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INVESTIGATION OF BACON FOR THE PRESENCE OF N-NITROSOTHIAZOLIDINE

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Introduction

We have recently identified N-nitrosothiazolidine (NTHZ) in fried bacon (1) and developed a dual column extraction method for its simultaneous determination with N-nitrosodimethylamine (NDMA) and -pyrrolidine (NPYR; 2). NTHZ was originally observed in occasional fried bacon samples when analyzed by the mineral oil distillation-Thermal Energy Analyzer method, which has been shown to artifactually form nitrosamines (NAs) during analysis (3,4), including NTHZ (1). Our dual column extraction method gave no evidence for artifactually forming NAs, including NTHZ, as a result of analysis.

N-Nitrosothiazolidine, derived from the nitrosation of thiazolidine, a Browning-type product of the reaction of cysteamine-D-glucose-water (5), has been shown to be a direct acting mutagen by the Ames Salmonella Test (6,7). Its possible carcinogenic properties toward laboratory animals have not yet been determined.

Analysis of a limited number of cured meat products of various types for NTHZ indicated this NA was present primarily in bacon. The reason NTHZ appeared in only a small number of fried bacon samples was not readily apparent, thus prompting us to determine the factors responsible for the formation of this nitrosamine. Results of these studies are reported herein.

Materials and Methods

Cured meat samples. Bacon samples were obtained from either local processors or purchased from local retail stores.

Reagents. Dichloromethane (DCM), pentane, and hexane were "Distilled in Glass" solvents from Burdick and Jackson Laboratories; all other reagents were purchased from commercial suppliers and used without further purification. A complete list of reagents needed for the dual column method, and the preparation of the nitrosamine standards have been published (2,3) elsewhere.

Analyses for nitrosamine. A general flow diagram of the procedure used for the isolation and detection of NTHZ in cured meats is shown in Figure 1. The details of the procedures used for the quantitation and confirmation of NTHZ have been reported elsewhere (1,2). The repeatability and reproducibility for the method have been shown to be 1.20 and 1.55 ppb, respectively, and the average recovery and standard deviation of the 10 ppb added N-nitrosothiomorpholine (NTMOR) internal standard was 93.3% ± 6.03% (2). The procedure for the determination of NTHZ in bacon drippings was similar to that reported for NTHZ in cured meats, except for the following: To 10.0 g bacon drippings in a 50 ml beaker, add 10 ml hexane and the NTMOR internal standard. The sample was transferred to the alumina column and 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 $NaNO_2$ of 38 ppm before frying. No detectable N-nitrosomorpholine was obtained. The average recovery and standard deviation of the NTMOR, added at the 10 ppb level, was 81.8% ± 6.28%. All the NTHZ values reported herein have been corrected for the recovery of NTMOR internal standard in each individual sample. "N.D." connotes none detected which is <1 ppb, the minimum level of reliable measurement.

Sodium nitrite. The bacon samples were analyzed for residual $NaNO_2$ by the procedure described by Fiddler (8).

Results and Discussion

The addition of several potential precursors to ground bacon prior to frying and the resulting levels of NTHZ detected are shown in Table 1. Only thiazolidine and cysteamine had a significant effect on NTHZ formation; whereas, the other reactants had no effect since the NTHZ levels were approximately the same as that for the original untreated bacon sample. Thiazolidine can readily react with residual nitrite in the bacon during frying to form NTHZ whereas cysteamine can react with formaldehyde, a fragmentation product of added or endogenous sugar or lipid oxidation, and residual nitrite upon frying of the bacon to form NTHZ. The fact that no increase in NTHZ was observed upon addition of cysteine suggests that the direct reaction of cysteine with formaldehyde to form thiazolidine-4-carboxylic acid is not the mechanism of NTHZ formation. Alternately, if present, the cysteamine will not be expected to result from the decarboxylation of endogenous cysteine since adding cysteine to the bacon before frying did not increase the NTHZ level after frying. The samples were also analyzed for 2-methyl-N-nitrosothiazolidine (NMTHZ). Concentrations of 21.3 and 7.9 ppb NMTHZ for samples 1 and 2, respectively, were detected only when cysteamine was added. NMTHZ can result during bacon frying from the reaction of added cysteamine, residual nitrite and endogenous acetaldehyde, produced from the fragmentation of sugars and oxidation of lipids. The absence of NMTHZ upon addition of acetaldehyde suggests that cysteamine or other amine precursors is present only in very small concentrations and is therefore the limiting factor in NMTHZ production.

In the approximately 30 samples analyzed to date, typically when NTHZ was absent in the uncooked bacon, none was detected after frying under the standard conditions (177°C for 6 min). Since NTHZ was found in some fried bacon samples, a study was undertaken to determine the effect of varying cooking conditions and frying times on the amount of NTHZ formed. The results are summarized in Table 2. All of the samples contained residual nitrite even for those which did not contain NTHZ. For those bacon samples containing NTHZ, unexpectedly, the uncooked controls contained higher levels than the cooked samples. This may have been due to loss of NTHZ by

volatilization during cooking. If the latter is not true, these results indicate that NTHZ was not formed during frying, baking, or broiling, but that it was already present in the bacon. This differs from the two volatile NAs normally found in bacon, NDMA, and NPYR, which are formed during frying and are absent in the uncooked product (9,10). No NTHZ was detected in samples 3 and 4 that were also subjected to the same cooking conditions despite the fact they contained 13 and 36 ppm residual $NaNO_2$, respectively.

Residual $NaNO_2$ in bacon is known to decrease upon storage (11). Since a high positive correlation exists between residual nitrite prior to frying and NPYR after frying (12), a corresponding decrease in NPYR has been observed after cooking as nitrite decreased during storage. A similar study in which commercial bacon (purchased in retail stores) was stored under refrigeration conditions was carried out and analyzed for NTHZ. The results in Table 3 show that for any given sample, the NTHZ levels for either the raw or fried bacon showed no upward or downward trend, but instead the variation in the NTHZ levels was not significantly different over 35 days at 5°C. This was despite the average decrease in residual $NaNO_2$ from 12 to 1 ppm. Therefore, refrigerated storage of the bacon did not effect the level of NTHZ found. No significant decrease was noted when 10 ppb NTHZ was added to nitrite-free bacon then heated at 185°C for 6 min. in either an open or closed Wheaton flask and then analyzing the entire flask contents. This indicates that under the bacon frying conditions employed in other experiments, NTHZ is not destroyed by heating and that the slightly lower NTHZ observed between the raw and fried product may be either due to slight volatization of the NA or some solubility in the bacon drippings that were not analyzed.

Subsequent development of a method for NTHZ determination in bacon drippings and analyses of 14 samples, enabled us to compare the NTHZ values in raw and fried bacon and its drippings. The results are shown in Table 4. In the five samples where NTHZ was not detected in the raw bacon, none was detected in the fried bacon or its drippings. Comparison of the NTHZ levels in raw bacon with fried bacon and its drippings would indicate an increase in the NTHZ levels as a result of frying. However, based on an average of 34% edible fried product and 31% drippings yield from frying the corresponding whole raw bacon, calculation of the amount of NTHZ shows that it is higher in raw bacon than in the combined fried product and drippings. Therefore, these results suggested that NTHZ was not formed during frying. The residual $NaNO_2$ levels for the 14 samples in Table 4 varied from 7 to 44 ppm, which was independent of the NTHZ concentration.

For the purpose of these investigations, bacon was selected primarily from a few processors in which NTHZ was found consistently, not from the majority where NTHZ was always absent. In conclusion, the data presented show that frying is not responsible for NTHZ formation. This is because NTHZ is absent in fried bacon and its drippings when not present in the raw whole bacon and when present, NTHZ was higher in the latter compared to the combined total of the fried product and its drippings. It appears that NTHZ and its precursor(s) are already present in the bacon prior to frying. The results suggest that NTHZ formation in bacon is associated with the processing step, however, the precise cause is not known at this time. Studies in this area are currently in progress.

References

1. The Isolation and Identification of N-Nitrosothiazolidine in Fried Bacon. W. I. Kimoto, J. W. Pensabene, and W. Fiddler. J. Agric. Food Chem., in press (1982).

2. The Determination of N-Nitrosothiazolidine in Fried Bacon by a Dual Column Chromatographic Method. J. W. Pensabene, and W. Fiddler. J. Assoc. Off. Anal. Chem., November issue, in press (1982).

3. Rapid Dry Column Method for Determination of N-Nitrosopyrrolidine in Fried Bacon. J. W. Pensabene, A. J. Miller, E. L. Greenfield, and W. Fiddler. J. Assoc. Off. Anal. Chem., 65, 151-156 (1982).

4. Confirmation of Low µg/kg Amounts of Volatile N-Nitrosamines in Foods by Low Resolution Mass Spectrometry. J. H. Hotchkiss, L. M. Libbey, and R. A. Scanlan. J. Assoc. Off. Anal. Chem., 63, 74-79 (1980).

5. Formation of Heterocyclic Compounds From the Reaction of Cysteamine and D-Glucose, Acetaldehyde, or Glyoxal. M. Sakaguchi, and T. Shibamoto. J. Agric. Food Chem., 26, 1178-1183 (1978).

6. Mutagenicity of Products Obtained From Cysteamine-Glucose Browning Model Systems. S. Mihara, and T. Shibamoto. J. Agric. Food Chem., 28, 62-66 (1980).

7. Mutagenicity of 2-Alkyl-N-Nitrosothiazolidines. J. Sekizawa, and T. Shibamoto. J. Agric. Food Chem., 28 781-783 (1980).

8. Collaborative Study of Modified AOAC Method for Analysis for Nitrite in Meat and Meat Products. R. N. Fiddler. J. Assoc. Off. Anal. Chem., 60, 594-599 (1977).

9. Nitrosopyrrolidine in Cooked Bacon. T. Fazio, R. H. White, L. R. Dusold, and J. W. Howard. J. Assoc. Off. Anal. Chem., 56, 919-921 (1973).

10. Nitrosopyrrolidine and Dimethylnitrosamine in Bacon. N. P. Sen, B. Donaldson, J. R. Iyengar, and T. Panalaks. Nature, 241, 473-474 (1973).

11. Effect of Nitrite and Other Factors on the Physico-chemical Characteristics and Nitrosamine Formation in Bacon. H. K. Herring. Proceedings of the Meat Industry Research Conference, American Meat Institute Foundation, March 22-23, Chicago, Illinois, U.S.A., pp. 47-60 (1973).

12. Belly Composition and Nitrite Level on Nitrosamine Formation in Fried Bacon. J. W. Pensabene, J. I. Feinberg, C. J. Dooley, J. G. Phillips, and W. Fiddler. J. Agric. Food Chem., 27, 842-845 (1979).

Figure 1 SCHEMATIC OF DUAL COLUMN METHOD

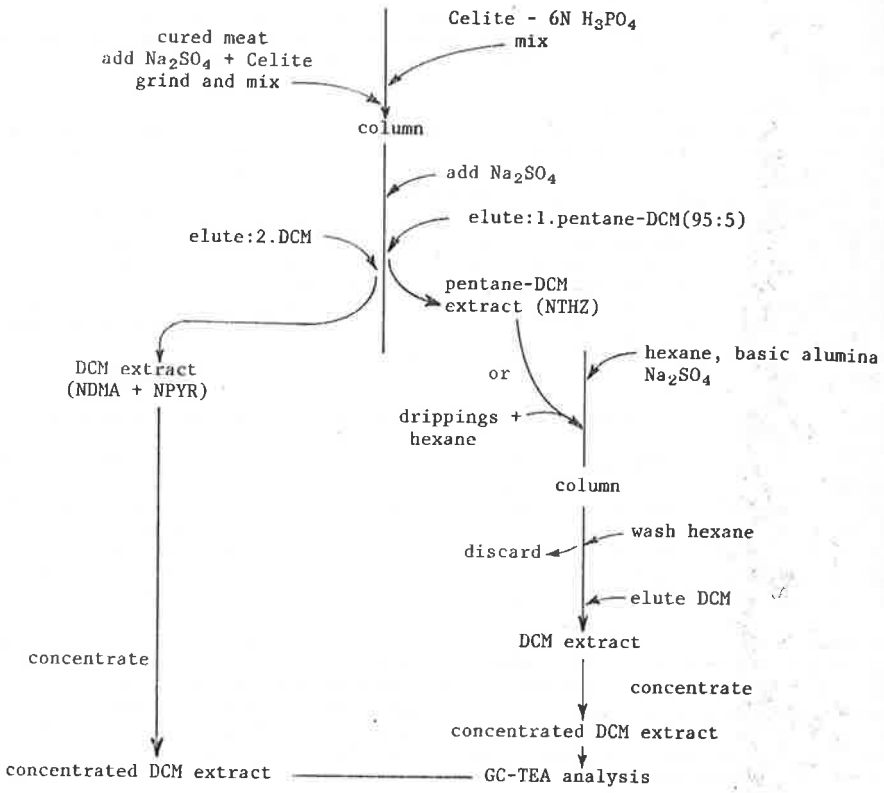

Table 1. Effect of addition of potential precursors on NTHZ formation in bacon.

Added reactant[a]	NTHZ (ppb) Sample 1[b]	Sample 2[c]	Sample 3[d]
None	5.0	3.8	N.D.
Thiazolidine	941	1802	38.6
Cysteamine	34.1	30.1	2.1
Cysteine	3.4	3.5	N.D.
Cystine	2.2	3.0	N.D.
Methionine	3.0	3.3	N.D.
Formaldehyde	6.3	6.6	N.D.
Acetaldehyde	3.9	3.5	N.D.

[a] Reactants (1000 ppm) added to ground commercial bacon containing [b]36 ppm, [c]34 ppm, and [d]1 ppm residual $NaNO_2$ then fried at 177°C for 6 min.

Table 2. Effect of cooking on NTHZ formation in bacon.

Sample no.	Residual $NaNO_2$ (ppm)	Cooking treatment; NTHZ (ppb)					
			Frying time (min.)[a]				
		None	6	9	12	Bake[b]	Broil[c]
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, 4	29, 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: [a] 177°C; [b] 204°C for 13 min.; [c] 1.6-2.4 cm below source (305°C) for 5 min.

Table 3. Effect of storage on NTHZ formation in commercial bacon.

Sample no.	Bacon storage[a] (days); NTHZ (ppb)					
	0		14		35	
	Raw	Fried[b]	Raw	Fried[b]	Raw	Fried[b]
1	3.7	4.0	3.5	4.0	4.1	3.1
2	5.2	4.1	5.5	4.1	5.1	4.8
3	6.3	5.3	7.9	4.5	6.7	6.2
4	7.5	4.7	7.9	6.3	8.5	5.1
5	5.6	3.8	5.8	4.6	5.4	3.7

[a] Storage temperature, 5°C.
[b] Fried at 177°C for 6 min.

Table 4. Comparison of NTHZ content in raw bacon, its fried product, and drippings.

Sample no.	N-Nitrosothiazolidine (ppb)		
	Raw	Fried[a]	Drippings
1-5	N.D.	N.D.	N.D.
6	6.3	5.3	8.0
7	5.6	3.8	5.5
8	7.5	4.7	6.6
9	4.5	3.6	2.5
10	3.8	5.8	5.1
11	4.2	3.9	6.2
12	2.6	2.5	2.0
13	4.9	4.3	3.4
14	2.4	2.6	2.7

[a] Fried at 177°C for 6 min.

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

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.