

Investigations of Edible Oils for Volatile Nitrosamines

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ABSTRACT

Twenty-one commercial edible oils and five margarine samples were purchased locally and analyzed for nitrosamines (NAs) by a gas-liquid chromatograph interfaced with a Thermal Energy Analyzer. N-nitrosodimethylamine (NDMA), at levels of 0.22-1.01 ppb, was the only volatile apparent NA detected. These levels were found in 7/7 corn, 6/6 olive, 2/2 sunflower, 1/3 soybean, 2/3 undesignated oils, and 4/5 margarine samples. The other three oils and one margarine sample showed no NDMA at the lower limit of 0.1 ppb. These levels of nitrosamine detected in the edible oils tested were much lower than the levels previously reported.

INTRODUCTION

THE CONSUMPTION of edible vegetable oils has increased dramatically in the past 20 years, while the use of fats from animal sources has concurrently decreased (USDA, 1978). Reasons for this shift include public concern with the role of saturated fats and cholesterol and their etiology in cardiovascular disease. Several studies have suggested a link between a high polyunsaturated fatty acid diet with cancer of the breast, colon, and stomach. However, according to Heyden (1975), none of the data showed a convincing cause and effect of polyunsaturated fatty acids and cancer.

In 1972, Hedler et al. investigated the possible presence of N-nitrosamines (NAs) in soybean oil based on an earlier observation that rats fed soybean oil had an increased incidence of tumors. These investigators reported the presence of 290 ppb of apparent N-nitrosodibutylamine, and 380 ppb N-nitrosodimethylamine (NDMA) which was confirmed by mass spectrometry. After this finding the U.S. Food & Drug Administration conducted a limited survey for NAs in edible oils; no confirmable levels were found (White et al., 1974). In a subsequent study Hedler and Marquardt (1974) detected 15-50 ppb apparent NDMA in the repeated analyses of a single sample of soybean oil but none in several other samples of soybean oil analyzed by GLC. A more thorough study was published by the same group (Hedler et al., 1979) utilizing a number of improved analytical techniques, including GLC-Thermal Energy Analysis (TEA). These investigators found apparent NDMA and N-nitrosodiethylamine (NDEA) in concentrations up to 23 and 28 ppb, respectively, in one-third of the 61 oil samples tested. Because of the contradictory results of previous reports, the potential health hazard posed to consumers of edible oils and the resulting adverse impact on the United States oil seed industry, a study was undertaken by our laboratory to investigate the presence of nitrosamines in these commodities. The results are reported herein.

EXPERIMENTAL

A NUMBER of commercial edible oils and margarine were purchased at local supermarkets. They included six olive oils, seven corn oils, three soybean oils, three undesignated oils, and five

margarines.

Nitrosamine analysis

Volatile NAs in the edible oils was isolated and separated by the method of White et al. (1974) except that N-nitrosomethylethylamine (NMEA) and N-nitrosohexamethylenimine (NHMI) were used as internal standards and the clean-up step with silica gel was deleted. This latter procedure is employed only when the sample is to be analyzed by mass spectrometry. The concentrated extracts were analyzed for NAs with a Varian-Aerograph Model 2700 gas chromatograph containing a 9 ft x 1/8 in. stainless steel column packed with 15% Carbowax 20M-TPA on 60/80 Gas Chrom P and interfaced with a TEA. The helium flow rate was 42 ml/min, the injector port temperature was 220°C, and the oven was programmed from 125 to 220°C at 4°/min. The TEA was operated under conditions similar to those reported by Fine and Rounbehler (1975). The lower limit of detection for the edible oils and margarine was 0.1 ppb or 2.5 ng/25g sample.

Amine analysis

To 100g of the edible oil was added 100 ml dichloromethane. This solution was extracted with 3 x 100 ml of 0.5N HCl. The combined aqueous extracts were then freeze-dried. The residue was dissolved in 5 ml of distilled water and made alkaline with 1N NaOH to a pH of 12-14. The amines were separated and detected with a Hewlett-Packard Model 5710A GLC equipped with a N-P detector and containing a 6 ft x 1/4 in. (o.d.) glass column packed with 12% Amine 220 + 8% KOH on 100/120 Chromosorb WAW. The following conditions were used: injector and detector temperatures, 150 and 250°C, respectively; He flow rate, 15 ml/min; H₂ flow rate, 3.7 ml/min; air flow rate, 100 ml/min; and oven temperature, 60°C. The amines that were analyzed included methylamine, dimethylamine, trimethylamine, ethylamine, and diethylamine. However, after a short period of use, the column packing could no longer resolve methylamine and dimethylamine.

Nitrosating ability of oils

To 100g of the edible oil was added 9.5 mg doubly distilled nitrosamine-free piperidine. The mixture was allowed to stand at room temperature for 7 days and then was worked up by the procedure previously described. The dichloromethane concentrate was analyzed for NAs.

RESULTS & DISCUSSION

INITIALLY, apparent NDMA and N-nitrosomethylvinylamine (NMVA) were detected in several oil samples in concentrations of up to 8 ppb. Although NMVA was not previously reported in edible oils (Hedler et al., 1972; 1979), the precursors for NMVA—trimethylvinylammonium ion, dimethylvinylamine, or methylvinylamine—could possibly arise from the decomposition of choline, which is the nitrogen base of lecithin. However, when the TEA trap temperature was changed from -115°C (ethanol-liquid N₂ slurry) to -196°C (liquid N₂), a profound change in the size of the chromatographic peaks occurred. An example is shown in Figure 1. The concentrations of apparent NAs were reduced significantly by the cold trap. Nonnitrosamines have been shown previously to be sensitive to TEA furnace and trap temperatures (Fiddler et al., 1978). Analysis of the same sample by a second GLC-TEA system equipped with a -115°C trap yielded no apparent NMVA and little NDMA. These results suggested that the apparent NDMA and

NMVA detected were non-NA artifacts.

After an evaluation of possible sources of contamination, it was observed that the TEA-responsive peaks were associated with the degradation of oil components in the GC injection port liner. These TEA responsive compounds, probably unsaturated components, react with ozone to give a chemiluminescent response. Cleaning the liner with organic solvents did not solve the problem, but after it was thoroughly cleaned with chromic acid cleaning solution and steel wool, followed by several solvent washings, tests showed no artifactual peaks (Fig. 2).

The results of the analyses of 21 edible vegetable oils and 5 margarine samples are shown in Table 1. NDMA, at levels of 0.22-1.01 ppb, was the only NA detected. These values were significantly lower than those reported by Hedler et al. (1972; 1979). Low levels of apparent NDMA were detected in all of the corn, olive, and sunflower oils, in one out of three soybean oils, and in four out of five margarine samples.

The refining of edible oils requires a variety of steps. The oil seed is first cooked, then pressed or solvent-extracted to obtain the crude oil. The crude oil may be treated with water to remove the phosphatides (degumming), followed by an alkali wash to remove the free fatty acids, or it can undergo a distillative deacidification procedure. This is followed by bleaching with either natural bleaching earth

or activated carbon, or a heat treatment in the presence or absence of air. The final step is deodorization, which is a vacuum steam distillation procedure (Carr, 1976; Cowan, 1976; Goebel, 1976). Crude edible oils contain variable levels of phosphatides, which, on decomposition or hydrolysis, can liberate amines. It may then be possible for NAs to be formed during the numerous steps involved in the processing of edible oils, since an amine source and a nitrosating species, nitrogen dioxide (an environmental contaminant), may be available. However, the deodorizing step should reduce or remove most of the volatile amine and NA contaminants, if present, to very low levels in the refined edible oil.

Six edible oils were analyzed for low molecular weight amines. The amines—methylamine, dimethylamine, trimethylamine, ethylamine, and diethylamine—when present were detected at concentration of <4 ppb. The potential for spontaneous nitrosation in the same edible oils was determined by adding nitrosamine-free piperidine, allowing the mixture to stand at room temperature for 7 days, and then analyzing for N-nitrosopiperidine (NPIP). The percent conversion of piperidine to NPIP is considered the "nitrosating ability" and this value varied from 0.0001%

Table 1—Volatile nitrosamines in edible oils (ERRC)^a

Type	Positive/total	NDMA, ppb (Corr.)	
		Range	Mean
Corn	7/7	0.22 - 0.84	0.49
Soybean	1/3	0.26	0.26
Olive	6/6	0.23, 0.95	0.51
Sunflower	2/2	0.31, 0.96	0.64
Other	2/3	0.84, 1.01	0.93
(Margarines)	4/5	0.24 - 0.81	0.52

^a Minimum detectable level: 0.10 ppb.

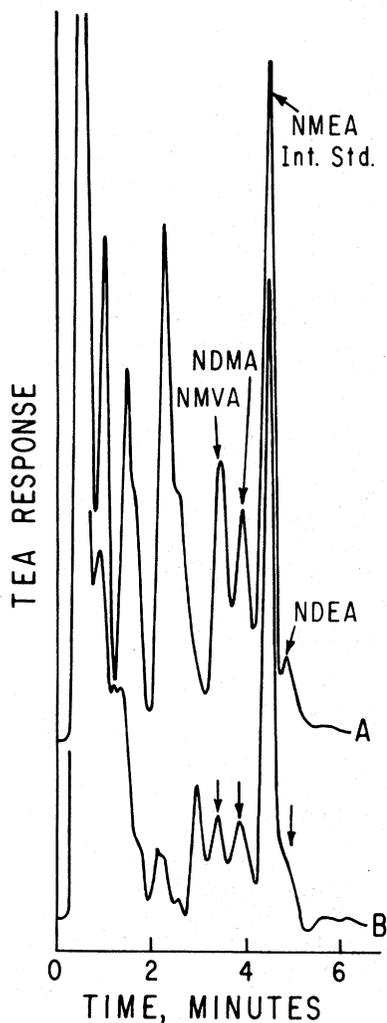


Fig. 1—TEA chromatogram of apparent nitrosamines in olive oil with varying cold trap temperatures: (A) -115°C, (B) -196°C.

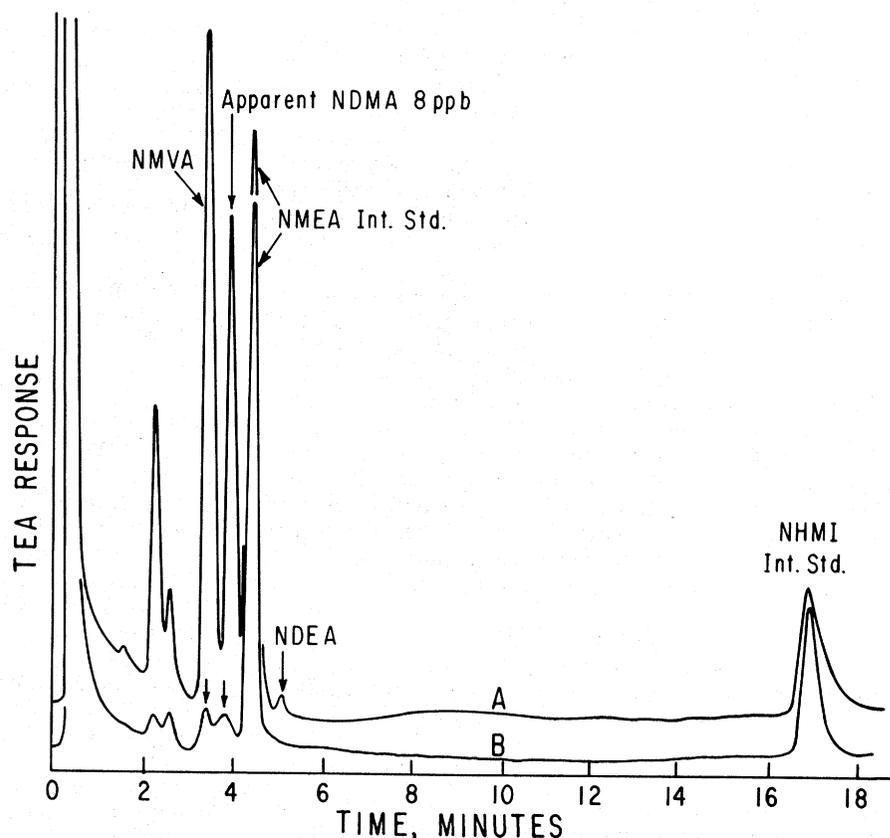


Fig. 2—TEA chromatogram of corn oil extract: (A) before, (B) after injector port liner cleaning.

to 0.006%. Although these values are extremely low, they indicate that nitrosation can occur in these edible oils, but that the concentrations of amines and nitrosating species are present in too low a level in the oils as examined to form appreciable quantities of NAs.

In summary, a variety of domestic and imported brands of edible oils representing a cross section of the different variations in processing, were analyzed for nitrosamines. In all of the oil samples tested, N-nitrosodimethylamine was the only apparent nitrosamine detected and the levels were 1 ppb or less. The results are in agreement with that of White et al. (1974), but not with those of Hedler et al. (1972; 1979). After our investigations were completed, it was called to our attention that in a letter to the editor Preussmann (1980) also indicated doubts about the results found in the paper by Hedler et al. (1979). He suggests, as we do, that contaminations or artifactual formation may have occurred and confirmation by other procedures, like mass spectrometry, are necessary.

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Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

NOTE: Precaution should be exercised in the handling of nitrosamines since they are potential carcinogens.
