NITROSAMINES OCCURRENCE IN SOME FOOD PRODUCTS

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Abstract

We take samples of foodstuffs from different sources, The studied products where meat, fish and dairy products, vegetables, fruit and soups have been examined for the presence of nitrosamines. Meals food stuff were studied in order to assess the possibility of interactions of the constituents which could result in nitrosamine formation. We use gas chromatography and high resolution mass spectrometry for precise determination. The most frequently nitrosamines founded were N-nitrosodimethylamine and N-nitrosopyrrolidine present in meat samples and dairy products. Other foods had level of nitrosamines below the compulsory limits.

Key words: n-nitrosamines, nitrite, foods products, meat products, dairy product

INTRODUCTION

The formation of N-nitrosamines from the interaction of nitrite and secondary and tertiary amines is well documented and their presence in various foodstuffs has been detected in this laboratory and elsewhere. The carcinogenic properties of N-nitrosodimethylamine were established and consequently it is important to ensure that the concentration present in food is below a level which is hazardous to man. Animal studies are in progress elsewhere to determine the effects of low concentrations of nitrosamines in the diet and to identify, if possible, guidelines for maximum permissible levels.

Published work on nitrosamines has concentrated on a few particular foodstuffs such as bacon and other cured meats. In this paper, we present data on the amounts of nitrosamines found in a wide range of foods over the period 2008-2010.

As the analytical method for traces of nitrosamines in food is time consuming and sophisticated, the number of samples which can be analysed is limited. Therefore, whilst aiming for an overall assessment it has been necessary to concentrate our efforts on those foodstuffs which have high nitrate, nitrite and amine contents, which could by interaction give rise to nitrosamines.

A significant source of nitrite in the diet is cured meat which it is used to impart colour, flavour and microbiological stability. Nitrite can be formed from the reduction of nitrate and data have been published on the nitrite and nitrate content of vegetables, milk and dairy products, and other

basic dietary constituents. Nitrite has been shown to increase during the storage of fresh vegetables with high nitrate content. Many canned foods contain nitrate, from which nitrite can be formed under some conditions of sterilization on of corrosion. Nitrate is also present in varying amounts in domestic water supplies.

The amine and amino acid content of foods is less well documented and in many cases only a total rather than individual amine content is available. Many amines, including amino acids, could give rise to non-volatile nitrosamines but by degradation, simple volatile nitrosamines can result. For example, the formation of N-nitrosopyrrolidine has been observed from praline and it could also arise from the breakdown of a peptide containing this amino acid. Useful reviews of the occurrence of amino-compounds have been published. Numerous pesticides are capable of nitrosation, and antibiotics such as the tetracyclines which have been used in poultry husbandry, will nitrosate. Residues of such substances in food could, therefore, form N-nitrosocompounds by reaction with nitrite under suitable conditions. Such residue levels are, however, far lower than the levels of many amines naturally present in food.

From a knowledge of the nitrate, nitrite and amine content of foods, it was decided to concentrate on meat and meat products, fish, dairy produce and canned foods (including baby foods), together with a range of appropriate miscellaneous foods to extend the coverage. Ready prepared meals and cooked complete meals have also been examined in those cases where interaction between constituents or the action of cooking could give rise to the formation of nitrosamines.

MATERIAL AND METHODS

Foodstuffs were prepared for analysis by the standard procedure used at this laboratory. The meals were cooked conventionally and ready prepared meals treated as directed by the manufacturers. Clean-up of the samples was by steam distillation and extraction into dichloromethane, followed by evaporation to small bulk to concentrate any nitrosamines. The resulting extracts were screened by gas chromatography using a Coulson electrolytic conductivity detector, and the presence of nitrosamines confirmed where appropriate by combined gas chromatography and high resolution mass spectrometry. Quantitative results are based on parent ion measurement after calibration against standard nitrosamine solutions and correction for recoveries. Each sample was examined for the following nitrosamines: N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP) and N-nitrosopyrrolidine (NPYR).

NDPA was used as an internal standard for the majority of samples to enable recovery corrections to be made. In the case of composite foods, such as the ready prepared meals and cooked meals, sufficient of each of the major constituents of the meal was taken to ensure that the detection limit was maintained for each of these constituents.

Our analytical procedure has been used for the determination of volatile nitrosamines in collaborative exercises with other laboratories and has given satisfactory results.

RESULTS AND DISCUSSION

General

The foods examined in this laboratory are discussed below and the results compared with data reported in the literature. It is generally accepted that confirmation of analytical results for nitrosamines by mass spectrometry is essential, and any data derived from other techniques alone should be treated with caution. The detection limit for the nitrosamines in our study is 1 μg/kg. Over 500 samples analysed by this laboratory are included, nearly all of which were obtained from normal retail outlets in **Bihor county**.

Cured meat products

There have been many reports in the literature of the presence of volatile nitrosamines in bacon, some of which have been substantiated by mass spectral data. In particular NPYR has been found to occur frequently in bacon after cooking. Bacon has been studied extensively in this laboratory, and the distribution of nitrosamines in cooked bacon, its cooked-out fat and the cooking vapours has shown that a high proportion of volatile nitrosamine is lost in the vapour, although measurable amounts remain in the cooked rasher. The effects of varying the nitrate and nitrite levels in the bacon and the conditions of storage prior to cooking have also been investigated, but for the purposes of this report only results from bacon containing normal permitted levels of nitrate and nitrite and not subject to storage abuse are included. Reports on the presence of nitrosamines in other cured meat products have been less consistent, and few have been confirmed by mass spectrometry. There have, however, been recent reports of products containing up to five volatile nitrosamines simultaneously, and of some cured meats containing NDMA and NDEA but not NPYR. These observations have for the majority of samples been confirmed by mass spectrometry. Many cured meat products contain added spices, and the use of spice premixes containing nitrite for curing has given rise to the formation of NPIP and other nitrosamines in the premixes. The presence of NPIP in meat products using such premixes is not unexpected and has been

observed. The current manufacturing process both in the UK and elsewhere is to store the curing agents and spices separately prior to use to eliminate interaction. However, a number of spiced meat products have been included in the present work as interaction in the end product is still possible.

Table 1 lists the meat products with the numbers of each commodity examined and the numbers containing the specified nitrosamines. The levels of NDMA and NPYR observed for bacon are similar to values found by other workers and by this laboratory in earlier studies. In one sample of fried bacon 1μg/kg of NDEA was detected, and in one sample 1 μg/kg of NPIP was found. The canned products covered chopped pork, corned beef, luncheon meat and frankfurters, all of which contained NDMA on occasions and one contained NDEA. NDMA was also detected in two samples of salami. The cooked cured meats were grilled gammon and fried corned beef, but no volatile nitrosamines were detected in these or in the cold cured meats. Only one cured meat product other than bacon contained either of the volatile heterocyclic nitrosamines above 1 μg/kg. This was a heavily spiced canned luncheon meat which contained 2 μg/kg of NPIP.

TABLE 1

Occurrence of volatile nitrosamines in meat and meat products

Commodity	Examined	Number of samples: Containing NDMA 1-5 (μg/kg)	Containing NDMA 5-10 (μg/kg)	Containing NPYR 1-20 (μg/kg)	Containing NPYR 20-200 (μg/kg)	Containing other nitrosamines 1-20 (μg/kg)
Bacon, uncooked	10	Not determined[a]		0	0	0
Bacon, uncooked	13	0	0	0	0	0
Bacon, fried	10	Not determined[a]		0	10	0
Bacon, fried	23	11	0	10	2	1(NDEA) 1 (NPIP)
Bacon, fried	23	2	0	18	3	Not determined[a]
Cured meats, canned	34	8	1	0	0	1 (NDEA)
Salami	11	2	0	0	0	0
Sausages	11	0	0	0	0	0
Cured meats, cooked	10	0	0	0	0	0
Cured meats, cold	9	0	0	0	0	0
Fresh meat, uncooked	13	0	0	0	0	0
Poultry, uncooked	2	0	0	0	0	0
Poultry, cooked	9	1	0	0	0	1 (NDEA)
Liver	4	0	0	0	0	0
Meat extracts	4	0	0	0	0	0

[a] Not determined= sample not examined for this nitrosamine.

Uncured meat

There have been occasional reports of nitrosamines in fresh (i.e. uncured) meat and offal. In those uncured meats examined in this laboratory, there was only one instance when any volatile nitrosamines were detected (see Table 1). Further fresh meat samples were covered by the complete meals.

Fish and fish products

Fish contain an abundance of amines, including simple aliphatic amines, and some of these have been shown to produce NDMA with nitrite. There have been several reports of nitrosamines in fish from the Western hemisphere and from Japan. Most instances of occurrence were in smoked or salted fish, but only in a few instances have any oh these findings been confirmed by mass spectrometry. Mass spectral confirmation of the presence of NDMA in fresh fish, both uncooked and fried, has been reported previously from this laboratory, and further evidence for the presence of NDMA in several different types of uncooked sea fish is given in Table 2, which includes cod, haddock, coley, hake, mackerel, plaice and skate. Cooked fish includes fried and boiled fish and fish cakes. Following the observation that a substantial proportion of volatile nitrosamines is lost during cooking, the analysis of fish cooked in plastic bags as recommended by the manufacturers seemed particularly important, but no nitrosamines were detected in these samples. The treated fish includes salted, pickled, canned and smoked fish.

TABLE 2

Occurrence of volatile nitrosamines in fish and fish products

Commodity	Examined	Number of samples: Containing NDMA	
		1-5 (μg/kg)	5-10 (μg/kg)
Fish, uncooked	61	17	6
Fish, cooked	9	0	0
Treated fish	24	4	1
Roe and caviar	16	0	0
Fish paste	2	0	0

Dairy products

Mass spectral confirmation of the presence of NDMA in several types of cheese was obtained in laboratory and this work has been extended to include 21 different varieties. Cheeses to which nitrate is added during manufacture were included in the study. The occasional occurrence of NDMA was observed, but with no greater regularity than in the cheeses without added nitrate. The levels of NDMA were similar for all the cheeses. A total of 16 other milk products having a high amine content, such as yogurt and dessert dishes, were also examined (see Table 3). All products were analysed within the manufacturers' recommended date for consumption. The fruit yogurts included black-currant, cherry, strawberry, pear, banana, pineapple, orange and raspberry. A hazelnut yogurt was interesting in that on screening it gave an apparent positive result for NDEA on an otherwise clean chromatogram in two different stationary phases. However, NDEA was not detected in this product by mass spectrometry. Indeed no volatile nitrosamines were found in any of these milk products.

Milk based beverages are included under miscellaneous samples (section 3.15).

TABLE 3

Occurrence of volatile nitrosamines in dairy products

Commodity	Examined	Number of samples: Containing NDMA	
		1-5 (µg/kg)	5-15 (µg/kg)
Cheese	58	9	1
Yogurt	8	0	0
Dessert dishes	7	0	0

Eggs

No volatile nitrosamines were detected in 25 samples of eggs which included both raw eggs and some cooked in various ways.

Vegetables

Several different types of common fresh and frozen vegetables were examined, totaling 16 samples. Two cans of spinach were also examined. No volatile nitrosamines were found. A further selection of vegetables is covered under cooked complete meals.

Fruit

Several canned fruits and canned jams of high amine and nitrate levels were examined, but in 14 samples no volatile nitrosamines were detected.

Baby foods

Some attention was paid to baby foods because they form a significant part of the diet of very young children. Sixteen canned foods were studied. The foods examined were those containing vegetable, fruit, cheese and cured meat products. No volatile nitrosamines were detected.

Soups

No volatile nitrosamines were detected in a selection of 20 canned and powdered soups.

Ready prepared meals

Commercial ready prepared meals have in recent years become a significant part of the diet in some sections of the community and some of these meals have been studied. Seven ready prepared meals, cooked in plastic containers as directed by the manufacturers, were included. Six canned ready prepared meat dishes containing spices were also analysed. No volatile nitrosamines were found in any of these products after cooking.

Pastry based foods

Early reports in the literature on the presence of nitrosamines in wheat and flour have not been substantiated. Experiments in which wheat was treated with high levels of nitrate fertiliser and secondary amines did not give rise to nitrosamine formation. In addition to the possible formation

of nitrosamines in the constituents of pastry, the pastry could act as an absorbing or entrapping medium for nitrosamines present within a pie. A total of 36 cured meat pies, cheese flans and other pastry based foods have been examined. Both the pastry and the pie contents were analysed and in neither the commercial nor home prepared products were any volatile nitrosamines detected, except in the special case of a pork pie prepared using a spice/curing salt premix.

Cooked complete meals

A total of 23 complete meals including stews and casseroles made from both fresh meat and cured meat, and fried and grilled meals, were prepared in the laboratory. The casseroles were cooked at 190ºC for 2 h and the fried dishes at 160 ºC. The stews and casseroles contained carrot, onion, tomato, celery, parsnip, turnip, potatoes, mushroom, herbs and spices; the fried and grilled meals contained onions, tomatoes and mushrooms, as appropriate. No volatile nitrosamines were detected in any of these meals.

Salads

Interaction of the constituents of cold meals prior to ingestion is less likely than in cooked meals, but a few salads consisting of lettuce, cucumber, tomato, beetroot, radish, cured meat, fish, salad cream, vinegar and other constituents, as appropriate, were analysed. No volatile nitrosamines were detected. Nitrosamine formation after ingestion of salad constituents is possible and analysis of stomach contents after such meal is the subject of a separate study.

Miscellaneous samples

This covered bread, flour, rice, fats, nuts, sauces, stuffing, frying oil and beverages, totaling 31 samples. The beverages were prepared using dried milk, as early reports on nitrosamines implicated spray dried non-fat milk as a potential source of nitrosamines. Subsequent work found no evidence of nitrosamines in dried milk, and no volatile nitrosamines were detected in the beverages or in any of the other commodities listed here.

CONCLUSIONS

A wide range of foodstuffs including cooked complete meals have been analysed in this survey and, except for fish, cheese and cured meats, the concentration of volatile nitrosamines was below the detection limit of 1 µg/kg. Most samples of fried bacon and several other cured meat products contained NDMA in the 1-5 µg/kg range. NPYR also occurred frequently in fried bacon in the 1-20 µg/kg range and sometimes up to 200 µg/kg. Fish often contained NDMA in the range 1-10 µg/kg and it was also present in some samples of cheese at the 1-5 µg/kg level. Except for one sample each of fried bacon, canned chopped pork and cooked chicken, which contained

traces of NDEA, and one sample of fried bacon and one pork pie made from a spiced premix which contained NPIP, other volatile nitrosamines were not detected in food.

For the detection of nitrosamines significantly below 1 μg/kg, the only technique available is that based on chemiluminescence in which nitrosamines are converted into nitric oxide which reacts with ozone to give an infrared emission. The foods included in this survey in which no nitrosamines were detected will be re-examined by this procedure at a later date.

REFERENCES

1. Bosnir J, Smit Z, Puntarić D, Horvat T, Klarić M, Simić S, Zorić I. 2003, Presence of N-nitrosamines in canned liver patty, Department of Health Ecology, Public Health Institute, Zagreb, Croatia. Coll Antropol.;27 Suppl 1:67-70.
2. Campillo N, Viñas P, Martínez-Castillo N, Hernández-Córdoba M., 2011, Determination of volatile nitrosamines in meat products by microwave-assisted extraction and dispersive liquid-liquid microextraction coupled to gas chromatography-mass spectrometry., J Chromatogr A.;1218(14):1815-21.PMID:21376329,
3. Chukhlebova LM., 2010, Hygienic evaluation of the safety of commercial type of fishes from the Amur River,Gig Sanit.;(4):43-6. Russian., PMID:20873385, [PubMed - indexed for MEDLINE]
4. Michaud DS, Holick CN, Batchelor TT, Giovannucci E, Hunter DJ., 2009 , Prospective study of meat intake and dietary nitrates, nitrites, and nitrosamines and risk of adult glioma., Am J Clin Nutr. 2009 Sep;90(3):570-7.PMID:19587083, [PubMed - indexed for MEDLINE]
5. Ozel MZ, Gogus F, Yagci S, Hamilton JF, Lewis AC., 2010 , Determination of volatile nitrosamines in various meat products using comprehensive gas chromatography-nitrogen chemiluminescence detection., Food Chem Toxicol. 2010 Nov;48(11):3268-73. PMID:20816717[PubMed - indexed for MEDLINE]
6. Pozzi R, Bocchini P, Pinelli F, Galletti GC., 2011, Determination of nitrosamines in water by gas chromatography/chemical ionization/selective ion trapping mass spectrometry., J Chromatogr A.;1218(14):1808-14, PMID:21377686,
7. Zeilmaker MJ, Bakker MI, Schothorst R, Slob W., 2010, Risk assessment of N-nitrosodimethylamine formed endogenously after fish- with-vegetable meals., Toxicol Sci. 2010 Jul;116(1):323-35., PMID:20351056, [PubMed - indexed for MEDLINE]