Analysis of *N*-nitrosamines in a nitric oxide gas cured comminuted pork product

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BACKGROUND

Concern over the use of nitrite in curing meat has been the subject of many investigations because of its reaction with secondary amines and amino acids that are present in meat. The reaction of nitrite with the naturally occurring amine compounds in meat can, in principle, give rise to *N*-nitrosamines (Sen et al., 1973; Gray and Randall, 1979), of which *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosopyrrolidine and *N*-nitrosopiperidine are the most commonly occurring in cured meat products. Furthermore, these compounds and nitrosamines, in general, have been shown to be potent carcinogens in animals (Sen et al., 1979). The aim of this study was to develop a sensitive and selective method to detect the presence of *N*-nitrosamines in a nitric oxide - cured pork product.

MATERIALS AND METHODS

The assay for nitrosamines in cured meat was performed using the Likens-Nickerson technique (Likens & Nickerson, 1964), in which dichloromethane (DCM) was used as the solvent. In the extraction procedure, the comminuted product was placed in a flask with sodium chloride, sodium carbonate and Milli Q water on one side of the apparatus and DCM on the other side. A cold finger was inserted in the middle to effect condensation. DCM being heavier than water ensured continual circulation of both solvents, because as they condensed, they ran to their respective sides of the apparatus. Extraction was carried out for 3 hours and the temperatures on either side of the apparatus were controlled for complete extraction. Two external standards were used for the purpose of recovery determinations along with one internal standard, each at a level of 10µg/ml. The compounds used were chlorobenzene and 1,2,4-trichlorobenzene as the two recovery standards and 1,3,5-trichlorobenzene as the internal standard for quantitation. The extract was obtained in DCM and was subsequently analysed by Gas Chromatography Mass Spectrometry (GC-MS).

A 5ml ampoule of a standard nitrosamine mixture in methylene chloride (Catalog Number: N-8270M, Alltech, Australia) was used to spike samples and determine detection limits. This nitrosamine standard was a nine-component mixture containing 2000µg/ml of each of the following nitrosamines: (*N*-nitrosodi-n-butylamine, *N*-nitroso-diethylamine, *N*-nitrosodimethylamine, *N*-nitrosodiphenylamine, *N*-nitrosodi-n-propylamine, *N*-nitrosomethylethylamine, *N*-nitrosomorpholine, *N*-nitrosopiperidine, *N*-nitrosopyrollidine).

Various concentrations of this nitrosamine standard were prepared (0, 1, 3, 5, 8 and 10µg/ml) with the addition of the internal standard at a fixed level of 10µg/ml. These were analysed by GC-MS to obtain retention times for the nine nitrosamines and to establish the lowest achievable detection limits for identification of each nitrosamine by GC-MS.

Extraction efficiencies for the Likens-Nickerson apparatus were determined using the standard nitrosamine mixture. Recovery tests included extraction of the nitrosamines from both water and water with the comminuted pork product. In all recovery experiments a level of 10µg/ml of the nitrosamine standard, the two recovery standards and the internal standard were used.

Gas chromatography was performed on a Hewlett-Packard (HP) HP Wax fused-silica capillary column (30 m x 0.2 mm x 0.02 µm) housed in a 5890 series II gas chromatograph. Helium was used as the carrier gas at a constant head pressure of 2.1 kg/cm^2. The oven temperature was initially set at 40°C and, after 4 min, was ramped at 15°C/min to a final temperature of 220°C, which was maintained for 24 mins. Sample injections were performed in the splitless mode using a HP 7673 autoinjector and a purge activation time of 1.5 min. The injector and detector temperatures were set at 250 and 280°C, respectively.

The capillary column was directly interfaced to a HP 5972 mass-selective detector, which was operated in the electron impact mode. The energy of the electron beam was 70 eV. The ion source temperature was 180°C. The abundance of the ions selected for monitoring the nitrosamines and the internal standard were optimised by using the most abundant ion from a cluster of ions separated by 0.05 amu in the range of ± 0.5 amu of the ion chosen in the full scan mode. In the case of *N*-nitrosodi-n-butylamine, *N*-nitroso-diethylamine, *N*-nitrosodimethylamine, *N*-nitrosodiphenylamine, *N*-nitrosodi-n-propylamine, *N*-nitrosomethylethylamine, *N*-nitrosomorpholine, *N*-nitrosopiperidine, *N*-nitrosopyrollidine the ions were 84.00, 116.00, 158.00, 102.00, 42.00, 44.00, 74.00, 42.00, 169.00, 168.00, 70.00, 130.00, 42.00, 88.00, 42.00, 56.00, 86.00, 116.00, 114.00, 42.00, 100.00, respectively, and in the case of the internal standard the ions were 180.00, 182.00. The dwell time for each ion was set so as to achieve a scan rate of 3 Hz to reduce imprecision of area measurement.

RESULTS AND DISCUSSION

The Likens-Nickerson extraction that was used in this study has the important advantage over other methods in that it was capable of extracting the volatile nitrosamines from the fat in the meat samples. Both the recovery standards and the internal standard were satisfactory for this work because they have similar retention times to the nitrosamine standards and were extracted in the Likens-Nickerson technique in the same way that the nitrosamine were.

The use of sensitive and highly specific detectors has made it possible to confidently quantitate trace levels of nitrosamines in complex mixtures with minimal sample preparation. Most analytical procedures for the analysis of nitrosamines involve the use of a chemiluminescent detector such as the Thermal Energy Analyser detector, which is also referred to as the TEA detector. This method is not as selective as GC-MS and some non-*N*-nitroso compounds can respond to the TEA. Thus confirmation of nitrosamines requires gas chromatographic mass spectrometric analysis.

The lowest achievable detection limit for identification of each nitrosamine by GC-MS was determined to be 1µg/kg. This compares favourably with previous studies where Gough and Webb (1972) reported detection limits of 2 and 5 µg/kg, while Hotchkiss et al., (1980) published a method for the confirmation of volatile nitrosamines in foods at levels of 1 to 10 µg/kg. A literature survey reveals that the recoveries of nitrosamines are quite variable and dependent on the matrix, the analyte and its concentration. Extraction efficiencies for the Likens-Nickerson technique were determined and are summarised below in Table 1. Analysis of the various nitric oxide-cured comminuted pork products showed no nitrosamines were detected above the detection limit of 1µg/kg.

CONCLUSIONS

In conclusion, the method developed has the specificity and sensitivity required to detect the presence of nitrosamines in the product at a detection limit of 1µg/kg. The results obtained with this method suggest that the application of nitric oxide gas in the curing process has the potential to eliminate the concerns of carcinogenic risk associated with the consumption of cured pork-based smallgoods.

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Table 1: Nitrosamine Recoveries.

Nitrosamines	Percentages			
	Water	Nitric Oxide Product 1	Nitric Oxide Product 2	Averages
N-nitrosodi-n-butylamine	24	94	66	61
N-nitrosodiethylamine	72	102	68	81
N-nitrosodimethylamine	34	35	22	30
N-nitrosodiphenylamine	46	31	16	31
N-nitrosodi-n-propylamine	47	119	89	85
N-nitrosomethylethylamine	60	63	38	54
N-nitrosomorpholine	11	18	21	17
N-nitrosopiperidine	37	34	21	31
N-nitrosopyrrolidine	13	19	13	15