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THE EVALUATION OF MEAT AND MEAT PRODUCTS EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS IN ROMANIAN FOODSTUFFS

EVALUAREA EXPUNERII CĂRNII ȘI PRODUSELOR DIN CARNE LA HIDROCARBURI POLICICLICE AROMATICE ÎN PRODUSE ALIMENTARE DIN ROMÂNIA

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds with a pronounced carcinogenic potential. The formation of PAH in food depends on the thermal processing methods, correlated with the chemical composition of the product, its packaging, and the structure of the coating surface. Suitable markers of the foodstuffs contamination with the potentially carcinogenic PAHs are benzo(a)pyrene and the sum of the four polycyclic aromatic hydrocarbons (PAH4): benzo(a)pyrene ($C_{20}H_{12}$), benzo(a)anthracene ($C_{18}H_{12}$), benzo(b)fluoranthene ($C_{20}H_{12}$) and chrysene ($C_{18}H_{12}$). In this survey, 206 samples of meat and smoked meat products were analyzed in order to investigate the levels of the benzo(a)pyrene and PAH4. The High Performance Liquid Chromatography (HPLC) method was used for the qualitative analysis of foodstuff samples. The values obtained were between undetectable and 3.00±1.2 µg/kg for benzo(a)pyrene, and between undetectable and 18.01±7.48 µg/kg for the PAH4.

The highest values of the potentially carcinogenic PAHs were obtained in samples of smoked pastrami, smoked pork breast and smoked sausages for benzo(a)pyrene and in samples of smoked ham, smoked pastrami and smoked pork muscles for PAH4.

Keywords: PAHs, benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, chrysene

REZUMAT

Hidrocarburile aromatice policiclice (HAP) sunt compuși organici cu potențial carcinogen pronunțat. Formarea HAP în alimente depinde de modalitățile de prelucrare termică, corelate și cu compoziția chimică a produsului, modul în care se ambalează și structura învelișului. Markeri adecvați pentru evaluarea contaminării produselor alimentare cu HAPi potențial carcinogene sunt benzo(a)pirenului și suma celor patru hidrocarburi aromatice policiclice (HAP4): benzo(a)piren ($C_{20}H_{12}$), benzo(a)antracen ($C_{18}H_{12}$), benzo(b)fluoranten ($C_{20}H_{12}$) și crisen ($C_{18}H_{12}$). În acest studiu au fost analizate 206 probe carne și produse din carne afumate pentru determinarea nivelelor de benzo(a)piren și PAH4. Pentru analiza calitativă a probelor de produse alimentare a fost utilizată cromatografia lichidă de înaltă performanță (HPLC). Valorile obținute au fost cuprinse între nedetectabil și 3,00±1,2 µg/kg pentru benzo(a)piren și între nedetectabil și 18,01±7,48 pentru HAP4.

Cele mai mari valori ale HAP potențial carcinogene au fost obținute în probele de pastramă afumată, piept de porc afumat și cârnați afumați pentru benzo(a)piren și în probele de șuncă afumată, pastramă afumată și mușchi afumat pentru HAP4.

Cuvinte cheie: HAP, benzo(a)piren, benzo(a)antracen, benzo(b)fluoranten, crisen

Aromatic Polycyclic Hydrocarbons (PAHs) are a class of organic compounds containing two or more combined (condensed) aromatic nuclei. They include a number of carcinogenic and genotoxic substances, of which the most important are benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(i)pyrenelene, chrysene, cyclopenta(c,d)pyrene, dibenzo(a,h)anthracene, dibenzo(a,e)pyrene, dibenzo(a,h)pyrene, dibenzo(a,l)pyrene, indeno(1,2,3-cd)pyrene and 5-methylcrisene (17).

PAHs have a pronounced carcinogenic potential, which has been described over the last 200 years. In 1775, for the first time, the English physicist Percival Pott highlighted a correlation between the incidence of scrotum cancer at chimney sweepers and continuous contact with PAH-rich ash (3).

The formation of PAHs in food depends on the thermal processing methods, correlated with the chemical composition of the product, the way it is packaged and the structure of the coating. In this regard, it should be noted that most food preparation processes are performed at temperatures between 370°C and 390°C but also at temperatures higher than 400-600°C (e.g., fat frying). At temperatures between

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400°C and 1000°C, the PAHs content increase linearly, and the phenols and fatty acids reach the maximum amount at 600°C. The smoking temperature, between 20°C and 55°C, does not significantly affect the production of PAHs, and the one of 40°C allows for superior flavors than those of 20°C or 55°C (11, 12).

In roasted meat (roasting with charcoal grilling, frying) PAHs are formed during the incomplete combustion process or by the pyrolysis of the fat when oxygen and carbon are released, which ultimately lead to the formation of polycyclic compounds, which given the resulting smoke is deposited on the meat. PAHs concentration in meat roasted at a higher distance from coal is lower than in meat roasted closer to coal. In order to avoid large amounts of PAHs, grilled meat must not be in contact with the flame, the process should last longer, and the fat should be as small as possible (9, 13). Another PAH-generating process is smoking, a measure of food preservation, which has been correlated with the increased incidence of pharyngeal cancer, observed in Icelandic fishermen, large smoked fish consumers, even if they were detected in low concentrations. Accumulation of PAHs in smoked foods depends on how they are smoked, the nature of the fuel, the temperature and duration of the smoking. Wood quality plays an important role, so pyrolysis of hardwood generates smoke poorer in PAHs than softwood containing resins (19, 20). PAHs are mainly formed from carbohydrates in food, at high temperatures and in the absence of oxygen. The contaminant marker is the benzo(a)pyrene and the sum of 4 PAH (Table 1).

Depending on the number of cores contained, the hydrocarbons can be divided into:

- Light PAHs (2-4 aromatic nuclei): chrysene; benzo(a)anthracene;

- Heavy PAHs (with 5 or more aromatic nuclei): benzo(a)pyrene; benzo(a)fluoranthene; dibenzo(a)-anthracene.

Benzo(a)pyrene is a polycyclic aromatic hydrocarbon found in coal tar. Its metabolites are mutagenic and highly carcinogenic (classified as a Carcinogenic Group 1 by IARC), as a result of incomplete combustion at temperatures between 300-600°C, following the transformation of starch, amino acids and fatty acids (8, 10). The content is expressed in micrograms / kg (µg / Kg = ppb). The carcinogenic action of benzo(a)pyrene can be achieved by contact (dermal), but especially by ingestion. The maximum permitted level laid down in Regulation (EC) 1881/2006 for benzo(a)pyrene is 2.0 µg/kg and for the sum of benzo(a)-pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene is 12.0 µg/kg (5).

Annually, the National Sanitary Veterinary and Food Safety Authority establishes a sampling plan for both animal and non-animal food probes in order to monitor (screening and confirmation methods) benzo(a)pyrene in food and to prevent possible risks for smoked meat and smoked meat products, in processing units and marketing units (1).

Sampling of meat and smoked meat products aims to keep contaminants at acceptable levels, within the limits of admissibility, by applying and observing good manufacturing practices. In the case of PAHs, effective consumer health protection is ensured by eliminating from consumption meat products and smoked meat products not in conformity, consumed as such or used as a blend in other food products.

The aim of this survey was the investigation of the benzo(a)pyrene and PAH4 levels in various samples of meat and smoked meat products by using the High Performance Liquid Chromatography (HPLC) method, in 25 Romanian counties and Bucharest.

MATERIALS AND METHODS

Food samples and sampling method

A number of 206 samples (Table 2) of meat and smoked meat products, from 25 counties and Bucharest, were analyzed for the detection of PAH.

All samples were taken within the official control and self-control program (1).

Table 1

Chemical formula of Aromatic Polycyclic (2, 18)

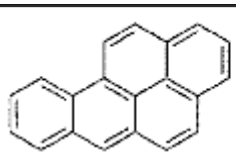	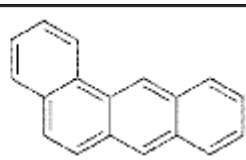	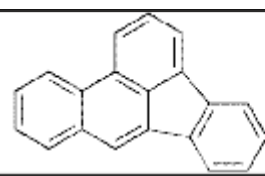	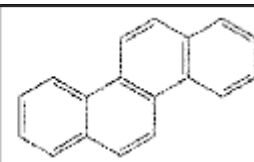
benzo(a)pyrene ($C_{20}H_{12}$)	benzo(a)anthracene ($C_{18}H_{12}$)	benzo(b)fluoranthene ($C_{20}H_{12}$)	chrysene ($C_{18}H_{12}$)

Table 2

Counties origin of food samples

Nr.crt.	County	Samples
1.	Alba	3
2.	Arad	3
3.	Argeş	12
4.	Botoşani	2
5.	Braşov	12
6.	Brăila	1
7.	Caraş Severin	1
8.	Covasna	20
9.	Cluj	14
10.	Dâmboviţa	2
11.	Dolj	2
12.	Harghita	3
13.	Ilfov	10
14.	Maramureş	17
15.	Mureş	3
16.	Neamţ	2
17.	Olt	8
18.	Prahova	7
19.	Satu - Mare	9
20.	Sibiu	9
21.	Suceava	6
22.	Timiş	2
23.	Tulcea	9
24.	Vaslui	3
25.	Vrancea	22
26.	Bucharest city	24

The sampling was carried out in accordance with the provisions of EC Regulation 333/2007 (6). The overall sample was about 1 kg, unless this was not possible, for example, when the sample consisted of a package or a unit. The minimum number of incremental samples taken from the lot or sublot is presented in Table 3.

Table 3

Minimum number of incremental sample to be taken from batch or sublot

Lot / sublot weight or volume (in kg or liter)	Minimum number of incremental sample to be taken
< 50	3
≥ 50 and ≤ 500	5
> 500	10

If the batch or sublot was made up of separate packages or units, the number of packages or units to be sampled to form a bulk sample was established according to Table 4.

Table 4

Number of packages or units (incremental samples) to be taken to constitute the bulk sample if the lot or sublot was made up of separate packages or units

Packages or units in the lot/sublot	Number of packages or units sampled
≤ 25	At least 1 pack or 1 unit
26-100	Approximately 5%, at least 2 packages or units
> 100	Approximately 5%, maximum 10 packages or units

After sampling, each sample was placed in a clean and inert container (aluminum foil) which provides adequate protection against contamination, loss of adsorption substances on the inside of the container and damage during transport. The samples taken were sealed and coded, keeping records of each sampling.

High Performance Liquid Chromatography (HPLC)

The methods of analysis for the official control of the levels of Pb, Cd, Hg, 3-MCPD and PAHs in foodstuffs was carried out in accordance with the provisions of EC Regulation 333/2007 (6).

HPLC is a qualitative analysis method used in biochemistry and analytical chemistry for the separation, identification and quantification of compounds (14).

The method consists in the PAHs being extracted with the acetonitrile/acetone mixture followed by two purifications on the C18 reverse phase cartridge and on the florisil cartridge. After separation, liquid chromatography is determined, and fluorescence measured at different excitation and emission wavelengths, according to the standardized and optimized working method. In order to obtain replicable results, the ambient temperature in the laboratory was stable (≤ 20 °C), higher temperatures increase the solubility of short chain fatty acids. The detection program is presented in Table 5.

Table 5

Detection program

Component	Time min	Excitation wavelength nm	The emission wavelength nm
Benzo (a)anthracene	**12.5**	**270**	**385**
Chrysene	14.0		
Benzo (b)fluoranthene	21.8	256	446
Benzo (a)pyrene	31. 2	292	410
Dibenzo(a,h) anthracene	44.1		

After the exact weighing of 2g of sample, 5 stages (0 - 4) were carried out for the analytical process followed by the actual determination (optimization of the apparatus, calibration of the calibration curve, reading, calculation and expression results).

Stage 0

The sample to be analyzed, chopped and well homogenized, was extracted with methanol and then with chloroform in two steps. The last bottom layer was collected in the same vial and then evaporated to dryness.

Stage 1

The sample to be analyzed was subjected to a three-stage Mixture 1 (acetonitrile and acetone) extraction, the supernatant (3 x 4 ml) being collected in the same vial, then evaporated to dryness. The fatty residue was thus obtained.

Stage 2

The fat residue was purified by successive extractions with the same Mixture1 in three steps, the supernatant (3 x 2 ml) being collected in the same vial, and then purified on the C18 cartridge. Prior to switching to the cartridge, this was conditioned according to the manufacturer's operating instructions.

After passing elute, Mixture 1 was added to elute all the particles of interest. All the eluate thus obtained was evaporated to dryness. The resulting oily residue was treated with hexane and thus extracted with hexane, which was kept for 24 hours at -18 ° C.

Stage 3

The hexane extract was subjected to successive extractions with Mixture 2 (hexane and dichloromethane) in three steps, the supernatant (3 x 2 ml) was collected in the same vial and then purified on the florisil cartridge. Prior to switching to the florisil cartridge, it was conditioned following the manufacturer's instructions. After passing the eluate, Mixture 2 was added to elute all the particles of interest.

Stage 4

All the eluate thus obtained was concentrated to 1 ml. It was then treated with 0.5 ml of toluene and evaporated to 50 µl, internal standard added and made up to the volume of interest (250 µl, 500 µl) with acetonitrile. Note that all of the fat residues were weighed and the evaporation to dryness was made with nitrogen in a water bath at 35°C.

The Determination

Optimizing the device

Before the determination was made, the Varian LC MS was opened, and optimization of the working parameters was done.

Drawing the standard curve

PAHs were prepared at concentrations of 1, 2, 3, 4, 5 ppb, with which the calibration curve was drawn. The curve shall be linear and pass through the origin, and the correction coefficient shall be at least 0.995.

Reading

After adjusting the device, the identification data of the samples to be analyzed was entered into the program. After the device was initialized, the samples were injected, following all the steps required by the machine's reading program. The reading was completed by the individual chromatogram for each sample read and by an analysis report, which identifies the concentration of each substance at the retention times specific to the PAHs analyzed. In the determination set was also read a fortified test at the maximum value allowed according to the legislation (5).

Calculation and Results

Before the calculation, the following aspects were pursued: the recovery percentage of the fortified sample to be in accordance with the regulations in force (16); retention times are appropriate for the analyzed PAHs; the internal standard (SI) area is the appropriate one; in the case of dilutions, take into account the dilution factor; issuing the analysis report and processing the data by correcting the value with the recovery ratio for benzo(a)pyrene and the PAH4, respectively (6).

RESULTS AND DISCUSSION

The results obtained from the laboratory examinations of meat and smoked meat products are presented in Table 6.

The recovery rate is the actual percentage of the concentration of a recovered substance during the analytical procedure. It is determined during validation if no certified reference material is available and it refers only to the benzo(a)pyrene, and not to the PAH4. For undetectable and non-quantifiable values, the recovery rate does not apply.

The obtained values were between undetectable and 3.00 ± 1.2 µg / kg for benzo(a)pyrene in smoked pastrami and between undetectable and 18.01 ± 7.48 for PAH4 (corrected with recovery) in smoked ham, some of the maximum values being higher than the maximum permitted levels laid down in the European Regulation (5).After applying the correction index (±), finally the values were below the maximum acceptability levels.

Table 6

Results obtained from the analysis of samples of smoked meat and smoked meat products

	Matrix	Samples	Values		
			Benzo(a)pyrene µg/kg	Benzo(a)pyrene recovery %	PAH4 µg/kg
1.	Smoked bacon: traditional, frozen	4	Undetectable*	-	0.72±0.31 – 2.43±1.01
2.	Smoked sausages: homemade, traditional, country side, rose, from sheep and calf, Plescoi, semi-smoked extra, for grill, for grill spicy, smoked ham, bacon, garlic, for Christmas, „Carniprod", „Stauder", „Bătrânu Sas" „Tătăran" „Baroni", "Montbeliard" ,"Udvarhelyi" , "Deak", "Hajdu".	56	Undetectable* Unquantifiable** - 2.5±1.00	57.1 -107.4	0.56±0.23 – 11.08±4.60
3.	Smoked pork neck	5	Undetectable* - 0.75±0.30	73.6	0.45±0.19 – 8.16±3.39
4.	Smoked bone: pork, homemade, boned, halfboned	4	Undetectable* - Unquantifiable**	73.6	0.70±0.29 – 2.31±0.86
5.	Smoked sausages : from Cristur, with chicken breast	2	Undetectable* - 0.81±0.32	73.6	3.43±1.42
6.	Smoked pork ribs	9	Undetectable* - Unquantifiable**	86.8	0.57±0.24 - 7.00±2.91
7.	Smoked ribs	2	Undetectable* - 0.81±0.32	73.6	3.57±1.440 – 6.85±2.84
8.	Smoked pork chops	4	Undetectable* - 0.87±0.35	107.4	5.8932
9.	Smoked pigtails	1	1.677±0.67	107.4	11.92±4.95
10.	Pork jowl with pepper	1	Undetectable*	-	2.2512
11.	Smoked ham : Pădurea Neagră, with turkey breast	8	Undetectable* - 1,0490±0,3429	70.7	0.25±0.10 – 11.23±4.66
12.	Smoked Kaizer: pork breast, extra.	9	Undetectable* - 2,584+0,8448	70,7	0.47±0.20 – 10.9200
13.	Smoked Leber	1	Undetectable*	-	1.76±0.73
14.	Smoked pork tenderloin: file, raw smoked, smoked pork, "Azuga", "Vilica" Delikatesa	16	Unquantifiable** - 0.97±0.39	67.1 – 107.4	0.73±0.30 – 13.8±5.73
15.	Smoked garf bones	1	Unquantifiable**	-	1.8859
16.	Pancitta Affumicata	1	Undetectable	-	1.62±0.67
17.	Smoked pastrami: sheep, pork, Aurora, turkey breast.	17	Nedetectabil* Unquantifiable** - 3.00±1.2	86.8 – 104.4	0.107±0.04 – 15.65±6.5
18.	Smoked pork legs	1	Nedetectabil*	-	Undetectable
19.	Hunter's smoked pork breast	5	Unquantifiable** Undetectable* - 0.82±0.33	67.1	3.79±1.57 - 7.26±3.01
20.	Smoked pulp : pork Transylvanian	3	Undetectable* Unquantifiable** 1.22±0.49	70.7 – 95.2	0.90±0.37 5.43±2.26
21.	Smoked Salami: dry, Baranya, Deli, winter's , summer's, of Banat, Sibiu, peasant salami with ham, Selmont, Sinaia, raw smoked, Carniprod, Paprika, homemade, rustic, sax, French, Lunghetto, Brio, Victoria, Bucureşti, ,	27	Undetectable* Unquantifiable**	69.1 – 107.4	Undetectable 0.48+0.20 – 6.37±2.65
22.	Smoked bacon	3	Undetectable* Unquantifiable**	67.1 - 107.4	0.48±0.20 – 1.7369
23.	Pork Shoulder Bega	1	Undetectable*	-	1.4966±0.6211
24.	Pork fillet specialty	1	Undetectable*	-	1.34±0.56
25.	Smoked ham: Pădurea Neagră, "Miska" from Deva, Şugatag, homemade, Transylvanian, raw	19	Undetectable* Unquantifiable** - 0.84±0.34	86.8 – 99.1	2.31±0.96- 18.01±7.48
26.	Smoked bacon: Transylvanian, traditional	5	Undetectable* Unquantifiable**	-	0.81±0.34 - 6.58±2.73
	TOTAL	206			

* **Undetectable < LOD** (Limit of detection, smallest measured content, from which it is possible to deduce the presence of the analyte with reasonable statistical certainty).
** **Unquantifiable < LOQ** (Limit of quantification, lowest content of the analyte which can be measured with reasonable statistical certainty).

In the case of the benzo(a)pyrene, the maximum value was 3.00±1.2 g/kg for smoked pastrami, 2.584±0.8448 for smoked pig breast, 2.5±1.00 for smoked sausages, when the maximum allowable value is 2.0 µg/kg. Values near the maximum allowable limit were also recorded in the smoked pork chest (2.584±0.8448) and smoked pork loin (1.22±0.49).

For the PAH4, the maximum value was 18.01± 7.48 µg/kg for smoked ham, 15.65±6.5 for smoked pastrami and 13.8±5.73 for smoked pork tenderloin, over the maximum admissible value of 12.0 µg/kg. Values close to the maximum admissible limit were also recorded in smoked pigtails (11.92±4.95), smoked ham (11.23±4.66), smoked sausages (11.08 ± 4.60), smoked kaizer (10.92) and smoked pig head (8.16 ± 3.39).

The values obtained from other food products were lower for both parameters. Thus, it was found that, for benzo(a)pyrene, the lowest values were found in smoked leber, smoked garf bones, smoked bones, smoked bacon, smoked sausages, smoked legs, Bega backs, smoked pig fillet and some smoked salami varieties. Even if for these products and for others specified in Table 6 the obtained values are within the admissible limits, they have to be evaluated and controlled, and setting maximum levels for benzo(a)pyrene in certain foods, where the processes of smoking can cause high levels of contamination, are extremely important because they have the role of protecting public health. Various authors reported different levels of PAHs in various food products depending on their processing (charcoal, gas, oven grilling).

Farhadian *et al* (2010) found fluoranthene in all samples, with the highest concentration of total PAHs in beef satay (132 ng/g) and the lowest in oven grilled chicken (3.51 ng/g) (7). In the non-marinated meat (kebab, Kofta) the mean values of Anthracene, Fluranthene, Chrysene, Benzo(a)anthracene and Benzo(a)-pyrene were 18.2 ± 11.2; 57 ± 28.59; 18.6 ± 1.69; 16.8 ± 7.0 and 9.2 ± 5.67 µg / kg, respectively. The total concentration values of the existing PAHs in the examined non-marinated charcoal grilled Kebab and Kofta were 119.8 ± 54.15 and 59.2 ± 16.9 µg/kg, respectively. Thus, marinating the meat prepared for charcoal grilling will greatly reduce the most hazardous carcinogenic PAHs compound such as benzo(a)-pyrene resulting in more safe charcoal grilled meat for human consumption (4). In traditional products the PAH value is lower, with 32.46 µg/kg benzoin(a)pyrene values in smoked bacon and 15.49 µg/kg in smoked sausage. The values are lower than twice their value for traditionally smoked products than for commercially smoked products (15, 16).

CONCLUSION

In the case of benzo(a)pyrene, the maximum value was obtained from smoked pastrami, smoked pig chest and smoked sausages. Values close to the maximum allowable limit were recorded in smoked kaiser, smoked pork chest and smoked pulp. In the case of PAH4, the maximum value was obtained from smoked ham, smoked pastrami and smoked tenderloins. Values close to the maximum admissible limit were also recorded in smoked pigtails, smoked ham, smoked sausages, smoked kaiser, and smoked pig neck. Values obtained from other food products were lower for both parameters. Thus, it was found that, for benzo(a)pyrene, the lowest values were found in smoked leber, smoked garf bones, smoked bones, smoked bacon, smoked sausages, smoked legs, Bega backs, smoked pig fillets and some smoked salami varieties. Even if the obtained values are within the limits of admissibility, meat and smoked meat products must be checked by laboratory tests, as smoking processes can lead to high levels of contamination with Polycyclic Aromatic Hydrocarbons, compounds with recognized carcinogenic potential.

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