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Polycyclic aromatic hydrocarbons in smoked cheese

Marie Suchanová,[1] Jana Hajšlová,[1]* Monika Tomaniová,[1] Vladimír Kocourek[1] and Luboš Babička[2]

[1]*Institute of Chemical Technology, Prague, Faculty of Food and Biochemical Technology, Department of Food Chemistry and Analysis, Technická 3, 166 28 Prague 6, Czech Republic*

[2]*Czech University of Life Sciences, Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Quality of Agricultural Products, Kamýcká 129, 165 21 Prague 6 - Suchdol*

Abstract

BACKGROUND: Polycyclic aromatic hydrocarbons (PAHs) represent a group of organic compounds containing two or more aromatic rings. Their control in the human food chain is required due to the mutagenic and carcinogenic potential, exhibited in vertebrates. In the present study, the occurrence of PAHs in 36 cheeses smoked by various processes was investigated.

RESULTS: PAH concentrations (sum of 15 US EPA PAHs) found in samples smoked under controlled industrial conditions were at level 0.11 μg kg^{-1}, whereas in 'home-made' cheeses, the PAH content was up to 10 times higher. A similar trend was observed for B[*a*]P, a marker compound representing carcinogenic PAHs. While its levels in commercial products prepared by controlled smoking technologies were close to the limit of quantification (0.03 μg kg^{-1}); in household samples, the B[*a*]P content ranged from 0.6 to 0.9 μg kg^{-1}. Significantly higher amounts of PAHs (up to three to six times) were found in surface layers as compared to internal parts of cheese.

CONCLUSION: Although smoked cheese is a popular food, only several papers have focused on PAH levels in these products. This paper evaluates the contribution of different smoking technologies to PAH contamination of several cheeses and thus can help in a risk assessment associated with their consumption. Moreover, the study shows the concentration ratios of selected PAHs, from which the type of smoking technology can be indicated. The results obtained in this study also supported the suggestion of the EU Scientific Committee on Food to use benzo[*a*]pyrene as an indicator of the occurrence of higher-molecular mass PAHs.
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Keywords: Polycyclic aromatic hydrocarbons (PAHs); smoked cheese; HPLC/FLD

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) constitute a large group of organic compounds containing two or more fused aromatic rings. PAHs occur widely in the human environment, mainly as a result of incomplete combustion of organic matter; for example, it may take place during fires, in various industrial processes or in car engines. In toxicological studies, several PAHs have been demonstrated to be carcinogenic, and therefore they represent an issue of a great concern to health.

Although air or drinking water may be responsible for some human exposure, the highest PAHs intake is typically associated with the occurrence of these hazardous chemicals in diet. Contamination of food crops by PAHs may be caused by environmental emissions; nevertheless, fairly high levels in some food commodities may be due to the processing practices such as drying and/or smoking. Also grilling, roasting and frying are high temperature processes potentially generating 'food-borne' PAHs. For instance, in barbecued meat the total PAHs were found to be present at levels up to 164 μg kg^{-1} with benzo[*a*]pyrene (B[*a*]P) being present at levels as high as 30 μg kg^{-1}, whereas in uncooked foods the average background values are usually in the range of 10^{-1} to 1 μg kg^{-1}.[1]

The health risk associated with dietary PAHs has recently been evaluated by Scientific Committee on Food (SCF).[2] In European Commission (EC) recommendations[3] the member states have been advised to monitor 15 SCF priority PAHs together with one additional PAH benzo[*c*]fluorene, recommended by the JECFA (Joint FAO/WHO Expert Committee on Food Additives).[4] The maximum levels recently set by EC on B[*a*]P for smoked meat products is 5 μg kg^{-1}; however, no limits are given for smoked cheese products at present.[5]

* Correspondence to: Jana Hajšlová, Institute of Chemical Technology, Prague, Faculty of Food and Biochemical Technology, Department of Food Chemistry and Analysis, Technická 3, 166 28 Prague 6, Czech Republic
E-mail: jana.hajslova@vscht.cz
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Although smoked cheese is a popular delicacy in many countries, only a few papers published in recent two decades have focussed on PAH levels in these products.[6–12] As a widely used contamination marker, carcinogenic B[*a*]P was determined in all the studies. In addition to B[*a*]P, another 15 US EPA PAHs were determined by Bosset *et al*.[11] in a set of 67 smoked cheeses. Pagliuca *et al*.[9] reported on the occurrence of six heavy US EPA PAHs (B[*a*]P, B[*a*]A, Chr, B[*b*]F, B[*k*]F). The broadest spectrum of PAHs in smoked cheeses manufactured from various types of milk (cows', sheep's, goats') was monitored by Guillén and Sopelana.[6,7] Besides the 'classic' analysis of PAHs in this smoked commodity, there is also the possibility of their 'head-space' determination being examined. Until now, none of studies were concerned with determination of the whole range of 'SCF PAHs' in smoked cheese.

As regards analytical strategy, several approaches for PAH isolation from smoked cheeses have been described in literature: (1) alkaline treatment followed by liquid–liquid extraction,[6,10,11] (2) extraction of freeze dried cheese by organic solvent supported by sonication[12] or (3) pressurised liquid extraction (PLE).[8] Clean-up of crude extracts can be performed using either SPE cartriges[6,9–11] or gel permeation chromatography (GPC).[8] For the final identification/quantification step, high-performance liquid chromatography with fluorescence detection (HPLC–FLD)[8–12] or gas chromatography with mass spectrometric detection (GC–MS)[6,7] are mostly used. An overview of methods employed for analysis of PAHs in food and environmental matrices and their relevance to control new EU regulations has been critically discussed in the recent paper by Wenzl *et al*.[13]

In the current study, a wide range of smoked cheeses available at a Czech market has been examined for 15 PAHs. The aim was to assess the increase (if any) of these hazardous chemicals due to the various (industrial and household) smoking processes. In order to document the distribution of PAHs within the cheese, surface parts were analysed separately.

EXPERIMENTAL

Cheese samples

Altogether, 36 smoked cheese samples were analysed in this study. Twenty-four samples of smoked cheeses were obtained from three Czech cheese producers (in the following text identified as cheese companies A, B and C) and three samples from small, private manufacturers (home-made products, D) employing traditional household practices. Nine samples were obtained from a common market (E). Smoking conditions and other cheese characteristics are summarised in Table 1. An unsmoked cheese product (Edam) obtained from the common market was used as the reference sample. When supplied, samples were stored at −18 °C.

Chemicals and materials

The standard mixture NIST 1647d of 16 priority PAHs: naphthalene (Naph), acenaphthene (Ace), fluorene (Fln), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benz[*a*]anthracene (B[*a*]A), chrysene (Chr), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), dibenz[*a*,*h*]anthracene (DB[*ah*]A), benzo[*g*,*h*,*i*]perylene (B[*ghi*]P) and indeno[1,2,3-*cd*]pyrene (I[1,2,3-*cd*]P) dissolved in acetonitrile was supplied by National Institute of Standards and Technology (NIST, Gaithesburg, MD, USA). Working standard solutions (concentrations in the range 0.01–60 ng mL^{-1}) were prepared in acetonitrile and stored at 4 °C.

Before use, all glassware was washed with detergent, rinsed with distilled water and acetone and then dried at 220 °C.

Chloroform and acetone (analytical reagent grade, Lach-Ner, Neratovice, Czech Republic) were redistilled in glass before use. Acetonitrile (gradient grade, for chromatography), *n*-hexane (for organic trace analysis), dichloromethane (for gas chromatography; all Merck, Darmstadt, Germany), were used as supplied. Deionised water was obtained from Milli-Q water purification system (Millipore, Bedford, MA, USA). Anhydrous sodium sulfate (Penta Praha, Prague, Czech Republic) was dried at 500 °C for 5 h and then stored in a tightly capped glass bottle.

Equipment

A laboratory blender (Waring blender, 38BL-40; Waring Commercial, New Hartford, CT, USA) and stainless-steel grater were used for homogenisation of cheese samples. A Soxhlet extractor with cellulose extraction thimbles (Filtrak, Niederschlag, Germany) was used for sample extraction.

An automated gel permeation chromatography (GPC) system consisting of 305 MASTER pump, fraction collector, automatic regulator of loop 231 XLI, microcomputer (software 731 PC via RS232C), dilutor 401C (GILSON, Paris, France) and stainless steel column 500 × 8 mm i.d. packed with gel Bio-Beads S-X3, 200–400 mesh (Bio-Rad Laboratories, Philadelphia, USA) was used for clean-up of extracts.

A vacuum evaporator (Büchi Rotavapor R-114 a Waterbath B-480, Postfach, Switzerland) was used for concentration of extracts. A high-performance liquid chromatographic system (HPLC), Hewlett-Packard 1100 Series, composed of a quarternary pump system with a degasser, an autosampler, a column thermostat, a fluorescence detector (FLD) (Hewlett Packard, Palo, Alto, CA, USA) and a Supelcosil™ LC-PAH (250 mm × 4.6 mm i.d.; 5 μm) column with the guard column Supelcosil™ LC-18 (20 mm × 4.0 mm i.d., 5 μm; Supelco, Bellefonte, USA) was used for analysis of sample extracts.

Analytical procedure

PAH analysis consisted of following steps.

Table 1. Overview and characteristics of the smoked cheese samples examined in this study

Category of cheese producer/source	Sample code	Cheese commercial name	Cheese type	Weight of single cheese package (g)	Dry matter/fat content (%, w/w)	Smoking conditions	Type of smoking process
A (Industrial)	A1–A3	Mozzarella	Pasta filata	2000	50/42	4 h at 32 °C, beech wood	Friction smoke technique
	A4–A15	Edam	Semi-hard	1500	56/45	4 h at 32 °C, beech wood	Friction smoke technique
	A16–A17	Jadel	White, brined, steamed	200	54/40	4 h at 32 °C, beech wood	Friction smoke technique
	A18	Smoke processed cheese	Processed, bar-shaped	1500	42/45	4 h at 32 °C, beech wood	Friction smoke technique
	A19	Smoke processed cheese	Processed, bar-shaped	1500	42/45	N/A	Dry smoke flavouring powder
B (Industrial)	B20	Tizian	Processed	90	45/45	N/A	Liquid smoke flavouring
C (Industrial)	C21–C24	Edam	Semi-hard	2000	57/45	4–5 h at 36–38 °C, smoke from beech wood	Smoke generation from burning wood – industrial
D (Household products)	D25–D26	Gazdovsky ostepek	Semi-hard	600	50/40	4 h, cherry tree and beech wood	Smoke generation from burning wood – household
	D27	Klobucik	Semi-hard	500	50/40	4 h, cherry tree and beech wood	Smoke generation from burning wood – household
E (Samples collected from retailed market)	E28	Edam	Semi-hard	260	56/45	–	–
	E29	Akawi	White cheese with smoked flavour	280	43/38	N/A	Liquid smoke flavouring
	E30	Smoked processed cheese	Processed	100	43/44	–	–
	E31	Jadel	Steamed	100	54/37	–	–
	E32	Koliba-mini	Semi-hard, steamed	200	48/27	–	–
	E33	Parenica	Semi-hard, steamed	115	48/25	–	–
	E34	Gazdovsky ostepek	Semi-hard	330	50/40	–	–
	E35	Koliba	Semi-hard, steamed	125	50/40	–	–
	E36	Tizian	Processed	90	45/24	–	–

N/A, not applied; –, information not available.

Soxhlet extraction
Ten grams of homogenised cheese sample obtained by homogenisation either of the whole sample or the cheese rind (represented typically by 1–2 mm thick surface layer) was thoroughly mixed with 25 g of anhydrous sodium sulfate in a grinding mortar, then placed into the extraction cellulose thimble, covered with glass wool and inserted into the Soxhlet extractor. Prior to use, the thimbles were pre-extracted for 2 h with an extraction solvent to minimise PAHs procedure blank. Extraction was carried out with 170 mL of hexane–dichloromethane mixture (1:1, v/v) for 7 h (10 cycles h^{-1}). The Soxhlet apparatus was covered with an aluminium foil to avoid access of daylight (to prevent the risk of photodegradation). The obtained extract was then carefully evaporated by rotary vacuum evaporator at 40 °C, just to dryness, and the residue was quantitatively transferred into a 10-mL volumetric flask by chloroform.

Liquid–liquid extraction of liquid smoke flavouring agent
For extraction of liquid smoke flavouring agent, modified procedure published by Pagliuca *et al.*[9] was used. Briefly, 10 g of sample were extracted with 100 mL of *n*-hexane in a separatory funnel; after 4 min of vigorous shaking the mixture was allowed to separate into two phases. The lower phase was re-extracted with 50 mL of *n*-hexane. This process was repeated twice. The three combined hexane extracts were concentrated by a rotary vacuum evaporator at 40 °C, just to dryness, and the residue quantitatively transferred into a 10-mL volumetric flask by chloroform.

Clean-up of crude extracts

The clean-up step (separation of lipids) was carried out by GPC employing gel Bio-Beads S-X3. The flow rate of the mobile phase (chloroform) was set at 0.6 mL min^{-1}; and the volume of sample injected onto the GPC column was 1 mL. After discarding the first 15.5 mL of eluate, the next 15.5 mL were collected. The purified extracts were subsequently subjected to concentration by rotary vacuum evaporator at 40 °C just to dryness. The residue obtained after evaporation of solvent was dissolved in 0.5 mL of acetonitrile before the HPLC–FLD determinative step; this solution was then transferred into a 2 mL amber vial.

Identification and quantification
The HPLC–FLD was carried out under the following conditions: The high performance liquid chromatography with fluorescence detection (HPLC-FLD) was carried out under the following conditions: gradient elution (0 min–55% acetonitrile + 45% water, 20 min–100% acetonitrile, 32 min–100% acetonitrile), mobile phase flow rate 1 ml min-1, injection volume 20 µl, column temperature 35 °C, FLD settings are shown in Table 2. The external standard calibration method based on peak heights was used for quantification of PAHs.

Table 2. FLD settings for PAH detection

Target PAHs	Time window (min)	Excitation wavelength (nm)	Emission wavelength (nm)
Naph	7.2–10.7	216	336
Ace, Fln	10.7–11.1	240	320
Phe	11.1–12.2	248	368
Ant	12.2–13.2	248	404
Flt	13.2–14.3	232	448
Pyr	14.3–16.0	236	384
B[*a*]A, Chr	16.0–19.3	270	388
B[*b*]F, B[*k*]F, B[*a*]P	19.3–23.6	250	430
DB[*ah*]A, B[*ghi*]P	23.6–25.8	295	405
I[1,2,3-*cd*]P	25.8–30.5	248	484

Performance characteristics and quality assurance
Since suitable matrices with certified concentrations of PAHs (CRM) are not available commercially; spiked samples at 0.5, 5 and 10 µg kg^{-1} were analysed within the validation study (50 µL, 50 µL and 100 µL of standard solution containing 100, 1000 and 1000 ng mL^{-1} of PAHs, respectively, were carefully incorporated into 10 g of cheese before extraction). Recovery was obtained as a slope of dependence of the measured values and the theoretical values (multiplied by 100). Repeatability of method was calculated as a relative standard deviation (RSD, %) from six parallel measurements of cheese with native PAHs content (0.1–45 µg kg^{-1}). The overview of selected performance characteristics is shown in Table 3.

RESULTS AND DISCUSSION

In the first phase of our experiments, we had to decide on a choice of optimal analytical strategy enabling

Table 3. Method performance characteristics obtained in validation study

PAH	Recovery (%)	Repeatability* (%)	Limit of detection (µg kg^{-1})
Naph	52	34	0.05
Ace	68	16	0.05
Fln	57	20	0.05
Phe	94	9	0.25
Ant	86	11	0.09
Flt	87	11	0.23
Pyr	73	11	0.12
B[*a*]A	71	13	0.05
Chr	72	14	0.04
B[*b*]F	70	14	0.08
B[*k*]F	75	14	0.01
B[*a*]P	71	14	0.01
DB[*ah*]A	78	15	0.02
B[*ghi*]P	70	12	0.04
I[1,2,3-*cd*]P	94	16	0.07

* Repeatability was calculated as a relative standard deviation (RSD, %), $n = 6$.
Limit of quantification (LOQ) was not lower than three times of LOD level.

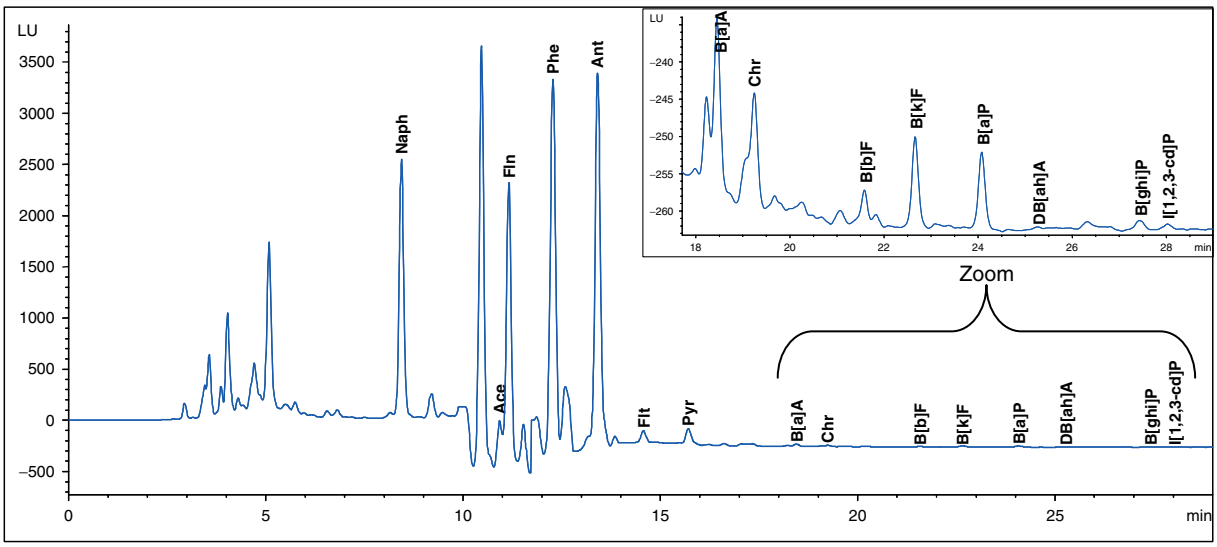

Figure 1. Example of an HPLC–FLD sample chromatogram obtained by analysis of sample D26 (PAH concentrations: 0.03–60 μg kg^{-1}).

to obtain accurate data even at low concentration levels of PAHs potentially occurring in smoked cheese. Considering our experience regarding limits of detection attainable either by GC–MS employing unit resolution mass analyser (quadrupole operated in selected ion monitoring mode) or HPLC–FLD, the latter technique was the preferred option, because of a better potential to detect even very low levels of carcinogenic PAHs. An example of cheese sample chromatogram obtained by this procedure is demonstrated in Fig. 1.

Regarding the key analyte, B[*a*]P, all the quality assurance criteria required by EU Directive 2005/10/EC[14] were met. Also, for most of the other PAHs (three-, four- and five-ring) the recoveries and repeatability of measurements were in a satisfactory range (70–94% and 9–16%, respectively). On the other hand, lower recoveries (52–68%) and poor RSDs (16–34%) were obtained for the most volatile PAHs such as Naph, Ace and Fln (see Table 3). It should be noted that these species are not a health concern in terms of carcinogenicity and therefore no modification of analytical procedure was carried out since it would have increased both time and labour demands. According to the data, results for Naph, Ace and Acy are semi-quantitative.

PAH levels (in μg kg^{-1}; values not corrected for recovery) determined in the set of examined cheeses are shown in Table 4. The sum of 12 PAHs (sum of all target PAHs except volatiles, Naph, Ace and Fln) and the sum of eight carcinogenic PAHs (B[*a*]A, Chr, B[*b*]F, B[*k*]F, B[*a*]P, DB[*ah*]A, B[*ghi*]P and I[1,2,3-*cd*]P) are presented in Fig. 2. The 'background level' obtained for the unsmoked reference sample, relevant to the latter PAH group, is shown in this figure as a dashed line. In commercial smoked cheese samples (categories A, B, C and E in Table 1), the concentrations of 12 PAHs and eight carcinogenic

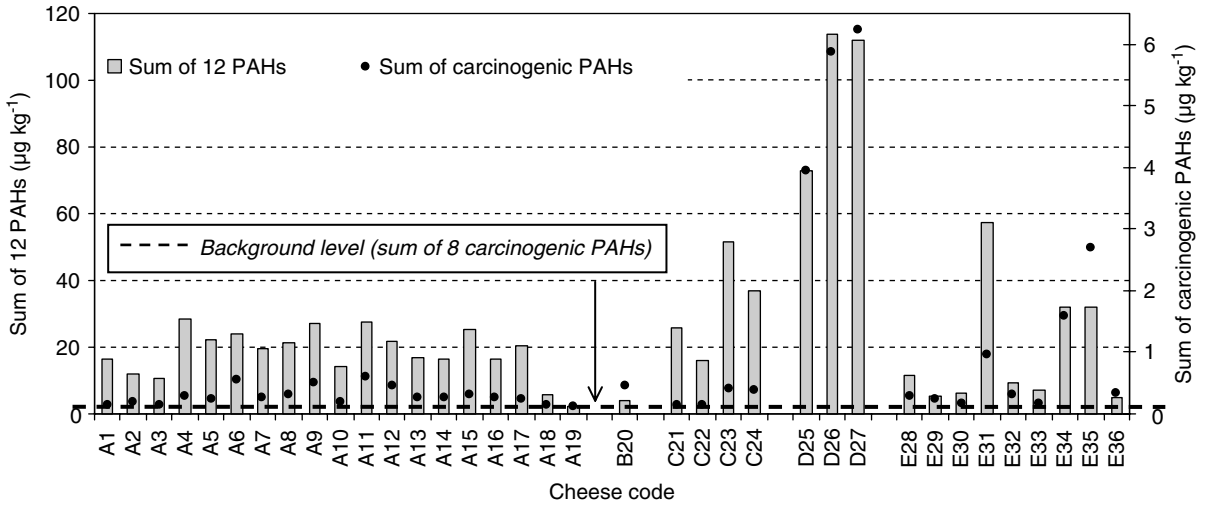

Figure 2. PAH content in smoked cheeses (homogenate taken for analysis was prepared from the whole cheese sample, including cheese rind), for cheese codes see Table 1. Sum of 12 PAHs = sum of Phe, Ant, Flt, Pyr, B[*a*]A, Chr, B[*b*]F, B[*k*]F, B[*a*]P, DB[*ah*]A, B[*ghi*]P and I[1,2,3-*cd*]P. Sum of eight carcinogenic PAHs = sum of B[*a*]A, Chr, B[*b*]F, B[*k*]F, B[*a*]P, DB[*ah*]A, B[*ghi*]P and I[1,2,3-*cd*]P.

Table 4. PAH content in smoked cheese and reference unsmoked sample (mean value, $n = 3$) ($\mu g\,kg^{-1}$)

Cheese code	Naph	Ace	Fln	Phe	Ant	Flt	Pyr	B[*a*]A	Chr	B[*b*]F	B[*k*]F	B[*a*]P	DB[*ah*]A	B[*ghi*]P	I[1,2,3-*cd*]P
A1	5.1	1.3	5.0	12	1.4	1.5	0.8	0.08[a]	0.06[a]	ND	ND	0.02[a]	ND	ND	ND
A2	2.7	1.1	5.0	8.5	1.2	1.3	0.8	0.08[a]	0.1	ND	ND	0.01[a]	ND	ND	ND
A3	13	1.4	5.7	7.7	1.3	1.1	0.6	0.08[a]	0.06[a]	ND	ND	0.01[a]	ND	ND	ND
A4	40	5.1	19	20	3.8	2.2	1.6	0.08[a]	0.2	ND	0.01[a]	0.03	ND	0.02[a]	ND
A5	55	5.2	17	15	4.0	1.7	1.0	0.08[a]	0.1	n.d	0.01[a]	0.03	0.01[a]	ND	ND
A6	14	2.6	11	17	2.6	2.2	1.3	0.2	0.3	n.d	0.02[a]	0.05	ND	0.02[a]	ND
A7	58	4.1	12	14	2.7	1.7	0.9	0.08[a]	0.2	ND	ND	0.03	ND	ND	ND
A8	9.8	2.4	11	15	2.6	2.2	1.4	0.08[a]	0.2	ND	0.01[a]	0.05	ND	0.02[a]	ND
A9	28	4.0	17	19	3.8	2.2	1.3	0.2	0.2	0.04[a]	0.02[a]	0.05	ND	0.02[a]	ND
A10	34	2.2	7	11	1.6	1.2	0.6	0.08[a]	0.1	ND	ND	0.01[a]	ND	ND	ND
A11	35	3.9	15	19	3.6	2.5	1.4	0.2	0.3	ND	0.02[a]	0.04	ND	0.06[a]	ND
A12	56	4.6	14	15	2.8	2.1	1.2	0.08[a]	0.3	ND	ND	0.04	ND	0.06[a]	ND
A13	9.2	1.9	8.7	12	2.0	1.7	0.9	0.08[a]	0.1	ND	ND	0.01[a]	ND	0.06[a]	ND
A14	9.0	1.8	8.6	12	1.9	1.7	0.9	0.08[a]	0.1	ND	ND	0.01[a]	ND	0.06[a]	ND
A15	53	5.0	15	18	3.4	2.1	1.2	0.08[a]	0.2	ND	ND	0.03	ND	0.02[a]	ND
A16	27	2.4	8.6	12	1.8	1.6	0.8	0.08[a]	0.2	ND	ND	0.02[a]	ND	0.02[a]	ND
A17	7.3	2.0	11	15	3.1	1.4	0.9	0.08[a]	0.2	ND	ND	0.02[a]	ND	ND	ND
A18	7.6	0.4	0.9	4.8	0.1[a]	0.3[a]	0.4	ND	ND	0.04[a]	0.01[a]	0.01[a]	ND	0.06[a]	ND
A19	15	0.5	0.9	1.6	0.1[a]	ND	0.4	ND	ND	0.04[a]	0.01[a]	0.01[a]	ND	0.06[a]	ND
B20	10	0.5	0.8	2.6	0.1[a]	0.3[a]	0.4	0.08[a]	ND	0.1[a]	0.06	0.07	0.01[a]	0.1	0.1[a]
C21	22	3.0	6.7	19	3.2	1.9	1.7	0.08[a]	0.06[a]	ND	ND	0.01[a]	ND	ND	ND
C22	29	1.9	3.9	11	2.2	1.2	1.1	0.08[a]	0.06[a]	ND	ND	0.01[a]	ND	ND	ND
C23	14	7.2	17	34	10.4	3.6	3.4	0.2	0.1	ND	0.02	0.03	ND	ND	ND
C24	19	4.1	8.6	26	5.6	2.5	2.3	0.2	0.1	0.04[a]	0.04	0.02[a]	0.01[a]	ND	ND
D25	36	4.1	18	40	14	7.1	7.1	1.3	0.8	0.3	0.2	0.6	0.01a	0.6	0.3
D26	60	7.1	30	63	23	12	11	1.9	1.2	0.4	0.3	0.6	0.03a	0.8	0.5
D27	24	5.4	27	60	23	12	12	2.2	1.3	0.5	0.3	0.9	0.01[a]	0.7	0.5
E28	14	1.3	4.9	9.0	1.2	0.7	0.5	0.08[a]	0.06[a]	0.04[a]	0.01[a]	0.04	ND	0.1	ND
E29	0.5	0.4	1.0	4.4	0.3	0.3[a]	0.3	ND	0.06[a]	0.04[a]	0.01[a]	0.03	ND	0.06[a]	ND
E30	8.5	0.9	1.8	5.0	0.3	0.3[a]	0.2[a]	ND	0.06[a]	ND	0.01[a]	0.02[a]	ND	0.06[a]	ND
E31	0.2	2.1	16	39	8.1	5.7	3.7	0.3	0.3	0.04[a]	0.06	0.1	0.01[a]	0.1	0.1[a]
E32	2.1	0.2	1.1	7.0	0.1[a]	1.1	0.7	0.08[a]	0.06[a]	0.04[a]	0.3	0.03	ND	0.06[a]	ND
E33	0.2	0.4	1.1	5.3	0.1[a]	0.9	0.5	ND	0.06[a]	ND	0.01[a]	0.02[a]	ND	0.06[a]	ND
E34	3.8	2.7	12	18	4.7	4.3	2.8	0.6	0.5	0.1[a]	0.07	0.2	0.01[a]	0.1	ND
E35	3.9	1.2	11	17	5.6	3.6	3.1	0.9	0.7	0.2	0.1	0.5	0.03[a]	0.3	0.3
E36	1.2	0.4	1.2	3.9	0.3	0.1[a]	0.2[a]	ND	0.1	0.04[a]	0.02[a]	0.05	0.01[a]	0.1	ND
Reference	12.9	0.6	1.1	4.1	0.1[a]	0.1[a]	0.4	ND	ND	0.04[a]	0.01[a]	0.01[a]	ND	ND	ND

[a] Estimate; analyte concentration below limit of quantification.
ND, not detected (for LODs see Table 3).

PAHs ranged from 2.3 to 57 $\mu g\,kg^{-1}$ and from 0.1 to 2.7 $\mu g\,kg^{-1}$, respectively. Significantly higher PAHs levels were found in 'home-made' samples (category D), where the content of total PAHs was in the range from 73 to 114 $\mu g\,kg^{-1}$ and the sum of carcinogenic PAHs ranged from 3.9 to 6.2 $\mu g\,kg^{-1}$. Higher contamination of these 'traditional' cheeses was obviously due to deposition of PAHs-containing solid particles on their surface. Purification of the smoke generated within industrial process under carefully controlled conditions allows only flavouring compound to reach the cheese, while most of hazardous products of wood pyrolysis are removed.

In addition to examination of the whole cheese, the rinds of selected samples were analysed separately (Table 5). The PAH levels ($\mu g\,kg^{-1}$; values not corrected for recovery) detected in these surface layers (thickness, 1–2 mm) were three to six times higher (Fig. 3) regardless the type of smoking technique used. Fairly lower overall contamination, similar to 'background levels' detected in unsmoked cheese was found in edible parts of samples that were aromatised either by dry smoke flavouring powder (A18) or under the controlled pyrolysis conditions (A19). Both these bar-shaped cheeses were coated by inedible waxy coating, which accumulated most of PAHs (i.e. served as contamination barrier). Compared to edible rind (cheese layer under the waxy coating), the PAHs content in the coating was 14 times higher; the sum of carcinogenic PAHs even 18 times higher. In summary, according to our estimates, in most cases, removing the 1–2 mm surface layer from the smoked cheese eliminates approximately 50–100% of PAHs detectable in particular product. These observations are in agreement with results of study published by Guillén and Sopelana.[6]

Table 5. PAH content in smoked cheese rind samples (μg kg^{-1})

Cheese code	Naph	Ace	Fln	Phe	Ant	Flt	Pyr	B[*a*]A	Chr	B[*b*]F	B[*k*]F	B[*a*]P	DB[*ah*]A	B[*ghi*]P	I[1,2,3-*cd*]P	Rind portion taken for analysis (% of cheese total weight)
A4–A15	184	24	101	93	28	8.8	7.2	0.9	0.4	0.2	0.1	0.3	0.06	0.1	0.2	7–13
A19	9.2	0.8	1.7	6.5	0.3	1.2	1.1	0.2	0.06[a]	0.1[a]	0.01[a]	0.07	0.06	0.1	0.1[a]	13
B20	16	1.0	2.7	8.1	0.4	0.7	0.9	0.08[a]	0.1	0.04[a]	0.01[a]	0.01[a]	0.01[a]	ND	ND	7
C21–C22	346	46	267	148	42	11	8.0	0.8	0.5	0.1[a]	0.08	0.2	ND	0.3	ND	8–9
C23–24	326	61	164	204	58	24	23	1.3	0.8	0.3	0.1	0.5	ND	0.4	0.4	8–9
D25	98	23	108	244	88	47	44	8.6	5.0	1.7	1.04	3.4	0.09	3.0	1.9	10
D26	167	28	137	371	132	77	75	14	7.9	2.9	1.6	5.4	0.1	4.2	2.8	10
D27	249	43	191	339	119	51	48	9.4	5.5	2.1	1.3	4.4	0.1	3.7	2.5	10
E28	60	12	62	65	17	6.5	5.0	0.5	0.3	0.2	0.1	0.3	0.03[a]	0.3	ND	6
E29	6.8	1.3	3.4	20	1.0	3.4	3.2	0.2	0.2	0.1[a]	0.08	0.4	ND	0.2	ND	13
E30	10	0.4	2.2	16	0.7	3.1	2.4	0.3	0.4	0.2	0.1	0.1	ND	0.2	ND	20
E31	137	23	120	270	61	37	25	3.0	1.2	0.6	0.3	0.7	ND	0.5	ND	16
E32	1.2	0.8	3.3	17	0.5	2.8	2.3	0.2	0.2	0.1[a]	0.06	0.08	ND	0.2	ND	13
E33	3.3	1.2	3.9	20	0.6	3.0	2.4	0.2	0.2	0.1[a]	0.07	0.08	ND	0.2	ND	10
E34	39	15	83	99	29	19	14	3.6	2.3	0.7	0.4	1.2	ND	0.5	ND	10
E35	84	9.2	66	85	25	18	14	4.4	2.8	1.0	0.7	2.1	ND	0.9	0.9	20
E36	ND	0.2	1.6	16	0.6	3.0	2.4	0.2	0.2	0.2	0.09	0.1	0.03[a]	0.1	ND	11

[a] Estimate; analyte concentration below limit of quantification.
ND, not detected.
A4–A15; C21–C22; C23–24: pooled samples.

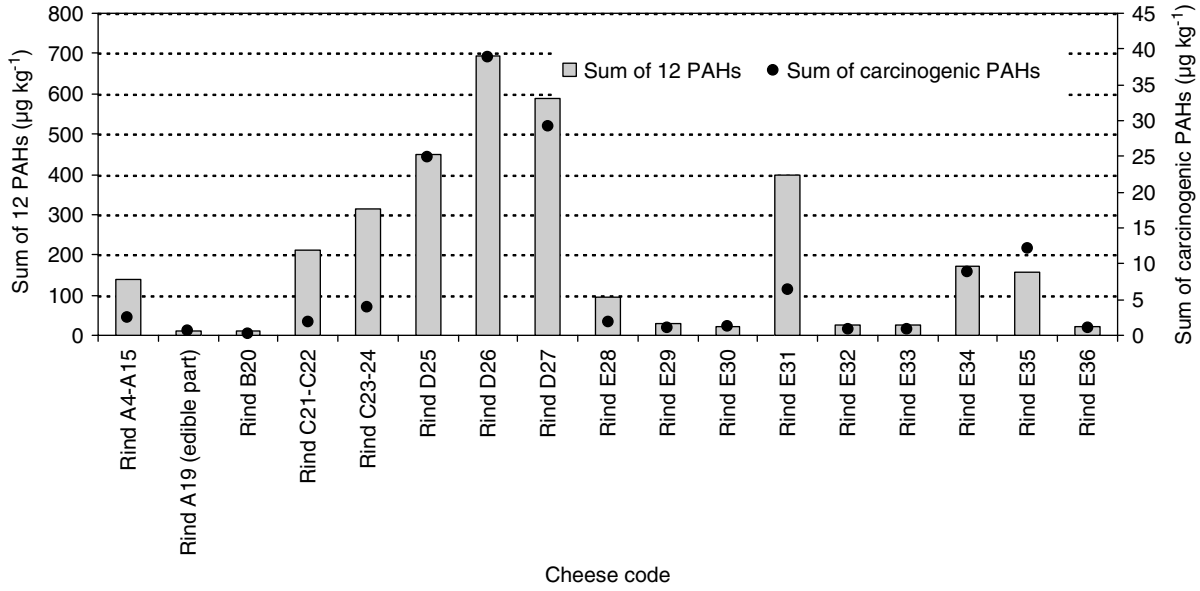

Figure 3. PAH content in smoked cheese rinds (pooled samples A4–A15, C21–C22 and C23–24), for cheese codes see Table 1. Sum of 12 PAHs = sum of Phe, Ant, Flt, Pyr, B[*a*]A, Chr, B[*b*]F, B[*k*]F, B[*a*]P, DB[*ah*]A, B[*ghi*]P and I[1,2,3-*cd*]P. Sum of eight carcinogenic PAHs = sum of B[*a*]A, Chr, B[*b*]F, B[*k*]F, B[*a*]P, DB[*ah*]A, B[*ghi*]P and I[1,2,3-*cd*]P.

Table 6. Comparison of B[*a*]P levels found in different studies

B[*a*]P content (μg kg^{-1})	Subject of study/ type of sample	Reference
0.01–0.88	Commercially and home-made smoked cheeses	This study
ND–0.55	Rind of commercial smoked cheeses	6
<0.1–3.8	Home-made smoked cheeses	8
<0.2–1.7	Effect of different smoking conditions on B[*a*]P	8
0.03–0.39	Effect of different smoking conditions incl. smoke-flavoured and liquid flavoured cheeses on PAHs	9
0.1–0.75	Investigation of different smoking conditions on B[*a*]P	10
<0.1–4.2	Investigation of different smoking conditions on PAHs	11
ND–0.91	Commercially smoked cheeses	12

Considering for comparison the maximum level 5 μg kg^{-1} given in EC Regulation No 1881/2006[5] for B[*a*]P in smoked meat, we classify the contamination as low. As shown in Table 6, concentrations of B[*a*]P documented in smoked cheese from the Czech market were comparable to levels reported for similar products by Guillén and Sopelana[6], Garcia Falcon *et al.*[12] Anastazio *et al.*[10] and Pagliuca *et al.*[9] from Spanish and Italian markets and even lower than reported by Bosset *et al.*[11] for Swiss cheeses and home-made products from Slovakia examined by Michalski and Germuska.[8]

Relative abundances of individual PAH groups, when applying classification based on the number of fused aromatic rings (see Table 7), varied largely among examined cheeses, reflecting differences in smoking practices, as shown in Fig. 4. Regardless the cheese type, 70–90% of total 12 PAHs were those with three aromatic rings: Phe and Ant (i.e. more volatile PAHs). PAHs containing four rings typically contributed to 10–20% of the total PAH content. In unsmoked cheeses and those smoked under the industrial conditions (friction technique and/or controlled wood burning) carcinogenic five- and six-ring species accounted only for 0.1–0.2% of PAH content. In home-smoked products and cheeses aromatised by liquid and/or dry smoke flavourings, the contribution of this hazardous fraction to the overall contamination was 2–12%. The analysis of liquid smoke-flavouring agent, which was used for sample B20 aromatisation, showed the presence of a high total PAH concentration (182 μg kg^{-1}), even those carcinogenic (10 μg kg^{-1}). PAH levels found in the liquid smoke flavouring agent are very similar to those published for liquid flavouring agents by Guillén *et al.*[15] but rather higher than those found by Pagliuca *et al.*[9] It should be noted that in spite of this relatively high PAH content, only the background level was found in the final cheese product (see Fig. 2).

Several authors in earlier studies concerned with a similar topic reported on a correlation between concentrations of Pyr and B[*a*]P in some matrices, such as smoke flavourings,[15,16] smoked cheese[6,7] or charbroiled hamburgers.[17] Similarly, a relationship between Phe/Pyr[6,7] and Phe/B[*a*]P[7] was investigated.

Table 7. PAH groups according to number of aromatic rings

Number of rings	PAH
2,3	Naph, Ace, Fln, Phe, Ant
4	Flt, Pyr, B[*a*]A, Chr
5,6	B[*b*]F, B[*k*]F, B[*a*]P, DB[*ah*]A, B[*ghi*]P, I[1,2,3-*cd*]P

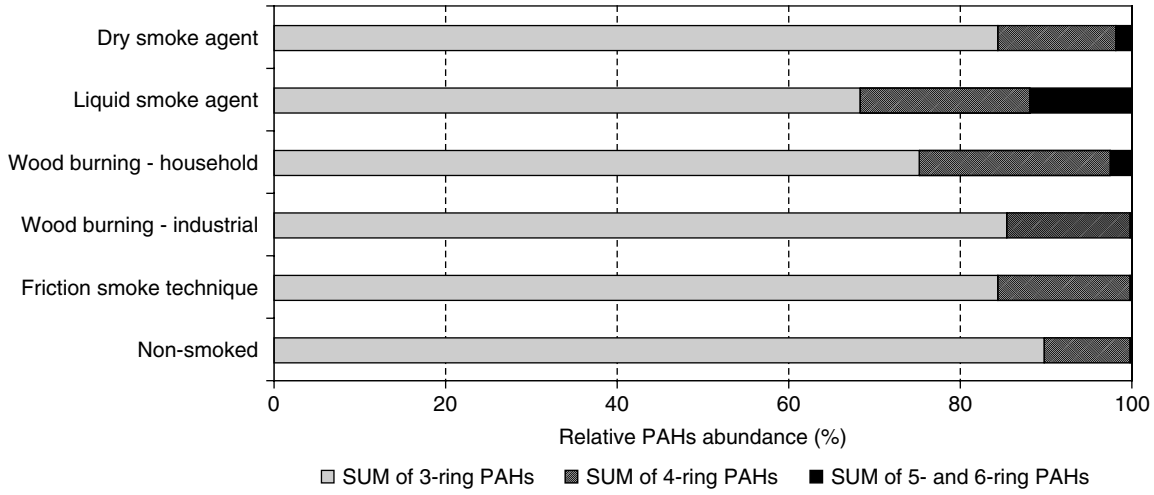

Figure 4. Relative abundances of individual PAH groups in tested cheeses. Data are aggregated based on the smoking technology.

Table 8. Ratios of selected PAH concentrations calculated for smoked cheese samples

PAH ratio	Smoking technology	Average	Median	Min.	Max.
Phe/Pyr	Unsmoked cheese	11	10	8	12
	Friction smoke (A5–A9; A11–A12)	14	14	11	15
	Wood burning – industrial (C21–C24)	11	11	10	11
	Wood burning – household (D25–D27)	6	6	5	6
	Liquid smoke (B20)	7	7	–	–
	Dry smoke (A19)	4	4	–	–
Pyr/B[*a*]P	Unsmoked cheese	40	40	33	45
	Friction smoke (A5–A9; A11–A12)	30	30	26	35
	Wood burning – industrial (C21–C24)	127	114	110	170
	Wood burning – household (D25–D27)	14	13	12	18
	Liquid smoke (B20)	6	–	–	–
	Dry smoke (A19)	40	–	–	–
Phe/B[*a*]P	Unsmoked cheese	405	410	390	431
	Friction smoke (A5–A9; A11–A12)	408	382	298	510
	Wood burning – industrial (C21–C24)	1359	1220	1117	1880
	Wood burning – household (D25–D27)	79	67	67	104
	Liquid smoke (B20)	37	–	–	–
	Dry smoke (A19)	160	–	–	–

Samples with B[*a*]P content close to the limit of detection were discarded from the calculation.

According to Franklach and Warnatz,[18] such relations typically should exist, since heavy PAHs are derived through pyrosynthesis from the lighter PAHs by addition of small units (i.e. acetylene or aryl radicals) what means that an increase of a precursor group during the smoking is accompanied by originating of final reaction products at higher degree. This observation could be useful for predicting the high molecular PAH levels based on the concentrations of lighter PAHs, whose determination is easier, due to their higher concentrations usually presented in analysed matrices. Under these conditions, uncertainty of measurement is lower and accuracy of data is better. Table 8 shows concentration ratios of Phe and Pyr, Pyr and B[*a*]P, and Phe and B[*a*]P found for each group of cheeses (grouping based on the smoking procedure). In the case of the Phe/Pyr ratio, relatively consistent results were obtained, both within the each individual group and between the groups (with median values in the range of 4–14), which is in a good agreement with values published by Guillén and Sopelana[7] (2.4–12). On the other hand, as regards Pyr/B[*a*]P and Phe/B[*a*]P concentration ratios, a distinct diversity between individual cheese groups representing different smoking procedures was found. However, ratios obtained within each group were relatively consistent, at least in terms of orders of magnitude. These results indicate that predicting the levels of high molecular PAHs (typically carcinogenic) from the concentrations of lighter PAHs is not a straightforward approach; nevertheless, it seems that availability of these ratios might be useful to identify the type of smoking technology. Similar strategy employing various PAHs ratios for the identification of the emission sources responsible for environmental pollution was reported in some studies.[19,20]

Recently, the EU Scientific Committee on Food concluded, that B[*a*]P can be employed as an indicator

Table 9. Correlations between B[*a*]P concentrations and other PAH groups

PAH groups	Correlation coefficient
Sum of 5- and 6-ring PAHs	0.993
Sum of 4-ring PAHs	0.947
Sum of 3-ring PAHs	0.848
Sum of 8 carcinogenic PAHs	0.995
Sum of 15 PAHs	0.728

of occurrence and concentrations of higher-molecular mass PAHs (from benzofluoranthenes upwards) in food, whereas it cannot be used as an indicator of lower-molecular mass PAHs.[3] The possibility of using this approach was demonstrated in a study by Kazerouni *et al*.[21] where the correlation coefficient between concentrations of B[*a*]P and the sum of the carcinogenic PAHs was 0.98, while it decreased to 0.87 when B[*a*]P and the total of 15 PAHs were correlated.

Almost the same trend was obtained in our study. A high correlation coefficient (0.995) was found for B[*a*]P concentrations and the sum of eight carcinogenic PAHs (Table 9), while the correlation between B[*a*]P content and the total content of 15 PAHs was weaker (0.728).

CONCLUSIONS

In this study, we examined 36 cheese samples smoked by various smoking technologies for the presence of major PAHs. The conclusions based on generated data can be summarised as follows.

Firstly, both the use of smoke flavourings and smoking procedure achieved under industrial conditions (friction smoke and wood burning) led to only slightly elevated PAH levels as compared to unsmoked cheeses. Distinctly the highest PAH levels were determined in home-made smoked samples.

Secondly, the analysis of cheese rinds has shown that the surface layers are three to six times more contaminated by PAHs compared to the whole sample. Their removal reduced the total PAH content by approximately 50–100%.

Thirdly, B[*a*]P concentrations in smoked cheese strongly correlated with the sum of carcinogenic PAHs, what confirmed the applicability of suggestion of EU SCF to use this analyte as an indicator of the carcinogenic higher-molecular PAH fraction.

Lastly, PAH profiles expressed as Pyr/B[*a*]P and Phe/B[*a*]P concentration ratios differed according to the smoking technology with the lowest values for home-smoked and liquid smoke flavouring treated cheeses. On the other hand Phe/Pyr ratios were similar for all smoking technologies, and hence not diagnostic.

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