Bull. Environ. Contam. Toxicol. (1985) 35:466–470

Bulletin of Environmental Contamination and Toxicology

Contamination of Dairy and Tobacco Products by Trace Quantities of Nitrosodiethanolamine (NDELA)

F. E. Okieimen and E. O. Akintola

Department of Chemistry, University of Benin, Benin City, Nigeria

The widespread interest in the presence of N-nitroso compounds within the environment and consumer products is due to the known carcinogenicity and mutagenicity of many of these chemicals (Krull et al. 1979). N-nitroso compounds can be divided into four main groups:

(i) volatile compounds, e.g. nitrosodimethylamine (NDMA),

(ii) low polarity, non-volatile compounds, e.g. N-nitroso benzylphenylamine,

(iii) high polarity, non-ionic, non-volatile compounds, e.g. N-nitrosodiethanolamine (NDELA), and

(iv) high polarity, ionic, non-volatile compounds, e.g. N-nitrosoamino acids.

Early studies concentrated on the determination of relatively simple volatile N-nitrosamines such as dialkyl and hetero cyclic compounds using gas chromatography - mas spectrometry (GC-MS) as the determinative step. The development of thermal energy analyser (TEA) linked with gas chromatography or high performance liquid chromatography widened the range of N-nitroso compounds which would be studied to include the less volatile compounds (Fine et al. 1976).

Although the TEA and GC-MS gave unambiguous answers at relatively low nitrosamine levels, the need to have a rapid and reliable screening technique which could be used as quality control/evaluation method where sophisticated analytical instrumentation was not available led to the development of a colorimetric method (Telling and Dunnett, 1981) for determining the total level of N-nitrosamines in the environment and consumer products.

In a previous report (Okieimen and Akintola, 1984) the level

of N-nitrosamine contamination of alcoholic beverages and meat products determined by the colorimetric method was discussed. The relatively high levels of contamination of these products by N-nitrosamines required that a survey of other consumer products be carried out. In this communication the total levels of N-nitrosamine contamination of dairy and tobacco products in Nigeria are examined.

MATERIALS AND METHODS

Dairy products (evaporated milk, powdered milk, infant feed, milk drink, ice cream, yoghurt, cheese) were purchased from supermarkets in Benin City. Tobacco products (tobacco leaf, cigarettes, cigars, pipe tobacco and snuff) were purchased from retail shops in Benin City. All the reagents used were of analytical grade.

The samples were analysed for N-nitroso compounds using the method developed by Telling and Dunnett (1981). The underlying principle of this method is the extraction of nitrosamines from the sample, denitrosation of the extracted nitrosamines and estimation of the nitrite produced by a colorimetric method.

Between 5 g and 20 g of the sample was weighed into a 100 ml conical flask; 500 mg ammonium sulphamate added and the mixture stirred thoroughly. A 20 ml aqueous sodium chloride solution (20% $^{w}/v$) was added and mixture shaken for 15 minutes. The aqueous mixture was quantitatively transferred to a 300 ml separating funnel using a 2 x 5 ml aliquots of the sodium chloride solution to rinse the flask. The solution was then extracted with 2 x 50 ml hexane and the hexane layers discarded.

The aqueous phase was extracted with 3 x 50 ml ethyl acetate and after each extraction, the organic phase was filtered through a 20 g anhydrous sodium sulphate. The combined filtrates was concentrated to less than 0.5 ml on a rotary evaporator using a water bath at 50°C. The residue was made up to 1 ml with glacial acetic acid and transferred to a reaction tube; 0.5 ml of this mixture was transferred to a second reaction tube (the control blank). A 0.2 ml aliquot of denitrosation reagent (3% $^{v}/v$ HBr in glacial acetic acid) was added to the test aliquot and the tube placed in a 50°C water bath for 5 minutes. The control blank had 0.2 ml of distilled water added to it. A 0.2 ml sulphanilamide solution (0.2% $^{w}/v$ in HCl) was added to each tube and the content mixed by shaking. After 5 minutes, 0.1 ml N-1 naphthyl reagent (0.1% $^{w}/v$ N-1 naphthyl ethylenediamine dihydrochloride) was added to each tube. The absorbance of the test sample was measured at 540 nm against the control blank after 25 minutes. Using a sodium nitrite standard,

the total level of N-nitroso compounds in the test samples measured as nitrosodiethanolamine (NDELA) was calculated from the Telling and Dunnett (1981) relationship

$$\frac{\mu g/ml\ NaNO_2 \text{ from the calibration graph}}{\text{mass of sample (g)}} \times \frac{1}{0.5} \times \frac{30}{69} \times \frac{100}{2.4}\ \mu g/g$$

RESULTS AND DISCUSSION

The levels of N-nitrosamine contamination of dairy and tobacco products are shown in table 1. It would be seen that the tobacco products presented higher levels of nitrosamine contamination than the dairy products. This may be due to the relatively high levels of ascorbic acid (present naturally and/or added as antioxidant) in milk products. It is generally well known that the presence of ascorbic acid suppresses nitrosation reactions leading to lower levels of N-nitrosamines.

Table 1. Levels of total N-nitroso compounds in dairy and tobacco products

	Products	N-nitroso compounds as NDELA[a] (µg/kg)
A.	Dairy Products	
	evaporated milk (3)	ND-1.57
	powdered milk (3)	ND
	infant feed (6)	ND
	ice cream	5.15
	yoghurt	1.53
	cheese (hard)	7.04
	milk drink	ND
B.	Tobacco products	
	toabcco leaf	3.88
	cigarettes (10)	8.31-21.35; 15.00[b]
	cigars (2)	21.41[b]
	pipe tobacco (2)	23.34[b]
	snuff	34.1[b]

Number of brands analysed in brackets

a average of duplicate absorbance measurements was used to calculate the amount of nitrosamine in each brand

b average values

ND means less than 1.0 ug/kg NDELA contamination

Of the 16 dairy samples examined, only 2 samples, (12.5%) gave more than 5 µg/kg NDELA; while 12 samples (75%) did not show detectable levels of N-nitrosamine. Cheese gave the highest NDELA content (7.04 µg/kg).

The level of nitrosamine contamination of the tobacco products ranged from 3.88 to 34.16 µg/kg (table 1) with a mean value of 17.3 µg/kg. Of the 5 types of tobacco products examined, tobacco leaf gave the lowest NDELA value of 3.88 µg/kg, while tobacco snuff gave the highest value (34.16 µg/kg). The mean value for cigarettes, cigars and pipe tobacco are 15.0 µg/kg; 21.41 µg/kg; and 23.34 µg/kg respectively. The observed difference between NDELA levels in tobacco leaf and tobacco products leads to the suggestion that tobacco processing additives might contain nitrosamine precursors or that tobacco processing accentuates nitrosation reaction.

Table 2 shows a comparison of the levels of nitrosamine contamination of some consumer products in Nigeria. It would be seen that the tobacco products gave the highest average level of nitrosamine.

Table 2. Comparison of the levels of nitrosamine contamination of some consumer products in Nigeria

Product type	N-nitroso compounds as NDELA (µg/kg)	Mean NDELA content (µg/kg)
dairy products	0.3-5.5	3.4
alcoholic beverages	7.8-19.2	8.5
meat products	0.6-68.9[c]	-
tobacco products	3.9-34.2	17.3

c) 4 meat products were examined, 3 of which gave between 0.6 and 0.9 ug/kg; only one meat sample consistently gave high NDELA values. Detailed study on this meat product will be discussed in future communication.

Although the tobacco products showed a relatively high level of nitrosamine contamination, it is unlikely that the risk of nitrosamine - induced cancer from the consumption of tobacco products (with the exception of tobacco snuff which is used to relieve tooth ache by oral application) would be as high as the cancer risk from the consumption of some of the other consumer products e.g. alcoholic beverages; which showed lower average values of nitrosamine contamination.

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Received January 28, 1985; accepted February 20, 1985