Quantitative Determination of Polycyclic Aromatic Hydrocarbons in Barbecued Meat Sausages by Gas Chromatography Coupled to Mass Spectrometry

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A method is described for the analysis of the 16 polycyclic aromatic hydrocarbons (PAHs) prioritized by the USA EPA in meat sausages grilled under common barbecue practices. Quantification was done by GC−MS using perdeuterated internal standards (IS). Validation was done by spiking the matrix at the 0.5 and 1.0 μg/kg levels. The average of expected values ranged from 60 to 134% (median 84%) at the 0.5 μg/kg level and from 69 to 121% (median 96%) at the 1.0 μg/kg level. The median of the limits of detection and quantification were 0.06 and 0.20 μg/kg, respectively, for a 4-g test portion. The carcinogenic PAHs were below the quantification limit in all products except one lamb sausage. Comparison of estimates when either 1, 5, or 16 perdeuterated PAHs were used as IS showed that the most accurate determination of PAHs required that each compound be quantified against its corresponding perdeuterated analogue.

Keywords: *Polycyclic aromatic hydrocarbons (PAHs); meat sausage; gas chromatography−mass spectrometry (GC−MS)*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds composed of two or more fused aromatic rings (Figure 1) resulting from the incomplete combustion or high-temperature pyrolysis of coal, oil, and other forms of organic materials. PAHs comprise the largest class of known chemical carcinogens and have been detected in the environment, especially in air, water, soils, and foods. Foods may be contaminated through different routes, which include the following: direct deposition of PAHs from the atmosphere as environmental contaminants and contamination from packaging materials and production of PAHs during the thermal processing of foods, e.g., drying, baking, grilling, and smoking (Dafflon et al., 1995). Over 100 PAHs have been identified and occur as complex mixtures, never as individual components. The two most common methods to measure PAHs are by HPLC with fluorescence detection (Perfetti et al., 1992; Dafflon et al., 1995; Chen et al., 1996) and gas chromatography with mass spectrometry detection (GC−MS) (Nyman et al., 1993; Chen and Lin, 1997; Chiu et al., 1997). Given the complexity of cooked food matrixes such as grilled meats, which contain numerous aromatic and heterocyclic aromatic compounds, in addition to PAHs, highly selective and specific methods are required for unequivocal analyte detection. Although variable excitation and emission wavelengths may be chosen for detection of different PAHs by fluorescence, the method still lacks specificity and false positive results may occur. GC−MS combined with the use of stable, isotopically labeled IS ensures high-resolution chromatography with unambiguous identification of PAHs and quantification by mass detection. In previous studies, one to six perdeuterated IS were used for the analysis of 16 or more PAHs (Nyman et al., 1993; Karl and Leineman, 1996; Baumard et al., 1997a). However, the recoveries of even structurally similar PAHs from complex food matrixes may be very different and lead to inaccurate estimates. The aim of our study was three-fold: (a) to develop a rapid analytical procedure for the detection of 16 PAHs in meat sausages by GC−MS; (b) to assess the importance of using the corresponding IS or surrogate IS for different PAH aromaticity groupings for reliable PAH quantification; and (c) comparison of the specificity between GC−MS and HPLC−UV-fluorescence detection methods.

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MATERIAL AND METHODS

Samples and Preparation. Sausages were either pork, beef, lamb, or turkey meat based items and obtained from local supermarkets in Lausanne. The sausages were grilled under standard barbecue practices (charcoal grilling; distance between fire and meat: 10 cm; temperature at the surface of the meat: 200−250 °C; percent weight loss: 15−40%), The sausages were then thoroughly homogenized with a Büchi B-400 mixer (Büchi, Flatwil, Switzerland) and stored in the dark at −40 °C. As a positive control, a spicy lamb sausage was barbecued under prolonged time to obtain a heavily contaminated sample.

Two coconut oil reference materials (CRM 458 and CRM 459) containing six PAHs (pyrene, chrysene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, and benzo[*g,h,i*]-perylene) with certified concentrations at the μg/kg levels were obtained from the European Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) and used to check the accuracy of the method.

Chemicals. *Caution: PAHs are carcinogenic and should be handled with care.* Cyclohexane, acetonitrile, *N,N*-dimethylformamide (HPLC grade), potassium hydroxide, and anhydrous sodium sulfate (p.a. grade) were obtained from Merck (Darmstadt, Germany). Ethanol was purchased from Fluka (Buchs, Switzerland). Distilled water was obtained from a

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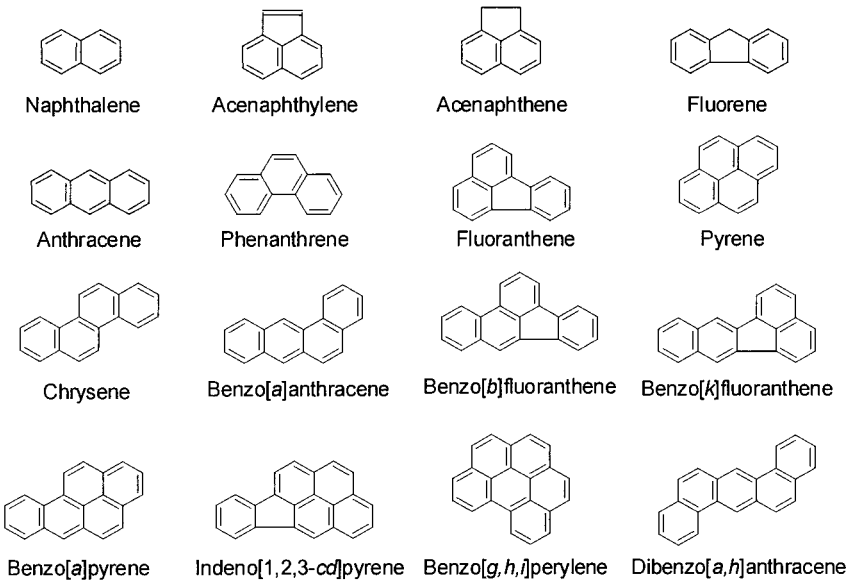

Figure 1. Chemical structure of the 16 PAHs listed by the US Environmental Protection Agency as priority pollutants.

Milli-Q water purification apparatus (Millipore, Bedford, MA). C_{18} end-capped (ec) solid-phase extraction (SPE) columns (1000 mg/6 mL) were obtained from Macherey Nagel (Oensingen, Switzerland). Isolute aminopropyl SPE columns (500 mg/3 mL) were purchased from IST (Hengoed, UK). A deuterium-labeled IS PAH mix containing 16 PAHs (chemical purity > 98%; isotope purity > 99%): naphthalene-d_8, acenaphthylene-d_8, acenaphthene-d_{10}, fluorene-d_{10}, anthracene-d_{10}, phenanthrene-d_{10}, fluoranthene-d_{10}, pyrene-d_{10}, chrysene-d_{12}, benzo[*a*]anthracene-d_{12}, benzo[*b*]fluoranthene-d_{12}, benzo[*k*]fluoranthene-d_{12}, benzo[*a*]pyrene-d_{12}, benzo[*g,h,i*]perylene-d_{12}, indeno[1,2,3-*cd*]-pyrene-d_{12}, and dibenzo[*a,h*]anthracene-d_{14} were obtained from Cambridge Isotope Laboratories (Andover, MA). Corresponding unlabeled PAHs were purchased from Sigma (Buchs, Switzerland). These are standards listed by the US-EPA as priority pollutants (EPA, 1982). Both labeled and unlabeled PAH standards were diluted to the appropriate concentrations with acetonitrile and stored in the dark at −20 °C before use. Radiolabeled ^{14}C-benzo[*a*]pyrene (^{14}C-B(a)P) (26.6 mCi/mmol) was purchased from Sigma (Buchs, Switzerland). All glassware was rinsed with hexane before analysis.

Extraction and Purification Procedures. (a) Meat Sausages. A well-homogenized cooked meat sample (4 g) was weighed to the nearest 10 mg into a 50 mL amber flask. Perdeuterated IS, 1 μg/kg final concentration (40 μL of a 100 ng/mL solution containing 16 PAHs), followed by 2 M potassium hydroxide in ethanol/distilled water (9:1, v/v) (10 mL) and several boiling chips were then added, and this slurry was refluxed for 1 h. After being cooled to room temperature, the solution was transferred into a glass tube. The saponification flask was further rinsed first with cyclohexane (10 mL) and then with distilled water (5 mL), and the washes were transferred into the glass tube. The combined liquid phases were mixed thoroughly by hand for 1 min, followed by centrifugation at 180*g* for 1 min (Centrifuge Mistral 2000, MSE Scientific Instrument, Leicestershire, England). The upper organic phase was removed, and the extraction step was repeated with additional cyclohexane (10 mL). The cyclohexane fractions were combined, dried over anhydrous sodium sulfate (ca. 3 g), and concentrated to 2.0 ± 0.5 mL by rotary evaporation under reduced pressure (45 °C, 180 mbar). The extract was applied to an Isolute aminopropyl SPE column previously conditioned with cyclohexane (6 mL). The flask was rinsed with cyclohexane (1 mL), and the PAHs were eluted with cyclohexane (10 mL). The collected fraction was concentrated under reduced pressure (45 °C, 180 mbar) to a 0.2 ± 0.1 mL volume. The purification of PAHs was completed by applying the extract to a C_{18} SPE column previously conditioned with acetonitrile (10 mL). The flask was rinsed with acetonitrile (1 mL), and the PAHs were eluted with acetonitrile (10 mL). A Supelco Visiprep water-aspirated vacuum manifold (Fluka, Buchs, Switzerland) was used to force the elution of the extracts through SPE columns at a flow rate of 1.5 mL/min. The collected fraction was evaporated under reduced pressure (45 °C, 180 mbar) to ca. 0.5 mL, transferred into a GC vial, and evaporated under a light stream of nitrogen to a 50 ± 20 μL final volume. Extreme care was exercised to avoid concentration of the extract to near dryness since low molecular weight PAHs are easily volatilized during the concentration process (Tamakawa et al., 1992). A blank assay was performed for each series of samples analyzed.

(b) Reference Oil Samples. Oils (4 g) were weighed to the nearest 10 mg into a glass tube. Perdeuterated IS (1 μg/kg final concentration, 40 μL of a 100 ng/mL solution) followed by 15 mL of cyclohexane were then added and the solution thoroughly mixed. *N,N*-Dimethylformamide/distilled water (9:1, v/v) (15 mL) was added, and the mixture was mixed thoroughly by hand for 1 min and then centrifuged at 180*g* for 1 min. The upper cyclohexane layer was discarded, and distilled water (15 mL) was added to the *N,N*-dimethylformamide phase, which was then extracted twice with cyclohexane (15 mL) and centrifuged (180*g*, 1 min). The collected cyclohexane phases were dried over anhydrous sodium sulfate (ca. 5 g) and concentrated to 1.0 ± 0.2 mL by rotary evaporation under reduced pressure (45 °C, 180 mbar). Solid-phase cleanup steps were then performed as described above.

(c) Recovery. Recovery experiments were conducted using ^{14}C-labeled B(a)P spiked in a meat sausage at a level of 5.9 μg/kg to optimize the extraction procedure. Radioactivity was measured by liquid scintillation counting with a LKB-Wallac 1219 Rackbeta counter (Wallac, Turku, Finland). For this purpose, an accurately measured volume of the eluant (1.0 mL) was thoroughly mixed with Ultima Flo M scintillator (10 mL) (Packard, Meriden, USA) prior to counting.

HPLC Method. Analyses were also performed by HPLC with UV and fluorescence detection to determine whether the identification and quantification of PAHs in meat sausages could be achieved. Perdeuterated IS were not used for these assays. A Hewlett-Packard 1050 series HPLC system with a diode-array detector (DAD) was interfaced to a Hewlett-Packard 1046A series programmable fluorescence detector. The column was a Supelcosil LC-PAH (150 × 4.6 mm i.d., 5 μm) (Fluka, Buchs, Switzerland). A mobile phase of acetonitrile/distilled water (40:60, v/v) was maintained for 5 min, and then the percentage of acetonitrile was increased linearly to 100% at 30 min and maintained at 100% for 6 min. The flow rate was 1.5 mL/min. The UV detector was set at 254 nm. The settings for the programmable fluorescence detector

were as follows (excitation/emission wavelength): $\lambda_1 = 270/340$ nm for 13.5 min (naphthalene); $\lambda_2 = 320/533$ nm for 2 min (acenaphthylene); $\lambda_3 = 270/340$ nm for 2 min (acenaphthene, fluorene); $\lambda_4 = 254/375$ nm for 1.5 min (phenanthrene); $\lambda_5 = 260/420$ nm for 2.5 min (anthrancene, fluoranthene); $\lambda_6 = 254/390$ nm for 6.5 min (pyrene, benzo[*a*]anthracene, chrysene); $\lambda_7 = 260/420$ nm for 6 min (benzo[*b*]fluoranthene, benzo[*k*]-fluoranthene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, benzo-[*g,h,i*]perylene); $\lambda_8 = 293/498$ nm for 2 min (indeno[1,2,3-*cd*]-pyrene). The injection volume was 10 μL. A library database containing 31 PAH UV spectra was provided with the Hewlett-Packard HP-CHEM software and used to confirm the identity and purity of endogenous PAHs.

GC−MS System and Operating Conditions. A Hewlett-Packard 6890 series II gas chromatograph system interfaced with a 5973 series mass selective detector and a 7683 automatic sample injector was used for chromatographic analyses. The column was a Supelco SPB-5 (25 m × 0.20 mm i.d., film thickness 0.33 μm), which was coupled to a J&W deactivated fused silica precolumn (1 m × 0.53 mm i.d.). Perfluorotributylamine was used for mass calibration at *m/z* 69, 219, and 502 using the Auto Tune option. Data acquisition and processing were carried out with the Hewlett-Packard HP-CHEM data system. Operating conditions were as follows: injector port temperature 250 °C, injection volume 2 μL in pulsed splitless mode (pulsed pressure 40 psi for 1 min) turning to split mode after 0.5 min; carrier gas He at a constant flow 40 cm/s; GC−MS interface temperature 280 °C; ionization by electron impact (70 eV); oven program temperature: 80 °C (0.5 min), increased at 8 °C/min to 230 °C, then to 300 °C at 5 °C/min and held for 2 min at 300 °C.

Identification of PAHs. Analysis was performed by selected ion monitoring (SIM). The molecular ion ($M^{\bullet+}$) of each PAH was used as the target ion for detection. For confirmation of peak identity and purity, a qualifier ion was also acquired during data acquisition. Qualifier ions were $(M - H_2)^{\bullet+}$ and $(M - D_2)^{\bullet+}$ for unlabeled and perdeuterated PAH, respectively. Fluorene was an exception where $(M - H)^{\bullet+}$ was selected as qualifier ion for the unlabeled species due to its higher abundance. The target ion for fluorene-d_{10} (IS) was $(M - 2D + 2H)^{\bullet+}$ (*m/z* 174) since a quantitative exchange of two deuterium atoms from the molecule occurred during the saponification step. Furthermore, no qualifier ion was used for this isotopically labeled derivative. Confirmation of analyte identity required that the ratio of the area of the qualifier ion to that of the corresponding target ion for each PAH was within ±20% of the calibration standard's ion ratio.

Quantification of PAHs. Each PAH was quantified by means of an external calibration curve (response ratio vs amount ratio) built from standard solutions containing unlabeled PAH concentrations ranging from 0 to 80 μg/kg and corresponding perdeuterated IS concentration set to 1 μg/kg (concentrations expressed in μg per kg of meat). Calibration curves were then shortened to achieve a linear fit over the appropriate concentration of the samples (coefficient of determination (r^2) for the different calibration curves ranged from 0.986 to 0.999). A minimum of five PAH concentrations were used to generate the calibration curve. Because of a potential variation of response factors of unlabeled PAHs to those of the perdeuterated IS (Baumard et al., 1997b), calibration standards were injected before and after analyses of the samples and both data sets were used to build the calibration curve. Responses of the calibration standard solutions were within 10% before and after sample injections.

RESULTS AND DISCUSSION

Extraction Procedure. Extraction of PAHs from foods has traditionally relied on a three-step procedure including saponification, liquid−liquid partition, and final cleanup using solid-phase extraction (SPE) columns (Grimmer and Böhnke, 1975; Dennis et al., 1983; Karl and Leinemann, 1996; Chen and Lin, 1997). The shortcoming of these methods is the large quantities of samples, organic solvents, and prolonged time required for purification and analysis. Recent investigations have led to alternative methods that allow multiple extractions to be done simultaneously and include: supercritical fluid extraction (SFE) (Yeakub Ali and Cole, 1998); accelerated solvent extraction (ASE) (Wang et al., 1999); and focused microwave extraction (Letellier et al., 1999). These extraction methods have given comparable results to those given by the traditional procedure, while eliminating the need for saponification and reducing the amount of solvents required. However, subsequent cleanup steps are still necessary in these novel procedures, which so far have been mainly used for the detection of PAHs at high levels (>300 μg/kg). Furthermore, expensive apparatus are needed. Our goal was to improve upon existing solvent extraction procedures by minimizing the amount of sample, organic solvent and time required for workup and detection of PAHs at and below the μg/kg (ppb) levels.

Preliminary studies were conducted to optimize solvent and solid-phase extraction conditions to recover PAHs from meat sausages using radiolabeled ^{14}C−B(a)P. The overall recovery of ^{14}C−B(a)P spiked at a concentration of 5.9 μg/kg was 60 ± 3% ($n = 3$). The principal loss occurred by partial partitioning of B(a)P into the alkaline ethanolic saponification solvent. The recovery of B(a)P in the cyclohexane phase could be augmented either by increasing the polarity of the alcohol phase by addition of water or by additional solvent extractions with cyclohexane. However, larger quantities of nonpolar compounds and solvent would also have been collected, leading to a more extensive cleanup step. The use of both aminopropyl and C_{18} SPE columns was an effective means of further purifying PAHs from these extracts, which still contained large amounts of impurities, such as fatty acids and other lipophilic components that resulted in rapid saturation and degradation of the capillary column performance. This extraction procedure provided chromatograms of PAHs with relatively few interfering compounds and flat baselines (Figure 2). Our extraction procedure required less than 70 mL of organic solvent per analysis and allowed six to eight test portions to be extracted within 8 h, whereas current procedures are usually less favorable in terms of both solvent consumption and time (Nyman et al., 1993; Karl and Leineman, 1996; Chen and Lin, 1997). Several precautions were required to ensure reproducible recovery of PAHs during the extraction and SPE cleanup steps and include (a) photodecomposition of PAHs due photoirradiation and oxidation and (b) volatility of the lower molecular weight PAHs during solvent concentration under a nitrogen stream or by rotary evaporation (Tamakawa et al., 1992). Thus, care was exercised to protect solutions containing PAHs from light and to avoid concentration to dryness.

Chromatographic and Analytical Detection Techniques. An attempt was made to quantify PAHs in meat sausages by HPLC with fluorescence detection. However, due to impurities that coeluted with PAHs, analyte identification based on the peak retention time was insufficient to confirm the presence and purity of many PAHs. To circumvent this uncertainty of analyte identity, a UV diode array detector (DAD) was connected in series with the fluorescence detector (FLD). Fluorescence peaks were used for the quantification of PAHs, whereas the DAD detector allowed the UV spectrum to be obtained for a single peak, which could

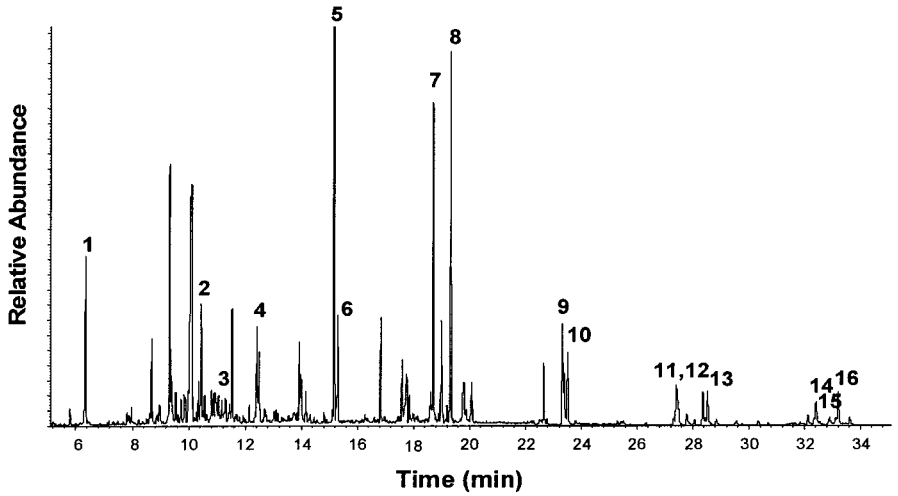

Figure 2. GC−MS/SIM profile of a meat sausage extract. Chromatogram represents the sum of the monitored ions of endogenous PAHs and internal standards spiked at a concentration of 1 μg/kg in barbecued lamb sausage. Peaks are numbered as indicated in Table 1.

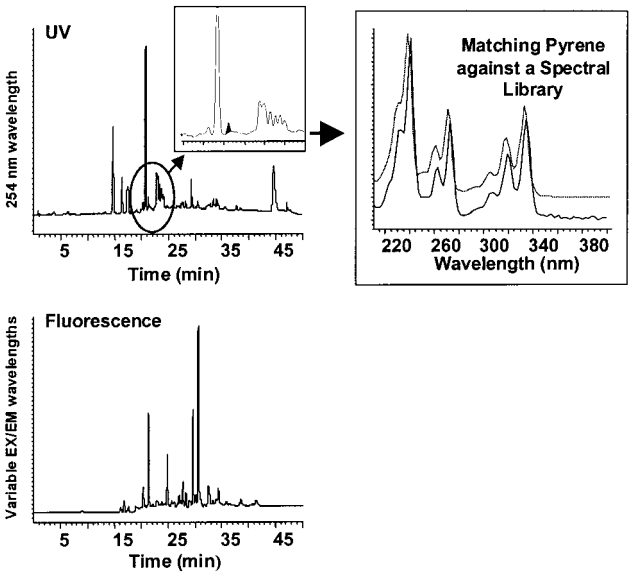

Figure 3. Analysis of PAHs by HPLC−UV and HPLC−Fluorescence. Detectors are coupled in series. The UV spectrum of a peak can be obtained by the UV-diode array detector to determine peak purity, and the spectrum may be compared to a spectral library database for PAH confirmation. The fluorescence detector, more sensitive than the UV-DAD, is used to quantify PAHs.

be compared to a spectral library database for evaluation of peak purity and structural confirmation. An example of this procedure is shown in Figure 3. In this extract, the on-line UV spectrum of pyrene was correctly matched with the library database; however, the UV spectra of many other PAHs were poorly matched with the database. The use of UV spectral analysis for analyte confirmation was restricted by the lower limit of detection (LLD) of UV detection, which is 20−320 times higher than that of the fluorescence detection (Chen et al., 1996). Therefore, relatively high PAH levels were required for corroborative on-line UV spectral analysis. We concluded that it was not possible to unambiguously establish the identity and purity of PAHs in these meat samples and to reliably quantify these chemicals by HPLC coupled with UV and fluorescence detectors.

Our GC−MS method makes use of a SIM mass detector and isotopically labeled standards for identification and quantification of the respective analytes (Johnstone and Rose, 1996). The mass spectra of PAHs, both labeled or unlabeled, have a characteristic fragmentation pattern under electron impact ionization (EI): the molecular ion ($M^{\bullet+}$) is the most abundant peak in the spectrum ($\geq$ 80% of total ions), whereas other fragments are present in low relative intensity. This weak fragmentation of PAHs enhances the sensitivity of the molecular ion for quantification, but limits the effective use of qualifier ions, due to their low abundance. The ratios of the qualifier ions to the target ions were well within ±20% of the calibration standard's ion ratio for all PAHs present at >1 μg/kg; however, when the amounts of PAHs approached the limit of quantification (i.e., <0.2 μg/kg), this ratio deviated from that of the pure standards by more than 20%. In such cases, identification of the PAHs was based upon the retention times of both molecular and qualifier ions and peak homogeneity is tentative. An example of this limitation of qualifier ion is shown in Figure 4. The ratios of the qualifier ions to the target ions of all PAHs were within the range of calibration standard ratios with the exception of benzo[*a*]anthracene. Thus, the identity of this PAH, which is just below the limit of quantification (LOQ), is tentative.

GC−MS Analysis: Validation of Quantitative Analyses and PAH Content in Meat Sausages. The limits of detection (LOD) and quantification (LOQ) were calculated as 3 times and 10 times, respectively, the standard deviation of the mean of 10 blank samples that underwent the entire workup procedure (ACS subcommittee on environmental analytical chemistry, 1980). For these 16 PAHs, the median of the LODs and LOQs were, respectively, 0.06 and 0.20 μg/kg. Traces of some PAHs were found within the analysis blank and were attributed to solvent contamination. Strongest contamination was observed for naphthalene (1.5 μg/kg), phenanthrene (0.4 μg/kg), pyrene (0.5 μg/kg), and fluoranthene (0.2 μg/kg). Each remaining PAH was found at a <0.2 μg/kg level. Thus, a blank solution was systematically run with each series of samples, and the concentration of PAHs found in the blank samples was subtracted from that of the meat samples. The entire procedure was validated by analyzing one sausage spiked at PAH concentrations of 0.5 and 1.0 μg/kg ($n = 3$). The results of the experiment are summarized in Table 1. With the

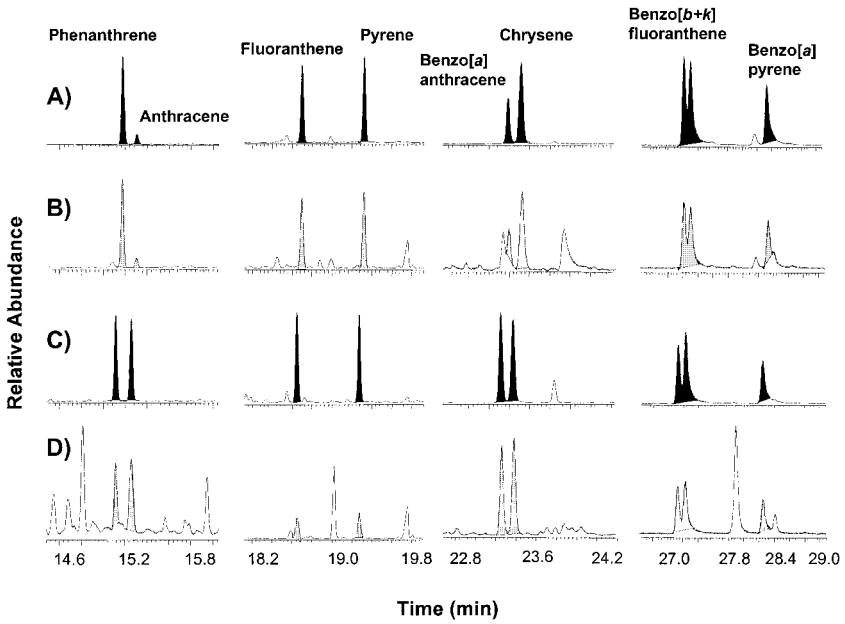

Figure 4. Chromatograms of some PAHs found in two barbecued meat sausages. The first 6 PAHs (14−24 min) were detected in the pork/beef A sample, whereas the extracted chromatograms of benzo[*b*+*k*]fluoranthene and benzo[*a*]pyrene (26−29 min) were from the Lamb B sausage: (A**)** target ion of endogenous PAHs (molecular ion); (B**)** qualifier ion of endogenous PAHs; (C**)** target ion of perdeuterated internal standards (molecular ion); (D**)** qualifier ion of internal standards.

Table 1. Percentage (%) of Expected Values of PAHs Spiked at 0.50 and 1.00 μg/kg into a Meat Sausage[a]

no.	PAHs	unspiked sausage (μg/kg)	sausage spiked at a 0.50 μg/kg level (μg/kg)	sausage spiked at a 1.00 μg/kg level (μg/kg)	mean percentage (%) of expected values at the 0.50 μg/kg level[b]	mean percentage (%) of expected values at the 1.00 μg/kg level[b]
1	naphthalene	6.55 ± 1.29	7.33 ± 0.51	8.56 ± 0.70	156	201
2	acenaphthylene	0.62 ± 0.04	1.06 ± 0.02	1.48 ± 0.01	88	86
3	acenaphthene	0.34 ± 0.03	0.91 ± 0.07	1.37 ± 0.03	115	103
4	fluorene	0.98 ± 0.13	1.27 ± 0.01	1.67 ± 0.02	60	69
5	phenanthrene	6.95 ± 0.40	7.33 ± 0.41	7.94 ± 0.11	75	99
6	anthracene	0.76 ± 0.02	1.13 ± 0.07	1.55 ± 0.03	75	79
7	fluoranthene	1.34 ± 0.03	1.69 ± 0.05	2.12 ± 0.18	71	78
8	pyrene	1.22 ± 0.38	1.63 ± 0.10	2.43 ± 0.12	84	121
9	benzo[*a*]anthracene	<0.20 (0.18)[c]	0.53 ± 0.01	0.87 ± 0.02	70	69
10	chrysene	0.46 ± 0.03	1.13 ± 0.07	1.55 ± 0.08	134	109
11	benzo[*b*]fluoranthene	<0.20 (0.11)[c]	0.50 ± 0.01	0.87 ± 0.03	78	76
12	benzo[*k*]fluoranthene	<0.20 (0.08)[c]	0.57 ± 0.01	1.04 ± 0.02	98	96
13	benzo[*a*]pyrene	<0.20 (0.07)[c]	0.46 ± 0.07	0.95 ± 0.03	78	88
14	indeno[1,2,3-*cd*]pyrene	ND[d]	0.51 ± 0.06	1.01 ± 0.04	102	101
15	dibenzo[*a*,*h*]anthracene	ND	0.51 ± 0.04	0.99 ± 0.02	101	99
16	benzo[*g*,*h*,*i*]perylene	<0.20 (0.07)[c]	0.59 ± 0.01	1.10 ± 0.10	104	103

[a] Mean and standard deviation of three independent extractions and single injection of each by GC−MS. [b] Results were corrected by the concentration of PAHs found within the unspiked sausage. [c] Concentrations in brackets are below the LOQ but were nevertheless used to calculate the mean percentage of expected values. [d] ND, not detected.

exception of naphthalene, the average of expected values ranged from 60 to 134% (median 84%) at the 0.5 μg/kg level and from 69 to 121% (median 96%) at the 1.0 μg/kg level. Results for naphthalene were consistently too high, which appears to be attributed to variable contamination of solvents and solid-phase support resins.

Two coconut oil reference materials (CRM 458 and CRM 459) containing six selected PAHs with certified concentrations at the μg/kg levels were also analyzed to further corroborate the accuracy of our method. We adapted the extraction procedure for the analysis of PAHs in these reference oils: a saponification was not done, and a back-extraction using *N*,*N*-dimethylformamide was performed to decrease the amount of lipid before the solid-phase cleanup steps. The comparison of results obtained by our analytical procedure with certified concentrations are given in Table 2, and an excellent correlation was found between the two data sets.

Table 2. PAHs in Two Reference Coconut Oils (in μg/kg)[a]

	CRM 458		CRM 459	
PAHs	certified values	exptl values	certified values	exptl values
pyrene	9.40 ± 1.50	8.07 ± 0.14	<0.90	<0.20
chrysene	4.90 ± 0.40	4.69 ± 0.04	<0.60	<0.20
benzo[*k*]fluoranthene	1.87 ± 0.18	1.78 ± 0.20	<0.20	<0.20
benzo[*a*]pyrene	0.93 ± 0.09	0.91 ± 0.06	<0.30	<0.20
indeno[1,2,3-*cd*]pyrene	1.00 ± 0.07	0.87 ± 0.03	<0.20	<0.20
benzo[*g*,*h*,*i*]perylene	0.97 ± 0.07	1.04 ± 0.03	<0.20	<0.20

[a] Comparison of values given by the present method with certified values. Mean and standard deviation of our data are the result of three independent extractions and single injection of each by GC−MS.

The results obtained from the analysis of PAHs in barbecued meat sausages are summarized in Table 3. The major PAHs present in these products did not include the compounds listed as carcinogenic by

Table 3. PAH Concentration (in μg/kg) in Barbecued Meat Sausages[a]

PAHs	turkey	beef	pork/beef A	pork/beef B	pork	lamb A	lamb B[b]
naphthalene	3.40 ± 0.92	3.38 ± 0.29	6.55 ± 1.29	29.97 ± 1.38	15.33 ± 2.20	26.26 ± 7.10	22.82 ± 1.51
acenaphthylene	0.33 ± 0.10	0.54 ± 0.12	0.62 ± 0.04	1.13 ± 0.20	1.32 ± 0.06	13.82 ± 1.31	5.29 ± 0.06
acenaphthene	0.21 ± 0.10	0.36 ± 0.01	0.34 ± 0.03	0.51 ± 0.01	0.90 ± 0.28	1.59 ± 0.67	1.22 ± 0.06
fluorene	0.89 ± 0.07	1.36 ± 0.10	0.98 ± 0.13	6.12 ± 0.10	3.13 ± 0.23	6.02 ± 0.09	4.68 ± 0.17
phenanthrene	1.41 ± 0.36	1.05 ± 0.07	6.95 ± 0.40	8.54 ± 0.99	9.19 ± 0.41	18.14 ± 0.71	38.59 ± 0.43
anthracene	0.20 ± 0.02	0.25 ± 0.06	0.76 ± 0.02	0.67 ± 0.06	1.43 ± 0.10	1.38 ± 0.12	5.63 ± 0.29
fluoranthene	<0.20	0.25 ± 0.07	1.34 ± 0.03	1.02 ± 0.09	1.77 ± 0.22	3.77 ± 0.10	15.00 ± 0.07
pyrene	<0.20	<0.20	1.22 ± 0.38	0.88 ± 0.25	1.95 ± 0.78	4.37 ± 0.32	19.26 ± 0.12
benzo[*a*]anthracene[c]	<0.20	ND	<0.20	ND	<0.20	0.44 ± 0.03	2.49 ± 0.14
chrysene	<0.20	ND	0.46 ± 0.03	0.20 ± 0.01	0.51 ± 0.02	1.51 ± 0.36	7.09 ± 0.26
benzo[*b*]fluoranthene[c]	ND[d]	ND	<0.20	ND	<0.20	0.34 ± 0.07	2.41 ± 0.05
benzo[*k*]fluoranthene[c]	ND	ND	<0.20	ND	<0.20	0.48 ± 0.06	2.97 ± 0.06
benzo[*a*]pyrene[c]	ND	ND	<0.20	ND	ND	0.32 ± 0.02	2.81 ± 0.04
indeno[1,2,3-*cd*]pyrene[c]	ND	ND	ND	ND	ND	0.30 ± 0.08	2.12 ± 0.10
dibenzo[*a*,*h*]anthracene[c]	ND	ND	ND	ND	ND	ND	0.37 ± 0.03
benzo[*g*,*h*,*i*]perylene	ND	ND	<0.20	<0.20	ND	0.45 ± 0.08	4.60 ± 0.24

[a] Mean and standard deviation of three independent extractions and single injection of each by GC−MS. [b] Sample barbecued under severe conditions. [c] Compounds listed as carcinogenic by IARC. [d] ND, not detected.

Table 4. Comparison of Results (in μg/kg) Obtained When either 16, 5, or 1 Internal Standard (IS) Is Used for Quantification

no.	PAHs	**16 IS**[a]	**5 IS**[b]		**1 IS**[c]	
1	naphthalene	22.82 ± 1.51	11.83 ± 2.15	**−48**[d]	12.33 ± 1.57	**−46**[d]
2	acenaphthylene	5.29 ± 0.06	7.29 ± 0.21	**+38**	7.09 ± 0.22	**+34**
3	acenaphthene	1.22 ± 0.06	1.22 ± 0.06	**0**	1.03 ± 0.10	**−16**
4	fluorene	4.68 ± 0.17	4.96 ± 0.26	**+6**	1.19 ± 0.05	**−74**
5	phenanthrene	38.59 ± 0.43	38.59 ± 0.43	**+0**	51.60 ± 2.05	**+34**
6	anthracene	5.63 ± 0.29	6.65 ± 0.28	**+18**	8.04 ± 0.15	**+43**
7	fluoranthene	15.00 ± 0.07	18.78 ± 0.36	**+25**	25.96 ± 0.50	**+73**
8	pyrene	19.26 ± 0.12	25.58 ± 1.00	**+33**	25.58 ± 1.00	**+33**
9	benzo[*a*]anthracene	2.49 ± 0.14	5.81 ± 0.31	**+133**	5.81 ± 0.31	**+133**
10	chrysene	7.09 ± 0.26	7.05 ± 0.26	**0**	7.05 ± 0.26	**0**
11	benzo[*b*]fluoranthene	2.41 ± 0.05	2.93 ± 0.12	**+22**	5.80 ± 0.21	**+141**
12	benzo[*k*]fluoranthene	2.97 ± 0.06	1.49 ± 0.01	**−50**	3.27 ± 0.19	**+10**
13	benzo[*a*]pyrene	2.81 ± 0.04	2.81 ± 0.04	**0**	5.85 ± 0.29	**+108**
14	indeno[1,2,3-*cd*]pyrene	2.12 ± 0.10	5.82 ± 0.43	**+174**	6.25 ± 0.36	**+195**
15	dibenzo[*a*,*h*]anthracene	0.37 ± 0.03	0.54 ± 0.04	**+47**	0.64 ± 0.05	**+75**
16	benzo[*g*,*h*,*i*]perylene	4.60 ± 0.24	4.60 ± 0.24	**0**	5.15 ± 0.16	**+12**

[a] Each PAH was quantified against its own deuterated analogue. Estimates presented as mean ± standard deviation ($n = 3$). [b] Nos. 1−4 quantified against acenaphthene-d_{10}; nos. 5−7 (phenanthrene-d_{10}); nos. 8−10 (chrysene-d_{12}); nos. 11−13 (benzo[*a*]pyrene-d_{12}); nos. 14−16 (benzo[*g*,*h*,*i*]perylene-d_{12}). [c] PAHs were all quantified against chrysene-d_{12}. [d] Deviation (%) to results obtained when each PAH is quantified against its own deuterated analogue.

the IARC (IARC, 1987) (benzo[*a*]anthracene, benzo[*b*]-fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene and dibenzo[*a*,*h*]anthracene). The exceptions to these data on PAH content were the high fat content lamb sausage (lamb A), where the sum of carcinogenic PAHs was 1.88 μg/kg, and the heavily barbecued lamb B sausage where the sum of carcinogenic PAH content was 13.17 μg/kg. These results are in accordance with previous findings, where PAH formation during charcoal grilling was shown to be dependent upon the fat content of the meat, the time of cooking and the temperature (Lijinsky and Ross, 1967; Doremire et al., 1979).

Choice of the Number of IS To Use for Reliable PAH Quantification. Some investigators (Baumard et al., 1997b) used either 1 or 6 perdeuterated PAHs for the analysis of PAHs in marine sediments and demonstrated the necessity of several IS of varying degrees of aromaticity when PAHs of different structures were analyzed. It was concluded that at least one IS per class of aromaticity should be used for an accurate quantification of PAHs. We followed this idea and compared results obtained when either 1, 5, or 16 IS were used. Our data were reprocessed to build the adapted calibration curves and the PAHs found within the heavily contaminated lamb sausage (lamb B sample) recalculated as shown in Table 4. As expected, larger deviations occurred when only one IS (chrysene-d_{12}) was used, indicating that the recovery of perdeuterated chrysene from the extract is significantly different from some of the other PAHs. When 5 IS were used, deviations still occurred. The effect of using an insufficient number of IS may be important when calculating the sum of the IARC listed carcinogenic PAHs, i.e., for risk assessment studies. In our example, this sum varies from 13.17 μg/kg when each PAH is quantified relative to its own perdeuterated analogue up to 27.62 μg/kg when only 1 IS is used. Therefore, due to the different physicochemical properties of PAHs, an accurate quantification of the 16 PAHs listed as priority pollutants by the EPA requires that each PAH be quantified against its own perdeuterated analogue.

ABBREVIATIONS USED

PAH, polycyclic aromatic hydrocarbon; IS, internal standard; GC−MS, gas chromatography−mass spectrometry; SPE, solid-phase extraction; DAD, diode array detector; FLD, fluorescence detector; B(a)P, benzo[*a*]-pyrene; SIM, selected ion monitoring; SFE, supercritical fluid extraction; ASE, accelerated solvent extraction; LOD, limit of detection; LOQ, limit of quantification.

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