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

John E. Newell* and Harold R. Sisken

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,

Uniroyal Chemical, Division of Uniroyal, Incorporated, Naugatuck, Connecticut 06770.

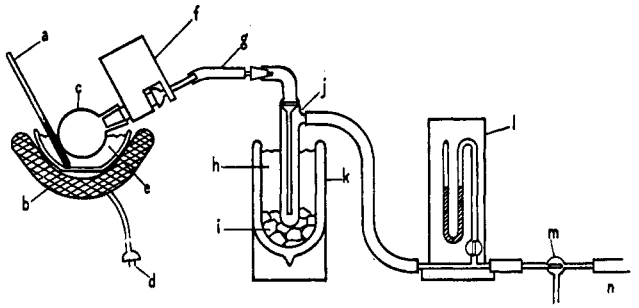

Figure 1. Vacuum distillation apparatus. Key: a. Thermometer; b. Heating mantle; c. 309-ml round-bottomed flask; d. To variable transformer; e. Evaporating dish containing water; f. Rotary evaporator; g. Teflon tubing; h. Acetone; i. Dry Ice; j. Vacuum trap; k. Dewar flask; l. Manometer; m. Three-way stopcock; n. To vacuum pump

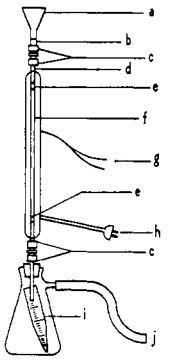

Figure 2. Scavenging assembly. Key: a. Funnel; b. Teflon connection; c. Swagelok fittings; d. Quartz tube; e. Glass wool; f. Porapak Q; g. Thermocouple leads; h. Heater plug-to variable transformer; i. centrifuge tube; j. To mild vacuum

and g-2 and g-3 denote valves having bellows instead of packing or O-rings, designed for leak-tight, moderately high temperature service. Valves g-2 and g-3 and the interconnecting T-joint and tubing were wound with asbestos-covered, No. 23 gauge, Nichrome wire and then covered with a wrapping of asbestos tape. By means of a variable transformer, the valve section was maintained at 50–60°C to eliminate condensation in this zone.

The scavenging column was a 19-in. length of 0.24 in. o.d., 0.16 in. i.d. quartz tube inserted in a 17-in. length of $^3/_8$-in. o.d., $^1/_4$-in. i.d. stainless steel tube wound with asbestos-covered 23 gauge Nichrome wire. A thermocouple was included between the Nichrome winding and the stainless steel tube. The winding was covered with several thicknesses of asbestos tape until the wrapped section was about 1 in. in diameter. The quartz tube had Swagelok fittings on the ends and the middle 11 in. was filled with 100/120 mesh Porapak Q held in place with quartz wool plugs.

The gas chromatograph was a Perkin-Elmer 881 instrument with the flame detector by-passed. The analytical column was a 6-ft × $^1/_8$-in. o.d. stainless steel column filled with 80/100 mesh Porapak Q-S.

Newly packed columns and scavenger tubes were preconditioned by heating to 175°C overnight with a low helium flow.

The analytical column in the chromatograph was connected to a quartz reducing tube in a furnace. The quartz reducing tube, micro reduction furnace, and titration cell enclosure have been described previously by Sisken and Newell (1971).

The detector was a Dohrmann C-200-A microcoulometer with a T-400 titration cell. The microcoulometer was operated with a Range Ohms setting of 100 or 200.

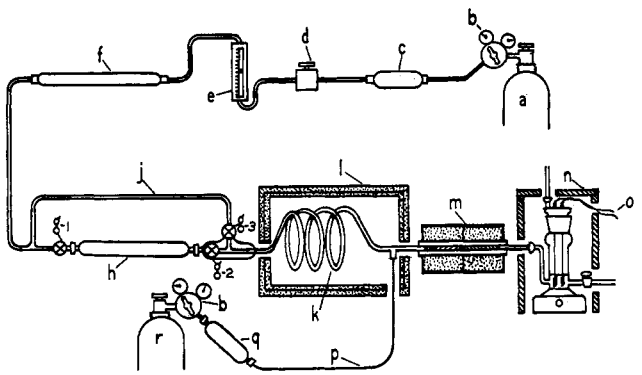

Figure 3. Gas chromatograph–microcoulometric titration assembly. Key: a. Helium; b. Pressure regulator; c. Molecular sieve drier; d. Constant flow regulator; e. Flow meter; f. Porapak Q precolumn; g-1. Valve 1; g-2. Valve 2; g-3. Valve 3; h. 19-in. Porapak Q scavenging column 0.24 o.d. with Swagelok fittings; j. Bypass; k. 6-ft × $^1/_8$-in. Porapak Q-S; l. Programmed temperature oven; m. Two-zone furnace; n. Titration cell; o. Electrical connections to microcoulometer; p. 0.010-in. i.d. S.S. capillary tubing; q. Molecular sieve moistener; r. Ultrapure hydrogen

The carrier gas was "High Purity Helium" (J. T. Baker Co.). Before entry into the chromatograph, the carrier gas passed through a cartridge containing Molecular Sieve 5A and a 23-in. × $^1/_2$-in. o.d. stainless steel tube filled with Porapak Q. This pre-column was conditioned at 150°C with a slow stream of helium passing through it for 16 hr, then cooled to room temperature. When in service, the helium flowed through the precolumn in the opposite direction to its flow during conditioning. Its flow rate was 20 ml/min when the analytical column was at 225°C. The hydrogen was "Ultra High Purity" (Matheson Co.) and its flow rate was 52 ml/min. Both flow rates were measured at the vent stack of the titration cell.

To maintain maximum sensitivity of the analytical column to nitrosodimethylamine, the system was treated to a relatively massive amount (1 μg in 1 μl of water) of NDMA (CAUTION: Carcinogen) at the beginning of each day's work. This was accomplished with a tube similar to the scavenger tube but containing no Porapak Q. The evaporation at 225°C, collection on the analytical column at 80°C, and temperature programming were as described below.

PROCEDURE

A 10-g portion of apple homogenate was weighed into a 300-ml round-bottomed flask containing 2 g of potassium carbonate and 2 ml of water, or 10 g of milk were introduced into the flask which contained 2 g of potassium carbonate. After connection to the rotary evaporator, the flask was partially immersed in a 10–15°C water bath which was a large evaporating dish supported in a heating mantle (Figure 1). The vacuum trap was chilled in a Dry Ice–acetone bath for several minutes, the vacuum line connected, the rotary evaporator tuned on, and vacuum slowly applied until an ultimate pressure of 1 mm was reached in 5 min. The variable transformer connected to the heating mantle was turned to a setting which raised the temperature to 50°C (40°C for milk) in 15 min. During the vacuum distillation, the vacuum was maintained at 1 mm or less.

The temperature of the bath was kept steady and the distillation continued for 30 min for apples. The apple pulp became almost dry. For milk, the distillation was discontinued when about 2 ml remained. Then air was slowly admitted to the system, and the cold trap was removed and warmed to room temperature. The distillate was rendered mildly alkaline (pH 8–9, with pH indicating paper) by adding 2 drops of 2% aqueous K_2CO_3.

Table I. Analytical Results

Untreated	Apples Raw	Apples Cooked	Milk, Raw
ppb NDMA found[a]			
High	1.3	1.7	2.7
Low	0.0	1.0	1.3
Avg	0.7	1.4	1.9
95% confidence limits	1.2		1.2
Number of analyses	12	4	11
Fortified (10 ppb)			
ppb NDMA recovered[b]			
High	12.4	7.1	9.3
Low	8.0	7.1	6.7
Avg	10.2	7.1	7.8
Avg % recovery		71	78
95% confidence limits	3.0		2.3
Number of analyses	12	2	7

[a] Small interference peak, calculated as NDMA. [b] Corrected for untreated sample.

Valves g-1 and g-2 of the apparatus (Figure 3) were closed, valve g-3 was opened, and the scavenger tube was removed from the apparatus. It was connected by a Swagelok fitting to a vacuum flask (Figure 2) and a small funnel was connected to the upper end with Teflon tubing. Using mild vacuum, the distillate was passed through the Porapak Q beads in the quartz tube, followed by 2 ml of water rinsing. The filtration was very slow, taking 30 or more minutes. Then clean air was sucked through the scavenger tube for 3 min.

The tube was removed from the filter flask and the uppermost end was connected to the assembly at valve g-1 (Figure 3), leaving the other end venting to the atmosphere. Valve g-1 was opened to permit helium to flow through the tube. The temperature of the stainless steel sleeve was slowly raised (in 8–10 min) to 100°C to expel water. A cold watch glass or metal sheet was held against the exhaust end and fogging showed the emission of water vapor. The open end of the tube was occasionally dried by insertion of cotton-tipped swabs. After a few minutes at 100°C, the fogging stopped and the heater was turned off and the helium was permitted to flow for another minute. Valve g-1 was closed and the tube was disconnected, reversed, and then reconnected, gas tight, at both ends so that the end to which the funnel had been attached was near valve g-2.

The programmed temperature oven, which had been maintained at 225°C to this point, was now cooled to 80°C and allowed to stabilize. With minimum loss of time, valves g-1 and g-2 were opened, valve g-3 was closed, and the scavenger tube variable transformer was turned on. The temperature of the scavenger tube was raised to 225 ± 5°C quickly and the variable transformer turned back to a setting that would hold this temperature. After 14 min, the recorder was started and the analytical column programmed from 80 to 225°C at 6°C/min. The upper temperature was held for 30 min thereafter. Nitrosodimethylamine recorded as a peak on the chart at a retention time of 26 ± 1 min. At 10 min into the programmed temperature run, the heater on the scavenger tube was turned off and the helium allowed to flow through it as it cooled.

The areas of peaks from the sample and from nitrosodimethylamine standards were found by tracing them on a piece of tracing paper. The peaks were then cut out and weighed. From these areas, the weight of the sample and Range-Ohms setting of the microcoulometer, the parts per billion of NDMA was calculated.

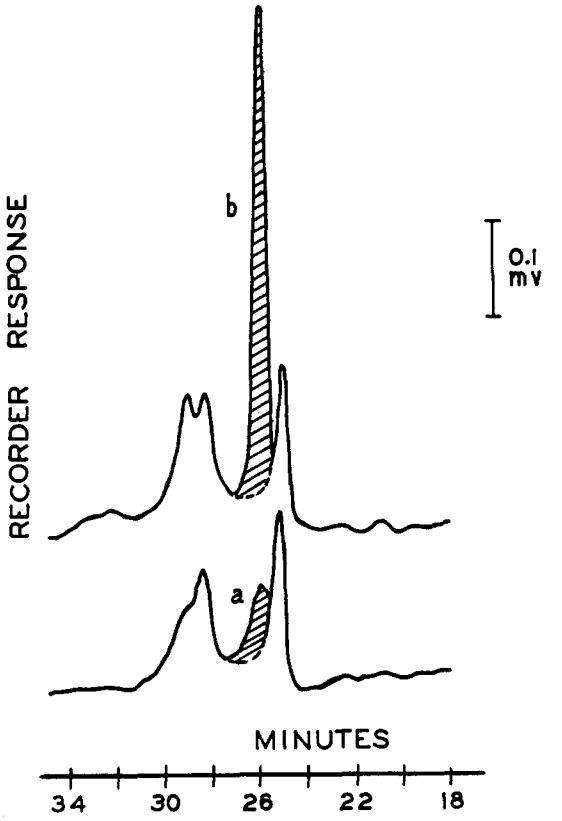

Figure 4. Chromatograms of untreated and NDMA-fortified cooked apples. Chromatogram b has been displaced upward for clarity

RESULTS

The samples of apples came from seven widely separated areas in the United States. Small positive blank results in untreated samples were due to a small peak of the same retention time as NDMA, caused by a naturally occurring nitrogenous component. The milk samples were from six cows. The chromatogram from untreated milk samples also showed a naturally occurring interference. However, no implication is intended that apples or milk contain nitrosodimethylamine. To find the efficiency of recovery, samples of cooked apples and milk were fortified with 10 ppb of NDMA prior to vacuum distillation. The data for raw apples were obtained as a part of an earlier project in which the efficiency of recovery was not measured. Several samples fortified with 10 ppb were analyzed and the average response of the equipment was set at 10. Therefore, the data for raw apples should be evaluated only as an indication of precision. The results of analyses are given in Table I. Typical chromatograms from untreated cooked apples, and cooked apples to which had been added 10 ppb NDMA, are shown in Figure 4.

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