Quantitative and Selective Gas Chromatographic Analysis of Dimethyl- and Trimethylamine in Fish

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The selective gas chromatographic separation of methylamines was accomplished using columns containing Graphon and tetraethylenepentamine with an alkali flame ionization detector (AFID). Trimethylamine (TMA, 10 ppm) and dimethylamine (DMA, 50 ppm) added to fish were easily detected by equilibrium vapor analysis. Greater sensitivity (25 ppb TMA and 100 ppb DMA) was obtained by using the AFID in conjunction with a gas entrainment, on-column trapping procedure.

Although the methods of Dyer (1945) and Dyer and Mounsey (1945) are commonly used in the assessment of seafood quality, there is a definite need for a more specific and rapid method for the quantitative determination of dimethylamine (DMA) and trimethylamine (TMA) in fish. Hashimoto and Okaichi (1957), Tozawa *et al.* (1970), and Castell *et al.* (1970) recognized the fact that DMA contributed significantly to the TMA value in certain species of the gadoid family when the picrate color reaction of Dyer (1945) was employed. The Japanese workers suggested the use of KOH rather than K_2CO_3 to eliminate the error due to DMA. TMA values obtained with KOH compared reasonably well with values obtained by gas–liquid chromatography (Tozawa *et al.*, 1970).

The importance of an accurate, reproducible method for determining amine contents, especially DMA, cannot be overemphasized since DMA, rather than TMA, is characteristically the principle methylamine produced in frozen fish of the gadoid species (Castell *et al.*, 1971; Tokunaga, 1964). In addition, Fazio *et al.* (1971) recently demonstrated the presence of *N*-dimethylnitrosamine in smoke-processed marine fish such as sable, salmon, and shad. Apparently, DMA and nitrite served as precursors for the carcinogenic nitroso derivative.

Several reports (Burks *et al.*, 1959; Hughes, 1959; Issoire and Chaput, 1961; James *et al.*, 1952; Lindsay Smith and Waddington, 1968; O'Donnell and Mann, 1964; Sze and Borke, 1963; Wick *et al.*, 1967) have dealt primarily with the gas chromatographic analysis of methylamines. A variety of supports and liquid phases have been investigated and reasonable separations have been reported. Recently, Di Corcia *et al.* (1970) used Graphon, a partially graphitized carbon black, for the separation of methylamines, as well as a variety of other polar, low-boiling compounds. A column, containing Graphon coated with tetraethylenepentamine (TEP), gave symmetrical peaks for methylamine (MA), DMA, and TMA with good resolution.

This study was initiated to further examine the detection and separation of methylamines using Graphon and TEP in conjunction with an alkali flame ionization detector (AFID) which is sensitive and selective to nitrogen compounds. The results presented herein describe rapid analytical procedures which can be used for quantitative and qualitative determinations of methylamines in fish, as well as other food products or biological materials.

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EXPERIMENTAL

Graphon (Cabot Corp., Billerica, Mass.) was sieved through 40–60 mesh sieves. Known amounts of liquid phase (TEP) were dissolved in methylene chloride (CH_2Cl_2) and the solution was poured over weighed amounts of support. Additional CH_2Cl_2 was added to make a slurry. Excess solvent was evaporated under a stream of nitrogen and the packing was thoroughly dried in an oven at 35 to 40°C. The coated material was resieved and stainless steel columns (5.5-m × 3-mm o.d.) were packed and conditioned for 24 hr at 70°C with a nitrogen flow rate of 10 ml/min.

DMA and TMA hydrochlorides were obtained from Baker Chemical Co., Phillipsburg, N.J., and were used without further purification. Methylamine was available as a 40% aqueous solution and trimethylamine oxide (TMAO) was purchased from E. H. Sargent & Co., Anaheim, Calif.

Standard curves were constructed for DMA and TMA from data obtained by equilibrium vapor analysis. Known amounts of amine hydrochlorides were added to screw-capped vials (Kimble No. 60957 size No. 1) containing 5 g of fresh fish that had been ground through a 3–6 mm plate in a Universal meat grinder, and the amines were liberated from their salts by addition of 10% NaOH. The vials were sealed with screw caps that had been modified as described by Morgan and Day (1965). All samples were heated at 60°C for 10 min and equilibrium vapor samples (1 ml) were removed and immediately injected into the gas chromatograph. Preliminary experiments indicated that treatment with 10% NaOH for 10 min at 60°C was sufficient for complete release of amines under our experimental conditions. Isopropylamine was used as an internal standard. Excluding the addition of amine hydrochlorides, frozen (−12 to −14°C) and spoiling fish samples were examined as described above.

Varian Aerograph (series 1200 and 1400) gas chromatographs, equipped with a flame ionization detector (FID) and an AFID, were used for all analyses. The column oven temperature was maintained at 60°C and the nitrogen gas flow rate was 30 ml/min. The detector and injector port temperatures were 210 and 190°C, respectively. The AFID was routinely tuned for optimum performance as described by the manufacturer (Varian Aerograph, Walnut Creek, Calif.).

An F&M model 810 gas chromatograph was used in conjunction with an Atlas CH-4 mass spectrometer (ms) for mass spectral analyses. The operating conditions for the ms were: filament emission, 70 eV; source, 12 μA; electron voltage, 70 eV; accelerating voltage, 3.00 kV; analyzer pressure, 2×10^{-6} Torr; multiplier voltage, 1.6 kV; and scanning speed, 2 sec for *m/e* 12 to *m/e* 100.

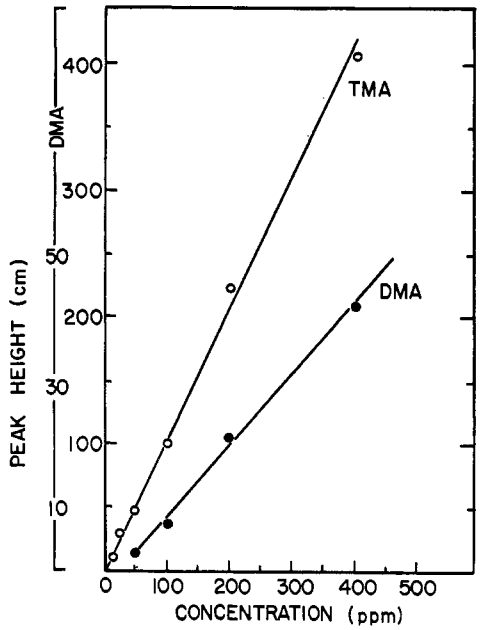

Figure 1. AFID and recorder response for various concentrations of TMA and DMA added to fresh fish. Column: Graphon and TEP (2%) 5.5 m × 3 mm o.d.

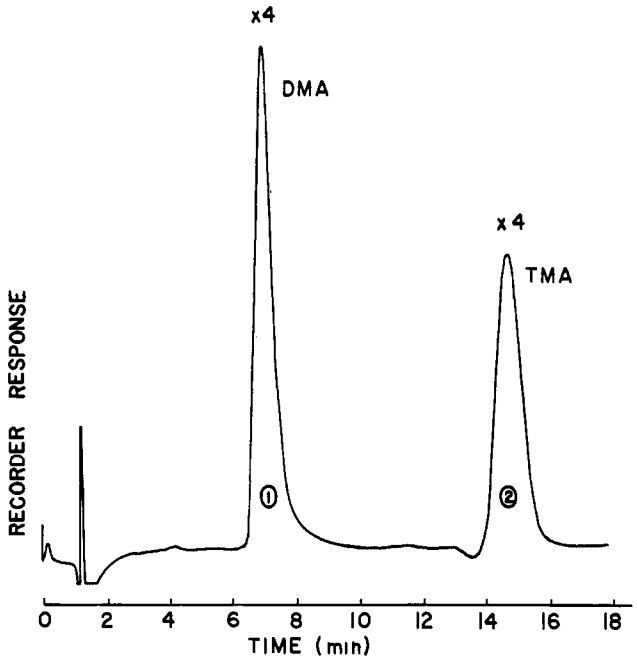

Figure 2. AFID response to volatile components from hake frozen at −12°C. Column: Graphon and 2% TEP (5.5 m × 3 mm o.d.)

RESULTS AND DISCUSSION

Peak heights obtained by equilibrium vapor analysis for various concentrations of TMA and DMA added to fish were plotted and the standard curves are shown in Figure 1. The limits of detection for TMA and DMA were 10 and 50 ppm, respectively. Since the linearities of response (Figure 1) were highly reproducible, the analytical procedure was used to determine TMA and DMA contents of various species of fish which had been frozen at −30°C and stored at −12 to −14°C for extended periods. Pacific hake (*Merluccius productus*) and true cod (*Gadus macrocephalus*) produced significant amounts of DMA, while Dover sole (*Microstomus pacificus*), Ling cod (*Ophiodon elongatus*), and *Sebastodes flavidus* and *S. alutus*

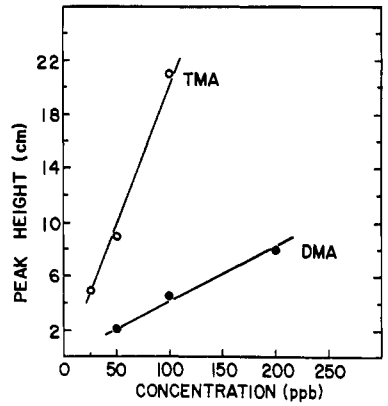

Figure 3. Standard response curves for TMA and DMA obtained by on-column trapping. Column: Graphon and 2% TEP (5.5 m × 3 mm o.d.)

Table I. Trimethylamine and Dimethylamine Contents of Various Species of Fish Stored at −12 to −14°C

Species	Days frozen	Concentration, ppm DMA	TMA
Pacific hake (*Merluccius productus*)	750	>400	30
Pacific hake (*M. productus*)	545	>400	35
Pacific hake (*M. productus*)	180	395	12
Dover sole (*Microstomus pacificus*)	290	...	40
True cod (*Gadus macrocephalus*)	150	75	12
Green rockfish (*Sebastodes flavidus*)	310	...	160
Ocean perch (*Sebastodes alutus*)	142	...	95
Ling cod (*Ophiodon elongatus*)	150	...	12

produced TMA but no DMA (Table I). Castell *et al.* (1971) also observed DMA formation in frozen hake (*M. bilinearis*). The above results substantiate previous reports (Castell *et al.*, 1971; Tokunaga, 1964) that nonmicrobial formation of DMA in fish tissue is characteristic of the gadoid species. Figure 2 is a typical AFID response to volatile components from hake (2 g) stored at −12°C. Peaks 1 and 2, DMA and TMA, respectively, were confirmed by retention times of authentic compounds, peak enhancement, and mass spectral data. Although TMA and DMA can be derived from TMAO under a variety of conditions, no apparent increase in either amine value was observed in the presence of excess oxide under the experimental conditions employed.

If increased sensitivity in the analysis of volatile amines is desired, the AFID can be used in conjunction with the gas entrainment, on-column trapping procedure of Morgan and Day (1965). The operating parameters for the gas chromatographic analyses were the same as described in the experimental section. However, each sample was purged with nitrogen (10 ml/min) at 60°C for 10 min. In addition, make-up carrier gas was supplied to the AFID by means of a micro-volume switching valve (Carle Instruments, Inc., Fullerton, Calif.) installed between the end of the column and the detector. The make-up gas was supplied to the AFID while the sample was being purged in order to maintain a constant flow rate (30 ml/min) through the detector. These conditions were necessary to maintain a relatively stable baseline throughout the analysis. Under the above conditions, TMA (25 ppb) and DMA (100 ppb) were easily detected and the responses were linear over the concentration range examined (Figure 3). If greater sensitivity is desired, the purge time may be increased accordingly.

Comparison of FID and AFID responses to volatile components in the equilibrium vapor over fish that had reached an advanced stage of spoilage demonstrated the advantage of the selective nitrogen detector. Although some volatile nonnitrogenous spoilage compounds masked DMA in the FID analysis, both DMA and TMA were easily quantitatively determined by the more specific alkali flame detector.

The procedures described above are more rapid and, in the case of on-column trapping, more sensitive than methods (Dyer, 1945; Dyer and Mounsey, 1945; Nonaka *et al.*, 1967) presently used for the quantitative analysis of TMA and DMA in fish.

Although columns, containing Graphon and TEP (2%), were used exclusively throughout this investigation, comparable separations and resolution of MA, DMA, and TMA were obtained using Graphon coated with 0.5% TEP (3.7-m × 3-mm o.d.). Contrary to results obtained by Di Corcia *et al.* (1970; Figure 1C), TMA and DMA could not be separated using Graphon and 5% TEP.

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Determination of Nitrosodimethylamine in the Low Parts Per Billion

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Nitrosodimethylamine is measured in apples and milk in the low ppb range by vacuum distillation, concentration, gas chromatography, and microcoulometric nitrogen determination. The concentration of the NDMA is achieved by percolation of the aqueous distillate through polymer beads. The method is sensitive to 3 ppb and the recovery is 70% or better.

About 20 ppb nitrosamines have been detected in tobacco smoke and food by Kroeller (1967) in Germany using thin-layer and gas chromatography. Mayerhofer and Moehler (1967) and Sen *et al.* (1969) used these techniques to determine nitrosodimethylamine (NDMA) and nitrosodiethylamine in food. Rhoades and Johnson (1970) selectively pyrolysed *N*-nitrosamines to ammonia at 400–600° C. Howard *et al.* (1970) extracted NDMA from smoked fish and detected it by a gas chromatograph equipped with a modified thermionic detector. Fazio *et al.* (1971), also working with fish, determined NDMA by gas chromatography and confirmed its identity with a mass spectrometer. Lakata (1967) suggested the use of a Porapak column for the gas chromatographic resolution of NDMA from other volatile components of foods.

This paper presents an analytical method sensitive to 3 ppb of NDMA in raw apples, cooked apples, and in milk. The method should be applicable to the determination of NDMA in other foods as well, and also to the determination of other volatile nitrosamines. The use of Porapak to accumulate a few parts per billion of organic compounds from large volumes of water could also find applicability in water pollution analysis and urinalysis.

The method consists of five operations. (1) The NDMA and other volatile components are removed from the nonvolatile fraction of the sample by a vacuum distillation by a technique which minimizes loss by hydrolysis and volatility. (2) The NDMA and other organic materials in the distillate are removed from the water by percolation through a column of polymer beads. (3) The NDMA is removed from the polymer beads by heat and carried into a gas chromatographic column. (4) Programmed temperature gas chromatography separates the NDMA from other components. (5) The NDMA is catalytically reduced to ammonia, which is microcoulometrically titrated and continuously recorded. The area of a peak at a specific retention time established the amount of NDMA.

MATERIALS AND EQUIPMENT

The equipment for this method is shown in Figures 1, 2, and 3. The rotary evaporator had a Teflon drive shaft (Model 5001, Calif. Laboratory Equipment Co., Berkeley, Calif.). In Figure 3, valve g-1 denotes a toggle valve with a Teflon seat,

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