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Inhibition of Formation of Volatile Nitrosamines in Fried Bacon by the Use of Cure-Solubilized α -Tocopherol

W. Fiddler,* J. W. Pensabene, E. G. Piotrowski, J. G. Phillips, J. Keating, W. J. Mergens, and H. L. Newmark

Pork bellies were injected with a conventional cure formulation that would produce target levels of 125 ppm NaNO_2 and either 500 ppm sodium ascorbate or α -tocopherol alone or in combination. A mixture of α -tocopherol and Polysorbate 20 (1:0.4 w/w) dispersed in the cure produced a good distribution of α -tocopherol in the adipose tissue which is thought to be the source of nitrosopyrrolidine precursor(s). The volatile nitrosamines in fried bacon were detected by GLC-thermal energy analyzer and confirmed by GLC-high-resolution mass spectrometry. In a comparison of the same sections of each belly pair subjected to different treatments, a combination of α -tocopherol and sodium ascorbate or α -tocopherol alone was found to inhibit nitrosopyrrolidine formation more effectively than did ascorbate alone.

Fried bacon which contains dimethylnitrosamine (DMNA) and nitrosopyrrolidine (NPy) at the ppb ($\mu\text{g}/\text{kg}$) level is still considered a potential source of environmental carcinogens. Since W. Fiddler et al. (1973) demonstrated that sodium ascorbate (NaAsc) and sodium erythorbate (NaEry) markedly diminish formation of DMNA in frankfurters prepared with high levels of nitrite, a number of studies have been made with these reductants in bacon cure. An American Meat Institute-USDA-FDA collaborative study showed that concentrations of NaAsc up to 1000 ppm reduced, but not completely eliminated, NPy in fried bacon prepared with 170 ppm NaNO_2 (Greenberg, 1973). The USDA Expert Panel on Nitrosamines (Food Chemical News, 1976) has recommended the use of 125 ppm NaNO_2 and 550 ppm NaAsc or NaEry for curing bacon. A study of fried bacon prepared with these concentrations of NaNO_2 and NaAsc or NaEry by ten commercial processors showed the effectiveness of this treatment, since only a small number of samples were found to contain very low levels of nitrosamines (NAs) (Food Chemical News, 1977). The mechanism of the inhibitory activity of ascorbate (Asc) or erythorbate (Ery) is thought to be due to their ability to compete with the precursor amine for available N_2O_3 nitrosating species. The limited inhibitory action of these reductants in bacon

may be due to the fact that they are more soluble in water than in fat. Nitrosopyrrolidine formation, however, has been shown to be associated with adipose tissue (W. Fiddler et al., 1974; Patterson et al., 1976). Recently, studies have been carried out on the use of lipophilic reductants to inhibit NA formation. Several researchers have determined the effect of a number of compounds on the nitrosation of secondary amines. The compound most commonly used has been ascorbyl palmitate (AscP) on the basis that the palmitoyl ester made Asc more fat soluble. Pensabene et al. (1976) found that this compound and NaAsc inhibited the nitrosation of pyrrolidine in a bacon-like model system containing oil, aqueous buffer, protein, and salts. Mottram and Patterson (1977) in a similar experiment, however, reported an increase in NPy formation with NaAsc and a slight decrease with AscP. The apparent contradiction in results is probably due to the fact that the earlier work was carried out in an open system and the latter in a closed one. The nitric oxide evolved was lost in the open system, thereby making it unavailable for the production of additional nitrosating agent. Despite this, AscP has been found to be more effective than NaAsc in reducing the amount of NPy found in edible bacon, its cooked out fat (Sen et al., 1976a), and vapors during frying (Sen et al., 1976b). In our studies with AscP in bacon we were unable to demonstrate consistent NA inhibition compared to NaAsc (W. Fiddler, 1977). This was possibly due to the slight oil or fat solubility of this compound (Swern, 1949). Compounds more lipophilic than AscP have been investigated in model systems (Gray and Dugan, 1975; Sen et al., 1976a). Coleman (1976) claimed that 5-25 ppm ethoxyquin (6-ethoxy-1,2-di-

* Eastern Regional Research Center, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pennsylvania 19118 (W.F., J.W.P., E.G.P., J.G.P.) and Hoffmann-LaRoche Inc., Nutley, New Jersey (J.K., W.J.M., H.L.N.).

hydro-2,2,4-trimethylquinoline) solubilized in the curing brine with isopropanol was effective in lowering the levels of DMNA and NPy found in fried bacon. Walters et al. (1976) reported that reduced levels of NAs are found in vapors during the frying of bacon in fat containing α -tocopherol (α -Toc). Also, bacon treated with a cure suspension containing α -Toc, AscP, and citric acid gave both NA inhibition and enhancement after frying. We have found 500 ppm α -Toc dispersed with Polysorbate 20 to be effective in inhibiting the nitrosation of pyrrolidine in aqueous and model systems simulating bacon (Pensabene et al., 1977).

We have proceeded with further experiments in which bacon is cured with solubilized α -Toc and NA formation in fried bacon is inhibited. The results of this work are described herein.

EXPERIMENTAL SECTION

Bacon Processing. Matched pork bellies were purchased from a local supplier within 1 day of slaughter and stored for 1 week in a cooler at 1 °C. The bellies were cut into thirds (brisket, center, and flank portions) and pumped to approximately 10% of their green weight to achieve added target levels of 1.5% sodium chloride, 0.5% sugar, 0.3% sodium tripolyphosphate, and 125 ppm sodium nitrite in the finished product. In addition, concentrations of 500 ppm NaAsc and 500 ppm α -Toc were used, alone or in combination. α -Toc and Polysorbate (TWEEN) 20 were mixed in ratios of 1:6, 1:1, 1:0.4, and 1:0.2, then dispersed in the cure formulations. The pumped bellies were stored in polyethylene bags at 1 °C for 20–22 h, then processed in a smokehouse in a commercial program of increasing heat and controlled humidity: 1 h dry bulb (DB) 38 °C, wet bulb (WB) 0 °C; 1 h DB 50 °C, WB 0 °C; 3 h DB 50 °C, WB 47 °C. A medium to heavy smoke was introduced after 2 h of drying. The finished bacon reached an average internal temperature of 53 °C (128 °F) after 5 h. The bacon pieces were placed in polyethylene bags and stored at 1 °C for 18 h.

Bacon Sampling, Frying, and Handling. Representative portions of the bacon sections were sliced and fried in a preheated Presto Teflon coated electric frying pan for 6 min at 177 °C (350 °F). Both the edible portion and rendered drippings were retained for NA analysis. The remaining portion of the raw bacon was vacuum packaged and shipped by air freight, in an insulated container with cold packs, to the laboratories at Hoffmann-LaRoche, Nutley, N.J. These samples were analyzed for NaNO₂, NaAsc, dehydroascorbate, and α -Toc. All the samples were analyzed at the same time.

Reagents. All chemicals and solvents, reagent grade or better, were used without purification, except for the following: Ethanol was distilled from KOH (ca. 5 g/L) and KMnO₄ (ca. 3 g/L). Skellysolve B (bp 68 °C) was purified by passage through silica gel (grade 12) and distilled from KOH (ca. 5 g/L) and aluminum turnings (ca. 3 g/L). Florex XXS (60–80 mesh), from Floridin Co., Quincy, Fla., was purified by adding 50 g of stannous chloride (SnCl₂·H₂O) and 2 L of concentrated HCl to 500 g of Florex XXS and boiling in a fume hood for 15 min. This was cooled and, after the acid was decanted, washed six–eight times with anhydrous ethanol until the washings were colorless. The Florex was stored under ethanol until used.

Nitrosamine Analysis. *a. Extraction of Fried Bacon.* The fried bacon was ground and mixed thoroughly twice prior to analysis. A 25 g sample, to which 1 mL of 0.5 μ g/mL CH₂Cl₂ solution of methylethyl nitrosamine (MENA) internal standard was added and mixed, was placed in a Virtis flask; 80 mL of distilled water and 10

mL of CH₂Cl₂ were added. The mixture was homogenized for 15 min in a Virtis blender, transferred to a polypropylene bottle, and centrifuged 10 min at 5000 rpm, and the resulting supernatant liquid was transferred to a round-bottom flask containing 75 mL of 5 N NaOH and 8 g of Ba(OH)₂. The solids in the centrifuged bottle were shaken with 25 mL of distilled water for 1 min and re-centrifuged for 5 min. The supernatant was added to the flask and the contents were distilled until no more aqueous distillate was collected. After addition of 25 g of NaCl and 5 mL of 6 N HCl, the distillate was extracted three times with 125 mL of CH₂Cl₂. The combined extracts were washed with 50 mL of 6 N HCl, then with 5 N NaOH.

b. Extraction of Cooked-Out Fat. Nitrosamines in the fat drippings were isolated and separated by the method of White et al. (1974). The resulting CH₂Cl₂ extract concentrated (ca. 1.0 mL) was applied to a 10 mm \times 7 cm water cooled column containing silica gel 60 (70–230 mesh, Brockmann activity 2–3, E. Merck, Darmstadt, Germany), washed with 150 mL of CH₂Cl₂–pentane (25:75, v/v) and eluted with 125 mL of ether–CH₂Cl₂ (30:70, v/v).

The CH₂Cl₂ extracts of fried bacon and eluate from the cooked-out fat were dried by being passed through anhydrous Na₂SO₄ and concentrated to 1.0 mL in a Kuderna-Danish apparatus.

c. Determination. The volatile NAs were quantitated by GLC–TEA under conditions similar to those described by Fine et al. (1975) and confirmed by GLC–mass spectrometric analysis using high resolution (1:12000) under conditions previously reported (Pensabene et al., 1974).

Bacon Analysis. *a. Nitrite.* Residual nitrite values were obtained by the Griess-Saltzman reaction with the procedure described by R. N. Fiddler (1977).

b. Ascorbate and Dehydroascorbate. These analyses were carried out by the microfluorometric method described by Deutsch and Weeks (1965) and Newmark et al. (1974).

c. α -Tocopherol. Homogenized samples of lean and fat portions from the bacon bellies were saponified and extracted by the method of Bieri et al. (1961). The method was modified by use of 1.0 g of 4-hydroxyacetanilide instead of pyrogallol in the saponification. Samples (7 to 10 g) were dispersed in 75 mL of ethanol, and 1 g of potassium hydroxide per gram of fat was added for saponification. In the triple extraction procedure 75 mL, 75 mL, and 50 mL of hexane (Skelly B) were used. Florex chromatography and colorimetry were as described in the Analytical Methods Committee Report (1959).

RESULTS AND DISCUSSION

In initial studies, a level of 500 ppm α -Toc solubilized with Polysorbate 20 (1:6 w/w) was effective in inhibiting the nitrosation of pyrrolidine in model systems (Pensabene et al., 1977). Nitrosamine inhibition in bacon was not clearly demonstrated by this ratio of α -Toc:Polysorbate 20. The α -Toc was so water soluble that it migrated into the lean tissue rather than remaining in the adipose tissue as was intended. Only after a number of experiments were conducted with α -Toc solubilized with varying ratios of Polysorbate 20 (1:6, 1:1, 1:0.4, 1:0.2), was the ratio of 1 to 0.4 found to give the best distribution characteristics.

The distribution of NaNO₂, NaAsc, and α -Toc in lean and adipose tissue of pork bellies prior to frying is shown in Table I. This is representative data from one of three experiments in which two pairs of matched bellies were cut into sections and cured with levels of the cure ingredients described in the Experimental Section. More α -Toc, NaAsc, and NaNO₂ was found on the lean tissue than in the adipose tissue with approximately two-thirds

Table I. Distribution of Nitrite, Ascorbate, and α -Tocopherol in Bacon Prior to Frying

Belly pair	Section	Theoret. input, ^a ppm		Lean, ppm			Adipose, ppm			Calcd overall, ^c ppm		
		NaAsc	α -Toc ^b	NaNO ₂	NaAsc	α -Toc	NaNO ₂	NaAsc	α -Toc	NaNO ₂	NaAsc	α -Toc
1	Brisket	0	0	87	<i>d</i>	3	37	<i>d</i>	4	56	<i>d</i>	4
1A	Brisket	0	500	86	<i>d</i>	350	28	<i>d</i>	192	57	<i>d</i>	272
1	Center	500	0	84	171	2	24	75	3	47	149	2
1A	Center	500	500	76	255	331	23	48	175	53	135	242
1	Flank	0	0	115	<i>d</i>	4	31	<i>d</i>	3	61	<i>d</i>	3
1A	Flank	500	0	72	250	3	29	98	5	47	162	4
2	Brisket	0	500	67	<i>d</i>	319	33	<i>d</i>	316	46	<i>d</i>	313
2A	Brisket	500	500	42	205	399	10	82	229	24	136	304
2	Center	0	0	64	<i>d</i>	4	25	<i>d</i>	3	42	<i>d</i>	3
2A	Center	0	500	88	<i>d</i>	342	29	<i>d</i>	197	53	<i>d</i>	256
2	Flank	500	0	86	239	7	20	77	4	44	131	5
2A	Flank	500	500	46	174	266	13	59	220	23	93	232

^a 125 ppm NaNO₂ in all samples. ^b α -Toc-Polysorbate 20 (1:0.4 w/w). ^c Based on actual lean and adipose tissue weight. ^d Not analyzed.

Table II. *N*-Nitrosamine Content of Fried Bacon

Belly pair	Section	Theoret. input, ppm ^a		Nitrosamines, ppb ^{b,c}			
		NaAsc	α -Toc ^d	Edible		Drippings	
				DMNA	NPY	DMNA	NPY
1	Brisket	0	0	4	16	7	19
1A	Brisket	0	500	6	4	7	7
1	Center	500	0	7	16	17	22
1A	Center	500	500	3	6	10	10
1	Flank	0	0	5	16	10	15
1A	Flank	500	0	3	8	10	18
2	Brisket	0	500	5	4	8	8
2A	Brisket	500	500	2	4	9	5
2	Center	0	0	5	13	13	25
2A	Center	0	500	3	6	10	14
2	Flank	500	0	3	8	16	17
2A	Flank	500	500	3	4	10	7

^a NaNO₂, 125 ppm. ^b Corrected for recovery of methylethyl nitrosamine internal standard. ^c Confirmed by GLC-high-resolution MS. ^d α -Toc-Polysorbate 20 (1:0.4 w/w).

of the α -Toc and one-third NaAsc in the lean compared to the adipose tissue. The addition of Asc to the cure lowered residual NO₂⁻ in the lean, and thus in the overall bacon, more extensively than did the addition of α -Toc.

The results of NA analyses of the corresponding two pairs of matched bellies are shown in Table II. Direct comparisons were made on slices derived from the same sections of each belly subjected to the different treatments. There was little inhibition of DMNA compared to NPY. Treatment with added α -Toc was more effective than with Asc, particularly in inhibiting NPY. This was further borne out when the results of all three experiments were considered.

To determine whether the difference in NA formation between two treatments was significantly different from zero, comparisons were made by Student's *t* test with the 15 differences in the replicate experiments which involved

the three sections of the five matched pairs of bellies (Table III). With α -Toc alone, in the absence of NaAsc (line 2), there was a significant inhibition of NPY in the fried edible portion and its drippings ($p = 0.05$). In the most important comparison (line 4), the Asc- α -Toc treatment exhibited statistically greater inhibition (with at least $p = 0.10$) than Asc alone for both NAs in all portions of the samples. The most significant difference ($p = 0.01$) was observed for NPY in the drippings. The difference in inhibition between Asc- α -Toc and Asc alone was larger for NPY than for DMNA. This was also true when α -Toc was compared to the zero control (line 2). This might result from the greater volatility of DMNA since more is lost in the fumes during frying. Also the results may suggest that a different mechanism might operate for the production of DMNA than for NPY. This study with its limited data base shows no clear cut difference between the inhibitory effect on NPY formation due to α -Toc alone compared to the combination of α -Toc with Asc or Asc alone (line 5 and 6).

In one experiment in which Asc was used in two treatments, DMNA formation was enhanced to variable degrees. These unexpected results were not statistically significant because of a large variation due to inhibition which occurred in other experiments. The analytical data were highly repeatable, thereby suggesting the bacon itself being the source of the variability. The precise cause of this NA enhancement with Asc cannot be explained at this time.

The entire collection of data without regard to matched pair or section were analyzed to determine the correlation of NA content with the amounts of added and residual Asc, α -Toc, and NO₂⁻ in raw bacon. These tests were performed at a significance level of $p = 0.05$ with 27 degrees of freedom, which resulted in all correlations in which $|r| \geq 0.367$ being judged significant (Snedecor and Cochran, 1974). Dimethylnitrosamine and NPY were correlated significantly with each other in the edible fried bacon (r

Table III. Results of Significance Testing on Various Portions of Sample

Comparisons		Replicates	Average difference, ppb						
(NaAsc- α -Toc) minus (NaAsc- α -Toc)			Dimethylnitrosamine			Nitrosopyrrolidine			
			Edible	Drippings	Total	Edible	Drippings	Total	
1	0 - 0	500 - 500	2	-18.00	0.00	-18.00	7.50	8.00	15.50
2	0 - 0	0 - 500	4	3.00	4.00 ^b	7.00 ^c	6.75 ^b	9.00 ^b	15.75 ^b
3	0 - 0	500 - 0	1	2.00	0.00	2.00	8.00	-3.00	5.00
4	500 - 0	500 - 500	4	2.50 ^c	3.75 ^c	6.25 ^b	4.25 ^c	10.00 ^a	14.25 ^b
5	0 - 500	500 - 500	2	3.00 ^d	-1.00 ^d	2.00 ^d	0.50	2.00	2.50 ^c
6	0 - 500	500 - 0	2	-22.50	-1.00	-23.50	0.00	-2.50 ^c	-2.50

^a Significant at $p = 0.01$. ^b Significant at $p = 0.05$. ^c Significant at $p = 0.10$. ^d Based on one value.

= -0.426). Added or residual α -Toc and NPy levels were significantly correlated ($r = -0.546$ to -0.689) with each other in the lean and adipose tissues and overall section; thus there was a diminution in NPy formation with increased α -Toc concentrations. There was no evidence of similar relationship in the case of DMNA and α -Toc treatment ($r = -0.040$ to -0.187). Residual NaNO_2 in lean tissue was correlated with NPy in edible fried bacon ($r = 0.531$), and increased residual NaNO_2 concentrations led to increased NPy formation. This provides additional evidence for the relationship between residual NO_2^- and NPy reported by Gough and Walters (1976). In our investigation there was no significant correlation between added or residual Asc with both NAs ($|r| < 0.256$) despite the indication that Asc had the most effect on residual NO_2^- in the paired comparison treatments. It appears that α -Toc inhibits NA formation while having little effect on residual NO_2^- ($|r| < 0.248$).

Although tocopherols are not currently used in cured meat products, they are permitted for use as antioxidants in rendered animal fat, either alone or in combination with vegetable fats (Code of Federal Regulations 1973). The Polysorbate 20 solubilizing agent, although not in common usage is permitted in bakery products as a flavor dispersing agent according to "good manufacturing practice" (Code of Federal Regulations, 1975).

In summary, the injection of 500 ppm α -Toc into bacon with the cure was found to be effective in reducing nitrosamine formation. The importance of good distribution of α -Toc in the adipose tissue cannot be overemphasized.

NOTE: Precaution should be exercised in the handling of nitrosamines since they are potential carcinogens.

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