Absence of Volatile N-nitrosamines in Margarines

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ABSTRACT

Samples of margarines, butter and vegetable oils were analyzed for volatile N-nitrosamines by the vacuum steam distillation method followed by dichloromethane extraction of the distillate and gas chromatographic-thermal energy analysis (GC-TEA) of the extracts. No volatile nitrosamines in excess of a practical limit of determination of 0.5 ppb were found in any of the samples. The problem of artifact formation during analysis of low levels of nitrosamines and the meaning of a "practical limit of determination" is briefly discussed.

INTRODUCTION

In 1979, Hedler et al. (1) had reported findings of nitrosamines, i.e., N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), in margarines and edible oils. Later, Eisenbrand (2) and Preussmann (3), who had analyzed some of Hedler's extracts by gas chromatography-thermal energy analysis (GC-TEA), dissociated themselves from Hedler's conclusions. Subsequent to Hedler's publication, in 1979 and 1980, we also had analyzed a number of margarines, fats and oils for volatile nitrosamines.

Recent papers by Sen and Seaman (4) and by Fiddler et al. (5) now prompt us to report our own findings on volatile N-nitrosamines in margarines, fats and oils which were analyzed during 1979-80. The negative result of this study has already been mentioned briefly in a review (6).

EXPERIMENTAL

The analytical procedure used was similar to that of Sen and Seaman (4) in their 1980 study. To a 20-g sample, 20 mL of water, 2 mL 0.1 N NaOH and 10 ppb (200 ng) of N-nitroso-dipropylamine (NDPA) were added, and the usual vacuum steam distillation was carried out. The aqueous distillate (ca. 15-20 mL) was acidified by addition of 1 mL 0.1 N H_2SO_4 and 1 g of ascorbic acid.

The volatile nitrosamines were then extracted from the acidified distillate by dichloromethane using an Extrelut® extraction aid as described before (7). The extracts were concentrated using a Kuderna-Danish type concentrator and a stream of nitrogen to a final volume of 1 mL (to 0.2 mL when levels below 0.5 ppb were to be detected). Five μL of the extract was injected into the gas chromatograph. The GC and TEA conditions were the same as described (7).

The NDPA added in the first step was carried through the whole clean-up and preconcentration procedure; it was used as an internal standard and to check the overall recovery by comparison with an identical aliquot of NDPA made up to 1 mL. Extracts showing a recovery of less than 70% were discarded.

The GC-TEA system used will detect trace amounts down to below 0.5 ppb of the following nitrosamines: NDMA, NDEA, N-nitrosodiisopropylamine (NDiPA), NDPA (often added as an internal standard because it is normally not found in foods), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), N-nitrosomorpholine (NMOR), and other volatile nitrosamines, e.g., unsymmetrical ones. Typical recoveries for nitrosamines at the 1 ppb level were 75-85%.

RESULTS

By vacuum steam distillation and GC-TEA analysis, the results were negative (below the "practical limit of determination" of 0.5 ppb—a discussion of this is given later) for all the volatile nitrosamines tested upon repeated analysis. The samples analyzed are mentioned in Table I. Typically, 3-5 replicas were run.

TABLE I

Margarines and Other Oil and Fat Samples Analyzed for Volatile Nitrosamines

Sample	Origin	Results (volatile nitrosamines, ppb)
Margarines		
Brand A	USA	all < 0.5
Brand B	USA	all < 0.5
Brand C	USA	all < 0.5
Brand D	USA	all < 0.5
Brand E	USA	all < 0.5
Brand F	USA	all < 0.5
Brand G	Great Britain	all < 0.5
Brand H	Great Britain	all < 0.5
Brand J	W. Germany	all < 0.5
Brand K	W. Germany	all < 0.5
Brand L	W. Germany	all < 0.5
Brand M	Austria	all < 0.5
Brand N	W. Germany	all < 0.5
Soft margarines		
Brand O	USA	all < 0.5
Brand P	USA	all < 0.5
Brand Q (1979)	W. Germany	all < 0.5
Brand Q (1980)	W. Germany	all < 0.5
High PUFA margarine		
Brand R	Austria	all < 0.5
Brand S	W. Germany	all < 0.5
Liquid margarine		
Brand T	W. Germany	all < 0.5
Halvarine		
Brand U	W. Germany	all < 0.5
Butter		
Brand A	USA	all < 0.5
Brand B	USA	all < 0.5
Frying fats		
Brand A	W. Germany	all < 0.5
Brand B	W. Germany	all < 1[a]
Soft frying fat		
Brand C	W. Germany	all < 0.5
Pure vegetable oils		
Soybean oil	USA	all < 0.5
Palm oil	USA	all < 0.5
Sunflower oil	USA	all < 0.5
Liquid frying fats and salad oils		
Brand A	W. Germany	all < 1[a]
Brand B	W. Germany	all < 1[a]
Salad oil		
Brand C	W. Germany	all < 1[a]

[a] True steam distillation clean-up (spattering/foaming problems with vacuum steam distillation). Practical limit of determination, 1 ppb.

Very low traces of NDMA and NDEA (below 0.1 ppb) have been observed in a number of cases. They may have been artifacts in amounts too low to warrant additional investigation. Typical GC-TEA analyses are illustrated by Figures 1 and 2.

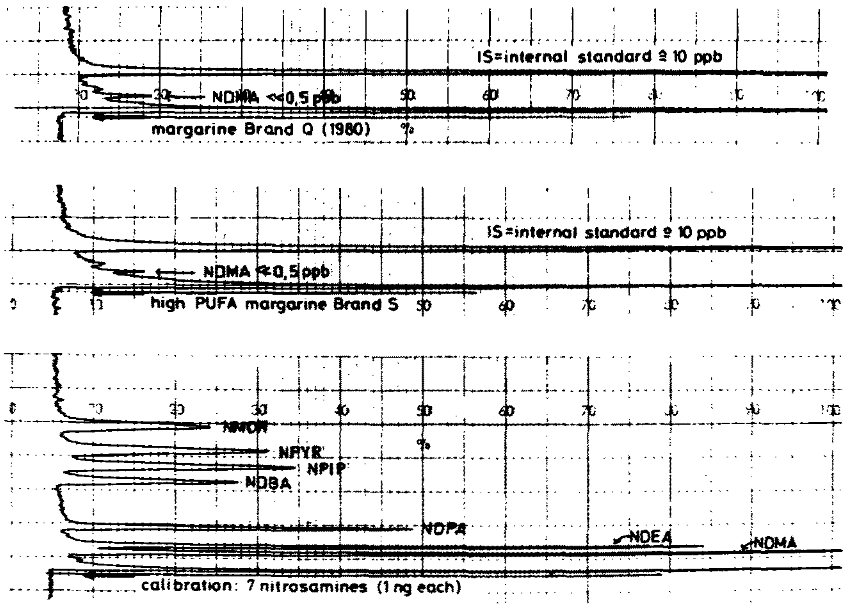

FIG. 1. GC-TEA analyses of margarines.

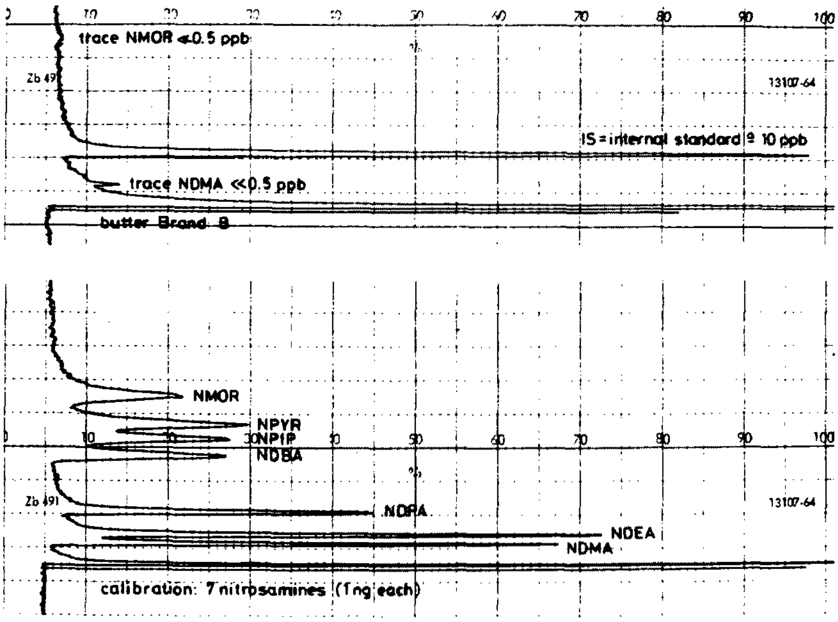

FIG. 2. GC-TEA analysis of butter.

In this laboratory, confirmation procedures are normally carried out by GC-mass spectroscopy (GC-MS) for levels above 10 ppb and by ultraviolet (UV) photolysis plus GC-TEA as well as by GC-TEA on a different column for TEA-active peaks above 2 ppb. However, no such peaks were found.

DISCUSSION

In their paper, Sen and Seaman (4) observed small amounts (between 1.0 and 3.8 ppb) of N-nitrosomorpholine (NMOR) in earlier samples of butter and margarine, but no NMOR in the later samples. Traces of NDMA were also observed (4), up to 0.3 ppb in the earlier samples.

Small amounts (0.5-2 ppb) of N-nitrosomorpholine (NMOR) were also observed by us in a few cases during the first series of experiments. However, in our case, this could be traced to contamination from reagents, especially the dichloromethane used. When selected batches of dichloromethane were carefully redistilled before use, these initial results could not be repeated and no NMOR peaks were detected. In these first experiments, we occasionally also observed traces of NDMA (around 0.1-0.7 ppb) in margarine extracts. These, however, were most likely artifacts and could never be verified by repeated analyses of the same sample.

In nitrosamine analysis in the low ppb range, there is always the danger of contamination from reagents or artifact formation from NO_X plus amines. Like Eisenbrand et al. (8), we also sometimes found artifact formation during clean-up on Extrelut® (Merck) that apparently contained adsorbed NO_X from air (6). Artifact formation on adsorbents and extraction aids such as Extrelut® was most prominent when amine-containing distillates (e.g., from cheese samples) were added to the extraction aid at an acidic pH (6,7,9). Preussmann (3) has also pointed out the danger of artifact formation in his letter to the editor.

Although contamination from reagents can largely be corrected, the danger of artifact formation is more persistent, particularly at levels below 0.5 ppb. Actually, we believe that one can never really be sure that a nitrosamine peak of 0.1 or 0.2 ppb was really present as such in the original sample—it may have been formed as an artifact during the analysis. We therefore would like to adhere to a tacit agreement that formerly existed among a number of European laboratories (including laboratories in Germany, Holland, England and Denmark) not to quote results below 0.5 ppb. Although the TEA detector is able to detect nitrosamines down to even below 0.1 ppb (if the extracts or distillates can be reduced sufficiently in volume), 0.5 ppb is considered a "practical limit of determination" (Table I) that is different from the "detection limit" of the GC-TEA system and takes into account the increased danger of artifact formation (e.g., from adsorbed atmospheric NO_X on glass surfaces) at levels below 0.5 ppb.

We have since learned that artifact formation can largely be avoided—at levels above 0.5 ppb—by heating the adsorbent or extraction aid before use (200 C overnight), by addition of ascorbic acid and by a reversal of the acid and alkaline steps, so that the aqueous distillate is alkaline when it comes into contact with the adsorbent used for preconcentration (6-9).

We believe that the earlier positive results reported by Hedler et al. (1) may also have been the result of inadvertent contamination or artifact formation during sample clean-up or nitrosamine preconcentration. Since Hedler et al. used silica in a preconcentration step, it may be possible that the silica used by them also caused artifact formation by adsorbed NO_X. The fact that we find no nitrosamines above a "practical limit of determination" of 0.5 ppb is in agreement with the findings of White et al. (10), Fiddler et al. (5), Preussmann (3) and also with the more recent samples analyzed by Sen and Seaman (4) (cf. the discussion section and the footnote to Table II of Sen and Seaman's paper [4]). We therefore believe that nitrosamines are really not a problem with food fats and oils, butter and margarines.

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