Nitrosamines Produced in Selected Foods under Extreme Nitrosation Conditions

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Beer, nonfat dry milk, and microwave-baked fish were subjected to extreme nitrosation to illustrate the upper limits of nitrosamine formation from amines found in certain foods. Beer produced approximately 1 ppm of *N*-nitrosodimethylamine (NDMA), as well as lower levels of *N*-nitrosodiethylamine (NDEA), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosomorpholine (NMOR). Nonfat dry milk produced parts per billion levels of NDMA, NPYR, and NMOR, as well as *N*-nitrosopiperidine. Nitrosated cooked fish contained 10 ppm levels of NDMA and parts per billion levels of NPYR. This study demonstrates that the limiting factor in volatile nitrosamine formation in these foods is not the availability of amine precursors in the foods but the amount of nitrosating agent applied.

INTRODUCTION

Certain foods have been found to contain various nitrosamines, many of which are known carcinogens (Tricker and Preussmann, 1991). Over the past decade, a number of investigators have demonstrated nitrosamine formation in food–nitrite mixtures, mainly in an attempt to determine the extent of formation under human gastric conditions (Siddiqi et al., 1988; Atawodi et al., 1991). Other investigators have shown increased levels of volatile nitrosamines (Poirier et al., 1989) and total *N*-nitroso compounds (Chen et al., 1992) in food–nitrite mixtures in which the level of nitrite exceeds the amounts found in the normal human fasting stomach.

The purpose of this study was to find the limits of volatile nitrosamine formation in certain foods. We exposed samples of domestic beer, nonfat dry milk, and microwave-baked fish to conditions of extreme nitrosation to determine a maximum for volatile nitrosamine formation in these foods.

EXPERIMENTAL PROCEDURES

Reagents and Samples. All chemicals were of analytical grade. Beer samples represented 35 commercial brands sold at retail outlets. Twenty-five brands were purchased in Oregon, four in Illinois, and six in North Carolina. Six brands of nonfat dry milk and five kinds of fish (salmon, tuna, halibut, cod, and rockfish) were purchased at retail outlets in Oregon. The beer was stored at 4 °C until nitrosated. Pieces of raw fish (450 ± 25 g) were wrapped in Saran Wrap and baked to a well-done condition in a Tappan microwave oven (Model PN 560T762P01) set on high for 15 min. The pieces of cooked fish were then stored at −12 °C.

Extreme Nitrosation. The reaction mixtures were prepared by mixing 15 ± 0.2 g of food with 5 mL of 0.58 M $NaNO_2$. Preliminary experiments indicated that no additional nitrosamine formation occurred beyond this amount of $NaNO_2$. For the nonfat dry milk and the fish samples, 25 mL of distilled water was also added. The fish samples were minced prior to addition to the reaction flash. The reaction mixtures were adjusted to pH 3.25 with glacial acetic acid for the beer and with 2.5 M HCl for the nonfat dry milk and fish samples. One milliliter of 1.5 μg/mL *N*-nitrosodipropylamine in ethanol was added for an internal standard. Teflon-lined screw-cap Erlenmeyer flasks (125 mL) were used for the nitrosation reaction. The reaction containers were sealed and incubated for 1 h at 100 °C. After the containers were cooled in ice water, the excess nitrite was destroyed by adding 5 mL of 1.54 M ammonium sulfamate (brought to about pH 1.0 with H_2SO_4).

The nitrosated beers were analyzed for nitrosamines according to the Celite column procedure described by Hotchkiss et al. (1981). The nonfat dry milk samples were analyzed using procedures described in Havery et al. (1982). Nitrosamine analysis of microwave-baked fish was accomplished by methods described in Hotchkiss et al. (1980). Nitrosamines are carcinogens in many animal species, and extreme care should be exercised in handling these compounds.

RESULTS

To determine background nitrosamine levels, samples of each food were analyzed for volatile nitrosamines as purchased from the retail outlets. *N*-Nitrosodimethylamine (NDMA) was the only nitrosamine found in beer. The range was from none detected (<0.1 ppb) to 0.8 ppb with a mean of 0.1 ppb for all samples. NDMA was the only nitrosamine found in the nonfat dry milk samples. It ranged from 0.3 to 1.2 ppb with a mean of 0.7 ppb. None of the fish samples contained nitrosamines above the detection limit of 0.1 ppb.

Results of nitrosation of samples of the beer, nonfat dry milk, and microwave-baked fish are summarized in Table I. Beer produced relatively large amounts of nitrosamines upon nitrosation. NDMA levels ranged from 496 to 2405 ppb with a mean of 1060 ppb. *N*-Nitrosodiethylamine (NDEA), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosomorpholine (NMOR) were produced at lower levels than NDMA, with NMOR being found in only 9 of the 35 samples.

Upon nitrosation, four nitrosamines, NDMA, *N*-nitrosopiperidine (NPIP), NPYR, and NMOR, were observed in all six samples of nonfat dry milk. NDMA levels ranged from 57 to 193 ppb with a mean of 142 ppb. The other three nitrosamines occurred at levels below 100 ppb.

Nitrosation of the five types of fish consistently yielded two peaks, identified as NDMA and NPYR. NDMA levels ranged from 1230 to 18 915 ppb with a mean of 10 919 ppb. NPYR levels ranged from none detected to 55 ppb.

DISCUSSION

These experiments were intended to produce large quantities of volatile nitrosamines by nitrosation with a large amount of nitrite. Indeed, the levels of volatile nitrosamines in the nitrosated samples exceeded the

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Table I. Mean Levels of Nitrosamines Found by Extreme Nitrosation of Domestic Beer, Nonfat Dry Milk, and Microwave-Baked Fish Samples

	no. of samples	ppb				
		NDMA[a]	NDEA[b]	NPIP[c]	NPYR[d]	NMOR[e]
beer	36	1060	54	nd[f]	267	50
nonfat dry milk	6	142	nd	55	34	25
fish	5	10919	nd	nd	14	nd

[a] *N*-Nitrosodimethylamine. [b] *N*-Nitrosodiethylamine. [c] *N*-Nitrosopiperidine. [d] *N*-Nitrosopyrrolidine. [e] *N*-Nitrosomorpholine. [f] Not detected; detection limit 0.1 ppb.

background nitrosamine levels by several orders of magnitude in a number of instances.

The relatively large amounts of NDMA and lesser amounts of NDEA, NPYR, and NMOR found in nitrosated beer are in conflict with results from Walker et al. (1979), who reported little or no increase in nitrosamines in beer under extreme nitrosation conditions. However, they used lower amounts of nitrite and conducted their experiments at 37 °C, while ours were at 100 °C.

Under extreme nitrosation conditions, microwave-baked fish samples exhibit a pattern similar to that in beer and nonfat dry milk in that NDMA was found in far larger amounts than the other nitrosamines. The relatively high levels of NDMA were not surprising since fish can contain high levels of the NDMA precursors dimethylamine and trimethylamine (Miller et al., 1972).

We recognize that the experimental conditions used in this study are not comparable to those in gastric or food-processing environments. Furthermore, although it does not seem likely, it should be recognized that the nitrosation conditions may have increased the levels of amines in the food samples. Nevertheless, this research demonstrates that relatively large amounts of volatile nitrosamines can be formed in these foods when large amounts of nitrosating agents are present. We conclude that the limiting factor in nitrosamine formation in these foods is not the availability of amine precursors but, rather, the amount of nitrosating agent.

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