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MAIN SUBJECT HEADING:

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ANALYTICS	HUMAN EFFECTS	ANIMAL TOXICITY	WORKPLACE PRACTICES- ENGINEERING CONTROLS	MISCELLANEOUS

SECONDARY SUBJECT HEADINGS: AN HU AT IH M

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Transportation/Handling/
Storage/Labeling

*Not
Relevant*

MR 427

EFFECT OF FRYING AND OTHER COOKING CONDITIONS ON NITROSOPYRROLIDINE FORMATION IN BACON

INTRODUCTION

RECENT SURVEYS have demonstrated that the presence of nitrosamines in very low concentrations in various types of cured meat products occurs in a random manner (Fazio et al., 1971a; Sen, 1972; Wasserman et al., 1972; Crosby et al., 1972; USDA, 1972). In bacon, however, nitrosopyrrolidine (NO-Pyr) has been found and confirmed in a very high percentage of fried, ready-to-eat samples (Crosby et al., 1972; Sen et al., 1973; Fazio et al., 1973). This is the first instance of the consistent occurrence of a nitrosamine in a food product. Since approximately 1.5 billion pounds of bacon are consumed annually, the presence of a nitrosamine—even in low concentrations—is a matter for concern.

The origin of NO-Pyr in bacon is still unknown; however, it has been suggested that it could be produced by the decarboxylation of nitrosoproline, which is present in the tissues (Lijinsky and Epstein, 1970). We are reporting the effect of various cooking methods and frying temperatures on the formation of NO-Pyr in bacon and on a possible mechanism for the formation of NO-Pyr from a precursor in bacon.

EXPERIMENTAL

Decarboxylation of nitrosoproline

Powdered nitrosoproline (0.05g, 0.347 mmoles) was added to a 100 ml single neck round-bottom flask containing 25 ml silicone oil. The flask was heated at various temperatures with stirring for 10 min in a constant temperature oil bath. After cooling, the silicone oil reaction mixture was extracted in a 125 ml separatory funnel three times with 30 ml H₂O using a 3 min shake, and the extracts were combined in a 250 ml separatory funnel. The water extracts were in turn extracted three times with 100 ml CH₂Cl₂ with gentle shaking for 3 min and the CH₂Cl₂ layers combined. The CH₂Cl₂ extracts were washed by shaking for 1 min with 50 ml 6N HCl followed by 50 ml 5N NaOH then concentrated to 1 ml using a Kuderna-Danish apparatus equipped with a three-section Snyder column. Nitrosopyrrolidine was then determined by gas-liquid chromatography.

To test the efficiency of the extraction procedure 5 mg of NO-Pyr was added to 25 ml of silicone oil and carried through the entire process. A final recovery of 50% was achieved.

Commercial survey

One-pound packages of commercial bacon were purchased in local retail markets. The bacon was fried until medium-well done in a preheated Presto teflon coated electric frying pan Model PC04AT, requiring 5–6 min at a calibrated thermostat setting of 176.7°C. The bacon slices were turned at intervals to ensure even cooking. The drippings and a portion of the uncooked bacon were saved for subsequent nitrosamine analyses.

Cooking methods

In studying the various methods of cooking, bacon was fried for 6 min in a preheated frying pan at 176.7°C or for 9 min starting with a cold pan set at 176.7°C. Bacon was also broiled for 4.5 min on a rack set 5–6 in. below the heat source (ca 305°C) or baked for 13 min at 204°C in a shallow tray placed in the center of a preheated Tappan Visualite electric oven Model EOKLV-31-3C. Using a Westinghouse "baconer" Model HBB202A, bacon was draped over a teflon-coated heating element and cooked for 7 min with the thermostat dial set between "crisp" and "more crisp" (ca 241°C). Microwave cooking of the bacon was done with a Litton Industries microwave oven Model 550 for 1 min in a rectangular cardboard tray. In all cases bacon was cooked until medium well done.

Analytical procedures

The bacon samples were analyzed for the following nitrosamines: dimethylnitrosamine, methylethylnitrosamine and diethylnitrosamine, nitrosopiperidine, nitrosopyrrolidine and nitrosomorpholine using a modification of the method of Fazio et al. (1971b) for the multi-detection of volatile nitrosamines. Basic alumina (Camag, Brockman No. 1) washed with 5 to 15% CH₂Cl₂ in hexane and a CH₂Cl₂ elution solvent was used instead of silica gel in the column chromatographic clean-up procedure. The average recovery of nitrosopyrrolidine in an aliquot of the same sample with 20 ppb added was 70% for both the fried bacon and the fat drippings.

Gas-liquid chromatography

The nitrosamines were quantitated by gas-liquid chromatography using a Varian-Aerograph Model 1740-1 modified for use as an alkali flame ionization detector with a potassium chloride coated coil (Howard et al., 1970). The sample was separated on a 10 ft x 1/8 in. o.d. stainless steel column packed with 13% Carbowax 20M-TPA on 60–80 mesh Gas Chrom P equipped for on-column injection. The flow conditions used were: helium 50-, hydrogen 58- and air 200 ml/min. Hydrogen flow and, to a lesser extent, air flow were adjusted slightly from time to time in order to

maintain desired detector sensitivity. Electrometer range used was 10⁻¹² amp/mv injector port and detector temperatures were 185 and 220°C, respectively; column temperature was programmed from 105 to 200°C at 4°/min for the determination of the six nitrosamines. For the model system experiment involving the formation of NO-Pyr from NO-Pro an isothermal temperature of 170°C was used.

GLC-mass spectrometric analysis

For confirmation of the identity of the nitrosamines a Varian-Aerograph Model 1740-1 gas-chromatograph equipped with a 5 ft x 1/8 in. o.d. stainless steel column packed with 13% GC SE-30 on 100–120 mesh Varaport 30 was connected to a DuPont Model 21-492 mass spectrometer. The gas flow rates used were helium 38-, hydrogen 40-, and air 400 ml/min. The temperatures used were: column-programmed from 100 to 170°C at 6°/min; detector, 200°C; and injector port, 190°C. The column effluent was split 1:1 with one-half going into a flame ionization detector and the other half passed via an inlet line heated at 190°C into the mass spectrometer operated in the peak matching mode adjusted to a resolution of 1 in 12,000 as described by Dooley et al. (1973). The mass spectra were obtained at an ionizing voltage of 70 ev and an ion source temperature of 150°C. The mass-to-charge ratio (m/e of 100.06366) for NO-Pyr was determined using the m/e 99.99361 reference peak of perfluorokerosene by measuring the difference in m/e. The signal was recorded on both an oscilloscope and a recording oscillograph.

All samples having a gas chromatography alkali flame ionization detector response with the same retention time as an authentic sample of NO-Pyr were checked by mass spectrometry. In general, 10 ppb NO-Pyr or greater could be confirmed using this procedure. Where there was no positive confirmation due to an insufficient concentration of nitrosamine or the presence of interfering background components, values are presented as apparent NO-Pyr and are used for comparative purposes only, with the understanding that the material may not be NO-Pyr.

RESULTS & DISCUSSION

THE RESULTS of analyzing bacon from six processors for the six volatile nitrosamines are shown in Table 1. Only NO-Pyr was found in confirmable concentrations in bacon fried at 176.7°C and in drippings. Other workers (Sen et al., 1973; Fazio et al., 1973) have found a major portion of the NO-Pyr in the cooked-out fat. Our observation, and that of Fazio et al. (1973), that no NO-Pyr

present in this cooking stage. The fact greater for (NO-Pro) is decarboxylation NO-Pyr. Under which and establish a precursor heated for different to Figure 1. production which is a recommendation NO-Pyr heating was reducing the 185°C still Pyr/mole study indicating frying would precursor for The course of frying Bacon from from a normal process

Table 1—
in commerce

Samples	Res Nal (ppb)
A	
B	
C	
D	
E	
F	

^a Confirmed

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...ent in uncooked bacon suggests that
... compound is formed during the fry-
... stage.

The fact that the rate of nitrosation is
... for proline than pyrrolidine (Mir-
... 1973) suggests that nitrosoproline
... (NO-Pro) is formed first followed by
... carboxylation during frying to yield
... NO-Pyr. To determine the conditions
... under which decarboxylation does occur,
... and establish the possibility of NO-Pro as
... precursor of NO-Pyr, NO-Pro was
... heated for 10 min in silicone oil at
... different temperatures. The results, in
... Figure 1, indicate that the maximum
... production of NO-Pyr occurs at 185°C,
... which is approximately the temperature
... recommended for frying bacon. Little or
... NO-Pyr formed at 100°C even when
... heating was carried out for 20 min. Re-
... placing the heating time to 5 min at
... 185°C still produced more than 2g NO-
... Pyr/mole NO-Pro. This model system
... study indicates that the conditions of
... frying would be important if NO-Pro is a
... precursor for NO-Pyr in bacon.

The correlation of time and tempera-
... ture of frying with the amount of NO-Pyr
... formed in bacon is shown in Table 2.
... Bacon from two bellies was obtained
... from a normal production run of a local
... meat processor to minimize differences

... due to composition or processing condi-
... tions. Samples from one belly, immedi-
... ately after production, were fried at
... various time and temperature combina-
... tions until they were medium well done,
... or they were fried for 10 min at each of
... several temperatures. When bacon was

... fried to a visually determined degree of
... medium well done, increasing the tem-
... perature from 99–204°C required times
... varying from 105 to 4 min, respectively.
... No NO-Pyr was formed at 99°C, but
... higher temperatures produced increasing
... concentrations, reaching a level of 17
... µg/kg (ppb) at 204°C. Bacon fried for 10
... min at 99°C was still raw and contained
... no NO-Pyr. Increasing the temperature, at
... a constant exposure of 10 min resulted in
... darker, crisper bacon with formation of
... increasing concentrations of NO-Pyr. The
... bacon fried at either 99° or 204°C for 10
... min would be unacceptable to most
... people on the basis of sensory quality.

The effect of the age of the bacon was
... also investigated by repeating the tests
... with the bacon from the second belly
... stored at 1.7°C for 2 wk. Although the
... concentrations of NO-Pyr formed after 2
... wk storage appear to be somewhat lower
... than the amounts found in fresh bacon, it
... is not possible at this time to draw any
... conclusions from the effect of storage.

Even though bacon is usually prepared
... by pan frying, other methods of cooking
... have been recommended. A number of
... processors include directions on the pack-
... age for baking or broiling bacon. The
... results of an investigation of the effects
... of various cooking methods on NO-Pyr
... formation in bacon prepared until medi-
... um well done are shown in Table 3. Our
... standard frying procedure again produced
... high yields of NO-Pyr; essentially none
... was formed with microwave cooking.
... This may be the result of the very short
... exposure time of bacon to heat in the
... microwave oven. Our data confirmed the
... results recently reported by Herring
... (1973): Baking, broiling and the "bacon-
... er" produced variable quantities of NO-
... Pyr in the bacon. However, it appears
... that the "baconer" may produce slightly

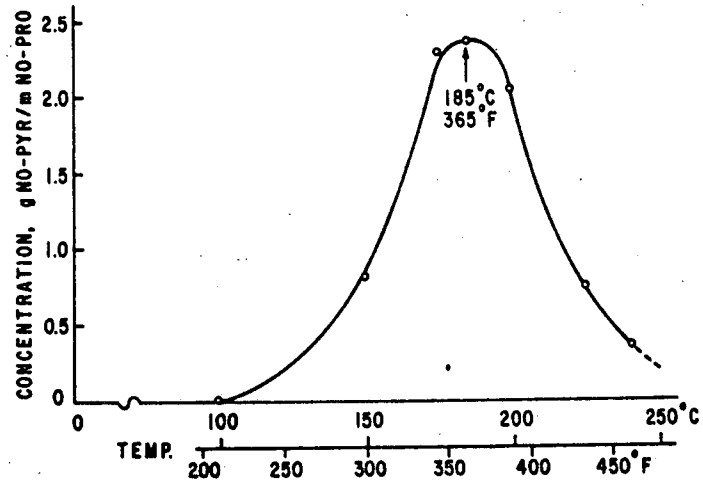


Fig. 1—Decarboxylation of nitrosoproline (NO-Pro) to nitrosopyrrolidine (NO-Pyr) in a model system.

Table 1—Nitrosopyrrolidine (NO-Pyr) found in commercial bacon

Samples	Residual NaNO ₂ (ppm)	NO-Pyr, ppb (Uncorr) ^a		
		Raw	Fried	Drippings
A	89	0	11	16
B	96	0	13	39
C	97	0	11	24
D	39	0	19	23
E	53	0	29	19
F	—	0	38	32

^a Confirmed by M.S.

Table 3—Effect of cooking methods on nitrosopyrrolidine (NO-Pyr) formation in bacon

Cooking method	NO-Pyr, ppb (Uncorr)		
	1	2	3
Raw	0	0	—
Fried (cold pan)	9	17 ^a	11 ^a
Fried (hot pan)	5	20 ^a	19 ^a
Bake	35 ^a	13 ^a	12 ^a
Broil	12 ^a	12	14 ^a
Baconer	9	7 ^a	16 ^a
Microwave	2	0	3

^a Confirmed by M.S.

Table 2—Effect of frying conditions on nitrosopyrrolidine (NO-Pyr) formation in bacon

Frying conditions				NO-Pyr, ppb (uncorr)	
Temperature (°C)	Temperature (°F)	Time (min)	Degree	Storage time—wk @ 1.7°C	Storage time—wk @ 1.7°C
99	210	105	Med. well	0	0
135	275	30	Med. well	8	5
176.7	350	6	Med. well	10 ^a	6
204	400	4	Med. well	17 ^a	7
99	210	10	Raw	0	0
135	275	10	V. light	0	0
176.7	350	10	Well	15	7
204	400	10	Burned	19 ^a	16 ^a

^a Confirmed by M.S.

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less NO-Pyr as shown in samples 1 and 2.

It is important to note that the 35 ppb value reported for the first baked bacon sample was confirmed by high resolution mass spectrometry. However, the mass spectrometer response for NO-Pyr, on a semiquantitative basis, was not as great as expected for this concentration of nitrosamine. It is possible that the gas chromatograph was responding to an additional component in the bacon sample which eluted at the same time as NO-Pyr. This points up the need for specific confirmation procedures to avoid erroneous interpretations of nitrosamine detection data alone.

(Precautions should be exercised in the handling of nitrosamines, since they are potential carcinogens.)

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