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Polycyclic aromatic hydrocarbons in smoked fish – a critical review

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Abstract

Among hundreds of components, wood smoke also contains at least 100 polycyclic aromatic hydrocarbons (PAH) and their alkylated derivatives. Many of them are carcinogenic. Benzo[α]pyrene (BaP) is regarded as a marker of the carcinogenic PAH in smoke and smoked fish, although in olive residual oil the maximum level of 2 μg/kg for each of the eight most carcinogenic PAHs, including BaP, has been set. Contemporary analytical procedures based on extraction of the hydrocarbons from the matrix, clean-up procedure, separation by gas chromatography (GC) or high performance liquid chromatography (HPLC), followed by detection and quantification by mass spectrometry (MS) or fluorescence detectors (FLD), respectively, make it possible to determine individual PAH in smoked foods at concentrations of the order of 0.1 μg/kg or even 0.01 μg/kg. Heavily smoked fish from traditional kilns, especially their outer parts, may contain up to about 50 μg BaP/kg wet weight, while the meat of mild hot-smoked fish, from smokehouses supplied with conditioned wood smoke from external generators, contains only about 0.1 μg/kg, or even less. Some older data on the contents of BaP in smoked fish should be treated with caution, if the analytical procedures used did not guarantee unequivocal separation and identification of the individual PAH.

Keywords: Polycyclic aromatic hydrocarbons; PAH; Benzo[α]pyrene; Smoking; Wood smoke; Smoke composition; Smoked fish; Smoke analysis

1. Introduction

Smoking is one of the oldest methods of food preservation and is still widely used in fish processing. In Europe about 15% of the total quantity of fish for human consumption is offered on the market in the form of either cold- or hot-smoked products. Traditional smoking involves treating of pre-salted, whole, eviscerated or filleted fish with wood smoke. The smoke is produced by smouldering wood and shavings or sawdust in the oven, directly below the hanging fish or fillets, laid out on mesh trays. The flow rate and distribution of smoke depends upon the natural draft as affected by the construction of the kiln and by the weather conditions. In modern, automatic smokehouses, the smoke develops in an external generator under controlled conditions of temperature and air access, while the circulation is forced and controlled by mechanical equipment. The temperature of the smoke is in the range 12–25 °C during cold-smoking and 25–45 °C in warm-smoking. In hot-smoking, the process may be carried out in different stages, during which the temperature of the smoke ranges from about 40–100 °C and that in the centre of the product may reach up to 85 °C.

The rate of deposition of different components depends upon the temperature, humidity, flow rate, and density of the smoke, the water solubility and volatility of the particular compounds, as well as on the properties of the surface of the fish (Foster, 1957). Recently, different smoke condensates or preparations are used in the form of aerosols or solutions applied as dips. In electrostatic smoking, a high voltage of about 30 kV accelerates

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the directed movement, deposition, and sorption of smoke particles, droplets, and vapours (Miler & Sikorski, 1990; Sikorski, 1956).

The composition of the smoke and the conditions of processing affect the sensory quality, shelf life, and wholesomeness of the product. Potential health hazards associated with smoked foods may be caused by carcinogenic components of wood smoke – mainly PAH, derivatives of PAH, such as nitro-PAH or oxygenated PAH, and to a lesser extent also N-nitroso compounds and heterocyclic aromatic amines. The contents of N-nitroso compounds in hot smoked fish generally does not exceed several μg/kg of product, that is lower than in many other foods (Röper, Heyns, Günther, & Steinig, 1981). According to Mondagere (1986), smoked-cured poultry products may contain N-nitrosothiazolidine carboxylic acid in amounts even as high as 1 mg/kg wet weight. Heterocyclic aromatic amines were found at concentrations below 1 μg/kg in heavily smoked, dried mackerel (Kato, Kikugawa, & Hayatsu, 1986).

2. The composition of wood smoke

2.1. The main components

The smoke for smoking of food develops due to the partial burning of wood, predominantly hardwood, such as beech, hickory and oak, but also softwood, such as pine and fir, or in some areas even bagasse, the refuse from sugar manufacture. It is a mixture of air, water vapour, CO_2, CO, and at least several hundred organic compounds, present in the aerosol at different concentrations either in the gaseous/vapour phase, or dispersed as tiny liquid droplets or particles, such as fly ash. They develop as a result of thermal degradation of wood, followed by the oxidation of some of the products of pyrolysis under limited oxygen supply. The thermal degradation of hemicelluloses, cellulose, and lignin of wood proceeds at 180–300, 260–350, and 300–500 °C, respectively. Oxidation of some of these decomposition products occurs at temperatures reaching up to 900 °C and, in large excess of oxygen, even 1200 °C. Smoke produced at 650–700 °C is the richest in components responsible for imparting the desirable sensory properties to the products. The temperature of smoke generation can be decreased by increasing the humidity of wood (Tóth & Potthast, 1984).

Among the very large number of constituents separated from different smoke condensates and extracts, about 380 organic compounds have been unequivocally identified as early as 20 years ago (Tóth & Potthast, 1984). This mixture is composed mainly of phenols, aldehydes, ketones, organic acids, alcohols, esters, hydrocarbons and various heterocyclic compounds. The suitability of smoke for treating fish and meat depends primarily on the contents of phenols, since they are mainly responsible for imparting the desirable sensory properties to the products and are valuable as antioxidants. The quantitative composition of smoke depends upon the kind of wood used and predominantly on the temperature and air supply, but also on the cleaning procedure applied after generation.

2.2. PAH in wood smoke

Wood smoke contains a large number of PAH (Obiedziński & Borys, 1977); at least 61 of them, with a wide range of molecular mass, from indene, 116 Da, to dibenzopyrenes, 302 Da have been unequivocally identified (Potthast, 1979; Tóth & Potthast, 1984). According to the Scientific Committee on Food (2002), 15 PAH (Table 1) "show clear evidence of mutagenicity/genotoxicity in somatic cells in experimental animals in vivo. They may be regarded as potentially genotoxic and carcinogenic to humans"; their carcinogenicity depends on their structure (Bartoszek, 2002). The light PAHs, of molecular mass below 216 Da, are regarded as not carcinogenic. Very mutagenic and carcinogenic is BaP (252 Da); it has been accepted as a marker of carcinogenic PAHs in wood smoke, smoked products, and environmental samples. It has been found that the ratio of the concentration of the carcinogenic PAHs to that of BaP in smoked products is generally not higher than 5. If the carcinogenicity of individual heavy PAHs is also taken into consideration, the carcinogenic potency of total PAHs contained in a food product is about 10 times higher than would result from the content of BaP alone (Scientific Committee on Food, 2002). However, BaP is often accompanied by benzo[e]pyrene, known to be significantly less carcinogenic (Howard & Fazio, 1980). The separation of these two isomers is a difficult analytical task and may not be achieved in all BaP assays. According to regulations in different countries regarding drinking water and foods, the determination of several other PAHs is also required.

The most important factor affecting the formation of PAH is the temperature of smoke generation. According to Tilgner and Miler (1963), BaP is not formed if the temperature of wood pyrolysis in a two-stage smoke generator is below 425 °C and that of oxidation of the volatile products of pyrolysis below 375 °C. By lowering

Table 1
Polycyclic aromatic hydrocarbons regarded as potentially genotoxic and carcinogenic to humans (Data from European Scientific Committee on Food, 2002)

Benz[a]anthracene	Benzo[a]pyrene	Dibenzo[ah]pyrene
Benzo[b]fluoranthene	Chrysene	Dibenzo[ai]pyrene
Benzo[j]fluoranthene	Cyclopenta[cd]pyrene	Dibenzo[al]pyrene
Benzo[k]fluoranthene	Dibenzo[ah]anthracene	Indeno[1,2,3-cd] pyrene
Benzo[ghi]perylene	Dibenzo[ae]pyrene	5-Methylchryzene

the temperature of the smouldering pile of wood shavings or sawdust to 300–400 °C and using filters, the content of PAH in the smoke can be decreased about 10-fold.

Smoke flavourings, which have been produced commercially since about the middle of the last century for use in the meat and fish industries contain only trace amounts of PAHs. These flavourings are generally smoke extracts, filtered and separated from the resinous material that contains most of the PAHs. Some flavourings are obtained by distillation of pyroligneous liquids. They are available as aqueous solutions or in dry form on different supports, e.g., salt, yeast, or other free-flowing material (Miler & Sikorski, 1990; White, Howard, & Barnes, 1971).

3. Methods for determination of PAHs in smoke and smoked fish

3.1. Introduction

The methods for determination, used in research laboratories and for routine monitoring of PAH in foods, have undergone marked improvements during the past 50 years. At present, there is still no official procedure accepted by all concerned, that would solve the difficulties associated with quantitative isolation of PAHs from the food material, clean-up of the extract without significant loss of the analyte, separation of all individual PAHs contained in the purified extract, detection of the separated components, unequivocal identification of the PAHs, and quantification of the identified compounds.

3.2. Isolation and recovery of PAHs from the food matrix

Actually, about 660 different PAHs have been described (Sanders & Wise, 1997). In smoked fish, Grimmer and Böhnke (1975) found about 100 PAHs and their alkylated derivatives. Thus it is a very rich mixture of compounds that are similar in chemical character, difficult to analyse, especially if accompanied also by other nonpolar components. All PAHs present in the wood smoke are very hydrophobic. The solubility of BaP in water is 1.5×10^{-8} mol/dm^3; thus they accumulate in the food materials preferentially in the lipid fraction. This also contains naturally occurring hydrocarbons, such as squalene $C_{30}H_{50}$, that is abundant, e.g., in some fish oils. The behaviour of these hydrocarbons during the extraction procedures is similar to that of PAH and complicates the further steps of analysis.

Because of the very low contents of individual PAHs in foods, of the order of 1 μg/kg, (which corresponds to 1 mm/1000 km, that is easier to visualize) and the requirement to determine BaP, with a reproducibility not lower than 48% of the value tolerated in the products (International Standard, 1998), one would be tempted to use considerably large samples for extraction. However, this may result in erroneous results because of the PAH contained naturally in the dry residue after evaporation of many pure organic solvents, e.g., hexane, cyclohexane, isooctane, methanol, and dimethylsulfoxide. Increasing the sample size leads to use of larger volumes of the solvents, and, in consequence, to a decrease in the accuracy of the analysis. Thus a strict adherence to the AOAC requirement of rigorous purification of the solvents should be obligatory (Fazio, 1990) and preferably very sensitive detectors should be used.

The efficiency of extraction of PAH depends upon the polarity of the solvent, on the nature of the matrix, and on the preparation of the sample (Jarvenpaa, Huopalahti, & Tapanainen, 1996; Moret, Conte, & Dean, 1999; Wang, Lee, Lewis, & Archer, 1999). The recovery of PAH can be high when the samples are totally soluble in the organic solvents used for extraction, e.g., some dairy products. However, in meat and fish, although the PAHs accumulate preferentially in the lipid tissue, they diffuse also into the muscles, where they may be bound by some structural elements. In animal tissues, BaP is known to form covalent bonds with nucleic acids (Scientific Committee on Food, 2002). It has been shown by Grimmer and Böhnke (1975), that alkaline hydrolysis of samples previously extracted with boiling methanol increased (about 3-fold) the total recovery of PAHs from meat. On the other hand, prolonged alkaline hydrolysis may lead to some loss of BaP due to degradation (Takatsuki, Suzuki, Sato, & Ushizawa, 1985).

The recovery of PAHs added to the analysed samples, as standards for calibration, may be higher than that of the hydrocarbons absorbed and bound by the food matrix due to smoking and storage. The PAHs added to the sample just prior to analysis remain rather unbound and are easier to extract. It is rational for analytical purposes, to use certified reference materials (CRM) from the EU Commission, Brussels, or standards from the US National Institute for Standards and Technology (NIST), containing strictly defined quantities of individual PAHs. Presently, such standards are available, e.g., SRM 1974 Mussel frozen NIST containing 15 PAHs, SR 2974 Mussel freeze-dried NIST with 14 PAHs, or SRM 2978 Mussel freeze dried NIST containing 9 PAHs (De Boer & Law, 2003). In a recent investigation regarding PAH in smoked cheese, the use of such reference materials has significantly contributed to the accuracy of the results (Michalski & Germuska, 2003).

3.3. Clean-up procedures

Whatever solvent is used for extraction, the extract from such complicated matrices as foods contains not

only PAHs, but also numerous other hydrophobic and slightly nonpolar compounds. These components must be removed in further steps of analysis in order to facilitate the separation and quantification of individual hydrocarbons. Since the properties and composition of the matrices are very diversified, there is no ''best'', most selective solvent, suitable for all applications.

From among a number of clean-up procedures, e.g., solvent:solvent fractionation, solid phase extraction, thin-layer and column chromatography on suitable stationary phases, and size exclusion chromatography, probably the most efficient and selective is column chromatography on $Al_2O_3 \cdot H_2O$, of activity IV. An eluent of low solvent strength, preferentially hexane, extracts all nonpolar aliphatic and aromatic hydrocarbons, polychlorinated biphenyls, some pesticides, and PAHs from such a column, while the components that make up the major mass of the dissolved material, such as triacylglycerols and more polar lipids, remain adsorbed in the column. Therefore this procedure, often treated as preliminary clean-up, has been suggested as a method of choice in the actual ISO standard (International Standard, 1998).

The extract purified on $Al_2O_3 \cdot H_2O$ is suitable for analysis directly by GC or HPLC, if selective detectors are available, making it possible to identify the separated compounds, not only by the retention time, but also by other measurements. Confirmation of the identification can be made, e.g., by on-line acquisition of UV spectra (DAD), emission spectra (FLD), or molecular ion in MSD. If such selective detectors are not used, further purification of the extract is necessary.

3.4. Separation, detection, and quantification of PAHs

3.4.1. HPLC

For HPLC separation of PAHs specially designed columns are commercially available. By using, e.g., the column Vydac 5 μm C18, 150 × 4.6 mm, at 30 °C and programmed eluent from 50:50 v/v water:acetonitrile to acetonitrile in 30 min, at flow rate 1 ml/min, separation of 16 PAHs from a pure, standard mixture can be achieved during not more than 32 min (Fig. 1). Most frequently, a FLD with controlled wavelength of excitation and emission and simultaneous on line scanning of fluorescence spectra, is used. This makes it possible to optimise the limit of detection by programmed selection of the excitation (Ex) and emission (EM) wavelengths. Thus PAHs can be detected simultaneously in several detection channels, e.g., at the basic Ex/Em 290/430 nm and additionally at 260/520, 270/440, and 250/500 nm. Under such conditions, the limit of detection of each of 15 PAH can be decreased to the low picogramme range.

While working with FLD the solvents have to be oxygen-free to avoid quenching of fluorescence of some PAHs, e.g., pyrene in the presence of oxygen in the mobile phase (De Boer & Law, 2003). Generally, fluorescence measurement makes it possible to detect most PAHs concentrations at least of 100 times below the DAD. However, acenaphthylene is not detected by FLD, because it does not emit any fluorescence (Shuster & Schulenberg-Schell, 1998). In such a case it is convenient to use, additionally, simultaneous DAD to register on-line the UV–VIS spectra of the individual PAHs. The UV spectra of PAH are more precise than the FL spectra, since they have a larger number of characteristic points. Therefore, the UV spectra offer higher reliability of the mach factor in identification of the PAHs by using library data.

The chromatograms of samples isolated from smoked fish contain many more components than the standards of 16 PAH. Thus, not all peaks are well separated on HPLC columns, which at 15 cm length have a theoretical plate number not exceeding 15,000–16,000. Nevertheless, some, not totally separated, components can be identified and quantified if they differ significantly in their Ex and Em values.

A large improvement of the analysis of PAHs in complex matrices can be achieved by using HPLC/MS. However, because of the high cost of this instrumentation, this technique cannot at present be applied generally for routine control in food monitoring.

3.4.2. GC/MS

Capillary columns used in GC have an efficiency of the order of 60,000 plates/30 m (ID 0.25 mm). Thus the components of natural extracts can be much better separated than by using HPLC. If the system of ''cool on column'' injection is used, over 100 peaks can be separated from smoked fish extracts containing PAHs, alkylated PAHs, and several other interfering compounds. This large number of peaks in extracts from smoked fish and meat products which can be detected by the FID cannot be identified by their retention times only, without further, often multistage clean-up procedures to remove interfering substances. Therefore, MS was used for identification and quantification of PAHs in smoke and smoked products already about 25 years ago (Lawrence & Weber, 1984; Potthast, 1979) and actually MSD are applied as a rule. By using the selected ion mode (SIM) technique, the individual PAHs can be identified at concentrations at least 100 times lower than is possible by HPLC and FLD. Recently, a GC/MS method has been successfully applied to determine 10 PAHs with 4–6 condensed carbon rings in smoked meats and liquid smokes. The method involved accelerated solvent extraction, gel permeation chromatography and quantification using ^{13}C-labelled PAHs. The repeatabilities of the results of six determinations of different PAHs were 3–12% (Jira, 2004).

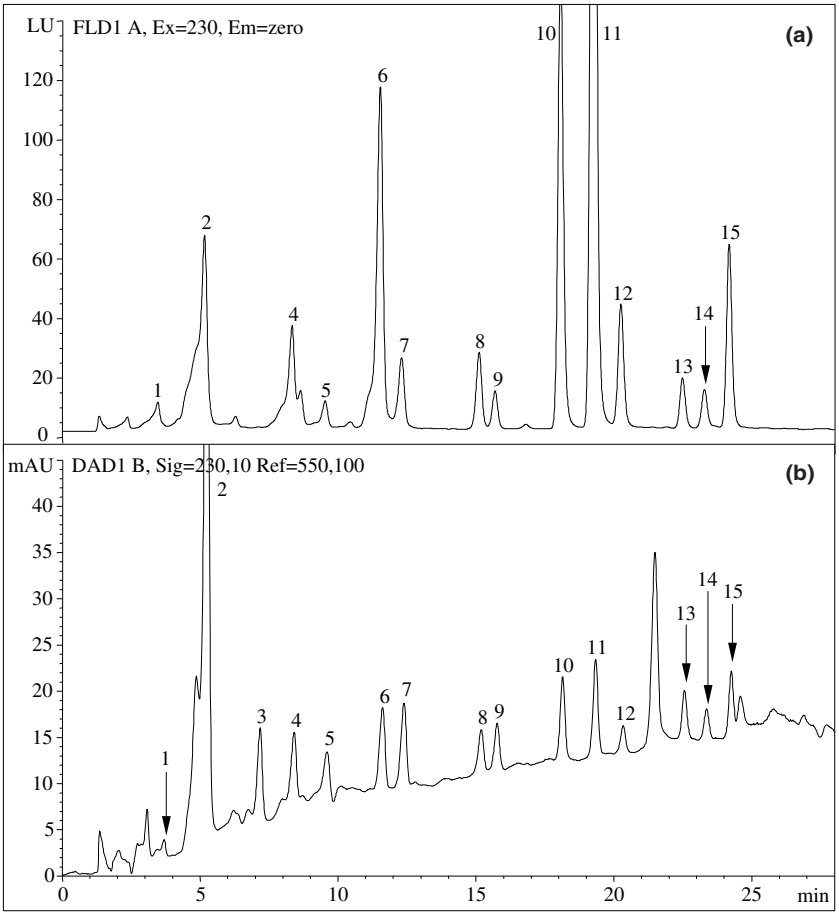

Fig. 1. HPLC chromatograms of a reference standard mixture of PAHs. Vydac column 201TP5415 (5 μm, C18, 150 × 4.6 mm); eluent: acetonitrile/water1:1 (v:v) – to 100% acetonitrile in 30 min, flow rate 1 ml/min, column temperature 30 °C. (a) FLD, (b) UV–Vis (DAD): 1, naphthalene; 2, acenaphthene + acenaphthylene; 3, fluorene; 4, phenanthrene; 5, anthracene; 6, fluoranthene; 7, pyrene; 8, benz[a]anthracene; 9, chrysene; 10, benzo[b]fluoranthene; 11, benzo[k]fluoranthene; 12, benzo[a]pyrene; 13, dibenzo[a,h]anthracene; 14, benzo[g,h,i]perylene; 15, indeno[1,2,3-cd]pyrene.

4. The contents of PAH in fish

4.1. The background level

Fish and marine invertebrates may naturally contain small amounts of different PAHs absorbed from the environment. Seawater is contaminated with PAH due to oil spills, atmospheric pollution resulting from incomplete combustion of fuels, industrial and urban effluents, and leaking from creosoted wharfs and pilings. The average natural content of PAHs is lower in fish musculature than in the liver and the tissues of molluscs, since fish, in contrast to bivalves, have the ability to oxidize and further metabolise PAHs to water–soluble compounds that are excreted by the living organism. Mussels and oysters absorb large quantities of PAHs accumulated on particles from atmospheric pollution, predominantly those with five and more aromatic rings, which associate with the dispersed phase. Depuration, which is applied to clean the alimentary tract of live oysters, does not significantly decrease the pollution with PAHs.

The average concentration of BaP in molluscs is generally much below 10 μg/kg wet weight; however, samples taken from creosote-polluted waters may contain as much as 215 μg/kg. Oysters from a gulf contaminated by hydrocarbons from oil spills contained 17 different PAHs; the total average concentration in 57 samples was about 90 μg/kg wet weight, that of carcinogenic PAHs 19 μg/kg, and of BaP 0.8 μg/kg (Iosifidou, Kilikidis, & Kamarianos, 1982). In the edible parts of shellfish, the content of BaP is below the detection limit or no higher than a few μg/kg. However, in the tail meat and digestive gland of lobsters from a commercial storage facility built of creosoted wood, as much as 280–2300 μg BaP/kg was found (Dunn & Fee, 1979). The edible parts of fish from unpolluted seas generally do not contain detectable amounts of BaP (Rainio, Linko, & Routsila, 1986). The concentrations of 27 individual PAHs in the raw muscles of trout from the Canadian Great Lakes ranged from below the detection limit to 1.24 μg/kg in the lean fish and from below 0.05–3.86 μg/kg in the fat trout, with the total contents of these compounds being not higher than 7.61 μg/kg wet weight.

The limit of detection of PAH was 0.05 μg/kg (Zabik, Booren, Zabik, Welch, & Humphrey, 1996).

4.2. PAH in smoked fish

Most of the PAHs in smoked foods come from the wood smoke. However, in smoked fish canned in oil, the contamination may be carried by the vegetable oil. Some oils contain high amounts of PAH, of the order of 50 μg/kg. The source of PAH may be combustion gases used for direct drying of oilseeds or of the residual olive mass after pressing (Slayne, 2003). Grape seed oil may contain BaP at concentrations of about 20 μg/kg (Moret, Dudine, & Conte, 2000). In canned smoked sardine, the content of BaP was, according to Lawrence and Weber (1984), about five times higher in the oil than in the fish. In order to decrease the contamination of oils with PAHs it is now required to add to the bleaching earth, in the refining process, about 20% of activated carbon.

Cold- and hot-smoked fish contain much more PAH than the raw material – from about 0.05 to about 60 μg of BaP/kg of product, depending on the properties of the fish, method and parameters of smoking, composition of the smoke and exposure of the edible parts to the smoke (Lawrence & Weber, 1984; Nistor, 1985; Petrun & Rubenchik, 1966; Steinig & Meyer, 1976; Tilgner & Daun, 1969; Wierzchowski & Gajewska, 1972). The meat of fish from modern, automatic chambers, supplied with smoke produced under controlled conditions, contains generally about 0.1 μg/kg BaP, and products from traditional kilns up to several μg/kg (Table 2). Petrun and Rubenchik (1966) found from 4.2 to 60 μg/kg of BaP in different hot- and cold-smoked fish from commercial smokehouses, while in kilka, smoked in an electrostatic apparatus supplied with smoke generated at 25–300 °C, the content of BaP was 1.7 μg/kg. Recently, BaP has not been detected in commercially smoked sardine, silver carp, squid, or tuna (Kannappan, Indra Jasmine, Jeyachandran, & Tamil Selvi, 2000). Storelli, Stuffler, and Marcotrigiano (2003) found, that the concentration of total PAHs in smoked seafood ranged from 46.5 μg/kg in swordfish to 124 μg/kg in herring, while that of BaP, undetected in several fish, reached 0.7 μg/kg in Scottish salmon. However, lean and fat trout fillets, hot-smoked with 30 min heating to an internal temperature of the fish of 82 °C, contained, on average, 5.12 and 8.43 μg BaP/kg, respectively (Zabik et al., 1996). In oil sardines, smoked for 6 h at 45–70 °C in a traditional kiln using smoke generated at 400–600 °C, the concentration of BaP was about 12 μg/kg wet weight. When smoking at 45 °C in filtered smoke generated at 300–400 °C lasted 3.5 h and was followed by sun-drying for 4–5 h, the fish contained only about 1.6 μg BaP/kg (Changrasekhar & Kaveriappa, 1985). A typical example of a product highly contaminated with PAHs is smoked-dried bonito (katsuobushi). It is made in repeated cycles of smoking for several hours at 80–

Table 2
The contents of benzo(a)pyrene in hot-smoked fish from various smokehouses

Smoked fish	Traditional kiln	Smokehouse with external smoke generator	Source
	BaP (μg/kg)	Wet weight of product	
Bückling, muscle		0.3	Karl and Leinemann (1996)
	0.5–1.4		Steinig and Meyer (1976)
Skin	22–43		Steinig and Meyer (1976)
Dogfish, skinned	2.6–3.7	1.1	Steinig and Meyer (1976)
		0.4–2.0	Karl and Leinemann (1996)
Eel, muscle	2.6–3.3	0.3–0.5	Steinig and Meyer (1976)
	0.3–3.9	n.d.–0.1	Karl and Leinemann (1996)
Skin	49–74	1.8–4.0	Steinig and Meyer (1976)
Halibut, muscle	1.5–3.7	0.6–0.8	Steinig and Meyer (1976)
	3.6	n.d–0.2	Karl and Leinemann (1996)
Mackerel, muscle	0.5–2.4	0.5–0.9	Steinig and Meyer (1976)
	0.5–2.4	n.d.–2.0	Karl and Leinemann (1996)
Skin	19–30	1.3–2.4	Steinig and Meyer (1976)
Salmon, muscle	3.1	0.4	Karl and Leinemann (1996)
Sprats, muscle	1.1–2.4		Steinig and Meyer (1976)
	1.6–6.8	1	Karl and Leinemann (1996)
Skin	9–28		Steinig and Meyer (1976)

n.d. – Not detectable.

120 °C, followed by overnight drying. The layer of tar that forms on the surface, up to about 3% of the fish weight, contains 20–40 times more BaP than the meat of the deeper layers (Kikugawa, Kato, & Hayatsu, 1986).

The content of BaP in various smoked meat products was, according to early German data (Potthast, 1978) from about 0.01–1.11 μg/kg in mild-smoked ham, through 0.18–2.08 μg/kg in cooked sausages, and 0.14–56.04 μg/kg in black smoked ham.

Although some assortments of smoked fish products may contain considerably large amounts of PAHs, smoked meats and fish do not generally contribute much to the human intake of these compounds. According to the Scientific Committee on Food (2002), in the average diets of several European countries, the estimated share of BaP from smoked fish makes only a small fraction of the total dietary intake. In the UK, oils and fat, cereals and vegetables contribute about 90% of the total while, in the Dutch diet, 97% of the total BaP intake comes from oils and fats, cereals, sweets and sugar. However, in local communities, where fish smoked traditionally constitutes a large proportion of the diet, the intake of BaP from these sources may be significantly higher.

Information on the persistence and distribution of smoke components, including PAHs, in different parts of smoked products during storage is scarce. The rate of diffusion in the fish is controlled by the character of the surface, as well as by the properties of the meat and of the deposited compounds. Most smoke phenols accumulate on the skin and in a layer of the product about 6 mm deep, especially in the fatty tissue. In some products, however, particularly in lean fish, even as much as 60% of the total mass of phenols can penetrate deeper layers (Kurko & Mezenova, 1985). According to Simko (1991), the concentration and distribution of BaP in smoked fish may change during storage due to diffusion and degradation, affected by the properties of the product and environmental factors. In the presence of light, PAHs are sensitive to photo-degradation and oxidation. The susceptibilities of individual PAHs are different, the half life for degradation being from a few hours to several days. Simko (1991) found that, immediately after smoking, the surface and internal layers of smoked fish contained 10.6 and 0.0 μg/kg BaP, respectively and, after seven days storage, 1.3 and 0.1 μg/kg. In fish hung freely at 18 °C, unlimited air access and daylight, the concentration of BaP decreased after four days from the initial 0.6 μg/kg to about 0.1 μg/kg.

5. Regulations regarding PAHs in smoked foods

According to German regulations, in force since 1973, the content of BaP in smoked meat products should not exceed 1 μg/kg. However, for meat products treated with smoke preparations, the upper limit of 0.03 μg/kg has been set by the European Union (Hartmann, 2000; Simko, 2002). Since the ratio of the contents of BaP and the other carcinogenic PAHs is rather constant, the use of BaP as a marker for the contamination of smoked meats by PAHs may be justified (Potthast, 1979).

Various PAHs differ in carcinogenic potency, but even those that are regarded as not being carcinogenic, may function in living organisms as synergists, increasing the carcinogenicity of other PAHs. Attempts have been made to express the potency of different PAHs relative to that of BaP, as toxic equivalency factors (TEF). This would allow estimation of the total equivalent exposure (TEQ) to PAHs, relative to BaP in various foods (Jira, 2004; Scientific Committee on Food, 2002). The EU countries producing olive residual oil have introduced, in 2001, into their national legislation, the maximum level of 2 μg/kg for each of the eight most carcinogenic PAHs, including BaP, but not higher than 5 μg/kg for the combined amount of them, as well as a maximum content of 25 μg/kg for all PAHs (Slayne, 2003). The last requirement, however, is unrealistic, since in practice only 16 PAHs are usually determined, because only few CRM are available.

6. Concluding remarks

Smoked fish constitute a significant part of the human diet, important because of their desirable sensory properties, high nutritional value and abundance, in fatty species, of lipids rich in $n-3$ fatty acid residues. The wood smoke used in smoking of fish may contain, depending predominantly on the temperature of generation, a large variety of PAHs, including the most carcinogenic ones. Smoking under mild conditions, in modern smokehouses supplied with filtered smoke from external generators, does not lead to significant contamination of the products with carcinogenic PAHs. The contents of BaP in the meat of hot-smoked fish is, on average, not higher than the limit set by different national and European regulations. However, heavily smoked products from traditional kilns, particularly the outer parts of such commodities, may contain up to about 50 μg BaP/kg wet weight. Most of the data regarding the contents of PAH and BaP in smoked fish, published in some older literature, should be treated with caution, if the procedures applied to the determination did not guarantee unequivocal separation and identification of the carcinogenic hydrocarbons.

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