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Volatile Nitrosamines in Various Cured Meat Products: Effect of Cooking and Recent Trends

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A study was carried out to determine the effect of cooking on the levels of volatile nitrosamines in various cured meat products. Of 64 samples tested, 39 were negative (<0.1 ppb) both before and after cooking. The majority of the positive samples contained extremely low levels (<1 ppb) of nitrosamines; the highest level detected was 8.6 ppb NPIP in a sample of spiced smoked beef. Only in two cases were significant increases in the levels of nitrosamines observed after cooking. Ten samples of baby foods containing various meat products were all found to be negative. In addition, 12 samples of fried bacon and 10 of cooked-out bacon fats were analyzed; all were positive for *N*-nitrosopyrrolidine, *N*-nitrosodimethylamine, and in some cases *N*-nitrosodiethylamine. The results indicate a continuously lowering trend in the levels of volatile nitrosamines in all types of cured meat products, except fried bacon.

The formation and occurrence of traces of volatile *N*-nitrosamines in various cured meat products have been well established (Eisenbrand et al., 1977; Fazio et al., 1973; Gough et al., 1977; Groenen et al., 1976, 1977; Sen, 1972; Sen et al., 1976a, Wasserman et al., 1972). The most commonly occurring nitrosamines in these foods are *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosopiperidine (NPIP), all of which are potent carcinogens (Magee et al., 1976). Although the levels of various nitrosamines detected in most cases are in the low parts per billion range, fairly high levels (50–300 ppb) have occasionally been detected in some products, especially those prepared with the addition of spice–nitrite premixes (Sen and McKinley, 1974; Wasserman, 1978) and fried bacon and cooked-out bacon fats (Fazio et al., 1973; Gough and Walters, 1976). Among cured meat products bacon is unique in the sense that it is generally free of nitrosamines in the raw stage; nitrosamines are formed only during the high-heat frying.

Previous studies from this and other laboratories suggest that although the levels of these nitrosamines in cured meat products have been decreasing steadily during the past few years, traces are still detected (Gough, 1978; Havery et al., 1978a,b; Eisenbrand et al., 1978; Sen et al., 1977).

Fried bacon still remains a problem item which consistently contains NPYR and NDMA. Various studies have shown that the addition of nitrite-scavenging chemicals such as ascorbate, ascorbyl palmitate, α-tocopherol, etc. to bacon can significantly reduce the concentration of nitrosamines in fried bacon, but these studies are still at

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a preliminary stage (Fiddler et al., 1978). It is not certain whether such treatments will result in a successful commercial method of producing bacon which will be free of nitrosamines.

There is also a possibility that similar high-heat cooking of other cured meat products might lead to the formation of increasing levels of nitrosamines. For example, Eisenbrand et al. (1977) have reported a significant increase in the levels of NDMA (1–12 ppb), NPIP (1–64 ppb), and NPYR (3–46 ppb) in some spiced meat products after frying. Similar increases in the formation of NPYR during frying of country-style ham have been observed (Greenberg, 1977). However, these studies were limited to the analysis of only a small number of samples. This study was therefore undertaken with a larger number and wider varieties of cured meat products to determine whether cooking significantly increases the nitrosamine levels. Such data would also provide valuable information about the trends in the levels of nitrosamines in uncooked products when compared to earlier results. The paper also contains the results of the analysis of ten baby food samples containing various meat products.

EXPERIMENTAL SECTION

Samples. The samples were purchased locally in the Ottawa area and stored at 4 °C (maximum storage, 1 week) prior to cooking and analysis.

Cooking. Bacon slices were fried in a Teflon-coated electric fry pan with the thermostat set at 340 °F. Starting with a cold pan, the slices were fried for 13–14 min; the slices were turned every 2–3 min. To ensure proper frying conditions, the temperature of the hot cooked-out fat was occasionally monitored with a thermometer. At the end of frying, the heat was turned off and the pan tilted to drain off the excess fat from the cooked lean. Suitable aliquots (10–25 g) of the cooked-out fats and the homogenized cooked lean were analyzed for nitrosamines.

Sliced salami, sausages, wieners, pepperoni, and bologna samples were fried as above but for a shorter duration (5–12 min) depending on the thickness of the slices. The cooking was stopped as soon as the slices appeared well cooked. All the smoked meat and the corned beef samples were wrapped in aluminum foil and cooked in an oven (160 °C) for 30 min. Weight loss during cooking was recorded for all samples. All samples, both uncooked and cooked, were homogenized thoroughly prior to analysis.

Apparatus and Reagents. All reagents used were analytical grade and the solvents were glass-distilled. Commercially available glass-distilled methylene chloride (Caledon Laboratories, Georgetown, Ontario) was redistilled before use.

A Varian gas chromatograph (Model 2700) coupled to a thermal energy analyzer (TEA) detector (Fine et al., 1975) was used for the analysis of volatile nitrosamines. A 6 ft $^1/_8$ in. (i.d.) stainless steel column packed with 25% Carbowax 20M and 2% NaOH on 60–80 mesh Chromosorb P was used for the GLC analysis. Carrier gas (Ar) flow rate was 30 mL/min and the oven temperature was set at 170 °C.

A Varian Mat (Model 311A) mass spectrometer equipped with an electron impact ionization source and coupled to a Varian Aerograph gas chromatograph (Model 1400) was used for the mass spectrometric confirmation. The column was similar to the one used above.

Nitrosamine Analysis. *Distillation and Cleanup.* An aliquot (20–50 g) of the sample was taken into a 2-L round-bottom distillation flask and mixed with 200 mL of 3 N potassium hydroxide solution and 500 ng of *N*-nitrosodipropylamine (internal standard). The mixture was distilled under vacuum using an all-glass vertical standing type flash evaporator (Buchler Instrument, NJ) (Sen et al., 1974; Sen, 1978) until 170–180 mL of distillate was collected. [During the distillation, ice-cold water must be passed through the condenser and the collection flask must be immersed in ice.] The collection flask containing the aqueous distillate was changed and about 400 mL of methylene chloride was distilled in the same manner (this second distillation step was carried out so as to rinse down any nitrosamines that may have been trapped inside the condenser). The methylene chloride distillate was used for the extraction as described below.

Table I. Nitrosamine Contents of Fried Bacon and Cooked-out Bacon Fat (ppb)[a]

cooked lean			cooked-out fat		
NDMA	NDEA	NPYR	NDMA	NDEA	NPYR
1.9	N[b]	14.9			
4.4	N	21.3			
Tr	Tr[c]	5.2	6.9[d]	N	26.2[d]
Tr	1.6	5.2	6.4	N	19.7[d]
17.2	6.0	10.9[d]	7.3[d]	6.2[d]	24.0[d]
Tr	2.1	2.0	7.8[d]	N	22.5[d]
4.7[d]	Tr	4.3	5.8	N	16.9
1.6	1.3	5.4[d]	2.6	N	22.5
3.0	Tr	21.8[d]	2.7	N	20.0
N	Tr	1.7	6.0	N	15.1
5.4[d]	Tr	14.6[d]	11.9	N	34.0
2.5	1.0	4.8	7.0	N	18.0
3.4[e]	1.0	9.3	6.4	0.6	21.9

[a] Results uncorrected for recovery losses. [b] N = negative (<0.1 ppb). [c] Trace (<0.2 ppb). [d] Confirmed by GLC–high-resolution mass spectrometry. [e] Mean values.

The aqueous distillate was made alkaline by mixing with 5 mL of 3 N potassium hydroxide solution and the mixture was extracted with two 200-mL portions of methylene chloride (obtained from the step above). The combined methylene chloride extract was washed once with 50 mL of glycine buffer (Sen, 1978) and then back-washed with 50 mL of 0.3 N potassium hydroxide. Finally, the methylene chloride extract was dried over anhydrous sodium sulfate and filtered, and the filtrate was concentrated to 1.0 mL in specially designed Kuderna-Danish type concentrators (Sen, 1978).

Reagent blanks were carried out regularly to ensure the absence of any contamination. Because of the small sample no antifoam was necessary during distillation.

GLC–TEA Analysis. A 5-μL aliquot of the final concentrated extract was analyzed by the GLC–TEA technique (Fine et al., 1975) which is highly specific for nitrosamines.

GC–Mass Spectrometric Confirmation. Prior to GLC–MS analysis the extract was further cleaned up on a basic alumina column as described previously (Sen, 1978). About 2–5-μL aliquots were injected, and the mass spectrometer was operated in the specific ion monitoring mode for the respective molecular ion peaks at a resolution of 5000. The operating conditions were similar to those reported earlier (Sen et al., 1976b). Since the GLC–TEA technique is highly selective, mass spectrometric confirmation was carried out only occasionally to double-check the identity of the nitrosamines detected by the TEA technique.

RESULTS AND DISCUSSION

The nitrosamine contents of the fried bacon and the cooked-out bacon fat and of the other cured meat products are given in Tables I and II, respectively. Since previous studies have shown that raw bacon is usually free of volatile nitrosamines only fried bacons were analyzed in this

Table II. Nitrosamine Contents of Various Meat Products Analyzed in the Survey

kind	no. of samples found positive[a]/no. analyzed		nitrosamine levels, ppb NDMA	NDEA	NPYR	NPIP
bologna	3/6		0.8	3.5	Tr[b]	4[c]
			Tr	Tr	3.2	N[d]
			Tr	N	N	N
ham	0/3					
mock chicken, turkey roll	1/3	(turkey roll)	Tr	Tr	N	N
salami	3/9		4.7[c]	N	N	N
			Tr	N	N	N
			N	N	Tr	N
sausages	6/20		Tr	Tr	N	N
			0.5	N	N	N
			N	0.9	N	N
			N	Tr	N	N
			Tr	N	N	N
			Tr	N	N	N
pepperoni	2/2		0.8	N	0.8	N
			N	1.6	N	N
smoked meats, some heavily spiced	6/8		N	N	N	1.5
			2	N	4.5	8.6[c]
			N	Tr	N	N
			Tr	Tr	N	N
			N	1.5	N	N
			0.2	Tr	Tr	1
wieners	2/7		Tr	N	N	N
			0.9	N	N	N
miscellaneous products (meat loaf, peameal bacon, etc.)	0/4					
corned beef (spiced)	2/2		Tr	N	N	7.3[c]
			Tr	N	N	N
baby foods (ham omelette, beef stew, ham broth, beef liver broth, ham and egg breakfast, etc.)		0/10				

[a] Mostly positive by GLC-TEA; some confirmed by GLC-MS as indicated below. [b] Trace (<0.2 ppb). [c] Confirmed by GLC-high-resolution mass spectrometry. [d] N = negative (<0.1 ppb).

Table III. Effect of Cooking on the Nitrosamine Levels in Various Cured Meat Products

kind		ppb[a] NDMA	NDEA	NPYR	NPIP
smoked meat (spiced beef)	uncooked	2	N[b]	4.5	8.6[d]
	cooked	1.2	N	3.3	6.7[d]
sausage (pepperettes)	uncooked	0.5	N	N	N
	cooked	1.2	N	N	N
salami	uncooked	4.7[d]	N	N	N
	cooked	7.6	N	5.3	N
corned beef	uncooked	Tr[c]	N	N	7.3[d]
	cooked	Tr	N	N	5.0[d]
bologna	uncooked	0.8	3.5	Tr	4[d]
	cooked	Tr	4.9	6.1[d]	6.5[d]
bologna	uncooked	Tr	Tr	3.2	N
	cooked	N	N	3.2	N
wiener	uncooked	0.9	N	N	N
	cooked	1.1	N	N	N
smoked meat (spiced)	uncooked	0.2	Tr	Tr	1
	cooked	Tr	Tr	0.2	0.9

[a] Results uncorrected for recovery losses. [b] N = negative (<0.1 ppb). [c] Trace (<0.2 ppb). [d] Confirmed by GLC-high-resolution mass spectrometry.

study. The baby food samples were analyzed without any further cooking for they are already well cooked. All other samples were analyzed both before and after cooking.

As indicated earlier, the efficiency of the distillation and clean-up steps was routinely checked by spiking each sample with 10 ppb of NDPA (internal standard). The recoveries of the internal standard were found to vary in the range of 75–85%. Similar recovery studies were occasionally carried out using a mixture of five volatile nitrosamines (NDMA, NDEA, NPIP, NPYR, and nitrosodibutylamine), and the results were highly satisfactory (70–90% recovery). These recovery values compare favorably well with those reported by other investigators (Sephany et al., 1976; Havery et al., 1978a; Elgersma et al., 1978).

Although the GLC–TEA technique is regarded as a reliable and specific method for determining *N*-nitroso compounds in foods, other chemicals may interfere and give a false positive result, especially at low levels. Therefore, those results which have not been confirmed by mass spectrometry should be considered as "apparent nitrosamines contents" only, and not as unequivocal proof of the presence of nitrosamines.

All the fried bacon samples and the cooked-out bacon fats were positive for nitrosamines, mainly NPYR and NDMA. The levels of nitrosamines detected are slightly lower than those observed in a previous survey carried out in December 1975 (Sen et al., 1977). At that time the average levels of NPYR in the cooked lean and the cooked-out fats were found to be 15 and 25 ppb, respectively. Although other workers (Groenen et al., 1976; Stephany et al., 1976) have reported the finding of significant levels of NDEA (up to 43 ppb) and NPIP (up to 8.2 ppb)

in fried bacon, only very low levels (trace to 6 ppb) of NDEA and none of NPIP were detected in this study. The levels of NPYR detected in our present study appear to be lower than those reported in two recent U.S. studies (Havery et al., 1978a,b) where an average of about 20 ppb NPYR was detected in fried bacon. A combination of various factors such as processing conditions, frying methods, etc., may account for this difference.

Of the 64 samples of various other cured meat products tested, 39 were negative (<0.1 ppb) both before and after cooking. Typical examples of the positive findings are shown in Table II. All ten baby food samples were negative. As can be seen (Table III), only in two cases (one salami and one bologna) one could observe a definite increase in the levels of nitrosamines (mainly NPYR) after cooking. In all other cases, cooking did not significantly change the nitrosamine contents of the samples. This is in contrast to the findings of Eisenbrand et al. (1977) who detected consistent and significant increases in the levels of nitrosamines in various meat products after cooking. More recent results from the same laboratory (Preussmann et al., 1979), however, are in agreement with the findings presented in this report.

Comparison of the data presented in this paper with those of earlier surveys (Sen et al., 1974, 1976a) indicates a continuous lowering trend in the levels of volatile nitrosamines in various cured meat products. The discontinuation of the use of nitrite–spice premixes around 1973–1974 and a better control of the input of nitrite additives are probably the two major factors responsible for this improvement. Increasing use of ascorbate or erythorbate, which are knwon to inhibit the formation of nitrosamines, in the preparation of various cured meat products may also be another contributing factor. Fried bacon appears to be the only cured meat product that continues to contain significant levels of NDMA and NPYR.

SAFETY NOTE

Since most volatile nitrosamines are potent carcinogens, proper precautions should be taken in handling these chemicals.

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