An Improved Procedure for the Determination of Volatile *N*-Nitrosamines in Bacon Grease by Using the Mineral Oil Distillation–Thermal Energy Analyzer Method

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An improved method is described for determining volatile *N*-nitrosamines in rendered bacon grease at the part-per-billion level. A distillation aid comprised of silica gel and kaolin greatly improves *N*-nitrosamine recoveries for the mineral oil distillation procedure. The *N*-nitrosamines are analyzed by gas chromatography with a Thermal Energy Analyzer detector. This procedure is a fast, selective, and reliable method for determining *N*-nitrosamines in bacon grease.

Over the last 10 years, *N*-nitrosamines in the environment have been the subject of intensive investigations. Many environmental and consumer matrixes have been shown to contain measurable levels of *N*-nitrosamines (IARC Scientific Publications).

The presence of *N*-nitrosamines in food systems has especially been of interest. Cured meat products, including bacon, are items of major concern due to the presence of fairly high levels of sodium nitrite. It is generally recognized that *N*-nitrosamines in cured meats are formed during cooking from the reaction of sodium nitrite with naturally occurring amines (Crosby, 1976; Fiddler, 1975; Gray, 1976). An excellent, up-to-date review on the nitrite/*N*-nitrosamine problem in cured meats is given by Gray and Randall (1979) and references therein.

Much of the effort in determining volatile *N*-nitrosamines in bacon has been concerned with the more edible and lean portions. However, bacon grease is used in frying many types of foods, and *N*-nitrosamines are more soluble in the fat (or grease) than in the lean portions of the bacon (Fazio et al., 1973). Several authors have reported that the levels of *N*-nitrosamines in the grease portion are roughly twice the levels found in the bacon (Patterson et al., 1976; Pensabene et al., 1974, 1979; Fazio et al., 1973; Bharucha et al., 1979; Snider and Johnson, 1979). Fat content of bacon ranges from 30 to 85% (Robach et al., 1980; Bharucha et al., 1979; Schroder and Rust, 1974; Cassens et al., 1979), with bacon in the United States averaging about 55% fat before frying.

Several papers have reported the analysis of *N*-nitrosamines in bacon grease by various distillation methods followed usually by gas chromatography–mass spectrometry (GC–MS) for confirmation (Fazio et al., 1973; White et al., 1974; Pensabene et al., 1974; Patterson et al., 1976). The work by White and Pensabene reported recoveries from grease of 60–90% and 70%, respectively. These methods are usually long and require careful sample preparation.

Cross et al. (1978) and Cross and Bharucha (1979) determined *N*-nitrosamines in bacon and bacon grease samples by determining the corresponding amines by thin-layer chromatography/fluorescent densitometry (TLD). They reported recoveries in grease of approximately 84% for *N*-nitrosodimethylamine (NDMA) and 74% for *N*-nitrosopyrrolidine (NPYR). This procedure is attractive because it is less expensive than the GC–MS method, but there are several time-consuming steps involved.

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Snider and Johnson (1979) reported recoveries of *N*-nitrosamines in bacon grease of 50% using a photoelectroanalyzer. Sample preparation was done by the relatively fast and simple mineral oil distillation method of Fine et al. (1975a).

We report here a rapid, simple, and reliable method for determining volatile *N*-nitrosamines in bacon grease using mineral oil distillation followed by analysis with the Thermal Energy Analyzer (Fine et al., 1975b).

EXPERIMENTAL SECTION

Safety Note. Many *N*-nitrosamines have been shown to be carcinogenic to laboratory animals, and should therefore be considered suspect to man. Direct contact with these chemicals should be avoided. Safety gloves should be worn whenever *N*-nitrosamines are being handled. If possible, obtain standards which have been diluted considerably (e.g., to 100 μg/mL) to avoid handling neat materials. All experimental work should be done in a hood or well-ventilated area.

Isopentane is very volatile and extremely flammable. Great care should be taken when handling this solvent. Keep away from open flames and sparking electrical devices, especially when the solvent is at or near room temperature.

Reagents. Diethylene glycol (for oil bath), mineral oil, and isopentane were used as received from Fisher Scientific Co. Methylene chloride (Distilled-in-Glass, Burdick and Jackson Laboratories, Muskegon, MI) was also used. The methylene chloride was checked for purity by injection into the GC–TEA system. Silica gel (6–16 mesh, sieved to ca. 10 mesh) and kaolin (N.F.) were obtained from Fisher Scientific Co. Analytical standards were purchased from Thermo Electron Corp., Waltham, MA. The stock standard solution was a six-component mixture containing 10 μg/mL each of *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosomorpholine (NMOR) in isooctane. The stock internal standard solution was 10 μg/mL *N*-nitrosodipropylamine (NDPA) in isooctane.

Apparatus. Glassware and other hardware used for distillations and concentrations were similar to those described for the mineral oil distillation procedure (Fine et al., 1975a). PREPTUBES (Thermo Electron Corporation, Waltham, MA) were used in the extraction step instead of separatory funnels, sodium sulfate, etc.

Metal containers filled with diethylene glycol and large enough to surround the boiling flask were used as oil baths. The oil was heated by Corning PC-35 hotplates.

The GC–TEA system was comprised of a Hewlett-

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Packard Model 5710A gas chromatograph coupled to a Thermal Energy Analyzer Model 502/LC (Thermo Electron Corporation, Waltham, MA) via a $^1/_8$-in. glass-lined stainless steel transfer line. The GC column was a 4 m × 3 mm i.d. glass column packed with Carbowax 20M + 5% KOH on 80/100 mesh Chromosorb W (Varian Associates). Raw data were collected and processed by a Hewlett-Packard Model 3353 Lab Automation System. A Linear Instruments Model 361 strip chart recorder was used to record the chromatograms. A Sunbeam Vista electric skillet and a Kitchen Aid Model K5-A grinder were also employed.

Working Standards. Solutions of the mixed standard contained 0.05 μg/mL, 0.1 μg/mL, 0.2 μg/mL, 0.5 μg/mL, and 1.0 μg/mL, with each one containing 0.4 μg/mL of the internal standard, NDPA.

Preparation of Distillation Aid. For promotion of smooth boiling and gradual release of moisture during the distillation process, a distillation aid was prepared by mixing two parts by weight each of kaolin and silica gel in a large, preweighed evaporating dish. One part by weight water was added slowly from a disposable pipet and the slurry thoroughly mixed with a spatula. The mixture was air-dried until about a 25% water loss, on the basis of amount of water added, was observed. This drying process took approximately 2 h depending on room temperature and humidity conditions. The mixture was stored in a tightly sealed jar. The resulting mixture had a water content of about 15% w/w determined after weighing a small portion before and after drying in an oven at 175 °C. This mixture has remained stable (water content) for over 6 months.

Skillet Calibration. The electric skillet was calibrated using a thermocouple attached to a Teflon paddle. Approximately 0.25-in. bacon grease (from blank bacon—no nitrite or nitrate used in curing) or a suitable cooking oil was placed into the skillet. The thermostat was set to 340 °F (171 °C). The temperature was recorded every minute while stirring in a "figure 8" motion. High and low temperatures were noted, and the thermostat was adjusted to produce a minimum temperature of 171 °C. The oil was poured off and the excess was removed with paper towels. The procedure was repeated at least two times daily if bacon was being cooked all day.

Bacon Frying. The skillet was turned on and the temperature allowed to cycle at least 10 min. As many strips of bacon as possible were placed into the skillet without overlapping but with room for turning. The bacon was fried on each side for 3 min, removed, and drained on paper towels. The remaining grease was decanted into a glass beaker (preventing as much loose, carmelized material from coming over as possible), covered with aluminum foil, and placed in a freezer at −30 °C.

Distillation, Extraction, and Concentration. The method of Fine et al. (1975a) as modified by Robach et al. (1980) was used for distillation, extraction, and concentration. Twenty-five grams of cold bacon grease was placed into a distillation flask. One gram of the distillation aid was added. Mineral oil was then added to the flask and the distillation was begun. After distillation, the cold trap containing the distillate was rinsed with methylene chloride, and the solution was poured into a prewashed Preptube for extraction. The methylene chloride extractant, containing the N-nitrosamines, was collected in a Kuderna–Danish concentrating apparatus. The sample was concentrated and brought to 0.5 mL with methylene chloride, transferred into a 1-mL conical-shaped vial with Teflon-lined cap, and placed in a freezer (−30 °C). A vibrating device (e.g., Wen Electric Pencil Engraver) attached to the support rod for the microconcentrator aided in keeping the boiling solution from bumping, preventing possible loss of sample.

Table I. N-Nitrosopyrrolidine Recoveries for Grease Extractions Using Silica Gel–Kaolin Distillation Aid

N-nitrosopyrrolidine spiking concentration, ppb	N-nitrosopyrrolidine recovery, %
4	67 ± 14
10	73 ± 12
15	77 ± 10
22	73 ± 10

Table II. Concentrations of N-Nitrosopyrrolidine in Bacon and Bacon Grease

sample	bacon, ppb[a]	grease, ppb[a]
1	5	13
2	8	12
3	4	9
4	4	7
5	2	4
6	12	26
7	3	4
8	8	22

[a] Not corrected for recovery.

GC–TEA Analysis. Quantitative determination of the volatile N-nitrosamines was conducted by using the coupled GC–TEA system under the following conditions: GC carrier gas and flow rate, helium at 40 mL/min; GC injection port temperature, 150 °C; GC column temperature, 180 °C isothermal; TEA GC pyrolyzer furnace, 425 °C (Hansen et al., 1979); TEA reaction chamber pressure, 1.5 torr; TEA attenuation, as appropriate; ice bath temperature, −160 °C (isopentane/liquid nitrogen slush bath); GC–TEA heated transfer line, 175 °C. The strip chart recorder was operated at 1 cm/min.

An internal standard program in the chromatography data system was used to calculate the N-nitrosamine concentrations in the samples.

RESULTS

The average recoveries using the silica gel–kaolin distillation aid for various levels of N-nitrosamine spiking (given as concentrations based on 25 g of bacon grease) are presented in Table I. Recovery was determined as described below by injecting N-nitrosopyrrolidine into the distillation flask prior to distillation. In most cases, spikes at each level were analyzed in triplicate.

These recoveries are lower than those using blank bacon as a distillation aid but are considerably higher than those in the absence of any distillation aid (see Discussion). The recoveries are consistent with most of those obtained by the workers cited earlier.

Table II presents some typical results for various bacon samples (analyzed by the mineral oil distillation–TEA procedure) and their corresponding greases (analyzed by the same procedure with addition of the silica gel–kaolin distillation aid). Recoveries for the bacon samples averaged 92%, while those for the grease samples averaged 78%. Recoveries were determined by spiking known amounts of N-nitrosopyrrolidine into the distillation flask containing 25 g of grease sample prior to distillation. A portion of the same sample was run simultaneously without spiking. The difference between results of the spiked and unspiked samples was compared to the known amount spiked to obtain the recovery value. Spiking experiments were usually analyzed in triplicate. Note that in most cases, the concentrations of N-nitrosopyrrolidine in the

grease samples are roughly twice those found in the bacon samples.

DISCUSSION

We first began analyzing for *N*-nitrosamines in bacon grease by using the mineral oil distillation procedure of Fine et al. (1975a). Recoveries were extremely low, ranging from 10 to 17% for *N*-nitrosopyrrolidine. After several attempts with consistently low recoveries, we noticed that during the distillation stage the distillation mass gave little or no bubbling or foaming, much unlike that for bacon samples. A driving force was apparently needed to remove the volatile nitrosamines.

We added a magnetic stirring bar and stirred the mixture rapidly during distillation. This procedure increased recoveries to levels ranging from 10% to 42%. A stream of nitrogen bubbled through the mixture increased recoveries to the 40% to 55% range.

Since the presence of bacon caused the distillation to proceed smoothly with even boiling and foaming, small amounts of "blank" bacon (bacon made with no sodium nitrite) were added to the distillation flask. Recoveries increased significantly, ranging from 90% to 115%. The use of "blank" bacon as a distillation aid seemed ideal. However, certain questions regarding such usage could arise, especially since *N*-nitrosamine levels in meat are a regulatory concern. Therefore, an alternative distillation aid was needed.

We noticed that several milliliters of water are distilled over during the distillation of bacon samples. The role of water therefore seemed important in carrying over the volatile *N*-nitrosamines. Bacon is composed of about 30–40% water (Pensabene et al., 1979). Much of this water, however, is removed during the cooking process (Bharucha et al., 1979).

Very little water would be expected in grease samples due to its lipid nature. Water added directly to grease samples boiled off immediately after the vacuum and heat were applied, with little or no improvement in the recovery. We concluded that although water was important for efficient distillation of volatile *N*-nitrosamines, it must be somehow physically combined with the sample matrix and slowly released throughout the distillation process.

A matrix was sought which contained some water and which would release this water slowly throughout the distillation. Previous experience with silica gel indicated that it might be an ideal distillation aid.

Silica gel and kaolin both have large capacities for water. Both materials are very hygroscopic. In addition, the water is rather difficult to remove unless heat and/or vacuum is applied. Both heat and vacuum are gradually applied during the mineral oil distillation step. Therefore, the use of silica gel and kaolin seemed to offer the desired characteristics.

CONCLUSIONS

Because the mineral oil distillation method is relatively quick and simple to use, it is ideal as a sample preparation procedure when the samples are analyzed by the TEA. The TEA is a very sensitive and selective detector for *N*-nitrosamines when used in conjunction with a gas or liquid chromatograph (Fine et al., 1975b). Although the cost of the TEA is relatively high compared to some other detectors, it is certainly less expensive than a GC–MS system. The TEA is becoming more readily available in many academic, governmental, and industrial laboratories. The procedure described in this paper using a silica gel–kaolin mixture as a distillation aid offers a simple and reliable method for determining volatile *N*-nitrosamines in bacon grease samples.

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