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Determination of heterocyclic aromatic amines (HAs) content in samples of household-prepared meat dishes

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Abstract

Aminoazaarene content was investigated in 10 meat samples (including pork, beef, turey and chicken) thermally processed at home according to common recipes used by residents of Upper Silesia region in Poland. The clean-up procedure included tandem solid-phase extraction (SPE) using Extrelut-type columns filled with diatomaceous earth, propylsulphonic acid and chemically bounded phase-C_{18}. Identification and quantitative analysis of HAs fraction was carried out using a HPLC system with DAD-type detector. Separation was achieved using TSK-gel ODS 80-T_M column and a mixture of 5% acetonitrile and 95% triethylamine phosphate buffer (pH 3.3) as a mobile phase. The results of qualitative determinations were confirmed by GC–MS method. To achieve this, HAs fractions were derivatized to pentafluoropropionic acid (PFPA) amide derivatives. The summary content of five aminoazaarenes determined in investigated meat samples, i.e. 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQ$_x$), 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine (PhIP) falls within the range of 1.9–77.4 ng/g of sample. The calculated values of theoretically daily human exposure to five determined HAs were in the range of 0.2–7.7 μg per day per person.

Keywords: Food analysis; Heterocyclic aromatic amines; Aminoazaarenes

1. Introduction

The rising cancer incidence has been recently noted among populaces of highly developed countries, especially with respect to diseases of colon, large intestine, prostate, liver and kidneys. To a large extent, it is related to rising consumption of meat as well as preserved meat. This has been confirmed by epidemiological studies carried out mainly by Swedish and Japanese investigators [1–4]. In their studies, they tried to determine the impact of etiological factors (such as eating habits, type of ecotoxin, place of domicile) upon the frequency of a given type of cancer occurrence and resulting mortality.

The average consumption of 15 tons of food (per dry mass) by man within his/her lifetime suggests that foodstuffs may be one of the major potential sources of biologically active exogenous compounds [5]. It has been demonstrated that the influence of diet type upon risk of acquiring cancer depends on the content of cancerous organic compounds that may form in high-protein food products subject to thermal treatment beyond 150 °C [6,7]. Among these compounds are biologically active heterocyclic aromatic amines with imidazopyridine, imidazoquinoline or imidazoquinoxaline moieties. Such amines form in fried, smoked or grilled meat and fish [8,9]. Model studies have shown that precursors of these amines, i.e. free amino acids, creatine, creatinine and hexoses are all present in meat and fish and aminoazaarenes may form from them as a result of Maillard reaction [10,11]. Biological studies of aminoazaarenes thus far identified in various foods, carried out using microsomal Ames test on bacterial strains as well as mammalian tissue cultures in vitro, allowed to conclude that these compounds possess high mutagenic activity [12,13]. It turns out that some aminoazaarenes are mutagenically 100-fold more active (Ames test) compared to food-contained genotoxic aflatoxins B_1 and more than 2000-fold more active than the strongly mutagenic hydrocarbon benz(a)pyrene [7].

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Additionally, it has been shown that aminoazaarene metabolites, the majority of which are hydroxylamine derivatives, may form adducts with a complementary DNA base—guanine at the C-8 position and exerting thus a strongly genotoxic effect [14–16]. Studies involving experimental animals resulted in rating all known food-originating aminoazaarenes as potential cancerogens [17,18]. In 1993, the International Agency for Research on Cancer (IARC) listed three of them, i.e. 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) as compounds that may directly cause cancer growth in human body [19].

The goal of the present study was to determine qualitatively and quantitatively, for the first time, aminoazaarenes forming in meat dishes prepared according to traditional cooking recipes from the Upper Silesia region of Poland. This permitted making initial risk assessment of exposure to food-ingested exogenous carcinogens by the populace of this most environmentally polluted region in Poland, where several components of the natural environment (airborne particulate matter, drinking water, sewage sludge) are contaminated with numerous organic ecotoxicants [20,21]. In addition, standard coefficients of cancer incidence and mortality found within the past 10 years in this region became exceptionally high pointing to a particularly grave public health problem [22].

2. Experimental

2.1. Chemicals

The following aminoazaarenes were used as standards: 2-amino-3-methylimidazo[4,5-*f*]quinoline(IQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline (MeIQx), 2-amino-3,4-dimethylimidazo[4,5-*f*] quinoline (MeIQ), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Toronto Research Chemicals Ontario, Canada).

HPLC-grade organic solvents: dichloromethane, *n*-hexane, methanol, acetone, acetonitrile, toluene, ethyl acetate, ammonium hydroxide (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 and ammonium acetate (analytical-reagent grade) were purchased from POCH, (Gliwice, Poland). Triethylamine (Fluka, Buchs, Switzerland) and 85% phosphoric acid (Merck, Darmstadt, Germany) were used for buffer preparation. Diatomaceous earth extraction columns (Extrelut, 20 ml) were obtained from Merck. Propylsulphonic acid (PRS, 500 mg) and octadecylsilane (C_{18}, 500 mg) SPE columns were from J.T. Baker, Gross-Gerau, Germany. PRS columns were preconditioned with dichloromethane (4 ml) while C_{18} columns with methanol (10 ml) and water (10 ml), respectively. Pentafluoropropionic acid anhydride (Aldrich, Dorset, UK) was used for derivatization of aminoazaarenes to amides.

2.2. Meat sampling

Some of the meats most commonly eaten in the region of Upper Silesia (Poland) were investigated: pork chops (coated in bread crumbs and eggs), beef collar, pork neck, beef/pork minced chops, turkey breast, pork fillet, pork joint and chicken breast. The raw meat products were obtained from a local “Tesco” supermarket store. Every prepared portion of the meat (according to the Table 1: 0.5 kg, 0.7 kg, 0.75 kg, and 1 kg) was drained off with filtration paper (to remove pan-residues and gravies), chopped, minced and thoroughly stirred. Crumb and crust were taken off the pork chop, and stuffing taken out of the beef collar before. Pan residues and gravies were not mixed with the meat samples. Twenty-five grams of meat was taken from such an averaging sample each time and analysed. Details regarding meat samples, frying fats used and cooking conditions employed are given in Table 1.

2.3. Clean-up procedure

To obtain aminoazaarene fractions, the clean-up procedure used previously by Gross and Grüter [23] was applied after necessary modifications. Our own findings were applied as well [24].

In order to determine aminoazaarenes in meat samples, four portions of the same sample were separated simultaneously and the obtained HAs fractions were combined and analysed qualitatively as well as quantitatively. To do this, 25 g of meat was homogenised for 1 min, with 75 ml of cold 1 M NaOH solution. From the dense suspension obtained, 20 g (contained 5 g of meat) was sampled four times (equivalent to the total of 20 g of meat matter). Cold 1 M NaOH solution (10 ml) and 15 g of loose Extrelut were added to each portion. After thorough mixing, each portion was loaded onto columns. Elution of aminoazaarenes was carried out directly from Extrelut columns onto PRS columns by means of 60 ml of CH_2Cl_2 containing 5% toluene. After drying, the PRS columns were washed with 6 ml of 1 M HCl solution, and then with 2 ml of water. Next, C_{18} columns were connected to PRS columns and washed with 20 ml of 0.5 M ammonium acetate solution (pH 8). As a result, aminoazaarenes were eluted from PRS columns directly onto C_{18} columns. These columns were then washed with 10 ml water, dried under slight vacuum and blown through with nitrogen. Finally, aminoazaarenes were eluted with 2 ml of CH_3OH–$NH_3{\cdot}H_2O$ (9:1 (v/v)) and the fractions from four separations combined. After evaporation to dryness, they were dissolved in 100 μl acetonitrile for the HPLC analysis.

To evaluate the percentage recovery of HAs separated and analysed using this multistep procedure, as well as to prevent

Table 1
Specifics of meat samples' preparation in household conditions

Meat type[a]	Cooking method	Doneness level	Household cooking conditions
Pork chop (coated in bread crumbs and egg)	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 in eggs and bread crumbs, fried for 15 min. on each side on teflon-coated frying pan using "Planta" margarine. Meat was placed in fat preheated to 230 °C. Frying temperature measured in the pan center was between 190–200 °C. After frying, the fat was drained off using filtration paper.
Beef collar	Pan-fried	Medium	Meat slices (150 g, 1.5 cm thick) were pounded into thinner slices, next covered with filling, i.e. smoked bacon, onions and pickles. After wrapping, the roulades were fried using peanut oil on a teflon-coated frying pan. Roulades were placed in fat preheated to 200 °C and were fried without cover for 20 min. Temperature during frying ranged between 150–160 °C. Next, water was added and the whole was simmered under cover for 1 h at 90–95 °C. The filling 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 of salt and the whole was put in the 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	To 0.5 kg of beef/pork minced meat one egg, two tablespoons of bread crumbs and one tablespoon of sour cream were added. Burgers of 4 cm diameter and 1.5 cm thick were then formed and covered with bread crumbs. The burgers were fried 12 min on each side on a teflon-coated frying pan using "Planta" margarine. The meat was placed in fat preheated to 230 °C. Frying temperature measured in the pan center was between 190–200 °C. After frying, the fat was drained off using filtration paper.
Turkey breast (no. 1)	Pan-fried	Well-done	Meat (0.75 kg) was sliced into 150–200 g, 1.5 cm thick portions and slightly pounded. The slices were fried 15 min on each side on a teflon-coated frying pan using "Planta" margarine. The meat was placed in fat preheated to 230 °C. Frying temperature measured in the pan center was between 190–200 °C. After frying, the fat was drained off using filtration paper.
Turkey breast (no. 2)	Roasted	Well-done	Meat (0.75 kg) was sliced into 150–200 g, 1.5 cm thick portions and slightly pounded, brushed with vegetable oil and wrapped in aluminum foil and 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 15 min. on each side using a garden-type grill fuelled with charcoal.
Pork joint	Roasted	Well-done	Boneless pork (0.70 kg) loin meat was slightly pounded and roasted under cover in the gas oven preheated to 250 °C using "Planta" margarine and a little butter. The meat was roasted at 240 °C for 25 min. on each side. Towards the end of roasting a small amount of water was added. After frying, the fat was drained off using filtration paper.
Chicken breast	Grilled	Very well-done	Six pieces of chicken breast meat (1 kg) were grilled 15 min. on each side using a garden-type grill fuelled with charcoal.

After frying the meat was stored in the freezer following drainage of excess fat. Frying fats: "Planta" margarine—a vegetable fat (11% polyunsaturated, 28% monounsaturated and 55% saturated). Peanut oil, containing per 100 ml 27.8 g polyunsaturated fat, 45.8 g monounsaturated fat and 18 g saturated fat.
[a] The meat samples were obtained from a local supermarket (Tesco). All meats were fried without seasoning except for small amount of salt.

matrix effect influence on peak positions in the HPLC and GC–MS chromatograms, spiked and unspiked samples were analysed under the same conditions.

Spiked samples were prepared by adding 40 ng of each of five known standards: IQ, MeIQ, MeIQx, 4,8-DiMeIQx and PhIP to 1 g of minced meat samples at the beginning of the homogenisation and extraction step.

2.4. High performance liquid chromatography

HPLC analyses of aminoazaarenes were performed using a Hewlett-Packard HP 1090 chromatograph equipped with a DAD system and a 20 μl loop injector.

The analytical system included TSK gel ODS 80-T_M column (5 μm particle size), 250 mm × 4.6 mm i.d. (Toso Haas, Stuttgart, Germany) and a mixture of 5% acetonitrile and 95% triethylamine-phosphate buffer (pH 3.3) as a mobile phase. The separations were performed with the following gradient elution programme: the mixture described above was initially used for 2 min, then it linearly increased to 25% acetonitrile within 20 min, then to 55% acetonitrile within 10 min and remained at 55% acetonitrile for 10 min. The optimised HPLC conditions were selected as the result of our earlier study [25].

All of the studied fractions were passed through a 0.45 μm filter (Bakerbond, Darmstad, Germany) before injection onto the HPLC system.

All separations were carried out at 40 °C using a 1 ml/min flow-rate. The UV detection of all aminoazaarenes was conducted at 254 nm and additionally at 274 nm (IQ, MeIQ, 4,8-DiMeIQx), 263 nm (MeIQx) and 315 nm (PhIP).

Compound identities were established by comparing retention factors (k) of the peaks with those of the corresponding standard mixture and spiked samples run under the same conditions.

The comparison of the online recorded UV spectra of IQ, MeIQ, MeIQx, 4,8-DiMeIQx and PhIP identified in meat dishes to UV spectra of standards was used to confirmation of HPLC data.

Quantitative determination was performed using an external calibration curve method. Correlation coefficients (r^2) for HA standard curves were: 0.985 for IQ, 0.961 for MeIQ, 0.990 for MeIQx, 0.972 for 4,8-DiMeIQx and 0.994 for PhIP.

2.5. Identification of aminoazaarenes by gas chromatography–mass spectrometry

The presence of HPLC-determined aminoazaarenes was confirmed by GC–MS analysis of standards and HAs fractions separated from spiked and unspiked meat samples derivatized to amides. Identification procedure consisted of comparing retention times and appropriate mass spectra. Aminoazaarenes were derivatized to amides by acylation reaction with pentafluoropropionic anhydride (PFPA), according to the procedure described by Campbell [26] for reduced nitro-polycyclic aromatic hydrocarbons. Details about the derivatisation of aminoazaarenes and their identification as PFPA amides were described earlier [27].

A mass spectrometer (QP 2000-Shimadzu) connected to a gas chromatograph (GC-14) was used. The samples were analysed by 2 μl split-less injection onto a 25 m × 0.2 mm (film thickness 0.25 μm) HP Ultra 1 fused-silica capillary column. Conditions for the analysis of amide-derivatized aminoazaarenes were as follows: electron impact (EI) 70 eV; helium flow rate 1 ml/min; temperatures: injector 270 °C, interface 280 °C, ion source 250 °C; GC temperature programme: 60 °C heating at 4 °C/min to 280 °C (held for 20 min).

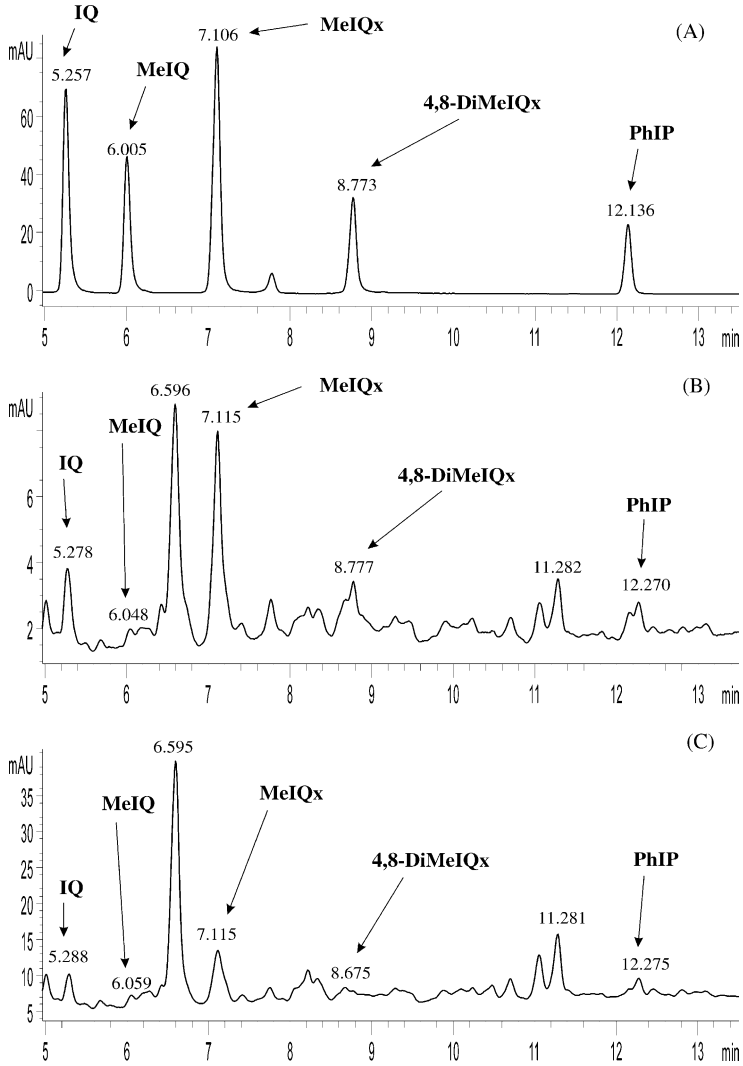

Fig. 1. HPLC chromatograms (λ = 254 nm) of aminoazaarenes: (A) standard mixture; (B) fraction separated from spiked; (C) fraction separated from unspiked grilled pork neck (no. 1) sample.

3. Results and discussion

Samples of meat products most commonly consumed in households of the Upper Silesia region of Poland were analysed in this study. Investigated products were prepared by department staff at their home according to recipes most favoured by residents of the region studied. The temperature and time of the meat dish preparation were measured. Table 1 shows kinds of meat dishes prepared, cooking methods used and degree of meat doneness.

Aminoazaarene content of heat-treated meat and its preserves depends on many factors: temperature and time of heat exposure, method of roasting/frying, type of fat used in the cooking process, spices added, etc [28–32]. With most recipes used throughout this study "Planta" margarine, a popular local brand, was used. No spices were added to prepared meats and conditions of thermal treatment of meat were adjusted in such a way as to obtain the degree of internal meat doneness corresponding to the "well-done" or "very well-done" levels.

In Figs. 1 and 2, exemplary HPLC chromatograms are shown for two out of ten investigated meat samples. Each Figure contains chromatogram of the standard mixture, HAs fraction separated from spiked and from unspiked samples. The analyses were performed consecutively on the same day to assure uniformity of conditions during determination. The value of hold-up time (t_M) was checked every time.

In Fig. 3 are presented the exemplary UV spectra of aminoazaarenes identified in unspiked grilled pork neck (no. 1) sample and UV spectra of aminoazaarene standards.

Table 2 lists the values of retention factor (k) for standards and aminoazaarenes identified in spiked and unspiked samples, the HPLC chromatograms of which are shown in Figs. 1 and 2. In the majority of investigated samples, five of the most commonly determined azaarenes were identified: IQ, MeIQ, MeIQx, 4,8-DiMeIQx and PhIP. The UV

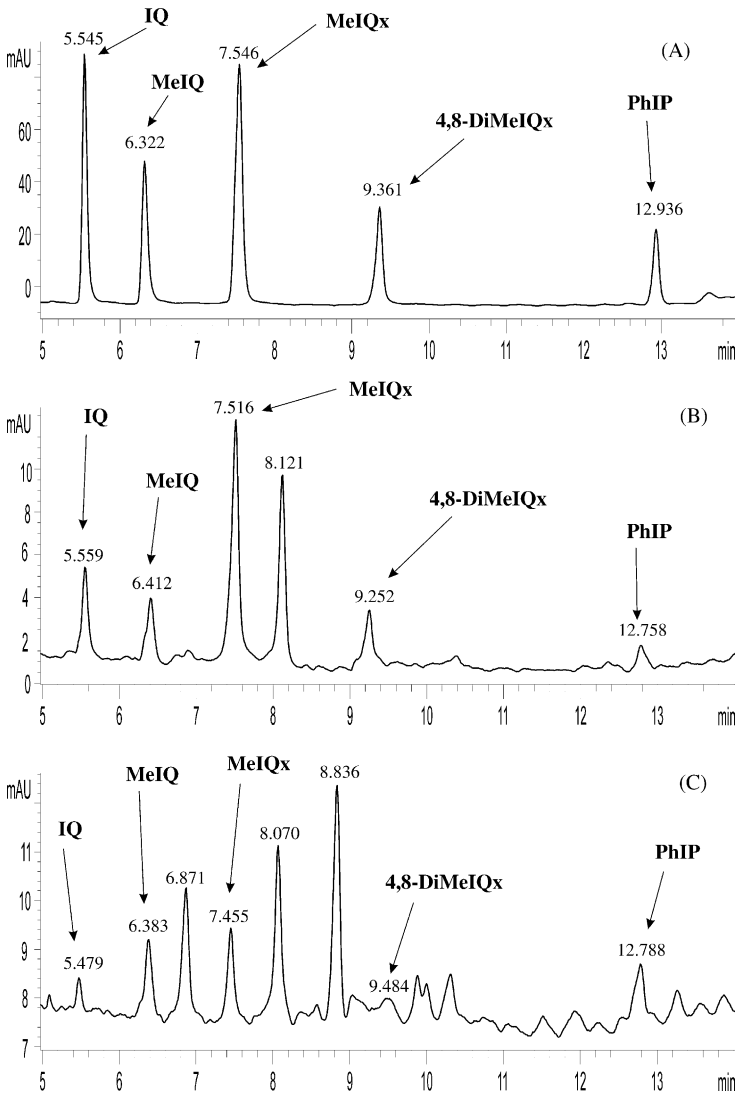

Fig. 2. HPLC chromatograms (λ = 254 nm) of aminoazaarens: (A) standard mixture; (B) fraction separated from spiked; (C) fraction separated form unspiked roasted turkey breast (no. 2) sample.

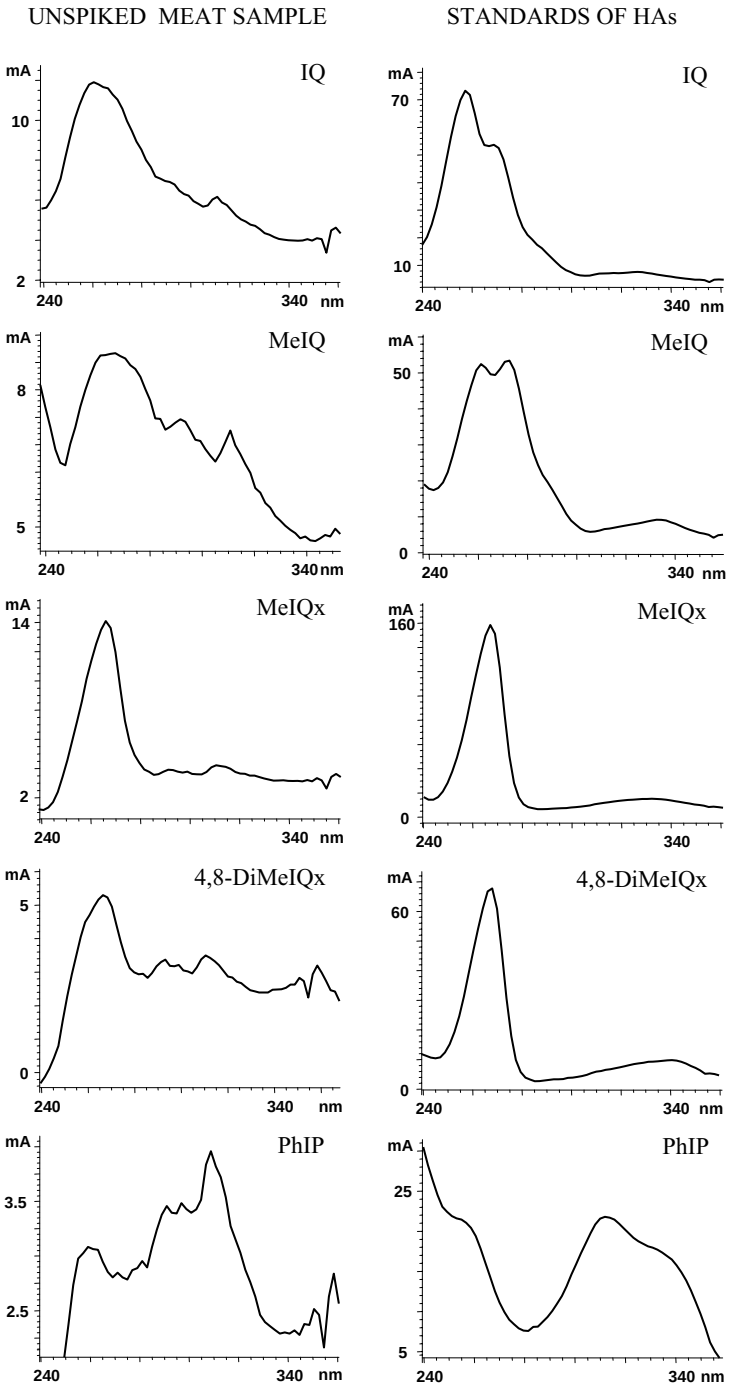

Fig. 3. UV-spectra of aminoazaarenes. Left side: the plot shows the online recorded UV spectra of IQ, MeIQ, MeIQx. 4,8-DiMeIQx and PhIP in unspiked grilled pork neck (no. 1) sample. Right side: the plot shows the UV spectra of aminoazaarene standards.

data confirmed the results of identification analysis obtained using HPLC method.

The results of identification analysis based on comparison of retention factor (k) were also confirmed by data gathered from GC–MS analyses. To this end, HAs standards and fractions separated from spiked and unspiked samples were derivatized to pentafluoropropionic acid amides (PFPA) and as such were loaded onto a GC–MS column [27]. The obtained results are summarized in Table 3 and in Figs. 4–6 which show exemplary GC–MS chromatograms (Fig. 4) of aminoazaarenes fractions separated from spiked and uspiked meat samples analysed as PFPA amide derivatives as well as mass spectra (Figs. 5 and 6) of some of the identified derivatives. The results of aminoazaarene determination by using HPLC method are presented in Table 4. GC–MS data obtained reveals that grilled pork neck (no. 1), pan-fried

Table 2
Retention factor (k) of standards and aminoazaarenes identified in spiked (A) and unspiked meat samples (B) the HPLC chromatograms of which are shown in Figs. 1 and 2; HAs detection limits

Compound	Standard (3.092)[a]	Pork neck (no. 1) (Fig. 1)		Standard (3.098)	Turkey breast (no. 2) (Fig. 2)		Detection Limits[b] (ng)
		A (3.086)	B (3.074)		A (3.088)	B (3.095)	
IQ	0.70	0.71	0.72	0.79	0.80	0.77	0.4
MeIQ	0.94	0.95	0.95	1.04	1.07	1.06	0.4
$MeIQ_x$	1.30	1.31	1.31	1.44	1.43	1.40	0.8
4,8-$DiMeIQ_x$	1.84	1.85	1.82	2.02	1.99	2.06	1.0
PhIP	2.91	2.98	2.99	3.18	3.13	3.12	2.0

[a] Values of hold-up time (t_M) (min.) determined before each consecutive analysis are listed in parantheses.
[b] Detection limits (based on a $S/N = 3$) were determined using HAs standard mixtures, loaded directly onto a column using a 20 μl loop injector.

Table 3
GC–MS identification of aminoazaarenes in the samples of investigated foods following derivatization to pentafluoropropylamides

Meat type	IQ (t_R = 46.50 min)	MeIQ + $MeIQ_x$ (t_R = 47.50 min)	4,8-$iMeIQ_x$ (t_R = 52.00 min)	PhIP (t_R = 50.60 min)
Pork chop	+[a] (225)[b]	+ (239, 240)	+/− (254)	−/+ (251, 370)
Beef collar	+/− (225)	+/− (239)	+/− (254, 373)	−/+ (251, 370)
Pork neck (no. 1)	+ (225)	+ (240, 359)	+ (254)	+ (251, 370)
Pork neck (no. 2)	+/− (225)	+/− (239)	–	−/+ (251)
Beef/pork minced chop	+ (225, 344)	+ (239, 240)	+ (254)	+(251, 370)
Turkey breast (no. 1)	+ (225, 344)	+(239, 240)	+/− (254)	+/− (251)
Turkey breast (no. 2)	n.a.	n.a.	n.a.	n.a.
Pork fillet	+ (225, 344)	+ (240)	+ (254)	−/+ (251)
Pork joint	+/− (225)	+/− (239, 240, 359)	+/− (254, 373)	−/+ (251, 370)
Chicken breast	+ (225)	+ (239, 240, 358)	+ (254, 373)	−/+ (251)
Detection limit[c] (ng)	50	50 and 6.0	7.5	6

n.a.: not analysed.
[a] (+) mass spectrum (at appropriate t_R) contains intensive peaks corresponding to masses of amide derivative fragments of aminoazaarenes; (−) no peaks corresponding to amide derivatives of aminoazaarenes; (+/−) mass spectrum contains peaks characteristic for fragmentary ions originating from aminoazaarenes and additional peaks that may originate from fragmentation of other compounds;(−/+) mass spectrum contains peaks corresponding to amide fragments of derivatives but their intensity is comparable to intensity of other ions (noise).
[b] The m/z values of characteristic ions for PFPA amide derivatives of aminoazaarenes. IQ: 225 (base peak, BP) and 344 (molecular ion, M^+); MeIQ: 239 (BP) and 358 (M^+); $MeIQ_x$: 240 (BP) and 359 (M^+); 4,8-$DiMeIQ_x$: 254 (BP) and 373 (M^+); PhIP: 251 (BP) and 370 (M^+).
[c] Amount (ng) of the derivatized standard introduced onto the column.

beef/pork minced chop, grilled pork fillet and grilled chicken breast samples unequivocally show the presence of almost all HPLC-identified aminoazaarenes (+) (Table 3). GC–MS analysis of amide derivatives in the remaining samples also confirmed presence of the majority of HAs determined by HPLC. In the cases when the contents of the investigated HAs were near to or lower than their HPLC detection limits (n.q. or n.d. in Table 4), the results of GC–MS analysis were ambiguously (e.g. IQ, MeIQ, MeIQx, 4,8-DiMeIQx in pork neck (no. 2) and beef collar or IQ, MeIQ, MeIQx in pork joint). The recorded mass spectra of these compounds revealed, besides fragment ions characteristic for amides, additional peaks resulting from fragmentation of other substances accompanying amides (+/−) (Table 3). The results of GC–MS analysis of all samples for PhIP amide derivative are puzzling. As can be seen from Table 3, mass spectra revealed the presence of fragmentary ions of this derivative, albeit, of very small intensity (−/+). Literature data suggest, that PhIP is formed in highly proteinaceous heat-treated food in amounts frequently greater than the remaining HAs [18,29]. Problems with the interpretation of this compound GC–MS may have resulted from too low yield of PhIP derivatization reaction as well as from the presence of excessive amounts of other matrix-coeluated substances in the aminoazaarene fraction.

As can be seen in Table 4, summarising the results of quantitative determinations, the total content of the five determined HAs falls within the range of 1.9–77.4 ng/g examined meat. It should be stressed that according to the literature data thermally treated meat dishes may contain also other biologically active aminoazaarenes such as: 2-amino-*n*,*n*-dimethylimidazopyridine (DIMP), 2-amino-*n*,*n*,*n*-trimethylimidazopyridine (TMIP), 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1), etc.[39].

A particularly high content of the five HAs determined in this work was found in investigated samples of red meat. This includes pan-fried beef/pork minced chops (77.4 ng/g) analysed together with their very well-done crust, a very well-done grilled pork fillet (56.1 ng/g) and a very well-done grilled pork neck (no. 1) (48.7 ng/g).

In some cases, despite seeing peaks in HPLC chromatograms at proper retention times and k values, reflecting thus the presence of corresponding aminoazaarenes, no quantitative determinations were made. Very small surface

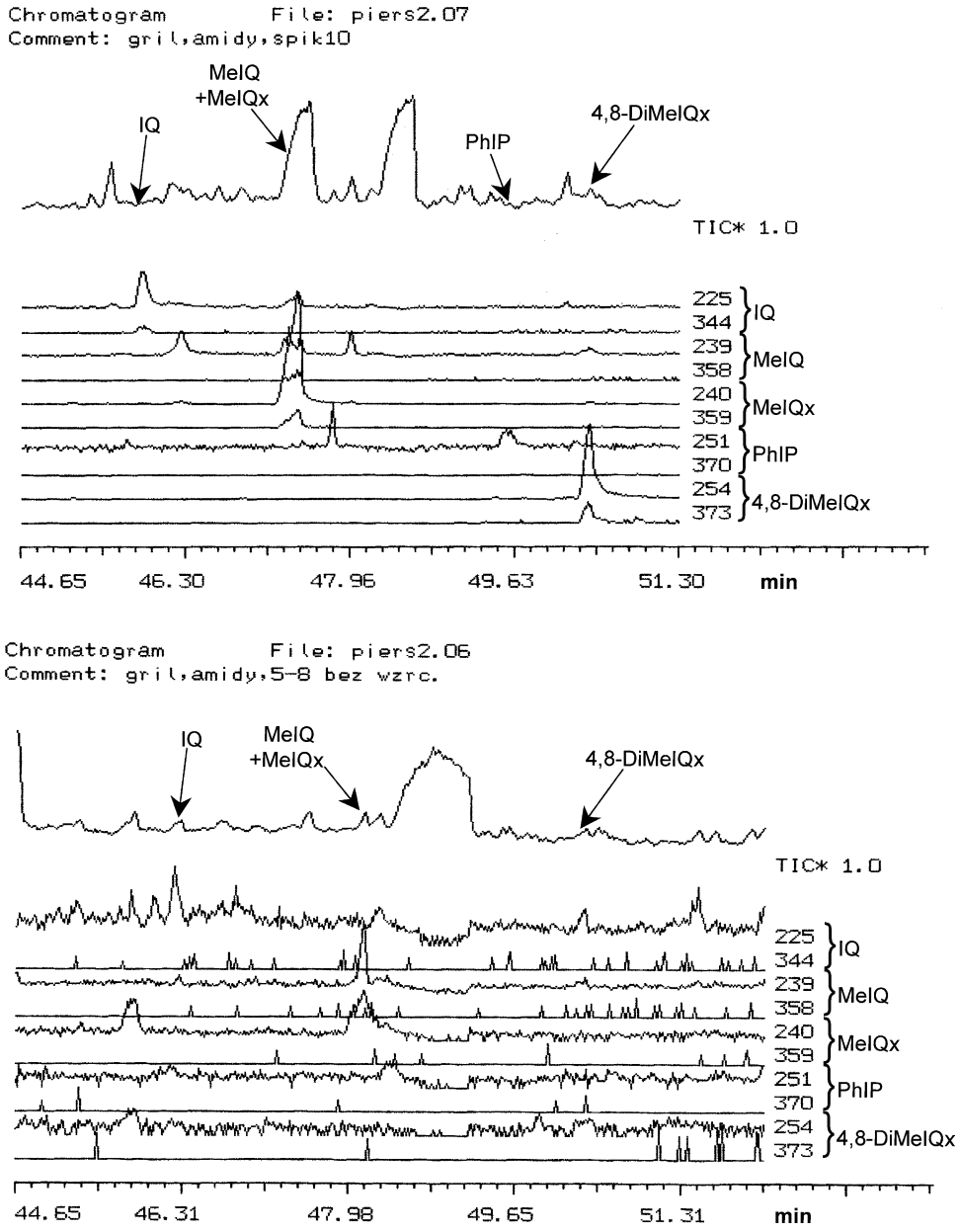

Fig. 4. GC–MS chromatograms of PFPA amide derivatives of aminozaarenes seperated from grilled chicken breast: (A) spiked and (B) unspiked samples.

areas under these peaks indicated their HAs content close to detection limit, i.e. their concentration falling below the determination limit, (0.1 ng/g of sample). For these aminoazaarenes "n.q" label was used in Table 4.

Comparison of our results with those of other investigators is rather difficult, as studied meats may have had various origins (content) and their thermal treatment, specific for local recipes, may have been significantly different. Also, the results strongly depend on clean-up procedure as well as HPLC detection method used. Considerable part of the results published so far pertains to commercially-available meat extracts or meat and its processed forms prepared in laboratory conditions, restaurants and "fast food" bars and not to meats prepared in household conditions. Nonetheless, the overall HAs content in samples investigated throughout this study (Table 4) is considerably higher than that reported by other authors previously [30,33–35]. Moreover, only a few investigators have found the presence of IQ and MeIQ in examined meat [31,34,36–39] while PhIP content was, as a rule, higher [18,29,37] than that in our samples (Table 4).

The analyses carried out in this study demonstrate that the overall content of the dominating and the most strongly mutagenic aminoazaarenes MeIQx, 4,8-DiMeIQx and PhIP falls in the 1.9–58.0 ng/g range. For comparison, Richling et al. [33] found, for example, 2.3–8.5 ng/g, Pais et al. [31] 2.8–38.2 ng/g and Johansson and Jägerstad [36] 0.2–18.5 ng/g, respectively.

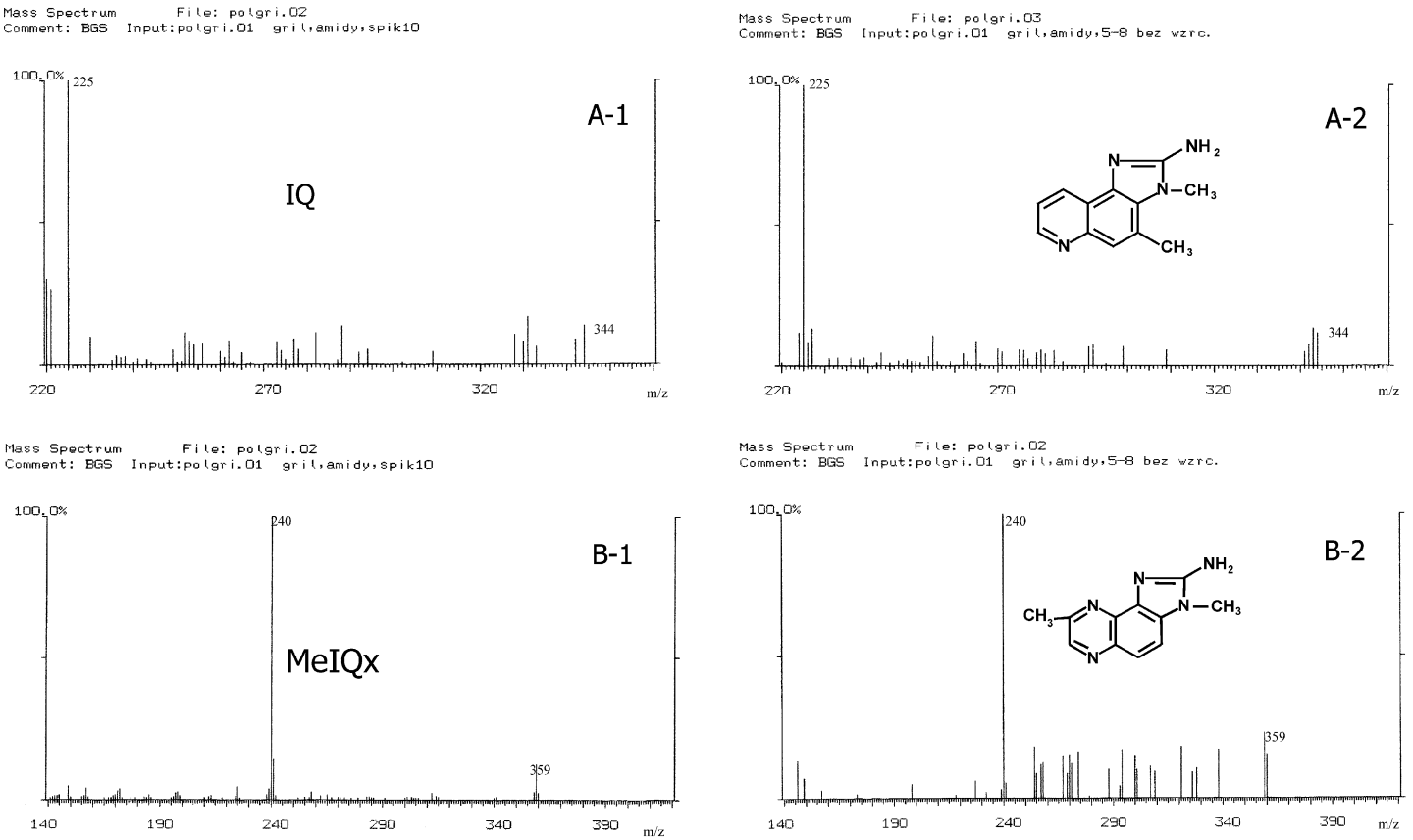

Fig. 5. Mass spectra of PFPA amide derivatives of aminoazaarenes identified in grilled pork fillet: (A-1) IQ in spiked sample; (A-2) IQ in unspiked sample; (B-1) MeIQx in spiked sample: (B-2) MeIQx in unspiked sample.

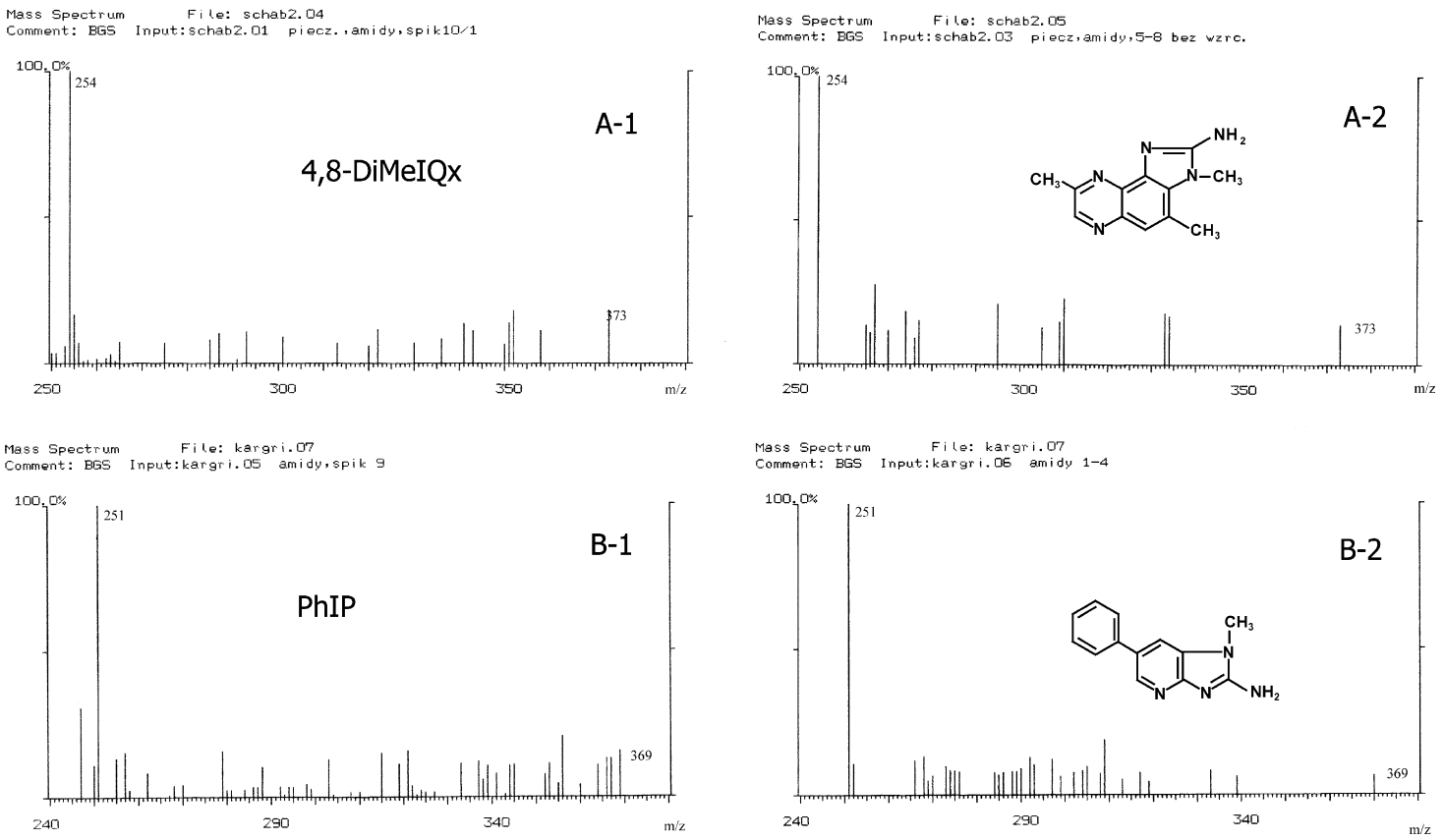

Fig. 6. Mass spectra of PFPA amide derivatives of aminoazaarenes identified in pan-fried pork chop: (A-1) 4,8-DiMeIQx in spiked sample; (A-2) 4,8-DiMeIQx in unspiked sample; (B-1) PhIP in spiked sample; (B-2) PhIP in unspiked sample.

Table 4
Heterocyclic aromatic amines content in the meat samples [ng/g cooked meat[a]] and theoretically daily human exposure [μg per day per person[b]]

Meat type[c]	IQ	MeIQ	$MeIQ_x$	4,8-Di-$MeIQ_x$	PhIP	Total HAs amount	Theoretically daily human exposure to HAs
Pork chop	1.1	1.8	3.2	7.5	2.1	15.7	1.6
Beef collar	n.q.	n.q.	n.q.	n.q.	1.9	1.9	0.2
Pork neck (no. 1)	5.8	4.4	9.1	17.4	12.0	48.7	4.9
Pork neck (no. 2)	n.q.	n.q.	n.q.	n.q.	2.0	2.0	0.2
Beef/pork minced chop	6.8	12.6	18.3	29.5	10.2	77.4	7.7
Turkey breast (no. 1)	n.q. (11)[d]	n.q. (9)	9.5 (6)	n.d. (4)	1.8 (8)	11.3	1.1
Turkey breast (no. 2)	n.q.	2.3	0.9	3.2	4.7	11.1	1.1
Pork fillet	6.7	n.d.	9.5	28.2	11.7	56.1	5.6
Pork joint	n.d. (7)[d]	n.d. (8)	n.q. (12)	2.2 (6)	1.3 (10)	3.5	0.3
Chicken breast	n.d.	4.9	1.8	2.1	7.4	16.2	1.6
Recovery (%), mean ± S.D.	83.7 ± 8.1	77.5 ± 14.5	60.6 ± 5.7	77.0 ± 12.1	61.7 ± 10.4		

n.d.: not detected; n.q.: analyte nearly its detection limit, i.e. detected in the background but not quantified.
[a] Content in ng/g, recovery corrected values.
[b] Theoretically daily human exposure to five HAs was calculated on the basis of their content in 100 g meat consumed daily.
[c] Cooking methods and doneness level are presented in Table 1.
[d] The values in parentheses are R.S.D. values (%) obtained from replicate day-to-day analyses of spiked samples: $n = 6$ for turkey breast (no. 1) and $n = 4$ for pork joint.

The IQ and MeIQ content in the investigated samples was from n.q. to 6.8 and to 12.6 ng/g, respectively. For comparison, Rivera et al. [38] have found in grilled beef 7.0 ng/g of IQ and 8.0 ng/g of MeIQ; Zimmerli et al. [35] have shown no IQ presence in grilled pork chop, and the content of MeIQ found was 1.4 ng/g. Toribio et al. [40,41] investigated a lyophilised beef extract, depending on clean-up procedure and detection method used (UV or MS), the IQ content was in the range of 32.5–37.5 ng/g while that of MeIQ reached 17.3 ng/g of extract, respectively.

Particularly noteworthy is, on the other hand, a surprisingly low aminoazaarene content in medium pan-fried beef collar meat (1.9 ng/g) (Table 4), quite often consumed in the Upper Silesia region. Using for frying the low-boiling point peanut oil rich in unsaturated fats and adding water after 20 min of frying results, in this case, in creating conditions unfavorable for HAs formation. Interestingly, the concentration of HAs in well-done roasted pork neck (no. 2), prepared without fat according to a traditional Silesian recipe called “on salt”, was approximately 20 times lower compared to a very well-done grilled pork neck (no. 1), (2.0 and 48.7 ng/g, respectively). Similarly, a well-done roasted pork joint contained 16 times less HAs than a very well-done grilled pork fillet (3.5 and 56.1 ng/g), respectively. It has been found that grilled meat, compared to meats prepared otherwise (except for beef/pork minced chop), contained more HAs. Comparison of aminoazaarene content between red meat (pork, beef) and poultry (turkey, chicken) reveals like values. This may suggest that aminoazaarene formation in meats depends above all on the kind of thermal treatment applied, i.e. on household cooking practices, and not on meat kind itself.

Table 4 shows values of theoretically daily human exposure to five determined HAs, calculated on the basis of their content in 100 g of meat consumed daily. These values fall within the range of 0.2–7.7 μg per day per person. Wakabayashi et al. [42] estimated the daily human exposure to PhIP and MeIQx to be between 0.1–13.8 and 0.2–2.6 μg per day per person, respectively. On the basis of findings of Johansson and Jägerstad [36], the human daily exposure to five different HAs resulting from a diet including 100–200 g fried meat or fish, may range from 0.04 to 7.0 μg per day per person, depending of cooking conditions and helping sizes. Although the amounts of aminoazaarenes in cooked foods are low, epidemiological studies have shown an association between cancer of the colon, rectum, bladder, prostate or kidney and meat consumption [1,18,36,43].

4. Conclusions

The presented study shows that the majority of thermal treatment procedures used in preparing meat for consumption results in the formation of promutagenic aminoazaarenes IQ, MeIQ, MeIQx, 4,8-DiMeIQx and PhIP. These compounds, very dangerous to human health, were all identified in samples of the examined meats (pan-fried, roasted or grilled pork, beef, turkey and chicken). The dominating ones were MeIQx, 4,8-DiMeIQx and PhIP.

The highest overall content of the five HAs determined was found in grilled meat. Noteworthy is the particularly high content of aminoazaarenes in beef/pork minced chop and grilled pork fillet.

Assuming a daily consumption of a 100 g helping of one of the examined meats, the calculated values of theoretically daily human exposure to five HAs fall in the range of 0.2–7.7 μg per day per person. This suggests that a diet rich in meats prepared according to recipes investigated in this study may contribute to a higher cancer incidence observed among the populace of the Upper Silesia region in Poland.

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