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Potential Precursors of *N*-Nitrosopyrrolidine in Bacon and Other Fried Foods

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The possibility of producing *N*-nitrosopyrrolidine from several compounds in a system designed to simulate the frying of fatty foods containing small amounts of water was investigated. With the exception of tests on *N*-nitrosoproline, equimolar amounts of the compound to be tested and sodium nitrite were added to the system prior to heating. With a heat-up time of 10 min plus 10 min at 170°, *N*-nitrosopyrrolidine was produced from *N*-nitrosoproline, pyrrolidine, spermidine, proline, and putrescine in yields of 2.6, 1.0, 1.0, 0.4, and 0.04% theoretical, respectively. *N*-Nitrosopyrrolidine was not produced from glutamine, glutamic acid, and hydroxyproline.

N-Nitrosopyrrolidine (NPy), a carcinogen, has been isolated from fried bacon as reported in FDA Papers (1972), by Crosby *et al.* (1972), and by Sen *et al.* (1973). The first reference indicates that preliminary studies on four different brands of bacon showed that NPy was formed when the bacon was cooked in a conventional manner but was not present in raw bacon. Levels ranged from 30 to 160 ppb in the cooked product. Crosby *et al.* found NPy in 13 of 24 samples of fried bacon of various origins. In the positive samples, concentrations ranged from a trace (<1 ppb) to 40 ppb. In addition to NPy, Crosby *et al.* also reported finding the *N*-nitroso derivatives of dimethylamine, diethylamine, and piperidine in some samples of fried bacon. In 8 of 16 samples of fried and raw bacon, Sen *et al.* found NPy in concentrations of 4 to 25 ppb and dimethylnitrosamine in six samples in concentrations of 2 to 30 ppb. NPy was not found in any uncooked samples but dimethylnitrosamine was found in one.

Lijinsky and Epstein (1970) speculated that the cooking of foods might result in the formation of certain secondary amines. Pyrolysis of protein could yield proline. Putrescine, to the extent that it might be present in a whole-some food product, could be converted to pyrrolidine. In the presence of nitrite, such secondary amines may be nitrosated under certain conditions to form the corresponding *N*-nitroso derivative.

The purpose of this investigation was to evaluate some possible precursors for the production of NPy under simulated conditions of pan-frying meat products such as bacon.

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EXPERIMENTAL PROCEDURES

Heating System. To simulate the physical conditions present in a system such as bacon being pan-fried at a pan temperature of 170°, a 200-ml balloon flask was fitted with a reflux condenser. Into the flask was introduced 100 ml of steam-stripped Wesson oil (processed soybean and cottonseed oil), 1 ml of water, a boiling chip, an internal standard (40 mg of methyl myristate), and 0.005 mol of the compound to be tested. With the exception of tests for the production of *N*-nitrosopyrrolidine from *N*-nitrosoproline, 0.005 mol of sodium nitrite was also added to the system. The balloon flask was immersed in a bath of silicone oil heated on a hot plate. The temperature of the contents of the flask was brought to 170° in about 10 min and held at 170° for an additional 10 min. During the heating period the water in the system was under constant

reflux and provided a "spattering" action within the system.

Distillation and Extraction. Following the heating process, contents of the balloon flask were transferred to a steam distillation apparatus and distilled with steam at atmospheric pressure until 150 ml of distillate had been collected. The distillate was saturated with sodium sulfate, transferred to a 500-ml separatory funnel, and extracted with two 100-ml portions of redistilled dichloromethane. The dichloromethane extract was dried over anhydrous sodium sulfate and concentrated to a volume of 10 ml by means of a rotary evaporator. The extract was further concentrated under a stream of nitrogen to a volume of 0.05 ml in a Chromaflex sample tube (Kontes no. 422560).

Gas Chromatographic Conditions. A Varian gas chromatograph (series 1400) equipped with a flame ionization detector was used in all analyses. A stainless steel column (⅛ in. o.d. × 10 ft) packed with 5% Carbowax 20M on Chromosorb G was employed. Temperatures of the injector, column, and detector were 200°, 180°, and 290°, respectively. The flow rate of the carrier gas was 25.5 ml/min.

Identification of NPy. NPy was identified by coincidence of gas chromatographic retention time with the authentic compound and by mass spectrometry. The mass spectrometer was an Atlas CH-4 operated under the following conditions: filament current, 20 μA; electron voltage, 70 eV; accelerator voltage, 3 kV. The effluent of a gas chromatograph, operated under the previously described conditions, was introduced into the mass spectrometer ion source through a Carle valve which was used to shunt excess solvent to the atmosphere. Spectra of authentic NPy and unknowns were compared for the purpose of identification.

Estimation of NPy Produced. An internal standard, 40 mg of methyl myristate, was added to the system each time prior to heating. A factor for relating the weight and peak area of the internal standard to the weight and peak area of NPy was determined from three runs in which authentic NPy also was added to the system and carried through the entire procedure along with the internal standard. The addition of an internal standard at the start of the procedure provided a means to compensate for losses of NPy that undoubtedly occurred during distillation, extraction, and concentration without the necessity of determining percent recovery.

Compounds Tested. The compounds tested were as follows: *N*-nitrosoproline, synthesized in our laboratory; pyrrolidine, Aldrich; spermidine-HCl, National Biochemicals; proline, J. T. Baker; putrescine, Pfaltz and Bauer; glutamic acid, Eastman; glutamine, J. T. Baker; hydroxyproline, Eastman. Compounds were tested for purity by thin-layer chromatography of the compound and by gas chromatography of a dichloromethane extract of the compound. Neither NPy nor pyrrolidine was found as impurities in any of the compounds.

RESULTS AND DISCUSSION

The compounds tested for the production of NPy are listed in order of decreasing yield in Table I. NPy was not produced from glutamic acid, glutamine, or hydroxyproline. Hydroxyproline may have yielded hydroxy-*N*-nitrosopyrrolidine, but we did not separate and identify it under the gas chromatographic and mass spectral conditions employed.

N-Nitrosoproline, pyrrolidine, spermidine, proline, and putrescine all yielded quantities of NPy that were easily detected and identified.

Table I. *N*-Nitrosopyrrolidine Production at 170° in an Oil–Water System

Compound tested	*N*-Nitrosopyrrolidine produced[a,b]	
	mg	% theoretical yield
N-Nitrosoproline	13	2.6
Pyrrolidine	5	1.0
Spermidine	5	1.0
Proline	2	0.4
Putrescine	0.2	0.04
Glutamic acid	0	0
Glutamine	0	0
Hydroxyproline	0	0

[a] From 0.005 mol of starting material and 0.005 mol of sodium nitrite (except sodium nitrite not used with *N*-nitrosoproline). [b] Data reproducible to approximately ±20% between replicate experiments.

Proline is a natural component of many foods and is especially abundant in connective tissue. Although most of the proline is found within protein molecules, hydrolysis or pyrolysis of protein under conditions of heating is likely to yield free proline (Lijinsky and Epstein, 1970). *N*-Nitrosoproline has not been reported as a component of cured meats, but it is not unlikely that this compound could occur at low levels, since proline and nitrite are present in the system.

Spermidine has been quantitated in a number of mammalian tissues, as reported in a review by Tabor and Tabor (1964). Levels of spermidine reported in muscle and various other tissues ranged from 0.1 to 8.6 μmol/g. Muscle tissue appears to contain the lowest levels of spermidine. Cohen (1971) points to the ubiquity of putrescine (the precursor of spermidine) and spermidine in both procaryotic and eucaryotic cells. Cohen further observed that only a few organisms are unable to synthesize putrescine and that such organisms grow slowly or not at all when it is not available to them. Thus, one can conclude that pork or other meat products will contain low levels of spermidine and putrescine as natural components. Microbial growth in meat prior to cooking and consumption could result in significantly higher levels of polyamines, since many microorganisms are capable of producing these compounds in relative abundance (Cohen, 1971).

It is of interest to note that spermidine yields as much NPy as pyrrolidine itself. This observation further serves to demonstrate that the formation of NPy from starting compounds other than pyrrolidine did not result from traces of pyrrolidine as an impurity in the compounds.

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