ACTA CHROMATOGRAPHICA, NO. 14, 2004

ORGANIC COMPOUNDS FORMED IN THERMALLY TREATED HIGH-PROTEIN FOOD PART I: POLYCYCLIC AROMATIC HYDROCARBONS

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SUMMARY

This work concerns the use of analytical procedures to determine polycyclic aromatic hydrocarbons (PAH) in heat-treated meat dishes prepared under household cooking conditions. These compounds have not yet been analysed in food in Poland. Application of tandem solid-phase extraction (SPE) with columns filled with Extrelut diatomaceous earth and C_{18} phase, and column chromatography on silica gel enabled selective isolation of PAH fractions from the meat sample matrix. Identification and quantitative analysis of the individual compounds were achieved by HPLC and GC–MS. Five PAH were identified and quantified in nine meat (beef, pork, and poultry) dishes prepared according to recipes used for cooking in Upper Silesia (roasted, fried) and in grilled dishes. The total PAH content was within the range 2.43–16.10 ng g^{-1} meat.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH), which can be found everywhere in the environment, are very well known ecotoxicants which are harmful to human health [1–3]. In mammalian cells PAH undergo metabolic activation to diol epoxides that bind covalently to cellular macromolecules, including DNA, thereby causing errors in DNA replication and mutations that initiate the carcinogenic process [4–6].

Food can be one source of PAH. Although the compounds can be taken up from the environment, i.e. from water, soil, and air, and can accumulate in plants, fruit, vegetables, grain, fats, meat, and seafood, and can accumulate in food during its transport and storage, they are primarily

formed as a the result of thermal treatment of the food [7]. When food, particularly meat, meat products, and fish, is smoked, roasted, barbecued, and grilled, PAH are formed as a result of incomplete combustion or thermal decomposition (pyrolysis) of the organic material. If the meat is in direct contact with the flame, pyrolysis of the fats in the meat generates PAH that become deposited on the meat. PAH production by cooking over charcoal (barbecued, grilled) is a function of both the fat content of the meat and the proximity of the food to the heat source [8–10]. It should be stressed that a Polish Standard for permissible PAH or benzo(*a*)pyrene (B(a)P) levels in food has not yet been established.

Isolation, identification, and quantitative determination of PAH in a complex food matrix suffers from three main problems:

- most PAH so far identified occur in food at microtrace levels, i.e. ppb or ppt levels, which makes their selective separation very difficult;
- many other organic components are co-extracted from the matrix with the PAH and make identification of the PAH by chromatographic and spectral methods difficult; and
- PAH are characterised by structural similarity and many occur as isomers, which again makes identification of individual compounds extremely difficult.

In samples of meat and meat products, especially thermally treated, the presence of such components as proteins, lipids, and compounds similar to PAH (e.g. azaarenes, aminoazaarenes) makes identification of PAH difficult. For this reason the first stage of any procedure for analysis of these compounds is successful removal from the matrix. Most methods used for isolation of PAH from homogenized meat samples involve saponification of lipids by methanolic KOH solution. Methods of extraction are subsequently used to isolate the PAH-containing fraction [8]. These methods include liquid–liquid extraction [11–14], Soxhlet or sonication extraction [15–17], solid-phase extraction (SPE) [11–14,18], and, more recently, supercritical-fluid extraction (SFE) [19,20], microwave-assisted extraction (MAE) [21,22], and accelerated solvent extraction (ASE) [23, 24]. For enrichment of the PAH fraction chromatographic methods such as column chromatography with SiO_2 or Florisil as stationary phase [11, 12,15,18,23], gel-permeation chromatography [12,17,24], or preparative HPLC [13] are most often used as clean-up procedures.

Thin-layer chromatography with UV detection [25] has been widely used for identification of PAH. More recently TLC with spectrofluorimetric detection has been used for quantitative determination of individual

PAH [10]. Capillary gas chromatography–mass spectrometry has also been widely used to determine PAH in food samples [12,14,23,26,27].

Although GC has high resolving power, most PAH can be decomposed by exposure to high temperature during the separation. Another problem is that some isomeric PAH are difficult to separate. High-performance liquid chromatography (HPLC) with fluorescence or UV diode-array detection has recently been more often used for quantitative and qualitative determination of PAH [11,13,15,17,18,24]. Although the resolving power of HPLC is lower than that of GC, HPLC is still an ideal means of fractionation of PAH for subsequent analysis by spectroscopic techniques. Reversed-phase columns can readily separate several PAH isomers that are difficult to separate by GC and the application of spectrofluorimetric detectors results in greater sensitivity and selectivity, which is essential for quantification of PAH separated from food samples [28,29].

This report describes the analysis of PAH formed in meat samples prepared under household cooking conditions characteristic of the Silesian region of Poland. Solid phase extraction and column chromatography were used for isolation of the compounds and reversed-phase HPLC and GC–MS were used for qualitative and quantitative analysis.

EXPERIMENTAL

Materials, Reagents, Samples

The compounds studied were fluoranthene, benzo(*a*)anthracene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, and benzo(*g*,*h*,*i*)perylene (Promochem, Wesel, Germany). The abbreviations used, the structures of the compounds, and the detection limits achieved by HPLC are given in Table I.

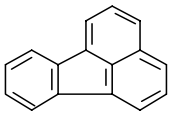
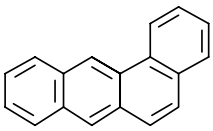
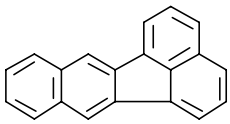
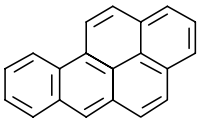
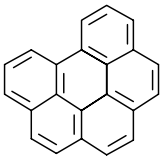
The solvents used in mobile phases (acetonitrile, dichloromethane, toluene, *n*-hexane, and methanol) were HPLC grade (J.T. Baker, Groß-Gerau, Germany).

Diatomaceous earth extraction columns (Extrelut, 20 mL) and refill material were from Merck (Darmstadt, Germany). Propylsulphonic acid (PRS, 500 mg) SPE columns (J.T. Baker) were preconditioned with dichloromethane (4 mL). Silica gel, 70–230 mesh, (Merck) was used for column chromatography. It was activated at 200°C for 12 h and preconditioned with *n*-hexane (25 mL).

Ten samples of grilled meat and meat thermally treated under household conditions in accordance with the recipes most often used by

Table I

The structures and detection limits of the polycyclic aromatic hydrocarbons determined

Compound	Abbreviation	Structure	Detection limit (ng)[a]
Fluoranthene	Fln		0.04
Benzo(*a*)anthracene	B(a)A		0.02
Benzo(*k*)fluoranthene	B(k)Fln		0.04
Benzo(*a*)pyrene	B(a)P		0.01
Benzo(*g*,*h*,*i*)perylene	B(g,h,i)P		0.01

[a]Detection limits (based on $S/N = 3$) were determined by loading standard mixtures of PAH directly on to a HPLC column by use of a 20-µL loop injector

the inhabitants of Silesia, i.e. pork chops (coated in bread-crumbs and egg), beef collar, pork neck, beef/pork minced chop and turkey breast were investigated. Pork neck, pork fillet, and chicken breast were also grilled. The list of meat dishes and the conditions used for preparation are given in Table II.

Methodology

The PAH fraction was isolated by use of procedures based on tandem solid-phase extraction on diatomaceous earth and use of SPE columns

Table II

Details of procedures used to prepare meat samples under household conditions

Type of meat	Cooking method (and amount of cooking)	Household cooking conditions
Pork chop	Pan-fried (well done)	Boneless meat (0.5 kg) was sliced into portions (150 g, 2 cm thick) and pounded into thinner slices which were then coated with eggs and bread crumbs and fried for 15 min on each side in a Teflon-coated frying pan using 'Planta' margarine. Meat was placed in fat preheated to 230°C. Frying temperature measured in the centre of the pan was between 190 and 200°C. After frying the fat was drained off by use of filter paper.
Beef collar	Pan-fried (medium)	Meat slices (150 g, 1.5 cm thick) were pounded into thinner slices then covered with smoked bacon, onions, and pickles. After coating the roulades were fried using peanut oil in a Teflon-coated frying pan. Roulades were placed in fat preheated to 200°C and were fried without covering for 20 min. Temperature during frying ranged between 150 and 160°C. Water was then added and the meal was simmered under cover for 1 h at 90–95°C. The coating was removed before analysis.
Pork neck (no. 1)	Grilled (very well done)	Neck pieces (150 g, 2 cm thick) were grilled for 30 min (15 min each side) on a common garden-type grill fuelled with charcoal. A total of 1 kg of meat was grilled.
Pork neck (no. 2)	Roasted ('on salt'; well done)	Neck meat (1 kg) was placed on a steel sheet previously covered with 1 kg salt and the whole preparation was put in an electric oven preheated to 220°C. The meat was roasted at 180°C for 3 h. This recipe yields the so-called pork neck 'on salt' roasted without additional fat.
Beef/pork minced chop	Pan-fried (very well done)	Minced beef/pork (0.5 kg) was mixed with 1 egg, 2 tablespoons breadcrumbs, and 1 tablespoon sour cream. Burgers 4 cm in diameter and 1.5 cm thick were then formed and covered with breadcrumbs. The burgers were fried for 12 min on each side, in 'Planta' margarine, in a Teflon-coated frying pan. The meat was placed in fat preheated to 230°C. Frying temperature measured in the centre of the pan was between 190 and 200°C.
Turkey breast (no. 1)	Pan-fried (well done)	Meat (0.75 kg) was sliced into 150–200 g, 1.5-cm-thick portions and lightly pounded. The slices were fried for 15 min on each side, in 'Planta' margarine, in a Teflon-coated frying pan. The meat was placed in fat preheated to 230°C. Frying temperature measured in the centre of the pan was between 190 and 200°C. After frying the fat was drained off by use of filter paper.
Turkey breast (no. 2)	Roasted (well done)	Meat (0.75 kg) was sliced into 150–200 g, 1.5-cm thick portions which were lightly pounded, brushed with vegetable oil, wrapped in aluminium foil, then placed in the oven preheated to 220°C. The meat was roasted at 160°C for 1 h.
Pork fillet	Grilled (very well done)	Four pork fillets (1 kg) were grilled for 15 min on each side using a garden-type grill fuelled with charcoal.
Chicken breast	Grilled (very well done)	Six pieces of chicken breast (1 kg) were grilled for 15 min on each side using a garden-type grill fuelled with charcoal.

filled with propylsulphonic acid (PRS). The eluted PAH fraction was cleaned by column chromatography with SiO_2 as stationary phase.

To evaluate the recovery of PAH separated and analysed by use of this procedure, and to prevent matrix effects affecting peak positions in HPLC or GC–MS chromatograms, spiked and unspiked meat samples were analysed under the same conditions. At the beginning of the homogenisation and extraction step minced meat samples (5 g) were spiked by addition of 40 ng of each of the five standards Fln, B(a)A, B(k)Fln, B(a)P, and B(g,h,i)P.

Clean-Up Procedure

The separation procedure, presented schematically in Fig. 1, involves four steps. Meat (25 g) was homogenised for 1 min with 75 mL cold 1 M NaOH solution. From the dense suspension obtained, 20 g (containing 5 g meat) was sampled four times (equivalent to a total of 20 g meat). The standards were added to the remaining portion (20 g suspension, i.e. 5 g meat + standards) to furnish a spiked sample. Each sample was mixed separately with Extrelut refill material (approx. 20 g) and the mixture obtained was placed in a 20-mL Extrelut column. After filling, each of the five columns was connected to a PRS SPE column and the PAH fraction was eluted with dichloromethane. The dichloromethane extract was evaporated to dryness and the residue was re-dissolved in *n*-hexane (1 mL) and applied to the top of a glass column packed with deactivated silica gel (10 g). The column was eluted with *n*-hexane (25 mL, discarded) and 60:40 (*v*/*v*) *n*-hexane–dichloromethane (60 mL, PAH fraction). The PAH extract was evaporated and the residue dissolved in acetonitrile (250 µL).

High-Performance Liquid Chromatography

HPLC analysis of PAH was performed with a Knauer (Berlin, Germany) liquid chromatograph equipped with a fluorescence detector (λ_{ex} = 360 nm, λ_{em} = 460 nm) and a 20-µL loop injector. Compounds were separated on a 250 mm × 4.6 mm i.d., 5 µm particle size, ChromSpher PAH column (Varian, Candela, Warsaw) with 84:16 (%, *v*/*v*) acetonitrile–water as mobile phase. Separations were performed under isocratic conditions (flow rate 2.5 mL min^{-1}). Every fraction studied was passed through a 0.45-µm filter (Bakerbond, Darmstadt, Germany) before injection on to the HPLC system.

The identities of the compounds were established by comparing the retention factors (*k*) of the peaks with those obtained from a standard

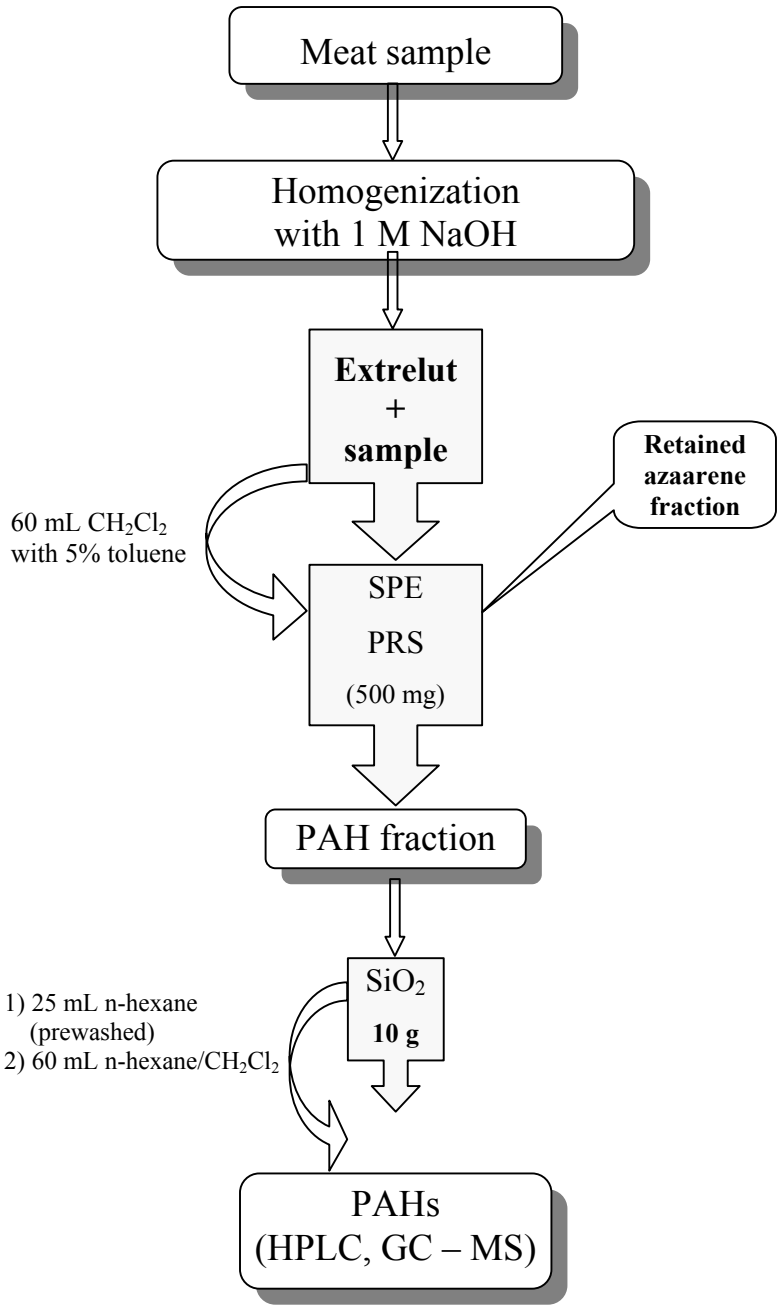


Fig. 1

Schematic diagram of the clean-up procedure

mixture of PAH and from spiked samples analysed under the same conditions.

Quantitative determination was performed by use of an external calibration curve method. The coefficients of determination (r^2) for the PAH standard calibration plots were 0.988 for fluoranthene, 0.982 for benzo(*a*)anthracene, 0.991 for benzo(*k*)fluoranthene, 0.975 for benzo(*a*)-pyrene, and 0.989 for dibenzo(*g*,*h*,*i*)perylene.

Capillary Gas Chromatography–Mass Spectrometry

The identities of peaks in the PAH fractions were confirmed by GC–MS (Shimadzu, Kyoto, Japan, QP-2000, GC 14). The compounds were separated on a 25 m × 0.2 mm fused silica capillary column coated with a 0.25-µm film of Ultra 1 (Hewlett–Packard, Vienna, Austria). The injector and interface were operated at 280°C. The column oven temperature was maintained at 120°C for 7 min after injection then programmed linearly at 20° min^{-1} to 200°C, then at 3° min^{-1} to 280°C, which was maintained for 25 min. Helium, at 1 mL min^{-1}, was used as carrier gas. The Shimadzu quadrupole mass spectrometer was operated in electron-impact mode (electron energy 70 eV, source temperature 250°C).

RESULTS AND DISCUSSION

Identification of five PAH often determined in food [8] was achieved on the basis of comparison of HPLC retention factors (k') with those of standards and of PAH determined in spiked and unspiked meat samples. Typical HPLC chromatograms of PAH are presented in Fig. 2 and results from quantitative determination of PAH in meat dishes by HPLC are listed in Table III. It is apparent from these data that only in the sample of roasted ('on salt') pork neck (Table II, no. 2) were no PAH identified. Carcinogenic benzo(*a*)anthracene, benzo(*k*)fluoranthene, and benzo(*a*)pyrene were found in every other meat sample. Data from GC–MS analysis, used to confirm results from HPLC, are listed in Table IV. They show that fluoranthene, benzo(*a*)anthracene, and benzo(k)fluoranthene were found in almost every meat dish. In a very few samples the presence of PAH was not confirmed by GC–MS, probably because of the higher detection limit (several orders of magnitude) of GC–MS compared with HPLC.

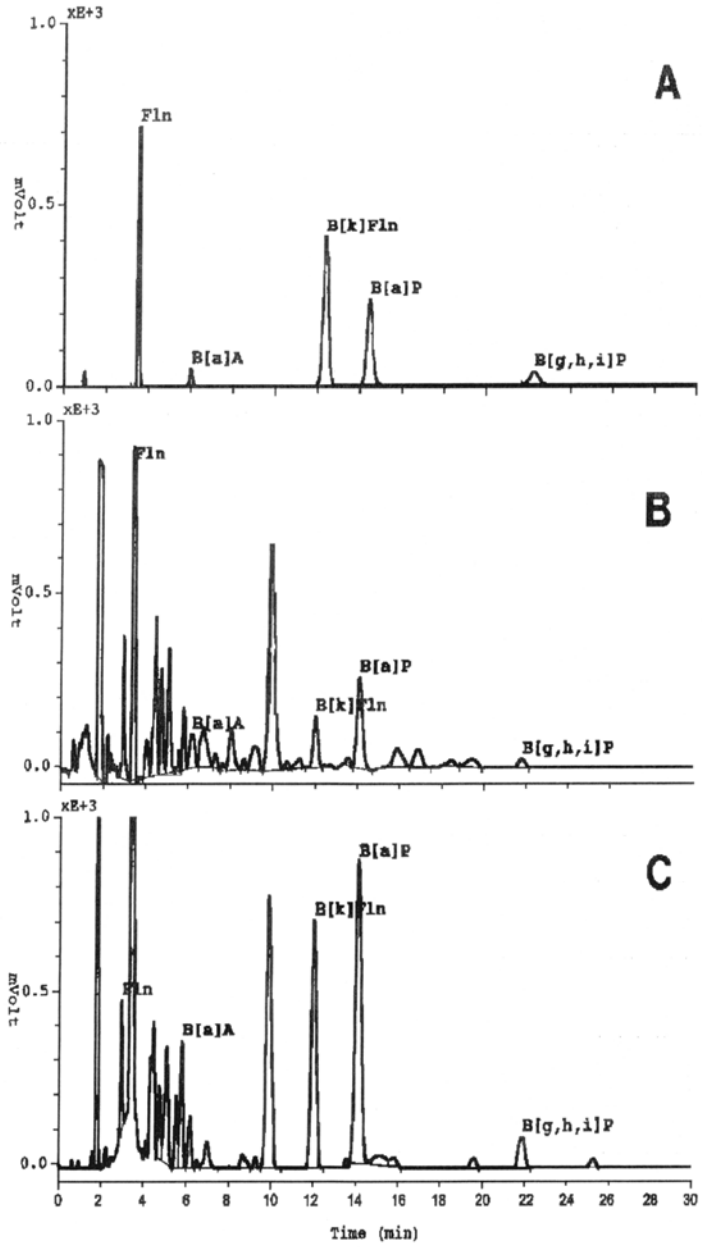


Fig. 2

Typical HPLC chromatograms obtained from analysis of PAH: (A) standard mixture; (B) fraction isolated from spiked grilled pork neck (no. 1 in Table II) and (C) fraction isolated from unspiked grilled pork neck

Table III

Polycyclic aromatic hydrocarbon content of meat samples (ng g^{-1} cooked meat)[a], and daily human exposure (μg day^{-1} $person^{-1}$)[b]

Type of meat[c]	Fln	B(a)A	B(k)Fln	B(a)P	B(g,h,i)P	Total content	Daily human exposure to PAH
Pork chop	2.10	11.90	0.32	0.41	0.99	15.72	1.57
Beef collar	0.62	0.75	0.21	0.38	0.81	2.77	0.28
Pork neck (no. 1)	0.63	10.78	1.58	2.25	0.86	16.10	1.60
Pork neck (no. 2)	n.d.[d]	n.d.	n.d.	n.d.	n.d.	–	–
Beef/pork minced chop	2.21	n.q.[e]	0.05	0.12	0.27	2.65	0.27
Turkey breast.(no. 1)	0.88	1.28	0.16	0.11	n.d.	2.43	0.24
Turkey breast.(no. 2)	1.04	6.21	1.54	3.93	n.d.	12.72	1.27
Pork fillet	2.34	2.76	0.21	0.71	1.26	7.28	0.73
Chicken breast	0.57	6.64	0.27	0.15	0.63	8.26	0.83
Mean recovery, %	70.0	60.0	60.4	66.5	53.0		

[a]Values are corrected for recovery
[b]Daily human exposure to five PAH was calculated on the basis of amounts in 100 g meat consumed daily
[c]Cooking methods and amount of cooking are listed in Table II
[d]Not detected
[e]Analyte nearly at its detection limit, i.e. detected in the background but not quantified

Table IV

Results from GC–MS identification of PAH in the meat samples

Type of meat	Fln (178[a]), t_R = 17.50 min	B(a)A, t_R = 25.20 min	B(k)Fln, t_R = 32.03 min	B(a)P, t_R = 33.90 min	B(ghi)P, t_R = 42.20 min
Pork chop	+	+	+/–	+	–
Beef collar	+	+	–	–	–
Pork neck (no. 1)	+	+	+	–	–
Pork neck (no. 2)	+	–	–	–	–
Beef/pork minced chop	+	+	–	–	–
Turkey breast (no. 1)	+	+	+/–	+/–	–
Turkey breast (no. 2)	+	+	+	+	+/–
Pork fillet	+	+/–[b)]	+	+	+
Chicken breast	+	+	+/–	+	+
Detection limit (ng)[b]	1.25	1.25	1.25	1.25	2.00

[a]Molecular ion (and base peak) for the determined PAH
[b]Amount of PAH standard (ng) introduced on to the column
'+' denotes compound identified on the basis of the mass spectrum and appropriate t_R of the PAH
'+/–' denotes mass spectrum contains peaks characteristic of PAH and additional peaks that might originate from fragmentation of other compounds
'–' denotes absence of peaks corresponding to the investigated PAH

Typical mass spectra obtained from the PAH identified in the meat samples are presented in Fig. 3.

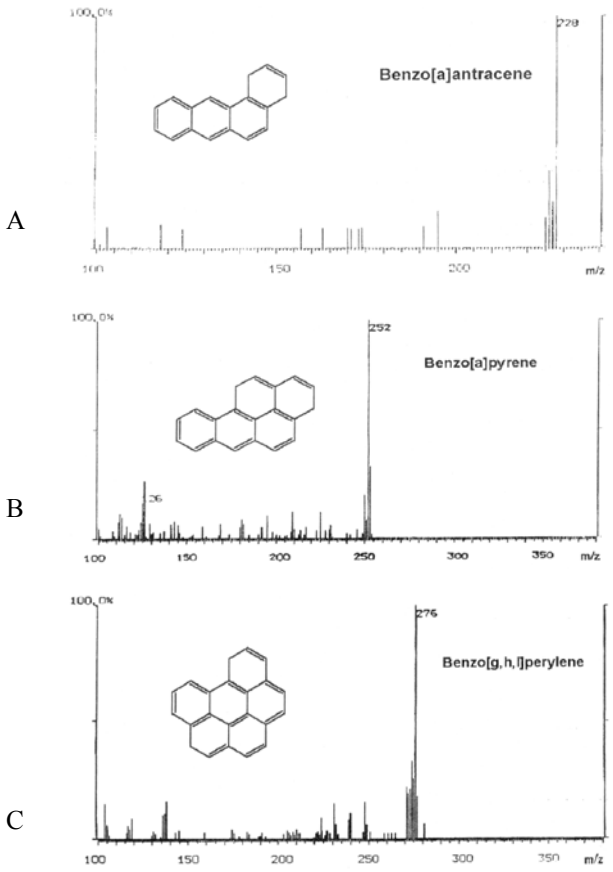


Fig. 3

Typical mass spectra of PAH identified in the meat samples investigated: (A) grilled chicken breast; (B) grilled pork fillet; (C) roasted turkey breast

It is apparent from Table III that the total PAH content of the meat examined falls within the range 2.43–16.10 ng g^{-1}. Recoveries of the individual PAH were from 53 to 70%. The total amounts of the compounds investigated in samples of grilled meat were: pork neck (no. 1) 16.10 ng g^{-1}; chicken breast 8.26 ng g^{-1}; well-done pan-fried pork chop 15.72 ng g^{-1}; well-done roasted turkey breast 12.72 ng g^{-1}. The high levels of carcinogenic benzo(*a*)anthracene – in the range 0.75–11.90 ng g^{-1} in almost every

meat dish – should be particularly stressed. Similar results were obtained by Chen et al. [15], who analysed samples of grilled pork neck, in which the concentration of B(a)A was up to 31.8 ng g^{-1}. It should, however, also be noted that levels of the most carcinogenic compound, benzo(*a*)pyrene, in most of the meat dishes investigated were not very high, in the range 0.11–3.93 ng g^{-1} (Table III).

On the basis of these results it seems that levels of particular PAH and their total concentration depend mainly on the preparation of the meat dish and the amount the meat is cooked. Other scientists have obtained similar results. For example, Wu et al. [12] proved that the highest quantities of the PAH fluoranthene, benzo(*a*)anthracene, and benzo(*k*)fluoranthene were formed in pork meat barbecued on an iron grill over an open charcoal flame. Kazerouni et al. [10], who investigated the benzo(*a*)pyrene content of 200 different meat dishes, found levels in thermally treated food to be highest in very-well-grilled or barbecued meat. For example, depending on the type of meat, its method of preparation, and the amount of cooking, the B(a)P concentration fell within the ranges: beef 0.01–4.86 ng g^{-1}, boneless chicken 0.1–0.4 ng g^{-1}, and pork 0.01–0.2 ng g^{-1}. Philips et al. [8] found the benzo(*a*)pyrene content and total PAH content were highest in grilled and barbecued food. In such meat dishes the total concentration of PAH can reach even 164 ng g^{-1} and the level of benzo(*a*)pyrene can be up to 30 ng g^{-1}.

Table III shows values for daily human exposure to the five determined PAH, calculated on the basis of their levels in 100 g of meat, consumed daily. These values fall within the range 0.24–1.60 μg day^{-1} $person^{-1}$. Studies conducted by Schoket [4] prove that frequent consumption of large amounts of grilled or barbecued beef, e.g. 280 g meat every day in one week, can result in human exposure to benzo(*a*)pyrene at a level of 0.72 μg day^{-1} $person^{-1}$, and that for non-smokers who eat huge amounts of hamburgers that value can reach 1.5 μg day^{-1} $person^{-1}$ [30]. Epidemiological studies conducted in highly developed countries to estimate the health hazard of PAH ingested in the diet show that, assuming daily consumption of 1.46 kg food, the daily dose of PAH can be 3.7 μg in Great Britain, 5–17 μg in Germany, 1.2 μg in New Zealand, and 3 μg in Italy [8].

In Table V results from PAH determination are compared with literature data. This comparison shows that food prepared using Upper Silesian recipes contains smaller quantities of PAH than indicated in the literature.

Table V

Comparison of the results from determination of PAH with literature data

PAH	Amounts in the meat samples investigated (ng g^{-1})	Literature data (ng g^{-1} in meat and sausages) [8,10,12,14,15,23]
Fluoranthene	0–2.34	0–42.2
Benzo(*a*)anthracene	0–11.90	0.2–31.8
Benzo(*k*)fluoranthene	0–1.58	0–11
Benzo(*a*)pyrene	0–3.93	0.01–4.86
Benzo(*g*,*h*,*i*)perylene	0–1.26	0–3

CONCLUSIONS

The multistep clean-up procedure used, based on tandem solid-phase extraction, enabled separation of PAH fractions from the cooked meat samples. Food prepared according to traditional recipes of Upper Silesian cuisine contained PAH in the range 2.43–16.10 ng g^{-1} meat; these concentrations are not high compared with literature data. The benzo(*a*)pyrene content did not exceed 10 ng g^{-1}, the value fixed in 1987 by the FAO and the WHO as the maximum permissible concentration of B(a)P in food. It has been shown that high daily PAH intake can result from consumption of well-done pan-fried pork chop (1.57 μg day^{-1} $person^{-1}$), very well-done grilled pork neck (1.60 μg day^{-1} $person^{-1}$), and well-done roast turkey breast (1.27 μg day^{-1} $person^{-1}$).

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