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The Occurrence of Volatile *N*-nitrosamines in French Foodstuffs

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ABSTRACT

Volatile N-*nitrosamines were estimated in 258 food samples and 53 beverages purchased in Eastern France in 1987–88.* N-*nitrosodimethylamine (NDMA) was found in 278 samples (89%) with a maximum level of 16 µg/kg. The highest values were found in stone fruit spirits. Nitrite-cured meats, smoked meats and smoked fishes were contaminated with appreciable amounts of this* N-*nitrosamine. The other* N-*nitrosamines were found more rarely, and usually below 0·5 µg/kg. Beer NDMA content (mean: 0·28 µg/litre) was lower than in the past due to the efforts of the brewing organizations.*

INTRODUCTION

The development of methodologies and instrumentations capable of detecting volatile *N*-nitrosamines at the ppb (µg/kg) level and less has revealed the contamination of numerous foods and beverages by these toxic compounds. Through active cooperation with the manufacturers, considerable progress has been made with a view to reducing *N*-nitrosamine-content in beer, malt, bacon and also in rubber nipples.

Many types of *N*-nitrosamines (dialkyl *N*-nitrosamines), well-known potent environmental carcinogens, have been shown to be oncogenic. Some of these *N*-nitrosamines are of importance in food toxicology. The principal precursors are the various amines, amides and nitrites (Mirvish, 1975) but also amino acids and other compounds which can undergo various reactions.

In attempting to study odour components in foods, Golovnya (1982) has identified more than 70 organic bases (including secondary aliphatic amines and heterocyclic compounds) in foods, and possible amine precursors of

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carcinogenic *N*-nitrosamines. Many nitrosable substances are derived from innocuous precursors which are commonly found in foodstuffs in appreciable amounts, free or in a combined form, such as proline, arginine, lysine, lecithin and choline. For instance, proline can be decarboxylated to pyrrolidine (Sen *et al.*, 1973*a*,*b*) or can form *N*-nitrosoproline first and then be decarboxylated to *N*-nitrosopyrrolidine (Nakamura, 1976). Arginine may give rise to derivatives which may become mutagenic after their nitrosation under acidic conditions (Endo & Takahashi, 1973*a*,*b*). Piperidine and pyrrolidine can be obtained from lysine by a bacterial metabolism (Saito & Sameshima, 1956). Cadaverine and putrescine can be obtained from lysine and ornithine, respectively, after decarboxylation. Both may undergo deamination and cyclization to piperidine and pyrrolidine, respectively, which lead to the corresponding carcinogenic *N*-nitrosamines (Bills *et al.*, 1973); Lijinsky & Epstein (1970) suggest that these modifications might occur during cooking. Lecithin can give rise to choline which, when heated, is converted into trimethylamine, one of the precursors of *N*-nitrosodimethylamine (NDMA) (Fiddler *et al.*, 1972). These few examples show us the complexity of the reactions occurring during food processing.

Many studies have been carried out in different countries to establish food and beverage contamination by volatile *N*-nitrosamines: USA (Scanlan, 1975; Gray, 1981), Canada (Sen *et al.*, 1978), United Kingdom (Gough *et al.*, 1978), France (Klein *et al.*, 1980), West Germany (Spiegelhalder *et al.*, 1980), The Netherlands (Stephany *et al.*, 1980), USSR (Aidjanov *et al.*, 1982), Poland (Karlowski & Bojewski, 1982, 1983), Japan (Kawabata *et al.*, 1984; Yamamoto *et al.*, 1984), Italy (Airoldi *et al.*, 1988; Gavinelli *et al.*, 1988), Sweden (Osterdahl, 1988*a*), etc.

The results of our survey of volatile *N*-nitrosamine contamination are presented and discussed here. This survey was made on food and beverages on the French market (East of France) with the much appreciated help from the Direction Générale de la Consommation, de la Concurrence et de la Répression des Fraudes who collected the foodstuffs.

MATERIALS AND METHODS

Collection of samples

All the samples (258 food samples and 53 beverages) were purchased between November 1987 and June 1988 from stores and supermarkets in the East of France (Moselle, Meurthe et Moselle, Meuse and Vosges). Before examination, all the samples were stored under usual household conditions. Some samples were directly analyzed, others were analyzed after appropriate storage in a cold room (where only food samples were admitted). In any case, storage did not exceed four weeks. Three determinations were carried out for each sample. It should be noted that products 'under suspicion' were not the only ones analyzed.

Analysis of samples

Materials

—chromatographic-grade quality dichloromethane (DCM) (S.D.S., Villeurbanne, France) used without prior distillation (a blank test for each bottle used revealed no contamination with volatile *N*-nitroso compounds);

—mineral crude oil: liquid paraffin (low viscosity, water white) (Merck, Nogent-sur-Marne, France);

—ascorbyl palmitate (FLUKA, Buchs, Switzerland;

—water, bidistilled (in glass apparatus) and tested for contamination;

—analytical grade sodium sulfate (anhydrous) (PROLABO, Paris, France);

—mixed *N*-nitrosamine reference standard: stock standard solution of 10 μg/ml ethanol of each of the following: *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodi-*n*-propylamine (NDPA), *N*-nitrosodi-*n*-butylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosomorpholine (NMOR) (ISCONLAB, Heidelberg, FRG). Working mixed standard solution: 0·31 μg each nitrosamine/ml, dilution of the stock solution 1:32 with dichloromethane. Stock and working solutions were stored in the dark at −20°C.

—*N*-nitrosomethyl-*n*-pentylamine (NMPA) internal standard solutions (from I.A.R.C., Lyon, France); Stock solution, 2 mg/ml ethanol. Working standard solution (for calibration): 10 μg/ml, dilution stock solution 1:200 with ethanol. Working internal standard solution: 0·20 μg/ml, dilution stock solution 1:10 000 with ethanol. Stock and working solutions were stored in the dark at −20°C.

Apparatus

Gas chromatographic analyses were performed using an isothermal gas chromatograph (GC) (GIRDEL, Model 30) interfaced to a Thermal Energy Analyzer TEA^{TM} (Model 502LC, Thermedics Inc., Woburn, Mass., USA). Data acquisition and processing were controlled by a Shimadzu CR-3A data system.

The chromatographic conditions

The gas chromatographic analyses were carried out on a stainless steel column (15 ft × $\frac{1}{8}$ in o.d.) packed with 10% (w/w) Carbowax 20 M on Chromosorb WAW (80–100 mesh). The chromatographic column was operated isothermally at 185°C with carrier gas flow rate in the range

10–30 ml/min (to have a carrier gas head pressure of 110 psi). Commercial-grade argon was used as carrier gas. For quantification, 10 μl of the final concentrate was injected into the column and analyzed against the external standard (daily injection). The TEA operating conditions were: furnace temperature, 500°C; the original cold trap temperature replaced by CTR Gas Stream Filter (Thermedics Inc., Woburn, Mass., USA); reaction chamber pressure: 1·6 mm Hg.

Extraction and clean-up procedures

Solid samples. 30 g of the food sample to be analyzed was cut into thin slices or minced, and weighed into a 500-ml round-bottomed flask (containing about 10 ml of mineral crude oil). Ascorbyl palmitate was used as nitrosation inhibitor and added to give a 500 mg/kg final concentration.

Depending on the water content of the sample, up to 10 ml of bidistilled water was added to the dried sample. *N*-nitrosomethyl-*n*-pentylamine (NMPA) was used as an internal standard with a view to obtaining a level of 7 μg/kg in the final concentrate. 20 ml of mineral crude oil was added again, to cover the sample before analysis. The contents were heated slowly (to 65–70°C) under vacuum (< 2 mm Hg). The distillation was continued until no more water was left in the distillation flask (this took about 45 min). The distillate was collected in a receiver flask (glass finger) immersed in liquid nitrogen. After having reached the ambient temperature, the distillate was quantitatively transferred with the washings of the glass finger to a 125-ml separating funnel and 4 ml 0·1 M hydrochloric acid was added. The contents of the separating funnel were then extracted three times with 30 ml dichloromethane.

The combined extracts were dried over anhydrous sodium sulfate and concentrated on a 3-stage Kuderna-Danish evaporator (SUPELCO France, St-Germain-en-Laye, France) at 46°C (water-bath) to a final volume of 0·5 ml. A 10 μl sample of the concentrate was injected onto the gas chromatograph.

Liquid samples. For beverages containing more than 20% (v/v) alcohol, 20 ml of the sample were diluted with 40 ml of bidistilled water. Dilution was necessary to retain the sample on the Chem Elut cartridge.

Beverages containing less than 20% (v/v) alcohol were analyzed without prior dilution. The Chem Elut cartridge was pre-wetted with 10 ml dichloromethane. The internal standard (NMPA) was mixed with the sample before addition to the cartridge. Once the sample was fully absorbed, dichloromethane was added (5 × 30 ml), and the eluate collected in a 3-stage Kuderna-Danish evaporator, then concentrated at 46°C to a final volume of 0·5 ml.

RESULTS AND DISCUSSION

Artifactual responses

Special care has been taken to avoid adventitious contamination of the sample and the reagents used during the overall analytical procedure; all the materials (stoppers, tubing and solid phase extraction devices) were carefully selected and checked before analysis. A reagent blank determination and a blank assay (without the sample) were regularly performed to check the entire analytical procedure for the risk of artifactual contamination. The use of a marker amine (2,6-dimethyl morpholine, recommended by Eisenbrand *et al.*, 1983) during the blank assay and some of the assays with samples, was a good probe to demonstrate that artifactual formation had not occurred. The procedures and the vessel used revealed no artificial contamination, and hence were considered perfectly reliable and adequate.

Recovery and detection limit

The contents of volatile *N*-nitrosamines expressed as μg/kg (ppb) were corrected for recovery of the internal standard; the results obtained for the most volatile *N*-nitrosamines (NDMA, and NDEA are the *N*-nitrosamines encountered most often) were directly corrected for recovery by means of the internal standard (NMPA). With respect to the least volatile *N*-nitrosamines (NDBA, NPIP, NPYR, NMOR), the results obtained (for more accuracy) could be corrected for recovery by carrying out a separate analysis of the particular sample after adding known amounts of these *N*-nitrosamines; this tedious and time-consuming work on the various samples screened was not performed, since these *N*-nitrosamines are usually not encountered (except for some of them, e.g. NPIP in cured meat products). The mineral oil distillation procedure and the extraction procedures for liquid samples allowed us to reach adequate recoveries (80–100%). Samples with poor recoveries were reconsidered. All these results are reported here without correction. The detection limits (in 30·0 g solid samples) of the *N*-nitroso compounds were estimated as follows: 0·04 μg/kg for NDMA, 0·06 μg/kg for NDEA, 0·11 μg/kg for NDBA, 0·09 μg/kg for NPIP, 0·16 μg/kg for NPYR and 0·19 μg/kg for NMOR (with a signal/noise ratio of 3:1). The detection limits were about two-fold better for liquid samples. The repeatability of the methods used was estimated on three determinations; the CV (coefficient of variation) was 20–30% for levels of contamination $<0{\cdot}5$ μg/kg, and $<20\%$ for levels $\geq 0{\cdot}5$ μg/kg.

NDPA, which is usually not encountered in foods and used by some workers as an internal standard, was replaced by NMPA. In practice, and for some samples, an interfering peak was observed with the same retention time as NDPA. *N*-nitrodimethylamine (Walker & Castegnaro, 1980) was suspected to be the interfering compound (not verified). The magnitude of this interfering peak did not seem to correlate with observed NDMA levels (trace levels or $> 1{\cdot}0\,\mu g/kg$).

The nitrosamine values presented are variable; in each category, some but not all the samples contain detectable quantities of *N*-nitrosamines. Two *N*-nitrosamines, namely NDMA and NDEA were regularly found. Other volatile *N*-nitrosamines, such as NDBA (curiously found in some samples), NPIP, NPYR and NMOR were found in relatively low concentrations or at trace levels, except in pork products. The presence of some volatile *N*-nitrosamines (NDBA, NMOR) in some samples may seem surprising; checks of solvents and reagents during blank tests were all negative. It is known that some brands of DCM may cause artifactual formation of nitrosamines or contain TEA responsive peaks (Pensabene & Fiddler, 1988). Sen & Baddoo (1986) studying the origin of the *N*-nitrosomorpholine content in margarine, showed that packaging papers (especially waxed wrappings) were the source of contamination. In our study, most of the samples collected were packaged in plastic containers or in aluminium foil, usually considered as non-contaminated materials. In view of our findings and checks, it seems unlikely that the positive findings of NMOR which we report here are due to the use of NMOR-contaminated DCM.

The positive findings obtained can be supported by the improved and well-established analytical procedures, but the effective presence of some of these compounds would have to be confirmed by further investigations using mass spectrometry for unequivocal identification, since an artifactual formation seems to be improbable (blank assay).

Our attempt to reveal a possible artifactual contamination during the analytical procedure having failed, the occurrence of some *N*-nitrosamines was not always well explained.

The foodstuffs and beverages survey

A great part of the research of the occurrence of *N*-nitrosamines in foods involved alcoholic beverages (including beers), pork products (nitrite-cured meats, smoked meats, etc.) and cheeses which are well-known to consistently contain volatile *N*-nitrosamines. Other common products (cereals, canned foodstuffs, etc.) were also examined for contamination.

Low alcohol beverages (red wines called 'apéritif' in French, usually sold with % alcohol <20 (v/v)) contained trace levels of volatile *N*-nitrosamines (NDMA essentially) similar to those obtained in non-alcoholic beverages (fruit juices, lemonades, etc.) (20 samples checked).

The *N*-nitrosamine contents in beers have been investigated in 12 samples (domestic and imported beers). The overall mean level of the positives (100%) was 0·28 μg/litre NDMA and can be considered low compared with the contamination levels obtained in the past (especially in 1979, by the German scientists, Spiegelhalder *et al.*, 1979) with up to 68 μg/litre NDMA in some beer samples). In a similar study (in the same region) of the occurrence of volatile *N*-nitrosamines in food, Klein *et al.* (1980) found all the beer samples collected to be positive with NDMA with concentrations ranging from 0·1 to 21·3 μg/litre (mean 3·1 μg/litre) in pale beers and from 7·2 to 16·8 μg/litre (mean 10·1 μg/litre) in dark beers. Since 1980, the NDMA contamination of beer has been regularly checked; although the number of beer samples surveyed is rather low in the present study (not sufficient to reach a general conclusion), beer is nowadays, in order of magnitude, about 10 to 20 times less contaminated. Changes in the malt-drying process operated by the brewing industry, have resulted in considerably lower NDMA levels in beer, up to 98% (Spiegelhalder *et al.*, 1980). Kubacki *et al.* (1989) demonstrated the utility of indirect malt-drying processes. Low levels of *N*-nitrosopyrrolidine (mean value 0·18 μg/litre) were also detected in two samples. In addition to NDMA, Spiegelhalder *et al.* (1979) reported the occurrence of NDEA. In our small survey of domestic and imported beers, NDEA has not been detected. One of the beer samples surveyed (a French dark beer) had an NDMA concentration of 1·5 μg/litre.

In addition to beer, other alcoholic beverages, including malt products and distilled spirits, have been surveyed for volatile *N*-nitrosamines. The highest values have been found in distilled spirits from stone fruits, with a mean content of 4·1 μg/litre NDMA and up to 16 μg/litre for a distilled spirit from cherry plum. Large amounts of NDEA were found in nearly all the distilled spirits obtained from stone fruits (mean content 3·0 μg/litre; range ND–12 μg/litre). In their report, Walker *et al.* (1979), also reported NDMA and NDEA contamination for 19 out of 34 spirits samples.

The two samples of whisky and scotch whisky examined were both positive, with respective contaminations of 2·5 μg/litre and 0·68 μg/litre NDMA.

Other alcoholic beverages showed indications for NDMA content from undetectable levels to 0·2 μg/litre (cognac, vodka). The level of NDMA seems to diminish with the alcohol concentration.

Results from our survey on a wide range of pork products (nitrite-cured meats, smoked meats, etc.) are summarized in Table 1. This table gives the results of *N*-nitrosamine levels in pork products, the list of the encountered volatile *N*-nitrosamines, the number of samples collected, the number of contaminated samples and the contamination range, the mean values of the contamination expressed in μg/kg (on three determinations for each sample) and the range (the lower and the upper values). The contamination range is

TABLE 1
Levels of Volatile *N*-nitrosamines in 89 Samples of Pork Products, Expressed in μg/kg

Sample (type)	*Number of analysed samples*	*Detected nitrosamines*	*Number of contaminated samples*	*Nitrosamine levels*		*Number of samples with nitrosamine content in the range*			
				Mean	*Range*	$p \leq 0.5$	$0.5 < p < 1$	$1 < p < 10$	$p \geq 10$
Smoked bacon	8	NDMA	8	0·25	ND–1·3	7	1	0	0
		NDEA	7	0·97	0·05–3·8	2	3	2	0
		NDBA	7	0·17	ND–0·80	6	1	0	0
		NPIP	6	0·11	0·05–0·27	6	0	0	0
Smoked pork brisket	19	NDMA	17	0·53	ND–4·0	12	2	3	0
		NDEA	17	1·6	ND–9·5	6	4	7	0
		NDBA	17	0·45	ND–2·7	13	2	2	0
		NPIP	11	2·9	ND–37	9	1	0	1
		NPYR	7	1·2	ND–11	6	0	1	0
		NMOR	12	0·25	ND–2·1	9	3	0	0
Ham (smoked, dried)	11	NDMA	11	0·54	ND–4·8	9	1	1	0
		NDEA	8	1·1	ND–12	4	1	3	0
		NDBA	9	0·29	ND–1·1	8	1	0	0
		NPIP	10	0·24	ND–1·3	8	1	1	0
		NMOR	5	0·48	ND–2·0	4	1	0	0
Ham	4	NDMA	4	0·14	ND–0·72	4	0	0	0
		NDEA	2	0·03	ND–0·07	2	0	0	0
		NDBA	4	0·09	ND–0·23	4	0	0	0
		NPIP	3	0·25	ND–0·97	2	1	0	0
		NPYR	1	0·12	ND–0·23	1	0	0	0
Fat liver (duck)	1	NDMA	1	0·47	0·17–0·77	1	0	0	0
		NDEA	1	0·81	0·57–1·0	1	0	0	0
		NDBA	1	2·8	1·7–3·5	1	0	0	0
		NPIP	1	2·6	2·1–2·8	1	0	0	0
		NPYR	1	0·20	0·10–0·28	1	0	0	0
		NMOR	1	0·79	0·40–1·1	1	0	0	0

German sausage (Mettwurst), pate (non-smoked)	10	NDMA	10	0·26	ND–0·84	8	2	0
		NDEA	8	1·1	0·02–7·5	3	2	3
		NDBA	8	0·26	ND–1·3	7	0	1
		NPIP	6	0·06	ND–0·41	6	0	0
		NMOR	4	0·24	ND–1·7	3	1	0
Sausage	5	NDMA	4	1·5	0·04–4·5	2	0	2
		NDEA	5	3·0	0·02–7·9	1	1	3
		NDBA	5	0·21	ND–1·0	5	0	0
		NPIP	3	0·25	ND–0·90	2	1	0
Sausage with garlic	2	NDMA	2	0·32	ND–1·3	1	1	0
		NDEA	2	2·6	1·3–6·4	0	0	2
Sausage (salami type)	4	NDMA	4	0·45	ND–2·1	3	1	0
		NDEA	4	4·6	1·5–12	0	0	4
		NDBA	4	0·56	0·29–2·0	3	1	0
		NPIP	3	0·17	ND–0·58	3	0	0
Various smoked sausages	15	NDMA	14	0·91	ND–9·3	9	3	2
		NDEA	13	2·4	0·91–10·3	1	1	11
		NDBA	14	0·43	ND–2·0	9	3	2
		NPIP	11	0·18	ND–2·2	10	0	1
		NPYR	6	0·28	ND–3·7	5	0	1
		NMOR	9	0·74	ND–7·2	8	0	1
Frying and cooking sausage	8	NDMA	8	0·32	0·05–1·2	5	3	0
		NDEA	7	1·8	0·03–5·1	1	1	5
		NDBA	7	0·23	ND–1·0	6	1	0
		NPIP	6	0·07	ND–0·22	6	0	0
Pork meat (in salt)	2	NDMA	2	0·09	0·08–0·28	2	0	0
		NDEA	2	0·03	ND–0·07	2	0	0

ND: not detected.
NDMA: *N*-nitrosodimethylamine.
NDEA: *N*-nitrosodiethylamine.
NDBA: *N*-nitrosodi-*n*-butylamine.
NPIP: *N*-nitrosopiperidine.
NPYR: *N*-nitrosopyrrolidine.
NMOR: *N*-nitrosomorpholine.

given, taking into account the mean values (of 3 determinations). ND means not detected (probably no contamination or a contamination below the detection limit). 'Trace levels' must be understood as being 'near to the detection limit'.

A total of 89 samples of cured meat products has been analyzed. Out of these, 85 samples (95%) showed indications for NDMA content. However, 64 samples (72%) had concentrations below 0·5 μg/kg. In nearly all the samples surveyed, a peak assigned to NDEA (but not confirmed by Gas Chromatography–Mass Spectrometry) was present at a concentration level often higher than NDMA. NDBA and NPIP were detected in most pork meat samples, but NPYR was rarely found. The occurrence of NDBA (but also NDEA) may be due to elastic rubber nettings. These materials formulated with zinc dibutyl or zinc diethyldithiocarbamates (vulcanization accelerators) have been identified as a source of this contamination (Sen *et al.*, 1988). Gavinelli *et al.* (1988), presenting a preliminary survey on volatile *N*-nitrosamine content in foods and beverages of the Italian market, have found appreciable amounts of NDMA, NDEA and NDBA (consistently detected) in cured foods (including both canned and cured meat) and canned luncheon meat; in their survey, a meat sample was reported to contain 55·6 μg/kg NDBA and 62·8 μg/kg NDEA. NDBA was also found by Gavinelli *et al.* (1988) in salami at a 19·71 μg/kg level (mean value) (all of the 4 samples checked were contaminated in the range 0·73–50·12 μg/kg) and in rolled bacon with levels ranging from 9·27 μg/kg to 23·78 μg/kg (mean content 13·65 μg/kg); NDEA was also reported at 1·01 μg/kg level (range ND–7·75 μg/kg). In our study, NMOR was also found in some apples, most often at trace levels. The use of elastic rubber nettings for packaging was again found responsible for the occurrence of NDMA, and to a lesser extent of NMOR (Sen *et al.*, 1987).

In the survey of 15 smoked sausages, 11 samples showed contamination between 1 and 10 μg/kg NDEA with a mean value of 2·4 μg/kg, and 1 sample in the same range with 9·3 μg/kg NDMA. The presence of garlic in some sausages does not seem to justify the high content of NDEA observed, compared with the same sausages without garlic. A high contamination was observed in one sample of fat liver (duck) (2·8 μg/kg NDBA and 2·6 μg/kg NPIP). The chromatogram of this sample is given in Fig. 1 (showing the high contamination). NPIP, NPYR, NDMA and NDEA occurred consistently in smoked pork brisket; one sample from a local and artisanal factory showed a 37 μg/kg NPIP-content! It is known that several factors influence the *N*-nitrosamine contents of meat products, including the amount of added nitrite, storage conditions and the age of the meat, the fat to lean ratio, the presence of nitrosation inhibitors, the slice thickness and the smoking conditions (Sen *et al.*, 1974; Gray *et al.*, 1982). A factor which tends to

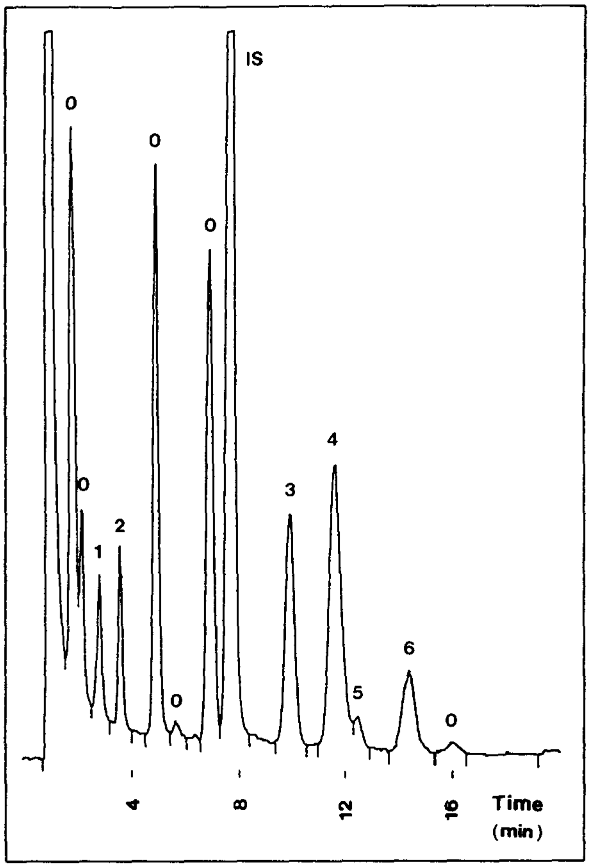

Fig. 1. Chromatogram of volatile *N*-nitroso compounds in a fat liver (duck) sample, obtained by gas chromatography coupled with a thermal energy analyser (TEA). The 6 most common volatile *N*-nitrosamines were identified with 6 unknown peaks (*N*-nitramines ? non-symetric *N*-nitrosamines? unsaturated *N*-nitrosamines?). 0: unidentified peak; 1: *N*-nitrosodimethylamine (NDMA); 2: *N*-nitrosodiethylnitrosamine (NDEA); 3: *N*-nitrosodi-*n*-butylamine (NDBA); 4: *N*-nitrosopiperidine (NPIP); 5: *N*-nitrosopyrrolidine (NPYR); 6: *N*-nitrosomorpholine (NMOR); IS: internal standard: *N*-nitrosomethyl-*n*-pentylamine (NMPA). Values obtained for this assay (in μg/kg): NDMA: 0·46; NDEA: 0·85; NDBA: 3·2; NPIP: 2·8; NPYR: 0·21; and NMOR: 0·86.

increase nitrosamine levels is cooking. Thus, one food sample may contain detectable nitrosamines but depending on the cooking (frying, boiling, baking, etc.), and under the effect of temperature, the levels can remain low or can jump from trace to high values (Pensabene *et al.*, 1974). For instance, *N*-nitrosothiazolidine and *N*-nitrosothiazolidine-4-carboxylic acid present in smoked bacon increased with an increase in frying temperature as well as with frying time (Sen *et al.*, 1986).

Fish (sardines in edible oil, fancy pink salmon, pilchards, salted cod, etc.)

was also surveyed for *N*-nitrosamine content. The 31 samples collected all showed indications for the presence of NDMA (mean concentration 0·48 μg/kg; range not detected to 3·5 μg/kg). Twenty-four samples (77%) contained NDEA from undetectable concentrations to 4·2 μg/kg (smoked fishes). The overall mean of the positives was 0·57 μg/kg. NDBA was also found in 16 samples (52%) (mean value 0·38 μg/kg) with up to 5·3 μg/kg for the most contaminated sample. The highest levels of *N*-nitrosamines were encountered, again, in smoked fish.

Nineteen products (fresh and frozen meats) were tested and showed only occasionally positive results, usually at the detection limit. Twelve samples (63%) were NDMA-contaminated from undetectable concentrations to 0·44 μg/kg (overall mean of the positives 0·07 μg/kg).

A total of 33 samples of canned foodstuffs has been analyzed. NDMA was regularly found (30 positives (91%)) in the range from undetectable to 1·9 μg/kg (mean value 0·19 μg/kg). NDEA was detected in 25 samples (76%) (mean value 0·52 μg/kg): 4 samples were found to be positive for NDEA in the range 1–5 μg/kg and 2 in the range 0·5–1 μg/kg. NDBA was detected in 13 samples (39%) (mean value 0·17 μg/kg) essentially in canned foodstuffs containing sausages.

The *N*-nitrosamine contents of cooked preparations (pizzas, moussaka, cod with potato flakes, etc.) were also investigated on 18 collected samples (7 freshly cooked and 11 frozen foods). NDMA was found in almost all the samples collected (15 samples (83%)), including pizzas and frozen vegetables cooked with meat and fish, in concentrations varying within the following limits: trace levels to 1·3 μg/kg (mean value 0·23 μg/kg). NDBA was surprisingly encountered in one sample of frozen vegetables at a level of 1·7 μg/kg and NDEA at 3·9 μg/kg. Poor storage at low temperatures (with many variations in temperature), from the manufacturing plant to the store, or the presence of high levels of nitrite-nitrate may justify these high values.

Foods for babies (2 samples examined) showed a sporadic occurrence of volatile *N*-nitrosamines (NDMA identified near the detection limit in both samples).

Fresh vegetables (6 samples examined) showed no significant pollution except for NDMA (mean value 0·41 μg/kg) and for one sample (tomatoes) with up to 3·3 μg/kg NDMA.

A number of cheeses (21 samples analyzed) have been shown to contain NDMA (21 samples (100%)) and NDEA (16 samples (76%)). The contamination observed was below 3 μg/kg for NDMA (mean value 0·45 μg/kg) and 4 μg/kg for NDEA (mean value 0·44 μg/kg). NPIP was also found in 4 samples (19%) up to 2·6 μg/kg in a camembert cheese (mean value 0·75 μg/kg for camembert type cheeses). Various cheeses were surveyed: camembert type, Saint-Paulin type, Edam type and fresh cheeses. Gough *et* *al.* (1977) who analyzed and found nitrosamines in 58 samples of cheeses including some to which nitrate had been added, noticed that there was no correlation between addition of nitrate and NDMA content in cheeses. Investigations by Danish scientists showed that no influence of production factors, e.g. nitrate addition, composition and type of cheese, storage or packaging conditions, could be established (Pedersen *et al.*, 1980). The presence of these contaminants in cheeses is still not well understood.

The remaining 37 samples of our survey were dried foods, various cakes, buns and pastries, chocolates and cereals. Dried prepared foods (9 samples), chocolates (15 samples) and cereals (8 samples) showed NDMA contamination. Levels from not detectable to 9·2 μg/kg were obtained.

13 chocolate samples were positive, as were 7 of the 8 cereals collected, with respective mean levels of 0·34 μg/kg and 0·41 μg/kg. The mean level in dried prepared food was 1·7 μg/kg. The most contaminated sample was a dried sauce for Chinese rice preparation with the NDMA level up to 9·2 μg/kg. These *N*-nitrosamine levels probably result from the drying process (and the temperatures reached). Weston (1984) found dried maize meal and wheaten corn flour samples to contain 2·6 and 4·2 μg/kg NDMA, respectively. Osterdahl (1988*b*) also reported the occurrence of NDMA in many dried foods: cocoa, cereal products and other products (soup powders, spices mixtures, etc.) with mean values for these products from less than 0·1 to 1·9 μg/kg. In our survey, appreciable amounts of NPIP were also detected in cocoa products (the 6 chocolates tested) with a mean value of 0·36 μg/kg with a level up to 2·1 μg/kg in a milk-free chocolate sample. A peak assigned to NDEA was observed in 30 samples (81%) tested, with NDEA content up to 4·2 μg/kg for a prepared dried food and up to 5·2 μg/kg in one sample of milk chocolate. The occurrence of NDEA (an artificial formation not having been observed) in such samples seems not to be reported; should a non-symmetrical *N*-nitrosamine, for instance, be suspected? The mean value for the 30 samples was 0·77 μg/kg.

Further investigations need to be conducted to confirm the occurrence of some *N*-nitrosamines, particularly diethyl *N*-nitrosamine, and other *N*-nitroso compounds, but also to try to identify the unknown peaks (see chromatograms 1 (duck fat liver sample) and 2 (an imported beer sample) (Figs 1 and 2), for example) which seem to be present in appreciable amounts too, and for some of them identified in some other foods. The use of GC-MS for unequivocal identification is recommended, but it will be generally necessary to clean up the sample extract further before analysis (and that will cause difficulties in identification at trace levels of these unknown peaks).

From the obtained results, the *N*-nitrosamine contents of French foodstuffs seem to have declined since the 1980s (since the last French survey). *N*-nitrosamines in foodstuffs can be considered to be a consequence

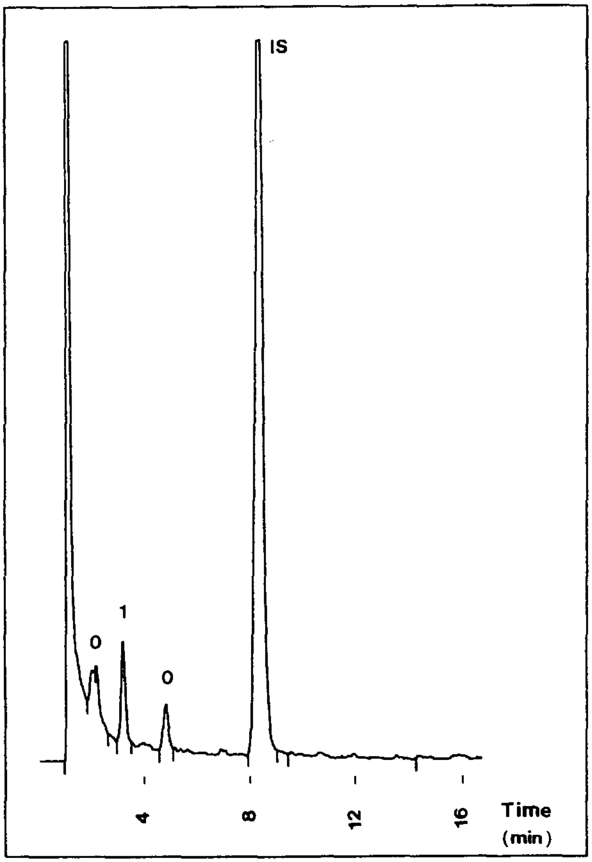

Fig. 2. Chromatogram of volatile *N*-nitrosamines in an imported beer sample (German beer) obtained by gas chromatography coupled with a thermal energy analyser (TEA), showing 0·27 μg/litre NDMA contamination. The chromatogram shows a peak not assigned with 4·8 min retention time (unknown peak). 0: unidentified peak; 1: *N*-nitrosidimethylamine (NDMA); IS: internal standard: *N*-nitrosomethyl-*n*-pentylamine (NMPA).

of the manufacturing processes. Changes or modifications of these processes (smoking, curing, drying, etc.) could eliminate or significantly reduce their levels. Efforts have been made to minimize these contaminations to the lowest feasible levels whenever possible, but efforts still have to be made. Although the levels of volatile *N*-nitrosamines are relatively low, the occurrence of non-volatile *N*-nitrosamines which can be present in large amounts in a sample must be borne in mind. It is not disputed that the amount of non-volatile *N*-nitroso compounds exceeds that of the volatile compounds by several orders of magnitude. Although there is considerable controversy with regard to methods of determining the apparent total *N*-nitroso compound contents (ATNC) of food matrix, these methods provide some information in the estimation of the non-volatile compound contents. Massey *et al.* (1988) have examined the contribution that simple volatile *N*-nitrosamines (NDMA and NPYR) and *N*-nitrosoamino acids (NSAR, NPRO, NHPRO) make to the ATNC content of cured meats and found that the volatile *N*-nitrosamines and the *N*-nitrosoamino acids account for less than 1% and 5%, respectively, of the ATNC. A whole meal might probably contain hundreds of μg/kg of these *N*-nitroso compounds, most of them probably potent carcinogens. Because of the lack of sufficient knowledge concerning quantitative extraction of these non-volatile compounds, cooperative efforts must be made between manufacturers and researchers.

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