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Note

Gas chromatographic determination of N-nitrosoamino acids by trimethylsilylation and single-ion mass fragmentography

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Experimental studies have shown that amino acids possessing secondary amino groups can be easily converted into their N-nitroso derivatives by nitrite under conditions similar to those prevailing in the human stomach and presumably also in some foods[1,2]. Such amino acids are present as natural constituents of many foodstuffs. Therefore, treatment of such foods with nitrite might lead to formation of N-nitrosoamino acids. Although most of these N-nitroso compounds have been reported to be non-carcinogenic by themselves in animals[3,4], they may be converted into well known carcinogenic nitrosamines by heat-induced decarboxylation[5]. N-Nitrosoproline for instance readily loses carbon dioxide and forms N-nitrosopyrrolidine under experimental conditions simulating the frying process[6,7]. N-Nitrosopyrrolidine is a well known liver carcinogen[8]. Levels of this nitrosamine found in fried bacon have been correlated with the amount of initially added nitrite and hence presumably with the amount of N-nitrosoproline formed during the curing process, but not with the amount of residual nitrite in the final product[9].

For a more detailed study of the possible relation between the content of N-nitrosoamino acids in foods and the levels of the corresponding nitrosamines found after cooking, we have developed a method for analysis of trace amounts of N-nitrosoamino acids. We selected the N-nitroso compounds of sarcosine, proline and 2-hydroxyproline for this investigation because of the widespread occurrence of these amino acids in foods[10–13]. The analytical procedure is based on trimethylsilylation and gas chromatographic (GC) separation of the volatile trimethylsilyl (TMS)-derivatives of the N-nitrosoamino acids. Both flame ionization and single-ion monitoring in a gas chromatograph–mass spectrometer were used for quantitative assessments.

EXPERIMENTAL

Analytical grade acetonitrile (E. Merck, Darmstadt, G.F.R.) was used as solvent and N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA; Macherey, Nagel & Co., Düren, G.F.R.) served as the silylating agent. The N-nitroso compounds of sarcosine, proline and 2-hydroxyproline were synthesized from the corresponding amino acids and nitrosyl tetrafluoroborate[14]. The purity of the N-nitrosoamino acids

was established by thin-layer chromatography (TLC) and by ultraviolet (UV), infrared, nuclear magnetic resonance and mass spectrometry (MS)[5].

Preparation of trimethylsilyl derivatives

Known amounts (0.2–20.0 μg) of the N-nitrosoamino acids, dissolved in acetonitrile, were placed in small reaction vessels (0.3-ml Reacti-Vial with Mininert valve®). The solvent was evaporated *in vacuo* in a desiccator, the vessel was closed and the dry residue was dissolved in 200 μl of MSTFA added by syringe through the valve. The mixture was allowed to stand at ambient temperature for 30 min; 1-μl amounts were injected into the gas chromatograph.

Gas chromatography

A Varian 2100–20 gas chromatograph, equipped with a flame ionization detector (FID), was used. Separations were carried out in glass columns (1.8 m × 1.7 mm I.D.) packed with 3% OV 17 on Gas-Chrom Q (100–120 mesh). Carrier gas (nitrogen) flow-rate, 40 ml/min. FID conditions: hydrogen flow-rate, 20 ml/min;

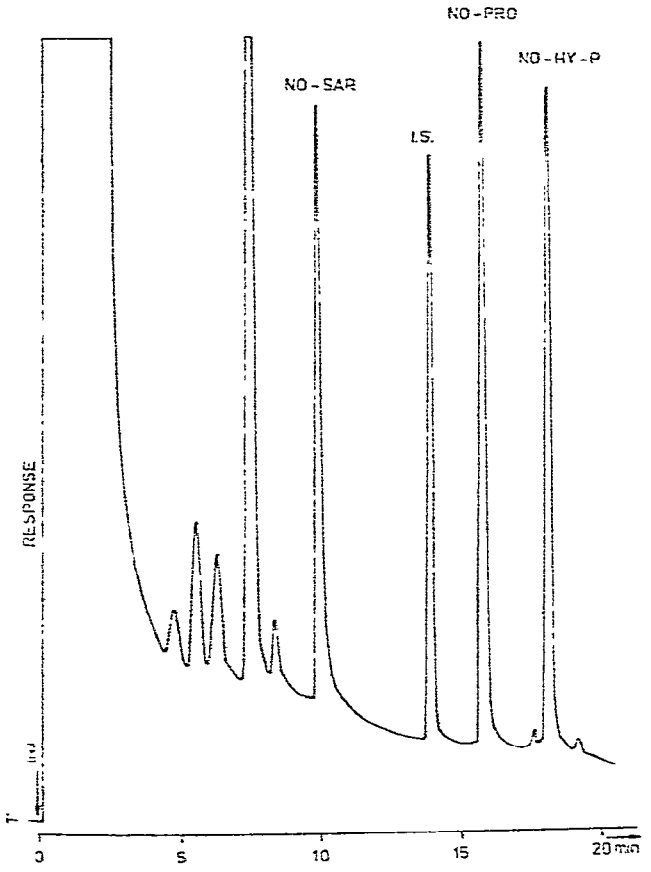

Fig. 1. Gas–liquid chromatogram of the TMS derivatives of the N-nitrosoamino acids and myristic acid as internal standard (I.S.). Sample size, 100 ng of each N-nitrosoamino acid.

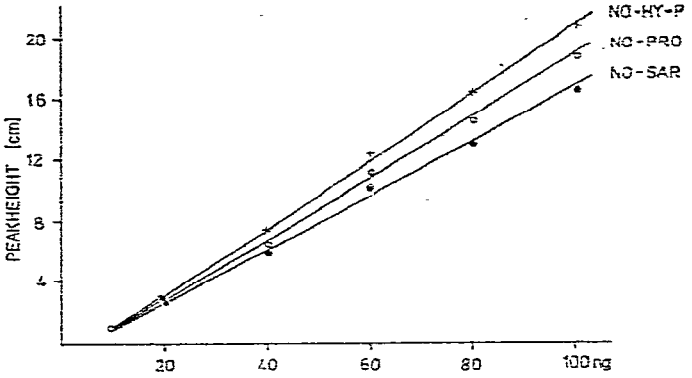

Fig. 2. Calibration graphs of the corrected peak heights against the amounts of the TMS derivatives (10–100 ng) of the N-nitrosoamino acids.

air flow-rate, 200 ml/min; temperature, 200°. Injection-port temperature, 170°. Oven-temperature, 4 min isothermal at 80°, then programmed at 6°/min to 170°.

Single-ion mass fragmentography

An LKB 9000 system, equipped with a multiple-ion detection (MID) unit was used. Separations were carried out on a glass column (1.2 m × 1.7 mm I.D.) packed with 6% OV-17 on Gas-Chrom Q (100–120 mesh). Carrier gas (helium) flow-rate, 30 ml/min. The temperatures of the injection port and oven were: N-nitrososarcosine (NO-Sar), 150° and 110°; N-nitrosoproline (NO-Pro), 160° and 150°; N-nitroso-2-hydroxyproline (NO-Hyp), 190° and 170°. Separator temperature, 230°. Ion-source temperature, 270°. Trap current, 60 μA. Electron energy, 70 eV. Accelerating voltage, 3.5 kV. Filter of the MID, 0.12 Hz. UV-recorder speed, 1 cm/min.

TABLE I

SOME ABUNDANT AND CHARACTERISTIC FRAGMENTS IN THE MASS SPECTRA OF THE TMS DERIVATIVES OF THE N-NITROSOAMINO ACIDS AT 70 eV

Assignment	*NO-Sar*		*NO-Pro*		*NO-Hyp*	
	m/e	*Relative abundance (%)*	*m/e*	*Relative abundance (%)*	*m/e*	*Relative abundance (%)*
M^+	190	1.4	216	1.6	304	0.4
$[M - CH_3]^+$	175*	5.5	201*	3.5	289	4.8
$[M - NO]^+$	160	4.7			274	1.3
$[M - CH_3 - CO_2]^+$	131	8.1	157	2.6	245*	12.4
$[M - COOSi(CH_3)_3]^+$			99	10.3	187**	4.1
$[M - OSi(CH_3)_3]^+$	101	11.4				
$[Si(CH_3)_2{-}C_4H_7N]^+$			142**	8.9		
$[Si(CH_3)_2{-}O{-}C_4H_6N]^+$					142**	9.5
$[COO{-}Si(CH_3)_2]^+$					102	3.8
$[HO{-}Si(CH_3)_2]^+$	75	18.9	75	18.3	75	21.2
$[Si(CH_3)_3]^+$	73	100	73	100	73	100

* Fragments chosen for mass fragmentography.
** Fragments determined by high-resolution MS.

RESULTS AND DISCUSSION

A typical gas chromatogram obtained by flame ionization detection of the TMS derivatives of the three N-nitrosoamino acids and of myristic acid used as an internal standard is shown in Fig. 1. The silylating agent and some unspecified by-products were rapidly eluted so that the peaks of interest could be recorded without interference. Under the conditions applied, effective baseline separation was achieved. As can be seen from Fig. 2, a linear relation exists between the corrected peak heights and amounts of the respective derivatives in the range 10–100 ng. Myristic acid was used as internal standard because its TMS derivative behaves very similarly to the corresponding derivatives of the N-nitrosoamino acids, especially with respect to sensivity to non-specific adsorption effects.

Analysis of trace amounts of N-nitrosoamino acids in foods requires a specific detection system. If amounts of less than 1–2 μg of a given N-nitrosoamino acid in a final food extract are to be reliably detected, the FID cannot be used because of its low specificity. In this case, advantage can be taken of the high specificity of a GC–MS combination using the mass spectrometer as an ion-specific detector. The most abundant and characteristic fragments of the mass spectra of the TMS derivatives are shown in Table I. Typical chromatograms obtained by single-ion detection of selected fragments are shown in Fig. 3. As can be seen, amounts of the TMS derivatives corresponding to 2 ng of the respective N-nitrosoamino acids can be easily detected as prominent peaks. In the range 1–10 ng, a linear relation was found between the peak heights and the amounts of the TMS derivatives (Fig. 4).

The narrow shape of the chromatographic peaks indicates the suitability of TMS derivative formation for analysis of trace amounts. The results of methyl-ester

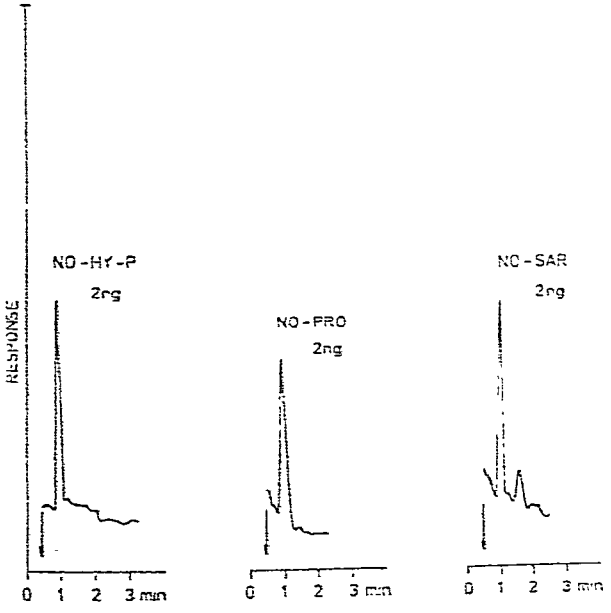

Fig. 3. Single-ion mass fragmentogram of the TMS derivatives of the N-nitrosoamino acids. The arrows indicate the opening of the valve.

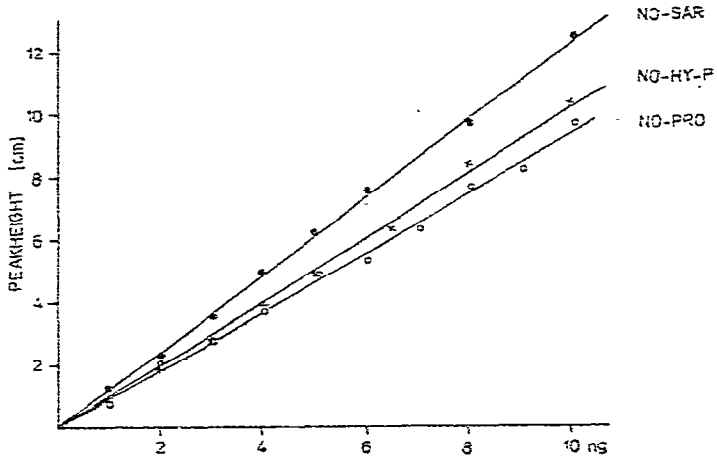

Fig. 4. Calibration graphs of the peak heights against the amounts of the TMS derivatives (1–10 ng) of the N-nitrosoamino acids. Detection: single-ion mass fragmentography at *m/e* 175 (NO-Sar), *m/e* 201 (NO-Pro) and *m/e* 245 (NO-Hyp).

formation on reaction with diazomethane were much less satisfactory[15]. The methyl ester of NO-Hyp gave strongly tailing peaks and low recoveries due to non-specific adsorption because of its free hydroxyl group. However in the above procedure, carboxyl and hydroxyl groups are silylated at the same time. This leads to products with satisfactory chromatographic properties. TMS derivative formation seems therefore to be the method of choice for quantitative determination of N-nitrosoamino acids in parts per billion in foods. Its application to the analysis of these compounds in food is currently being investigated.

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