Distribution of Some Volatile Nitrosamines in Cooked Bacon

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Bacon was fried in the normal UK domestic manner, and the rashers, the cooked-out fat and the vapour were analysed for N-nitrosopyrrolidine and N-nitrosodimethylamine. Quantitative results were based on high resolution mass spectral measurements, and it was found that for both of the nitrosamines by far the greatest proportion occurred in the vapour.

1. Introduction

It is now well established that cured meat products such as bacon contain trace amounts of N-nitrosodimethylamine and that on cooking, N-nitrosopyrrolidine is formed. The origin of the N-nitrosopyrrolidine is not conclusively established, but may be formed from proline by nitrosation and subsequent decarboxylation. The present study is restricted to the determination of these two nitrosamines, both of which are known to show carcinogenic activity. The distribution of these nitrosamines in cooked bacon, in the resulting cooked-out fat, and in the vapour produced during cooking has been measured.

2. Experimental

2.1. Sample preparation

Pork for the experiments was mild block cured using multi-needle injection. Nine batches each of two cuts were used, middle (back) of pH range 5.5–6.5 and collars of pH range 5.8–6.9. All blocks were given identical treatment in respect of brine composition (21% w/w NaCl), the amount of brine pumped into each block, the ratio of meat weight to cover brine, and immersion time. The injection brine contained 2% w/w sodium tripolyphosphate, but no ascorbate. Target NaCl content was 4% in the water contained in the bacon, and phosphate content 0.3% in the lean meat. The final heat process (smoking) was carried out under fixed conditions of temperature and humidity. The only variables were the levels of sodium nitrite and potassium nitrate. Nitrite levels in the brine were such as to provide 100 or 200 mg/kg of added nitrite in the bacon. Nitrate levels were zero, 250 or 500 mg/kg. The legal limits in the United Kingdom for nitrite and nitrate in bacon as purchased by the consumer are 200 and 500 mg/kg respectively. Bacon of the type used in this work typically contains 40–60 mg/kg of nitrite (as purchased). After smoking the blocks were matured for 6 days. They were then sliced, vacuum packed and stored at 5°C until cooked. Bacon slices for cooking were selected from the packs such that a sample representative of all the bacon in each cure was analysed. Bacon was fried following current domestic UK practice as closely as possible, to produce a crisp well-cooked commodity. Known weights of the representative slices from each cure were fried in preheated thermostatically controlled electric frying pans set at 171°C (340°F). The bacon rashers were turned after 4 min, giving a total cooking time of 8 min. The pans were fitted with ventilated lids over which was placed an inverted wide mouth funnel connected to a water
vacuum pump and collection vessel. In order to preserve a realistic domestic situation, some sacrifice of vapour trapping efficiency was necessary, and there were obviously losses of vapour during turning. However, since trapping was carried out throughout the cooking process, the concentration of nitrosamines in the condensed vapours was assumed to be representative of the concentration which would result from complete trapping of all vapours in an ideal situation. On completion of frying excess fat was drained from the rashers. The rashers and the cooked-out fat were weighed and stored at \(-18^\circ C\) pending analysis. The condensate was weighed and stored at \(+4^\circ C\).

### 2.2. Analytical procedure

A total of 36 bacon samples were analysed. Each sample of cooked bacon was frozen in liquid nitrogen, ground in a mortar and minced. 250 ml of water and 1 ml of a 2.5 mg/l aqueous solution of \(N\)-nitrosodipropylamine to check recoveries were added to 250 g of the minced bacon, and the mixture steam distilled at atmospheric pressure; 400 ml of distillate was collected, and 80 g of sodium chloride and 4 ml of 10 N sulphuric acid added to it. This was extracted with \(4 \times 40\) ml of redistilled dichloromethane, and the combined extracts washed with 70 ml of 1.5 N sodium hydroxide. The dichloromethane extract was dried over sodium sulphate, transferred to a Kuderna-Danish flask and evaporated to 2.5 ml at 45°C on a water bath. The flask was cooled, 800 \(\mu l\) of hexane added and evaporated to about 250 \(\mu l\). The volume was measured with a 500 \(\mu l\) capacity syringe used to transfer the extract to a septum-fitted vial, which was stored at 10°C prior to analysis. The cooked-out fat was treated in a similar manner, but it was not found necessary to freeze or mince this prior to distillation. Nitrosamines were extracted directly from the vapour condensates and thereafter treated as above. All extracts were examined by combined gas chromatography and mass spectrometry. A Pye 104 chromatograph was fitted with a short polar packed column connected to a high efficiency support-coated open-tubular column. A venting valve was placed between the two columns to prevent overloading of the high efficiency column by solvent and contamination of the mass spectrometer by extraneous material. A parallel reference flow system was incorporated to maintain a constant flow rate of carrier gas through the columns, irrespective of the switching valve mode. A detailed description of this apparatus has been published.\(^7\) The gas chromatograph was interfaced to an MS902 mass spectrometer via a silicone rubber separator.\(^8\) The mass spectrometer was operated under high resolution, and the nitrosamines were detected by monitoring their respective parent ions at the appropriate retention time, with reference to a suitable fragment ion of a fluorinated hydrocarbon. Calibration of the mass spectrometer for a quantitative assessment of the level of \(N\)-nitrosodimethylamine, \(N\)-nitrosodipropylamine and \(N\)-nitrosopyrrolidine, was carried out with standard nitrosamine solutions, four times each day. Precision of the mass spectral results was \(\pm 20\%\). The concentrations of nitrosamines in the extracts, as determined by mass spectrometry, were corrected for the losses incurred during the clean-up procedure.\(^9\) For \(N\)-nitrosodimethylamine, losses occurred mainly in the final stages of evaporation of the extract, whereas \(N\)-nitrosopyrrolidine was lost predominantly during the initial steam distillation of the foodstuff.

### 3. Results

The concentrations of nitrosamines in the cooked bacon, cooked-out fat and vapour, expressed in \(\mu g/kg\), were obtained from the corrected mass spectral results. The concentration ranges of \(N\)-nitrosodimethylamine in the cooked-out fat (0–30 \(\mu g/kg\)) and vapour (10–160 \(\mu g/kg\)) are shown in Figure 1. Levels of \(N\)-nitrosodimethylamine in the cooked bacon were all in the region of the detection limit, the highest value being 6 \(\mu g/kg\). The range of concentration (2–180 \(\mu g/kg\)) of \(N\)-nitrosopyrrolidine is shown in Figure 2. For both nitrosamines the levels in the cooked bacon are typical of those reported by other workers.\(^4\)\(^,\)\(^10\)\(^,\)\(^11\) No attempt was made to correlate the concentrations with the nitrite or nitrate levels, as this forms part of a larger programme, the results of which will be published later.

The weights of uncooked bacon, cooked bacon and cooked-out fat were known, and hence the
weight lost as vapour during cooking was calculated. From this the absolute quantities of nitrosamines present and their distribution between the cooked bacon, cooked-out fat and vapour were estimated. Results are shown in the form of histograms, for N-nitrosodimethylamine in Figure 3 and for N-nitrosopyrrolidine in Figure 4.

The distribution by weight of the cooked bacon itself, the cooked-out fat and the vapour for the collars and backs is shown in Figure 5. The backs gave rise to somewhat more cooked-out fat, but less vapour than the collars. No difference in the distribution of the nitrosamines in the backs and collars was detectable, although it is recognised that small variations would be masked by the limitations of the experimental procedure.
4. Conclusions

In bacon fried in a normal UK domestic manner, by far the greatest proportion of N-nitrosodimethylamine and N-nitrosopyrrolidine is lost in the vapours produced during cooking. Up to 10% of N-nitrosodimethylamine is found in the rasher itself and up to 20% in the cooked-out fat. For N-nitrosopyrrolidine the corresponding maximum figures are 25 and 30%, respectively.
Figure 5. Proportion by weight of cooked bacon, cooked-out fat and vapour in collars and backs.

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