N-NITROSOTHIAZOLIDINE AND N-NITROSOTHIAZOLIDINE-4-CARBOXYLIC ACID IN DRY-CURED BACON

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ABSTRACT

As part of a comprehensive study on dry-cured bacon for the presence of N-nitrosamines, this product was analyzed for N-nitrosothiazolidine (NTHZ) and N-nitrosothiazolidine-4-carboxylic acid (NTHZC) in addition to N-nitrosopyrrolidine (NPYR), which occurs frequently in fried bacon. Forty-seven samples were obtained from 16 different processors. NTHZ was isolated by a dual column method in which the sample was first extracted on a Celite-sodium sulfate column, then cleaned-up on a basic alumina column. The NTHZC was extracted from the sample with methanol containing sulfamic acid, followed by solvent partitioning into ethyl acetate and subsequent derivatization with diazomethane to form a volatile methyl ester. Both NTHZ and NTHZC were quantitated using a gas chromatograph interfaced with a chemiluminescence detector (Thermal Energy Analyzer). NTHZ values ranged from none detected (N.D.) to 22.3 ppb with an average of 6.3 ppb; NTHZC ranged from 7 to 6478 ppb with an average of 936 ppb. Generally, concentrations of NTHZ and NTHZC were higher than previously found in cure-pumped bacon. This suggests that the processing conditions used for dry-cured bacon vary and may favor the formation of NTHZC.

INTRODUCTION

N-Nitrosothiazolidine (NTHZ) and/or its 4-carboxylic acid derivative (NTHZC) have been detected in a variety of smoked, cured meat products, but principally in bacon, (Kimoto *et al.* 1982; Pensabene and Fiddler 1983a,b;

Helgason *et al.* 1984; Mandagere *et al.* 1984; Hotchkiss and Vecchio 1985; Sen *et al.* 1985, 1986, 1987; Ellen *et al.* 1986; Pensabene *et al.* 1985). Unlike N-nitrosopyrrolidine (NPYR), which is formed as a result of frying (Gray 1976; Pensabene and Fiddler 1983b), these nitrosamines result from smokehouse processing, namely the use of wood smoke. Most of the published studies have been carried out with bacon because wood smoke plays an important role in contributing to the unique desirable flavor of this product. As such, bacon is typically smoked longer than most other cured meat products. Pensabene and Fiddler (1983b) found that traditional direct wood smoking produced a greater amount of NTHZ in raw bacon than that produced with liquid smokes. Ikins *et al.* (1986) and Sen *et al.* (1986) confirmed this finding, and demonstrated it was also true for NTHZC. There is now ample evidence that formaldehyde generated during the combustion of wood reacts with either cysteamine or cysteine in the meat to form the nitrosamine. Nitrite present in the cured product is the principal source of the nitrosating agent and to a lesser extent, nitrogen oxides in the wood smoke. A recent paper by Pensabene *et al.* (1987) on the cysteamine and cysteine content of pork bellies and processed bacon indicated no correlation between these compounds and their counterpart nitrosamines, NTHZ and NTHZC. These authors and Sen *et al.* (1986) suggested that formaldehyde in either wood smoke or liquid smoke may be the limiting reactant, rather than thiazolidine or its 4-carboxylic acid (thioproline) or nitrite. For a specialty product such as dry-cured bacon there are lengthy curing and smoking times that would favor increased NTHZ and NTHZC formation compared to other cured meat products. The former represents an increased opportunity for microbial/enzymatic degradation of meat components for generation of precursor amines, particularly cysteamine and the latter for generating greater amounts for formaldehyde. To date, analyses of retail samples of dry-cured and pump-cured bacon of unknown history were inconclusive insofar as comparing the NTHZ results, and nonexistent for NTHZC in dry-cured bacon (Skrypeck *et al.* 1985; Pensabene and Fiddler 1983b). For this reason, we have surveyed dry-cured bacon for both NTHZ and NTHZC from commercial producers for which the processing conditions were available. The results are reported herein.

MATERIALS AND METHODS

Samples of dry-cured bacon were obtained from 16 processors immediately after production, and shipped to ERRC within 1 day (Fiddler *et al.* 1988). The procedure and requisite calculations for determining NTHZ and NTHZC have been described previously (Pensabene and Fiddler 1982, 1985). Residual $NaNO_2$ was determined in 10 g of uncooked sample by the modified Griess-Saltzman procedure (Fiddler 1977). We used the General Linear Models (GLM)

procedure of the Statistical Analysis System PC software distributed by SAS Institute, Inc., and interpreted the results according to the methods of Snedecor and Cochran (1979).

RESULTS AND DISCUSSION

Because of the possibility that fried dry-cured bacon may have a high incidence of NPYR exceeding the 17 ppb action level established by the USDA, Food Safety Inspection Service (FSIS), we recently carried out a survey on this product for NPYR (Fiddler *et al.* 1988). Having these samples, there was an opportunity to conduct a more comprehensive study on NTHZ and NTHZC in raw dry-cured bacon and to obtain additional information on the formation of these nitrosamines. In this study, "raw" includes thermal treatment during processing, but not cooking post processing. To help assure the reproducibility of the NTHZ and NTHZC values and determine if they were representative, analyses were performed in duplicate on 3 different dry-cured bacon samples obtained from each establishment. The Analysis of Variance of the data showed a repeatability of 0.1 ppb for NTHZ and 12.1 ppb for NTHZC. The days in cure, hours smoked, range and average of residual $NaNO_2$, NTHZ and NTHZC in dry-cured bacon from 16 processors are shown in Table 1. The overall range was N.D. to 22.3 ppb (av. 6.3 ppb) for NTHZ and 7-6478 ppb (av. 936 ppb) for NTHZC. The values obtained for NTHZ were not out of line with other published reports (Pensabene and Fiddler 1983b; Sen *et al.* 1985b). The NTHZC levels found were consistently higher than NTHZ (Helgason *et al.* 1984; Pensabene and Fiddler 1985; Sen *et al.* 1985b, 1986; Ikins *et al.* 1986). Thirty six percent of the samples contained NTHZC in excess of 1000 ppb, with the highest value being 6478 ppb. The NTHZC in these samples was confirmed by GC/MS after preparation of the methyl ester. Sen *et al.* (1986) found 3800 and 4300 ppb NTHZC in two raw dry-cured bacon samples and from 1400 to 9000 ppb NTHZC in six raw pumped bacon samples. Unlike our study, their study emphasized obtaining only products smoked in an "old fashioned" smokehouse. Most striking is that no NTHZ ($<$ 1 ppb) and low NTHZC levels were detected in the samples from companies #8 and 14. The samples obtained from company #14 were not typical dry-cured bacon, but an unsmoked, pepper coated dry-cured product called pork side meat, used for cooking and flavoring purposes. Thus the results can be explained. Because of the same 10 h smoking time and 4 day curing time for both company #8 and #12, one would expect similar nitrosamines results, particularly for NTHZC. Clearly this was not the case. Company #8 was the only producer who used a commercial cure premix that contained sodium ascorbate and sodium carbonate in addition to the normal curing salts. The latter compound, at the 1% level, served as a buffering agent to

TABLE 1.
N-NITROSOTHIAZOLIDINE AND N-NITROSOTHIAZOLIDINE-4-CARBOXYLIC ACID CONTENT OF DRY-CURED BACON

Co	Days in Cure	Hours Smoked	Residual $NaNO_2$-ppm Range	Avg.	NTHZ-ppb Range	Avg	NTHZC-ppb Range	Avg.
1	10	24	22.3— 33.6	27.9	9.0—10.0	10.6	586—1654	1036
2	10	2.5	4.2— 26.6	12.2	2.1— 4.4	2.9	154— 254	188
3	3	15	27.8— 49.3	38.8	9.5—11.6	10.4	652—1053	907
4	13	8	10.4— 30.7	20.2	2.5— 3.3	3.0	164— 351[c]	286
5	4.5	12	20.2— 39.6	29.5	4.1—12.4	8.9	1064—1793[c]	1351
6	7	22	102.3—288.7	181.5	11.2—22.3	18.3	1630—3626	2590
7	5	5.5	41.0— 53.6	45.9	1.7— 2.7	2.2	153— 197	175
8	4	10	21.6— 37.2	28.5	N.D.[b]	N.D.	7— 11	9
9	6	4	12.2— 19.8	15.4	3.8— 4.3	4.1	285— 319[c]	305
10	7	4	15.0— 29.7	20.9	5.5— 8.9	6.6	985—1729	1275
11	7	14	22.0— 36.2	26.6	4.8— 6.1	5.7	422— 546	478
12	4	10	13.6— 39.3	28.0	N.D.— 2.6	1.6	117— 212[c]	156
13	12	2	44.9— 50.8	47.9	15.1—16.0	15.6	2579—6478	4188
14	56	a	2.2— 5.8	3.4	N.D.	N.D.	17— 67[c]	38
15	33	60	11.3— 73.8	33.1	6.5—14.9	11.2	1566—1987	1789
16	14	4	9.4— 42.0	21.6	N.D.— 1.8	0.6	141— 308	200

[a]Smokehouse heated electrically-no smoke
[b]N.D. = none detected
[c]Samples confirmed by GC/MS as NTHZC methyl ester

prevent the destruction of nitrite in the dry-cured mix prior to use. The reductants, sodium ascorbate, or its isomer sodium erythorbate, are required for manufacturing pump-cured bacon at the ingoing level of 550 ppb (Federal Register 1975), to help reduce NPYR at the time of frying. These compounds, normally incorporated in the brine injected for pump-cured bacon, are not typically used in dry-cured formulations perhaps because of the longer curing times required for the application of this type of cure. Gray *et al.* (1984) obtained a 66% reduction of NTHZ in fried pump-cured bacons and a 32% reduction in dry-cured bacons prepared with 500 ppm sodium ascorbate and 500 ppm α-tocopherol when compared to the nontocopherol treated controls. In that study α-tocopherol coated salt was used. This treatment was previously reported to reduce NPYR in fried dry-cured bacon (Gray *et al.* 1982). While it was shown that this combination of ascorbate and α-tocopherol was effective in reducing NTHZ/NTHZC, ascorbate exhibited most of the inhibitory activity since the nitrosamine precursors were demonstrated to be water soluble and contained in the pork belly lean tissue (Fiddler *et al.* 1986). Therefore, it would be desirable to utilize ascorbate/erythorbate in the manufacture of dry-cured bacon to reduce these nitrosamines.

A significant correlation ($p < 0.01$) was found between residual $NaNO_2$ and NTHZ and NTHZC in these dry-cured bacon samples. These correlations with $NaNO_2$ have not been previously observed. This is particularly true for pump-cured bacon where extensive data is available. Unlike pump-cured bacon, it is thought that dry-cured bacon is smoked so extensively that formaldehyde is no longer the limiting reactant, but the amount of nitrite available is. This is supported, in part, by a significant correlation ($p < 0.01$) between the length of time the bacon is smoked and NTHZ formation, but not NTHZC. In this study, a significant correlation was also found between NTHZC and NTHZ. The significant correlation reported for NTHZC in raw and NTHZ in fried pump-cured can be explained by the frying temperature/time favoring the thermal decarboxylative conversion of one nitrosamine to the other (Sen *et al.* 1986). This correlation between the two nitrosamines in dry-cured bacon cannot be explained since there was no cooking post processing. There was no correlation between NTHZ/NTHZC and the time in cure. This factor would effect the bacterial/enzymatic conversion of cysteine to cysteamine and thus the amount of each nitrosamine that could form during smokehouse processing. The widespread use of hardwood smoke in the manufacture of dry-cure bacon clearly favors the formation of NTHZ/NTHZC.

With the amounts of NTHZ and NTHZC present in smoked cured meat products, particularly NTHZC, there is concern about the toxicological significance of these nitrosamines. Few animal feeding studies have been carried out with NTHZ or NTHZC. A computer-aided structure-activity evaluation system predicted NTHZ to be carcinogenic and NTHZC to be noncarcinogenic

(Rosenkranz and Klopman 1987). NTHZ, however, has recently been shown to be non or weakly carcinogenic to female Fischer 344 rats (Lijinsky *et al.* 1988). Helgason *et al.* (1984) induced hyperglycemia in young mice with NTHZC and associated this nitrosamine with the diabetogencity of Icelandic smoked and cured mutton. Of considerable interest is the detection of NTHZC and its 2-methyl derivative in the urine of humans (Ohshima *et al.* 1983; Tsuda *et al.* 1983). These nitrosoamino acids have been proposed as indicators of endogenous nitrosamine formation. While the biological significance of NTHZC in human urine is not yet evident, it is inferred that NTHZC is noncarcinogenic, like N-nitrosoproline (Mirvish *et al.* 1980). The thiazolidine precursors in cured meats could serve as effective nitrite scavengers, as has been proposed in subsequent *in vivo* urine studies. This is supported by Tahira *et al.* (1984) who found that thiazolidine-4-carboxylic acid (thioproline) is nitrosated up to 1000 times more rapidly than is proline under certain conditions. This kinetically favored reaction would help explain the high levels of NTHZC found in some of the samples. It is also possible that cysteine, which is ubiquitous in foods, exerts an indirect protective effect since thiazolidine-4-carboxylic acid could favorably compete for nitrite with other nitrosatable amines in meat to form a noncarcinogenic nitrosamine verses a carcinogenic one.

Our results and those of others suggest that the concentrations of preformed dietary NTHZC, as found in smoked cured meat products, would affect the urinary content of this nitrosamine. We, therefore, recommend that appropriate control experiments be conducted and care exercised in interpreting the results of *in vivo* studies because considerable urinary NTHZC could be due to dietary rather than endogenous formation.

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