Determination of Volatile N-Nitroso Compounds in Various Samples of Edible Vegetable Oils and Margarine (Commercially Available Products)

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ABSTRACT

The examination of samples of various commercially available vegetable oils (olive oil, sunflower oil, thistle oil, linseed oil, plant germ oil, etc.) and of various samples of margarine for the presence of volatile N-nitroso-compounds yielded the following results. By means of the above mentioned procedure (gas liquid chromatography – AFID gas liquid chromatography – TEA), N-nitrosodimethylamine (NDMA) was found to be present in 21 of 61 different samples of vegetable oil, in concentrations ranging from < 1 μg/kg to 23 μg/kg. 18 samples contained N-nitrosodiethylamine (NDEA) in concentrations varying between < 1μg/kg and 27.8 μg/kg. 37 out of 107 different samples of margarine were shown to contain N-nitroso compounds. N-nitrosodimethylamine was found to be present in 15 samples. The range of concentrations determined was between < 1 μg/kg and 5.8 μg/kg. 33 samples contained N-nitrosodiethylamine in concentrations varying between < 1 μg/kg and 7.5 μg/kg.

INTRODUCTION

It has become apparent in recent years that traces of volatile N-nitroso compounds, which are potent carcinogens, may occur in certain foodstuffs, especially in cured meat and fish products. In 1972 we reported on the presence of N-nitrosodimethylamine in one sample of soybean oil (1). This sample had been treated by Kaunitz et al. (Columbia University, Department of Pathology, New York) as follows. The soybean oil was transmethylated with sodium methylate in methanol. After methylation, the methyl esters of fatty acids were converted into urea aducts, and the nonadduct-forming material was recovered and concentrated. Nitrosodimethylamine (NDMA) was determined by gas liquid chromatography with a nitrogen selective flame ionization detector. The identity of the compound was confirmed by Dr. Fales (National Institute of Health, Bethesda) with the aid of mass spectrometry.

This paper is concerned with the examination of samples of various commercially available vegetable oils and of various samples of margarine for the presence of volatile N-nitroso compounds.

EXPERIMENTAL

Concentration of the N-nitroso Compounds

The samples (100 g) were steam-distilled from 350 ml 0.2 n sodium hydroxide in the presence of sodium chloride. The approximately 100 ml of distillate obtained were treated with sodium chloride and extracted with twice the quantity of dichloromethane; the dichloromethane extract was dried thoroughly with anhydrous sodium sulfate. Silica gel PF_{254} (1 g) was added to the absolutely dry dichloromethane extract, and the dichloromethane was removed by distillation in a rotary evaporator. The extraction of the compounds absorbed by the silicalgel was carried out in a Soxhlet apparatus using 100 ml ethyl acetate. The ethyl acetate extract was then concentrated to a measured volume of 5-10 ml by cold vacuum treatment in a rotary evaporator.

Gas Liquid Chromatography – Flame Ionization Detector (AFID)

The N-nitroso compounds were determined by gas liquid chromatography using a nitrogen selective sensitized flame ionization detector under the following conditions: Column A, 20% Carbowax 20M on Chromosorb WAW 80/100 mesh. Column B, 10% SP1000 on Chromosorb WAW 100/120 mesh. Carrier gas was helium. The temperature was raised from 80 to 160 C at 20 C per min, then held at 160 C for 4 min.

The evaluation of the extracts was performed with the aid of an electronic digital integrator. A solution containing 0.36 ng of NDMA, and 0.34 ng of NDEA per μl in ethyl acetate was used as a standard.

N-nitroso compounds exposed to ultraviolet light (360 nm) decompose to the corresponding dialkylamine and nitric oxide (2). In order to obtain further preliminary

TABLE I

Concentrations of NDMA and NDEA in Various Samples of Edible Vegetable Oils

Vegetable Oil	Number of samples	Concentration <1 μg/kg		Concentration 1-10 μg/kg		Concentration > 10 μg/kg		Highest concentration μg/kg	
		NDMA	NDEA	NDMA	NDEA	NDMA	NDEA	NDMA	NDEA
Olive oil	16	1	1	7	2	—	—	3.5	3.6
Sunflower oil	11	—	1	1	1	1	—	11.3	9.3
Plant germ oil	6	—	—	2	2	1	1	14.8	27.8
Linseed oil	3	—	—	—	—	—	—	—	—
Thistle oil	4	—	—	2	2	—	—	7.1	7.0
Soybean oil	5	—	—	3	4	1	1	20.0	19.0
Vegetable oil without special declaration	16	—	—	1	2	1	1	23.0	21.0
	61	1	2	16	13	4	3	23.0	27.8

TABLE II

Recoveries for NDMA and MDEA from Thinlayer Chromatograms (Silicagel)

Sample	Amount applied µg		Recoveries RF 0.2-0.4 %		Recoveries RF 0.4-0.6 %		Theoretical RF	
	NDMA	NDEA	NDMA	NDEA	NDMA	NDEA	NDMA	NDEA
Standard	1.80	1.70	34.2 ± 6.9 n=6	—	—	29.4 ± 6.8 n=5	0.24	0.49
Extract from vegetable oils	0.70	1.14	40.4 ± 7.0 n=12	—	—	28.4 ± 5.1 n=5		
Extract from margarines	1.08	3.06	32.8 ± 5.7 n=5	—	—	40.2 ± 2.1 n=5		

TABLE III

Concentrations of NDMA and NDEA Obtained by 2 Detector Systems:
a) AFID (Prior to and Following Irradiated with UV light 360 nm)
b) TEA

Sample	AFID		TEA	
Sample	NDMA	NDEA	NDMA	NDEA
Mixture of extracts from vegetable oils	0.35 µg/ml	0.57 µg/ml	0.17 µg/ml	0.37 µg/ml
Plant germ oil	14.8 µg/kg	27.8 µg/kg	10.1 µg/kg	17.1 µg/kg
Plant germ oil	4.0 µg/kg	2.6 µg/kg	5.1 µg/kg	10.4 µg/kg
Sunflower oil	1.8 µg/kg	—	1.2 µg/kg	1.4 µg/kg
Olive oil	3.3 µg/kg	—	1.9 µg/kg	1.7 µg/kg
Margarine	2.5 µg/kg	5.5 µg/kg	1.7 µg/kg	1.9 µg/kg
Margarine	3.0 µg/kg	1.4 µg/kg	3.3 µg/kg	4.1 µg/kg
Margarine	5.8 µg/kg	—	5.6 µg/kg	4.1 µg/kg

evidence as to the identity of these compounds, parts of the extracts exhibiting peaks corresponding to the retention times of the N-nitroso compounds were exposed to ultraviolet light (360 nm 24 hr) in a closed quartz glass. Both the unirradiated and the irradiated extract were compared with the standard. Peaks corresponding to the N-nitroso-compounds have to be disappeared from the chromatograms of the irradiated extracts.

Preparative Thinlayer Chromatography

Standard and extracts of the oils and margarine were further separated by thin layer chromatography, and the chromatograms were developed using hexane/ether/dichlormethane (4:3:2) (3). The areas corresponding to the R_f values of NDMA and NDEA were extracted with ethyl acetate. Prior to and following irradiation with ultraviolet light the extracts were again evaluated by gas liquid chromatography.

Gas Liquid Chromatography – Thermal Energy Analysis (TEA)

In addition to the AFID, certain extracts of vegetable oils and of margarine were tested using the N-nitroso group selective thermal energy analyzer (4). The tests were carried out by Preussmann et al. (Deutsches Krebsforschungszentrum, Institut für Toxikologie und Chemotherapie, Heidelberg, F.R.G.).

RESULTS

Determination of NDMA and NDEA in Various Samples of Vegetable Oils

The recoveries of 1.8 µg NDMA and 1.7 µg NDEA obtained from spiked samples of vegetable oils by means of the above mentioned procedure (n = 13) amount fo 61.6 ± 12.5 % and 68.5 ± 17.9%, respectively.

The group of 61 samples consisting of olive oil, sunflower oil, plant germ oil, linseed oil, thistle oil, and soybean oil were treated by the method described above.

In gas liquid chromatograms, some of the final extracts exhibited peaks corresponding to the retention times of NDMA and NDEA. These peaks disappeared from the chromatograms of the irradiated extracts. Exposure to UV light had no effect on peaks of compounds with other retention times. The concentrations of NDMA and NDEA determined are shown in Table I. N-nitrosodimethylamine and N-nitrosodiethylamine were found to be present in concentrations of more than 1 µg/kg in 32.8 and 26.2% of the investigated oils, respectifully. Plant germ oil and soybean oil seem to be favored to contain N-nitroso compounds.

Determination of N-nitrosodimethylamine and N-nitrosodiethylamine in Various Samples of Margarine

Vegetable oils are the source for the manufacture of margarine. According to this fact, it seemed to be necessary to investigate various samples of margarine for the presence of N-nitrosodimethylamine and N-nitrosodiethylamine.

The recoveries of 1.8 µg NDMA and 1.7 µg NDEA obtained from spiked samples of margarine (n = 6) amount to 62.2 ± 10.2% and 63.3 ± 7.5% respectively.

107 samples of 35 different products were cleaned up corresponding to the method. In gas liquid chromatograms 15 of the extracts exhibited peaks corresponding to the retention time of NDMA (9 with < 1 µg/kg; 6 between 1 and 10 µg/kg and 5.8 µg/kg as the highest) and 33 of the extracts exhibited peaks corresponding to the retention time of NDEA (24 with < 1 µg/kg; 9 between 1 and 10 µg/kg and 7.5 µg/kg as the highest). These peaks disappeared from the chromatograms of the irradiated extracts. Only 5.6% and 8.4% of the investigated samples were shown to contain NDMA and NDEA, respectively, in concentrations of more than 1 µg/kg.

Preparative Thinlayer Chromatography

Extracts from vegetable oils and margarine were sepa-

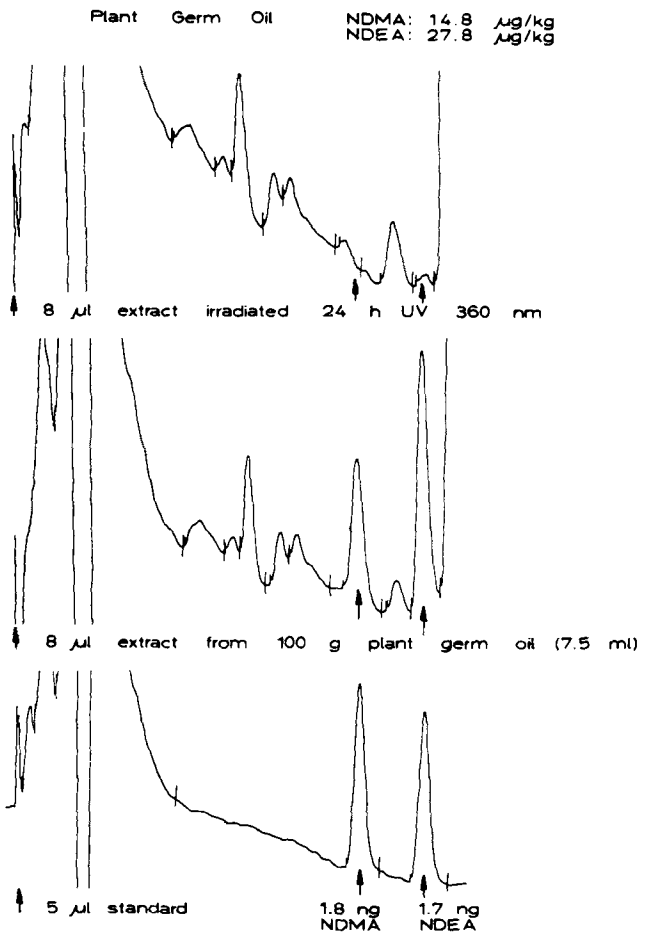

FIG. 1.

rated by preparative thinlayer chromatography in comparison with NDMA- and NDEA-containing standard solutions to provide further evidence of the presence of N-nitroso compounds. Areas corresponding to the R_f values of NDMA and NDEA were eluted and again evaluated by gas chromatography (AFID). The results of these investigations are shown in Table II.

These results reveal that the separation of standard and extracts by TLC leads to substantial losses of NDMA and NDEA. Average recovery of the compounds used was ca. 35%. This is due to the volatility of these substances. On the other hand, both in the standard and in the extracts, NDMA and NDEA were only found in the areas corresponding to the R_f values of these N-nitroso compounds, after separation by TLC. This result can be regarded as additional information concerning the identity of the N-nitroso compounds contained in the extracts.

Results by Gas Liquid Chromatography — Thermal Energy Analysis

Following separation by gas chromatography, the thermal energy analyzer (TEA) was used to examine certain extracts, in addition to the alkali flame ionization detector (AFID). The results of these investigations are shown in Table III.

Thus, the presence of NDMA and NDEA in the samples examined was qualitatively confirmed by means of the thermal energy analyzer. With regard to NDMA, even the quantities obtained from both detectors were comparable.

Figure 1 shows chromatograms of standard extract prepared from a vegetable oil (plant germ oil) and the same extract exposed to UV light (AFID). The irradation of the

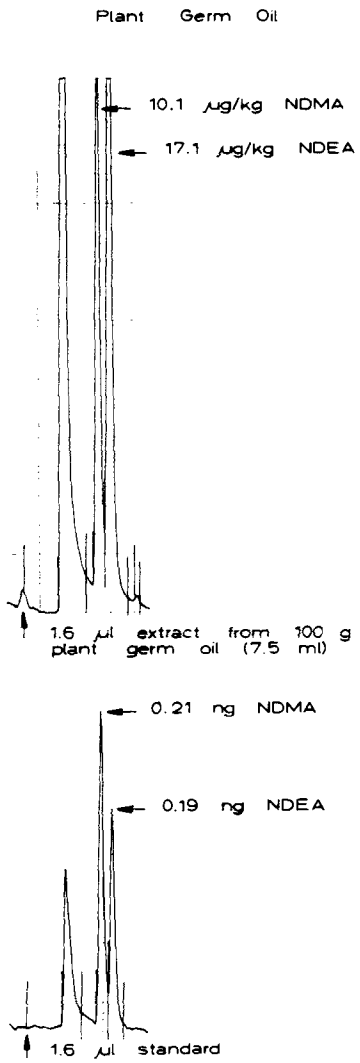

FIG. 2.

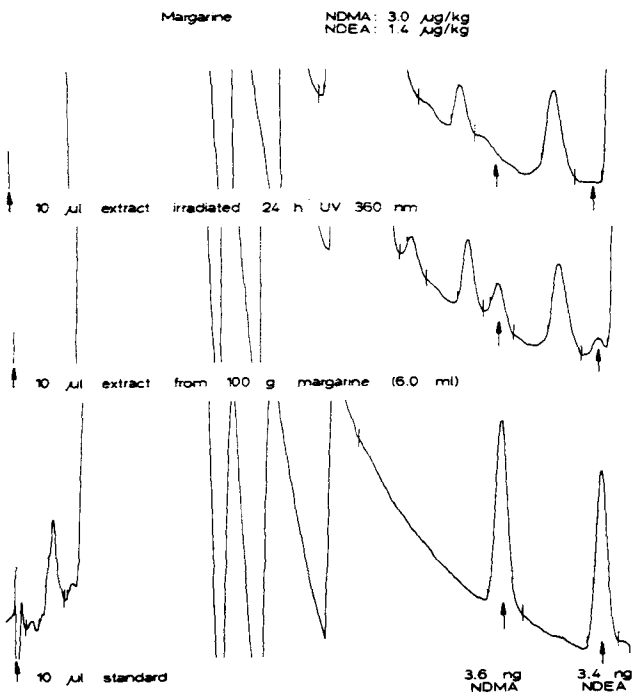

FIG. 3.

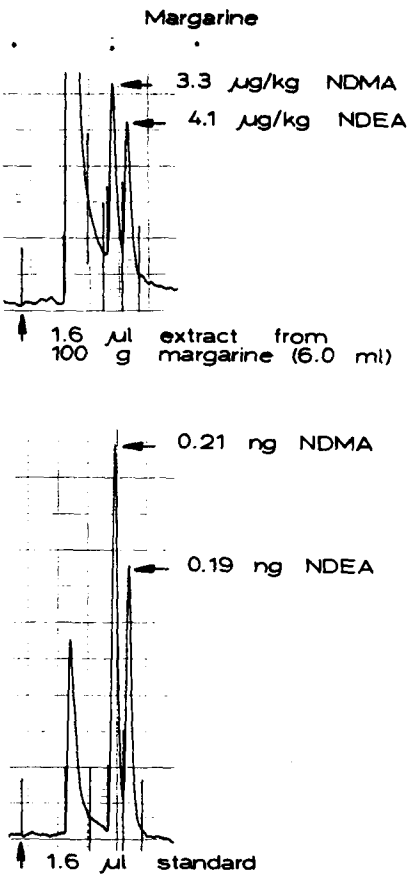

FIG. 4.

extract at 360 nm for 24 hr causes disappearance of the two peaks corresponding to NDMA and NDEA.

Figure 2 shows the chromatogram of the same extract examined with the thermal energy analyzer, and Figure 3 demonstrates chromatograms of standard extract prepared from a sample of margarine and the same extract exposed to UV light (AFID).

UV irradation of the extract (360 nm, 24 hr) causes disappearance of the two peaks corresponding to NDMA and NDEA. The chromatogram of the same extract examined with the thermal energy analyzer is shown in Fig. 4.

The comparative investigation of vegetable oils and margarine have revealed that the apparent occurrence of the N-nitroso compounds is predominant in the vegetable oils.

ACKNOWLEDGMENTS

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