

## Gas Chromatographic Method for Determination of Dimethylamine, Trimethylamine, and Trimethylamine Oxide in Fish-Meat Frankfurters

WALTER FIDDLER, ROBERT C. DOERR, and ROBERT A. GATES

*U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118*

**A method is described for analysis of minced fish-meat and surimi-meat frankfurters for dimethylamine (DMA), trimethylamine (TMA), and trimethylamine oxide (TMAO) using a headspace-gas chromatographic technique. After simple acid extraction and addition of NaOH, the headspace was directly injected into a gas chromatograph by a gas-tight syringe. DMA and TMA were separated on a Chromosorb 103 column and detected by a flame ionization detector. TMAO was measured as TMA after Zn reduction. Repeatability of the method for DMA, TMA, and TMAO was 6.6, 1.0, and 18.8 ppm, respectively. The method was applicable to Alaska pollock-meat and Atlantic menhaden-meat frankfurters, unwashed, and washed mince and surimi.**

Based on advances in food technology, use of minced fish (mechanically separated flesh) and surimi (a washed form of mince, comprised primarily of stabilized myofibrillar protein) has been proposed as a partial substitute for meat in heretofore formulated all-meat products (1-3). Use of fish in nitrite-cured products raises concerns regarding formation of the potent carcinogen, *N*-nitrosodimethylamine (NDMA), because fish generally contains more dimethylamine (DMA) than meat (4, 5). The presence of NDMA at the low ppb level, particularly in salt-dried fish and other seafoods not exposed directly to nitrite, has been noted in several reviews (6, 7). Deterioration of fresh fish through microbial spoilage is accompanied by a parallel decomposition of trimethylamine oxide (TMAO) to trimethylamine (TMA) to the extent that the latter compound is used as an indicator of freshness (8).

In fish of the gadoid family, TMAO principally forms DMA and formaldehyde by endogenous enzymes (9), with the maximum formation below the freezing point of fish (-5 to -10°C) (10) and under refrigeration conditions in the absence of oxygen (11). One member of the gadoid family, Alaska pollock, is the preferred raw material for shellfish analogues made from surimi. While DMA can be nitrosated directly to form NDMA, both TMAO and TMA have also been shown to form NDMA (12-16). Under certain conditions, NDMA forms more readily from TMAO than TMA (17). Therefore, any investigation of NDMA in frankfurters containing meat in combination with fish mince or surimi requires accurate measurement of all 3 compounds—TMAO, TMA, and DMA.

For reasons of specificity, gas chromatography (GC) has been the common means for direct analysis of DMA and TMA, despite problems associated with its use. Analysis of volatile amines by GC has been hampered by loss of sample response, the ghosting phenomena, and badly tailed peaks because of adsorptive effects between the aliphatic amines

and the chromatographic support or adsorbent (18, 19). Most efforts to overcome these difficulties have involved deactivating the column packing material by adding a strongly basic material such as KOH or by adding ammonia to the carrier gas with noticeable improvement. Lack of published statistical data on the quantitative aspects of measuring low ppm of methylamines in seafood samples, except for a few fortification-recovery studies, also suggests problems in using GC for amine analysis. Lundstrom and Racicot describe an apparently successful method for determining both DMA and TMA in seafood (20). Despite this and other investigations on different sample preparations and GC column packings and detectors, there remains a need for a simple, specific, accurate method for methylamines as recently discussed (21).

The present paper describes an improved method applicable to fish-meat frankfurters containing Alaska pollock and Atlantic menhaden unwashed and washed mince and surimi.

### METHOD

#### Reagents

(a) *Hydrochloride salts of DMA and TMA*.—(Aldrich Chemical Co.) Dry to constant weight in a vacuum oven at 70°C.

(b) *Hydrochloric acid (HCl)*.— Concentrated, 2.0 and 0.5N (J.T. Baker).

(c) *Sodium hydroxide (NaOH)*.—15N (J.T. Baker).

(d) *Zinc powder*.—(Fisher Scientific) Purify 12 g by stirring for 1 min with 30 mL of 2N HCl, then transfer to a Buchner funnel and wash with 30 mL water, 20 mL ethanol and 20 mL acetone. Dry in a vacuum oven at 165°C for 1 h, then place in a suitable container.

(e) *Amine standard solution*.— Prepare 1 mg/mL individual standard solutions from dried crystals of DMA-HCl and TMA-HCl with 0.5N HCl. Prepare a combined working standard by taking 100  $\mu$ L of each amine solution and adding it to 25 mL volumetric flask and diluting to volume with 0.5N HCl. Final concentration of DMA and TMA is 4  $\mu$ g/mL, equivalent to 10 ppm in a 10 g sample.

(f) *Fish-meat samples*.—Frankfurters in which 15% or 50% of the meat was substituted with fish were prepared by the National Marine Fisheries Service and shipped to Eastern Regional Research Center as described previously (22). Samples of fish were either the raw ingredients for the frankfurters or ones purchased at local retail stores.

#### Apparatus

(a) *Tissumizer*.—Tekmar model SDT18/10 with a model SDT100EN shaft or equivalent.

(b) *Vortex mixer*.—Lab-Line Instruments Super-Mixer or equivalent.

(c) *Centrifuge tubes*.—Oak Ridge style, 50 mL polypropylene (Sorvall Instruments, Dupont Co.) or equivalent.

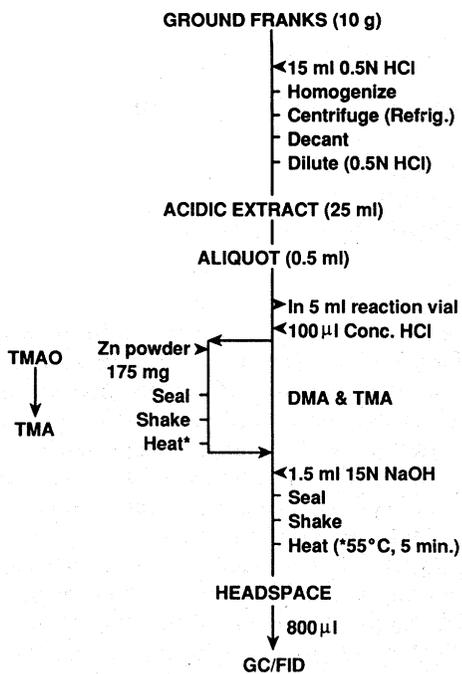


Figure 1. Schematic of amine method.

(d) *Refrigerated centrifuge*.—Sorvall RC-5B with an SA-600 rotor or equivalent.

(e) *Water bath*.—Exacta-Heat constant temperature bath or equivalent.

(f) *Reaction vials*.—Kontes microfex 5 mL vials, No. 749000-0005 or equivalent.

(g) *Gas-tight syringe*.—(Precision Sampling Corp.) 1 mL syringe.

(h) *Gas chromatograph*.—Hewlett-Packard model 5880A gas chromatograph equipped with a flame ionization detector and a controller-electronic integrator terminal level four. A 3.3 m × 2 mm id glass column packed with Chromosorb 103 was preconditioned at 250°C overnight with helium flow. Operating conditions: oven, 125°C isothermal; injector, 150°C; detector, 250°C; helium, 20 cc/min; hydrogen, 30 cc/min; air, 200 cc/min.

#### Procedure

Figure 1 is a schematic of the method.

**Sample preparation.**—Grind fish-meat frankfurters or fish twice through a 1/8-in. plate before analysis. Mix thoroughly. Weigh 10 g comminuted fish-meat or fish sample into a centrifuge tube. Add 15 mL of 0.5N HCl to tube and homogenize sample for 7 min with Tissumizer power setting at 70. After homogenization, centrifuge sample for 45 min at 15 000 rpm and 0°C. Decant liquid from tube into 25 mL volumetric flask and dilute to volume with 0.5N HCl. This is the sample solution.

**Amine determination.**—Pipette 0.5 mL DMA/TMA standard solution into a 5 mL vial containing 100 µL concentrated HCl, then add 1.5 mL of 15N NaOH. Immediately cap vial and agitate on vortex mixer for 1 min. Heat for 5 min in 55°C water bath. Inject, on column, 800 µL of the vial's headspace gases into GC. (*Caution:* do not permit any liquid contained in vial to be injected into column). Repeat standard determinations until response is reproducible—that is, at least 4 injections in a row that yield peak areas within 10% of each other. An average of the usable injections is used in the calculations.

Pipette 0.5 mL sample solution into a 5 mL vial containing 100 µL concentrated HCl and determine DMA and TMA as above.

For TMAO, pipette 0.5 mL sample solution into a 5 mL vial containing 100 µL concentrated HCl. Add a spatula tip amount (ca 175 mg) of powdered zinc, cap, agitate, and heat as above. Add 1.5 mL 15N NaOH, cap, agitate, heat, and inject headspace sample as above.

**Calculation.**—Concentrations of DMA and TMA are each calculated as follows:

$$C_x = \frac{A_x}{A_s} * C_s$$

where  $C_x$  is the concentration of amine in the liquid phase of the sample;  $C_s$ , the concentration of amine in the liquid phase of the standard; and  $A_x$  and  $A_s$  are the corresponding peak areas. TMAO is calculated by subtracting the TMA value from total TMA after reduction, then multiplying the result by 1.28 to express the concentration based on the oxide. All values in the paper are discussed in terms of amount of amine present in the sample, not in the liquid or vapor phase.

**Statistical analysis.**—The general linear models (GLM) procedure of the Statistical Analysis System PC software (version 6.04, SAS Institute, Inc., Box 8000, SAS Circle, Cary, NC 27512) was used to analyze results. Results were interpreted according to the methods of Snedecor and Cochran (23).

#### Results and Discussion

The method initially evaluated was as described by Lundstrom and Racicot (20); it had been successfully used to measure DMA and TMA in a wide variety of fish and seafood products. Under the same conditions, including use of nitrogen phosphorus specific detector (NPD), we could not obtain repeatable responses for a standard concentration of DMA in benzene. The high degree of variability precluded application of this method. Substitution of benzene by 2-propanol as used by Zeisel et al. (24), also produced erratic results. Our change to the more commonly available flame ionization detector (FID) improved repeatability of the DMA response in organic solvents. To lessen problems associated with repeated injections of extraction solvents containing amines and other sample components, we considered a headspace method. Concentrations of amines in the combination fish-meat frankfurters were sufficiently high so that the greater sensitivity of the NPD vs FID was not a factor. This meant that a less rigorous isolation/cleanup procedure might be employed. Miller and coworkers developed an equilibrium vapor analysis method in which an aliquot of the headspace was removed from a sealed screw-cap vial after addition of NaOH and heating (25). Using this approach, we employed a 5 mL reaction vial with Teflon-coated septa and a gas-tight syringe that permitted analysis of the products containing fish without loss of volatile amines.

In addition to the porous polymer, Chromosorb 103, selected for this study, a few other packings were evaluated. One, also a porous polymer, HayeSep B was recommended for separation of  $C_1$  and  $C_2$  amines and ammonia (26). A 3.3 m (10 ft) × 2 mm id glass column packed with 60–80 mesh HayeSep B was operated isothermally at 125°C. Helium carrier flow rate was 20 cc/min. Under these recommended conditions, DMA and TMA had long retention times of >7 min, did not produce sharp peaks, and produced erratic peak areas upon repeated injection of amine standard.

Changing operating conditions failed to improve the per-

**Table 1. Recovery of dimethylamine and trimethylamine in fish-meat frankfurters**

| Amine added, ppm | Av. Recovery, % |            |
|------------------|-----------------|------------|
|                  | DMA             | TMA        |
| 5                | 100.2 ± 4.7     | 94.2 ± 6.9 |
| 50               | 92.1 ± 3.0      | 92.6 ± 5.5 |
| 500              | 88.1 ± 5.2      | 91.7 ± 3.1 |

*n* = 4.

formance of this column. We also tested another packing material, 4% Carbowax 20M/0.8% on KOH, on Carbowax B (27), specifically designed for analysis of volatile amines. After packing and conditioning as recommended, both DMA and TMA were resolved and separated under 4 min with sharp peaks. Unfortunately, amine levels below 5 ppm gave unpredictable results, with the DMA peak often disappearing. Because we needed to measure lower concentrations of this amine, this packing was not suitable. Despite successful application of Chromosorb 103 to determine DMA and TMA in fish and fish products (20, 28), Chromosorb 103 was not entirely problem-free. The columns packed with this material had to be conditioned daily with several injections of amine standards before peak areas reached a plateau and were reproducible. After conditioning, the column could be used the entire day.

Typically, the Chromosorb 103 column could be used for injection of at least 2000 samples and standards. Then, a sudden, unexplained deterioration of the column could be observed in which the DMA peak became very small or disappeared. Conditioning by injection of *n*-nonylamine to reduce the active sites did not improve column performance with respect to DMA repeatability. On occasion, new columns conditioned overnight at 250°C with carrier gas flow exhibited similar behavior and necessitated repacking. We found Chromosorb 103 to be the best column packing available for our use.

With the GC operating conditions described in the *Experimental* section, monomethylamine, DMA, and TMA were well resolved with retention times of 1.4, 2.3, and 2.8 min, respectively. Only the latter 2 volatile amines were quantitated in the samples tested. A peak for acetone, the solvent used to clean the syringe, was occasionally observed at 5.2 min.

The GC chromatographic detector linearity response to DMA and TMA was calculated from the means of triplicate determinations from standards ranging from 1 to 1000 ppm. This would cover anticipated concentrations in the product type tested. Calibration curves were plotted as amine concentration in ppm vs peak area. For  $y = ax + b$ , DMA had a slope of 0.00777 ppm/unit area and intercept of +9.52 ppm,  $r^2 = 0.997$ ; TMA had  $a = 0.00443$  ppm/unit area,  $b = 4.01$ ,  $r^2 = 0.996$ . Both correlations were highly significant ( $P < 0.001$ ).

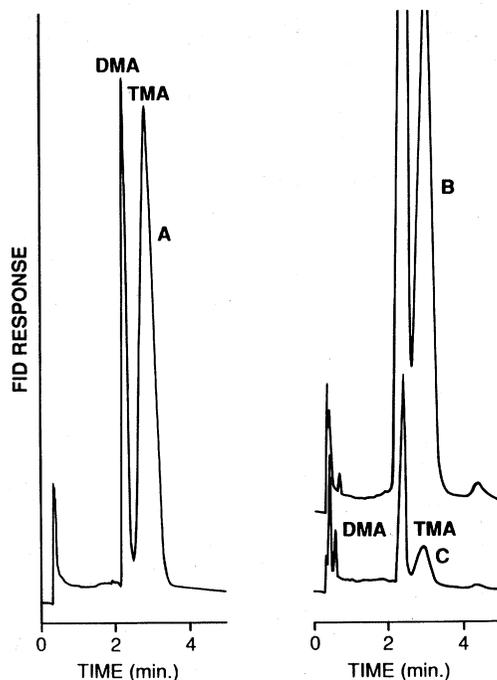
To measure TMAO as TMA and keep the method simple, ca 175 mg Zn powder was added to the sample extract containing 100  $\mu$ L conc. HCl. The acid was needed for the Zn to work effectively. In fish-meat frankfurters fortified with 10, 100, and 1000 ppm TMAO, 100% was converted by Zn to TMA.

Overall recovery studies were performed in duplicate on DMA and TMA where fortification levels, added to samples before analysis, were representative of concentrations found in this type of cured product.

Results of recovery studies are shown in Table 1. These recoveries and standard deviations show that the method was satisfactory for this type of analysis.

Figure 2 shows a chromatogram of (A) a standard containing the equivalent of 10 ppm DMA and TMA; curve (B) is from a frankfurter sample containing 50% unwashed Alaska pollock mince. Because the peak areas were measured, no attempt was made to attenuate the signal to keep peaks on scale. Curve (C) is the corresponding washed mince sample. While not indicated in the figure, these 2 samples also contained 1112 and 207 ppm TMAO, respectively. Concentrations of DMA were higher than TMA in all Alaska pollock samples and for most sample frankfurters containing Atlantic menhaden samples. No interfering peaks were observed in either the fish-meat frankfurters after processing and broiling or in raw fish. This was also true for the TMAO reduced samples, although 2 noninterfering peaks whose retention times corresponded to methanol and ethanol were sometimes noted.

To date, this method has been used to analyze more than 250 samples of minced fish (unwashed and washed) and surimi-meat frankfurters, in which the meat has been substituted at the 15 and 50% levels. The fish source was comprised of Alaska pollock (high amine) and Atlantic menhaden (low amine). This accounts for the wide range of concentrations of DMA, TMA, and TMAO shown in Table 2. Repeatabilities and coefficients of variation were estimated from the error term of the ANOVA. The CV for DMA is acceptable for our uses, given the known difficulty in accurately measuring this amine. The minimum detectable level was 0.1 ppm for DMA and 0.05 ppm for TMA. This method was also applied to all-meat frankfurters and the minced fish and surimi used in the preparation of the frankfurters. Limited analysis indicated that this approach may be effective for analysis of other fish species and seafoods.



**Figure 2. Chromatograms of DMA and TMA: (A) standards (10 ppm), frankfurter sample containing 50% Alaska pollock; (B) unwashed mince (190.5 and 11.4 ppm); (C) washed mince (4.5 and 0.9 ppm, respectively).**

**Table 2. Amine ranges and repeatabilities in fish-meat frankfurters**

| Amine | Range                  | Repeatability, ppm | CV, % |
|-------|------------------------|--------------------|-------|
| DMA   | ND <sup>a</sup> -711.7 | 6.6                | 20.5  |
| TMA   | ND-120.8               | 1.0                | 16.0  |
| TMAO  | ND-1785.7              | 18.8               | 12.7  |

<sup>a</sup> ND = not detectable.

n = 255 samples in duplicate.

### Conclusion

The direct equilibrium headspace GC method described is rapid and easy to perform requiring no organic solvents directly in the analysis and a reasonable array of reagents and equipment readily available in the analytical laboratory. As a result, other preparation and sampling techniques that cause column deterioration are avoided. The method is applicable for routine analysis of methylamines in fish-meat samples and fish.

The authors recommend that this method be evaluated by others as an alternative to other proposed procedures.

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