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Analysis of nonpolar heterocyclic amines in cooked foods and meat extracts using gas chromatography–mass spectrometry

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Abstract

Heat processing of muscle foods gives rise to the formation of mutagenic and carcinogenic heterocyclic amines, often at ng/g levels. A gas chromatographic–mass spectrometric (GC–MS) technique was introduced for the analysis of nonpolar heterocyclic amines in common cooked meats, pan residues, and meat extracts after solid-phase extraction. The mutagenic heterocyclic amines 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-9H-pyrido[2,3-*b*]indole (AαC) and 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole (MeAαC) were identified in several samples in amounts up to 8 ng/g. Also the comutagenic substances 1-methyl-9H-pyrido [3,4-*b*]indole (harman) and 9H-pyrido[3,4-*b*]indole (norharman) were detected in the samples in amounts up to almost 200 ng/g. The GC–MS method can be applied without derivatisation of the sample. The technique offers high chromatographic efficiency, yielding detection limits for pure references in the range 0.1–2 ng per injection. © 1998 Elsevier Science B.V.

Keywords: Food analysis; Amines, heterocyclic; Indoles

1. Introduction

Our diet plays an important role in the prevention but also in the cause of diseases. We are exposed to complex mixtures of compounds which meet nutritional demands as well as some compounds which may constitute health risks. One class of hazardous substances consists of heterocyclic amines, which are predominantly formed during cooking of meat and fish. About 20 years ago, these substances were identified as potent mutagens in the Ames/*Salmonella* test [1,2]. Many heterocyclic amines have been identified and synthesised and have been shown to cause tumours in animal experiments (for reviews see [3–7]). Based on results from long-term animal studies on mice, rats and nonhuman primates [8–12] several of these compounds have been classified as carcinogens [13]. Heterocyclic amines are primarily found in cooked muscle foods at low ng/g levels (for reviews see [14,15]). The low concentration of heterocyclic amines and the complex sample matrix of cooked foods make the analysis of these compounds very difficult. In most food surveys, only polar heterocyclic amines e.g. MeIQx, DiMeIQx and PhIP have been determined [16–21]. The presence of nonpolar heterocyclic amines (Trp-P-1, Trp-P-2,

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AαC and MeAαC) is not often reported. These compounds have been thought to be formed exclusively under extreme cooking conditions [22], and consequently not to be found in the Western diet [23]. However, Trp-P-1 and Trp-P-2, have been detected in broiled sardines, beef and chicken, meat extract and bouillon cubes [24–29]. AαC has been detected in barbecued or broiled beef, chicken, mutton, salmon, meat extract and bouillon cubes [12,26,28,30–36] and MeAαC in grilled beef, chicken and mutton [30].

Trp-P-1 and Trp-P-2 have been shown to induce liver tumours in mice and rats [4,5,37,38], and Trp-P-2 is suspected in the etiology of cancer [39]. Furthermore, neonatal mice have been found to be particularly sensitive to heterocyclic amines [11,40]. When Trp-P-1 was administered to pregnant mice, increased levels of Trp-P-1 were observed in the foetal liver and gastrointestinal content, and Trp-P-1 was also bound to the pigmented parts of the foetal eyes [41]. The amino-α-carbolines AαC and MeAαC induce tumours in liver and the vascular system [[5], review], and recently MeAαC was shown to be metabolised by human cyt P4501A2 [42]. Simultaneous administration of five heterocyclic amines, including at least one of the aforementioned compounds, showed a synergistic effect on the carcinogenicity [43–45]. Enhancement of the carcinogenesis in rats was also observed following simultaneous administration of ten heterocyclic amines at low doses [46]. Thus, the presence of nonpolar heterocyclic amines in cooked foods should not be ignored.

In a recently published study, foods prepared under household cooking conditions were analysed for heterocyclic amines using HPLC with UV detection [29]. Several of the food items were shown to contain the nonpolar heterocyclic amines Trp-P-1 and Trp-P-2, and the β-carbolines harman and norharman. AαC and MeAαC were only tentatively identified, as coeluting impurities made reliable identification impossible. These results led us to search more extensively for nonpolar heterocyclic amines in our subsequent experiments and to investigate alternative analytical methods. Here we present data on nonpolar heterocyclic amines in cooked foods and meat extracts using a new gas chromatography–mass spectrometry (GC–MS) method without derivatisation of the compounds.

2. Materials and methods

2.1. Chemicals

All commercially available chemicals, reference compounds (Trp-P-1, Trp-P-2, AαC, MeAαC, harman and norharman) and solvents were of analytical or HPLC grade, and have been described previously [27]. According to the manufacturers, the chemical purity of the reference compounds was higher than 99%; this was also confirmed using HPLC with UV detection and multi-component analysis (Varian, PolyView, version 4.0) for each of the reference compounds. The concentrations of the different standard solutions were calculated from absorbance measurements using known molar extinction coefficients ([47]; G.A. Gross, personal communication).

2.2. Food samples — cooking, extraction and purification

Reindeer meat, fillet of pork and pork loin in the form of pork chops (referred to as pork chops later) were fried in a pan at 225°C for 4–9 min. Meat loaf was roasted in an oven at 175°C for 45 min. Pan residues were prepared from the pork chops and the meat loaf as earlier described [21]. Two meat extracts (A and B) were obtained from a company. The heterocyclic amines in the food samples were extracted using a modification [21] of the solid-phase extraction method described by Gross and Grüter [32]. After purification, only the extracts containing the heterocyclic amines Trp-P-1, Trp-P-2, AαC, MeAαC, harman and norharman, were analysed. The samples were evaporated to dryness under nitrogen and were then dissolved in 25 μl caffeine in methanol (0.3 μg/ml) serving as an internal standard.

2.3. Gas chromatography–mass spectrometry

A combined gas chromatograph–quadropole mass spectrometer (HP 6890 gas chromatography system with mass-selective detector) was used for the analysis of nonpolar heterocyclic amines. The gas chromatograph was equipped with a capillary column (Rtx-50, 50% phenyl–50% methyl polysiloxane, 30 m×0.32 mm I.D., 0.50 μm film thickness, Restek, USA) which was routed through the separator oven directly into the mass spectrometer

ion source. Helium was used as a carrier gas at a flow-rate of 1.0 ml/min and a pressure of 0.7 p.s.i. (1 p.s.i.=6894.76 Pa). The gas chromatograph was fitted with a Grob-type capillary injector in the splitless mode and maintained at 270°C. The injection volume was 1 μl. The initial oven temperature was maintained at 100°C for 2 min, then raised to 320°C, at 20°C per min, and maintained at 320°C for 7 min. The mass spectrometer was operated in the negative ion mode with an electron energy of 70 eV. The temperature of the electron impact ion source was maintained at 250°C. Analyses were performed using single-ion monitoring (SIM). Six reference mixtures, each containing Trp-P-1, Trp-P-2, AαC, MeAαC, harman and norharman at increasing concentrations between 0 and 20 ng in caffeine in methanol (0.3 μg/ml) were prepared and analysed, and the standard curves were used for the quantification of the heterocyclic amines in the food samples. The recovery of the extraction was estimated by the addition of accurately measured amounts of each standard to two out of four samples at the beginning of the extraction procedure.

3. Results and discussion

A chromatogram from the GC–MS analysis of a standard solution, containing all six nonpolar heterocyclic amines, together with their retention times, is shown in Fig. 1a. The GC–MS method with the detection of negative ions provides high chromatographic efficiency and the analytes can easily be separated from each other. Fig. 1b shows a chromatogram from the analysis of meat extract A. Table 1 presents the averages of duplicate determinations of the concentrations of Trp-P-1, Trp-P-2, AαC, MeAαC, harman and norharman in the food samples (ng/g dry matter).

The highest concentration of Trp-P-1, 5 ng/g, was found in meat extract A. Trp-P-1 was detected at levels below 1.0 ng/g in all the other samples but the pork chop pan residue. The presence of Trp-P-2 could only be established in one sample, meat extract B, 6.4 ng/g. The amounts of Trp-P-1 and Trp-P-2 are at the same level as those found in our previous study [29]. Trp-P-1 and Trp-P-2 have earlier been detected in cooked foods at concentrations below 1 ng/g [12,28] and at 5.5–53 ng/g [24–27]. Trp-P-1 and Trp-P-2 have been regarded as high-temperature mutagens [22], but in our study they were detected in foods cooked at 225°C, and also in the meat extracts that are normally prepared at boiling temperature [48].

AαC and MeAαC were detected in low amounts in the food samples; the concentrations ranging from not detectable up to 8.1 ng/g. AαC has earlier been detected at high levels (100–650 ng/g) in grilled or barbecued meat [30,36], but also at lower levels, below 10 ng/g in fried meat and fish [12,28,32,35]. To the best of our knowledge, only one report on the presence of MeAαC in cooked foods has hitherto been published. MeAαC was detected at 5.4–63.5 ng/g in beef, chicken and mushroom grilled for 4–6 min [30]. In our previous studies involving the screening of cooked foods for nonpolar heterocyclic amines using HPLC, poor peak shapes or coeluting impurities have made reliable identification and quantification of the amino-α-carbolines impossible.

The comutagens, harman and norharman, were detected at levels up to almost 200 ng/g in all the samples analysed. This is in agreement with results from earlier reports on the presence of ng/g levels of harman and norharman in cooked foods and meat extracts [21,26,27,29,31–33,49]. However, our quantifications of harman and norharman are tentative, due to varying degrees of recovery of these compounds. Norharman and harman are not mutagenic towards *Salmonella typhimurium* TA98 and TA100, but norharman enhances the mutagenic effects of Trp-P-1 and Trp-P-2 [50] and other aromatic amines [48]. The presence of norharman and harman in cooked foods should therefore not be ignored. Contradictory results have been observed regarding the comutagenic properties of harman and norharman, and data are lacking with respect to their possible effects on human health [51].

3.1. Analytical considerations

The detection limits in the SIM mode based on a signal-to-noise ratio of 3:1 were calculated for the six compounds, and for an injection volume of 1 μl, they were found to be 0.1 ng for Trp-P-1, MeAαC, harman and norharman, 0.5 ng for AαC, and 2 ng for Trp-P-2. Experiments with a purified extract using low-level spiking of reference standards showed that

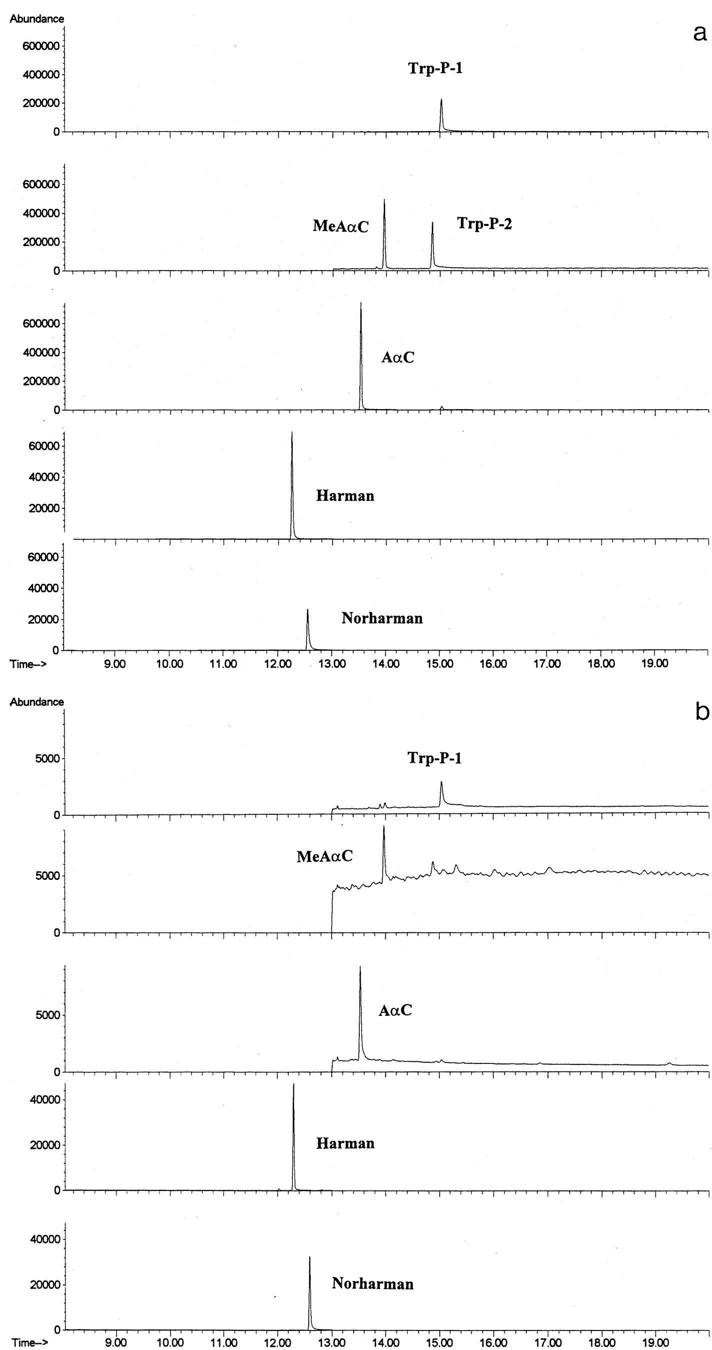

Fig. 1. Single-ion monitoring traces of (a) reference compounds (b) meat extract A. For the reference ions (m/z) the retention times are Trp-P-1 (m/z 211) 15.0 min, Trp-P-2 (m/z 197) 14.9 min, AαC (m/z 183) 13.5 min, MeAαC (m/z 197) 14.0 min, harman (m/z 182) 12.3 min, and norharman (m/z 168) 12.6 min.

Table 1
GC–MS analysis of nonpolar heterocyclic amines (ng/g dry matter of the raw meats) in cooked meat products

Sample	Cooking method	Temperature (°C)	Time (min)	Trp-P-1	Trp-P-2	AαC	MeAαC	Norharman[a]	Harman[a]
Reindeer, crust	Fried	225	5	0.24	Trace	ND[b]	ND	80	10
Fillet of pork, crust	Fried	225	7	0.8	Trace	Trace	3.2	200	6
Pork chop, pan residue	Roasted	175	60	ND	ND	0.04	0.08	0.04	0.04
Meat loaf, pan residue	Roasted	175	45	0.08	ND	0.28	ND	0.2	0.04
Meat extract A[c]				5.0	ND	8.1	4.0	100	200
Meat extract B[c]				0.3	6.4	ND	1.6	100	20

[a] The values for norharman and harman are not corrected for recovery due to varying degrees of recovery.
[b] ND=not detected.
[c] ng/g dry product.

the detection limits of the heterocyclic amines in the food samples were somewhat higher than those obtained for standard solutions. The detection limits varied with the compound analysed and the complexity of the sample matrix, and Trp-P-2 was especially difficult to determine at low levels in the food samples. This is evident from Fig. 1b, showing a very small peak at the retention time of Trp-P-2, however, we could not properly distinguish this peak from the background noise. Values between 1 and 6 ng/g have been obtained for the detection limits of nonpolar heterocyclic amines, using liquid chromatography–mass spectrometry [27]. Using GC–MS with derivatisation of the compounds, detection limits for polar heterocyclic amines were reported to be in the range 0.03–0.2 ng/g [52,53]. In the analysis of meat extracts, the detection limits reported by different workers using UV detection ranged from 1 ng/g [32] to 2–5 ng/g [47] for polar heterocyclic amines. When process flavours were analysed using UV detection, detection limits of 50 ng/g were obtained [54].

The average recovery in the extraction of heterocyclic amines in the food samples ($n=12$) was 42% for Trp-P-1 and 44% for Trp-P-2, which is consistent with earlier data from our laboratory [29], but lower than those reported by Gross [31], and 52% and 58% for AαC and MeAαC, respectively, which is higher than our earlier data and at about the same level as found by Gross [31]. The variation between duplicate determinations was less than 15%.

Analysing low levels of heterocyclic amines means that coeluting impurities and high levels of chromatographic interference can make peak identification impossible; this was demonstrated when some of the samples were analysed using HPLC with UV detection and none of the compounds could be properly identified (data not shown). Generally, when foods are cooked at high temperatures, the sample matrix becomes very complex, and for the analysis of pan residues and meat extracts, an additional purification step is often needed [55]. This new method employing GC with the detection of negative ions using MS offers high chromatographic efficiency and provides an alternative method of analysing nonpolar heterocyclic amines in complex food samples. However, it causes contamination of the ion source through the deposition of nonvolatile material, and column lifetime is not as great as with HPLC columns.

The presence of nonpolar heterocyclic amines in common cooked meat and fish has more or less been neglected in analysing Western diets. In this study we have focused on the nonpolar heterocyclic amines with the aim to investigate alternative analytical methods for their determination. Results from other recent studies on the carcinogenicity of nonpolar heterocyclic amines, emphasise the need to pay more attention to their presence in cooked foods. Interestingly, most of the literature data have been produced during the past five years, which indicates that there is an increasing interest in, together with the successful development of analysis methods for, the screening of nonpolar heterocyclic amines in cooked foods.

4. Abbreviations

Abbreviation	Systematic name	CAS registry number
AαC	2-Amino-9H-pyrido[2,3-b]indole	26148-68-5
DiMeIQx	2-Amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline	
Harman	1-Methyl-9H-pyrido[3,4-*b*]indole	486-84-0
MeAαC	2-Amino-3-methyl-9H-pyrido[2,3-*b*]indole	68006-83-7
MeIQx	2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline	
Norharman	9H-Pyrido[3,4-*b*]indole	244-63-3
PhIP	2-Amino-1-methyl-6-phenylimidazo[4,5-*f*]pyridine	
Trp-P-1	3-Amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole	62450-06-0
Trp-P-2	3-Amino-1-methyl-5H-pyrido[4,3-*b*]indole	62450-07-1

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