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# COMMUNICATIONS

## *N*-Nitrosopyrrolidine Collected as a Volatile during Heat-Induced Formation in Nitrite-Containing Pork

Ground pork belly containing 200 ppm of sodium nitrite was heated to 177 °C and the condensate was collected and analyzed for *N*-nitrosopyrrolidine using gas chromatography and mass spectrometry. Nitrosopyrrolidine in an amount equivalent to 19 ppb (average) in the initial ground pork sample was detected in the condensate. When the total amount of nitrosopyrrolidine in the cooked pork, rendered fat, and condensate was determined, it was found that 20–40% of the total nitrosopyrrolidine produced was given off as a volatile during the cooking process. The amount of nitrosopyrrolidine produced was increased when 0.1% putrescine was added to the ground pork prior to heating. The addition of an equal amount of proline resulted in the production of even higher levels of nitrosopyrrolidine.

The occurrence of *N*-nitrosopyrrolidine in cooked bacon at levels up to 108 ppb has been reported (Crosby et al., 1972; Fazio et al., 1973; Sen et al., 1973). Proline, putrescine, and collagen, all naturally occurring in pork, have been shown to react with nitrite at high temperatures to produce nitrosopyrrolidine (Bills et al., 1973; Huxel et al., 1974; Gray and Dugan, 1975). Nitrosoproline, a compound which decarboxylates at bacon-frying temperature to yield nitrosopyrrolidine, has recently been isolated from raw bacon (Kushnir et al., 1975). Model systems consisting of heated oil (Bills et al., 1973; Pensabene et al., 1974; Kushnir et al., 1975) or high-temperature dry systems (Huxel et al., 1974; Gray and Dugan, 1975) have been used, rather than actual meat systems, to evaluate and compare various amines as nitrosopyrrolidine precursors. In addition, the study of nitrosopyrrolidine formation in heated bacon has involved analysis of cooked bacon and the rendered fat, but the possibility of nitrosopyrrolidine being given off as a volatile during the cooking process has not been investigated.

The purpose of this investigation was to compare proline and putrescine as nitrosopyrrolidine precursors in heated nitrite-containing pork and to determine the relative amount of nitrosopyrrolidine given off as a volatile during the cooking operation.

EXPERIMENTAL PROCEDURES

**Sample Preparation.** Two matched pork bellies were obtained fresh from a commercial processor. To ensure a homogenous sample for cooking experiments, the pork was ground, mixed, and reground with a Hobart meat grinder fitted with a $^3/_8$ in. plate. The ground pork was stored at −23 °C until used in the experiments. In preparation for cooking, sodium nitrite was mixed into the pork at a level of 0.02% (200 ppm). Putrescine dihydrochloride (K and K Laboratories, Inc.) at levels of 0.10 and 0.40% (calculated as putrescine) was incorporated in the same manner into portions of the pork containing 200 ppm of added sodium nitrite. Proline at a level of 0.10% was mixed into other portions of pork containing 200 ppm of added sodium nitrite. The pork was reground with a meat grinder at least three times to ensure adequate mixing of the added compounds.

**Cooking Method.** One hundred grams of the material was placed in a 500-ml boiling flask which was fitted with a distillation head, condenser, and receiver. Thermocouple

leads were placed through the distillation head into the ground pork. Heat was applied with a prewarmed heating mantle which was removed when the temperature of the meat reached 177 °C, the recommended temperature for frying bacon. The heating process normally took about 12 min. During the cooking process, 30–35 ml of condensate was collected.

**Analysis for Total Amount of Nitrosopyrrolidine.** The cooking apparatus was disassembled after cooling and the contents of the cooking flask were transferred to a Waring Blendor jar with 100 ml of pH 7.0 citrate–phosphate buffer (McIlvaine, 1921). Two grams of ammonium sulfamate was added, and the contents were blended for approximately 15 s. The blended material was transferred with another 100 ml of buffer to a 1-l. boiling flask. The distillation head and condenser were rinsed with buffer and this rinse was added to the boiling flask along with the condensate obtained during cooking. Approximately 10 ml of redistilled dichloromethane was used to rinse the cooking flask and this was also added to the boiling flask.

The material in the boiling flask was steam distilled until 200 ml of distillate was collected. The distillate was saturated with sodium sulfate, acidified to a pH of approximately 1 with HCl, and extracted twice with 200-ml portions of dichloromethane. The extract was dried over sodium sulfate and concentrated to 4 ml in a Kuderna-Danish apparatus. The concentrator tube was then fitted with a micro-Snyder column and the sample was further concentrated with a slow stream of nitrogen gas to a volume of 2 ml. One milliliter of Nanograde hexane (Mallinckrodt) was added and the concentration was continued to a volume of 0.2–0.3 ml.

Percent recovery was estimated by adding 100 μg of nitrosopyrrolidine to samples of cooked pork that contained no added putrescine or sodium nitrite. Two samples spiked in this manner were analyzed according to the above procedure. The analysis of raw pork was carried out by blending 100 g of pork containing 0.02% nitrite and 0.40% putrescine with 2 g of ammonium sulfamate and 200 ml of buffer. The blended material was then steam distilled and analyzed in the same manner as the cooked samples.

**Analysis of Condensate Only.** After the pork sample was cooked, the distillation head and condenser were rinsed with distilled water which was combined with the cooking condensate. After acidifying with HCl and saturating with sodium sulfate, the cooking condensate was extracted twice with equal volumes of dichloromethane. The extract was dried over sodium sulfate and concentrated to 1 ml in a Kuderna-Danish apparatus.

Concentrated extracts of the condensate were subjected to a column chromatographic clean-up procedure similar to that described by Pensabene et al. (1974). Five grams of 80–200 mesh absorptive alumina (Fisher Scientific Co.) was placed in a 10 × 200 mm glass column plugged with glass wool. The alumina was washed with 30 ml of hexane, and the 1-ml dichloromethane concentrate was then placed on the column. Five milliliters of hexane was used to rinse the concentrator tube and wash the sample onto the column. One hundred milliliters of hexane containing 10% dichloromethane was passed through the column and discarded and nitrosopyrrolidine was then eluted with 100 ml of dichloromethane. The dichloromethane eluent was concentrated to 0.2–0.3 ml in the manner described above. Recovery was estimated using 50 μg of nitrosopyrrolidine.

**Gas Chromatography.** A Varian 1400 gas chromatograph (GC) equipped with a flame ionization detector and a stainless steel column (12 ft × 0.13 in. i.d.) packed with 7% Carbowax 20M on Chromosorb G was used to analyze the concentrated extracts for nitrosopyrrolidine. Operating conditions were as follows: injector temperature, 190 °C; oven temperature, 170 °C; detector temperature, 280 °C; flow of nitrogen carrier gas, 30 ml/min. Nitrosopyrrolidine was quantitated with the aid of an internal standard, ethyl undecanoate, added prior to the final concentration of the dichloromethane extract.

Table I. Nitrosopyrrolidine in the Condensate of Pork Heated to 177 °C

| Added sodium nitrite, % | Added amine, % | Nitrosopyrrolidine produced,[a,b] trials A | B | Av |
|---|---|---|---|---|
| 0.00 | | 0 | 0 | 0 |
| 0.02 | | 23 | 15 | 19 |
| 0.02 | 0.10, putrescine | 89 | 31 | 60 |
| 0.02 | 0.40, putrescine | 151 | 127 | 139 |
| 0.02 | 0.10, proline | 536 | 330 | 433 |

[a] Corrected for 81% recovery. [b] Units are parts per billion relative to the original 100-g pork sample prior to heating.

**Gas Chromatography–Mass Spectrometry.** A Finnigan Model 1015C quadrapole mass spectrometer (MS) in combination with a Varian 1400 GC was used to identify nitrosopyrrolidine. In some cases the extracts were further concentrated to 0.05 ml before MS analysis. Samples were introduced into the MS through the GC operated under the conditions described, but with helium as the carrier gas. Operating conditions for the MS were as follows: filament current, 400 μA; electron voltage, 70 eV; analyzer pressure, $5 \times 10^{-7}$ Torr; scan time, 1 s. Identification of nitrosopyrrolidine was confirmed by comparing the spectra obtained to that of standard nitrosopyrrolidine.

**Safety Precautions.** Nitrosopyrrolidine is a potent carcinogen and care should be exercised in the handling of this compound.

## RESULTS AND DISCUSSION

The amount of nitrosopyrrolidine in the condensate of various cooked pork samples is shown in Table I. Without added nitrite, no peaks were observed on the gas chromatogram at the retention time of nitrosopyrrolidine. The column chromatographic clean-up procedure removed an interfering compound, identified as 2-tridecanone, that eluted at approximately the same retention time as nitrosopyrrolidine. The levels of nitrosopyrrolidine collected from samples cooked with nitrite but without added amine were low and could not be confirmed by MS. Increased amounts of nitrosopyrrolidine in the condensate were detected by GC and confirmed by MS when the pork contained added nitrite and proline or putrescine.

Putrescine has been reported in fresh and cured pork (Spinelli et al., 1974) and levels could increase with aging as in the case of dry sausage (Dierick et al., 1974). The lowest level of putrescine used in this study was, however, about 30 times higher than the maximum level found in pork (Spinelli et al., 1974). When equal amounts of proline and putrescine are compared, proline results in a much higher level of nitrosopyrrolidine in the condensate. Although putrescine may make a contribution to the amount of nitrosopyrrolidine formed during cooking of bacon, our conclusion supports the findings obtained with hot oil systems in which proline or nitrosoproline were determined on the basis of yield to be more likely precursors of nitrosopyrrolidine (Bills et al., 1973; Kushnir et al., 1975).

The total amount of nitrosopyrrolidine produced from heated pork was estimated by analyzing the combined cooked pork, cooked-out fat, and condensate. Recovery of nitrosopyrrolidine from the combined fractions was 30%, which is similar to the recoveries reported by others using steam distillation (Crosby et al., 1972; Telling et al., 1971). Nitrosopyrrolidine was not detected in uncooked samples of pork containing nitrite and putrescine. When corrected for recovery, three samples containing 0.02% sodium nitrite produced an average of 109 ppb of nitrosopyrrolidine. Three samples cooked with 0.02% sodium nitrite and 0.40% putrescine averaged 321 ppb. When relating the total amount of nitrosopyrrolidine produced to that determined in only the condensate of similar samples (Table I), it appears that approximately 20–40% of the nitrosopyrrolidine formed was given off as a volatile. Thus, it is likely that during the heat-induced formation of nitrosopyrrolidine in bacon, a significant portion of the nitrosamine may be volatilized from the product and not detected in the cooked bacon or rendered fat.

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# Rapid Analysis of Potassium Sorbate in Dried Prunes by Ultraviolet or Colorimetric Procedures

A rapid analysis procedure for the determination of potassium sorbate in dried prunes was developed. It utilizes a rapid double extraction procedure which gives high extraction efficiency for potassium sorbate in dried prunes. This procedure also eliminates most of the interfering compounds which are found in dried prunes and cause high blank values with many present potassium sorbate analysis procedures. The extract can be read directly by ultraviolet (uv) instrumentation or by a colorimetric procedure. The colorimetric procedure allows small laboratories with limited resources to run potassium sorbate analysis by this extraction procedure.

It has been previously determined at this laboratory that from 200 to 600 ppm of potassium sorbate will preserve processed dried prunes, depending on their moisture content. Recently, concern in the United States and other countries over the increased use of food additives and their safety has led many countries to place rigid controls on their use, including bans on certain additives. This development has led to the increased need for new rapid and accurate methods of analysis. In the United States potassium sorbate is classified as GRAS (generally recognized as safe). In other countries the tolerance ranges from 0 to 1000 ppm (Dada, 1975).

The analysis of potassium sorbate in dried prunes is complicated by the large quantities of sugars, acids, and other naturally occurring compounds. These compounds and those formed during dehydration cause high blank values in many currently used ultraviolet or colorimetric procedures. This inaccuracy can be reduced by determining a blank value on untreated prunes from the same lot, but these are not always available and, in any event, lead to an increase in the analysis time. Several analytical procedures are currently used by prune processors (Stafford and Nury, 1969); one is rapid with a high blank value, and the others are slow and time consuming. Because of these factors and the increased necessity for accurate and rapid potassium sorbate analysis, a new analytical procedure was developed. It utilizes a rapid double extraction procedure which gives high extraction efficiency for potassium sorbate and eliminates most of the interfering compounds in prunes. The extract can be read directly by uv instrumentation or analyzed by the slightly modified colorimetric procedure of Nury and Bolin (1962). The colorimetric method allows small laboratories with a minimum of equipment to run potassium sorbate analysis of prunes by this procedure.

EXPERIMENTAL SECTION

**Apparatus.** A Bausch and Lomb Spectronic 20 colorimeter was used for the colorimetric procedure. A Beckman DB spectrophotometer was used for the uv