This article was downloaded by:

On: 18 January 2011

Access details: Access Details: Free Access

Publisher Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-

41 Mortimer Street, London W1T 3JH, UK



International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information: http://www.informaworld.com/smpp/title~content=t713640455

Determination of the Total Level of Nitrosamine Contamination of Some Consumer Products in Nigeria

F. E. Okieimen^a; E. O. Akintola^a; T. C. A. Anucha^b; M. M. Ajibola^b

 $^{\rm a}$ Department of Chemistry, University of Benin, Benin City, Nigeria $^{\rm b}$ Department of Pharmaceutical Chemistry, University of Benin, Benin City

To cite this Article Okieimen, F. E. , Akintola, E. O. , Anucha, T. C. A. and Ajibola, M. M.(1985) 'Determination of the Total Level of Nitrosamine Contamination of Some Consumer Products in Nigeria', International Journal of Environmental Analytical Chemistry, 21: 4, 261-266

To link to this Article: DOI: 10.1080/03067318508077067 URL: http://dx.doi.org/10.1080/03067318508077067

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Intern. J. Environ. Anal. Chem., 1985, Vol. 21, pp. 261–266 0306-7319/85/2104-0261 \$18.50/0 © 1985 Gordon and Breach, Science Publishers, Inc. and OPA Ltd. Printed in Great Britain

Determination of the Total Level of Nitrosamine Contamination of Some Consumer Products in Nigeria

F. E. OKIEIMEN and E. O. AKINTOLA

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

T. C. A. ANUCHA and M. M. AJIBOLA

Department of Pharmaceutical Chemistry, University of Benin, Benin City

(Received October 30, 1984)

The total level of N-nitrosamine contamination of beer, tobacco products, dairy products, meat products and food seasoning items (Bouillon cubes) was determined by a colorimetric method. The dairy products presented the lowest level of nitrosamine contamination (mean value 0.003 mg/kg); while the bouillon cubes gave the highest range of nitrosamine contamination (0.078–2.585 mg/kg). The levels of nitrosamine in the meat products, alcoholic beverages and tobacco products were between 0.001 and 1.972 mg/kg; 0.008 and 0.019 mg/kg; and 0.004 and 0.023 mg/kg respectively.

KEY WORDS: Nitrosamine, contamination, consumer products, carcinogenicity, mutagenicity, gas chromatography-mass spectrometry.

INTRODUCTION

During the past few years, there has been considerable interest in the analysis of food for volatile N-nitrosamines because of the known carcinogenicity and mutagenicity of these compounds.¹ Although the early studies were mainly concerned with meat cured with nitrite,² volatile N-nitrosamines have been detected in other foods such as cheese and fish,^{3,4} in alcoholic beverages,^{5,6} and in various other

aspects of the environment.⁷⁻⁹ In most of these and similar other reports, ^{10,11} the emphasis has been on the detection, identification and quantification of volatile nitrosamines with relatively little information on the non-volatile compounds. However the development of Gas chromatography-Mass Spectrometry (GC-MS) and Thermal Energy Analyser (TEA) linked with GC or High Performance Liquid Chromatography (HPLC) analytical techniques widened the range of N-nitroso compounds that could be studied to include the less volatile nitrosamines.^{9,12}

Although the TEA gave unambiguous results at relatively low nitrosamine levels, the need to have a rapid reliabile screening technique that could be used as quality control/evaluation method where sophisticated analytical instrumentation is not available led to the development of a colorimetric method⁹ for determining the total level of N-nitrosamine within the environment. This communication reports on the survey of the level of nitrosamine contamination of some Nigerian consumer products.

EXPERIMENTAL

Materials

The consumer products used for this survey: dairy products, alcoholic beverages, tobacco products, meat products and bouillon cubes were obtained from retail shops in and around Benin City. All the reagents used were of analar grade.

Determination of N-nitroso compounds in the test samples

The samples were analysed for N-nitroso compounds using the method developed by Telling and Dunnett.⁹ The underlying principle of this method is the extraction of nitrosamines from the sample, denitrosation of the 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 cm³ conical flask; 500 mg ammonium sulphamate added and the mixture stirred thoroughly. A 20 cm³ aqueous sodium chloride solution (20% w/v) was added and the mixture shaken for 15 minutes. The aqueous

mixture was quantitatively transferred to a $300\,\mathrm{cm^3}$ separating funnel using $2\times5\,\mathrm{cm^3}$ aliquots of the sodium chloride solution to rinse the flask. The solution was then extracted with $2\times50\,\mathrm{cm^3}$ hexane and the hexane layers discarded.

The aqueous phase was extracted with $3 \times 50 \text{ cm}^3$ ethyl acetate and after each extraction, the organic phase was filtered through 20 g anhydrous sodium sulphate. The combined filtrates were concentrated to less than 0.5 cm³ on a rotary evaporator using a water bath at 50°C. The residue was made up to 1 cm3 with glacial acetic acid and transferred to a reaction tube, $0.5\,\mathrm{cm}^3$ of this mixture was transferred to a second reaction tube (the control blank). A 0.2 cm³ 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 cm³ of distilled water added to it. A 0.2 cm³ sulphanilamide solution (0.2% w/v in HCl) was added to each tube and the content mixed by shaking. After 5 minutes, $0.1 \,\mathrm{cm}^3 \,\mathrm{N} - 1$ naphthyl reagent $(0.1\% \,\mathrm{w/v} \,\mathrm{N} - 1$ napthyl 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 relationship.⁹

$$\frac{\mu \text{g cm}^{-3} \text{ of NaNO}_2 \text{ from calibration graph}}{\text{mass of sample (g)}} \times \left[\frac{1}{0.5} \times \frac{30}{69} \right] \times \frac{100}{2.4} \mu \text{g g}^{-1}$$

RESULTS AND DISCUSSION

All of the brands (22) of lager beer analysed were contaminated by N-nitrosamine (Table I). The mean nitrosamine contamination in the beer samples was 0.008 mg/kg and the highest value obtained was 0.019 mg/kg. The beer samples analysed were the pilsner type, and the level of nitrosamine reported from this study are about the same order of magnitude as, but are somewhat higher than the values reported for similar products.^{5,7}

The level of nitrosamine contamination of bouillon cubes ranged from 0.078 to 2.585 mg/kg (Table I) with a mean value of 1.327 mg/kg. Bouillon cubes are food seasoning items containing

TABLE I

Levels of total N-nitroso compounds in some consumer products in Nigeria.

Products	N-nitroso compounds as NDELA ^a (mg/kg)	Average NDELA content mg/kg
Alcoholic beverages (22)	0.0080.019	0.008
Boullion cubes (9)	0.078-2.585	1.327
Tobacco products (13)	0.004-0.034	0.017
Dairy products (16)	0.001-0.006	0.003
Meat products (4)	0.001-1.972	

Number of brands analysed in brackets.

meat extracts, salts, colourings, flavouring agent and preservatives. The innate chemical composition of the constituents of these seasoning items, and/or the chemical interaction of these constituents with one another and with the environment could be responsible for the relatively high levels of nitrosamine content of these products. Boullion cubes are sold in two sizes: 4 g and 10 g cubes. Because they are added to foods in relatively small quantities, it would seem from the levels of nitrosamine contamination reported from this study that the risk of nitrosamine induced cancer from the consumption of boullion cubes would be low.

The level of nitrosamine contamination of the tobacco products ranged from 0.004 to 0.034 mg/kg with a mean value of 0.017 mg/kg (Table I). Of the 5 types of tobacco products examined, tobacco leaf gave the lowest NDELA value of 0.004 mg/kg, while tobacco snuff gave the highest value (0.034 mg/kg). The mean values for cigarettes, cigars and pipe tobacco are 0.015 mg/kg, 0.021 mg/kg and 0.023 mg/kg respectively. The observed difference between NDELA levels in tobacco leaf and the tobacco product leads to the suggestion that tobacco processing additives could contain nitrosamine precursors and that tobacco processing could accentuate the nitrosation reaction. Although the tobacco products showed fairly high levels of nitrosamine contamination, it is unlikely that the risk of

^aAverage of duplicate absorbance measurement on each sample (brand) was used to calculate the level of nitrosamine in the product.

nitrosamine induced cancer from the consumption of tobacco products (except for tobacco snuff that is taken internally) whould be high.

The dairy products examined showed the lowest range of nitrosamine contamination (0.001–0.006 mg/kg) (Table I). Of the 16 samples analysed, nitrosamine was not detected in 7 samples (44%); 8 samples contained less than 0.002 mg/kg; only 1 sample of ice cream gave fairly high level (0.006 mg/kg) of nitrosamine. All of the infant food formulae and 3 powdered milk samples showed no detectable levels of nitrosamine. The relatively low levels of nitrosamine contamination of dairy products may be due to the ascorbic acid usually added to milk products as antioxidant. It is generally well known that the presence of ascorbic acid suppresses nitrosation reactions leading to low levels of N-nitrosamine. 13

The level of nitrosamine contamination of the meat products analysed ranged from 0.001 to 1.972 mg/kg. One sample of the meat products, Suya,† contained relatively high levels of nitrosamine (1.972 mg/kg), while the levels in other meat products varied from 0.001 mg/kg to 0.002 mg/kg. In order to establish the contribution of the various constituents to the level of nitrosamine in suya, analyses were carried out on the (i) spices; (ii) raw beef; (iii) beef coated with the spices (pregrilled suya); (iv) hot suya; and (v) suya left for various periods of time. The results (Table II), show that all the ingredients for preparing suya contain fairly high levels of nitrosamine. The nitrosamine content of the pre-grilled suya (1.779 mg/kg) is somewhat higher than the sum of the levels in the ingredients (1.384 mg/kg); indicating a possible chemical interaction between the constituents of the ingredients, leading to the increase in the nitrosamine level. The level of nitrosamine of the meat product dropped significantly on cooking (1.051 mg/kg) and then gradually increased for 18 hours to 2.018 mg/kg. Suya has been shown to contain a wide range of bacteria flora, 14 and it is thought that bacterial accentuated nitrosation reactions would lead to the high levels of nitrosamine in the cold meat products.

[†]Suya is prepared by coating meat (usually beef) with spices (brown and red pepper, groundnut cake, palm oil and salt) and then roasted around a glowing fire.

TABLE II

Contamination of suya by trace amounts of N-nitrosamine.

Suya ingredients	Total nitrosamine (as NDELA) content ^a mg/kg
Raw meat (beef)	0.311
Mixed spices	1.073
Suya (pre-grilling)	1.779
Grilled suya (0.00 hours)	1.051
Grilled suya after 6 hours	1.646
Grilled suya after 12 hours	1.754
Grilled suya after 18 hours	2.018
Grilled suya after 24 hours	2.019

^aAverage of duplicate absorbance measurement on each sample was used to calculate the level of nitrosamine in the products.

References

- 1. S. S. Mirvish, Toxicol. and Appl. Pharmacol. 31, 325 (1975).
- A. E. Weisserman, J. W. Pensabene and E. G. Poitrowski, J. Fd. Sci. 43, 276 (1978).
- J. R. Iyengar, T. Panalaks, W. F. Miles and N. P. Sen, J. Sci. Fd. Agric. 27, 567 (1976).
- G. Eisenbrand, B. Spiegelhalder, R. Preussmann, C. Janzowski and J. Kann, I.A.R.C. Publications 19, 311 (1978).
- R. A. Scalan, J. F. Barbour, J. H. Hotchkiss and L. M. Libbey, Fd. Cosmet. Toxicol. 18, 27 (1979).
- 6. B. Spiegelhalder, G. Eisenbrand, and R. Preussmann, Oncology 37(4), 211 (1980).
- 7. I. S. Krull, G. Edwards, M. M. Wold, T. Y. Fan and D. H. Fine, In N-nitrosamines (American Chemical Society, 1979), Chap. 11, p. 175.
- 8. L. Lakritz and W. Kimoto, Fd. Cosmet. Toxicol. 17, 31 (1980).
- 9. G. M. Telling and P. C. Dunnett, Int. J. Cosmet. Sci. 3, 241 (1981).
- 10. O. Bassir and E. N. Maduagwu, J. Agric. Food Chem. 26, 200 (1978).
- W. R. Bontoyon, M. W. Law and D. P. Wright, J. Agric. Food Chem. 27, 1979 (1979).
- D. H. Fine, R. Ross, D. P. Rounbehlar, A. Silvergleid and L. Song, J. Agric. Food Chem. 24, 1069 (1976).
- 13. C. L. Walters, In Vitamin C Recent Aspects of its Physiological and Technological Importance, Ed. G. G. Birch and K. J. Parker, (Applied Science Publishers, 1974).
- 14. J. O. Igene, Tropical Veterinarian 1, 85 (1983).