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Inhibition of Nitrosamine Formation in Fried Bacon by Propyl Gallate and L-Ascorbyl Palmitate

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It was shown that treatment, just prior to frying, of normal nitrite-cured bacon with 1000 ppm of propyl gallate, piperazine, sodium ascorbate, or ascorbyl palmitate markedly reduces the formation of nitrosopyrrolidine during cooking. Propyl gallate, piperazine, and ascorbyl palmitate were more effective than sodium ascorbate in this respect. When nitrosoproline was added to lard or nonnitrite bacon (bacon prepared without nitrite) and the mixture cooked, the formation of nitrosopyrrolidine was demonstrated but the yield was extremely low (0.06–0.21%). Addition of the above-mentioned additives did not inhibit the formation of nitrosopyrrolidine from nitrosoproline. It was, therefore, concluded that these additives inhibit nitrosopyrrolidine formation in normal bacon by interfering with reactions other than the decarboxylation step of nitrosoproline. The possibility of using ascorbyl palmitate in cured bacon is discussed.

Studies with laboratory animals have indicated that many *N*-nitrosamines are strong carcinogens (Magee and Barnes, 1956; Druckrey et al., 1967; Lijinsky et al., 1969). The reported occurrence (Sen, 1972; Crosby et al., 1972; Fiddler et al., 1974) of nitrosamines in food products, especially nitrite-treated meats, is, therefore, a matter of concern. Previous studies (Sen et al., 1973; Fazio et al., 1973; Crosby et al., 1972) have shown that traces of nitrosopyrrolidine (NPy) are formed during frying of bacon although none can be detected in the uncooked product. It is believed that NPy is formed by decarboxylation of nitrosoproline (NPro) which could arise from the interaction of added nitrite and the naturally occurring amino acid, L-proline (Lijinsky and Epstein, 1970; Sen et al., 1973). An alternative pathway would be via direct interaction of pyrrolidine and nitrite. The results of model system experiments by Pensabene et al. (1974) and Bills et al. (1973) have provided support to the theory that NPy can be formed from NPro under simulated conditions of bacon frying. It has also been established that putrescine, spermine, spermidine, and collagen, all of which are known to occur in pork bellies, can react with nitrite to form NPy (Bills et al., 1973; Huxel et al., 1974). Among all the precursors tested NPro seemed to produce NPy in highest yields.

Nitrite is used as a curing agent for bacon as it imparts an attractive red color to the meat. In combination with sodium chloride, it also induces a particular type of flavor (Brooks et al., 1940; Cho and Bratzler, 1970; Parr and Henrickson, 1970). In addition, the combination of salt and nitrite plays a significant role in controlling the outgrowth of *Clostridium botulinum* spores (Pivnick et al., 1967; Greenberg, 1972). Although the exact mechanism of action of nitrite against the growth of *botulinum* spores is not known, recent studies (Greenberg, 1972; Perigo and Roberts, 1968) have indicated that the initial level of nitrite used, and not the residual level, is the important factor. Nitrite-treated canned pork luncheon meat with only 2 ppm of residual nitrite has been shown to possess considerable antibotulinum effect (Pivnick and Chang, 1973).

Studies by Fiddler et al. (1973) have shown that the addition of 550–5500 ppm of sodium ascorbate (NaAsc) in wieners can markedly reduce the formation of dimethylnitrosamine (DMN). Similar studies (Herring, 1973) with bacon have indicated that NaAsc at 500–2000-ppm levels is effective in reducing the formation of NPy in cooked bacon but the results have been reported to be erratic. Moreover, the possibility exists that the addition of excess ascorbate at the initial stage of the curing process may destroy the added nitrite and reduce its inhibitory effect against *C. botulinum*. It was, therefore, thought that it would be more desirable to add the ascorbates or other nitrite-scavenging food additives at the end of the curing process, because by that time the bacon would already contain the *botulinum*-inhibitory factor which is believed to be formed (from nitrite) during the curing process. In this paper we wish to report the results of a study in which samples of commercial bacon prepared with normal levels of nitrite were treated with various food additives, and their effect on the formation of NPy during cooking was investigated.

MATERIALS AND METHODS

Bacon Samples. Vacuum-packed side bacons were purchased from the local supermarkets. The nonnitrite bacons were obtained through the courtesy of a commercial firm, and these were prepared by the company's standard method except that no nitrite was used during curing.

Chemicals. Propyl gallate (PG) (Nutritional Biochemicals Corporation), L-ascorbyl palmitate (AP) (ICN-K&K Laboratories, Inc.), and NaAsc (Hoffmann-La Roche Ltd.) were used without further purification. NPro

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Table I. Effect of Various Additives on the Formation of NPy in Fried Bacon[a]

Brand	Nitrite,[b] ppm	Cooking condition[e]	Additive[c]	NPy formed, ppb	% inhibition of NPy formation
A	64	1	Control	22[d]	
			Solvent blank	11[d]	
			PG + NaAsc	3[d]	86
			PG + Cys	6[d]	72
A	69	1	Control	15	
			Solvent blank	15	
			NaAsc	20	−33
			AP	3	80
			PG	3	80
			Cys	10	33
A	30	1	Control	14[d]	
		2	Control	12[d]	
		2	PG	3[d]	75
		2	AP	3[d]	75
		2	AP sprinkled	4[d]	66
B	25	1	Control	35	
			Solvent blank	25	
			Piz	3	91
			PG	2	94
B	101	1	Control	30[d]	
			Solvent blank	40[d]	
			AP	4[d]	86
B	25	1	Control	25[d]	
			Solvent blank	20	
			NaAsc	15	40
			AP	5	80
			PG	4	84
C	30	1	Control	15[d]	
			Solvent blank	15[d]	
C			NaAsc	10[d]	33
			PG	2[d]	86
C	86	1	Control	44[d]	
			Solvent blank	22[d]	
			PG	6[d]	82
			Piz	4[d]	90
C	59	1	Control	11[d]	
			NaAsc	5[d]	54
			AP	3[d]	73
D	90	1	Control	20	
			Solvent blank	15	
			PG	4	80
			PG + NaAsc	4	80
			PG + Cys	3	85
D	10	1	Control	21[d]	
		2	Control	22[d]	
		2	PG	7[d]	68
		2	AP	9[d]	59
E	32	1	Control	30[d]	
			Solvent blank	30[d]	
			PG + NaAsc	5[d]	83
			PG + Cys	Neg.	100
F	35	1	Control	40[d]	
			Solvent	40[d]	
			NaAsc	9[d]	77
			AP	5[d]	87
			PG	1[d]	97
G	76	2	Control	10	
			PG	5	50
			AP	10	0
			AP sprinkled	3	70

[a] All results are expressed on the basis of the weight of the uncooked bacon. [b] Analyzed before cooking. [c] For details, see text. Pg, NaAsc, and Cys were dissolved in 1:1 ethanol, AP in 95% ethanol, and Piz in ether. AP powder was sprinkled in two cases (see Materials and Methods). [d] Confirmed by GLC-high resolution mass spectrometry. [e] Cooking conditions: (1) 13 min at a setting of 340–350° F (starting from a cold pan); (2) 6 min at a setting of 400° F (preheated pan).

was a gift from Drs. W. Lijinsky and C. L. Walters. NPy was obtained from Adams Chemicals. All the solvents used for the analytical work were glass-distilled, and most of them were purchased from Caledon Laboratories Ltd.

Treatment and Frying of Bacon. Strips of bacon (100 g) were placed on an electric fry pan and given a light spray (on both sides) of the particular food additive (2%) dissolved in a suitable solvent (Table I). The amount of the solution used in each case was carefully controlled and kept close to 5 ml corresponding to 1000 ppm of the additive in the uncooked bacon. The samples were allowed to stand at room temperature for 30–60 min, and then fried. In the majority of the cases the bacon strips were cooked (starting from a cold pan) for 13 min at a setting of 340–350°F (setting recommended by the manufacturer of the fry pan). Some samples were cooked for 6 min (starting from a preheated pan) at a setting of 400°F. The maximum temperature, as monitored by a thermometer or a thermocouple, of the product during cooking by the two techniques varied between 134–142°C and 155–180°C, respectively. To ensure even cooking and maximum formation of NPy all samples were fried to a well-done stage and the strips turned over at regular intervals. In a few cases AP powder was applied by sprinkling (approximate level, 2000 ppm) from a salt shaker instead of spraying. The control samples did not receive any spray but the solvent blanks were sprayed with 5 ml of appropriate solvents (without any additive) and then treated exactly as the test samples.

Determination of Nitrite. The uncooked samples were analyzed for nitrite according to the method of Kamm et al. (1965).

Nitrosamine Analysis. The cooked bacon samples, including the cooked-out fat, were extracted with methylene chloride and the extracts analyzed for nitrosamines as described previously (Sen et al., 1974). The amounts of NPy in the final cleaned-up extracts were semiquantitatively estimated by a TLC–fluorometric method (Sen et al., 1973, 1974), and in the majority of the extracts the results were verified by GLC–high resolution mass spectrometric analysis using methods similar to those described by Telling et al. (1971) and Gough and Webb (1973).

A Varian Mat (Model 311 A) mass spectrometer equipped with an electron impact ionization source and coupled (all-glass Watson Biemann separator) to a Varian Aerograph gas chromatograph (Model 1400) was used for the mass spectrometric analysis. The mass spectrometer was operated in the specific ion monitoring mode for NO^+ (m/e 29.9980) at a resolution of 5000. Operating conditions were: source temperature, 250°C; emission current, 3 mA; electron voltage, 70 eV; accelerating voltage, 3 kV. GLC

Table II. Decarboxylation of NPro to NPy in the Presence or Absence of Various Food Additives[a]

Cooking medium (g)	NPro, mg	Additive (mg)	Cooking condition[b]	NPy formed, ppb	% yield of NPy
Lard (50)			3	N[d]	Control
Lard (50)	5		3	40[c]	0.06
Lard (50)	5	PG (50)	3	60	0.09
Nonnitrite bacon (100)			1	N	Control
Nonnitrite bacon (100)	5		1	40[c]	0.11
Nonnitrite bacon (100)	5	AP (100)	1	40[c]	0.11
Nonnitrite bacon (100)	5	Piz (100)	1	45[c]	0.13
Nonnitrite bacon (100)	5	PG (100)	1	45[c]	0.13
Normal bacon (100)			2	5	Control
Normal bacon (100)	1.68		2	30	0.21

[a] All results are based on the basis of the weight of the uncooked bacon or lard. [b] Cooking conditions: (1) 13 min at a setting of 340°F (cold pan); (2) 6 min at a setting of 400°F (preheated pan); (3) 13 min at a setting of 375°F (cold pan). [c] Confirmed by GLC–high resolution mass spectrometry. [d] N = negative.

conditions were: 6 ft × 1/8 in. o.d. stainless steel column packed with 25% Carbowax 20M and 2% NaOH on 60–80 mesh Chromosorb P; He flow, 30 ml/min; column temperature, 140°C for 4 min, then programmed at 10°C/min to 180°C. The operation of the GLC–mass spectrometer under these conditions allowed us to detect at least 1 ng each of DMN, diethylnitrosamine (DEN), dibutylnitrosamine (DBN), and NPy.

Before we acquired the above-mentioned mass spectrometer we carried out similar analysis for a few samples through the courtesy of the Laboratory of the Government Chemist, London, England. One milliliter of final cleaned-up extracts was sealed in glass ampules and sent by air mail. The procedure used by them can be briefly described as follows.

The chromatograph was a Philips Model R fitted with two 2 mm i.d. stainless steel columns in series. The first column was 2.4 m in length containing 15% Carbowax 20M, and the second column 5.4 m containing 5% Carbowax 20M. The support was AW-DMCS treated Chromosorb W in both columns. A peak cutting system (Gough and Webb, 1973) enabled solvent and other extraneous material to be vented on elution from the first column. NPy and co-eluted materials passed on to the second column for separation prior to entering the mass spectrometer. A silicone membrane separator (Gough and Webb, 1972) was used to interface the chromatograph to an AEI MS 902 mass spectrometer.

The GLC columns and separator were operated at 160°C and the carrier gas flow rate programmed from 6 ml/min to 25 ml/min, 5 min after injection. The retention time of NPy was 15 min, and it was detected by parent ion monitoring under a resolution of 7000. The detection limit was 0.2 μg/ml for an injected volume of 5 μl.

RESULTS AND DISCUSSION

The data (Table I) indicate that among all the compounds tested PG, AP, and piperazine hydrate (Piz) consistently inhibited the formation of NPy in cooked bacon. Sodium ascorbate (NaAsc) was also effective but to a lesser degree, and the results were inconsistent. It should be emphasized that we are not suggesting the use of Piz as an additive in bacon. Piz was used only as a test compound because of its well-known capacity (Mirvish, 1972) to react rapidly with nitrite thus making it (nitrite) unavailable for the formation of NPy. In a few cases, bacon samples were treated with a mixture of PG and cysteine (Cys) or PG and NaAsc but the results were not much different from those obtained with PG alone. It has been mentioned earlier that the levels of NPy reported in this study represent the total amounts (lean + fat) of NPy formed in each case. Previous studies (Fazio et al., 1973; Pensabene et al., 1974; Sen et al., 1973) have established that a larger concentration of NPy is present in the cooked-out fat than in the edible portion. Therefore, the concentration of NPy in the cooked lean portions of the treated bacons will be extremely small, probably in the 1–2 ppb range.

Figure 1. Proposed mechanisms for the formation of NPy in cooked bacon. The nitrosation reaction step of spermidine would also be expected to be inhibited by these additives.

The results on the formation of NPy from NPro after cooking with lard or nonnitrite bacon are presented in Table II. Both the lard and the nonnitrite bacon when cooked alone did not form any NPy, but when cooked with NPro formed detectable levels of NPy. This confirms the earlier model system findings of Pensabene et al. (1974) and Bills et al. (1973) that NPy can indeed be formed from NPro, but our yields (0.06–0.21%) are much lower than that observed by the above-mentioned investigators. Since NPy is volatile considerable amounts are probably lost during the normal high-heat cooking in an open fry pan. The effect of AP, PG, and Piz on the formation of NPy (via decarboxylation) from NPro was also investigated (Table II). As expected, these additives did not have any noticeable effect on the yield of NPy from NPro. The data in Table I and II, therefore, suggest that these additives inhibit NPy formation in normal cooked bacon by interfering with reactions other than the decarboxylation step of NPro. Since all of these additives are effective scavengers of nitrite (Cort, 1974) it is most likely that these compounds retard nitrosamine formation by competing for nitrite with various nitrosatable precursors of NPy as shown in Figure 1.

In view of the above-mentioned results it can be concluded that nitrosation reactions occurring during cooking of bacon play a much greater role in the formation of NPy

Table III. Correlation of TLC Data with That Obtained by GLC-High Resolution Mass Spectrometry[a]

NPy, μg[b]	
GLC-MS	TLC
4	4
0.4	0.4
2	3.5
0.6	0.5
2	2.2
1	2.7
0.5	0.6
N[c]	0.25

[a] High resolution mass spectrometric analyses were carried out by the Laboratory of the Government Chemist, London, England. [b] Amount present in the final methylene chloride extract of cooked bacon (corresponds to 90-g samples). [c] N = negative (detection limit, 0.5).

than has been thought previously. The low residual amounts of NPy in the additive-treated bacons (Table I) are probably originating from the decarboxylation of preformed (already present before cooking) NPro, and, therefore, its (residual NPy) formation not preventable by the additives. In sample F (Table I), for example, 87–97% of NPy is forming by the nitrosation reactions and the remaining 13–3% is formed by other mechanisms, probably via decarboxylation of NPro. If preformed NPro were the major precursor of NPy in cooked bacon it would not have been possible to inhibit the formation of NPy by treating the bacon with PG, AP, or Piz. However, the situation may vary with different types of bacon as well as the method of cooking. Under extreme cooking conditions the decarboxylation reaction of NPro may contribute proportionately more NPy than has been observed in this study.

From our previous experience (Sen et al., 1973, 1974; Panalaks et al., 1974) we concluded that the TLC–semiquantitative method used for the estimation of NPy is quite specific. The data in Table III provide additional support for the reliability of the technique, and show that our TLC results correlate very well with the high resolution mass spectrometric data obtained by the Laboratory of the Government Chemist. Our own GLC–high resolution mass spectrometric results also agreed well with the TLC data, and in no case did we obtain any false-positive result by the TLC method. Although the TLC technique is reliable, it would be advisable to confirm positive TLC findings by GLC–mass spectrometry, whenever possible. The recoveries of NPy added to cooked bacon ranged between 80 and 90%. In addition to NPy most of the cooked bacon samples contained traces (1–3 ppb) of DMN. As these levels were extremely low, it was not possible to determine with certainty the effect of the additives on the formation of DMN. A few examples of typical GLC–mass spectrometric tracings are shown in Figures 2 and 3.

Although we have shown that treatment of normal bacon with PG and AP can effectively inhibit the formation of NPy during cooking, the procedure of applying the additive just before frying is inconvenient to follow. Nevertheless, the approach appears to be very promising. Both PG and AP are permitted food additives, and both are widely used as antioxidants (Cort, 1974; Canadian Food and Drug Regulations, 1974; CRC Handbook of Food Additives, 1972) in many food products. On a molar basis 1000 ppm of AP is equivalent to 480 ppm of NaAsc. Many cured meat products are already being manufactured with the addition of 547 ppm of NaAsc or sodium erythorbate (U.S. Code of Federal Regulations, 1971). Therefore, it is not unreasonable to assume that the addition of equivalent amounts of AP to bacon, before or after curing,

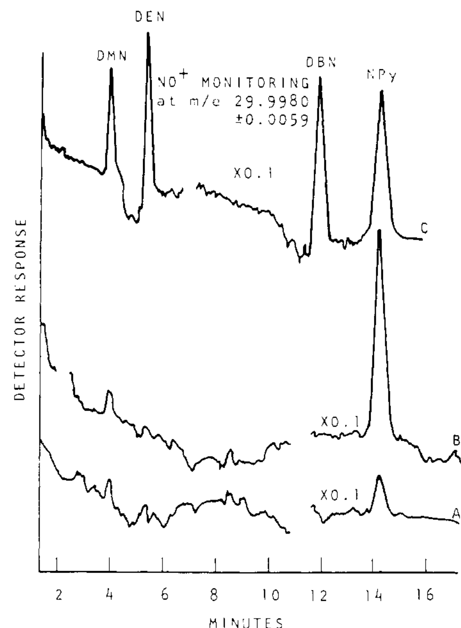

Figure 2. Tracings of specific ion monitoring for NO^+ by the GLC-mass spectrometric (Mat 311 A) technique: (a) a bacon treated with AP; (b) control for the same bacon; (c) 2.5–3 ng of each standard (bacon of brand B with 101 ppm of nitrite in Table I). Gaps in the tracings represent areas where the recorder pen was raised for rechecking the peak matching accuracy. For conditions see text.

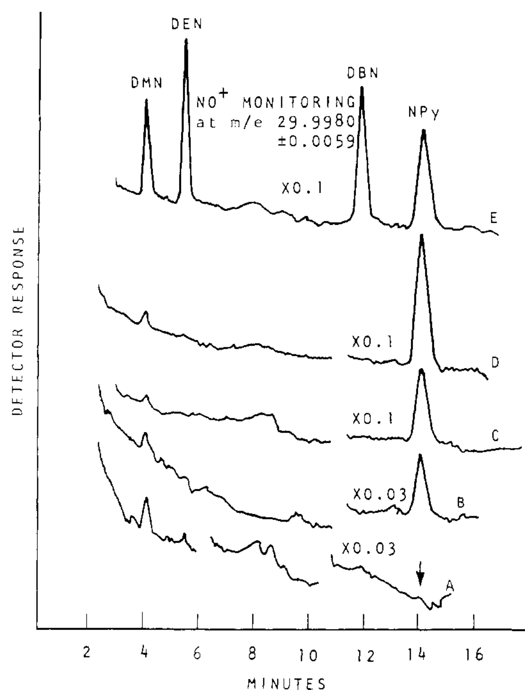

Figure 3. Specific ion monitoring tracings (Mat 311 A) of bacon brand F (Table I): (a) sample treated with PG; (b) with AP; (c) with NaAsc; (d) control; (e) 2.4–3 ng of each standard. Note that tracings a and b were carried out at a higher sensitivity setting.

will be acceptable. However, further research is needed to determine the feasibility and effectiveness of such treatment.

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An Investigation of the Surface Lipids of the Glabrous Cotton (*Gossypium hirsutum* L.) Strain, Bayou SM1

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The surface lipids of the glabrous cotton (*Gossypium hirsutum* L.) strain, Bayou SM1, were analyzed with an integrated gas chromatography–mass spectrometry system. *n*-Alkanes, C_{27}–C_{38}, account for 49.9% of the total wax, with *n*-nonacosane ($C_{29}H_{60}$) as the major wax constituent (28.7%). *n*-Primary alcohols C_{26}, C_{27}, and C_{28} account for 5.5% of the wax, with *n*-octacosanol ($C_{28}H_{58}O$) predominating (4.4%). Nineteen sterols and triterpenoids were detected and identities for nine are proposed: cholesterol (0.7%), 24ξ-methyl-$\Delta^{5,22}$-cholestadien-3β-ol (0.4%), stigmasterol (2.7%), fucosterol (4.5%), 24-methylenelophenol (3.4%), 4,4,14α-trimethyl-$\Delta^{7,9(11),24}$-cholestatrien-3β-ol (0.8%), 24-ethylidenelophenol (3.6%), 24-methylenecycloartanol (0.8%), and 24-methylcycloartanol (1.0%). The sterol and triterpenoid fraction accounts for 44.6% of the total wax, with an unidentified $C_{29}H_{48}O$ (M^+ 412) as the major constituent (6.5%).

Surface lipids (wax) of the glabrous cotton (*Gossypium hirsutum* L.) strain Bayou SM1 were examined by gas–liquid chromatography/mass spectrometry (GLC–MS) to investigate a possible chemical basis for the reported nonpreference of glabrous cottons by certain cotton insects (Lukefahr et al., 1968, 1970, 1971; Davis et al., 1973). The information may also be helpful in light of the report that cotton cuticular lipids are a potential factor in boll rot resistance (Wang and Pinckard, 1973).

All previous studies of cotton wax constituents have involved the extraction of ground plant tissue, and therefore were not concerned solely with lipids of surface origin (Power and Chesnut, 1925, 1926; Chibnall et al., 1934; Sadykov et al., 1963; Sadykov and Padkudina, 1964; Sadykov, 1965). Power and Chesnut (1925, 1926) reported the isolation of *n*-alkanes C_{31} and C_{35} from ground cotton foliage, squares (flower buds), and flowers, and based their identification on melting point and elemental analysis; however, Chibnall et al. (1934), using crystal spacing data, later showed that both solids were mixtures of paraffins.

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