

Factors Affecting the N-Nitrosothiazolidine Content of Bacon

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ABSTRACT

The analysis of raw and cooked bacon for the presence of N-nitrosothiazolidine (NTHZ), a recently identified nitrosamine in bacon, indicated that the levels found were unrelated to residual nitrite or refrigerated storage. Raw bacon contained higher levels of NTHZ than either fried, baked, or broiled bacon. Since the total amount of NTHZ in whole raw bacon was higher than that found in fried bacon and its drippings combined, it was concluded that NTHZ was not formed during frying as is N-nitrosopyrrolidine. The presence of NTHZ in bacon appears to be associated with smokehouse processing.

INTRODUCTION

N-NITROSOTHIAZOLIDINE (NTHZ) was recently identified in fried bacon (Kimoto et al., 1982) analyzed by the mineral oil distillation-Thermal Energy Analyzer procedure adopted by the AOAC in 1982 as a screening procedure for N-nitrosopyrrolidine. N-Nitrosothiazolidine has also been reported by Gray et al. (1982); however, details on the method of isolation were not described. Since the mineral oil distillation procedure formed nitrosamines during analysis (Hotchkiss et al., 1980; Pensabene et al., 1982), we developed a dual column extraction method that does not artifactually produce nitrosamines as a result of the analytical method (Pensabene and Fiddler, 1982). NTHZ was isolated by this procedure and subsequently confirmed by gas chromatography-mass spectrometry (Kimoto et al., 1982).

N-Nitrosothiazolidine has been reported to be a direct acting mutagen in the Ames Salmonella Test (Mihara and Shibamoto, 1980; Sekizawa and Shibamoto, 1980). Both N-nitrosothiomorpholine, the six-membered homolog of NTHZ (Garcia et al., 1970), and nitroso-1,3-oxazolidine, its oxygen analog (Wiessler and Schmahl, 1976), were found to be carcinogenic to rats. Therefore, it is likely that NTHZ might also be carcinogenic, although this has not yet been determined.

Occasionally, fried bacon sample analyzed in our laboratory and by the Food Safety and Inspection Service (FSIS) were found to contain NTHZ. Therefore, the various factors influencing the formation of this nitrosamine in bacon were investigated, and the results of these studies are reported herein.

MATERIALS & METHODS

Materials

N-Nitrosothiomorpholine (NTMOR) and NTHZ were synthesized from their corresponding amines and sodium nitrite, and purified by fractional vacuum distillation as described previously (Pensabene et al., 1972). A complete list of reagents needed for determining NTHZ in raw and fried bacon were reported elsewhere (Pensabene and Fiddler, 1982). Bacon samples, cured and nitrite-free bellies, cured pork butts, and ham were obtained from local processors or

purchased from local retail stores. Liquid smoke samples were obtained from FSIS or from a local processor. All other reagents were purchased from commercial suppliers and used without further purification.

Bacon processing

Pork bellies were stitch-pumped with a commercial cure to approximately 10% of their green weight to achieve added target levels of 2% NaCl, 0.25% sugar, 120 ppm sodium nitrite, and 550 ppm sodium erythorbate in the finished product. The pumped bellies were split in half lengthwise and each half processed into bacon in one of three smokehouses. In House No. 1 (commercial), the bellies were sprayed first with a commercial liquid smoke solution and then placed in a gas-fired smokehouse in which slab wood (sawmill trimmings) was used for smoke generation. The bellies were kept in the house with no air exchange for 12–14 hr until the internal temperature of the bellies reached 60°C. House No. 2 (commercial) was heated by electricity. Bellies were kept in the house for 1/2 hr at 54°C, sprayed with liquid smoke, heated for 1 hr at 54°C, resprayed with liquid smoke, heated for two more hours at 54°C, followed by 3–4 hr at 66°C until the bellies reached an internal temperature of 60°C. House No. 3 (laboratory) was a gas-heated smokehouse in which the processing schedule was: 1 hr at 38°C, 1 hr at 49°C, 5 hr at 57°C, and 1/2 hr at 66°C, with medium to heavy sawdust smoke introduced after the drying period (first 2 hr) and a constant air exchange exhaust. The bacon attained an internal temperature of 59°C at the end of the processing schedule.

Bacon sampling, frying, and handling

The raw bacon was ground and mixed thoroughly prior to analysis. Samples (300g) of the comminuted bacon were either fried in a preheated (177°C) electric pan for 6 min, 9 min, or 12 min, baked for 13 min in a preheated oven set at 204°C, or broiled 1.6–2.4 cm below a 305°C source for 5 min. The edible portion and rendered drippings were retained for nitrosamine analysis. For the storage study, bacon was held in a refrigerator maintained at 5°C. For the processing time study, half the samples were removed from House No. 1 after 6 hr; the others after 12 hr.

Sodium nitrite analysis

Residual sodium nitrite content was determined in 10g of cured meat sample prior to cooking by the Griess-Saltzman procedure as modified by Fiddler (1977).

Nitrosamine analysis

Unless noted, all cured meat samples were analyzed for NTHZ prior to cooking (raw). The complete details of the procedure for analysis of NTHZ in raw and fried bacon have been described elsewhere (Pensabene and Fiddler, 1982). The standard deviation for the repeatability of the method was determined to be 1.20 ppb when NTHZ concentration was corrected for the recovery of the internal standard. The average recovery and standard deviation of the 10 ppb NTMOR internal standard was 93.3% ± 6.0%. The procedure for the determination of NTHZ in bacon drippings was similar to that reported for NTHZ in cured meats except for the following: 10 ml of hexane was added to 10g bacon drippings in a 50-ml beaker followed by the NTMOR internal standard. The sample was transferred to the alumina column, the beaker rinsed twice with 4 ml hexane, then the procedure continued as described for cured meats. To test for artifactual nitrosamine formation, 10 ppm morpholine was added to bacon drippings obtained from bacon that had a residual NaNO₂ of 38 ppm before frying. No N-nitrosomorpholine was detected after analysis. The average recovery and

standard deviation of the 10 ppb NTMOR internal standard was $81.8\% \pm 6.3\%$. All NTHZ values reported were corrected for the recovery of NTMOR internal standard in each individual sample. "N.D." denotes "none detected" or <1 ppb, the minimum level of reliable measurement based on the gas chromatography-Thermal Energy Analyzer system (gc-TEA) response (Pensabene and Fidler, 1982).

Mass spectrometer confirmation

All samples over 10 ppb were confirmed by gas chromatography-mass spectrometry (Kimoto et al., 1982).

Liquid smoke samples

To 50g liquid smoke concentrate was added 50 ml H₂O and 100 ml DCM. The solution was kept at room temperature for 72 hr, then the DCM was removed from the mixture. The DCM was extracted once with 50 ml 5N NaOH, dried by passing through anhydrous sodium sulfate into a Kuderna-Danish flask, and concentrated to 1-4 ml. The concentrated DCM extract was added to a flask containing 10 ml of 0.2N NaOH and 25 ml mineral oil, and vacuum distilled to 130°C at 0.5 mm Hg. The aqueous distillate was extracted 3 x with 50 ml DCM each, the combined DCM washed once with 25 ml 5N NaOH, dried, and concentrated to 1 ml for gc-TEA analysis of NTHZ.

RESULTS & DISCUSSION

SINCE RESIDUAL NITRITE is known to decrease during bacon storage (Herring, 1973), and a positive correlation exists with N-nitrosopyrrolidine after frying (Pensabene et al., 1979) we conducted a similar storage study for NTHZ. Refrigerated storage of the bacon for 35 days did not affect the level of NTHZ found, although the mean residual nitrite content decreased from 12 ppm to 1 ppm.

When NTHZ was absent in uncooked bacon, none was detected after frying under the standard conditions (177°C for 6 min). Six samples of commercial bacon were cooked under various conditions (Table 1). NTHZ was present in four of the six irrespective of whether they were fried, baked, or broiled. These samples also contained NTHZ in the raw bacon. In the two samples containing no NTHZ in the raw material, no NTHZ formed. All raw bacon samples contained residual nitrite ranging from 13-36 ppm. When 10 ppb NTHZ was added to nitrite-free bacon and heated at 185°C for 6 min in either an open or closed Wheaton flask, no significant decrease in NTHZ was found, indicating it was not destroyed by heating under bacon frying conditions. Development of a method for NTHZ determination in bacon drippings permitted a comparison of NTHZ values in raw and fried bacon and the drippings (Table 2). In the five samples where NTHZ was not detected in the raw bacon, none was detected in the corresponding fried bacon or its drippings. Comparison of the NTHZ levels in raw bacon with that in combined fried bacon and drippings showed a higher level in raw bacon. Therefore, NTHZ was not formed during frying, baking, or broiling, but was already present in the raw bacon. This is in contrast to the two volatile nitrosamines, N-nitrosodimethylamine and N-nitrosopyrrolidine, which are formed during frying and are absent in the uncooked product (Fazio et al., 1973; Sen et al., 1973).

NTHZ was consistently found in bacon samples produced by one manufacturer who used a gas-fired, wood-burning smokehouse (House No. 1), suggesting that NTHZ may be formed during the processing procedure. In a preliminary experiment, NTHZ was not found in five cured, unprocessed bellies, but was present in the bellies after smokehouse treatment, again indicating that NTHZ was formed during processing. To determine whether processing time in House No. 1 had an effect on NTHZ formation, six cured bellies were split in half lengthwise to minimize differences due to composition, processed, and analyzed for NTHZ. NTHZ levels were 46% higher and NaNO₂

levels were 33% lower after 12 hr compared to 6 hr processing. When cured meat products were processed in Houses 1 and 2, NTHZ levels were consistently higher in products processed in the gas-fired, wood-burning house (House No. 1), even though NTHZ was detected in much lower concentrations in the products processed with liquid smoke in the electrically heated house (Table 3). Also, the role of nitrite in the formation of NTHZ is not clear, since the NTHZ content appeared to be independent of the amount of sodium nitrite present. This was particularly apparent in the case of nitrite-free bacon where little nitrite was present, but 3.9 ppb NTHZ was found after treatment in House No. 1. This contrasted with the 20.3 and 34.1 ppb NTHZ found in cured pork butts containing comparable low concentrations of sodium nitrite.

Green bellies were pumped, halved, and processed separately in Houses 1 and 3, which were both gas-fired, but differed in the source of smoke (liquid plus wood vs sawdust, respectively). NTHZ was detected only in bacon processed in House No. 1. This may be due to the higher uncontrolled smokehouse temperature and higher humidity resulting from limited air exchange in House No. 1 or from the liquid smoke sprayed on the bellies prior to processing. NTHZ was detected in 10 samples of liquid smoke, including the one used in Houses 1 and 2, in concentrations ranging from 1.1-58.7 ppb, with an average of 22.5 ppb, indicating that these solutions may contribute to the presence of NTHZ on the product. Bacon was then prepared using bellies that were split into thirds: one-third was processed normally (liquid + wood smoke); one-third only with wood smoke; and one-third only with liquid smoke, each prepared separately in House No. 1. The results (Table 4) indicated that liquid smoke infrequently, but only to a minor extent, contributed to NTHZ formation, perhaps due to the lower concentration of smoke components present

Table 1—N-Nitrosothiazolidine formation in bacon cooked under various conditions

Sample no.	Residual NaNO ₂ (ppm)	N-Nitrosothiazolidine (ppb)					
		Raw bacon	Frying time (min) ^a			Bake ^b	Broil ^c
			6	9	12		
1	36	11.4	10.5	10.5	7.4	8.9	10.0
2	13	4.7	3.8	3.8	2.5	3.7	3.7
3	29	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4	36	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5	34	4.1	3.1	2.0	2.4	4.7	4.1
6	13	5.1	4.8	3.5	3.2	4.4	3.4

Cooking conditions: ^a177°C; ^b204°C for 13 min; ^c1.6-2.4 cm below source (305°C) for 5 min.

Table 2—N-Nitrosothiazolidine in raw bacon, its fried product, and drippings

Sample no.	N-Nitrosothiazolidine (ppb)		
	Raw	Fried ^{a,b}	Drippings ^b
1-5	N.D.	N.D.	N.D.
6	6.3	1.8	2.5
7	5.6	1.3	1.7
8	7.5	1.6	2.0
9	4.5	1.2	0.8
10	3.8	2.0	1.6
11	4.2	1.3	1.9
12	2.6	0.9	0.6
13	4.9	1.5	1.1
14	2.4	0.9	0.8

^a Fried at 177°C for 6 min

^b Based on 34% fried and 31% drippings yield

Table 3—Effect of smokehouse on N-nitrosothiazolidine formation

Sample no.	Product type	N-Nitrosothiazolidine (ppb)		Residual NaNO ₂ (ppm)	
		House 1 ^a	House 2 ^b	House 1	House 2
1	Bacon	13.8	2.6	17	12
2	Bacon	13.7	3.4	11	15
3	Bacon	13.5	3.8	13	9
4	Nitrite-free bacon	3.9	N.D.	3	2
5	Pork butt	29.4	4.5	29	5
6	Pork butt	34.1	7.8	3	5
7	Pork butt	20.3	7.2	2	3
8	Ham	10.8	—	25	—

^a Gas-fired, wood + liquid smoke

^b Electric, liquid smoke

Table 4—Effect of liquid smoke on N-nitrosothiazolidine formation in bacon^a

Sample no.	N-Nitrosothiazolidine, ppb		
	Wood + liquid	Wood only	Liquid only
1	6.7	7.5	N.D.
2	6.2	10.6	N.D.
3	6.3	7.1	1.3
4 ^b	N.D.	N.D.	N.D.
5 ^b	6.1	4.8	3.3
6 ^b	3.6	3.4	3.5

^a House No. 1

^b Nitrite-free bellies

in comparison with those obtained by the more lengthy smoking process generated by the wood over a 12-hr period. The presence of NTHZ in the samples tested appeared to be associated with wood smoke itself, and the source of nitrosation species may not only be the residual nitrite in the cured meat product, but also the nitrogen oxides in the wood smoke and/or gas used in heating.

Experiments were then conducted to determine whether NTHZ formation occurred only on the surface of bacon after it was processed. In the samples analyzed to date, the NTHZ content was found to be higher on the exterior than in the less exposed interior portion of the bacon.

In conclusion, the results of these experiments indicate that the presence of NTHZ in bacon appears to be associated with the smoking step in smokehouse processing. The results also indicate that NTHZ can be present in cured meat products other than bacon, suggesting that bacon does not have a unique composition that would favor formation of this nitrosamine. Whether NTHZ is formed during wood smoking and is deposited on the meat during processing, or whether one or more of the smoke components react with

meat constituents to form the nitrosamine is not known. Further research in this area is in progress.

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Note: Nitrosamines are potential carcinogens. Exercise care in handling these materials.

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