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Volatile Nitrosamines in Foods and Beverages: Preliminary Survey of the Italian Market

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The question whether humans suffer from cancer as a result of exposure to N-nitroso compounds has not been yet answered. Food is one of the sources of human exposure to these compounds, and during the past decade considerable attention has in fact been paid to the identification and quantitative determination of N-nitroso compounds in food, volatile N-nitrosamines being those studied most extensively (Scanlan 1975). Although the primary focus has been on meat cured with nitrite, volatile nitrosamines reportedly occur in other foods such as cheese and fish (Pedersen et al. 1980; Yamamoto et al. 1984).

Many studies have been made in different countries to establish the degree of contamination of foodstuffs typical for each country and to identify the efforts needed to remove or at least reduce their presence in the environment. So far, there are only a few reports of nitrosamines in Italian cheese and beers (Cerutti et al. 1975; Tateo and Roundbehler, 1983), but no extensive studies have been made in Italy. We present here a preliminary survey on volatile nitrosamine content in foods and beverages on the Italian market, including canned beef, pork, poultry, cured meat, milk products, malt products, domestic Italian canned wines and beers.

A recently developed method was used for measuring nitrosamines in solid food matrices by simultaneous distillation-extraction followed by high resolution gas chromatography-thermal energy analysis (HRGC-TEA) (Gavinelli et al. 1986). Liquid food matrices and beverages were screened for nitrosamine content after column extraction and HRGC-TEA. The following nitrosamines were considered: N-nitrosodimethylamine (NDMA); N-nitrosodiethylamine (NDEA); N-nitrosodipropylamine (NDPA); N-nitrosodibutylamine (NDBA); N-nitrosopiperidine (NPIP); N-nitrosopyrrolidine (NPYR) and N-nitrosomorpholine (NMOR).

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MATERIALS AND METHODS

Ready to eat canned 'luncheon' meats (beef, pork, poultry), vacuum packaged sliced cured meat, whole and low fat milk, baby formula milk products, malt products, domestic Italian canned wines and beers were purchased from local retail outlets.

Standard volatile N-nitrosamines were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.; Extrelut extraction columns were from Merck, Darmstadt, F.R.G.; all other reagents were of the purest grade available. Distilled water percolated through an activated charcoal column was used.

Nitrosamines were extracted from canned meats using a microsteam distillator-extractor as previously described (Gavinelli et al. 1986). Briefly, 20 g of canned meat were homogenized with 2 ml glycerol containing α-tocopherol (400 mg) to inhibit unwanted nitrosation and the internal standard N-nitrosodiisopropylamine (NDiPA, 5 ppb). The homogenate, 55 ml glycerol, 2 ml water, 0.5 ml Tween 80, 15 g sodium chloride and antibumping granules were added to the distillation flask. The temperature inside the distillation flask was kept at 140°C. The extraction chamber was supplied with 3 ml methylene chloride and 1.5 ml water; the collecting tube containing 5 ml methylene chloride and antibumping granules was immersed in a water-bath at 50°C. The distillation time was 150 min followed by 20 min of solvent reflux. The methylene chloride phase of the extraction chamber and that in the collecting tube were combined, O.5 ml isooctane were added, the volume was reduced to about 0.5 ml under an air stream and then analyzed by HRGC-TEA.

Because of the different matrix of cured meat, unwanted nitrosation was completely inhibited only when 600 mg of α-tocopherol and 600 mg of ammonium sulphamate in 5 ml 0.14 M sulfuric acid were added to the sample.

Milk, beer or wine (20 ml) were spiked with 5 ppb of internal standard (NDiPA) and added to dry Extrelut extraction columns. Nitrosamines were eluted with 40 ml methylene chloride, 0.5 ml of isooctane were added to the eluate and the volume was reduced to about 0.5 ml before HRGC-TEA analysis.

Baby formula powdered milk and malt-containing breakfast products were reconstituted with water (13% w/v as indicated on the package label) before being added to the extraction column.

A DANI 3800 gas chromatograph was used, coupled to a TEA 543 detector (thermal energy analyzer, Thermo Electron, Waltham, MA, U.S.A.). A wall-coated fused silica capillary column, CP WAX 57 CB, 10 m x 0.32 mm i.d., 1.27 μm film thickness (Chrompack, Middelburg, the Netherlands) was used. The oven temperature was kept at 50°C for 30 s and then raised to 105°C at a rate of 25°C/min; the injector temperature was 200°C, the carrier gas (He) head pressure was 0.5 bar and the split ratio 1:20. The GC-TEA interface and pyrolyzer temperatures were 250 and 500°C respectively.

RESULTS AND DISCUSSION

To establish the amount of α-tocopherol and ammonium sulphamate needed to inhibit unwanted nitrosations, various amounts of inhibitors and 150 ppm nitrite, representing the maximum allowed amount of nitrite in Italy, were added to nitrite-free canned and cured meat found to be nitrosamine free which were then processed as described above. 400 mg of α-tocopherol and a mixture of α-tocopherol and ammonium sulphamate (600 mg each) prevented any nitrosamine formation in canned and cured meat respectively.

Table 1. Volatile nitrosamines in canned luncheon meat produced in Italy

Meat Samples	Volatile Nitrosamine median ppb (range)			
	NDMA	NDEA	NDBA	NPYR
Beef with jelly 6/7 (*)	0.35 (n.d.- 5.53)	0.79 (n.d.-18.31)	43.05 (n.d.-90.24)	n.d. (n.d.- 5.88)
Ground beef and pork 1/1	8.70	n.d.	71.82	n.d.
Turkey 1/1	0.94	3.03	68.41	n.d.
Pork with jelly 1/1	n.d.	62.80	55.60	2.35
Chicken 0/1	n.d.	n.d.	n.d.	n.d.

(*) No. of positive results/Total no. of samples
n.d. < 0.3 ppb
NDPA, NPIP and NMOR = n.d.

Table 1 reports nitrosamine levels in the canned meats examined. Only meats with nitrite or nitrate added were found to be contaminated with nitrosamines. The most frequently detected nitrosamines were NDMA, NDEA and NDBA; NPYR and was only occasionally observed; NDPA, NPIP and NMOR were never detected.

Table 2 shows nitrosamine content in cured foods. All the different types examined contained at least one of the seven nitrosamines considered. Three

out of six different brands of prosciutto were found to be nitrosamine-free and in accordance with the package label, two of the negative samples did not contain nitrites or nitrates either.

Table2. Volatile nitrosamines in cured meat produced in Italy

Food Samples	Volatile Nitrosamine median ppb (range)			
	NDMA	NDEA	NDBA	NPIP
Prosciutto 3/6 (*)	0.54 (n.d.-1.88)	n.d. (n.d.-11.01)	n.d. (n.d.- 6.46)	n.d. (n.d.-2.43)
Ham 3/3	n.d. (n.d.-0.3)	n.d. (n.d.- 0.3)	5.73 (4.63-17.32)	n.d.
Bologna 4/4	4.76 (0.94-16.47)	0.41 (n.d.-1.01)	14.63 (10.24-21.82)	n.d.
Salami 4/4	5.35 (0.59-7.76)	0.35 (n.d.-4.04)	19.71 (0.73-50.12)	n.d. (n.d.-0.38)
Rolled bacon 3/3	0.59 (0.47-0.94)	1.01 (n.d.-7.75)	13.65 (9.27-23.78)	n.d.
Coppa 2/2	3.29-6.23	n.d.	n.d.-3.41	n.d.-0.64
Speck 1/1	n.d.	n.d.	4.51	n.d.
Bresaola 1/1	24.70	0.78	7.56	n.d.
Wurstel 1/1	n.d.	n.d.	14.15	n.d.

(*) No. of positive results/ Total no. of samples
n.d. < 0.3 ppb
NDPA, NPYR and NMOR = n.d.

The amounts of nitrosamines found in Italian canned and cured meat were of the same order of magnitude as earlier reports (Crosby et al. 1972, Sen et al. 1980, Spiegelhalder et al. 1980). In both canned and cured meat we consistently detected NDBA. Mass spectrometric analyses on pooled samples confirmed the identity of the gas chromatographic peak. NDBA was detected in several, but not all samples, suggesting that the presence of this nitrosamine was not due to an artifact. Moreover, reagent blank samples run together with meat samples were never found to be contaminated with any of the nitrosamines tested. NDBA was seldom detected in foods; in 1976 Stephany et al. reported the presence of this nitrosamine in different Dutch foodstuffs

(Stephany et al. 1976). Later on, the same laboratory failed to detect any NDBA in Dutch foods (Ellen et al. 1986).

Analyses reported in this study were run about one year ago. We recently performed extractions on canned meats of the same brand as analyzed before and found less nitrosamines, including NDBA, than in the earlier tests (data not shown). This is not surprising and suggests that nitrosamine content in foodstuff may vary over time, depending on several factors, including reduced amount of nitrite added or the manufacturers' sources of raw materials for canned and cured meat.

Milk (9 different types), baby formula powdered milk (5 types), malt products (6 types) and wines (7 types) were not found to contain any of the tested nitrosamines. Recoveries of known amounts of volatile nitrosamines added to liquid matrix food before column extraction were usually high, ranging from 60 to 90%. The detection limit of the method was 0.05 ppb.
As expected, the only volatile nitrosamine found in beers was NDMA (Table 3).

Table 3. NDMA in domestic Italian and imported beers

	type	+/Tot (*)	median ppb	range
Italian beer	light	7/8	0.39	n.d. - 0.71
	dark	6/7	0.22	n.d. - 0.42
Imported beer	light	5/5	0.13	0.07 - 0.19
	dark	3/3	0.30	0.15 - 0.36

(*) No. of positive results/Total no. of samples
n.d. < 0.05

Italian beer NDMA content was in agreement with data reported by Tateo and Roundbehler (1983) and similar to the NDMA content of imported beers. Earlier studies by Spiegelhalder et al. (1980) on German beers and by Goff and Fine (1979) on beers of different origin, reported higher NDMA levels ranging from <0.5 to 68 ppb and from 0.4 to 7 ppb respectively. Though our data are too few to permit any general conclusion, they do seem to indicate a trend toward lower NDMA content in beers especially imported ones, and this may be the result of changing the procedure for drying malt.

Though the levels of nitrosamines detected were relatively low, it must be borne in mind that a whole serving may contain hundreds of ppb. At the moment we do not know whether these concentrations pose a real hazard to man, but they may be sufficiently high to warrant concern. In the absence of data clearly relating nitrosamines to the causation of cancer in man, all possible efforts should be made to keep contamination low and in some instances we have the means to do it.

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