

pterin-6-carboxylic acid (Table I).

These differences in kinetic behavior may be directly related to the arrangement of the subunit globules described in Nagler and Vartanyan's molecular model (Nagler and Vartanyan, 1976) (Figure 3). For optimum catalytic efficiency, the electron transport chain at the active site of the enzyme requires that the molybdenum- and FAD-containing globules must be within a favorable proximity to each other. This arrangement is in agreement with the recent magnetic interaction model for xanthine oxidase proposed by Barber et al. (1982). If the protein segments described in Nagler and Vartanyan's model are represented as segments of the enzyme's primary structure that closely interact with the amino acid side chains of the globules, then it is possible that these segments might have a more significant structure-to-function relationship to the intact enzyme molecule than realized heretofore. The greater catalytic efficiency and higher affinity for both substrates and inhibitors observed in this study with the NPD XO preparation support this conclusion.

**Registry No.** Xanthine oxidase, 9002-17-9; P6C, 948-60-7; allopurinol, 315-30-0; folic acid, 59-30-3; xanthine, 69-89-6; hypoxanthine, 68-94-0.

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## Organic Nitrates and Nitriles in the Volatiles of Cooked Cured Pork

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Headspace volatiles from slices of cooked cured pork loin containing residual sodium nitrite concentrations between 63 and 1057  $\mu\text{g/g}$  were collected on Tenax GC and analyzed by gas chromatography-mass spectrometry. Benzonitrile, phenylacetoneitrile, and four alkyl nitrates were found in the headspace volatiles of all the bacon samples. Several alkanenitriles were also found in samples with high residual nitrite levels. All these nitrogen compounds were also found in a volatile extract of high residual nitrite bacon prepared by steam distillation-solvent extraction. Possible mechanisms for the formation of these compounds from reactions between lipid and sodium nitrite and the importance in cured flavor are discussed.

A number of studies using sensory panels have clearly demonstrated that sodium nitrite is essential for the formation of the characteristic flavor of cured meats (MacDougall et al., 1975; Gray et al., 1981). However, there are few reports on flavor volatiles from cured meats, a marked contrast to the wealth of literature relating to cooked beef flavor (MacLeod and Seyyedain-Ardebili, 1981). Recently, benzonitrile, phenylacetoneitrile, and several alkyl nitrates were tentatively identified in the headspace volatiles of cured pork (Mottram et al., 1984). None of the compounds

were found in control samples of uncured pork or in nitrite-free salt pork.

This paper reports further work to establish the extent of formation of nitriles and nitrates in cooked cured pork and to determine if the amount of sodium nitrite used in the cure affects the concentration of these compounds in the headspace volatiles.

#### MATERIALS AND METHODS

**Preparation of Cured Pork.** Slices (3 mm thick) of fresh pork loin were randomly distributed between treatment groups. Curing was carried out using a slice-cure based on the method described by Holmes (1960) using brines containing 200 g/L sodium chloride and so-

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dium nitrite concentrations between 650 and 6160 mg/L. Slices were immersed in brine for 2 min, allowed to drain for 10 min, and then partially dried in an oven at 36 °C for 5 min. A nitrite-free salt pork was prepared and slices of untreated pork were also taken.

Samples of minced *M. longissimus dorsi* from each treatment were analyzed for sodium nitrite and sodium chloride by using methods described previously (Mottram et al., 1984).

**Collection of Headspace Volatiles.** Lean minced meat from each treatment was cooked for 20 min, on a boiling water bath. Three replicate samples (50 g) of cooked meat were placed in 250-mL conical flasks, each equipped with a Dreschel bottle head and a glass tube packed with 100 mg of porous polymer absorbent (Tenax GC) attached via a screw joint on the head outlet (Mottram et al., 1984). Headspace volatiles from the flasks, maintained at 60 °C, were aspirated by nitrogen (50 mL/min) onto the Tenax traps. The collected volatiles were thermally desorbed from the Tenax at 250 °C under a flow of nitrogen and were condensed in a cooled capillary tube held at -100 °C. Headspace volatiles from three replicate samples were all desorbed into the same capillary; solvent (20  $\mu$ L) was added and the extract obtained was analyzed by combined gas chromatography-mass spectrometry (GC-MS).

**Isolation of Volatiles by Steam Distillation.** Minced lean meat (200 g) from the high-nitrite cure (6160 mg/L sodium nitrite) was homogenized in distilled water (1.5 L). Volatiles were extracted for 3 h by using simultaneous steam distillation-solvent extraction in a Likens-Nickerson apparatus with alkene-free, redistilled, diethyl ether (20 mL) as the solvent (Mottram and Puckey, 1978). After being dried with anhydrous sodium sulfate, the extract was reduced in volume to 1 mL in a Kuderna-Danish evaporator, followed by further concentration to 200  $\mu$ L by evaporation with a slow flow of nitrogen.

**Gas Chromatography-Mass Spectrometry.** A Finnigan 4000 GC-MS coupled to an Inco 2100 data system was used to analyze the extracts. A fused silica capillary column (50 m  $\times$  0.32 mm i.d.) coated with CP Wax 51 (Chrompak, Ltd.) was connected directly to the ion source. The column temperature was held at 60 °C for 5 min and then programmed at 4 °C/min to 190 °C. Helium was used as the carrier gas. The ion source temperature was 250 °C and the electron energy 40 eV. The GC-MS was operated in a continuous scan mode with a scan time of 1 s over a mass range of 33-400 amu. Replicate analyses of each treatment were carried out, and the area of a characteristic ion in each compound of interest was measured and quantified by comparison with the authentic compound.

**Reference Compounds.** Aryl- and alkanenitriles were obtained commercially. Alkyl nitrates were prepared by the reaction of silver nitrate with 1-bromoalkanes in acetonitrile (Ferris et al., 1953).

## RESULTS AND DISCUSSION

The residual sodium nitrite concentrations in the lean of the four cured porks ranged from 63 to 1057  $\mu$ g/g (Table I). Typically British back bacon prepared by tank curing in the traditional Wiltshire or similar process would contain between 50 and 150  $\mu$ g/g sodium nitrite, the maximum permitted level in bacon in the United Kingdom being 200  $\mu$ g/g.

The headspace volatiles from all the bacon samples contained benzonitrile, phenylacetonitrile, and the four alkyl nitrates that had been found previously. These were confirmed as pentyl, hexyl, heptyl, and octyl nitrates by

Table I. Concentrations of Nitriles and Nitrates in the Collected Headspace Volatiles of Cooked Bacons Containing Different Amounts of Residual Sodium Nitrite

compound	ion measured, m/e	quantity collected, ng/g of cooked meat, for bacons with residual NaNO <sub>2</sub> concns <sup>a</sup> of			
		63	132	223	1057
pentyl nitrate	46	0.2	1.6	1.6	0.3
hexyl nitrate	46	0.5	2.9	4.2	3.2
heptyl nitrate	46	0.1	0.8	0.3	0.1
octyl nitrate	46	1.5	3.4	1.9	0.4
benzonitrile	103	0.2	1.0	2.2	8.2
phenylacetonitrile	117	0.04	0.1	0.4	3.9
nonanenitrile	110	- <sup>b</sup>	-	1.0	1.8
decanenitrile	110	-	-	-	0.2
undecanenitrile	110	-	-	-	0.6
dodecanenitrile	110	-	-	-	0.1
tridecanenitrile	110	-	-	-	0.7

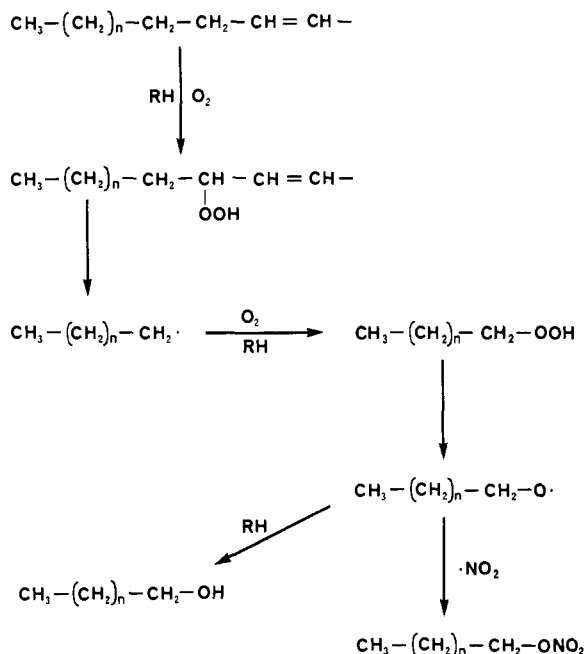
<sup>a</sup>  $\mu$ g/g, measured in uncooked lean tissue 3 days after curing. <sup>b</sup> Not found (detection limit approximately 0.01 ng/g of cooked meat).

comparison of their mass spectra and retention times with those of synthesised samples. In addition to benzonitrile and phenylacetonitrile, several alkanenitriles were found in the bacon with the highest residual nitrite levels (Table I). Nonane nitrile was also identified in the bacon containing 232  $\mu$ g/g residual sodium nitrite. As well as the nitriles listed in Table I, hexane- and octanenitrile were also found in the headspace volatiles from the high-nitrite bacon, but they eluted from the column on the tail of large octanal and decanal peaks and could not be accurately quantified. The higher molecular weight alkanenitriles were completely resolved from the corresponding aliphatic aldehydes. No trace of any of these nitrogen-containing compounds were found in the headspace volatiles of salt pork or untreated pork.

The low nitrite bacon contained smaller amounts of alkyl nitrates than the other samples, but otherwise there was no apparent relationship between residual nitrite concentration and the amounts of alkyl nitrates. However, benzonitrile and phenylacetonitrile both increased markedly with increasing nitrite concentrations, and the alkanenitriles were only found in bacons with higher nitrite levels.

A volatile extract of the bacon containing 1057  $\mu$ g/g residual nitrite was also prepared by steam distillation-solvent extraction in a Likens-Nickerson apparatus. The C<sub>5</sub> to C<sub>8</sub> alkyl nitrates, benzonitrile, and phenylacetonitrile were found in this extract, together with a series of alkanenitriles from hexanenitrile to hexadecanenitrile, with the later members of the series present in relatively large amounts. Since the organic nitrates and nitriles were detected by using two completely different methods of volatile extraction—steam distillation and headspace concentration—it can be assumed that these compounds are genuine volatile components of cured meat and are not artifacts of a particular extraction technique.

The aromas of alkyl nitrates and alkanenitriles were similar to the corresponding aliphatic aldehydes and alcohols, and a preliminary examination indicated that their odor thresholds were relatively high (greater than 1 part in 10<sup>6</sup> parts of water). Consequently, it is unlikely that at the levels detected these compounds will be important in the characteristic aroma of cured meats. Benzonitrile and phenylacetonitrile appear to have lower odor thresholds with almond-like aromas, very similar to that of benzaldehyde, and these compounds may contribute to cured meat aroma. However, this similarity to benzaldehyde, which is found in relatively large amounts in the



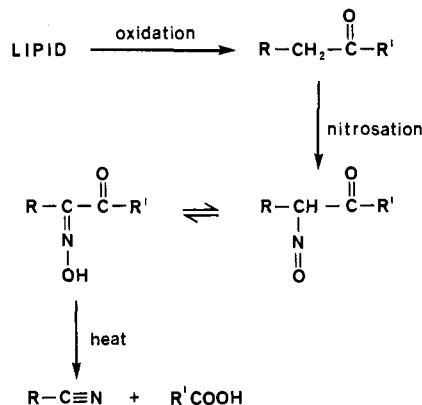
**Figure 1.** A possible mechanism for the formation of alkyl nitrates in cooked bacon.

headspace volatiles of all cooked meats (Mottram et al., 1982; Mottram and Edwards, 1983), suggests that it is unlikely that benzonitrile and phenylacetoneitrile alone are responsible for the aroma difference between cured and uncured meats.

Apart from the recent report from this laboratory (Mottram et al., 1984) none of these nitrogen compounds have been found previously in cooked meat volatiles. A recent paper by Ho et al. (1983) listed 135 volatile compounds found in fried bacon, but these included only one nitrile, 4-methylpentanenitrile, and an isocyanate, butyl isocyanate, both of which appeared to be minor components receiving no comment on their possible origin. Packed GC columns were used in this work and in earlier studies on cured flavor, whereas the present results were obtained by using fused silica capillary columns. The superior resolution and lack of adsorption on these capillary columns may explain why other studies have not found these nitriles and nitrates.

The most likely origin of these nitrogen compounds is from reaction between fatty acids in the lipid and sodium nitrite. Thermal oxidative decomposition of unsaturated lipids generally proceeds through free radical mechanisms, and alkyl nitrates could result from the participation of nitrous acid, or free radical species derived from nitrite, in these decomposition reactions. At ambient temperatures fatty acids react with nitrogen oxides to give various addition and oxidation products (Walters et al., 1979; Pryor et al., 1982). Nitrogen oxides, such as nitrogen dioxide, are known to be formed when cured meats are heated (Mottram and Puckey, 1978; Woolford et al., 1972), and their participation in the thermal oxidative degradation of lipids to give alkyl nitrates could be anticipated (Figure 1).

A mechanism for the formation of the nitriles involving nitrite is not readily obvious, but the long straight chain aliphatic nitriles must be derived from the fatty acids. Hexanenitrile has been reported to result from the thermal interaction of valine and the triglyceride tricaproin (Lien and Nawar, 1974), but nitrite was not involved and the nitrile was believed to be produced by the thermal decomposition of an intermediate amide. A possible alter-



**Figure 2.** A possible route for the formation nitriles in cooked bacon.

native route to nitriles involves the formation of an oxime and the subsequent thermal decomposition to the corresponding nitrile. Oximes can be formed by the C-nitrosation of a methylene group activated by an adjacent carbonyl or other electron-withdrawing group, and such an activating group may also facilitate the thermal degradation of the oxime (Sidgwick, 1966; Rogic et al., 1977). A possible route for the formation of nitriles from long chain fatty acid oxidation products is shown in Figure 2.

The isolation of these nitriles and nitrates in the headspace volatiles of cooked cured meat clearly demonstrates the reactivity of nitrite and suggests that other similar reactions between nitrite and flavor precursors may occur, giving rise to other compounds that may also contribute to cured meat flavor.

**Registry No.** Pentyl nitrate, 1002-16-0; hexyl nitrate, 20633-11-8; heptyl nitrate, 20633-12-9; octyl nitrate, 629-39-0; benzonitrile, 100-47-0; phenylacetoneitrile, 140-29-4; nonanenitrile, 2243-27-8; decanenitrile, 1975-78-6; undecanenitrile, 2244-07-7; dodecanenitrile, 2437-25-4; tridecanenitrile, 629-60-7.

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