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Analytical Methods

Analysis of azaarenes in pan fried meat and its gravy by liquid chromatography with fluorescence detection

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Abstract

A method for analysis of six azaarenes (benzo[*h*]quinoline, benzo[*a*]acridine, benzo[*c*]acridine, dibenzo[*a*,*c*]acridine, dibenzo[*a*,*j*]acridine and dibenzo[*a*,*h*]acridine) in thermally treated high-protein food has been described. The clean-up procedure used based on alkaline hydrolysis, tandem solid phase extraction on columns filled with Extrelut – diatomaceous earth and cation exchanger (propyl sulfonic acid), enabled a selective isolation of carcinogenic compounds belonging to benzoacridines and dibenzoacridines from samples of cooked meat and its gravy. The isolated fractions of aza-PAHs were analysed by high-performance liquid chromatography with fluorescence detection. The detection limits for the azaarenes were between 0.0001 ng and 0.005 ng loaded on column. The recoveries for the four-ring and five-ring azaarenes were from 55% to 67%. Two types of dishes prepared from pork by pan-frying were investigated. Total contents of the benzoacridines and dibenzoacridines determined in cooked meat were 1.57 and 2.50 ng/g in collar and chop samples, respectively; their gravies contained 0.34 and 0.59 ng of these azaarenes per g of cooked meat.
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1. Introduction

Azaarenes (aza-PAHs) are nitrogen heterocycles, analogues of polynuclear aromatic hydrocarbons (PAHs). The majority of them found in the environment originate from anthropogenic source (Bleeker et al., 2002; Wilhelm, Matuschek, & Kettrup, 2000). They are formed as a result of pyrolysis or incomplete combustion of organic matter, especially coal and crude oil products (Nito & Ishizaki, 1997). Azaarenes, particularly four- and five-ring compounds, in many cases express higher mutagenic and carcinogenic activity in comparison to their corresponding PAHs (Bleeker et al., 1999, 2002; Sovadinova et al., 2006). Most metabolites of azaarenes (dihydrodiols and diol-epoxides) have strong carcinogenic activity when tested on experimental animals (Kumar et al., 2001). Aza-PAHs were determined in vehicle exhaust, aerosols of urban atmosphere (Chen & Preston, 2004; Warzecha, 1993), soils (Koči, Petrovská, Šimek, VaraĎová, & Syslová, 2007; Švabenský, Koči, & Šimek, 2007), groundwater, river and lake sediments (Machala, Ciganek, Bláha, Minksová, & Vondráček, 2001; Wakeham, 1979), sewage sludges (Bodzek, Janoszka, & Warzecha, 1996). Some investigations on their microbial degradation as one of the most important factors in reducing concentration of these pollutants in the environment have been carried out (Kimura & Omori, 1995; Willumsen, Johansen, Karlson, & Hansen, 2005).

In cooked meat azaarenes may be formed as the result of pyrolysis of nitrogen containing organic matter, especially from aromatic amino acids (Galceran, Curto, Puignou, & Moyano, 1994; Grimmer & Naujack, 1986; Rivera, Curto, Pais, Galceran, & Puignou, 1996). Contents of azaarenes in grilled and home cooked meat were

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determined by us at the level of some ng/g (Janoszka, Warzecha, Błaszczyk, & Bodzek, 2004a) and was lower than those of PAHs in these samples (Janoszka, Warzecha, Błaszczyk, & Bodzek, 2004b). While the concentration of PAHs, mainly of benzo[*a*]pyrene (B[*a*]P), is often determined in different types of food (Fontcuberta et al., 2006; Kazerouni, Sinha, Che-Han Hsu, Greenberg, & Rothman, 2001), the analysis of azaarenes accompanying them is not common. Grimmer and Naujack (1986) as well as Joe, Salemme, and Fazio (1986) first described methods of aza-PAHs analysis in thermally treated meat. The methods developed by these authors consisted of liquid–liquid extraction of azaarenes by organic solvents and sulphuric or hydrochloric acid, purification by column chromatography and determination of benzoacridines, dibenzoacridines and their methyl derivatives by gas chromatography with flame ionisation detector (GC–FID) (Grimmer & Naujack, 1986). Galceran et al. (1994) described the procedure of isolating acridine derived compounds from charcoal-grilled meat, which includes saponification with NaOH, enrichment by liquid–liquid partition with cyclohexane, extraction of azaarenes with H_2SO_4 and column chromatography on alumina. The determination of aza-PAHs was performed by gas chromatography–mass spectrometry (GC–MS) with single ion monitoring (SIM) mode. Rivera et al. (1996) developed a chromatographic method that enabled a simultaneous determination of three groups of mutagenic and/or carcinogenic compounds in a grilled meat sample: PAHs, aza-PAHs and heterocyclic amines. This method includes coupling of solid-phase extraction (SPE) columns filled with diatomaceous earth, propyl sulfonic acid and RP-18 phase. Azaarenes isolated by this procedure were determined by HPLC–UV method.

Gravy – a by-product of thermally treated proteinaceous food – frequently consumed directly or used for sauce preparation, has not yet been investigated for azaarenes contents.

The purpose of our work was to determine quantitatively six procarcinogenic aza-PAHs in thermally treated meat as well as in its gravies by high-performance liquid chromatography with fluorescence detection (HPLC–FD). The clean-up procedure based on tandem-solid phase extraction used by us for isolation of azaarenes from meat matrix (Janoszka et al., 2004a) has been adapted for the isolation of these compounds from gravy samples. Concentration of individual azaarenes in meat can be very low (below 1 ng/g), so one of the purposes of the present investigations was to modify HPLC–FD method used so far and to improve the sensitivity of the azaarenes determination in high-protein and fat containing food matrix. The modification consisted of application of a fluorescence detection program of optimum excitation (Ex) and emission (Em) wavelengths selected for each individual compound being analysed. Two pork dishes prepared in the method commonly used and often consumed in Poland were investigated.

2. Materials and methods

2.1. Materials

Standards: six azaarenes were used for the study: benzo[h]quinoline (B[*h*]Q) (95%) was from Ultra Scientific (North Kingstown, USA), benzo[*a*]acridine (B[*a*]Ac) (99.5%), benzo[*c*]acridine (B[*c*]Ac) (99.7%), dibenzo-[*a*,*c*]acridine (DB[*a*,*c*]Ac) (99.8%), dibenzo[*a*,*j*]acridine (DB[*a*,*j*]Ac) (99.6%) and dibenzo[*a*,*h*]acridine (DB[*a*,*h*]Ac) (99.3%) were purchased from Promochem (Wessel, Germany). Standard mixture (of $1\ \mathrm{mg\ L^{-1}}$ in acetonitrile) was prepared from standard stock solutions (each concentration of $0.2\ \mathrm{g\ L^{-1}}$ in acetonitrile). HPLC-grade organic solvents: dichloromethane, *n*-hexane, methanol, acetonitrile, toluene (POCH, Gliwice, Poland) and water from a simplified water purification system (Millipore, Vienna, Austria) were used as the components of mobile phases and as extraction solvents. Sodium hydroxide, hydrochloric acid, ammonium hydroxide and ammonium acetate (analytical-reagent grade) were purchased from POCH (Gliwice, Poland). Diatomaceous earth extraction columns (Extrelut, 20 mL) and refill material were obtained from Merck (Darmstadt, Germany). The solid phase-extraction columns filled with propyl sulfonic acid (PRS, 500 mg, 3 mL) were purchased from J.T. Baker (Deventer, Holland). PRS columns were preconditioned with dichloromethane (4 mL). All solutions were passed through a 0.45 μm filter (Millipore, Bedford, USA) before the injection into the HPLC system.

2.2. Food samples

Pork joint was purchased from the local butcher market. Two pieces, coming from one pork weighing about 4 kg each (with bones), were bought. Bones and some small fat pieces were cut out before preparing the meat dishes. Two kinds of dishes (collars and chops) were prepared from pork joint by frying without fat and without additives (spices, salt) on a teflon-coated frying pan heated on a gas cooker. The meats were prepared in such a way as to obtain the degree of internal meat doneness corresponding to the "very well-done" levels.

Collars: four 1 cm thick meat slices (100 g) were pounded. After scrolling (without filling) the roulades (of ca. 3 cm diameter and 10 cm length) were fried for 20 min (5 min on each side) on a pan preheated to 200 °C. Pan temperature during frying was about 170 °C. Next, 200 mL of water was added and the whole was simmered under cover for 1 h at 95–98 °C.

Chops: 400 g of meat was sliced into 1 cm thick (100 g) portions and were slightly pounded into 0.7 cm slices, which were fried for 6 min on each side on a pan preheated to 200 °C. Pan temperature during frying was about 170 °C. Next, 100 mL of water was added and the whole was simmered under cover for 10 min at 95–98 °C.

An averaged sample was prepared from each kind of meat. For this the ready collars or chops were minced in an electrical mincer and next carefully mixed. From 400 g of raw meat 230 ± 5 g of cooked collar and 210 ± 5 g of cooked chop were obtained. The pan residues obtained from both types of meat dishes were collected and evaporated nearly to dryness in a vacuum evaporator, and two samples of gravy about 10 g each were received every time. The gravy samples had a form of dense brownish liquid.

2.3. Clean-up

The multistage clean-up procedure based on solid phase extraction and used to isolate the azaarenes fraction from thermally treated meat samples was described in detail elsewhere (Janoszka, 2007; Janoszka et al., 2004a).

Samples of 30 g meat were alkaline hydrolysed by homogenisation with 90 mL 1 mol L^{-1} NaOH for 3 h. From the suspension obtained, 20 g (containing 5 g of meat) were sampled four times. Into the fifth portion azaarenes standards mixture was introduced to furnish a spiked sample. 1 mol L^{-1} NaOH (10 mL) and 15 g of Extrelut were added to each portion which was loaded in a 20 mL polypropylene column. Elution of azaarenes was carried out directly from Extrelut columns onto propyl sulfonic acid (PRS)-SPE columns by means of 60 mL of CH_2Cl_2 containing 5% toluene. The PRS columns were dried and next rinsed with 6 mL of 0.1 mol L^{-1} HCl and 2 mL of water. Next they were washed with 20 mL of 0.5 mol L^{-1} ammonium acetate solution (pH 8) and 10 mL water. After drying azaarenes retained in the PRS columns were eluted using 4 mL of methanol–aqueous ammonia mixture (9:1; v/v). To determine the low contents of azaarenes in meat, the four fractions obtained for unspiked samples were combined to one more concentrated fraction (equivalent of 20 g of cooked meat) in which the azaarenes could be analysed both qualitatively and quantitatively. After evaporation to dryness the residues obtained were dissolved in acetonitrile (spiked sample in 200 μL and unspiked in 100 μL) before the HPLC analysis.

The procedure used for isolation of azaarenes from the gravy matrix was analogous: gravies (10 g samples) were hydrolysed for 3 h with 90 mL 1 mol L^{-1} NaOH. From the hydrolysate some portions 10 g each (equivalent of approximately 20 g of cooked meat) were collected. Into three of them azaarenes standards mixture was introduced to furnish spiked samples. 10 mL of 1 mol L^{-1} NaOH and Extrelut were added to each portion. The elution from Extrelut onto PRS columns was carried out with CH_2Cl_2 containing 5% toluene. The PRS columns were washed and azaarenes adsorbed on them were eluted in the same way and with the same volumes of solvents as described for meat samples. The residues obtained after evaporation of methanol–water mixture were dissolved in acetonitrile (spiked sample in 1200 μL and unspiked in 100 μL) before quantitative HPLC analysis.

To prevent matrix effect on peaks positions in the chromatogram as well as to evaluate the percentage recovery of azaarenes, spiked and unspiked samples were analysed under the same conditions. The meat samples were spiked with the standard mixture in amount of 200 ng of each standard/5 g of meat and the gravies samples – in amount of 400 ng/gravy sample (equivalent of 20 g of cooked meat).

To compare azaarenes contents in both kinds of samples (meat and gravies) the final results of the determinations were given in the same units, i.e. in ng/g of cooked meat. The obtained results of quantitative analysis were corrected by the recovery values.

2.4. HPLC

HPLC analyses of azaarenes were performed using a Knauer liquid chromatograph (Germany) equipped with a fluorescence detector (Shimadzu RF-10 Axl, Kyoto, Japan) and a 20 μL loop injector. The analytical system included Hypersil Green PAH column (5 μm particle size), 250 × 4.6 mm I.D. and guard column (5 μm, 10 × 4 mm), (Thermo, England) and a mixture of 85% acetonitrile and 15% water as a mobile phase. The separations were performed at 40 °C (thermostat HPLC column heater, Cluzeau-Info-Labo, Sainte-Foy-la Grande, France) under isocratic conditions (flow rate: 1.0 mL min^{-1} to 0–8 min; 0.7 mL min^{-1} to 8.01–15 min; 1.0 mL min^{-1} to 15.01–40 min). The fluorescent detection was performed by applying the following excitation (Ex) and emission (Em) wavelength program: 263/363 nm from 0 to 7 min (determination of B[*h*]Q), 285/407 nm from 7.01 to 12 min (B[*c*]Ac), 279/404 nm from 12.01 to 20 min (B[*a*]Ac), 275/388 nm from 20.01 to 26 min (DB[*a*,*c*]Ac), 291/410 nm from 26.01 to 30.50 min (DB[*a*,*j*]Ac), 290/408 nm from 30.51 to 40 min (DB[*a*,*h*]Ac). Qualitative analysis of aza-PAHs was based on the comparison of retention times values recorded for standard azaarenes with the values of appropriate components identified in the spiked and unspiked meat samples run under the same conditions. Quantitative determination was performed by

Table 1
Data from azaarenes determination by HPLC–fluorescence method

Azaarene	Detection limit (ng/column)[a]	RSD% for peak area[b]	Linearity[c] r
B[*h*]Q	0.005	3.30	0.999
B[*c*]Ac	0.0001	2.16	0.991
B[*a*]Ac	0.001	5.95	0.999
DB[*a*,*c*]Ac	0.001	6.11	0.999
DB[*a*,*j*]Ac	0.0001	2.06	0.998
DB[*a*,*h*]Ac	0.0001	4.34	0.998

[a] Detection limits (based on a $S/N = 3$) were determined using azaarene standard mixtures loaded directly onto a column using a 20 μL loop injector.

[b] Day-to-day analysis for 1 ng 20 μL^{-1}.

[c] Value of correlation coefficient r for plots recorded in the range of detection limit to 5 ng for each standard.

Table 2
Results of azaarenes determination in food samples

Azaarene	Content of azaarenes (ng/g of cooked meat[a]) determined in				Recovery (%)
	Collar		Chop		
	Meat	Gravy	Meat	Gravy	
B[*c*]Ac	0.83 ± 0.37	0.09 ± 0.01	0.99 ± 0.29	0.21 ± 0.01	54.73
B[*a*]Ac	0.54 ± 0.24	0.07 ± 0.01	0.13 ± 0.05	0.13 ± 0.03	63.21
DB[*a,c*]Ac	0.25 ± 0.18	0.06 ± 0.01	0.19 ± 0.03	0.06 ± 0.03	57.41
DB[*a,j*]Ac	0.36 ± 0.17	0.07 ± 0.01	0.09 ± 0.03	0.08 ± 0.03	66.82
DB[*a,h*]Ac	0.52 ± 0.21	0.06 ± 0.01	0.17 ± 0.02	0.11 ± 0.04	55.33

[a] Content in ng/g, recovery corrected values.

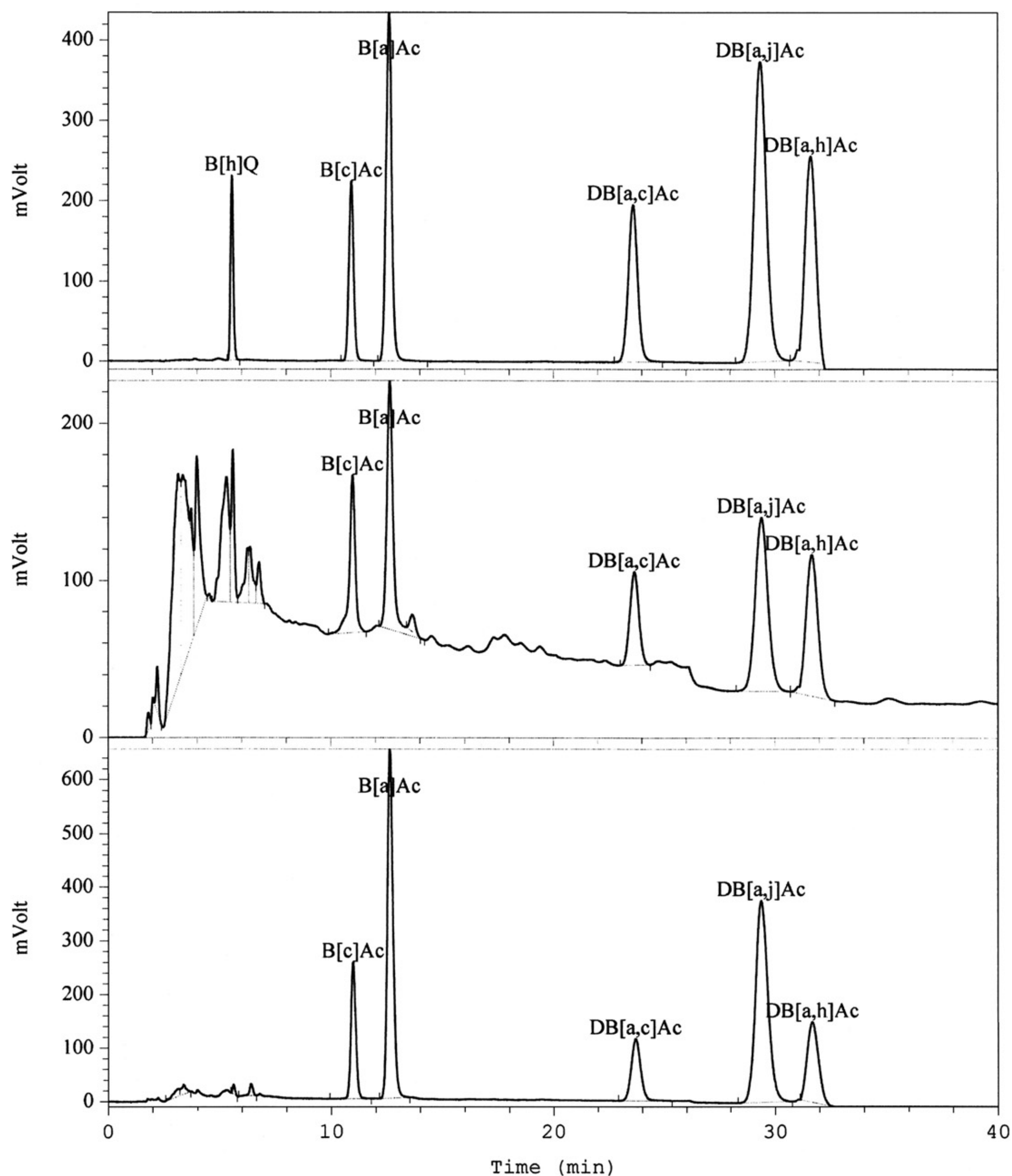

Fig. 1. HPLC–FD chromatograms of azaarenes: standards mixture for concentration of 2 ng 20 μL^{-1} (top); fraction separated from unspiked collar meat sample (injection: 20 μL from 100 μL) (middle); fraction separated from spiked with 200 ng of each standard/20 g of cooked collar meat sample (injection: 20 μL from 200 μL) (bottom).

using an external calibration curve method, by peak area measurement.

3. Results and discussion

3.1. HPLC-fluorescence analysis of azaarenes

For the azaarenes separation a column (Hypersil Green PAH) containing specially tailored alkyl bonded silica with a high carbon loading, designed specifically for the analysis of polynuclear aromatic hydrocarbons, was applied. Using this column and a mixture of acetonitrile–H_2O under isocratic conditions a complete resolution of azaarenes mixture containing B[*h*]Q, two benzoacridine isomers and three dibenzoacridines was achieved. The relative standard deviation (RSD, %) values of the retention times t_R (min) for these azaarenes were below 2.1% (day-to-day separations).

Azaarenes have characteristic Ex and Em wavelengths, at which fluorescence values are a maximum (Wilhelm et al., 2000). HPLC–FD analysis with the application of Ex and Em wavelength program that is optimal for the six investigated aza-PAHs enabled determining them at a very low concentration level. The detection limit for the azaarenes was between 0.0001 ng and 0.005 ng based on signal-to-noise ratio 3:1 (Table 1). The calibration plots recorded were linear in the range of detection limit to 10 ng injected onto the column ($r > 0.99$) for each of the investigated azaarenes.

Matching the fluorescence detection program and the HPLC elution program made selective determination of aza-PAHs possible, which was important as the method was developed to analyse azaarenes in a very complex matrix of high-protein food samples. Earlier we applied a method of HPLC with fluorescence detection at a constant wavelength of Ex (360 nm) and Em (460 nm) for azaarenes determination in thermally treated meat (Janoszka et al., 2004a). Using the HPLC−FD program at optimum Ex/Em waves for particular compounds enabled optimising azaarenes detection limits, especially for dibenzoacridines, for which the limits were lowered over one hundred times (Janoszka et al., 2004a). Similarly, the planar chromatography method with densitometric detection, used by us before to analyse meat samples, allowed rapid determination of some azaarenes (B[*c*]Ac, DB[*a*,*h*]Ac and DB[*a*,*c*]Ac) (Janoszka, 2007), but at the detection limit ten times higher than in the method described here.

3.2. Analysis of azaarenes in meat and gravy

The clean-up procedure is a part of a complex analytical scheme developed to isolate from meat samples three fractions of organic carcinogenic compounds: PAHs, azaarenes and heterocyclic amines (Janoszka et al., 2004a, 2004b; Warzecha et al., 2004). Rivera et al. (1996) were the first to use this procedure to analyse extracts of charcoal-grilled meat and after some modifications it was used by us to determine azaarenes in various meat dishes prepared in household conditions (Janoszka et al., 2004a). This procedure composed of an alkaline hydrolysis and a solid phase extraction on diatomaceous earth (Extrelut) and cation exchanger (PRS) enabled the isolation of azaarenes concentrate from a complex proteinaceous-fatty matrix of fried meat samples. Moreover, a qualitative–quantitative determination of individual components of this fraction by HPLC method was possible without additional cleaning-up of the samples.

The purpose of the present investigations was to determine six azaarenes in samples of meat thermally treated according to domestic conditions, and in gravies obtained from their frying as well. In order to check the suitability of the clean-up procedure for the investigated food samples both spiked with known amounts of standards and unspiked samples were analysed. The percentage recoveries are given in Table 2. The recoveries for the four-ring and five-ring azaarenes (benzoacridines and dibenzoacridines) determined for the investigated meat and gravy samples were between 55% and 67%. They were close to the values obtained by us before for other meat samples (Janoszka et al., 2004a). It is difficult to isolate particular classes of toxic compounds from a complex matrix of food samples. The recoveries we obtained are comparable to the data given by Rivera et al. (1996) for azaarenes isolated from meat extracts (24–95%) and recoveries of other xenobiotics, e.g., aminoazaarenes occurring in thermally treated high-protein food in amounts of ng/g of the sample (Janoszka et al., 2001; Skog, Augustsson, Steineck, Stenberg, & Jägerstad, 1997). Clean-up procedure applied in this work did not give satisfactory results in B[*h*]Q isolation from food sample. The recovery for this three-ring compound was very low (5.6%). Such a low recovery may result from a greater volatility of this compound and its better solubility in water in comparison to other azaarenes being determined (de Voogt, Wegener, Klaner, van Zijl, & Govers, 1988).

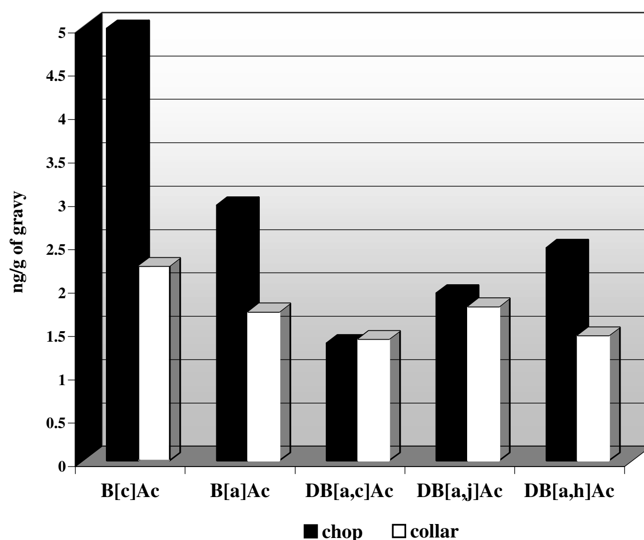

Fig. 2. Content of azaarenes in collar and chop gravies samples expressed in ng/g of gravy.

Application of Ex and Em wavelength program during azaarenes determination enabled determining them in a complex matrix of meat and gravy samples with a high sensitivity. The calculated quantification limits in real meat samples and gravies (assuming that LOQ = 6LOD) (Eurachem Guide, 1998) were: 0.0006 ng/g of cooked meat for B[*a*]Ac, DB[*a,h*]Ac, DB[*a,j*]Ac, 0.006 ng/g for B[*c*]Ac, DB[*a,c*]Ac and 0.03 ng/g for B[*h*]Q. Concentrations of azaarenes determined in the investigated meat dishes prepared from pork and in corresponding gravies are from 0.06 to 0.99 ng/g (Table 2). Contents of individual azaarenes determined in meat samples is of the same order as the concentrations of B[*a*]Ac, B[*c*]Ac, DB[*a,h*]Ac and DB[*a,c*]Ac determined by us in other meat dishes made of pork, poultry and beef being from 0.04 to 2.9 ng/g and presented in our earlier papers (Janoszka, 2007; Janoszka et al., 2004a). Concentration of B[*a,j*]Ac was determined by us in this kind of sample for the first time. Fig. 1 presents an example of HPLC–FD chromatogram of standard mixture, unspiked and spiked sample recorded for a collar sample.

Not only meat samples but also gravies that are formed during meat dishes thermal preparation were investigated for azaarenes contents. Up till now gravies have never been investigated as far as azaarenes content is concerned. As we expected aza-PAHs were also found in these samples. The

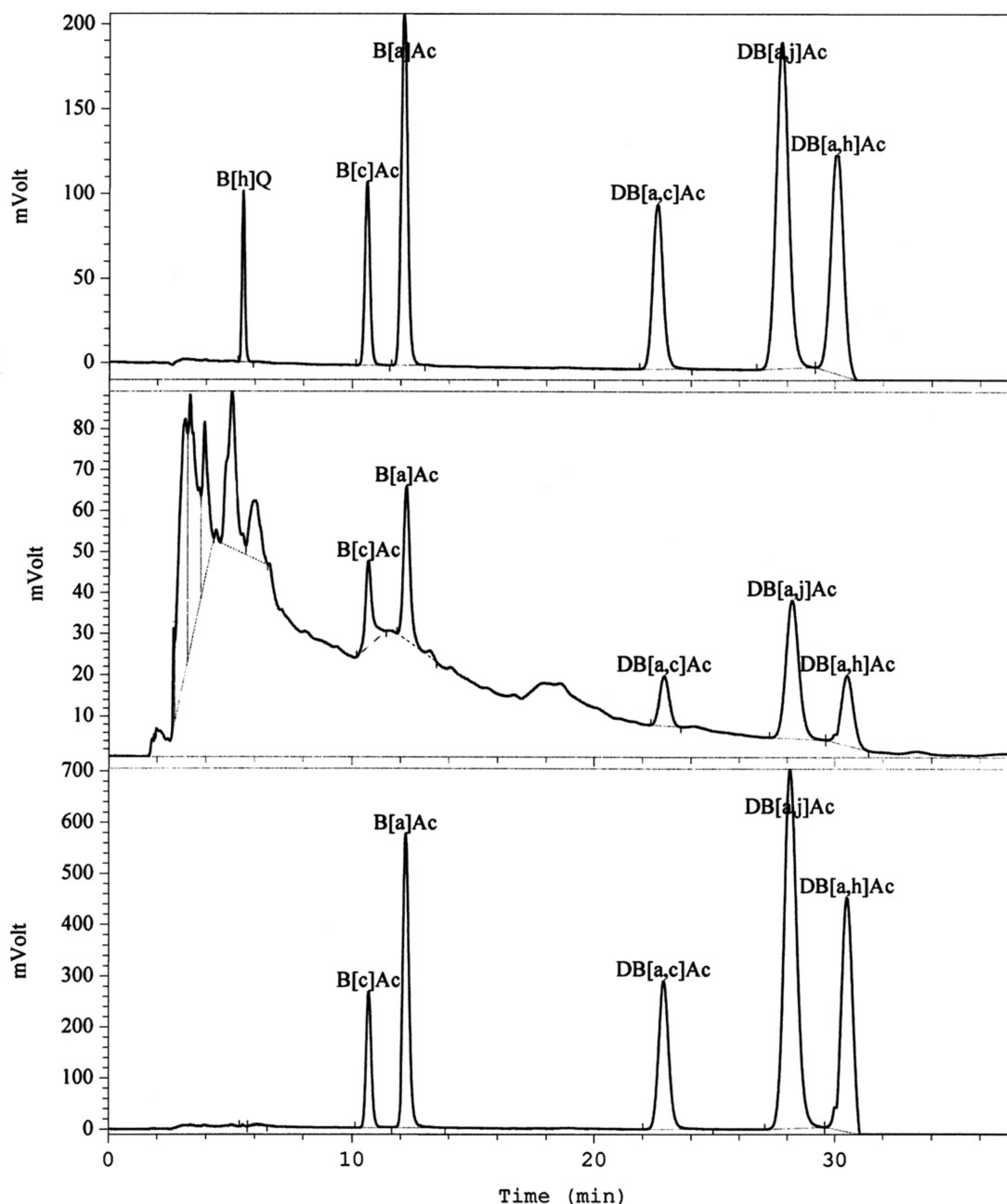

Fig. 3. HPLC–FD chromatograms of azaarenes: standards for concentration of 1 ng 20 μL^{-1} (top); fraction separated from unspiked collar gravy sample (injection: 20 μl from 100 μL) (middle); fraction separated from spiked with 400 ng of each standard/10 g of collar gravy sample i.e., equivalent of 20 g of cooked meat (injection: 20 μL from 1200 μL) (bottom).

results obtained in this study show that contents of individual azaarenes in gravies (expressed in ng/g of cooked meat) are lower in most cases than in meat samples from which the gravies come from (Table 2). They range from 0.06 to 0.21 ng/g of meat. To show these results in ng per 1 g of gravy they are from 1.40 (DB[*a*,*c*]Ac in chop) to 4.98 (B[*c*]Ac in chop) (Fig. 2). Such a data agrees with results of other xenobiotics analysis (e.g. aminoazaarenes) described by Skog, Steineck, Augustsson and Jägerstad (1995), who determined higher concentrations of analytes in pan residues than in meat samples. Fig. 3 presents HPLC chromatograms recorded for unspiked and spiked collar gravy. Comparing azaarenes concentrations in the investigated two kinds of gravies higher contents of some aza-PAHs (B[*c*]Ac, B[*a*]Ac, DB[*a*,*h*]Ac) in samples coming from chops were found. However the total contents, including the amount of azaarenes in meat and gravy, were higher for collar samples (2.85 ng/g of cooked meat) in comparison to chops (2.17 ng/g). This may be caused by the longer time of thermally treating the collars than chops.

Fig. 4 presents contents of five determined azaarenes in collar and chop meat and gravies samples expressed in ng/100 g of cooked meat. Assuming a daily consumption of 100 g helping of one of the examined meats, a theoretical human exposure to the total amount of five determined benzoacridines and dibenzoacridines is 250 ng/day/person (collars) and 157 ng/day/person (chops). Consumption of the gravy that is formed during frying this portion of meat may increase the exposure by 35 ng/day/person (collars) and 59 ng/day/person (chops), which corresponds to increase by 14% and 37%, relatively. The results obtained show that when assessing the dietary intake of muta-/carcinogenic compounds it is necessary to consider not only the amount of consumed meat dishes, but also the amount of the consumed sauces prepared from meat gravies.

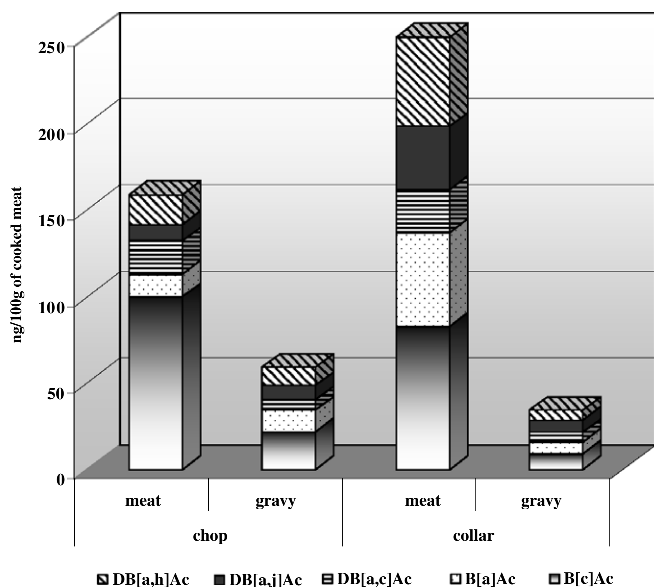

Fig. 4. Contents of determined azaarenes in collar and chop meat and gravies samples expressed in ng/100 g of cooked meat.

In our previous works the contents of four aza-PAHs (B[*a*]Ac, B[*c*]Ac, DB[*a*,*c*]Ac, DB[*a*,*h*]Ac) (Janoszka et al., 2004b), five heterocyclic amines (Warzecha et al., 2004) and five PAHs (Janoszka et al., 2004a) were determined in nine household prepared pork, beef and poultry dishes (without gravies) and the theoretical daily exposures assessed for azaarenes were low in the case of each type of dish. The determined total contents of these food mutagenes expressed in ng/100 g of cooked meat were respectively: for azaarenes from 80 to 330 ng; for heterocyclic amines: from 200 to 7700 ng and for PAHs: from 240 to 1600 ng/100 g.

According to the data presented by Jägerstad and Skog (2005) in a review on genotoxicity of heat processed food, the human daily exposure to HAs resulting from a diet may range from a few ng/day to some μg/day/person depending on dietary habits and cooking practices. The mean contribution from gravy and sauce to the daily exposure to heterocyclic amines was assessed for about 30% (Augustsson, Skog, Jägerstad, & Steineck, 1997).

The contents of PAHs in thermally treated fish and meat may be from 0 to over 100 ng/g (Fontcuberta et al., 2006; Janoszka et al., 2004a; Jägerstad & Skog, 2005). Sinha, Kulldorf, Gunter, Strickland, and Rothman (2005) estimated benzo[*a*]pyrene intake derived from meat in the range from 0.2 ng to 101 ng/day/person. The exposure to all polynuclear aromatic compounds can be higher, as B[*a*]P constitutes only 0.5–5% of the total amount of PAHs in food samples (Jägerstad & Skog, 2005).

Despite the concentration of azaarenes in thermally treated meat being lower than heterocyclic amines and PAHs, their presence in food can be harmful for human, as the studies carried out so far show that metabolites of some azaarenes, particularly dibenzoacridines, are highly carcinogenic to experimental animals (Kumar et al., 2001).

4. Conclusions

Clean-up procedure based on alkaline hydrolysis and solid-phase extraction using coupled columns packed with different adsorbents: diatomaceous earth (Extrelut) and propyl sulfonic acid (PRS) allows selective isolation of azaarenes fraction from the complex matrix of thermally treated meat and from its gravy as well.

The HPLC–fluorescence detection program being used and based on the application of the selected and optimum for each of the analysed compound wavelengths Ex/Em allows determining individual compounds in the complex proteinaceous-fatty matrix of food at the level of 0.0006–0.03 ng/g of cooked meat sample.

Total contents of the investigated benzacridines and dibenzacridines determined given in the same units, i.e., in ng/g of cooked meat were 1.57 and 2.50 ng/g in collar and chop samples, respectively; their gravies contained 0.35 and 0.59 ng.

The summary concentration of these compounds expressed in ng/g of gravy was 13.40 for collar and 8.40 for chop gravy samples.

It can be concluded that gravies are an important source of muta-/carcinogenic azaarenes, particularly for the people who often consume this by-product of meat frying or use them for sauce preparation. Thus the amount of consumed sauces should be considered for estimating human exposure to carcinogenic compounds occurring in food.

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