N-Nitrosamine Formation in Fried Bacon Processed with Liquid Smoke Preparations

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The incorporation of liquid smokes into curing brines resulted in fried bacon with lower concentrations of N-nitrosodimethylamine (NDMA), N-nitrosopyrrolidine (NPYR), and N-nitrosothiazolidine (NTHZ) than bacon processed by the traditional woodsmoke process. Levels of NDMA and NPYR in bacon treated with an atomized liquid smoke were not significantly lower than the woodsmoke control but did contain less NTHZ. An aqueous model system in which the smoke condensates were reacted with cysteamine and nitrite accurately reflected the trend in NTHZ concentrations in the corresponding fried bacon. N-Nitrosothiazolidinecarboxylic acid (NTCA) concentrations in raw bacon appeared to correlate with NTHZ concentrations in fried bacon. Residual nitrite levels in raw bacon treated with cure-solubilized liquid smoke decreased as the pH of the smoke condensate decreased.

INTRODUCTION

Recently, N-nitrosothiazolidine (NTHZ) and/or N-nitrosothiazolidinecarboxylic acid (NTCA) have been reported in smoked cured meats (Pensabene and Fiddler, 1983a,b; Helgason et al., 1984; Sen et al., 1985, 1986). The formation of these compounds in smoked meats appears to be the result of the reaction of cysteamine and cysteine, respectively, with formaldehyde in the smoke, followed by nitrosation (Mandagere et al., 1984; Sen et al., 1985). Using scanning calorimetry, Mandagere et al. (1984) determined that the decarboxylation of NTCA to NTHZ occurs at a lower temperature than that used in frying. Sen et al. (1985, 1986) reported that at least a part of the NTHZ detected in fried bacon is formed as a result of the decarboxylation of NTCA present in raw bacon.

Of the studies pertaining to the influence of liquid smokes on N-nitrosamine formation in meat systems, most have been concerned with the inhibition of N-nitrosopyrrolidine (NPYR) formation (Sleeth et al., 1982; Theiler et al., 1984). Relatively little attention has been directed to the influence of liquid smoke condensates on NTHZ and NTCA formation in bacon, although Pensabene and Fiddler (1983a) did demonstrate that some liquid smokes contain preformed NTHZ. Liquid smoke atomized onto the surface of bellies inhibited the formation of NTHZ during traditional smoking; however, the incorporation of liquid smokes in the curing brine did not significantly lower NTHZ formation (Pensabene and Fiddler, 1985). The major objective of our study was to investigate further the role of liquid smokes in NTHZ and NTCA formation in bacon and model systems.

MATERIALS AND METHODS

Safety. N-Nitrosamines are potent animal carcinogens and must be handled with appropriate safety precautions.

Bacon Processing. Thirty uniform pork bellies were obtained from a local commercial supplier within 48 h of slaughter and processed into bacon as described by Gray et al. (1982). The bellies were randomized, and five bellies were placed in each of six treatment groups (five liquid smoke treatments and a traditional woodsmoke control). The bellies were stitch pumped to 110% of their original weight to obtain target concentrations of 120 mg/kg nitrite, 1.5% sodium chloride, 0.5% sucrose, 550 mg/kg sodium ascorbate, and 0.35% sodium tripolyphosphate in the finished bacon. Four solubilized liquid smokes (obtained from commercial suppliers) were added to the brine at levels recommended by the manufacturers (liquid smoke B, 1.8%; C, 1.8%; D, 2.0%; E, 3.1%). Another liquid smoke preparation (A) was atomized in the smokehouse for two 30-min intervals during the cooking process. The control bacon was smoked with hickory sawdust woodsmoke in an Elek-Trol laboratory smokehouse as described by Reddy et al. (1982). All samples treated with the liquid smokes were also subjected to a standard 8-h cooking process in the smokehouse. The bacon samples were sliced, packaged, and stored at 4 °C as previously described (Reddy et al., 1982).

Model System Studies. The role of smoke in NTHZ and methyl-N-nitrosothiazolidine (MeNTHZ) formation in bacon was further evaluated in an aqueous model system consisting of nitrite and cysteamine. Cure-solubilized liquid smokes were included in the model system at the same concentrations as those employed in the curing brines of bacon. The liquid smoke that was atomized and a condensate from woodsmoke were included in the model system at a concentration of 3.5%. The condensate from woodsmoke was obtained by drawing smoke from the smokehouse through ice-cooled traps for 6 h with a vacuum pump. Solutions of nitrite, cysteamine, and liquid smoke condensates were combined, and the volume was adjusted to 100 mL with distilled water. The reaction systems contained 120 and 25 mg of nitrite and cysteamine, respectively. The samples were transferred to stoppered 250-mL Erlenmeyer flasks, and the samples were adjusted to pH 5.5 with concentrated NaOH. The samples were reacted for 1 h at 30 °C in a water bath and extracted with three 75-mL aliquots of methylene chloride. The extracts was dried over anhydrous sodium sulfate and concentrated in a Kuderna-Danish apparatus to a final volume of 100 mL.

N-Nitrosamine Analyses. Representative portions of the bacon samples were fried in a preheated General Electric Teflon-coated electric frying pan for 6 min at 171 °C (340 °F). As many strips of bacon were put in the frying pan as possible without overlapping but with room for turning. The bacon was fried on each side for 3 min, removed, and drained on paper towels. N-Nitrosamine concentrations in the fried bacon samples were determined by using the mineral oil distillation procedure as described by Reddy et al. (1982). Ammonium sulfamate (2 g) was added to the distilling flask in order to prevent artifactual

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Table I. N-Nitrosamine Formation in Fried Bacon Processed with Various Smoke Treatments

smoke treatment	mode of appl	N-nitrosamine concn,[e] μg/kg		
		NDMA[a]	NPYR[a]	NTHZ[a]
woodsmoke	traditional	4.7 ± 1.5 (2.8–6.7)	13.6 ± 7.3 (6.3–25.0)	5.1 ± 1.2 (3.1–6.4)
liquid smoke A	atomized	4.2 ± 1.7[b] (2.4–6.9)	9.3 ± 7.0[b] (4.6–21.4)	0.9 ± 0.8[d] (tr, 1.8)
B	in brine	2.9 ± 0.9[c] (1.5–4.1)	2.5 ± 1.5[d] (0.4–4.5)	tr[d,f]
C	in brine	3.9 ± 1.0[b] (2.4–5.2)	4.0 ± 2.6[d] (1.0–7.2)	tr[d]
D	in brine	2.3 ± 1.4[d] (1.1–4.5)	4.8 ± 4.2[d] (2.0–12.0)	2.1 ± 2.1[d] (tr, 5.5)
E	in brine	1.2 ± 0.4[d] (0.8–1.6)	2.5 ± 0.8[d] (1.6–3.7)	tr[d]

[a] NDMA, N-nitrosodimethylamine; NPYR, N-nitrosopyrrolidine; NTHZ, N-nitrosothiazolidine. [b] Not significantly different from woodsmoke control bacon ($p < 0.10$). [c] Significantly different from woodsmoke control bacon ($p < 0.05$). [d] Significantly different from woodsmoke control bacon ($p < 0.005$). [e] Five bellies were analyzed per treatment, and duplicate analyses of each belley were performed. [f] tr = trace, <0.2 μg/kg.

formation of N-nitrosamines. Addition of 2,6-dimethylmorpholine (100 μg/kg) to the distillation system indicated that N-nitroso-2,6-dimethylmorpholine was not an artifact. During the analyses, N-nitrosothiomorpholine (200 ng) was routinely added to each sample at the start of the analysis to monitor the efficiency of the overall analytical process. The results, however, were not corrected for percentage recoveries (90 ± 5%) of the internal standard. Previous studies in our laboratory indicated average NPYR and N-nitrosodimethylamine (NDMA) recoveries of 80% and 88%, respectively, from fried pork bellies spiked with respective N-nitrosamines (Bernthal et al., 1986). The average value for recovery of NTHZ from similarly spiked samples was 83%. NTHZ in the cook-out fat was quantitated by the method of Owens and Kinast (1980). Ammonium sulfamate (2 g) was again added to the fat prior to distillation. The average value for recovery of NTHZ was 78 ± 3%.

N-Nitrosamine levels were quantitated using a GC–TEA system comprised of a Varian 3700 gas chromatograph coupled to a TEA Model 502 LC (Thermo Electron Corp., Waltham, MA) (Gray et al., 1982). The N-nitrosamines were separated on a 3 m × 2 i.d. glass column packed with Carbowax 20 M on 80/100 Chromosorb W (Supelco Inc., Bellefonte, PA). Gas chromatographic conditions: temperature programming, 100–180 °C at 15 °C/min; carrier gas (nitrogen) flow rate, 30 mL/min; TEA pyrolyzer temperature, 475 °C; TEA vacuum, 1.5 mm.

Quantitation of N-Nitrosothiazolidinecarboxylic Acid in Bacon. Raw and fried bacon samples were ground and thoroughly mixed with a Hobart grinder (Model 84181D). N-Nitrosopipecolic acid [prepared by nitrosating pipecolic acid as described by Lijinsky et al. (1970)] was added (200 ng) to the bacon sample (25 g) that was then blended in 200 mL of distilled water containing 1 g of ammonium sulfamate. The mixture was centrifuged at 1800 rpm in a Model K centrifuge (International Centrifuge, Needham Heights, MA) for 10 min and the supernatant filtered through glass wool. The residue was reextracted with another 100-mL aliquot of distilled water and centrifuged and filtered as before. The total filtrate was treated with 5 mL of 20% ammonium sulfamate in 3 N H_2SO_4 and 15 g of NaCl. After sitting at room temperature for 10 min, the mixture was centrifuged for 10 min at 1800 rpm and the supernatant filtered as described above. The filtrate was transferred to a 500-mL separatory funnel and extracted with three 75-mL aliquots of ethyl acetate. The extract was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator to 2 mL. The concentrate was treated with diazomethane and further concentrated to 1 mL under a steady stream of nitrogen. NTCA levels were determined by a GC–TEA procedure using a 3 m × 2 mm i.d. glass column packed with a mixed phase, 1% OV-210 and 2% OV-17, on 80/120-mesh Chromosorb W (Supelco Inc., Bellefonte, PA). The gas chromatograph was temperature programmed from 100 to 180 °C at 8 °C/min.

Average recoveries of 78.6 ± 3.2% were observed for blank samples spiked with NTCA at 50, 100, and 150 μg/g levels, and average recoveries of 83.5 ± 4.6% were obtained for the internal standard.

Nitrite Analysis. Residual nitrite was quantitated according to the standard AOAC procedure (1984).

Analysis of Phenols in Liquid Smoke. Phenols in liquid smoke were analyzed by the procedure of Lustre and Issenberg (1970). The phenol fraction was contained in a final volume of 10 mL of acetone. The fraction was analyzed by a Hewlett-Packard 5840A gas chromatograph equipped with an fid detector and a 50 m × 0.25 mm i.d. 20 M Carbowax capillary column (Alltech Associates, Inc., Deerfield, IL). Gas chromatographic conditions: temperature programming, 50–190 °C at 10 °C/min; nitrogen flow rate, 25 mL/min; fid temperature, 350 °C; injection temperature, 225 °C. The identities of the phenols were confirmed on a Hewlett-Packard 5983A gas chromatograph–mass spectrometer unit equipped with a 2 m × 3 mm i.d. glass column packed with 1% SP 1240-DA on 100/120 Supelcoport (Supelco, Inc., Bellefonte, PA). Operation conditions: electron energy (eV), 70; actual source temperature, 200 °C; helium carrier gas, 25 mL/min.

Statistical Treatment of N-Nitrosamine Data. Statistical analysis of N-nitrosamine data for the different bacon treatment groups was performed using Bonferroni t-statistics (Gill, 1978).

RESULTS AND DISCUSSION

N-Nitrosamine Concentrations in Smoked Bacon. The N-nitrosamine concentrations in fried bacon from bellies processed by various smoke treatments are shown in Table I. Of the four groups of bellies that were treated with cure-solubilized liquid smokes, treatments B, D, and E resulted in fried bacon containing significantly lower ($p < 0.05$) NDMA concentrations than bacon produced by traditional smoking. NDMA concentrations in bacon treated with an atomized liquid smoke were not significantly different from those present in the woodsmoke controls.

A more pronounced reduction was observed in NPYR concentrations in bacon treated with cure-solubilized liquid smoke (Table I). Bacon processed in this manner had average NPYR concentrations at least 60% lower than those in traditionally smoked bacon. The most effective liquid smoke treatments were B and E, which produced bacon samples with an average NPYR concentration of 2.5 μg/kg. This value represents an 82% reduction relative to the woodsmoke samples where the average NPYR concentration was 13.6 μg/kg. Theiler et al. (1984) reported that bacon produced with liquid smoke dispersed in the curing brine at a concentration of 2.5% contained

much less NPYR (57% reduction) than unsmoked control bacon samples. These investigators also observed no inhibition of NPYR formation when liquid smoke was sprayed onto the outside of the belly. In the present study, fried bacon processed with atomized liquid smoke contained an average NPYR concentration of 9.3 μg/kg. The difference, however, was not statistically significant ($p < 0.05$) from the woodsmoke control samples and could be due in part to variations in composition of the pork bellies used in the study.

NTHZ concentrations in fried bacon produced from bellies that had been stitch pumped with liquid smoke were significantly lower (<0.005) than those in bacon produced by traditional woodsmoking. Bacon prepared with liquid smoke D had an average NTHZ concentration of 2.1 μg/kg. This was the only cure-solubilized liquid smoke preparation that produced greater than trace (<0.2 μg/kg) amounts of NTHZ in the fried bacon. The most likely explanation for the difference in these data is that liquid smoke D probably contains a higher concentration of formaldehyde than the other liquid smokes as this aldehyde is believed to be involved in the formation of the thiazolidine compound (Mandagere et al., 1984). However, no carbonyl analyses were performed on the liquid smokes to substantiate this hypothesis.

Atomizing liquid smoke A and depositing it on the surface of pork bellies resulted in fried bacon samples averaging 0.9 μg/kg of NTHZ. These results are similar to those obtained by Pensabene and Fiddler (1983a) who concluded that liquid smoke sprayed on the surface of bellies made only a minor contribution to NTHZ formation in fried bacon. However, a part of this contribution to NTHZ in fried bacon was thought to be due to the presence of preformed NTHZ in the liquid smoke. Pensabene and Fiddler (1983a) reported an average of 22.5 μg/kg NTHZ in 10 samples of liquid smoke. Analysis of the five samples of commercial liquid smokes used in the present study revealed no detectable levels of NTHZ. Similar results were reported by Sen et al. (1986) for four commercial liquid smokes. Our results therefore indicate that atomized liquid smoke spray did not deposit preformed NTHZ on the surface of the pork bellies and that the small levels of NTHZ in the smoked bacon most likely came from reaction of formaldehyde in the liquid smoke with meat components. Support for this is found in the fact that liquid smoke condensates have been shown to contain formaldehyde in concentrations ranging from trace amounts up to 1.68 g/L (Potthast and Eigner, 1985).

NTHZ was not detected in any of the samples of cook-out fat analyzed in this study. This observation agrees with those of Gray et al. (1982) who hypothesized that the absence of NTHZ in the cook-out fat might indicate that the major precursors of NTHZ in cured meats were water soluble and would most likely reside in the lean tissue of bacon. This was subsequently confirmed by Fiddler et al. (1986). In addition, NTHZ may be volatilized from the cook-out fat during frying (Hotchkiss and Vecchio, 1985). Pensabene and Fiddler (1983a) demonstrated that NTHZ does not undergo appreciable thermal decomposition during frying and reported low concentrations of NTHZ in the cook-out fat of fried bacon (2.5 μg/kg). Similarly, Hotchkiss and Vecchio (1985) reported a mean NTHZ value of 3.1 μg/kg in the cook-out fat of fried bacon.

Model System Studies. In order to confirm the relative contribution of various liquid smoke preparations to NTHZ formation in bacon, a model system study was carried out in which the liquid smokes were reacted with cysteamine and nitrite to promote *N*-nitrosamine formation. Liquid smoke concentrations in the model systems were similar to those in the brines used to produce the various bacon samples. A woodsmoke condensate was obtained by drawing smoke from the smokehouse for 6 h through traps that were cooled in ice water. The model system *N*-nitrosamine data generally reflect those of the bacon study (Table II). The reaction of woodsmoke condensate with cysteamine and nitrite in the model system resulted in the formation of NTHZ concentrations at least sixfold greater than those in the liquid smokes. Furthermore, liquid smoke D produced NTHZ concentrations that were at least 6 times higher than those produced by the other liquid smokes designed for stitch pumping (liquid smokes B, C, and E). This trend confirms the fried bacon data as liquid smoke D was the only cure-solubilized liquid smoke that produced bacon that, upon frying, contained greater than trace (0.2 μg/kg) amounts of NTHZ (Table I). Liquid smoke A produced relatively high concentrations of NTHZ in the model system, and the spraying of this smoke condensate on the surface of bellies also yielded fried bacon with detectable concentrations of NTHZ (Table I). MeNTHZ was detected when five of the six smoke condensates were reacted in the model system. This would indicate the presence of acetaldehyde in the liquid smokes and would support the hypothesis that formaldehyde in smoke is the precursor of NTHZ. Toth and Potthast (1984) reported that acetaldehyde and formaldehyde were present in woodsmoke at concentrations of 1150 and 200 mg/100 g of wood, respectively. However, the concentrations of MeNTHZ produced in the model system study were much lower than the NTHZ concentrations. This would indicate that formaldehyde reacts much more rapidly with cysteamine than does acetaldehyde. Consequently, MeNTHZ was not detected in any of the smoked samples, the limit of detection being 1 μg/kg.

Table II. *N*-Nitrosothiazolidine Formation in an Aqueous Model System Consisting of Smoke Condensate, Cysteamine, and Nitrite[a]

smoke condensates	amt *N*-nitrosamine, μg	
	NTHZ[b]	MeNTHZ[b]
woodsmoke[c]	2044.9	81.6
liquid smoke A	226.3	3.6
B	51.1	8.3
C	22.3	32.4
D	328.3	26.9
E	37.8	ND

[a] Model system consisted of cysteamine (25 mg), nitrite (120 mg), and smoke condensate at manufacturers recommended levels where applicable (woodsmoke = 3.0%; A = 3.0%; B = 1.8%; C = 1.8%; D = 2.0%; E = 3.1%). Total volume of 100 mL was reacted in flasks for 1 h at 30 °C. [b] NTHZ; *N*-nitrosothiazolidine; MeNTHZ = 2-methyl-*N*-nitrosothiazolidine; ND = not detected (level of detection 1.0 μg/kg). [c] Smoke from the smokehouse was pulled through ice water cooled aqueous traps for 6 h by a vacuum pump.

NTCA Concentrations in Smoked Bacon. The results of the analyses of NTCA in raw and fried bacon are summarized in Table III. The pattern of NTCA concentrations in the raw bacon samples was similar to that for NTHZ concentrations in the fried bacon. Traditionally woodsmoked bacon contained the highest concentrations of NTCA (average 87.3 μg/kg) followed by bacon processed with liquid smoke D (average 76.5 μg/kg) and liquid smoke A (average 37.8 μg/kg). The other liquid smoke treatments resulted in raw bacon with relatively low concentrations of NTCA as compared to the woodsmoke controls. Similar trends were reported by Sen et al. (1986) who indicated that most bacons produced by the old-fashioned direct-

Table III. N-Nitrosothiazolidinecarboxylic Acid and N-Nitrosothiazolidine Concentrations in Raw and Fried Bacon Produced by Different Smoke Treatments

smoke treatment	mode of appl	N-nitrosamine concn, μg/kg		
		NTCA[a] (raw)	NTHZ[a] (fried)	NTCA[a] (fried)
woodsmoke	traditional	87.3 ± 20.6 (67.6–113.1)	5.1 ± 1.2 (3.1–6.4)	128.2 ± 36.4 (90.0–179.2)
liquid smoke A	atomized spray	37.8 ± 9.1[b] (30.4–52.5)	0.9 ± 0.8[b] (tr, 1.8)	58.0 ± 24.6[b] (28.1–89.0)
B	in brine	24.6 ± 6.0[b] (18.8–32.6)	tr[b,d]	25.8 ± 5.4[b] (19.2–34.0)
C	in brine	12.1 ± 1.9[b] (10.5–15.1)	tr[b,d]	19.8 ± 7.4[b] (14.6–26.8)
D	in brine	76.5 ± 21.2[c] (51.2–107.5)	2.1 ± 2.1[b] (tr, 5.5)	127.2 ± 28.6[c] (98.8–168.0)
E	in brine	23.0 ± 5.6[b] (18.5–29.9)	tr[d]	36.6 ± 13.6[b] (12.6–45.6)

[a] NTCA, N-nitrosothiazolidinecarboxylic acid; NTHZ, N-nitrosothiazolidine. [b] Significantly different from woodsmoke control bacon ($p < 0.005$). [c] Not significantly different from woodsmoke control bacon ($p < 0.05$). [d] tr = trace, <0.2 μg/kg.

smoking process contained the highest levels of NTCA, whereas those processed with liquid smoke contained either undetectable or extremely low levels of NTCA. These investigators suggested that the smoke generated by the direct smoking process probably contained higher levels of formaldehyde than the liquid smoke. NTCA levels reported by Sen et al. (1986) were also much higher than those obtained in our study and by Sen et al. (1985).

When the average concentrations of NTCA in the fried bacon samples were adjusted to account for the loss of fat and moisture during frying, NTCA concentrations were 15–45% lower than the average NTCA concentrations in raw bacon. This adjustment was performed by frying several packages of randomized sliced bacon and calculating the loss in weight of the bacon as a consequence of frying. Sen et al. (1985) spiked raw bacon with known amounts of NTCA and ascertained that 1–3% of the added NTCA was converted to NTHZ under their frying conditions. However, this percent conversion of NTCA to NTHZ does not account for the differences in NTCA levels in the raw and fried (adjusted) bacon samples in our study. Further studies are necessary to establish the mechanism of NTCA decomposition and the products thus formed.

The data in Table III appear to support the observation of Sen et al. (1985) that the NTCA levels in the raw bacon correlate with those for NTHZ in the corresponding samples of fried bacon. This is particularly true for the woodsmoked bacon samples and those prepared with liquid smoke D. Further conclusions regarding these results are not warranted because NTHZ levels in raw bacon were not measured and NTHZ values in the fried samples were generally lower than those reported by other workers (Sen et al., 1985, 1986). These low NTHZ values could be due in part to the strict processing conditions used in the study. However, Sen et al. (1986) have provided data that indicated a positive correlation between NTCA levels in raw bacon and the NTHZ content of fried bacon, but not with that of raw bacon. These investigators reported that all raw bacon samples containing >1000 μg/kg NTCA produced >30 μg/kg NTHZ upon frying. Furthermore, most fried bacon contained higher levels of NTHZ than the corresponding raw bacons—a finding that conflicts with that reported by Pensabene and Fiddler (1983a). It was suggested that the different findings might be due to lower NTCA levels in the raw bacons as well as to differences in the methods of frying and analysis (for NTHZ). Sen et al. (1986) also reported that higher frying temperatures and longer frying times produced higher levels of NTHZ in the fried product. Hotchkiss and Vecchio (1985), on the other hand, reported that NTHZ levels in bacon, unlike NPYR levels, did not always increase with frying time.

Further studies are needed to further establish the mechanism of NTHZ formation in raw and fried bacon. Results of Sen et al. (1985, 1986) and Mandagere (1986) have implicated the heat-induced decarboxylation of NTCA during the frying of bacon. Decarboxylation of NTCA, however, cannot account for the small amounts of NTHZ in raw bacon as the normal smoking temperature (58 °C) is not high enough to effect the decarboxylation reaction (Skrypec et al., 1985). NTHZ in raw bacon is probably produced by direct nitrosation of thiazolidine, formed by the reaction between formaldehyde in the smoke and cysteamine in the pork belly. Studies are necessary to establish the concentration of cysteamine in meat products.

Table IV. Residual Nitrite Levels in Bacon Exposed to Various Smoke Treatments

smoke treatments	mode of appl	condensate pH	resid nitrite,[a] mg/kg
wood smoke	traditional		61.0 ± 10.9 (44.2–72.9)
liquid smoke A	atomized in smokehouse	2.2	66.6 ± 24.3[b] (50.4–107.8)
B	in brine	5.3	38.4 ± 7.0[c] (33.2–50.6)
C	in brine	5.5	46.4 ± 11.1[d] (38.9–65.6)
D	in brine	4.5	29.6 ± 4.2[c] (24.6–35.3)
E	in brine	4.6	37.2 ± 8.6[c] (31.1–51.1)

[a] Three determinations per belly were made; five bellies per treatment were used. [b] Not statistically different from woodsmoke control bacon ($p < 0.10$). [c] Statistically different from woodsmoke control bacon ($p < 0.005$). [d] Statistically different from woodsmoke control bacon ($p < 0.01$).

Residual Nitrite and Phenols in Smoked Bacon. The effect of smoke treatments on residual nitrite is summarized in Table IV. Bellies treated with liquid smoke in the curing brine (treatments B–E) all had significantly ($p < 0.01$) lower nitrite concentrations than bellies treated with either an atomized liquid smoke or woodsmoke. The acid nature of liquid smokes promotes the reduction of nitrite to volatile nitric oxides, thus lowering the residual nitrite concentration (Sleeth et al, 1982). The average residual nitrite concentration of the bellies stitch pumped with cure-solubilized liquid smoke increased as the pH of the smoke condensate increased.

The influence of liquid smoke preparations on residual nitrite and volatile N-nitrosamine levels in a cured ground pork belly model system has recently been investigated by Theiler et al. (1984). These investigators obtained data that revealed that the incorporation of liquid smoke containing unneutralized organic acids may lead to depletion in residual nitrite, thereby explaining the significant reduction in NPYR levels in the fried pork belly system. However, it was also demonstrated that N-nitrosamine inhibition can be achieved with solutions of buffered or neutralized smoke solutions without affecting the level of residual nitrite. Results of our study appear to support the observation that the lower the residual nitrite level in the raw bacon, the lower the levels of NDMA and NPYR in the fried bacon. However, some of the observed differences may again be due to variations in pork belly composition. NTHZ levels did not appear to be influenced by residual nitrite. Sen et al. (1986) also failed to observe

Table V. Major Phenol Concentrations from Ether Extracts of Liquid Smokes Used in the Curing Brines of Bacon

	μg phenol/mL liquid smoke			
liquid smoke	B	C	D	E
guaiacol	2692.5	1656.7	938.7	422.3
methylguaiacol	156.4	1056.6	600.8	294.6
phenol	1856.5	981.3	398.0	193.6
o-cresol	768.8	417.5	153.8	60.9
p-cresol	524.6	293.4	138.2	43.1
m-cresol, 2,4-dimethylphenol	839.3	482.2	160.2	28.4
eugenol	105.3	69.7	53.2	16.2
cis-isoeugenol	82.1	54.4	51.2	10.7
syringol	4075.7	2722.2	2211.3	1392.2
trans-isoeugenol	132.8	125.3	61.7	16.2
4-methylsyringol	2154.9	1575.3	967.4	548.2
4-allylsyringol	381.6	298.2	135.5	94.5
total measd phenol	15174.5	9732.8	5870.0	3120.9
manufac rec level in meat, %	0.19	0.19	0.20	0.31
theor concn in meat, μg/g	28.8	18.5	11.7	9.7

a correlation between residual nitrite and NTHZ levels in raw bacon and other smoked meats. These investigators speculated that a major factor influencing the levels of NTCA and NTHZ in smoked cured meats was the formaldehyde concentration in the smoke.

Another factor to consider in regard to the role of smoking on *N*-nitrosamine formation in bacon is the phenol content of the smoke. Phenols have been reported to be both inhibitors and catalysts of nitrosation reactions (Challis and Bartlett, 1975; Davies and McWeeny, 1977). Inhibition by phenols can be exerted either by the reduction of nitrite to a nonnitrosating species or by binding nitrite directly via C-nitrosation of the phenol (Challis, 1973). On the other hand, *p*-nitrosophenols can catalyze *N*-nitrosamine formation in aqueous systems (Davies and McWeeny, 1977). A high nitrite to phenol ratio favors the formation of the catalytic species, which is probably the quinone monoxide tautomer of the nitrosophenol (Davies et al., 1980). Furthermore, Pignatelli et al. (1980, 1982) have shown that 1,3-dihydroxyphenols, but not 1,2- and 1,4-dihydroxphenols, are potent catalysts of *N*-nitrosamine formation.

In order to ascertain whether there was a relationship between theoretical phenol concentrations in smoked bacon, residual nitrite, and *N*-nitrosamine concentrations of the fried product, ether extracts of the liquid smokes were analyzed (Table V). Thirteen major phenols of liquid smoke were identified and quantitated, and the theoretical concentrations of these phenols in the bellies were calculated. While the liquid smokes with lower phenol concentrations had slightly higher recommended usage levels, discrepancies between treatment groups in the theoretical phenol concentration of raw bacon still existed. There appears to be no relationship between phenol concentrations, residual nitrite levels, and *N*-nitrosamine concentrations. Failure to establish such a relationship could be due to the low levels of *N*-nitrosamine in the fried bacon samples of all treatment groups and to possible variation in composition of the pork bellies used in the study. Clearly, more definitive studies are required to determine the role of smoke phenols in *N*-nitrosamine formation in fried bacon. Theiler et al. (1984) have indicated that the precise mechanism whereby specific compounds present in liquid smoke inhibited *N*-nitrosamine formation has not been investigated. Similarly, Sen et al. (1986) suggested the presence of *N*-nitrosation inhibitors such as phenol and syringol in liquid smoke to explain the wide variations in NTHZ levels obtained on incubating liquid smokes and known precursors of NTHZ.

In conclusion, liquid smokes produce less NTHZ in fried bacon than does the traditional woodsmoking process. This study also describes a simple model system that can be used to evaluate the potential contribution of liquid smoke to NTHZ formation in bacon. Results of this study also support those of Theiler et al. (1984) in that bacon processed with liquid smokes contains less NPYR than traditionally woodsmoked bacon upon frying. The use of cure-solubilized liquid smokes can therefore be considered as a means of lowering *N*-nitrosamine levels in fried bacon.

Registry No. NDMA, 62-75-9; NPYR, 930-55-2; NTHZ, 73870-33-4; MeNTHZ, 70629-19-5; NO_2^-, 14797-65-0; $NH_2(CH_2)_2SH$, 60-23-1; PhOH, 108-95-2; *o*-MeC_6H_4OH, 95-48-7; *p*-MeC_6H_4OH, 106-44-5; *m*-MeC_6H_4OH, 108-39-4; guaiacol, 90-05-1; methylguaiacol, 91-16-7; 2,4-dimethylphenol, 105-67-9; eugenol, 97-53-0; *cis*-isoeugenol, 5912-86-7; syringol, 91-10-1; *trans*-isoeugenol, 5932-68-3; 4-methylsyringol, 6638-05-7; 4-allylsyringol, 6627-88-9.

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Solid-Phase Extraction and HPLC Determination of β-Cryptoxanthin and α- and β-Carotene in Orange Juice

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A simplified method is presented to determine three important orange juice carotenoids. Nonaqueous reversed-phase (NARP) high-performance liquid chromatography (HPLC) employing acetonitrile–methylene chloride–methyl alcohol (65:25:10, v/v) and a C-18 column was used to separate β-cryptoxanthin and α- and β-carotene from other juice components. Detection was at 450 nm. A photodiode array detection (PDAD) system was used to obtain UV–visible spectra for chromatographic peak identification and to determine chromatographic peak purity. Methanol was used to extract the carotenoids from centrifuged juice solids. A C-18 solid-phase extraction column was used to clean up the saponified sample prior to HPLC analysis. Ratios of β-carotene to α-carotene and β-cryptoxanthin to β-carotene from repeated (n = 5) injections were determined with relative standard deviations of 3.3 and 2.6%, respectively. Average carotenoid ratios for Valencia orange juice were 1.08 and 2.31, respectively. Murcott juices had average carotenoid ratios of 18.1 and 5.8, respectively.

INTRODUCTION

Certain carotenoids such as β-cryptoxanthin and α- and β-carotene are highly colored compounds that also exhibit provitamin A activity. β-Carotene has the highest vitamin A activity because it can be split through central enzymatic cleavage (at least in theory) into two molecules of vitamin A. Due to structural differences in part of the molecule, only half of each molecule of α-carotene, γ-carotene, and β-cryptoxanthin has the required intact β-ionone ring structure for vitamin A activity (Goodwin, 1980). Therefore, these carotenes exhibit lower vitamin A activity. Nevertheless, these carotenes are important dietary sources of vitamin A.

Carotenoids, located in juice vesicle plastids, are also the major source of color in orange juice and as such are an important quality factor. Orange juices with the expected deep yellow–orange color are preferred by most consumers and may even be perceived as being sweeter. The U.S. Department of Agriculture has set minimum color standards for orange juice and allots 40 of 100 quality points to juice color (USDA, 1983). In this grading system, color is weighted just as heavily as flavor.

Specific carotenoid patterns can be used to characterize different citrus cultivars and hybrids (Gross, 1977). Carotenoid patterns may have potential for use by citrus taxonomists to differentiate between different cultivars. However, before these patterns can be used with any certainty, a simple, accurate analytical method must be developed so that large numbers of samples can be analyzed and the natural variation in carotenoid levels determined.

Due to the relatively recent increase in the price of orange juice, certain juice processors have been tempted to dilute orange juice with inexpensive components such as sugars, citric acid, or washed orange solids (pulp wash) (Attaway, 1982). The net effect of these additions would be to reduce the natural yellow–orange color of orange juice. In order to mask the adulteration of orange juice, a coloring agent must also be added. Since β-carotene occurs naturally in orange juice, is commercially available, and is relatively inexpensive, it is a natural candidate to increase the color of adulterated juice to that of natural levels. At present, no rapid, simple method exists to determine this type of adulteration.

Whether an individual is interested in citrus carotenoids due to nutrition, taxonomy, or regulatory or quality control concerns, the need exists for a relatively simple, rapid procedure for determining citrus carotenoids that would lend itself to routine analysis.

The chromatographic isolation and identification of carotenoids are well represented in the literature (Nells and De Leenheer, 1983; Ritacco et al., 1984; Stewart and Wheaton, 1973; Calabro et al., 1978; Hsieh and Karel, 1983; Noga and Lenz, 1983; Will and Ruddat, 1984; Kamikura, 1961; Bushway and Wilson, 1982; Stancher and Zonta, 1982; Tsukida et al., 1982 and references therein). However, these procedures are not well suited for routine analysis often due to lengthy sample preparation procedures and the use of solvent gradients.

Sample preparation is the current bottleneck in citrus carotenoid analysis. All of the current procedures require lengthy liquid–liquid and or liquid–solid extractions. Artifact formation is a concern in any sample preparation

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