels ranging from 0 to 1300 ppm prior to being cold smoked. The flesh was analyzed for the presence of *N*-nitroso compounds by gas–liquid chromatography immediately after processing and again after 2 weeks storage at 40°F. Trace amounts of *N*-nitrosodimethylamine (<10 ppb) were detected in samples with nitrite levels ranging from 0 to 550 ppm. This value did not increase with higher nitrite levels. Storage at 40°F did reflect a slight decrease in concentrations. The identity of the isolated compound was confirmed by gas–liquid chromatography as the nitramine derivative and by GC–MS.

Howard et al. (1970) have reported finding *N*-nitrosodimethylamine (DMNA) in smoked chub. Gas chromatographic determination and mass spectrometric confirmation of DMNA ranging from 4 to 26 ppb in samples of raw, smoked, and smoked nitrite and/or nitrate treated sable, salmon, and shad have been described by Fazio et al. (1971a,b). High amounts of DMNA (0.12–0.45 ppm) were also reported by Sen et al. (1972) in fish meal which had been implicated in the liver disease of mink in Canada. Studies by Crosby et al. (1972) showed volatile nitrosamines and nitrite levels in cured and fresh fish. Kawabata et al. (1973) found no DMNA in salted roe products prepared with less than 0.09 m*M* nitrite, while DMNA was detectable when higher concentrations were used.

Concern about the possibility of DMNA in smoked, nitrite-treated fishery products prompted the National Marine Fisheries Service to investigate their possible occurrence. Sablefish was selected as a target species because, in preliminary studies conducted at this Center, smoked sable was found from among several smoked-processed fishery products examined to contain the highest level of DMNA (22 ppb). We have attempted in this investigation to correlate DMNA concentration to nitrite level in stored and unstored samples of cold-smoked sablefish. The results of these findings are presented in this article.

EXPERIMENTAL SECTION

Materials. The solvents, methylene chloride, pentane, methanol, and ethyl ether, were purified by distillation. Solvents, silica gel, and Celite 545 were tested prior to use to assure the absence of interfering peaks.

Gas Chromatographic Conditions. A Victoreen Model 4000 gas chromatograph equipped with a Coulson electrolytic conductivity detector and an Autolab System IV computing integrator was employed in the analysis of sablefish extracts. A 6 ft × 4 mm i.d. glass column coated with 10% Carbowax 1540 + 3% KOH on 100–120 mesh Gas-Chrom Q support was used. The following parameters were maintained throughout all analyses: temperature of injector block, 190°; carrier gas (helium) flow rate, 70 ml/min; GC oven temperature, 120° from 0 to 300 sec; 120–180° at a program rate of 5°/min.

Conditions of Coulson detector operated in reductive mode were: hydrogen flow rate, 83 ml/min; venting helium flow, 70 ml/min; furnace temperature, 820°; venting block temperature, 200°; conductivity bridge, 30 V; attenuation, 1.

GC–MS Apparatus. A 3200F–6103 Finnigan (Quadrupole) Automated GC–MS system was used in conjunction with a 5 ft × 2 mm i.d. glass column coated with 3% SP-2340 on 80–100 mesh Supelcoport. GLC parameters were: carrier gas flow rate, 20 ml/min; injector temperature, 200°; oven, 80° from 0 to 300 sec; 80–180° at a programmed rate of 5°/min. Separator and transfer lines were maintained at 200°. Ionization was by electron impact at 70 eV and by chemical ionization using methane as the reagent gas.

Analytical Procedures. For this investigation, the multidetection method of Fazio et al. (1971a,b) was used to determine volatile *N*-nitrosamines. Briefly, this procedure involved digestion of the sample in methanolic KOH, liquid–liquid extraction of an aliquot equivalent to 25 g with methylene chloride, and distillation of the nitrosamines

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Table I. DMNA, Nitrite, Chloride, and Moisture Levels in Sable Flesh Which Was Brined, Smoked, and Stored for 2 Weeks at 40°F

Sample	Proposed concn of $NaNO_2$, ppm	Initial values: DMNA, ppb	Initial values: $NaNO_2$, ppm	Initial values: NaCl, %	Initial values: Moisture, %	Values after storage: DMNA, ppb	Values after storage: $NaNO_2$, ppm	Values after storage: NaCl, %	Values after storage: Moisture, %
I	0	0	7	3.7	62	0	1	3.8	62
II	50	0.7	42	3.5	61	0.5	6	3.8	61
III	100	1.6	81	3.5	61	1.2	65	3.6	61
IV	200	3.5	199	3.8	59	2.5	198	3.5	60
V	400	8.2	530	4.3	58	6.5	550	4.5	59

from alkaline solution, with further cleanup by solvent partitioning and column chromatography on silica gel and Celite 545 columns followed by GLC analysis.

N-Nitrosodimethylamine, -diethylamine, -ethylpropylamine, -dipropylamine, -propylbutylamine, -dibutylamine, -piperidine, -pyrrolidine, and -diamylamine were used for calibration and recovery studies.

The method of Althorpe et al. (1970) was used to convert the DMNA to its corresponding nitramine derivative. GLC column and GC conditions were exactly the same as those described in the section on gas chromatography.

Moisture and nitrite determinations were made according to the official AOAC method. Chloride analysis was done according to the method of Greig and Seagran (1965).

Processing of Sablefish. Frozen sablefish purchased from a commercial supplier was thawed overnight at 38°F in fiberglass tanks of water. Prior to filleting, the entire fish was then immersed in a 100-ppm hypochlorite solution and rinsed with cold water. The fillets were also dipped in a 100-ppm hypochlorite solution, trimmed of the thin tail and belly sections, and then cut into pieces weighing from ⅓ to ½ lb each.

Brining. Five brine solutions containing 0, 150, 300, 600, and 1300 ppm of $NaNO_2$, respectively, were prepared. All had concentrations of 4% NaCl and 100 ppm of sodium hypochlorite to retard spoilage of the highly perishable sable. The solutions were maintained at 36°F.

For each of the five brining conditions, the solution and the fish were combined in a 1:1 ratio by weight in fiberglass tanks where they were held for 72 hr at 36°F. After brining, the fish was removed from the tanks and placed on oiled racks skin side down, coated with paprika, and cooled overnight at 33°F.

Smoking. Racks of fish were placed on a "DRY-SYS" equipment smokehouse with a Mepaco smoke generator. The fish was held at 125°F for 18 hr inside the smokehouse oven. The oven was cooled to 90°F, and the fish smoked at 90°F for 30 min. After cooling the fish to 40°F, the racks of fish were removed from the smokehouse and cooled for 24 hr at 33°F.

Sample Preparation. After the removal of skin and bones, the smoked sable was blended in a Hobart silent cutter. Samples were weighed immediately for the following analyses: moisture, chloride, nitrite, and *N*-nitrosamines. Samples not analyzed immediately were stored at −20°F until analyses. Smoked sable to be analyzed after storage was packed in polyethylene bags and held at 40°F for 2 weeks.

RESULTS AND DISCUSSION

Samples of sablefish were analyzed, and the results are presented in Table I. All samples contained less than 10 ppb of DMNA. Storage at 40°F reflected a slight decrease in concentration. Table I also summarizes moisture and chloride concentrations. The percentage of salt in the water phase of the product ranged from 3.5 to 4.5. For smoked fish, the recommended value is 3.5%. It is the value which is commonly referred to in relation to the safety of the product, and it is the value which will be of interest to the regulatory agency official. Nitrite concentrations which were actually attained and maintained during the processing and storage period are also shown in Table I. Column 2 shows the concentrations of sodium nitrite we were striving to attain in our samples. After the smoking process, a decrease in $NaNO_2$ is apparent in most of the samples which might be due to some loss by oxidation; however, additional $NaNO_2$ was probably lost with the large amount of drip which was exuded from the product. In sample I, there was no added $NaNO_2$ to be lost, only moisture, so the increase in $NaNO_2$ concentration is reasonable. In sample V, the increase can probably be explained by more moisture than nitrite being lost. In the stored samples, the lower the original concentration of nitrite, the greater is the loss during the storage.

In Figure 1, chromatograms obtained with a fortified and unfortified sablefish extract are shown after silica gel cleanup. A peak (shown by an asterisk) with the same retention time as nitrosopyrrolidine was removed by acid–Celite treatment. The arrow on the unfortified chromatogram illustrates a peak with a DMNA retention time. For recovery studies, a mixture of nine *N*-nitrosamines was used. Samples fortified at 5 ppb gave recoveries of *N*-nitrosamines ranging from 65 to 88%.

Figure 2 shows the effect of an acid–Celite column cleanup. Interfering peaks have been removed, but the suspected DMNA peak is still present. The bottom chromatogram shows the same extract after silica gel cleanup.

Figure 3 shows chromatograms of the nitramine derivative of DMNA and the corresponding nitramine derivative of suspected DMNA in the extract. Because the suspected DMNA underwent the specific reaction with peroxytrifluoroacetic acid and because of its exact retention time on the chromatogram with the derivative of DMNA, its identity is strongly affirmed.

An extract prepared from smoked sable containing more than 500 ppm was submitted to GC–MS analysis. The extract had previously undergone acid–Celite column treatment. Figure 4 shows a three-channel mass fragmentogram obtained with a 2μl injection of the extract. To enable detection of trace amounts of the suspected compound in the presence of interfering substances, the GC–MS was programmed to scan only the molecular weight ion (74^+), $CH_2{=}N^+{=}CH_2$ (42^+), and NO (30^+) rather than the entire mass range. The sensitivity increased dramatically for the identification of DMNA, e.g., normal operation was 50–500 pg. In this case, the scan number of the three ions correlated exactly with the retention time of DMNA.

To obtain a mass spectrum of the suspected nitrosamine in smoked sable containing more than 500 ppm of $NaNO_2$, the following was done. The multidetection method of Fazio et al. (1971a,b) was used to prepare eight samples. The eluents from eight silica gel columns were combined, concentrated to 1 ml, and analyzed by GLC (Figure 2). The extract was chromatographed on an acid–Celite col-

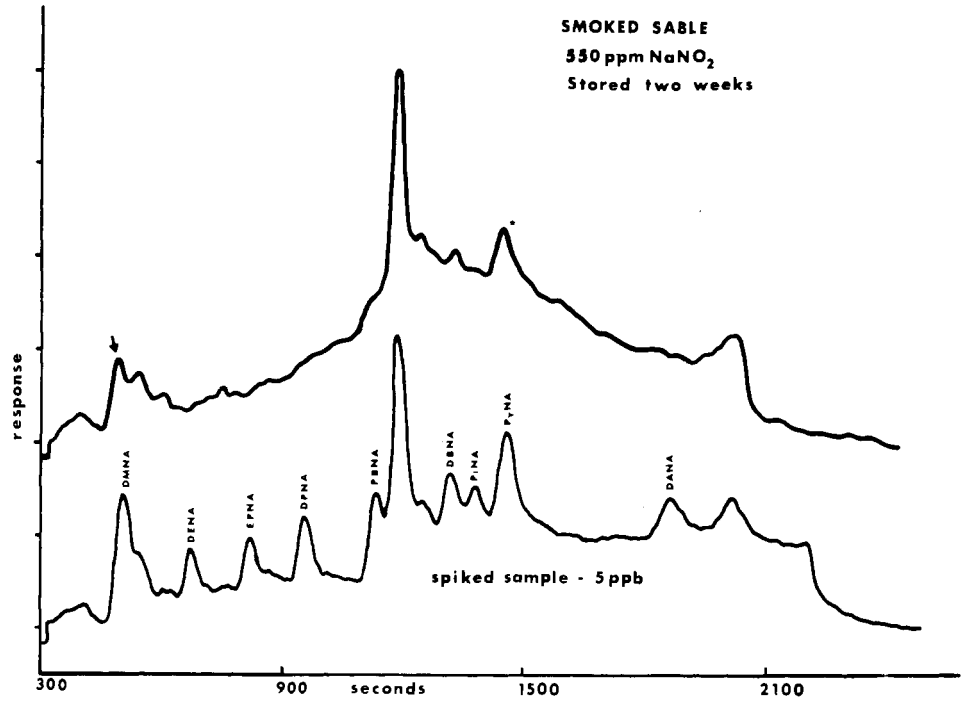

Figure 1.

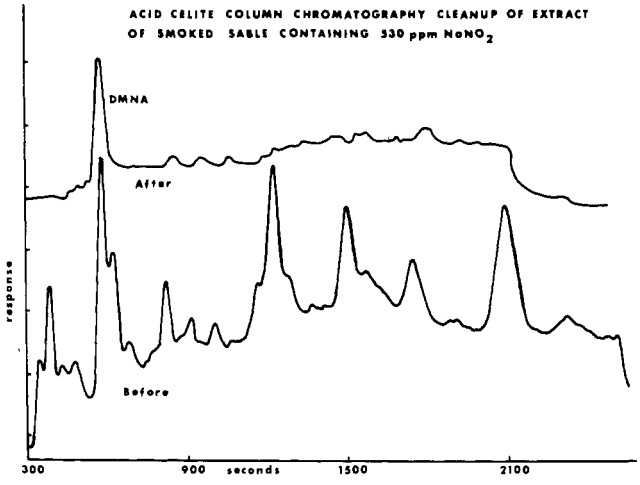

Figure 2.

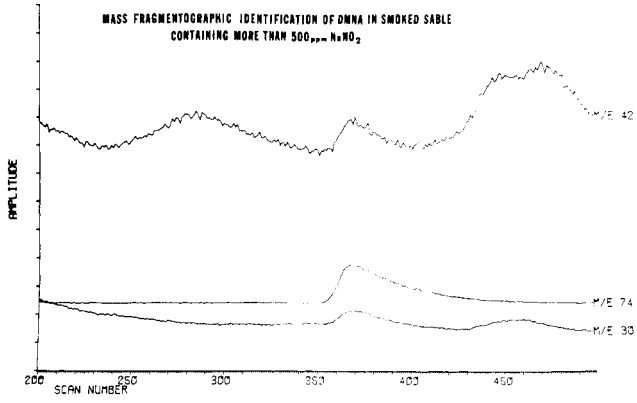

Figure 4.

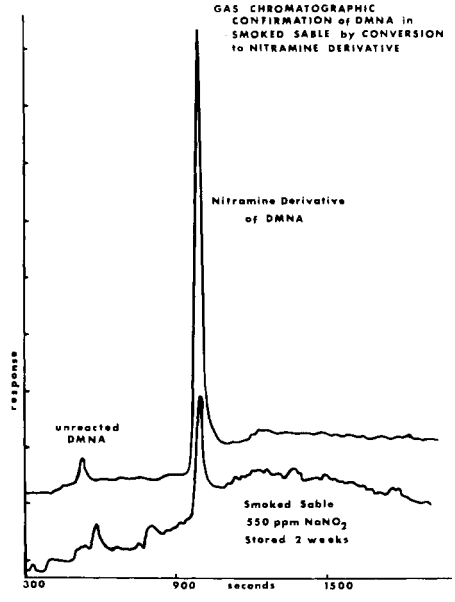

Figure 3.

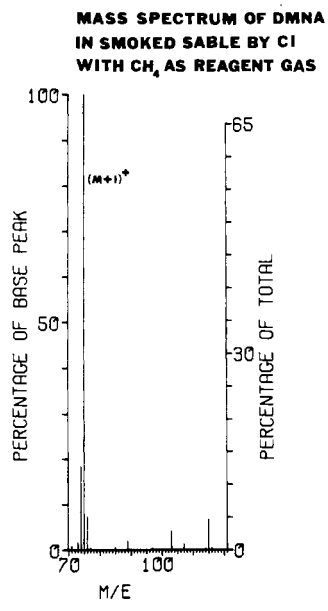

Figure 5.

umn. The eluent from this column was concentrated to 1 ml in a 250-ml Kuderna-Danish apparatus. For further concentration, it was transferred to a Chromaflex sample tube (K-422560) and carefully brought to 100 μl under a gentle stream of nitrogen. A proximate concentration of this extract was 10 ng/μl. Ten microliters of the extract was injected on the GC–MS column.

Figure 5 shows the analysis performed using a chemical ionization source with methane as the reagent gas. Chemical ionization offered the advantages of an intense protonated molecular ion 75^+ and a simpler fragmentation pattern. Specific ion monitor of the suspected 75^+ peak in the chromatogram established its retention time with that of the DMNA standard. In this case, the 6000 Interactive GC–MS data system subtracted the background caused by an interfering peak and recalled the spectral information stored on a high speed disk.

In view of the above findings, it can be concluded that less than 10 ppb of *N*-nitrosodimethylamine was found in cold-smoked sablefish, that this value was not increased when nitrite was used up to 550 ppm, and that no other nitrosamines were identified.

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Rapid Method for the Determination of Mercury in Fish Tissue by Atomic Absorption Spectroscopy

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A simple, rapid method for the determination of mercury in fish tissue by flameless atomic absorption spectroscopy is described. Digestion of the sample is done with sulfuric–nitric–hydrochloric acids. A digestion time of 15 min is sufficient to convert the mercury to an inorganic form so that it can be reduced to the elemental state and determined by flameless atomic absorption spectroscopy. Results using this method compare very favorably with those obtained using the Food and Drug Administration Official First Action Method for the determination of mercury in fish.

Several methods have been used for the decomposition of fish samples for mercury analysis (Holak et al., 1972; Malaiyandi and Barrette, 1970; Munns and Holland, 1971; Saha and Lea, 1972; Uthe et al., 1970). Problems associated with prevention of mercury losses, use of potentially explosive reagents, length of digestion time, reproducibility, excessive fumes, interference of fat in the tissue samples, and varying mercury values obtained when varying sample weights are used showed a pressing need for a convenient method to determine microquantities of mercury in biological materials. This paper describes a simple method for determination of mercury in fish tissue using a mixture of acids for digesting the samples. It also describes the optimum conditions for the digestion. The total mercury content in the digested samples is determined by the flameless atomic absorption spectroscopy technique.

EXPERIMENTAL SECTION

Apparatus. The equipment for this method is the same as that utilized in the Food and Drug Administration Official First Action Method for the determination of mercury in fish (Munns and Holland, 1971).

Reagents used included: (a) hydrochloric acid, 6 *N;* (b) sulfuric acid, 6 and 1 *N;* (c) dilute nitric–sulfuric acid mixture, 1 part nitric, 9 parts sulfuric, in 8 parts water; (d) reducing solution (in a 1000-ml volumetric flask containing 600 ml of 6 *N* sulfuric acid, 30 g of sodium chloride, 30 g of hydroxylamine sulfate, and 50 g of stannous chloride were dissolved; solution was diluted to mark with distilled water); (e) mercury standard solution: (1) 1000 μg/ml, 0.1354 g of mercuric chloride ($HgCl_2$) was dissolved in 100.0 ml of water; (2) 1 μg/ml, 1 μg/ml standard in 1 *N* sulfuric acid from standard solution was prepared daily.

Determination of Mercury. About 1 g of wet fish sample (about 400 mg of dry fish sample) was weighed into a digestion flask. To this was added 10 ml of dilute nitric–sulfuric acid mixture and 3–4 boiling chips. Condenser was connected and cold water circulated through it. To this was added 1 ml of 6 *N* HCl through condenser and gentle heat was applied for 15 min (about 10 min for dissolution of the sample and 5 min of gentle boil). The heat was removed and the contents were allowed to stand 15 min. Distilled water (90 ml) was added through the condenser while the contents of the flask were swirled. The flask was disconnected from the condenser and cooled to room temperature. Residual fumes from the flask were blown off using a

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