NITROSAMINE ANALYSIS AND INHIBITION STUDIES

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INTRODUCTION

Because of wide-spread concern over the formation of N-nitrosopyrrolidine (N-Pyr) in bacon, the U.S.D.A. issued a call for processing and safety information on a variety of nitrite-treated products in October, 1977. Based on data compiled by industry, government and university research on nitrite, nitrosamines and Cl. botulinum toxin the U.S.D.A. ruled that pumped-bacon must employ 120 ppm in-going sodium nitrite to prevent botulinum toxin formation and 550 ppm sodium ascorbate or erythrobate to inhibit nitrosamine formation. The objective was to reduce N-nitrosamine content to the "confirmable" level of 10 ppb.

Despite these efforts, occasional pumped-bacon samples are found to exceed the minimum acceptable limit for N-nitrosopyrrolidine. Furthermore, a recent survey of nitrosamine levels in dry cured bacon revealed that 46 percent of the dry-salt cured bacon analyzed by Thermal Energy Analyzer detection contained N-Pyr greater than 16 ppb.

There have been a number of studies designed to eliminate N-nitrosamine formation in foods. A variety of substitutes for nitrite have been proposed but none satisfactorily replaced all the functional properties of nitrite. Alternatives are to reduce the amount of nitrite added by altering the processing procedure and/or adding chemicals which would enhance the benefit of nitrite while inhibiting its harmful effects.

In view of the growing concern regarding the use of nitrite for curing of meat products, the following objectives were pursued in this research:

1. To study the effects of pH and heating time upon the N-nitrosation of L-proline by sodium nitrite in a closed system.
2. To study the effects of additives on the formation of N-nitrosopyrrolidine in the above model system and in bacon, and
3. To develop a simple and direct screening, confirmative analytical procedure for determining nitrosamines in bacon.

MATERIALS AND METHODS

Model System Studies of N-Nitrosopyrrolidine Inhibition.

Preparation of reaction mixtures. Eleven reaction mixtures were studied. They were prepared to contain 0.133M of each component studied. The control sample contained only L-proline and sodium nitrite. Components of the other mixtures added to the control mixture were as follows: dextrose; sodium ascorbate; sodium chloride; dihydroxyacetone; pyruvaldehyde; dextrose and sodium ascorbate; dihydroxyacetone and pyruvaldehyde; propyl gallate and L-cysteine.HCl; dextrose, sodium ascorbate and dihydroxyacetone; dextrose, sodium ascorbate, pyruvaldehyde and dihydroxyacetone.

Four sets of samples were studied. The variables were pH (5.5 and 7.5) and heating time at 180°C (15 or 30 minutes). The samples contained 0.1M phosphate buffer to maintain pH.

Extraction and concentration of N-nitrosopyrrolidine. The nitrosamines were extracted with methylene chloride as described by Huxel et al. (1974) and diethylnitrosamine added as internal standard.

Analysis of N-nitrosopyrrolidine in model system studies. N-Pyr contents of the model system samples were analyzed using the extraction system described by Lane et al. (1974) and the gas-liquid chromatography method of Lane and Bailey (1973). These methods were modifications of the procedure of Howard et al. (1970) for the extraction and determination of N-dimethylnitrosamine in fish.

N-Pry was also analyzed and its presence confirmed by the gas-chromatographic-mass spectrometric method of Lane et al. (1947).

Bacon Studies of N-Nitrosopyrrolidine Inhibition.

Bacon curing and cooking. Pork bellies were sliced into 4 mm thick slices and 500 g-samples cured with 1000 ml of curing solution containing curing ingredients and other chemicals listed in Table I. The belly slices were cured for 24 hrs. at 4°C and excess solution removed by blotting.

Cooking was carried out at 175°C in an electric frying pan for 8 minutes on both sides. Cooked bacon was blotted to remove excess drip.

Bacon N-nitrosopyrrolidine extraction, concentration and analysis. Extraction and concentration of cooked bacon samples for nitrosamine analysis was carried out by the method of Goodhead and Gough (1975) and analyzed by the GLC-MS method of Lane et al. (1973). The mass spectrometer was set at a resolution of 1/10,000 to scan for the

presence of parent ion peaks of DENA (m/e 102.079308) and N-Pyr (m/e 100.063659) by continuous scanning. The chromatographic column was 7% poly MPE on 60-80 mesh Tenax.

TABLE I

CURING INGREDIENTS FOR BACON

Sample No.	Ingredient
1	120 ppm Sodium nitrite[1] 2% Sodium Chloride
2	Sample 1 + 2% Dextrose
3	Sample 1 + 2% Dextrose + 550 ppm Sodium Ascorbate
4	Sample 1 + 500 ppm Dihydroxy- acetone + 500 ppm pyruvaldehyde
5	Sample 1 + 500 ppm Propylgallate + 500 ppm Cysteine. HCl

[1]Replicate samples were also prepared containing 0.1M phosphate buffer, pH 6.8.

Direct Sampling For Gas-Liquid Chromatographic-Mass Spectrometric Analysis of N-Nitrosopyrrolidine.

An external sampling device similar to that described by Legendre et al. (1979) was used to analyze N-nitrosopyrrolidine in cooked bacon. The direct sampler was connected to a Varian Aerograph 1520 gas chromatograph-C.E.C.-21-110C mass spectrometer equipped with a peak matching unit for high resolution analysis. High resolution peak matching was set at 1/10,000 (10 percent Valley). Perflurokerosene (m/e 99.993608) was used as reference to ensure accuracy. The presence of N-Pyr was determined by continuous scanning for the parent ion peak at 10.06359 and the internal standard DENA at m/e 102.079308 at the appropriate retention time. Ion responses (mv) of the nitrosamines were compared for quantitative purposes.

Cooked bacon analyzed for N-Pyr was separated from drip, frozen in liquid nitrogen and blended to a fine powder. Five hundred mg of sample was placed on glass wool packed in the sample tube and 10 ng (DENA (internal standard) injected onto the sample.

The sample in the external sampling tube was heated to 180°C for 20 minutes to elute the nitrosamines. The volatile nitrosamines were eluted from the sample onto the G.L.C. column which was maintained at ambient temperature. The chromatographic column was temperature-programmed at 20°C per minute from ambient temperature to 110°C and then to 215°C per minute.

RESULTS AND DISCUSSION

Inhibition of N-Pyr Formation in Model System.

Results of inhibition studies of N-Pyr formation in the model system are given in Table II. The data show the influence of various additives, pH, and heating time on the formation of N-Pyr. Generally, all inhibitors except NaCl were very effective in retarding N-Pyr formation. All inhibitors except NaCl and sodium ascorbate were added to reduce amine content in the reaction mixture. The results from GLC-MS determination were generally lower than those obtained by AFID (not shown), although the percent inhibition was found to be in excellent agreement in samples analyzed by the two methods. Heating for 30 minutes at 180°C instead of 15 minutes resulted in only slight increase in N-Pyr formation.

The data indicate that increase in pH from 5.5 to 7.5 causes a sharp decrease in N-Pyr. These results support the data of Mirvish (1970, 1975); Ender and Ceh (1971); Sander et al. (1972) and others who contend that increase in amine basicity reduces the degree of nitrosation. It was anticipated that dextrose would be more inhibitory at the higher pH since carbonyl amine interaction occurs more readily at alkaline pH than in acid.

Inhibition of N-Nitrosopyrrolidine Formation in Bacon.

Analysis by extraction and GLC-MS. Analysis of bacon for N-Pyr by extraction, purification and GLC-MS revealed data dimilar to those discussed for the model system. Results in Table III indicate that all compounds studied were very effective in reducing N-Pyr formation. A mixture of cysteine and propyl gallate was most effective in reducing N-Pyr formation, confirming results by Sen et al. (1976), but the bacon was of unacceptable quality because of discoloration.

Analysis by direct-sampling-GLC-MS. Apparent N-Pyr content in bacon was somewhat greater as determined by this method (Table IV) compared to analysis by the extraction method (Table III). This is explained partly by the superior recoveries of N-Pyr by this method and perhaps also due to the formation of the nitrosamine during direct sampling. The general effects of pH, inhibitors and heating time appear to be comparable to those obtained by extract analysis.

Except for the control which had no inhibitor, all samples would have been judged acceptable for N-Pyr content as measured by this method.

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TABLE II

INHIBITION OF N-NITROSOPYRROLIDINE BY VARIOUS ADDITIVES
IN MODEL SYSTEM DETERMINED BY GAS-LIQUID CHROMATOGRAPHY-MASS-SPECTROMETRY

pH	pH 5.5				pH 7.5		
Heating time	15 min		30 min		30 min		
Inhibition Conc. of N-Pyr	ug	%	ug	%	ug	%	% due to pH[2]
Control[1]	68.6	--	78.0	--	25.0	--	68.0
Dext.	18.1	73.6	22.6	71.0	12.6	49.6	44.3
Na Asc	10.3	85.0	13.2	83.0	9.3	62.5	29.3
DHA	14.7	78.5	14.0	82.0	7.3	70.8	48.0
Pyr. Ald.	28.7	59.2	29.8	61.8	8.9	64.2	70.0
Na Cl	65.1	5.2	72.8	6.7	21.3	14.8	70.8
Dext. + Na Asc	8.7	87.3	8.6	89.0	4.5	82.1	47.7
DHA + Pyr. Ald.	N.D.	N.D.	15.0	80.7	4.1	83.4	72.5
Pro. Gal + Cyt.HCl	N.D.	N.D.	6.2	92.0	1.2	94.9	79.5
Dext. + Na Asc + DHA	6.1	89.9	8.9	89.5	5.0	80.1	44.3
Dext. + Na Asc + Pyr. Ald. + DHA	N.D.	N.D.	8.6	88.9	3.2	87.0	62.5

[1] Control: Equal parts of 1M $NaNO_2$ and 1M L-Proline: for all other samples, 1 mL of additives were mixed with 2 mL of control sample and diluted to 7.5 mL with the appropriate buffer.

[2] % Reduction in N-nitrosopyrrolidine compared to pH 5.5 buffer samples.

DHA - Dihydroxyacetone; PA - Pyruvaldehyde; Pro. Gal. - Propyl Gallate; Cyt.HCl - Cysteine. Hcl.

TABLE III

INHIBITION OF N-NITROSOPYRROLIDINE BY VARIOUS ADDITIVES IN BACON AS DETERMINED BY GAS-LIQUID CHROMATOGRAPHY-MASS-SPECTROMETRY OF EXTRACTS[1]

	Normal Cured Bacon pH 5.5		Buffer pH 6.8 Cured Bacon		
	N-Pyr Con. ug/Kg(ppb)	% Inhibition	N-Pyr Con. ug/Kg(ppb)	% Inhibition	% Reduction[3] due to pH
Control[2]	40.2	--	33.6	--	16.4
Dextrose (2%)	12.4	69.2	8.0	76.2	35.5
Dextrose (2%) Sodium Ascorbate (550 ppm)	7.5	81.3	6.4	80.9	14.7
Dihydroxyacetone Pyruvaldehyde (500 ppm)	3.8	90.5	1.7	94.8	54.3
Cysteine.HCl Propyl Gallate (500 ppm)	2.2	94.5	1.5	95.5	32.8

[1]Bacon Sample size 100g cooked weight.

[2]Control-120 ppm $NaNO_2$ and 2% NaCl.

[3]% Reduction in N-nitrosopyrrolidine compared to normal cured bacon.

TABLE IV

INHIBITION OF N-NITROSOPYRROLIDINE BY VARIOUS ADDITIVES IN BACON[1] AS DETERMINED BY DIRECT SAMPLING GAS-LIQUID CHROMATOGRAPHY-MASS-SPECTROMETRY

	Normal Cured Bacon pH 5.5		Buffer pH 6.8 Cured Bacon		
	N-Pyr Con ug/Kg(ppb)	% Inhibition	N-Pyr Con ug/Kg(ppb)	% Inhibition	% Reduction[3] due to pH
Control[2]	46.0	--	42.0	--	8.7
Dextrose (2%)	8.0	82.0	3.0	92.6	62.5
Dextrose (2%) Sodium Ascorbate (550 ppm)	10.0	79.3	3.0	92.6	70.0
Dihydroxyacetone (500 ppm) Pyruvaldehyde (500 ppm)	15.0	67.4	2.0	95.4	86.6
Cysteine.HCl (500 ppm) Propyl Gallate (500 ppm)	16.0	65.2	12.0	71.4	25.0

[1]500 mg of bacon was analyzed.

[2]Control-120 ppm $NaNO_2$ and 2% NaCl.

[3] % Reduction in N-nitrosopyrrolidine compared to normal cured bacon.