

and C-6), 121.7 (d, C-5), 41.8, and 27.4 (t, $-\text{CH}_2-$), 34.1 and 27.0 (d, $-\text{CH}-$), 22.5, 17.9, and 11.6 (q, CH_3-).

Hydrolysis of Pigment 2. Working in the same way as described for pigment 1, neoaspergillilic acid (4) was isolated: MS, molecular ion peak at m/e 224 (8), fragment ions at m/e 207 (33), 193 (20), 182 (42), 166 (80), 153 (20), 123 (100); $^1\text{H NMR}$ (CDCl_3 , 100 MHz) δ 0.9–1.1 [12 H, $(\text{CH}_3)_2\text{CH}-$], 2.22 [2 H, m, $(\text{CH}_3)_2\text{CH}-$], 2.72 (4 H, m, $-\text{CH}_2\text{CH}-$), 7.35 (1 H, br s, H-5), 9.92 (1 H, $-\text{NOH}$).

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 For note X see Assante et al. (1981).

An Investigation into the Possible Presence of Volatile *N*-Nitrosamines in Cooking Oils, Margarine, and Butter

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Following a report of the occurrence of volatile nitrosamines in various vegetable oils and margarines in Germany, a study was carried out to determine the nitrosamine levels in such products sold in retail outlets in Canada. Thirty-eight samples of various vegetable oils, ten of butter, fourteen of margarine, and six of lard were analyzed. All the oils and lard were negative. Only one butter and five margarine samples contained trace levels (0.2–3.8 ppb) of *N*-nitrosodimethylamine and/or *N*-nitrosomorpholine. Further investigations at the plant level failed to uncover any definite source of the nitrosamine contamination detected in some of the margarine samples. Recent samples of margarine from these plants were, however, either negative or contained insignificant levels of volatile nitrosamines. It was concluded that nitrosamine levels in these products are either negative or negligible and, therefore, should not be a matter of concern.

Studies carried out during the last 10 years have shown that traces of certain volatile *N*-nitrosamines (simple called nitrosamines), which are potent carcinogens, are present in various foods and beverages such as cured meats, fish, cheese, fried bacon, instant skim milk powder, malt, beer, and whiskies (Fazio et al., 1979; Goff and Fine, 1979; Pensabene et al., 1974; Spiegelhalder et al., 1979; Hotchkiss et al., 1979; Sen et al., 1979). The nitrosamines in the above-mentioned products are believed to be formed by the interaction of amines and added nitrite or gaseous nitrogen oxides in hot flue gas used for the commercial processing of these items.

Recently, Hedler et al. (1979) of Germany reported the occurrence of fairly high levels (up to 27.8 ppb) of *N*-nitrosodimethylamine (NDMA) and/or *N*-nitrosodiethylamine (NDEA) in various vegetable oils such as olive oil, soybean oil, plant germ oil, sunflower oil, and a few unspecified vegetable oils. Of particular interest is the fact that a high percentage of the olive (8/16), soybean (5/5), and plant germ (6/6) oils analyzed were found to be positive for nitrosamines. Other oils were found to contain these nitrosamines only occasionally. To our knowledge, this appears to be the only documented evidence for the presence of nitrosamines in these products. The source of the contamination or the mechanism of formation of the

two nitrosamines is, however, unclear. In addition, the same workers noted the presence of NDMA (up to 5.8 ppb) and/or NDEA (up to 7.5 ppb) in 37/107 margarine samples examined.

The Canadian Health Protection Branch has been monitoring the situation since 1974. A brief summary of our up-to-date findings is presented in this report.

EXPERIMENTAL SECTION

Samples. Most of the samples were purchased in the Ottawa area and analyzed within 1–2 weeks. Some of the samples of margarine, butter, boiler additives, and miscellaneous ingredients of margarine were directly obtained from manufacturing plants located in various parts of Canada.

Nitrosamine Analysis. Since the study was carried out in two stages (1974 and 1980), the techniques used for nitrosamine analysis varied considerably between the two studies. These are described as follows.

(a) *1974 Study.* A mixture consisting of a 50-g aliquot of the oil and 350 mL of 3 N KOH was boiled under a reflux condenser for 6 h, and the mixture was then distilled (at atmospheric pressure) until ~200 mL of distillate was collected. The distillate was made alkaline and extracted with dichloromethane (DCM), and the DCM extract cleaned up on a basic alumina column as described previously (Sen, 1978). The purified extract was carefully concentrated (by using a Kuderna-Danish type concentrator) to 1.0 mL, and a 5–10- μL aliquot was analyzed by

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gas-liquid chromatography using a Coulson electrolytic conductivity detector (GLC-CECD) operating in the pyrolytic mode (Sen, 1978). It should be noted that in a few cases a small amount (1 g) of antifoam "A" (Dow Corning) was added to the oil-3 N KOH mixture to prevent excessive foaming. The detection limit of the overall method was ~ 10 ppb for NDMA and NDEA. The 1974 study was limited to the analysis of oils only; no margarine or butter was analyzed.

(b) 1980 Study. A 20-g aliquot of the sample (oil, butter, margarine, or other ingredients) was analyzed by the aqueous vacuum distillation method from 3 N KOH or 1% sulfamic acid as previously described in detail (Sen et al., 1979). The end determination was carried out by gas-liquid chromatography using a thermal energy analyzer (GLC-TEA) detector (Fine et al., 1975; Sen et al., 1979) which is highly sensitive to and specific for *N*-nitroso compounds. The detection limit of the overall method was about 0.2 ppb for NDMA and NDEA and 0.5 ppb for *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosomorpholine (NMOR). Since the GLC-TEA technique is highly specific for *N*-nitroso compounds, there was no need to clean up the sample extracts by alumina column chromatography. Also, no antifoam was used during vacuum distillation of the samples. Appropriate reagent blanks were regularly carried out to eliminate the possibility of contamination from reagents used in the study.

For the analysis of steam distillates, boiler water, and KOH additives (as obtained from various food processing plants), the aqueous vacuum distillation step was omitted. Instead, the samples were made alkaline (if necessary), extracted with DCM, and then processed in the usual manner.

High-Pressure Liquid Chromatographic-TEA (HPLC-TEA) Analysis. The identity of NDMA and NMOR in two samples of margarine was independently verified by HPLC-TEA analysis. The conditions used were as follows: column, 250 \times 2.1 mm Lichrosorb Si60 (5 μ m); solvent, 5% acetone in *n*-hexane; flow rate of solvent, 0.5 mL/min; TEA slush bath, acetone plus dry ice (-80°C); sample size, 50–100 μ L; attenuation, 8 or 16.

Storage Studies. Various oils were spiked with known levels of different nitrosamines, mixed well, and then allowed to stand in the dark at room temperature in a glass-stoppered flask for 1–4 weeks. The stored samples were then analyzed in the usual manner. The spiking levels in the two studies (1974 and 1980) ranged between 10 and 200 ppb for each nitrosamine. These studies were carried out to demonstrate the efficiency of the analytical procedures used to recover traces of nitrosamines from these products.

RESULTS AND DISCUSSION

In the 1974 study, a total of seven samples of various oils (four soybean oils, two soy plus rapeseed oils, and one mixed vegetable oil) were analyzed; all were negative. Various storage studies carried out (Table I) indicated good recoveries (80–90%) for both NDMA and NDEA. Although the detection limit of the method used at that time was much higher than that of the GLC-TEA method, any NDMA and NDEA if present at >10 -ppb levels would have been detected.

In the 1980 study a total of 31 oils were examined. These consisted of 11 samples of olive oil, 4 of soybean oil, 5 each of corn and sunflower oils, and 2 each of mixed vegetable, peanut, and hydrogenated soybean oils. Again, no NDMA or NDEA was detected (detection limit 0.2 ppb) in any of the samples tested. Percentage recoveries of

Table I. Percentage Recoveries of Added Nitrosamines from Various Cooking Oils after Storage

oil ^a	spiking level, ppb	storage period, ^b days after spiking	method used for analysis	% recoveries	
				NDMA	NDEA
mixed vegetable oil	200	30	GLC-CECD	80	80
soybean oil	200	30	GLC-CECD	90	95
corn oil	10	6	GLC-CECD	94	95
soybean oil	10	6	GLC-TEA	97	116
sunflower seed oil	10	11	GLC-TEA	103	105
corn oil	10	12	GLC-TEA	93	101
olive oil	10	14	GLC-TEA	86	95
soybean oil	10	14	GLC-TEA	82	91

^a All the unspiked oils were negative. ^b Stored in glass-stoppered flasks at room temperature in the dark.

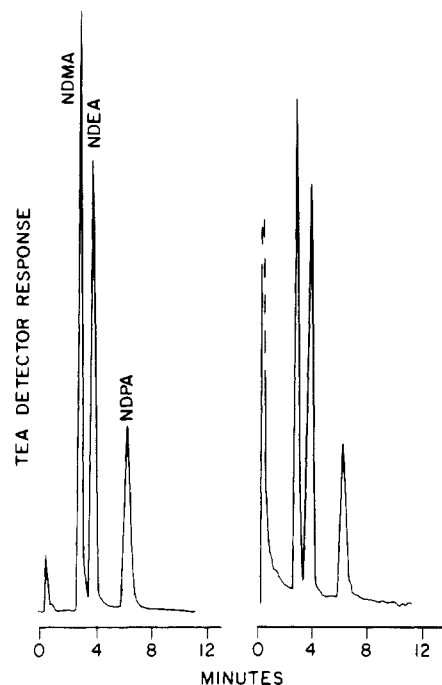


Figure 1. GLC-TEA diagram: (left) 1 ng of each standard nitrosamine; (right) 5 μ L/1.0 mL extract of a spiked (10 ppb for each nitrosamine) soybean oil analyzed after 14 days of spiking and storage at room temperature in the dark.

NDMA and NDEA each added to various oils (Table I) at a level of 10 ppb and stored in the dark for 6–14 days ranged between 82 and 116%. It should be noted that these recovery values are comparable or better than those observed by the above-mentioned German researchers, who reported an average recovery value of 61.6% for NDMA and 68.5% for NDEA at a spiking level of ~ 17 ppb for each of two nitrosamines. A typical GLC-TEA diagram obtained from the analysis of a spiked soybean oil is shown in Figure 1.

In addition to the cooking oils 10 samples of butter, 14 of margarine, and 6 of lard were analyzed for the presence of volatile nitrosamines. The results are given in Table II. As can be seen, most of the butter and all the lard samples were negative, but five of the margarine samples contained traces of NDMA and/or NMOR. Since these levels were too low to be confirmed by mass spectrometry, the identity of NDMA and/or NMOR in two samples (Table II) was independently verified by HPLC-TEA. Positive response by both the GLC-TEA and HPLC-TEA

Table II. Volatile Nitrosamine Contents of Butter, Margarine, and Lard Analyzed in the Study

kind	no. of positive/ total no. of samples analyzed	nitrosamine levels ^{a,b}	
		GLC-TEA	HPLC-TEA
butter	1/10	(a) NMOR, 1 ppb	
margarine ^b	5/14	(a) NMOR, 2.6 ppb	NMOR, 2.5 ppb
		(b) NDMA, 0.3 ppb	MDMA, 0.2 ppb
		NMOR, 1.7 ppb	NMOR, 1.7 ppb
		(c) NDMA, 0.2 ppb	
		(d) NMOR, 2.2 ppb	
		(e) NMOR, 3.8 ppb	
lard	0/6		

^a None of the results were confirmed by GLC-mass spectrometry. ^b More recent samples either gave negative results or contained insignificant levels of NDMA or NDEA; none contained NMOR.

techniques can be taken as a reliable proof for the presence of a particular nitrosamine (Fan et al., 1978).

The above results suggested that traces of nitrosamines could be present in some samples of margarine, but the source of the contamination was not apparent. One of the possible sources was thought to be the amine-containing boiler water additives which are commonly used in food processing plants. These amines could either serve as precursors of the nitrosamines or contain the nitrosamines as impurities. The nitrosamines could have also originated from various ingredients of margarine. An attempt was, therefore, made to uncover the source of the contamination. As a follow-up study (carried out ~1 year later), various ingredients of margarine (e.g., lard, vegetable oils, whey powder, etc.), boiler water additives, boiler water, and steam condensates from boilers were collected from three different plants and analyzed for volatile nitrosamines. At the same time, fresh samples of margarines were also procured from each plant and analyzed for nitrosamine contents. In all three plants investigated all the freshly collected samples of margarines were either negative or contained extremely low levels of (0.2–0.3 ppb) of NDMA or NDEA; none contained NMOR. These levels were extremely low (close to the detection limit of the method). The samples of the boiler waters and the steam condensates were negative. Only two boiler water additives (each from a different plant) were found to contain traces of nitrosamines (4.6 ppb of NMOR in one case and 1 ppb each of NMOR and NDEA in the other). These additives were, however, used only in extremely low concentrations (10 lb/2 × 10⁶ lb of steam), and therefore, nitrosamines would not be expected to be detected in the finished product. The respective companies were notified about

these results, and appropriate corrective actions were taken to remedy the situation.

It should be emphasized that these plants had used different types of boiler water additives in the past and that the composition of various additives may vary from time to time. Therefore, the positive results found a year earlier could have been due to combination of many factors which are difficult to pinpoint. The boiler additives used by these plants at the present time are highly satisfactory with respect to their nitrosamine content.

In conclusion, various cooking oils, butter, and lard analyzed in this study appear to be free of nitrosamines. Although traces of nitrosamines were detected in some samples of margarine, the situation seems to have improved a great deal. Our findings on cooking oils differ from those observed by the German workers. The reason for which is unclear at the moment.

Safety Precaution. Since most nitrosamines are potent carcinogens, proper precautions must be taken while handling or working with these compounds. All waste materials containing nitrosamines should be chemically treated (Williams, 1975) in order to destroy any residual nitrosamines.

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