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Polycyclic aromatic hydrocarbons in meat smoked with different types of wood

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ABSTRACT

The influence of the wood used for the smoking of meat on the formation of polycyclic aromatic hydrocarbons (PAH) has been studied. Ten types of wood and charcoal were used for preparation of smoked meat samples. The analytical sample preparation method implied extraction of PAH with cyclohexane, liquid–liquid extraction with *N*,*N*-dimethylformamide/water, back extraction with cyclohexane, followed by clean-up on silica solid phase extraction (SPE) column and quantification by gas chromatography–mass spectrometry. It was found that the type of wood has a significant influence on the amount of PAH in smoked meat. The samples smoked with apple-tree and alder contained the smallest PAH concentrations. The samples smoked with spruce had the highest concentrations of PAH. The difference in content of benzo[*a*]pyrene (from 6.04 till 35.07 μg/kg) and total PAH (from 47.94 till 470.91 μg/kg) indicates that choice of wood for smoking is one of the critical parameter to be controlled in order to diminish the contamination of food products.

1. Introduction

Smoking is one of the oldest methods of food preservation and is still widely used in fish and meat processing. Wood smoke contains a large number of polycyclic aromatic hydrocarbons (PAH) and their alkylated derivatives (Obiedzinski & Borys, 1977).

PAH occur as contaminants in different food categories including water, vegetables, fruit, cereals, oils, smoked meat and fish (Dennis, Massey, McWeeny, Knowles, & Watson, 1983; Moret & Conte, 2002; Moret, Conte, & Dean, 1999; Šimko, 2002). However, smoked products have traditionally received special attention because considerable amounts of PAH have been detected (Gomaa, Gray, Rabie, Lopez-Bote, & Booren, 1993; Karl & Leinemann, 1996; Larsson, Pyysalo, & Sauri, 1988).

Recently, the European Union established maximum levels for benzo[*a*]pyrene (BaP) for different food categories (Commission Regulation (EC) 208/2005). The maximum level of BaP 5 ng/g was set for the smoked meat. The Scientific Committee on Food concluded in its opinion of 4th December 2002 that a number of heavy PAH are carcinogens and that BaP can be used as a marker for the occurrence and effect of these carcinogenic PAH in food (Opinion of the Scientific Committee, 2002). According to the Commission Recommendation further analyses of the relative proportions of these PAH (benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*g*,*h*,*i*]perylene, benzo[*a*]pyrene, crysene, cyclopenta[*c*,*d*]pyrene, dibenz[*a*,*h*]anthracene, dibenzo[*a*,*e*]pyrene, dibenzo[*a*,*h*]pyrene, dibenzo[*a*,*i*]pyrene, dibenzo[*a*,*l*]pyrene, indeno[1,2,3-*cd*]pyrene, 5-methylchrysene) in foods is necessary, to inform a future review of the suitability of maintaining benzo[*a*]pyrene as a marker (Commission Recommendation 2005/108/EC).

Conditions of smoke generation can dramatically influence the level of PAH in smoked foods (Toth & Potthast, 1984). Results obtained by Potthast (1979) show that the PAH concentration found in smoke coming both from softwood (pine) and from hardwood (beech) are very similar. However, another study on PAH in herring samples smoked over smoldering spruce and juniper twigs showed a slight tendency that the high molecular PAH were relatively more abundant in spruce juniper smoked samples (Larsson, 1982).

There is only limited number of studies devoted to the influence of these factors on the level of PAH in food. In this paper the influence of species of wood on the PAH content in smoked meat has been studied. Ten different wood types and in market available charcoal were used for smoking of meat.

2. Materials and methods

2.1. Chemicals

For the sample preparation cyclohexane (ECD tested), *N*,*N*-dimethylformamide, methanol (HPLC grade), sodium chloride (ACS) were purchased from Acros, ethanol from J.T. Baker, sodium sulphate (ACS) from Fluka, potassium hydroxide from Avsista and silica solid phase extraction (SPE) tubes (500 mg) from Phenomenex. Ultra pure water was obtained with a MilliQ filter system.

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Mixture of 15 PAH standards: benz[*a*]anthracene (B*a*A), benzo[*b*]fluoranthene (B*b*F), benzo[*j*]fluoranthene (B*j*F), benzo[*k*]fluoranthene (B*k*F), benzo[*g*,*h*,*i*]perylene (B*ghi*P), benzo[*a*]pyrene (B*a*P), crysene (CHR), cyclopenta[*c*,*d*]pyrene (CPP), dibenz[*a*,*h*]anthracene (D*ah*A), dibenzo[*a*,*e*]pyrene (D*ae*P), dibenzo[*a*,*h*]pyrene (D*ah*P), dibenzo[*a*,*i*]pyrene (D*ai*P), dibenzo[*a*,*l*]pyrene (D*al*P), indeno[1,2,3-*cd*]pyrene (I*cd*P), 5-methylchrysene (5MC) and deuterated standard benzo[*a*]pyrene-d_{12} were purchased from Dr. Ehrenstrofer. The standard mix of PAH consisted of a solution in acetonitrile with concentration 50 mg/l and the concentration of deuterated benzo[*a*]-pyrene-d_{12} dissolved in cyclohexane was 1000 ng/μl. Mixtures were stored at 4 °C.

2.2. Samples and preparation

The pork obtained from local supermarket has been used for the experiments. It was smoked in home made smoking kiln (see Fig. 1) using 10 different wood types and commercial charcoal. Temperature in smoking chamber was maintained 80 °C that was controlled with ventilator and temperature regulator. By changes of temperature, the regulator switched off or on the ventilator, consequently the airstream to smoking chamber was interrupted or restored. Smoking time was 5 h.

The samples of meat were stored for one week at −18 °C in dark before the analysis. The meat was thoroughly homogenized. The sample preparation procedure was elaborated according to the Larsson (1982) with changes made in order to adapt to the gas chromatography–mass spectrometry (GC–MS) detection method. Twenty-five grams of sample were placed into round bottomed flask, 12 g of potassium hydroxide and 100 ml of ethanol were added. Then, 25 μl of internal standard benzo[*a*]pyrene-d_{12} solution with concentration 10 ng/μl and 125 μl of PAH mix with concentration 1 ng/μl were added, and the mixture subjected to an alkaline treatment with potassium hydroxide and ethanol by heating for 2 h (40 °C) under reflux and filtered. After cooling to room temperature solution was transferred to a 500 ml separating funnel, 100 ml of water and 100 ml of cyclohexane were added. The funnel was shaken and the layers were allowed to separate. The ethanol/water phase was transferred into a 250 ml separating funnel and shaken with another 50 ml of cyclohexane. The ethanol/water phase was discarded and the cyclohexane phases were combined. The cyclohexane solution was washed successively with 50 ml × 2 of water, 50 ml of methanol/water (4:1) and 50 ml × 2 of water. The cyclohexane extract was shaken with 50 ml of *N*,*N*-dimethylformamide/water (9:1) solution. The layer of *N*,*N*-dimethylformamide/water solution was transferred into a 250 ml separating funnel, 50 ml of 1% NaCl solution were added and PAH were extracted with 75 ml of cyclohexane. The cyclohexane phase was dried over anhydrous sodium sulphate and concentrated by rotary evaporator under reduced pressure (40 °C, 235 mbar). The extract was applied to a silica SPE column previously conditioned with cyclohexane (5 ml). The flask was rinsed with cyclohexane (3 ml), and the PAH were eluted with 6 ml (3 ml × 2) cyclohexane. The collected fraction was evaporated under a light stream of nitrogen at 40 °C temperature, dissolved in 50 μl of cyclohexane and transferred into a GC vial.

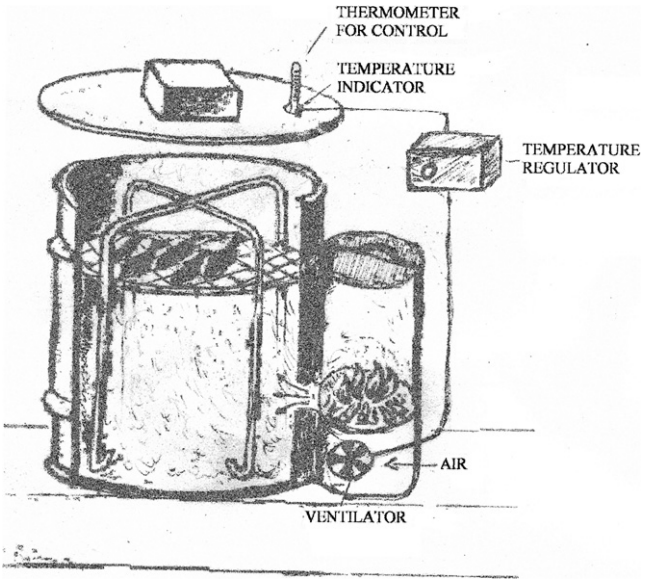

Fig. 1. Scheme of home made smoking kiln used for meat smoking.

2.3. Gas chromatography with mass selective detector (GC–MS)

A Hewlett Packard Model 6890 gas chromatograph equipped with a Model 5973 mass selective detector was employed for analysis. Operating conditions were as follows: Varian Factor Four capillary column 30 m × 0.25 mm with film thickness of 0.25 μm, helium carrier gas 1 cm^3/min, injector and detector temperature 280 °C, temperature program: 120 °C (1 min), 120→250 °C (15 °C/min), 250 °C (13 min), 250→280 °C (20 °C/min), 280 °C (1 min), 280→300 °C (35 °C/min), 300 °C (20 min). Total run time was 45.74 min. The ionizing voltage was 1941 V. One microlitre of the sample solution was injected into gas chromatograph. The data were acquired operating the MS in selected ion monitoring mode. Peak spectra were compared to the mass spectra of PAH standards and library supplied with the instrument. It was not possible to separate B*b*F and B*j*F form using this methodology. These compounds were determined together as the sum. A chromatogram of PAH standard solution is given in Fig. 2.

3. Results and discussion

As has been described above, the samples were subjected to an alkaline treatment, extracted with cyclohexane, than liquid–liquid extraction with *N*,*N*-dimethylformamide-water, back extraction with cyclohexane was carried out. Before the determination of PAH by GC–MS, the samples were cleaned up by silica SPE tubes. The limits of detection for all compounds were <0.10 μg/kg. The quality control of PAH analysis was performed by inclusion of control samples with known added amount of PAH to the analytical sequence. Average recoveries of individual PAH were from 75% to 110%.

First, it must be noticed that Table 1 shows that in samples were abundant 12 up to all 15 in EU recommendation listed PAH. The concentrations of PAH generally were higher for PAH with smaller molecular weight (*m*/*z* 226 and 228) and were smaller or below detection limit for PAH with the molecular weight *m*/*z* 302. Only in samples smoked with spruce all 15 PAH were detected however the concentrations of B*a*P in all samples were exceeding the maximum permitted limit in Europe.

The samples smoked with apple-tree and alder generally contained the smallest concentrations both of individual and of total PAH. However concentration of B*a*P in samples smoked with maple generally was similar in the samples smoked with alder, the total PAH concentration is much higher, due to higher concentrations of CPP, B*a*A and CHR.

Despite the fact that hardwoods including hazel and aspen are recommended for smoking (Курко, 1969), we obtained high concentrations of B*a*P in smoked meat using this type of wood (see Table 1). Bird-cherry as well indicated higher B*a*P concentration in products in comparison with apple-tree and alder.

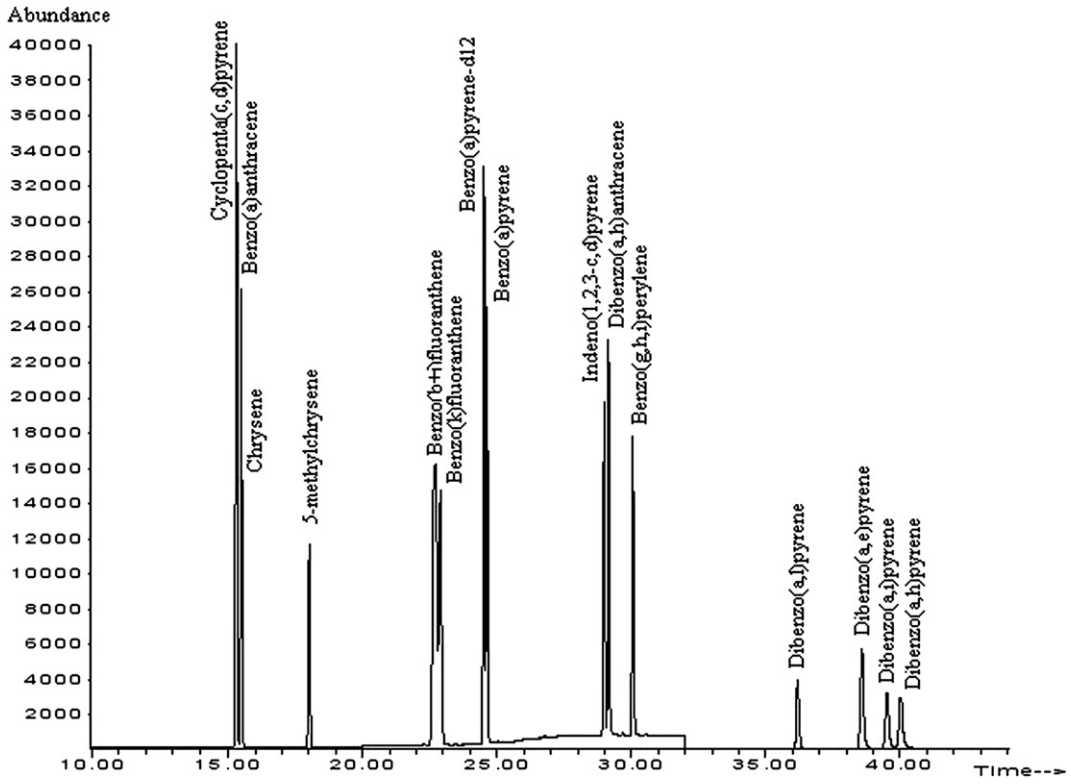

Fig. 2. Chromatogram of PAH standard solution (2.5 ng/μl).

Table 1
Mean concentration of PAHs determined in samples of meat ($n = 3$) smoked with different species of wood

PAHs	Apple	Alder	Alder + juniper	Spruce	Maple	Hazel	Plum	Aspen	Bird-cherry	Rowantree	Charcoal
Mean concentration found, μg/kg											
CPP	8.97	16.10	44.25	138.19	31.81	85.01	59.13	75.75	24.52	40.20	12.75
B*a*A	8.43	12.97	40.14	111.80	25.84	76.88	82.07	49.28	20.79	36.35	17.10
CHR	9.53	13.82	37.90	114.66	26.59	75.71	92.05	45.51	19.67	38.27	17.69
5MC	1.34	1.95	4.57	14.06	2.73	8.00	8.43	7.24	2.91	6.06	2.54
B*b*F + B*j*F	3.30	5.32	10.75	6.34	4.37	16.86	17.03	16.96	10.11	10.27	5.67
B*k*F	2.12	3.24	6.76	10.46	2.64	10.08	10.63	9.67	5.43	5.55	3.30
B*a*P	6.04	9.43	20.38	32.34	9.31	30.97	30.59	35.07	17.30	20.06	10.01
I*cd*P	2.62	4.22	9.83	13.78	3.05	13.11	9.18	20.89	9.32	10.43	5.09
D*ah*A	0.44	0.62	1.36	2.08	0.62	1.84	1.23	2.77	1.44	1.66	0.84
B*ghi*P	2.99	5.01	10.21	15.44	3.75	15.89	9.68	23.99	10.69	10.05	4.96
D*al*P	1.14	1.74	4.42	7.13	1.46	5.63	3.11	10.87	4.19	5.03	n.d.[a]
D*ae*P	0.50	0.45	1.09	1.89	0.44	1.58	0.86	3.03	n.d.	n.d.	n.d.
D*ai*P	0.52	0.43	1.01	1.95	0.34	2.10	0.68	n.d.	n.d.	n.d.	n.d.
D*ah*P	n.d	n.d.	n.d.	0.79	n.d.	n.d.	n.d.	6.04	n.d.	n.d.	n.d.
Sum of PAHs	47.94	75.30	192.67	470.91	112.95	343.66	324.67	307.07	126.37	183.93	79.95

[a] Below detection limit (<0.10 μg/kg).

Lately charcoal is often used as fuel in food processing, including the smoking of meat, besides charcoal sometimes are considered as less hazardous firewood. We performed the smoking using the commercial charcoal as well. Results show that meat smoked with charcoal contains concentrations of BaP similar to the meat smoked with alder, however no dibenzopyrenes were found.

Woods can be mixed in the smoking process to add flavour to meat. One of such wood type used in the smoking process is juniper, which is widely used to improve the flavour and taste of smoked product. We observed that smoking with alder with addition of juniper increases the concentration of BaP and total PAH more than two times.

One of reasons why softwoods are not advised for use in food smoking could be connected with high content of resin that causes high concentrations of PAH. Materials that contain resin can promote intensive origination of soot and wherewith smoking products can be polluted with PAH. Probably the reason of elevated concentrations of PAH found in meat smoked with plum can be a gum that is present on bole of plum.

The samples smoked with spruce has generally the highest values of individual and total PAH concentrations. That is due to higher concentrations of CPP, B*a*A, CHR and 5MC in samples smoked with this type of wood. In our study B*a*P content in meat smoked with spruce was five times higher in comparison with smoking with apple. Although percentage of B*a*P in meat smoked with spruce was less as by using another type of wood the significant quantities of other PAH were found. The total concentration of PAH in samples smoked with spruce was ten times higher as in samples smoked with apple.

The proportions of all PAH in smoked meat vary depending on the used type of wood. For example CPP amount (%, from total

PAH) in meat smoked with spruce and maple was 29% and 28%, respectively, however in meat smoked with charcoal only 16%. Relative proportion of sum of B*b*F and B*j*F found in meat sample smoked with spruce was only 1.3% that is significantly less as found in meat smoked with other type of wood. Only in meat smoked with aspen high concentrations of D*ah*P were found. In spite of the fact that total content of PAH in meat smoked with alder and apple-tree was less, relative ratios of D*ae*P and D*ai*P were higher in comparison with meat smoked with other type of wood.

4. Conclusions

The wood nature has a significant influence on the amount of PAH in smoked meat. The samples smoked with apple-tree and alder generally contain the smallest concentrations both of individual and of total PAH. The samples smoked with spruce has generally the highest values of individual and total PAH concentrations. The difference in content of B*a*P (from 6.04 till 35.07 μg/kg) and total PAH (from 47.94 till 470.91 μg/kg) indicates that choice of wood for smoking is one of the critical parameter to be controlled in order to diminish the contamination of food products.

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