REVIEW

Investigation of Polycyclic Aromatic Hydrocarbons in Foods

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Polycyclic aromatic hydrocarbons (PAHs) also known as polycyclic organic matter or polynuclear aromatic hydrocarbons are a class of carcinogenic chemical compounds originating from incomplete combustion of organic matter and geochemical processes. Exposure to PAHs is a major concern for human health. Breathing the air near coal-tar, asphalt production or applications, cigarette smoke, wood smoke, vehicle exhausts, fumes from chimneys, inhalation of polluted air, eating grilled or charred meats, any food with PAHs deposited on them during growing or processing, with drinking water and coal-tar-containing medications are the main sources of PAH exposure. Under the guidance of the latest literatures, the emphasis in this review will be placed on the general information about PAH compounds, contamination sources, human exposures, analytical methods and regulations.

Key Words: Polycyclic aromatic hydrocarbons, Food, Contaminant, Benzo[a]pyrene, Consumer health.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) also known as polycyclic organic matter or polynuclear aromatic hydrocarbons are a class of carcinogenic chemical compounds originating from incomplete combustion of organic matter and geochemical processes. Polycyclic aromatic hydrocarbons consist of two or more fused aromatic rings. These compounds which contain up to four fused aromatic rings are named as light PAHs and those containing more than four aromatic rings are named as heavy PAHs.

In 1775, Percival Pott found an association between exposure to soot and a high incidence of scrotal cancer in chimney sweeps. In 1920, Japanese scientists discovered that painting extracts of soot caused skin tumours in mice. The first pure chemical carcinogen dibenzo(a,h)anthracene which is an important PAH compound was isolated from soot extract by Kennaway in 1929.

Polycyclic aromatic hydrocarbons consist of several hundred compounds. Among these different PAHs, 16 compounds are evaluated as priority by US

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Environmental Protection Agency (USEPA) because they are considered to be more harmful than the others. These 16 toxic PAHs are: benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), cyclopenta[c,d]pyrene (CPP), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[j]fluoranthene (BjF), dibenzo[a,h]anthracene (DhA), indeno[1,2,3-cd]pyrene (IcP), benzo[g,h,i]perylene (BgP), dibenzo[a,l]pyrene (DIP), dibenzo[a,e]pyrene (DeP), dibenzo[a,i]pyrene (DiP), dibenzo[a,h]pyrene (DhP), benzo[c]fluorene (BcL), chrysene (CHR) and 5-methylchrysene (5MC). The molecular structures of these compounds are shown in Fig. 1.

The International Agency for Research on Cancer (IARC) classified three PAHs of these 16 compounds (BaA, BaP and DhA) as probably carcinogenic (group 2A) and 9 (5MC, BbF, BjF, BkF, IcP, DeP, DhP, DiP and DIP) as possibly carcinogenic to humans (group 2B).

Benzo[a]pyrene is the most known and identified as carcinogen compound among these PAHs. Because it is found in the environment and a wide variety of food stuff and can easily be detected. BaP is considered as a good marker for total...
PAHs in foods. Simko stated that BaP contributes 1-20% to the total carcinogenicity found in samples from the environment. In a study conducted by Kazerouni, the correlation coefficient between BaP levels and total PAHs has been reported as 0.87 and between the carcinogenic PAHs and BaP as 0.98. On the other hand, recent toxicological studies exposed that DIP has a much stronger carcinogenic effect than BaP.

Exposure to PAHs is a major concern for human health. Breathing the air near coal-tar, asphalt production or applications, cigarette smoke, wood smoke, vehicle exhausts, fumes from chimneys, inhalation of polluted air, eating grilled or charred meats, any food with PAHs deposited on them during growing or processing, with drinking water and coal-tar-containing medications are the main sources of PAH exposure. Polycyclic aromatic hydrocarbons are lipophilic compounds and can be absorbed by the lung and gastrointestinal tract tissues and by the skin. Once PAHs are enter the body, they mostly go to internal organs, especially those containing fat. Most PAHs are converted to non-toxic compounds and leave the body in a few days by urine, but some become activated and bind to DNA and form PAH-DNA adducts. These are markers of PAH exposure. This binding leads to errors in DNA replications and mutations. The formation of DNA adducts leads to the development of cancer. Because of their mutagenic and carcinogenic effects, PAHs have been included in several priority pollutant lists of the Agency of Toxic Substances and Disease Register (ATSDR), of the International Agency for Research on Cancer (IARC), of the European Community (EC) and of the Environmental Protection Agency (EPA). Several studies have been carried out to determine the levels of exposure of humans to PAHs.

Under the guidance of the latest literatures, the emphasis in this review will be placed on the general information about PAH compounds, contamination sources, human exposures, analytical methods and regulations.

**Polycyclic aromatic hydrocarbons in the environment**

PAHs in the environment arise from a number of potential natural and anthropogenic sources. They may react with O₃ and NOₓ in the atmosphere and generate more harmful components with effective carcinogenic activity. Polycyclic aromatic hydrocarbons enter the environment (especially air) mostly by volcanic eruptions, natural or forest fires, residential wood burning, thermal geological reactions, industrial processes such as coke ovens in the production of aluminium, iron and steel, foundries, heating in power plants and residences, waste incineration, combustion of fossil fuel, environmental tobacco smoke, transportation and exhaust from automobiles and trucks. Furthermore, crude oil and other petroleum based products contribute to contamination of significant amount of PAHs to the environment.

In soils, PAHs are most likely to stick tightly to particles and can evaporate from surface soils to air. They are present in air as vapours or stuck to the surfaces of small solid particles and can travel long distances before they return to earth in rainfall or particle settling. Certain PAHs in soils also contaminates underground
water sources. Addition to this they can also enter surface water through discharges from industrial plants and waste water treatment plants, sewage sludge and various kinds of sediments\textsuperscript{26-28}. After entering the environment, the movement of PAHs depend on properties such as how easily they dissolve in water and evaporate into the air. Because PAHs are lipophilic compound they do not easily dissolve in water\textsuperscript{29,30}.

Polycyclic aromatic hydrocarbons can break down to longer-lasting products by reacting with sunlight and other chemicals in the air, generally over a period of days to weeks. Breakdown in soil and water generally takes weeks to months and is caused primarily by the actions of microorganisms.

Typical concentrations for PAHs in forest soil and urban soil range from 5 µg to 100 µg/kg and 600 µg to 3000 µg/kg, respectively. Higher values (1000-3000 µg/kg) near areas of heavy transportation and industrialization have been observed. However, levels of 8000-336000 µg/kg have been reported for road dust\textsuperscript{31}. In the air, annual mean levels of BaP in rural background areas varied between 0.1 and 1 ng/m\textsuperscript{3}; for urban areas levels were between 0.5 and 3 ng/m\textsuperscript{3} (with traffic sites at the upper boundary of this range); levels up to 30 ng/m\textsuperscript{3} have been measured within the immediate vicinity of a cookery\textsuperscript{32}. In a study the mean outdoor concentration of BaP in air was 0.9 ng/m\textsuperscript{3} and indoor concentrations ranged from 0.1 to 8.1 ng/m\textsuperscript{3} in Phillipsburg, New Jersey that contains a metal pipe foundry\textsuperscript{33}. The pollution of waters with PAHs has been reported by a number of researchers. In various studies PAH concentrations ranged from 0 to 2621 ng/g in Kara Sea and adjacent rivers (Russia 1993), in Gao Ping River (Taiwan 2000), in Tonghui River (China 2002), in Biobio River (Chile 2003), in La Plata River (Argentina 2006) and in Yellow River (China 2007)\textsuperscript{34-39}.

**Polycyclic aromatic hydrocarbons in food**

Polycyclic aromatic hydrocarbon compounds are widespread in food stuffs not only as a result of the environmental pollution but also as a consequence of some thermal treatments which are used in the preparation and manufacturing of foods\textsuperscript{4,6,10,12,17,41}. Food smoking, grilling, frying, roasting, baking, toasting and drying are known as important sources of PAH contamination\textsuperscript{4,6,10,12,17,41}. Formation of PAHs in foods is affected by several factors such as the methods used for preparation of food (grilling, frying, smoking, roasting, etc.), temperature and time of cooking, distance from the heat source and drainage of fat\textsuperscript{42}.

Smoking is one of the oldest food preservation technologies and used to achieve the characteristic taste, colour and aroma to food (especially meat and meat products, fish and fish products)\textsuperscript{14}. In addition to this, smoking enhances preservation due to the dehydrating bactericidal and antioxidant properties of smoke such as phenol derivates, carbonyls, furan derivates, organic acids and their esters\textsuperscript{5}. Smoke is mostly generated from certain kinds of wood (hardwood such as beech, hickory and softwood such as pine and fir) in traditional smokehouses which are still used widely in many countries.
The smoke for smoking of food is a mixture of air, water vapour, CO₂, 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. 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. Smoke produced at 650-700 °C is the richest in components responsible for imparting the desirable sensory properties to the products. Temperature of smoke generally plays an important role, because the amount of PAHs in smoke formed during pyrolysis increases linearly with the smoking temperature between 400-1000 °C. Additionally, the PAH levels in smoked food depend on combustion temperature, the type of smoke generator and wood. Because of potential health hazards associated with smoked foods PAHs, derivatives of PAHs (nitro-PAH, oxygenated PAH), N-nitroso compounds and heterocyclic aromatic amines are the carcinogenic components of wood smoke.Incomplete wood combustion during smoking can produce considerable amounts of PAHs which can penetrate through the surface of products. There are several studies conducted by a number of researchers about the presence of PAHs in smoked fish, meat and meat products. In a study performed by Goma et al., the total PAH concentrations were detected between 2.6-29.8 and 9.3-86.6 µg/kg in smoked meat and fish, respectively. In another study conducted by Panalaks in Canada, smoked fish and meat samples were analyzed and PAH compounds were detected in 18 out of 25 smoked fish samples (max. 141 µg/kg) and in 19 out of 43 smoked meat samples (max. 13 µg/kg). Petrun and Rubenchik found the levels of BaP ranged from 4.2 to 60 µg/kg in hot and cold-smoked fish samples. In their study, Storelli et al. reported that the concentration of total PAHs in seafood varied from 46.5 to 124 µg/kg. Reinik et al. found the highest total PAH concentrations in smoked meat, sausage and chicken samples as 16, 19 and 6.5 µg/kg, respectively. In another study, Djinovic et al. stated that there are differences in PAH contents between final smoked beef ham samples from traditional smokehouse (3.9 µg/kg) and industrial smokehouse (1.9 µg/kg).

Another well known PAH formation mechanism is the grilling of various foods. When the foods especially fatty meat and meat products are in direct contact with the flame, due to high temperature, pyrolysis of the fats in the meat generates PAHs that can accumulate on the surface of meat. Additionally, fat dripping onto the flame or coals cause PAH formation which are then carried back on the meat. The thickness of the meat is another important factor on formation of PAHs, because less thickness may result in a greater surface area contacting with the open flame per weight. Normal roasted or fried foods do not produce abundant amount of PAHs as much as charred foods.
In a study, Reinik et al.\textsuperscript{10} reported that home-prepared meat products, especially those which were prepared using a disposable grill, contained higher concentrations of BaP and PAHs compared to commercial products. The mean concentrations of BaP in industrial and disposable grill meat were 0.17 and 1.0 µg/kg, respectively.

Kazerouni et al.\textsuperscript{13} reported that grilled/barbecued steak, well done grilled/barbecued chicken with skin and very well done grilled/barbequed hamburger had the highest concentrations of BaP. Grilled/barbequed meats contained higher levels of BaP compared to broiled or pan-fried meats. They stated that the concentration of BaP in grilled/barbequed meats is affected by the length of cooking time.

In a study, Aygun and Kabadayi\textsuperscript{50} determined that BaP levels in over grilled lamb (62.60 µg/kg) and beef (37.60 µg/kg) were found to be higher than grilled lamb (43.80 µg/kg) and beef (31.33 µg/kg) samples. Elhassaneen\textsuperscript{16} determined the range of PAHs between 0.31 and 14.95 µg/kg in charcoal broiled beef burgers. Benzo[a]pyrene levels of the samples varied from 0.99 to 4.8 µg/kg. In another study, Mottier et al.\textsuperscript{6} analyzed the contents of 16 PAHs in seven different barbequed meat sausages. According to the results the highest concentrations were found for phenanthrene and naphthalene. The sum of carcinogenic PAH content was 13.17 µg/kg in heavily barbequed lamb sausage and BaP levels were between ‘not detected’ and 2.81 µg/kg.

Like other thermally processed food products, toasted bread may also contain PAH compounds not only due to contamination of source but also during toasting step. In their study, Kayali-Sayadi et al.\textsuperscript{51} detected different PAHs (acenaphthylene, phenanthrene and dibenzo[a,h]anthracene) in toasted bread samples and the levels were ranged between 0.32-9.4 µg/kg. Some other researchers stated that the products which contain white granary flour may contain higher PAH levels than the products with white flour. Dennis et al.\textsuperscript{52} found the total PAH levels for products with granary flour as 5.6 µg/kg and for products with white flour as 1.5 µg/kg.

Because of environmental contamination, PAHs can be also found in water sediments. At the result the fresh water animals such as bottom feeding fish, filter feeding vertebrates and invertebrates are particularly prone to exposure and accumulation of the carcinogenic PAH compounds\textsuperscript{21,53}. Generally, vertebrates are able to metabolize the majority of absorbed PAHs by enzymes while in the invertebrates this metabolic capacity is inferior\textsuperscript{53,54}. In Hong Kong, Kong et al.\textsuperscript{55} determined PAH levels in tilapia (Oreochromis mossambicus) a fresh water fish as 76.5 ng/g - 60.1 ng/g wet weight.

A number of researchers announced that fats and oils may contain remarkable high concentrations of PAH compounds because of their lipophilic nature. Pupin and Toledo\textsuperscript{56} analyzed 40 olive oil samples in Brazil and they determined the highest BaP levels as 164 µg/kg. In a similar study, Moret et al.\textsuperscript{57} examined 51 olive oil samples and found that the total PAH concentrations varied from 2.94 to 143.12 µg/kg. Thomson et al.\textsuperscript{58} determined the BaP concentrations between 0.2-5.2 µg/kg in
examined margarine samples. Hopia et al.\textsuperscript{59} detected different levels of PAH compounds in Finnish butters, margarines and vegetable oils. In another study conducted by Stijve and Hischenhuber\textsuperscript{60} 12 samples of vegetable oils were tested and the highest BaP concentration was determined in coconut oil as 581.7 µg/kg.

Leafy vegetables can be a significant source of PAHs in the human diet. The most important contamination route is via atmospheric exposure rather than uptake from the soil or endogenous biosynthesis\textsuperscript{21,61}. Leaf features such as surface area and hairs have very important role in PAH uptake and accumulation\textsuperscript{62}. Hairs increase the leaf surface that is able to capture particulates from air. A study conducted by Howsam et al.\textsuperscript{63}, it was found that the hairy leaves contain higher PAH concentrations then hairless leaves. In another study, Kazerouni et al.\textsuperscript{13} declared that green leafy vegetables such as kale and collard greens had higher BaP content comparing to other vegetables. The author explained the difference by the greater contact surface of kale and collard green to the ambient air during growth. In a similar study, Greenberg et al.\textsuperscript{64} determined the BaP levels between 12.6-48.1 µg/kg in kale. Fiedler et al.\textsuperscript{65} examined green tea leaves and found that the total PAH concentrations of examined samples varied from 497 to 517 µg/kg, compared with brick tea leaves ranged from 1048 to 1162 µg/kg.

Other than these studies, it was demonstrated that milk derivates may contain different PAH concentrations according to environmental conditions (especially PAHs in grass, exposure to smoke, etc.). Kishikawa et al.\textsuperscript{66} determined the average concentrations of total PAHs in commercial milk and human milk as 0.99 and 0.75 µg/kg, respectively. In another study, it was reported by Somogyi and Beck\textsuperscript{67} that the human milk contained BaP at a level of 6.5 ng/kg.

**Determination methods of PAHs in food**

Generally, PAHs in food are expressed as µg/kg (ppb) and analysis steps are as follows: extraction/hydrolysis, liquid/liquid partition, clean-up procedures, concentration, chromatographic separation and determination\textsuperscript{5}.

The most frequently used analytical methods for determination of PAHs are high performance liquid chromatography (HPLC) with UV or fluorescence detection, liquid chromatography mass spectrometry (LC-MC), gas chromatography mass spectrometry (GC-MS). Because of its high separation efficiency, GC-MS methods provide higher sensitivity and selectivity than HPLC methods\textsuperscript{68}.

Several GC methods have been defined for PAHs analysis with different techniques of extraction and purification. Some of these are summarized in Table-1\textsuperscript{5}.

**Regulations about polycyclic aromatic hydrocarbons**

Since 1 April 2005, the Commission Regulation (EC) No 208/2005 of 4 February 2005, which provides maximum levels for BaP in different food groups. Nowadays, these maximum levels are part of Commission Regulation (EC) No 1881/2006 of 19 December 2006\textsuperscript{69} (Table-2). Also, the Scientific Committee on Food (SCF) recommended to the member states of European Union to analyze the contents of 15
<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Treatment and Preseparation</th>
<th>Column/Stationary Phase</th>
<th>Temperature</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbequed sausage</td>
<td>Saponification with mixture of ethanol, water and KOH, extraction with CE, preseparation by SPE on Isolute aminopropyl and C18 columns</td>
<td>25 m x 0.2 mm capillary column /SPB-5</td>
<td>80 °C for 0.5 min→230 °C at 8 °C/min→300 °C at 5 °C/min</td>
<td>MSD</td>
</tr>
<tr>
<td>Smoked sausage</td>
<td>Saponification in methanolic KOH, liquid-liquid extraction (methanol-water-CE and DMF-water- CE) precleaning on silica gel and GPC on Sephadex LH 20</td>
<td>10 m x 2 mm packed column/5% OV-101 on sorbent Gas Chrom</td>
<td>260 °C isothermal</td>
<td>FID</td>
</tr>
<tr>
<td>Smoked meat products</td>
<td>Saponification with mixture of ethanol, water and KOH, partition with DMF, precleaning on Kiesel gel 60</td>
<td>25 m x 0.28 mm capillary column /SE-54</td>
<td>240 °C isothermal</td>
<td>MSD</td>
</tr>
<tr>
<td>Smoked fish and fish products</td>
<td>Saponification in methanolic KOH, liquid-liquid extraction (methanol-water-CE and DMF-water- CE) precleaning by CC on silica gel and GPC on Sephadex LH 20</td>
<td>55 m x 0.3 mm glass capillary column /SE-54</td>
<td>165 °C for 6 min, 165→255 °C at 4 °C/min</td>
<td>FID</td>
</tr>
<tr>
<td>Smoked fish</td>
<td>Saponification methanol-water-KOH mixture under reflux, extraction into CE, extraction of PAHs with caffeine/formic acid, washing with NaCl solution, extraction into CE, preseparation on silica gel</td>
<td>30 m x 0.25 mm capillary fused silica column /SE-54</td>
<td>110 °C isothermal for 1.5 min→210 °C at 30 °C/min→290 °C at 3 °C/min→300 °C at 10 °C/min</td>
<td>MSD</td>
</tr>
<tr>
<td>Smoked meats</td>
<td>Saponification with methanolic KOH, extraction with CE partition with DMF/water clean up on silica gel and with GPC on Bio Beads S-X3</td>
<td>50 m x 0.25 mm capillary column /DB-5</td>
<td>70 °C→280 °C at 5 °C/min</td>
<td>MSD</td>
</tr>
<tr>
<td>Smoked meats</td>
<td>Saponification with methanolic KOH, extraction with n-hexane clean up by SPE on Florisil</td>
<td>30 m x 0.32 mm /DB-5</td>
<td>80 °C isothermal for 1 min→150 °C at 10 °C/min→280 °C at 4 °C/min</td>
<td>ITD</td>
</tr>
<tr>
<td>Smoked chicken</td>
<td>Extraction with methanol in Soxhlet app., +KOH, extraction into n-hexane, clean up on Pep-Pak Florisil</td>
<td>30 m x 0.32 mm /DB-5</td>
<td>70 °C isothermal for 1 min→150 °C at 10 °C/min→280 °C at 4 °C/min hold for 14 min</td>
<td>ITD</td>
</tr>
</tbody>
</table>

CE = cyclohexane, CC = column chromatography, DMF = dimethyl formamide, GPC = gel permeation chromatography, SPE = solid-phase extraction, MSD = Mass spectrometry detector, FID = Flame ionization detector, ITD = Ion trap detector.
TABLE-2
BENZO[a]PYRENE LIMITS IN FOODS ACCORDING TO EC 208/2005

<table>
<thead>
<tr>
<th>Foodstuffs</th>
<th>Maximum levels (µg/kg wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oils and fats (excluding cocoa butter) intended for direct human consumption or use as an ingredient in foods</td>
<td>2.0</td>
</tr>
<tr>
<td>Smoked meats and smoked meat products</td>
<td>5.0</td>
</tr>
<tr>
<td>Muscle meat of smoked fish and smoked fishery products, excluding bivalve mollusks. The maximum level applies to smoked crustaceans, excluding the brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (Nephropidae and Palinuridae)</td>
<td>5.0</td>
</tr>
<tr>
<td>Muscle meat of fish, other than smoked fish</td>
<td>2.0</td>
</tr>
<tr>
<td>Crustaceans, cephalopods, other than smoked. The maximum level applies to crustaceans, excluding the brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (Nephropidae and Palinuridae)</td>
<td>5.0</td>
</tr>
<tr>
<td>Bivalve mollusks</td>
<td>10.0</td>
</tr>
<tr>
<td>Processed cereal-based foods and baby foods for infants and young children</td>
<td>1.0</td>
</tr>
<tr>
<td>Infant formulae and follow-on formulae, including infant milk and follow-on milk</td>
<td>1.0</td>
</tr>
<tr>
<td>Dietary foods for special medical purposes intended specifically for infants</td>
<td>1.0</td>
</tr>
</tbody>
</table>

PAH compounds, which are classified as priority (15 SCF-PAH) and to check the suitability of BaP as a marker for the occurrence and impact of carcinogenic PAHs in food. Additionally, the European Food Safety Authority (EFSA) recommends to analyse benzo[c]fluorene assessed to be relevant by the Joint FAO/WHO Experts Committee on Food Additives (JECFA).

Conclusion

Because PAHs are widespread in the environment, generally foods including cereals, fats and oils, seafood, tea, coffee contain PAHs. Therefore, humans are exposed to these carcinogenic compounds mainly through foods. Especially, cooking methods which are used dry heating (such as roasting, barbecuing and grilling) cause the formation of higher PAH levels in food. So, people whose diet is rich in barbequed foods may take in a larger amount of PAHs. Also, the higher cooking temperatures and the closer distance to the heating source result in higher levels of PAHs in food. Furthermore, many people expose to PAHs via air (tobacco smoke, wood smoke, industrial smoke, traffic exhaust) and contaminated water.

In order to prevent from health risks of PAHs, it should be consumed less barbequed/grilled/smoked foods. Also, fatty foods such as meat, fish and their products should not be overcooked and contacted with flame directly. In addition to this, it should be avoided open burning of garbage, leaves and yard waste, tires, agricultural fields and it should not be smoked tobacco products.
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42. 1017 (2001).
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