ACKNOWLEDGMENT

We thank Joan B. Shumsky for her secretarial aid.

LITERATURE CITED

Anderson, H. J.; Lee, S. F. *Can. J. Chem.* **1965**, *43*, 409.
Chang, S. S.; Peterson, R. J. *J. Food Sci.* **1977**, *42*, 298.
Dumont, J. P.; Roger, S.; Adda, J. *Lait* **1974a**, *54*, 31.
Dumont, J. P.; Roger, S.; Cerf, P.; Adda, J. *Lait* **1974b**, *54*, 243.
Fishbein, L. *Sci. Total Environ.* **1979a**, *11*, 111.
Fishbein, L. *Sci. Total Environ.* **1979b**, *11*, 163.
Ho, C.-T.; Carlin, J. T.; Lee, K. N.; Hsieh, O. A.-L.; Hwang, L. S.; Chang, S. S. "Book of Abstracts", 182nd National Meeting of the American Chemical Society, New York, Aug 23–28, 1981; American Chemical Society: Washington, DC, 1981; AGFD 49.
Ho, C.-T.; Coleman, E. C. *J. Agric. Food Chem.* **1981**, *29*, 200.
Hodge, P.; Rickards, R. W. *J. Chem. Soc.* **1965**, 459.
Jackman, L. M. "Application of Nuclear Magnetic Resonance Spectroscopy on Organic Chemistry"; Pergamon Press: London, 1959; p 62.
Kahn, J. H.; Nickol, G. B.; Conner, H. A. *J. Agric. Food Chem.* **1972**, *20*, 214.

Received for review June 12, 1981. Revised manuscript received October 28, 1981. Accepted October 28, 1981. This work was performed as part of NJAES Project No. 10508, supported by the New Jersey Agricultural Experiment Station. This research was partially supported by the Rutgers Research Council. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903.

A Rapid Liquid–Liquid Extraction Cleanup Method for the Determination of Volatile *N*-Nitrosamines in Cooked-Out Bacon Fat

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A rapid liquid–liquid extraction method is described for the determination of volatile nitrosamines in cooked-out bacon fat. The method consists of partitioning of the nitrosamines between *n*-hexane and an acidic aqueous–methanol mixture containing small amounts of sulfamic acid. An aliquot of the aqueous phase is then extracted with dichloromethane, the dichloromethane extract concentrated, and an aliquot of the concentrated extract analyzed by a GLC–thermal energy analyzer. The average percentage recoveries of *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosopiperidine, *N*-nitrosopyrrolidine, and *N*-nitrosomorpholine when added to cooked-out bacon fat or lard at levels ranging between 5 and 20 ppb were 78.8, 77.8, 89.4, 100.3, and 97.4, respectively. The method has an overall detection limit of 1 ppb for each of the above five nitrosamines. The average levels (uncorrected) of *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine detected in the 11 samples of cooked-out bacon fat were found to be 4.8 and 21.1 ppb, respectively.

Studies during the past 10 years have shown that fried bacon is one of the food products that consistently contains traces of volatile *N*-nitrosamines (simply called nitrosamines), mainly *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosodimethylamine (NDMA). These studies (Sen, 1980) have also indicated that the concentration of volatile nitrosamines in the cooked-out bacon fat is approximately twice that present in the cooked bacon. Since both NDMA and NPYR are potent carcinogens, it would be desirable to have a rapid and sensitive analytical method for monitoring their presence in these and other food items. Although a variety of methods are presently available (Preussmann et al., 1978) for this purpose, many of them are too lengthy and some need expensive instrumentation such as high-resolution mass spectrometry. Of the available methods, the combined gas–liquid chromatographic–thermal energy analyzer (GLC–TEA) technique seems to be best suited for routine monitoring. In the GLC mode, the TEA detector is highly sensitive and specific for nitrosamines (Fine et al., 1975b) and has the added advantage that it can also be operated in the high-pressure liquid chromatographic (HPLC) mode which is sometimes useful for confirmation of the GLC data. The detector is expensive but not beyond the reach of most modern laboratories.

Because of the extremely high specificity of the TEA detector, it is not always necessary to carry out extensive sample cleanup prior to the end determination by GLC–TEA. For example, two rapid Celite column cleanup methods for the determination of volatile nitrosamines in fried bacon (Fiddler and Pensabene, 1980) and beer (Hotchkiss et al., 1981) have already been reported. The above-mentioned method for fried bacon, however, was reported to be unsatisfactory for the analysis of cooked-out bacon fat. It should also be noted that the mineral oil distillation method, which is used by many laboratories (Fine et al., 1975a; Havery et al., 1978) for the analysis of a variety of foods for their nitrosamine contents, has been reported to produce inconsistent and low results (as low as 17% recovery) when applied to cooked-out bacon fat (Owens and Kinast, 1980). We report here a rapid sample workup procedure suitable for the analysis of this product.

EXPERIMENTAL SECTION

Samples. The samples were purchased locally in the Ottawa area. Bacon slices were fried in a Teflon-coated electric frypan (340 °F setting) as described previously (Sen et al., 1979). After the slices were fried, the

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0021-8561/82/1430-0364$01.25/0

cooked-out fat was carefully decanted into a sealed jar, cooled to room temperature, and then stored at −20 °C until ready to analyze.

Apparatus and Reagents. All reagents used were of analytical grade, and the solvents were "distilled in glass" type. "Glass-distilled" dichloromethane (DCM) was purchased from Caledon Laboratories, Georgetown, Ontario, and was redistilled from an all-glass apparatus before use.

Acidic Methanol–Water–Sulfamic Acid Reagent (Hereafter Called "Methanolic Extraction Solvent"). This reagent was prepared fresh daily by mixing methanol plus water plus 1% sulfamic acid plus 3 N sulfuric acid (v/v) (50:40:8:2).

Reagent Blank. So that the absence of contamination could be ensured, a reagent blank was carried out with each batch of reagents.

Nitrosamine Standards. All except *N*-nitrosoazetidine (NAZET) were purchased from commercial suppliers (Fisher Scientific Co.; Aldrich Chemicals). The NAZET standard was a gift from Dr. W. Fiddler of the U.S. Department of Agriculture, Philadelphia, PA.

GLC–TEA System. A Varian gas chromatograph (Model 2700) coupled to a TEA detector (Model 502) was used for the analysis of volatile nitrosamines. The GLC conditions were as follows: column, 6 ft × 1/8 in (o.d.) Ni tubing packed with 20% Carbowax 20 M and 2% NaOH on 80–100-mesh Chromosorb P, acid washed: carrier gas, Ar, 30 mL/min; GLC oven temperature, 170 °C; injector port temperature, 220 °C; TEA furnace temperature, 450 °C; TEA vacuum chamber pressure, 1 mm; TEA cold trap, stainless steel trap, immersed one-third in liquid nitrogen; recorder, 1-mV span.

Procedure. The frozen or solidified bacon fat was melted in a hot water bath and mixed well. A 10-g aliquot was weighed in a 150-mL beaker and mixed immediately with 80 mL of *n*-hexane, and the mixture was transferred quantitatively to a 250-mL separatory funnel by using another 20 mL of *n*-hexane for rinsing the beaker. One-milliliter of NAZET internal standard (100 ng/mL in ethanol), and 100 mL of methanolic extraction solvent were added to the contents of the separatory funnel, and the mixture was shaken *vigorously for 5 min*. The entire contents were transferred (without rinsing) into a polyethylene-stoppered centrifuge tube and centrifuged for 5 min at about 2000 rpm.

A 50-mL aliquot of the bottom aqueous layer was carefully removed by using a 50-mL pipet and transferred into another 250-mL separatory funnel. About 25 mL of 1 N KOH solution was added to the contents of the separatory funnel, and the mixture was extracted with two 75-mL portions of DCM. The combined DCM extracts were dried by passing through a bed of 40 g of anhydrous sodium sulfate, and the dried extract was concentrated to 1.0 mL by using a Kuderna-Danish concentrator as described previously (Sen and Seaman, 1981).

GLC–TEA Analysis. The attenuation of the TEA detector was adjusted so that an injection of 30 pg of NDMA gave a definite peak (at least 3 times the noise level). By use of this setting (usually an attenuation of 4), 6-μL aliquots of the above concentrated extract were analyzed. The amount of various volatile nitrosamines present in the extract and the percent recovery of the added NAZET standard were calculated by comparison of peak heights obtained with the various standard solutions and the sample extract. The presence and concentrations of the following nitrosamines were studied: NDMA, *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA), *N*-nitrosodibutylamine (NDBA), NAZET, *N*-nitrosopiperidine (NPIP), NPYR, and *N*-nitrosomorpholine (NMOR).

RESULTS AND DISCUSSION

Twelve different samples of cooked-out bacon fat were analyzed by using the proposed method, and the results were compared with those obtained by an aqueous vacuum distillation method which has been used in this laboratory during the last 8 years (Sen et al., 1974, 1976, 1978, 1979). This distillation procedure has been shown to give reliable and accurate results in several international collaborative studies (Castegnaro and Walker, 1978, 1980). As can be seen in Table I the agreement between the two sets of data is quite good. The average percent recovery of the added internal standard in the rapid liquid–liquid extraction method was much superior to that obtained in the vacuum distillation method (Table I). This is to be expected because, in the latter method, there are always some losses of the volatile nitrosamines during the distillation step. However, the vacuum distillation method produces a much cleaner final extract than the rapid method and, therefore, would be the method of choice if mass spectrometric confirmation is to be carried out.

Recovery studies, using the liquid–liquid partitioning technique, were carried out on cooked-out bacon fat from nitrite-free bacon or lard spiked with seven volatile nitrosamines. These results (Table II) show good recoveries for five nitrosamines (NDMA, NDEA, NPIP, NPYR, and NMOR), which had been reported to occur in cooked-out bacon fat. These recovery values are comparable or better than those obtained with a modified mineral oil distillation method (Owens and Kinast, 1980). These workers reported an average recovery of 73% for NPYR at a spiking level of 10 ppb; they did not carry out recovery studies with other volatile nitrosamines.

Although the method gave poor or no recovery for NDPA and NDBA, for reasons explained below, this drawback would not limit the usefulness of the method since these two compounds seldom occur in cooked-out bacon fat or fried bacon. In fact, none of the samples analyzed by the aqueous vacuum distillation method, which works equally well for all the seven volatile nitrosamines in this study, contained any detectable levels of NDPA or NDBA. The minimum detection limit of the overall method is about 1 ppb (by using an attenuation setting that gives a signal to noise ratio > 3 for 15 pg of NDMA) for each of the five nitrosamines mentioned above.

The method is based on liquid–liquid partitioning of various volatile nitrosamines between the lipophilic hexane phase and the hydrophilic aqueous methanol phase. The partition coefficients (Eisenbrand et al., 1969) of most of the common volatile nitrosamines favor distribution in the aqueous phase. Only NDPA and NDBA, because of their high fat solubility, are not extracted into the aqueous–methanol phase. Dilute sulfuric and sulfamic acids were incorporated in the extraction medium to destroy any nitrosating agents which might otherwise lead to the artifactual formation of nitrosamines during the analysis. A sample of bacon fat was analyzed after the addition of 50 ppm of nitrite and 10 ppm of morpholine (one added immediately after the other). No evidence of artifactual formation of NMOR could be observed. Also, several samples of cooked-out bacon fat, which on previous analysis were shown to be negative for NMOR, were reanalyzed with the addition of 10 ppm of morpholine. Again, the results were negative for NMOR in all cases. Therefore, it is highly unlikely that the method is prone to artifactual formation of nitrosamines.

Table I. Comparison of Results by This Method with Those Obtained by an Aqueous Vacuum Distillation Method (Sen et al., 1979)

		levels of various volatile nitrosamines detected					
		this method			vacuum distillation method		
brand	nitrosamine	uncorrected, ppb	% recovery of NAZET	corrected, ppb	uncorrected, ppb	% recovery of NAZET	corrected, ppb
1	NDMA	11.5	79.1	14.5	9.4	76.7	12.2
	NPYR	32.3		40.8	32.9		42.9
2	NDMA	4.1	88.4	4.6	3.9	69.8	5.6
	NPYR	26.1		29.5	25.5		36.5
3	NDMA	3.2	100.0	3.2	2.0	75.8	2.6
	NPYR	8.6		8.6	7.2		9.5
4	NDMA	7.8	88.9	8.8	6.6	67.8	9.7
	NPYR	43.9		49.4	38.6		56.9
	NMOR	trace[a]		trace	trace		trace
5	NDMA	2.6	93.0	2.8	2.2	79.0	2.8
	NPYR	8.4		9.0	8.4		10.6
6	NDMA	1.6	–[b]		1.3	–	
	NPYR	5.2	–		4.3		
7	NDMA	6.4	94.3	6.8	4.8	62.9	7.6
	NPYR	36.8		39.0	27.3		43.4
8	NDMA	11.4	75.0	15.2	8.7	84.0	10.4
	NPYR	33.5		44.7	29.6		35.2
	NMOR	7.1		9.5	5.3		6.3
9	NDMA	9.9	108.0	9.2	9.0	80.0	11.2
	NPYR	18.6		17.2	14.7		18.4
10	NDMA	3.0	108.0	2.8	2.7	89.6	3.0
	NPYR	28.4		26.3	26.0		29.0
11	NDMA	1.9	108.0	1.7	1.8	77.3	2.3
	NPIP	trace		trace	trace		trace
	NPYR	25.5		23.6	17.8		23.0
12[d]	NDMA	ND[c]	91.9	0	ND	85.7	0
	NPYR	ND		0	ND		0
		mean recovery:	94.0			77.1	

[a] <2 ppb. [b] Omitted by mistake. [c] ND = none detected (detection limit 1 ppb). [d] From bacon made without any added nitrite.

Table II. Percentage Recoveries of Various Volatile Nitrosamines Added to Cooked-Out Bacon Fat or Lard

sample	spiking level, ppb of each nitrosamine	% recoveries by this method						
		NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
cooked-out fat from bacon made without nitrite	10	78.3	70.8	34.7	0	74.2	89.7	80.0
cooked-out fat from bacon made without nitrite	5	95.9	100.0	38.8	0	88.0	114.0	108.0
cooked-out fat from a bacon[a] that contained very low levels of nitrosamines	20	80.2	74.4	36.9	0	103.6	106.0	103.6
lard[b]	10	68.6	–[c]	–	–	–	98.2	–
lard[b]	10	70.9	65.9	32.3	0	91.7	93.6	98.0
	overall mean:	78.8	77.8	35.7	0	89.4	100.3	97.4

[a] Nitrosamine levels in the unspiked sample were subtracted before calculating recoveries. [b] Negative for nitrosamines. [c] Not spiked.

Table III. Precision of the Method As Determined by Five Replicate Analyses of a Cooked-Out Bacon Fat

		NDMA level, ppb		NPYR level, ppb	
no. of determinations	% recoveries of NAZET internal standard	uncorrected	corrected for % recovery of NAZET	uncorrected	corrected for % recovery of NAZET
1	82.9	3.4	4.1	9.9	11.9
2	87.8	3.3	3.8	10.4	11.8
3	78.0	3.1	4.0	9.0	11.5
4	98.5	3.2	3.2	10.8	11.0
5	92.2	3.1	3.4	9.7	10.5
mean ± SD	87.9 ± 8.0	3.2 ± 0.13	3.7 ± 0.39	10.0 ± 0.69	11.3 ± 0.59
CV,[a] %	±9.0	±4.0	±10.5	±6.9	±5.2

[a] Coefficient of variation.

Table III gives the precision of the method as determined by five replicate analyses of a sample. It should be of interest that for NDMA, the coefficient of variation of the uncorrected results (±4.0%) was slightly lower than that (±10.5%) of the corrected values, whereas the corresponding values for NPYR were much closer (6.9% vs. 5.2%). This may be due to the slight differences in the partition coefficients of different nitrosamines in the

system used in this study. Nevertheless, a coefficient of variation of 10.5% at a level of 3.7 ppb (for NDMA) can be considered highly satisfactory.

Alternatively, one may determine average percentage recoveries of each of the volatile nitrosamines of interest and use these values (instead of NAZET recoveries) for correcting the individual results. But, since the percentage recovery of a nitrosamine may vary slightly from sample to sample (see Table II), this method of calculation would not be 100% accurate either. Therefore, the correction method suggested above (using NAZET recoveries) is a simpler and reasonable approach. It should give results within 10–15% of the true values.

In this study, the average levels of NDMA and NPYR in the first 11 cooked-out bacon fat samples (Table I) were respectively 4.8 and 21.1 ppb as compared to average levels of 6.4 ppb of NDMA and 21.9 ppb of NPYR determined in a 1978 survey (Sen et al., 1979). It appears, therefore, that the levels of volatile nitrosamines in cooked-out bacon fat have not changed appreciably during this time.

In conclusion, the liquid–liqiuid partitioning procedure is very simple and rapid and does not involve the use of any cumbersome and complicated distillation as a part of the sample preparation step. The entire procedure can be completed within 1.5–2 h. If proper fume hood and handling facilities are available, a large number (8–12) of samples can be analyzed within a working day. It is hoped the technique will be useful for rapid screening of cooked-out bacon fats for the presence of various volatile nitrosamines, mainly NDMA and NPYR.

Safety Note. Since most volatile nitrosamines are strong carcinogens, adequate precautions should be taken in handling these chemicals.

ACKNOWLEDGMENT

We thank Dr. Walter Fiddler of the U.S. Department of Agriculture, Eastern Regional Research Laboratories, Philadelphia, PA, for providing us with the NAZET standard.

LITERATURE CITED

Castegnaro, M.; Walker, E. A. *IARC Sci. Publ.* **1978**, *No. 19*, 53.
Castegnaro, M.; Walker, E. A. *IARC Sci. Publ.* **1980**, *No. 31*, 445.
Eisenbrand, G.; Marquardt, P.; Preussmann, R. *Fresenius Z. Anal. Chem.* **1969**, *247*, 54.
Fiddler, W.; Pensabene, J. W., Eastern Regional Research Laboratories, U.S. Department of Agriculture, Philadelphia, PA, personal communication, 1980.
Fine, D. H.; Rounbehler, D. P.; Oettinger, P. E. *Anal. Chim. Acta* **1975a**, *78*, 383.
Fine, D. H.; Rufeh, F.; Lieb, D.; Rounbehler, D. P. *Anal. Chem.* **1975b**, *47*, 1188.
Havery, D. C.; Fazio, T.; Howard, J. W. *IARC Sci. Publ.* **1978**, *No. 19*, 41.
Hotchkiss, J. H.; Harvey, D. C.; Fazio, T. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 929.
Owens, J. L.; Kinast, O. E. *J. Agric. Food Chem.* **1980**, *28*, 1262.
Preussmann, R.; Castegnaro, M.; Walker, E. A.; Wassermann, A. E. *IARC Sci. Pub.* **1978**, *No. 18*, 1–212.
Sen, N. P. In "Safety of Foods"; Graham, H. D., Ed.; AVI Publishing Co.: Westport, CT, 1980; pp 319–349.
Sen, N. P.; Donaldson, B.; Seaman, S.; Iyengar, J. R.; Miles, W. F. *IARC Sci. Publ.* **1978**, *No. 19*, 373.
Sen, N. P.; Iyengar, J. R.; Donaldson, B.; Panalaks, T.; Miles, W. F. *IARC Sci. Publ.* **1974**, *No. 9*, 49.
Sen, N. P.; Iyengar, J. R.; Miles, W. F.; Panalaks, T. *IARC Sci. Publ.* **1976**, *No. 14*, 333.
Sen, N. P.; Seaman, S. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 933.
Sen, N. P.; Seaman, S.; Miles, W. F. *J. Agric. Food Chem.* **1979**, *27*, 1354.

Received for review May 8, 1981. Accepted November 5, 1981.

Degradation Products (*Z*)-11-Hecadecenal and (*Z*)-9-Tetradecenal, Components of a Sex Pheromone of the Tobacco Budworm

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Degradation of two components of virelure [(*Z*)-11-hexadecenal and (*Z*)-9-tetradecenal] occurred in hexane solution in sealed tubes under air and in fluorescent light. The products were isolated by column chromatography and preparative gas–liquid chromatography and identified by a combination of gas chromatography and infrared, nuclear magnetic resonance, and mass spectrometry. Both virelure components were degraded by the same major mechanisms—oxidation to cis and trans epoxides, oxidation of the aldehyde to an acid, and subsequent decarboxylation. A total of 12 degradation products were characterized from each compound.

Two components of the pheromone of the tobacco budworm, *Heliothis virescens* (F.), were isolated, identified, synthesized, and reported to be (*Z*)-11-hexadecenal [(*Z*)-11-HDAL] and (*Z*)-9-tetradecenal [(*Z*)-9-TDAL] (Roelofs et al., 1974; Tumlinson et al., 1975). The female tobacco budworm moth contains these two compounds in the approximate ratio of 16 parts of (*Z*)-11-HDAL to one part of (*Z*)-9-TDAL, and this ratio has been used to formulate an attractant (virelure) for field trapping studies to monitor insect populations (Hendricks et al., 1977). Permeation of the air with sufficient quantities of these chemicals also has potential for preventing males from locating and orienting to individual pheromone-releasing females (Gaston et al., 1967; Shorey et al., 1967). Klun et al. (1980a) isolated and identified five additional compounds from heptane washes of ovipositors of tobacco budworms and determined that a mixture of the seven chemicals exceeded the attractiveness of four virgin females. Klun et al (1980b) also identified four chemicals from ovipositors of the corn earworm moth, *Heliothis zea*, and (*Z*)-11-HDAL comprised 90–95% of this pheromone. Preliminary observations in our laboratory suggested that

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