Effect of Sodium Nitrite Concentration on the Formation of Nitrosopyrrolidine and Dimethylnitrosamine in Fried Bacon

Fried bacon samples prepared with 0-, 50-, 100-, 150-, and 200-ppm levels of sodium nitrite were analyzed for nitrosopyrrolidine and dimethylnitrosamine. The samples prepared without any added nitrite were negative, but all the fried samples with added nitrite contained detectable levels (2–20 ppb) of nitrosamines. The levels of nitrosopyrrolidine correlated well with the initial concentrations of nitrite but not with that of nitrite found in the raw bacon just prior to frying. The identity of nitrosopyrrolidine in a few samples was confirmed by glc–mass spectrometry.

Sodium nitrate and sodium nitrite are widely used as curing agents for a variety of meat products. These chemicals impart a pleasant red color to the meat and also induce a particular type of flavor to the product (Brooks *et al.*, 1940; Cho and Bratzler, 1970; Parr and Henrickson, 1970). Apart from this aesthetic aspect, nitrite acts as a preservative by inhibiting the outgrowth of *Clostridium* spores which might be present in the meat (Greenberg, 1972; Pivnick *et al.*, 1967).

The use of nitrite, however, as a curing agent for bacon has recently come under criticism for it has been implicated in the formation of carcinogenic nitrosamines, mainly nitrosopyrrolidine (NPy), during frying of bacon. Earlier reports indicated that fried bacon may contain traces to 106 ppb (μg/kg) of NPy and 0–5 ppb of dimethylnitrosamine (DMN) (Crosby *et al.*, 1972; Sen *et al.*, 1973). Although it is generally believed that nitrite is responsible for the formation of NPy in fried bacon no definite studies have been carried out to determine the effect of nitrite concentration on the final levels of nitrosamines in the cooked product. This communication presents the results of such a study.

EXPERIMENTAL SECTION

Bacon Samples. The experimental samples were prepared by a commercial firm in Canada using its normal curing procedure. A hand pumping maching was used to inject trimmed, fresh pork bellies with sufficient pickle to increase the weight by 9% of the fresh weight. Sodium nitrite concentrations in the pickle were chosen to give an initial level of 0, 50, 100, 150, or 200 ppm in the product. After pickling, the bellies were smoked for 8–10 hr to an internal temperature of 128°F. The products were then chilled for 48 hr at 26°F, pressed, sliced, and vacuum-packaged in nylon polyethylene laminate film. The samples were stored at −20° until fried and analyzed without additional storage in the freezer.

Reagents and Solvents. All reagents used were of analytical grade, and all solvents were distilled from an all-glass apparatus (Sen, 1971; Sen and Dalpé, 1972).

Determination of Nitrite. The samples were allowed to thaw at room temperature and then macerated by passing through a meat mincer. The cut pieces were mixed by hand, and a 10-g aliquot was analyzed for nitrite by the method of Kamm *et al.* (1965).

Extraction and Clean-Up for Nitrosamine Analysis. A 100-g aliquot of the sample was fried in an electric fry pan (temperature 340–350°F) for 8–10 min. The fried sample was cooled and the entire contents, including the rendered fat, were transferred to a blender. The material was extracted with methylene chloride and the extract purified by the method described earlier (Sen, 1971; Sen *et al.*, 1972) with minor modifications. Instead of collecting 150 ml of distillate, as was done previously, 170 ml was collected during the distillation step. Care was taken to distill the solution slowly so that about 4 hr was needed to collect the 170-ml distillate.

The aqueous distillate was made alkaline by adding 10–20% potassium carbonate, and extracted with two 170-ml portions of methylene chloride. Interfering amines were removed by washing the extract with glycine–hydrochloric acid buffer (pH 2.1). The methylene chloride extract was dried over anhydrous sodium sulfate, filtered, and concentrated (using a Snyder column) to 15 ml by heating the solution in a warm water bath. Exactly 20 ml of *n*-heptane was added and the solution again concentrated to 20 ml (water bath 80–90°). Excessive heating at this stage was avoided to prevent the loss of volatile nitrosamines.

The solution was allowed to cool and then chromatographed on a basic alumina (3% water content) column as described previously (Sen, 1971; Sen and Dalpé, 1972). After washing the column with 50 ml of anhydrous *n*-pentane the adsorbed nitrosamines were eluted with 75 ml of methylene chloride. The eluate was finally concentrated to 1.0 ml (using Snyder columns) and then used for gas–liquid chromatographic (glc) and thin-layer chromatographic (tlc) analysis.

Glc Determination of DMN. About 10–20-μl aliquots were analyzed by glc using a Coulson electrolytic conductivity detector (pyrolytic mode) as described earlier (Sen *et al.*, 1972, 1973).

Tlc Estimation of NPy. NPy was detected by a method similar to that published by Sen *et al.* (1973). Aliquots (20 and 100 μl) of the extract were analyzed by tlc using an acetic acid–ninhydrin combination as the spray reagent (Sen and Dalpé, 1972). The plate was heated in an oven (90–100°) for 15–30 min (excessive heating should be avoided), and then examined inside a Chromato-Vue chamber (Ultra-Violet Products Inc., San Gabriel, Calif.) using the top short-wave as well as the bottom long-wave transilluminator uv lamps for detecting purposes. The amount of NPy present in the extract was semiquantitatively estimated by visual comparison of the intensity and size of the orange fluorescent spots with that of known amounts of standards spotted alongside the bacon extract. The technique is both very sensitive and specific; 20 ng of NPy produces a clearly distinguishable spot. Other nitrosamines do not interfere with the analysis. The tlc data are normally within 10–20% of that obtained by the glc–mass spectrometric method.

Glc–Mass Spectrometric Confirmation of NPy. The final extracts from 200–300 g of bacon were further purified by preparative tlc (Sen, 1971, 1972). The glc-mass spectrometric confirmation was carried out as described previously (Sen, 1972; Sen *et al.*, 1973; Fiddler *et al.*, 1972).

RESULTS AND DISCUSSION

The results (Table I) clearly demonstrate a gradual increase in the levels of NPy in fried bacon with an increase in the concentration of sodium nitrite used in the preparation of the bacon. Even the samples prepared with the lowest level of sodium nitrite, namely 50 ppm, contained

Table I. Levels of DMN and NPy in Fried Bacon Samples Prepared with Different Levels of Sodium Nitrite

Sample no.	Sodium nitrite, ppm		Nitrosamines in fried samples,[e] ppb	
	Initial	Final[a]	DMN	NPy
1–5	0	10	All N[b]	All N
		0		
		0		
		0		
		7		
6–9	50	25	N	2
		15	N	2
		12	3	4
		20	N	4
10–14	100	27	N	8
		20	N	7
		37	2	8
		15	N	8
		32	3	8
15–19	150		3	10
		49	N	20
		54	3	10
		49	N	5
		49	N	5
20–24	200	32	N	20[c]
		49	3	20[c]
		104	N	20[d]
		113	5	12
		123	3	12

[a] Analyzed after storage for 6 weeks at −20°. [b] N = none detected (detection limit, 2 ppb). [c] Confirmed by glc–mass spectrometry (Sen, 1971, 1972). [d] Confirmed by combined glc–high-resolution (resolution 1 in 10,000 with 10% valley definition) mass spectrometry (Fiddler *et al.*, 1972). [e] Results expressed on the basis of the weight of the uncooked bacon.

minute traces (2–4 ppb) of NPy. Statistical analysis of the data by Kendall's rank correlation test (Siegel, 1956) indicated high correlations between the levels of NPy and that of sodium nitrite initially used to prepare the bacons. The approximate significance probabilities for the two-tailed test were as follows: $P < 0.001$ between initial sodium nitrite and final NPy. The correlation between the final levels of sodium nitrite (analyzed before frying) and that of the nitrosamines in the fried products was, however, negligible. No NPy was found in raw bacon (Sen *et al.*, 1973). This suggests that NPy in fried bacon may be originating from an intermediate nitroso compound which is probably produced during the early stages of the curing of bacon, and, therefore, its concentration is dependent on the initial nitrite concentration. As suggested previously (Sen *et al.*, 1973) this intermediate compound may be nitrosoproline which may undergo decarboxylation reaction to form NPy during frying of bacon. Preliminary studies by Fiddler and Wasserman (1973) suggest that such a decarboxylation reaction can take place in model experiments.

Results presented in Table II indicate that all the commercial fried bacon samples examined contained NPy and one contained traces of DMN. Although both the tlc and glc methods used for determining NPy and DMN are highly specific, we would like to emphasize that the results which were not confirmed by glc–mass spectrometry should be considered as tentative. It is noteworthy that none of the positive (by tlc) samples (five in this study and seven in other studies) tested so far have failed the glc–mass spectrometric confirmatory test for NPy. It is, therefore, highly likely that the values for NPy presented in both Tables I and II are a true measure of the NPy contents of the fried bacon samples. Since the initial nitrite concentration in the commercial products was not known no statistical analysis was carried out.

Table II. DMN and NPy Contents of Fried Bacon Purchased from Local Stores

Brand	Final sodium nitrite,[a] ppm	Nitrosamines in the fried samples,[d] ppb	
		DMN	NPy
A	30	N[b]	20
A	30	N	40[c]
A	76	N	18
B	44	N	75
B	32	3	25
B	54	N	21
C	12	N	15
C	32	N	30
C	94	N	21
D	20	N	25[c]
D	20	N	30
D	54	N	31

[a] Analyzed just before frying. [b] N = none detected (detection limit, 2 ppb). [c] Confirmed by glc–mass spectrometry (Sen, 1971, 1972). [d] Results expressed on the basis of the weight of the uncooked bacon.

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