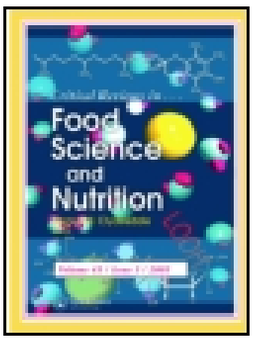

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Review of the quantification techniques for polycyclic aromatic hydrocarbons (PAHs) in food products

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Abstract

There is a growing need for accurate detection of trace-level PAHs in food products due to the numerous detrimental effects caused by their contamination (e.g., toxicity, carcinogenicity, and teratogenicity). This review aims to discuss the up-to-date knowledge on the measurement techniques available for polycyclic aromatic hydrocarbons (PAHs) contained in food or its related products. This article aims to provide a comprehensive outline on the measurement techniques of PAHs in food to help resolve problems associated with their accurate quantification and resulting deleterious impacts on human health. The main part of this review is dedicated to the opportunities and practical options for the treatment, detection, and quantification of PAHs in various food samples. Basic information regarding all available analytical measurement techniques is also evaluated with respect to their performance in terms of PAH detection.

Keywords

Analytical methods, Food products, Polycyclic aromatic hydrocarbons, GC-MS, HPLC

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1. Introduction

PAHs are found ubiquitously in all different media such as air, soil, water, and various food products that we encounter in our everyday life (Moon et al., 2010; Mastral et al., 2000; Pandey et al., 2011; Szulejko et al., 2014; Kim and Kim, 2015). Human beings are exposed to PAHs largely by intake of food and through environmental pollution (Xia et al., 2010, 2013; Kim et al., 2013) (**Figure 1**). Because each food consists of complex and heterogeneous matrices, the analysis of PAHs in food samples can be obstructed by various unintended analytical parameters such as maximizing the recovery of food analytes and minimizing accompanying interferences (e.g., through extraction and clean-up). Hence, it is of utmost importance to develop simple and accurate analytical methods, through which the concentration levels of PAHs can be assessed in different food types. The conventional techniques used to measure PAHs are complicated by many problems, especially in the extraction methods, which are the most common and popular methods for PAH measurement in food products, before separation and detection. As such, one of the main goals in improving the quantification of PAHs is to minimize the interference, and this can be achieved by proper pretreatment of samples with correct extraction methods and clean-up procedures.

In general, PAHs are not present individually but in the form of mixtures. That is why they have been listed as a group of hazardous compounds such as the 15 EU priority PAHs (2005): benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (BghiP), benzo[a]pyrene (BaP), chrysene (CHR), dibenzo[a,h]anthracene (DBahA), indeno[1,2,3-cd]pyrene (IP), benzo[a]fluoranthene (BAF), cyclopental[cd]pyrene (CPcdP), dibenzo[a,e]pyrene (DBaeP), dibenzo[a,h]pyrene (DBahP), dibenzo[a,i]pyrene (DBaiP), dibenzo[a,l]pyrene (DBalP), and 5-methyl

chrysene (MCH). The United States Environmental Protection Agency has also noted 16 PAHs as potential food contaminants (US-EPA, 2010). These include some of the 15 EU priority PAHs as well as some additional compounds including acenaphthene (ACP), acenaphthylene (ACY), anthracene (ANT), fluoranthene (FA), fluorene (FLR), naphthalene (NPH), phenanthrene (PHE), and pyrene (PYR) (Table 1).

To quantify trace-level PAHs contained in food samples, a number of analytical techniques have been developed through the combination of different experimental approaches available for extraction, separation, and identification of PAHs (showed in **Figure 2**). In principle, most of those methods have been applied for the determination of PAHs found not only in food but also in many other media (water and smoke) (Crosby et al., 1981). The extraction procedures for PAHs largely depend on the nature of the food matrices. Previously, researchers generally relied on saponification with KOH-methanol in conjunction with clean-up, Soxhlet, and liquid-liquid extraction (LLE) (Wenzl et al., 2006). Several upgraded techniques have been developed continuously, such as accelerated solvent extraction (ASE) (Jira, 2008), supercritical fluid extraction (SFE) (Zougagh et al., 2004), and solid-phase extraction (SPE) (Teixeira et al., 2007; Stumpe-Vīksna et al., 2008; Orecchio et al., 2009; Janoszka, 2011; Farhadian et al., 2012) with organic solvents to cover both liquid (vegetable oils, milk, and milk formulas) and solid food samples (meat, fish, cereals, and their products). Furthermore, the accuracy and coherence of the methods in each study were determined by the selected extraction methods for given food samples. All the sample preparations and the steps of clean-up require different approaches and procedures depending on the types of food samples and subsequent methods of separation and quantification.

The objective of this paper was to review the procedures used for quantification of PAHs in various food products across diverse experimental steps including sample collection, preparation, and analytical methods. An effort has also been made to develop newer bio-sensing techniques because of their advantages of delivering sensitive and rapid detection of PAH contamination in food. In our recent work, we provided a comprehensive evaluation regarding the general status of PAH contamination in various food categories (e.g., plant (fruits, vegetables, beverages, cereals, and nuts) and animal-based (land and water)) (Bansal and Kim, 2015). In the current article, we expanded our efforts to provide an overview of the up-to-date knowledge regarding the fundamental options available for the quantitation of PAHs in various food products.

2. Types of PAHs prevalent in food products

PAHs, as well-known carcinogenic and mutagenic compounds, are classified by their number of carbon rings into ‘heavy PAHs’ with five or more aromatic rings or ‘light PAHs’ with less than 5 rings (Lee and Shim 2007; Phillips, 1999; Zhang et al., 2009). In general, heavy PAHs tend to be more stable and toxic than lighter ones (Yebra-Pimentel et al., 2015). PAHs have been found in both non-processed and processed food samples. PAHs in food products can lead to the formation of biologically active metabolites (Guillen et al., 1997). Polluted environment is a major contributor to contamination of food products with PAHs by various routes such as (1) PAH accumulation on the waxy surfaces of plant-based foods such as cereals, vegetables, and fruits; (2) uptake of PAHs by plants through soil and water; and (3) exposure of crops to PAH sources such as nearby highways or heavy traffic sites (Hites et al., 1978; Wagrowski and Hites, 1996; Simon et al., 2005; Wenzl et al., 2006). Food processing activities such as grilling, roasting, smoking, drying, and heating are also key sources of PAH production or

enrichment in the products of meats, oil seeds, dairy products, chocolates, and wines (Christensen and Bzdusek, 2005). Furthermore, the sedimentation of PAHs in bodies of water can also contaminate seafood and fish as well (Fahnrich et al., 2002). Therefore, accurate evaluation of PAH levels in food is considered an urgent task by groups concerned with human health such as the European Union and others (EU, 2005).

The main routes of PAH formation or contamination can vary considerably between different food types. The occurrence of PAHs in vegetable oils is due to the thermal treatments used to dry the oil seeds, by which combustion gases come in contact with the seeds (Wegrzyn et al., 2006). The main entrance sources of PAHs in food are oils and fats. Fat has played a major role in the enhancement of PAH formation via thermal decomposition (Alomirah et al., 2011), which is why baking processes for flour products and processed breads are vulnerable to PAHs under common exposure conditions including high temperatures and addition of fat for enhanced palatability. Plant-based foods (fruits, leafy vegetables, and cereals) are apt to be contaminated via atmospheric transport of PAH under their growing conditions owing to the larger, waxy, and cuticle surface areas, which provide conditions conducive for the deposition of PAHs. Similarly, non-leafy vegetables like stems (cucumber, eggplant, and tomato) and tubers (potato, radish, and carrot) can easily take up PAHs from contaminated sites (Wennrich et al., 2002). Likewise, processed products like sweets, candies, and chocolates can be affected by drying, roasting, winnowing, blending, and fermenting of the cocoa seeds followed by blending of cocoa liquor (The World Atlas Class of Chocolate, 2003).

Consumption of meat and meat products (grilled chicken, chicken satay, and beef satay) is the most common route of PAH intake by humans (Farhadian et al., 2010). Beverages such as juices, milk, and

coffee are also susceptible to PAH contamination due to the addition of burnt sugar and heat processing (Tfouni et al., 2007). Apart from non-alcoholic beverages, accumulation of PAH in alcoholic beverages can occur if their ageing is carried out using an improper wood type (Chinnici et al., 2007). Therefore, contamination by all these varied processes and routes has necessitated the development of highly sensitive analytical procedures not only for accurate sampling of the food items but also for the detection of PAHs.

3. Preparation of food samples for PAH analysis

Sample collection and preparation is an essential step for accurate quantification of PAHs. Due to the complex nature of matrices involved in food, the sampling often requires extensive extraction and/or preparation steps (Ridgway et al., 2007). Optimal sample preparation provides numerous advantages in terms of reduction in analysis time, fewer sources of error, more sensitive identification, and more accurate quantification. A number of extraction approaches have been proposed by food scientists (Crosby et al., 1981; Kolarovic and Traitler, 1982). One of the oldest and foremost methods is the saponification with alcoholic-KOH medium (Kazerouni et al., 2011). The coupling of extraction and clean-up methods was also employed in several other studies (e.g. Duedahl-Olesen et al., 2010). Food samples take a wide variety of physical forms, such as the solid biological matrices of meats and fats, liquid or solutions, and dried powders. Solid samples are usually treated by solid or liquid extraction and extraction methods must be accompanied by additional clean-ups or concentration steps (Ridgway et al., 2007). Liquid samples are commonly treated by liquid-liquid extraction or sorption methods.

Because they are lipophilic compounds, PAHs can reach inside the tissue cells, into non-polar regions, via a concentration gradient, rather than by diffusion (Simko, 2002). For this reason, simple extraction with non-polar solvents poses some difficulty. Due to their highly non-polar nature, PAHs tend to be

enriched in fatty foods, while they are found at relatively low concentrations in cereals, fruits, and vegetables. In the case of cooked foods (e.g. meat and sausages) that contain many heterocyclic compounds, highly selective approaches are needed for the determination of analytes present (Mottier et al., 2000). It was found that only 30% of PAHs were extractable with methanolic KOH, while 60% of PAHs were brought out from meats using alkaline hydrolysis (Simko, 2002). Pressurized liquid extraction is one of the more successful methods being used. Numerous combinations of non-polar solvents (pentane and hexane) or solvents with slight polarity (such as DCM) have been used for the extraction of PAHs (Carabias-Martinez et al., 2005). However, liberation of other lipids along with the extracted PAHs was a major drawback of this method. Therefore, clean-up in the form of acid treatment or saponification is the most important step for the removal of lipids. In addition, as acid treatment involves a destructive process, researchers have also looked for non-destructive options to facilitate the recovery of analytes to some extent.

The introduction of carbon-based sorbents favorably removed or improved the clean-up steps through fractionation of the aromatic compounds. A number of retainer sorbents (florisil, silica gel, and alumina) were later tested for absorption of PAHs in high-lipid foods (Gomez-Ariza et al., 2002). Researchers also observed that PAHs present on the surface of foods required another treatment method such as solid-liquid extraction (solid phase extraction and super critical fluid extraction) for concentrated accumulation of analytes. SFE was helpful for the rapid extraction of organic analytes from food products (Hawthorne et al., 1988). Therefore, the selected extractions were able to lessen or reduce the disadvantages of lengthy clean-up steps, while decreasing the sample analysis time and requiring less labor.

Matrix-solid phase dispersion is a popular extraction method, particularly for liquid foods (Bogusz et al., 2004; Pensado et al., 2005). Likewise, researchers also relied on headspace solid-phase microextraction (HC-SPME) (Aguinaga et al., 2008; Ishizaki et al. 2010, Kumari et al., 2013) and SPE for such purposes (Naccari et al., 2011). In the case of solid foods, there is supercritical fluid extraction (SFE) (Zougagh et al., 2004; Veyrand et al., 2007) as well as pressurized liquid extraction (Houessou et al., 2007; Veyrand et al., 2007). Recently, microwave-assisted extraction has also been adopted for the determination of PAHs in smoked fish (Ghasemzadeh-Mohammadi et al., 2012), charcoal-grilled chicken (Farhadian et al., 2011), horse mackerel (Ramalhosa et al., 2012), and miscellaneous seafood (Zhang et al., 2010). These extraction techniques helped reduce the time in the procedures required for clean-up. The main sample purification techniques are SPE, column chromatography, and gel permeation chromatography (GPC), which has been commonly used for separating interfering mixtures from food samples. Currently, GPC is one of the most widely used techniques for PAH extraction (Ballesteros et al. 2006; Suchanová et al., 2008).

4. Treatment and analysis for determination of PAHs

4.1 Treatment of PAHs for GC-based analysis

The various analysis routes for plant- and meat-based PAH contamination are depicted in **Table 2**. PAH determination consists of 3 steps, which are (i) extraction, (ii) clean-up or purification, and (iii) detection. GC is the most commonly applied and popular technique after LC-MS for analysis of PAHs in all types of food samples. The GC-MS technique has commonly been recommended in many official methods for the analysis of PAH (Plaza-Bolaños et al., 2010). Due to the superiority of GC over LC in terms of resolution, GC is convenient for defining structural information in combination with mass spectra. GC-MS can also be applied to PAHs with less (NPH, ACY, and ACP) or no (CS and Ccdp)

fluorescence properties (Plaza-Bolaños et al., 2010). In addition, GC-MS employs the usage of single-step injection whereas LC-FL involves multi-reinjection for confirmation (Plaza-Bolaños et al., 2010).

For the GC-MS, the fragmentation of PAHs is not easy to achieve due to their stability. Hence, the MS-MS fragmentation technique was developed to allow the recognition of the specific product ions for the heavier PAHs by taking advantage of the high voltage generated from the collision of the gas cells. The GC-MS-MS can be used to overcome the shortcomings in the quantitation of heavier PAHs that tend to co-elute with other similar compounds. This detection technique removed the problems of signal noise interferences of lipid substances, while improving the separation of heavier PAHs (FA and PYR) with the improvement of chromatographic properties. The usage of high resolution gas chromatography (HRGC-MS) also offers the advantage of low fragmentation of PAH molecules, leading to higher yields of molecular ions. Therefore, for the analysis of PAHs with numerous isomers and a tendency to co-elute (e.g., dibenzopyrene), HRGC-MS is an appropriate solution.

4.2 Treatment of PAHs for HPLC-based analysis

In addition to GC-based analysis, analytical methods commonly recommended and employed for the detection of PAHs are HPLC-coupled with fluorescence detector (FLD) along with MS (**Table 3**). Liquid chromatography in combination with some other detectors, like ultra-violet visible and photo-diode array, has also been used previously. However, FLD is considered the first choice option due to its superior specificity and sensitivity (Dost and Ideli, 2012; Nasr et al., 2010; Ramalhosa et al., 2012). In fact, other detecting options tend to be subjected to interference by the complex matrices of food components (Plaza-Bolaños et al., 2010). With the introduction of LC-FLD, an analysis technique with high selectivity and sensitivity has been extended to a wider variety of targets in food matrices.

(Barranco, 2004; Ramalhosa et al., 2009). The seven PAHs found in pork meat were determined by LC-FLD coupled with tandem SPE clean-up, through which targets were isolated from the meat matrix with improved sensitivity (Janoszka, 2011). Farhadian et al. (2010) also reported on the analyses of three PAHs (FLR, BbF, and BaP) in Malaysian grilled meat (chicken and beef satay) using LC-FLD after homogenizing the sample in cold NaOH solution for 3-6 hr before coupling with SPE.

The SPE-based analysis of a single PAH, BaP, in Korean smoked foods was also reported (Cho and Shin 2012). Furthermore, Ishizaki et al. (2010) reported on the use of SPME in dried vegetables and determined the 16 PAHs present by LC-FLD with SPME pretreatment with increased sensitivity. However, in order to make the pretreatment less tangible, microwave-assisted extraction was also investigated with LC-FLD. Ramalhosa et al. (2012) reported on an optimized and sensitive method for the determination of 18 PAHs in fish (horse mackerel, chub mackerel, and sardine) using microwave-based extraction coupled with LC-FLD. A similar extraction technique was followed by Farhadian et al. (2011) for analyzing charcoal-grilled beef. Furthermore, Zhang et al. (2010) investigated microwave extraction coupled with LC-FLD for assessing the PAHs in a variety of seafood samples. Microwave extraction is generally advantageous because of its usage of a low amount of solvent. Similar studies have also been applied to some seafood, like mussels (Serpe et al., 2010), and commercial milk (Kishikawa et al., 2003).

In considering techniques used for analysis of PAHs, time consumption is a major factor that needs to be minimized for the facile assessment of a maximum number of compounds present in the food sample. Considering this factor, the feasibility of using other methods (e.g. quetchers, soxhlet extraction, and SPE) with LC-FLD also needs to be evaluated. Ramalhosa et al. (2009) reported on quetchers-based

extraction for seafood (fish). Although LC-FLD is remarkably superior over LC-UV detection, a demerit of the former is its insensitivity to isotopically labeled compounds (Dost et al. 2012; Ramalhosa et al., 2012), the latter is not limited in such respect. As a result, LC-FLD is not recommended for the identification of indigenous PAHs.

In light of such limitations of FLD, a mass spectrometer is a potent alternative that can be used to resolve various issues. The enhanced sensitivity of MS has been a major advantage as proven in the analysis of milk samples of raw, pasteurized, and ultra-heat-treated varieties (Naccari et al., 2011). 18 PAHs in seafood (salmon), steak, and pork chop were detected with LCMS (Gosetti et al., 2011). The issue of sensitivity has been resolved by the association of LC with MS while the matter of interference due to the complex matrices of the food and complications arising from multiple injections have been overcome considerably with the progress made in the GC-MS technique in this field (Houessou et al., 2006; Kumari et al., 2013).

5. Quantification methods of PAHs

5.1 GC-based methods

5.1.1 GC-based detection of PAH in plant-based foods

Plant-based foods are an important part of human nutrition. PAHs are significant contaminants that enter foods either through processing methods or absorption in growing sites via human activities (Culcotta et al., 2002; Culcotta et al., 2005; Gianguzza and Orecchio, 2006). Humans are never exposed to single PAH by consumption of food but rather, they are exposed to PAHs in the form of complex mixtures. This feature complicates the analysis of PAH and hinders the interpretation of their long-term consequences on human health. In an effort to overcome these complexities, quantitative approaches for

measuring PAHs have been improved substantially, especially in the GC method, to allow for their analysis in various food materials (Wenzl et al., 2006) (**Table 4A**).

Among the various plant foods, cereals and grains occupy an essential space. Orecchio and Pappuza, (2009) assessed the PAHs in baked bread employing GC-MS via LLE. The DL was found to be in a range of values <0.015 ng/g (or 0.01 pg in mass (IV = 1 μL)). Roasted bread was also analyzed using GC-MS with soxhlet extraction to yield a DL of PAHs (BAP, CHR, BAA, BKF, BBF, B(ghi)P, DB(ah)A, IP, ACP, ANT, FA, FLR, NPH, PHE, and PYR) in the range of 0.5-5 ng/g (Al-Rashdan et al., 2010) (**Table 4B**). Similar to cereals, vegetable oils and seeds is also an indispensable part of the human diet and are a potent source of PAH entry into human metabolism. Hossain and Salehuddin (2012) detected various PAHs (BAP, CHR, BAA, ANT, FLR, NPH, PHE, and PYR) in three types of edible oils (soybean oil, mustard oil, and coconut oil) using the coupling of LLE and GC-MS. The DLs measured in terms of absolute mass were between 1.9 and 2.5 ng. Olive oil was also reported to have BAP with a DL of 1.6 ng/g using GC-MS with SPE (1.6 pg (in terms of mass) with an IV of 1 μL or 8 pg (in terms of mass) with an IV of 5 μL) (Bogusz et al., 2004). In addition, Ballesteros et al. (2006) also evaluated the PAHs in olive oil using GC-MS preceded by solvent extraction. They reported that the DL was in the range of 0.05-0.07 ng/g (as 0.35-0.49 pg in terms of mass with an injection volume (IV) of 7 μL) (**Table 4B**). The levels of PAHs in other vegetable oils, such as rapeseed oil, sunflower oil, grape seed oil, and sesame oil, were determined by coupling GC-MS and SPE (Fromberg et al., 2007) and were found to have DLs in the range of 0.2-1.5 ng/g (0.4 – 3 pg in terms of mass (IV 2 μL).

Different types of beverages are also consumed by all age groups throughout the world. Coffee and fruit juices rate at the highest levels of consumption. Orecchio et al. (2009) analyzed brewed coffee samples with GC-MS and SPE. The DLs of the detected PAHs (BAP, CHR, BAA, BKF, BBF, B(ghi)P,

DB(ah)A, IP, ACP, ANT, FA, FLR, and PHE) were in the range of 0.0009-0.01 ng/mL (at IV 1 µL). An effective study was also done with HRGC-HRMS and LLE on human milk, yielding a DL of 0.02-2.93 ng/g (0.4 – 58.6 pg in terms of mass (IV 20 µL) (Cok et al., 2012). Additionally, vegetables are an important part of the human diet. Mo et al. (2009) assessed the PAHs present in assorted vegetables (such as bean, spinach, and cabbage) through the combination of GC-MS and soxhlet extraction. They found that PAHs (ACP, ACY, and PYR) were present with DLs of 0.2, 0.1, and 1.4 ng, respectively (in terms of mass).

5.1.2 GC-based detection of PAH in meat-based foods

Meat products require attention in PAH assessment because meats have one of the greatest potentials for transfer of PAHs to humans. People of many different regions of the planet consume meat and seafood cooked in various ways, including smoked, grilled, barbequed, and processed forms. Numerous researchers have detected critical levels of PAHs with the use of GC-MS. Stumpe-Vīksna et al. (2008) determined the level of PAHs in smoked pork and achieved a DL of 0.1 ng by GC-MS and LLE. Ghasemzadeh-Mohammadi et al. (2012) determined the PAHs in smoked fish with GC-MS and DLLE and reported DLs in the range of 0.21-0.53 ng/g (0.3 – 0.79 pg in mass (IV = 1.5 µL)). Similarly, an assessment of Danish smoked fish was made by GC-MS and DLE (Duedahl-Olesen et al., 2010). They reported the DL of the PAHs to be 0.3-2.6 ng in terms of mass (depicted in **Table 4B**). Grilled foods assessed via a GC-MS and homogenization method yielded DLs between 170 and 490 µg (IV = 2 µL) (Alomirah et al., 2011).

Furthermore, Reinik et al. (2007) determined the PAHs in smoked and grilled meat (sausage, ham, and chicken) by the application of GC-MS and solvent extraction. They found the DLs to be in the range of 0.1-0.3 ng/g. PAHs in smoked mackerel, salmon, and herring were quantified by GC-MS (via solvent

extraction) and their DLs were reported as 0.2-0.34 ng/g (2 – 3.4 pg in terms of mass (IV 10 µL)) (Yurchenko and Mölder, 2005). Jira et al. (2008) also analyzed the PAHs in smoked meat (ham and bacon) using the coupling of GC-MS and ASE. They reported the DL as being 0.01 ng with (IV 1 µL). Some seafood, like salmon and mussel, were also analyzed by GC-MS and ASE, with the DLs being in the range of 1200-1700 ng/g (or 1.2-1.7 ng in terms of mass (IV = 1 µL)) (Liguori et al., 2006). One of the most preferred seafood products, tuna, was evaluated with MS-SIS (selected ion storage) and soxhlet extraction (Al-Omair and Helaleh, 2004) and found to have DL values for various PAHs (shown in **Table 4B**) in the range of 0.75-3 ng/mL (0.7 – 3 pg in mass) (IV = 1 µL). Similarly, marine food was also measured by GC-MS and soxhlet extraction and the MDL was in the range of 0.05-0.1 ng/g (Wan et al., 2007). Recently, Forsberg et al. (2011) evaluated the PAH levels in salmon using GC-MS and quetchers. They reported the DLs as 1-5 ng/mL (or 1 – 5 pg in mass) using an injection volume of 1 µL.

5.2 HPLC-based methods

5.2.1 HPLC-based detection of PAH in plant-based foods

HPLC has been considered the foremost tool for the determination of PAHs. To date, various methods have been demonstrated to satisfy the validation procedures set for the assessment of PAHs in food. HPLC also provides fast, simple, and non-destructive methods for the direct sensing of PAHs in food samples (**Table 5A**). In addition, detection with FLD enables the on-line monitoring of PAHs based on the incremented selectivity and sensitivity of fluorimetry for molecular absorption (Zougagh et al., 2004). Ciecierska and Obiedzinski (2013) analyzed the PAHs in bread using LC-FLD via solvent extraction. The DLs of PAHs ranged from 0.05-0.29 ng/g (**Table 5B**) (or 1 - 5.8 pg in mass (IV of 20 µL)). HPLC methods in earlier studies were mostly directed towards the evaluation of vegetable oils. The screening and identification of PAHs from olive oil was performed using the combination of LC-

FLD with SFE (Zougagh et al., 2004). They found PAHs (BAA, BBF, ANT, and PYR) with DLs of 14, 16, 12, and 16 ng/g, respectively (or 7, 8, 6, and 8 ng in mass, respectively) at IV of 500 µL.

Similarly, Teixeira et al. (2007) analyzed sunflower, soybean, and virgin olive oils by employing LLE with LC-FLD. They reported DLs of 0.09 and 0.004 ng/g for IP and BKF, respectively, or 1.8 and 0.08 pg in mass (IV of 20 µL). Also, Bogusz et al. (2004) detected PAH in olive oil using SPE and LC-FLD. They reported the DL for BAP as 1 ng/g or 10-20 pg in mass (IV of 10-20 µL). Furthermore, sensitive detection of LC-UV was also reported by Dost and Ideli (2012), who also measured BAP, BAA, BBF, and BKF with DLs of 0.46, 0.61, 0.31, and 0.47 ng/mL, respectively (or 23, 30.5, 15.5, and 23.5 pg in terms of mass, respectively) at an IV of 50 µL. LC-FLD was also employed combined with solvent extraction (Pandey et al., 2004; Yusty and Devina, 2005). Pandey et al. (2004) evaluated several edible oils (such as coconut, groundnut, linseed, mustard, olive, safflower, sesame, soybean, palm, and rice bran oil). They evaluated several PAHs (BAP, CHR, B(ghi)P), ACP, ANT, PHE, and PYR) with MDLs in the range of 0.1 - 4 ng/g 2 – 80 pg in mass (IV = 20 µL). Likewise, Yusty and Devina (2005) also isolated various PAHs (BAP, BAA, BBF, BKF, B(ghi), DB(ah)A, and IP) (**Table 5B**) in vegetable oils with DLs in the range of 0.07-0.67 ng/mL (3.5 – 33.5 pg in mass at IV = 50 µL).

In addition to its successful use in the analysis of oils, LC-FLD showed remarkable detectability for PAHs in beverages as well. Kishikawa et al. (2003) assessed milk (skimmed milk, condensed milk, fortified milk, and whole milk) by employing LC-FLD with saponification (SPFN). They found the PAHs to have DLs in the range of 0.001-0.03 ng/g (or 0.02 – 0.6 pg (IV = 20 µL)). Similarly, Naccari et al. (2011) analyzed the heated milk (raw, pasteurized, ultra-high treated (UHT) semi-skimmed, and whole milk) using LC-FLD with SPE. They found the DLs to be in the range of 0.01-0.2 ng/g (for CHR, BAA, BKF, B(ghi)P, ANT, PHE, and PYR) with 1 - 20 pg in terms of mass (IV = 100 µL)

(**Table 5B**). In addition, sugarcane juice was also analyzed by Tfouni et al. (2009), who measured BAP, BAA, BBF, and BKF for DLs of 0.005, 0.009, 0.006, and 0.004 ng/g, respectively, or 0.1, 0.18, 0.12, and 0.08 pg, respectively in mass (IV = 20 µL). Similarly, Tfouni and Toledo, (2007) analyzed cane sugar with LC-FLD in association with homogenization. They found the DL to be in the range of 0.01-0.17 ng/g (or 0.3 - 5 pg in mass (IV = 30 µL).

5.2.2 HPLC-based detection of PAH in meat-based foods

Evidence indicates that large amounts of PAHs are formed in meat and meat products when cooked over grills, charcoal chips, or in the oven (Janoszka., 2011). The matter of investigating carcinogenic or mutagenic PAHs has been on the agendas of many regulatory organizations and researchers. Therefore, LC-FLD methods have been adopted for use on a large scale in order to confirm the effectiveness of the methods in isolating the mutagenic and carcinogenic PAHs. Farhadian et al. (2011) investigated the levels of PAHs in charcoaled-grilled meat by coupling SPE and LC-FLD. They reported DLs in the range of 0.01-0.03 ng/g (or 0.2-0.6 pg in terms of mass (IV of 20 µL)). Pork meat was also assessed with a similar combination of LC-FLD and SPE (Janoszka., 2011). The DLs were reported as 0.0005-0.001 ng/column (or 0.5-1 pg/column with an IV of 20 µL). Cooked meat products, such as grilled beef, kebab, and chicken were analyzed by Farhadian et al. (2012). They reported BKF and BAP with DLs of 10 and 30 pg/g (or 0.2-0.6 pg in mass with an IV of 20 µL). Furthermore, seafood exhibited trends of contamination somewhat similar to those of meats, such as having either environmental or cooking-generated PAHs. Seafood from the Gulf of Naples (Italy) including mussel, shrimp, mackerel, and squid were analyzed by employing saponification and FLD (Perugini et al., 2007). The DLs were found to be in the range of 0.01-0.21 ng/g (**Table 5B**) (or 0.2 - 4 pg in mass (IV = 20 µL)).

Likewise, Zhang et al. (2010) measured PAHs from marine organisms (fish, crab, and shrimp) using microwave-assisted extraction (MAE) with FLD. They evaluated the DLs as being in the range of 0.004 – 0.03 ng/mL (or 0.08-0.6 pg in mass (IV = 20 µL)). Ramalhosa et al. (2012) also analyzed Pelagic fish via MWAE and FLD and found that the BKF had a DL of 0.04 ng/g (with no information on IV). The PAH level of mussels was also measured by FLD with solvent extraction (Serpe et al., 2010). They reported that the PAHs (BAP, CHR, BAA, BBF, BKF, and DB(ah)A) had DLs of 0.2-0.8 ng/g (or 4 - 16 pg in mass (IV = 20 µL)). In addition, Ramalhosa et al. (2009) analyzed fish with the combination of quetchers and FLD. They detected DLs between 0.09 and 0.86 ng/mL (or 1.3-13 pg in mass (IV = 15µL)). Despite the remarkable and sensitive detection rendered by FLD, researchers also investigated the efficiency of UHPLC-MS. Gosetti et al. (2011) analyzed the PAHs in salmon, pork, and steak by coupling UHPLC -MS-MS with SPE and they found that the DLs were in the range of 0.02-0.76 ng/mL (or 0.4-15 pg in mass (IV = 20 µL)). Therefore, FLD and MS are more or less equally sensitive; however, MS sensitivity is superior in detection as well as providing more accurate qualitative information.

5.3 Bio-sensing methods

It was in the 1980s that environmental scientists developed the immunochemical methods for the detection of PAHs (Fukal, 1999). Currently, many commercial immunoassays are available for environmental applications. As such, a wide range of immunosensors is accessible as sensing assays for the determination of PAH analytes (Zhang et al., 2015). These assays take the form of potentiometric, amperometric, conductometric, fluorimetric, and UV detection. They aim to deliver quick detection with sensitive, selective, and reliable determination. The advantages of these assays over other analytical techniques are the miniaturization of the instrumental devices, non-tangible pre-treatment set-ups, the

small amount of testing sample, minimal consumption of organic solvents, omission of time-consuming sample preparation, and lack of extensive clean-ups, which all simplify the analysis.

5.3.1 Cytometric immunoassay

A recent method based on cytometric immunoassay was reported for use in wheat flour with detection limits of 800-1700 μg L^{-1} (Meimaridou et al., 2010). In this technique, color-encoded micro-beads were used to quantify BaP for the first time in a food product, although this technique has been commonly used for clinical diagnosis purposes. The BaP equivalents were found to be in the range of 96-100 μg L^{-1}, and these results were two times higher than those of the GC-MS (49.5 μg L^{-1}). The DL was reported as 0.3 μg L^{-1} (10 pg in mass (IV = 50 μL)).

5.3.2 Electrochemical immunoassay

In the domain of non-chromatographic sensing techniques, an approach based on bio-electrochemical immunoassay has been studied intensively. For such application, an enzyme-linked immunosorbent assay coupled with antibody-covered magnetic beads was employed for detection of PAHs in water samples (Lin et al., 2007). The voltametric profile was then used to monitor the change in analyte behavior with a DL of 50 pg mL^{-1} (or 5 pg in mass (IV = 100 μL) for horseradish peroxidase (HRP)-labeled PAH. As such, an electrochemical immunosensor can provide fairly high sensitivity for PAH detection. However, a number of issues (e.g., poor reproducibility of the antibodies and variable usage of methods for particular profiles of PAHs) remain to be addressed in order to further improve its applicability in food-based samples. Therefore, other methods are still necessary at this time. Although studies in quantification are still quite limited, more alternatives are likely to become available to increase the specificity of the targets for sensing.

5.3.3 Amperometric analysis

The use of an amperometric sensor for the analysis of PHE in tap water was reported using bovine serum albumin (BSA) coated with antigen (Fahnrich et al., 2003). The detection limit of the method was 0.25-23 ng/mL (or 25 pg - 2.3 ng in mass (IV = 100 μL) for PHE. However, it was not purely a PHE immunosensor and several other PAHs in the tap and river samples were also found to be bound together. Therefore, this type of sensor is not yet sufficiently reliable and can only be deployed as a field sensor for limited purposes.

5.3.4 Surface Plasmon Resonance (SPR) analysis

As a sensing technique, surface plasmon resonance (SPR) was employed for the detection of BaP with an MDL of 10 ppt-300 ppb (Gobi and Miura, 2004). The method was developed for use with the thin gold film sputtered glass plate as a chip for sensing application. The sensing of SPR appears to be superior, sensitive, and reusable in some senses, while lacking specificity for the simultaneous determination of multiple PAHs. As of now, the immunoassays for PAH analysis of food have been made in only a limited number of studies. The majority of the studies are, instead, based on testing the PAHs in environmental samples such as soil and water (Lin et al., 2007). Because all the analytical methods are different in their fundamentals, it is not practical to make direct comparisons of results from different media. However, the demand for rapid and sensitive methods will grow continuously for screening of different PAHs.

5.3.5 Sensors using novel materials

Recently, noticeable developments in materials science have been achieved, offering an excellent pathway for direct sensing techniques. Novel materials (such as nanomaterials, graphene, and metal organic framework (MOF)) are some great examples of these advances. These materials not only counter the drawbacks of conventional sensing techniques but also provide high practical utilities for sensing (Zhang et al., 2015). Although the use of these sensing approaches is still mostly limited to some environmental samples, they are likely to be expanded soon to food samples. For example, the use of zeolitic imidazole frameworks (ZIFs) developed for the novel bio-inspired polydopamine (PDA) method allowed the detection of six PAHs (FA, PYR, BAA, BAF, BAP, and DB(ah)A) in real samples like water or soil (Zhang et al., 2015). This MOF-based method exhibited excellent extraction efficiency for the six PAHs, with enrichment factors between 550 and 734, good linearity (10–5000 pg mL^{-1}), and low detection limits (0.5–5 pg/mL) in environmental samples (water and soil). Furthermore, its rates of recovery were seen in the range of 82.5–98.6% (Zhang et al., 2015).

Likewise, Wang et al. (2015) investigated a copper-centered metal-organic framework (Cu-MOF) for ultrasensitive and rapid electrochemical detection of bisphenol A (BPA) in plastic products. Relatively high sensitivity and selectivity of Cu-MOF were obtained by its large specific surface area and π–π stacking interactions. The electrochemical sensor exhibited a high sensitivity (0.2242 A M^{-1}) and low detection limit (13 nmol L^{-1} in less than 11 seconds) for BPA with a wide linear range from 5.0×10^{-8} to 3.0×10^{-6} mol L^{-1}. Furthermore, Cu-MOF-based sensors have rates of recovery in the range of 94.0–101.6% for practical applications (Wang et al., 2015). In addition, comparisons between different MOFs (HKUST-1, MOF-5, and MIL-53(Al)) were also carried out for the detection of parabens in environmental waters, cosmetic creams, and human urine (Priscilla et al., 2015). Accordingly, HKUST-

1 was found to be the most adequate due to its high affinity for parabens with low detection limits (0.1 μg/L) in a wide linear detection range of 0.5–147 μg/L (Priscilla et al., 2015).

6. Comparison of the QA performances among different approaches

It is widely agreed that quantitation of PAHs based on their accurate identification is the most crucial event for their analysis. Those techniques that enable the identification of numerous analytes in one injection of a small volume and at the same time provide reliable reproducibility in the method are the most desired. Because food and environment are the greatest biological elements for human survival, detection of contaminants such as PAHs in both of these systems are considered the topmost priority. Knowing that direct assessment of PAHs in biological matrices like food is more complex, various pre-treatments in terms of extraction methods became indispensable tools for the preparation of samples to break down the complex structures of food to release PAHs in a free form. Approaches like bio-sensing (immunoassay and enzymatic assay) and electrochemical (amperometric/voltametric) sensing have opened new avenues for analytical methods by reducing the number of steps for sample preparation. However, these techniques still cannot compete with chromatographic sensing, which allows multiple and specific identification of targets. Furthermore, these techniques have not been firmly established in the sector of food. Indeed, in the domain of environment, specificity is a prominent issue.

Overall we have observed that GC-MS has outperformed other methods in detecting PAHs in food (both plant-based and meat-based) with sensitive detection limits. The minimum DL of PAH was in the picogram range (e.g., 0.35 - 5 pg in olive oil). This method was followed by the HRGC-HRMS for human milk (0.4 – 58.6 pg). In terms of meat-based products, GC-MS detection was made at 0.2- 0.3 pg (IV of 10 μL) for smoked seafood (mackerel, salmon, and herring) and 0.01 ng in smoked ham and

bacon with an IV of merely 1 μL. Although LC-FLD also showed remarkably high sensitivity (DL of 6 - 8 ng) for olive and vegetable oils, its IV was relatively larger (500 μL). LD-FLD also showed a lower range of detection of 0.9 – 20 pg in the case of heated milk (IV = 100 μL). Similarly, in the case of meat-based foods, DLs for some seafoods (e.g., mussel, shrimp, and mackerel) were 0.2 - 4 pg with an IV of 20 μL. Moreover, UHPLC-MS-MS had DLs in the range of 0.4 - 15 pg using an IV of 20 μL. Therefore, it is apparent that the sensitivity of PAH analysis achievable by GC-MS is highly comparable to that of LC-FLD/MS, although the latter generally requires moderately higher quantities of sample for detection.

In the domain of bio-sensing, bio-electrochemical immunoassay yielded a DL of 5 pg, using 100 μL of sample. However, in this sensing approach, detection of specific PAH was not possible. Furthermore, amperometric analysis exhibited a DL of 25 pg in tap or river water for the detection of a single PAH (PHE) with an IV of 100 μL. In the case of newly emerged novel materials (hybrids of metal organic frameworks (MOFs)), ZIFs recorded DLs in the range of 0.5-5 pg/mL for some limited environmental samples (water and soil). The sample volume was 100 μL, which was further diluted to 20 mL before analysis.

Overall, chromatographic techniques (GC and UHPLC) with advanced detectors (MS and FLD) are still the leading choices both for sensitivity of detection and minimal sample requirement. GC-MS provides all the merits of accurate detection and quantification in a single injection. In contrast, LC is lacking only in terms of requiring a relatively large sample volume, with extra steps required for sample filtration and preparation. In addition, the area of bio-sensing, including use of novel materials, shows some possibilities with remarkably low detection limits. However, they are still confined to the

environment-based samples along with the drawback of being comparatively inferior in their ability to distinguish different PAHs. Therefore, more studies are needed to bring about the revolution in food analysis with the application of bio-sensing methods. It is worth noting that the reliability of chromatographic techniques can be strengthened further if novel sensing materials are incorporated in their stationary medium. This modification will directly improve their sensitivity, while helping simplify pre-treatment of food samples by assisting in the first hand separation using hybrid materials. This type of approach can eventually facilitate the quicker detection of PAHs with a trace amount of sample.

7. Conclusion

In this review, a comprehensive comparison was made between the quantitative approaches employed for the detection of PAHs in various food samples. This presentation was aimed to provide the complete and clear-cut generalization of PAH quantification analysis in all categories of food products in association with all the up-to-date analytical techniques. We have reviewed the most significant and recent experimental applications developed for GC, HPLC, and other miscellaneous techniques with respect to PAH quantification in food.

In today's world, there is a need for techniques for the detection of lethal and mutagenic contaminants. These techniques must meet the general need for environmental friendliness, cost effectiveness, minimal time consumption, non-tangible sample preparation, and superior sensitivity/specificity. Therefore, the supremacy of chromatography can be strengthened further with the use of functionalized novel materials. Future research can provide breakthroughs in food analysis so that issues can be resolved with direct solutions.

Moreover, legal standardization authorities always look for sensitive methods for the accurate quantification of contaminants in food products. All types of food products are the primary sources of human survival. Therefore, precise analysis with sufficient sensitivity will help the food regulatory bodies to link contaminants like PAHs with clinical studies in relation to human consumption of food. Analytical detection is always the backbone of clinical studies aiming to locate the exact human impacts at the molecular level. Therefore, improved knowledge of the sensitive detection methods for PAHs in each particular food type would considerably help humans to avoid the risk associated with PAH consumption.

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Table 1. Summary of most frequently analyzed PAHs in food samples (US-EPA and EU)

Order	PAH	Abbreviation	Chemical formula	CAS number	Molecular mass (g/mole)	Structure
1	Benzo[a]pyrene	BAP[a]	$C_{20}H_{12}$	50-32-8	252	
2	Chrysene	CHR[a]	$C_{18}H_{12}$	218-01-9	228	
3	Benz[a]anthracene	BAA[b]	$C_{18}H_{12}$	56-55-3	228	
4	Benzo[b]fluoranthene	BBF[b]	$C_{20}H_{12}$	205-99-2	252	
5	Benzo[k]fluoranthene	BKFc	$C_{20}H_{12}$	207-08-09	252	
6	Benzo[ghi]perylene	B(ghi)P[c]	$C_{22}H_{12}$	191-24-2	276.33	
7	Dibenzo[a,h]anthracene	DB(ah)A[c]	$C_{22}H_{14}$	53-70-3	278	
8	Indeno[1,2,3-cd]pyrene	Ip[c]	$C_{22}H_{12}$	193-39-5	276	
9	Benzo[a]fluoranthene	BAF	$C_{20}H_{12}$	203-33-8	252.3	
10	Cyclopental[cd]pyrene	CPcdP	$C_{18}H_{10}$	27208-37-3	226.27	
11	Dibenzo[a,e]pyrene	DBaeP	$C_{24}H_{14}$	192-65-4	302.37	
12	Dibenzo[a,h]pyrene	DBahP	$C_{24}H_{14}$	189-64-0	302.37	
13	Dibenzo[a,i]pyrene	DBaiP	$C_{24}H_{14}$	189-55-9	302.37	

14	Dibenzo[a,l]pyrene	DBalP	$C_{24}H_{14}$	191-30-0	302.37	
15	5-methyl chrysene	MCH	$C_{19}H_{14}$	3697-24-3	242.31	
16	Acenaphthene	ACP	$C_{12}H_{10}$	83-32-9	154.08	
17	Acenaphthylene	ACY	$C_{12}H_8$	208-96-8	152.19	
18	Anthracene	ANT	$C_{14}H_{10}$	120-12-7	178.23	
19	Fluoranthene	FA	$C_{16}H_{10}$	206-44-0	202.26	
20	Fluorene	FLR	$C_{13}H_{10}$	86-73-7	166.22	
21	Naphthalene	NPH	$C_{10}H_8$	91-20-3	128.17	
22	Phenanthrene	PHE	$C_{14}H_{10}$	85-01-8	178.23	
23	Pyrene	PYR	$C_{16}H_{10}$	129-00-0	202.25	

[a]PAH2

[b]PAH4 + PAH2

[c]PAH8 + PAH4

Table 2. GC-based analysis of PAHs in plant- and meat-based foods

Order	Extraction		Clean-up/Purification	Detection	Food sample	References
Gas Chromatography						
A.	**Plant-based foods**					
1	Soxhlet	DCM, hexane	Soaked silica with DCM	GC-MS	Bread	Al-Rashadan et al., 2010
2	LLE	Hexane, cyclohexane, iso-octane, acetonitrile, acetone	Soaked silica with DCM/ SPE	GC-MS	Vegetable oils	Wu and Yu, 2012
3	LLE		Column chromatography	GC-MS	Baked bread	Orrechio and Papuzza, 2009
4	LLE	Diethyl ether + Pentane	Evaporation	GC-MS	Human milk	Cok et al., 2011
5	Sox-wave	Hexane-	Solid phase	GC-	Coffee	Orrechio et

		acetone	extraction	MS		al., 2009
6	Soxhlet		Sonication	GC-MS	Fruits, vegetables	Mo et al., 2009
7	Ultrasonication assisted extraction		Solid phase extraction	GC-MS	Herbal tea infusion	Schulz et al., 2013
8	Vortexing with Carbon nano-tube		Washing via Ultrasonication	GC-MS	Edible oils: peanut, soybean, rapeseed, olive, maize	Zhao et al., 2011
9	SPME			GC-FID	Carrot, radish, potato, cucumber, lettuce	Lei et al., 2011
10	SPME			GC-FID	Chocolates	Kumari et al., 2013
11	Solvent extraction		GPC + SPE	GC-MS	Vegetables oils	Fromberg et al., 2007
B.	**Meat-based**					

	foods					
1	Microwave assisted	ACN, hexane, acetone, KOH, EtOH)	Dispersive liquid-liquid extraction	GC-MS	Smoked fish	Ghasemzaeh-Mohammadi et al., 2012
2	Refluxing with KOH	Centrifugation	Solid phase extraction	GC-MS	Barbequed meat sausages	Mottier et al., 2010
3	LLE	Hexane, cyclohexane, iso-octane, acetonitrile, acetone	Saponification	GC-FID	Fish residues, mullet	Nasr et al., 2012
	LLE	N,N-dimethyl fomaldehyde + Cyclyohexane	SPE	GC-MS	Smoked meat	Stumpe-Viksna et al., 2008
4	Saponification	KOH	Rotary evaporation + Silica gel	GC-MS	Smoked/Grilled chicken	Alomirah et al., 2011

			chromatography			
5	Saponification	KOH:EtOH-Water	Silica gel chromatography + Rotary evaporation	GC-MS	Liver and kidneys of sheep, goat, cow	Husain et al., 1997
6	Accelerated solvent extraction	Hexane	GPC (Rotary evaporation + Nitrogen drying) +Silica gel chromatography	GC-MS	Smoked meat	Jira, 2004
7	Accelerated solvent extraction	Hexane + Acetone	Chromatographic size exclusion	HRGC-HRMS	Fish (Sardine, hake, tuna,shelfish), Ham, chicken, lamb	Martorell et al., 2010
8	Quetchers		SPE	GC-MS	Salmon	Forsberg et al., 2011

Table 3. HPLC-based analysis of PAHs in plant- and meat-based foods

Order	Extraction		Clean-up/Purification	Detection	Food sample	References
High Performance Liquid Chromatography						
A.	**Plant-based foods**					
1	LLE	Hexane, DMF, Water	SPE	HPLC-FLD	Edible oils	Teixeira et al., 2007
2	Ultrasonication extraction		SPE	LC-FLD + LC-MS	Infant cereals: rice, barley, corn maize	Rey-Selgueiro et al., 2009
3	Solvent extraction		Column-chromatography	HPLC-FLD	Sugarcane juice	Tfouni et al., 2009
4	Solvent extraction		GPC	HPLC-FLD	Bread	Ciecierska and Obiedzinski,

						2013
5	Solvent extraction		SPE	HPLC-FLD	Heated milk	Naccari et al., 2011
6	SPME			HPLC-FLD	Tea	Ishizaki et al., 2011
7	Saponification	NaoH + MeOH-Toluene	Rotary evaporation	HPLC-UV	Vegetable oils	Dost and Ideli, 2012
B.	**Meat-based foods**					
1	Soxhlet	KOH, MeOH	Sep-Pak-Florisil Cartridge	HPLC-UV	Meat product	Chen et al., 1996
	Soxhlet	Rotary evaporation		HPLC-FLD	Fish species	Dhananjayan and Murlidharan, 2012
2	Homogenization	KOH	SPE	HPLC-FLD	Grilled Meat	Farhadian et al., 2012
3	Accelerate	Hexane,	Evaporation	HPLC-	Smoked	Kpoclou et al.,

	d solvent extraction	acetone		FLD	shrimp	1996
4	Saponification	KOH, MeOH	Sep-Pak Florisil cartridge	HPLC-UV	Beef + Pork	Chung et al., 2011
5	Quetchers	Centrifugation	Filtration	HPLC-FLD	Fish	Ramalhosa et al., 2009
6	Microwave assisted extraction	Nitrogen stream dryness	SPE	HPLC-FLD	Fish, shrimp, crab	Zhang et al., 2010
7	Ultrasonication assisted extraction	Hexane	Nitrogen stream dryness	HPLC-FLD	Charcoaled-grilled pork	Veigas et al., 2014
8	Microwave assisted extraction	Acetone + Hexane	PTFE Filtration	HPLC-FLD	Sardine, chub and horse mackerel	Ramalhosa et al., 2012
9	Alkaline hydrolysis		Solid phase extraction + Column Chromatograph	HPLC-FLD	Pork meat	Janoszka, 2011

			y			
10	SPE		Centrifugation	UPLC-MS-MS	Salmon, pork steal	Gosetti et al., 2011
11	SPE	Dichloro methane	Rotary evaporation + ACN	HPLC-FLD	Charcoaled-grilled meat	Farhadian et al., 2011
12	LLE	Cyclohexane	SPE	HPLC-FLD	Mussel	Serpe et al., 2010

Table 4A. Basic information of DLs for PAHs in plant- and animal-based foods by the GC method (Refer to Table 4B for the detailed information with the matching reference ID number)

Ref No.	Food	DM[a]	IV[b] (uL)	Ext[c]	Location of study	
Plant-based						
	Cereals					
1	Baked bread	MS	1	LLE + CC	Italy	Orecchio and Papuzza, 2009
2	Roasted bread	MS	NA	Soxhlet	Kuwait	Al-Rashdan et al., 2010
	Oils					
3	Oils	MS	1	LLE	Bangladesh	Hossain and Salehuddin, 2010
4	Olive oil	MS	1.0-5	Vortexing + SPE	Riyadh	Bogusz et al., 2004
5	Vegetable oils	MS	2	GPC+SPE	Denmark	Fromberg et al., 2007
6	Olive oil	MS	7	Solvent ext + Rotary evaporator	Spain	Ballestros et al., 2006
	Beverages					
7	Human milk	HRMS	20	LLE	Turkey	Cok et al., 2011
8	Coffee	MS	1	SPE	Italy	Orecchio et al., 2009
	Vegetables					
9	Vegetables (bean,	MS	1	Soxhlet	Pearl River	Mo et al., 2009

	cabbage, spinach, mustard)				Delta, China	
Meat-based						
10	Smoked pork	MS	1	LLE + SPE	Latvia	Stumpe-Viksna et al., 2008
11	Smoked fish	MS	1.5	DLLE		Ghasemzadeh-Mohammadi et al. 2012
12	Smoked fish (salmon, herring, mackerel)	MS	1	PLE + GPC	Denmark	Duedahl-Olesen et al., 2010
13	Grilled and smoked foods	MS	2	Hom koh +Rot ev	Kuwait	Alomirah et al., 2011
14	Smoked and grilled meat	MS	NA	Solvent extraction	Tartu, Estonia	Reinik et al., 2007
15	Smoked fish, mackerel, salmon, herring	MSD	10	Solvent extraction + GC	Estonia	Yurchenko and Molder, 2005
16	Smoked meat (ham and bacon)	MS	1	ASE	Germany	Jira, 2008
17	Salmon and mussel	MS	1	ASE + GPC	Norway	Liguori et al., 2006
18	Tuna	MS-SIS	1	Soxhlet	Kuwait	Al-Omair and Helaleh, 2003

19	Marine foods (fish, crab, mullet)	MS	NA	Soxhlet	Bohai Bay, China	Wan et al,. 2007
20	Salmon	MS	1	Quechers	United States	Forsberg et al., 2011

DM[a] Detection method

IV[b] Injection volume

Ext[c] Extraction method

Table 4B. Comparison of detection limits (LODs) between different PAHs (in both concentration and mass terms) using GC

Order	**PAH**	**Reference ID**																			
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
	DL	**ng/g (pg)**	**ng/g**	**ng**	**ng/g (pg)**	**ng/g (pg)**	**ng/g (pg)**	**ng/g (pg)**	**ng/mL**		**ng/g**	**ng/g (pg)**	**ng/g**	**ng/g (ng)**	**ng/g**	**ng/g (pg)**	**ng/g**	**ng/g**	**ng/mL (pg)**	**MDL (pg/g)**	**ng/mL (pg)**
				Plant-based foods							**Meat-based foods**										
1	**BAP[a]**	<0.015 (0.5	0.5	2.5	1.6 (1.	0.2 (0.	0.05 (0	0.003 (0	0.00009		0.1	0.21 (0	0.4	6000000 (12	0.1	0.24 (	0.01	1700	3 (3)	60	1 (1)

		01)			6)	4)	.35)	.6)				.3)		00)		2.4)				
2	**CHR[a]**	<0.015(0.01)	0.5	2.3	_	0.3(0.6)	_	0.18(3.6)	0.00021		0.1	0.26(0.4)	0.3	9800000(1966)	0.2	_	0.01	1100	1(1)	60	1(1)
3	**BAA[b]**	<0.015(0.01)	0.5	1.9	_	0.4(0.8)	_	0.008(1.6)	0.00022		0.1	0.36(0.5)	0.4	9500000(19000)	0.2	ND	0.01	1100	0.75(7)	50	5(5)
4	**BBF[b]**	<0.015(0.01)	1	_	_	0.6(1.2)	_	0.006(1.2)	0.00009		0.1	0.41(0.6)	_	5000000(5000)	_	0.34(3.4)	0.01	1700	0.91(0.91)	100	1(1)

5	**BKF[c]**	<0.015 (0.01)	0.5	_	_	0.5 (1)	0.006 (0.42)	0.002 (0.4)	0.00009		0.1	_	0.3	3400000 (680)	_	0.34 (3.4)	0.01	1300	1.43 (1.43)	100	1 (1)
6	**B(ghi)P[c]**	<0.015 (0.01)	1.5	_	_	0.2 (0.04)	0.007 (0.5)	0.1 (2)	0.00021		0.1	0.48 (0.72)	0.5	4800000 (960)	0.2	0.35 (3.5)	_	1200	3.33 (3.33)	_	5 (5)
7	**DB(ah)A[c]**	<0.015 (0.01)	0.5	_	_	0.9 (1.8)	_	<0.02 (0.4)	0.00021		0.1	0.46 (0.7)	0.3	3900000 (780)	0.2	_	0.01	1700	4.2 (4.2)	60	5 (5)
8	**Ip[c]**	<0	1	_	_	0.	_	0.	0.		0	0.	_	46	0	0.	_	1	2.	5	1

		.015 (0.01)				3 (0.6)		05 (1)	0021		.1	46 (0.7)		0000 (920)	.3	2 (2)		300	3 (2.3)	0	(1)
9	**BAF**	_	_	_	_	_	_	_	_		_	0.46 (0.7)	_	_	_	_	_	_	_	_	_
10	**CPcdP**	_	_	_	_	_	_	_	_		_	0.46 (0.7)	_	_	_	_	_	_	_	_	_
11	**DBaeP**	_	_	_	_	_	_	_	0.0034		_	_	0.4	_	0.2	_	_	_	_	_	_
12	**DBah**	_	_	_	_	_	_	_	0.01		_	_	0.	_	_	_	_	_	_	_	_

	P								1				3							
13	**DBaiP**	_	_	_	_	_	_	_	0.006		0.1	_	0.3	_	_	_	_	_	_	_
14	**DBalP**	_	_	_	_	_	_	_	0.004		0.1	_	0.3	_	_	_	_	_	_	5 (5)
15	**MCH**	_	_	_	_	_	_	_	_		0.1	_	0.3	_	0.2	_	0.01	_	_	_	_
16	**ACP**	<0.015 (0.01)	4	_		1.5 (3)		<0.02 (0.4)	0.001	0.2		0.53 (0.79)	_	6000000 (1200)				460	0.25 (0.25)	20	_
17	**ACY**	<0.015 (0.	4	_		0.4 (0.		<0.02	0.00007	0.1		0.36 (0	0.4	7300000 14				470	_	20	1 (1)

		01)				8)		(0.4)				.5)		60)						
18	**ANT**	<0.015 (0.01)	2	2.5		0.2 (0.4)		0.2 (4)	0.001	_		0.22 (0.33)	0.8	71000 00 (1420)				690	0.19 (0.19)	30	5 (5)
19	**FA**	<0.015 (0.01)	2.5	_		1.2 (2.4)		0.64 (13)	0.001	_		0.28 (0.42)	1	99000 00 (1980)				_	0.2 (0.2)	30	1 (1)
20	**FLR**	<0.015 (0.01)	5	2.3		0.6 (1.2)		<0.02 (0.4)	0.001	_		0.25 (0.37)	1.2	72000 00 (1540)				620	0.2 (0.2)	20	1 (1)

21	**NPH**	_	3	2		_		<0.02 (0.4)	_	_		0.12 (0.18)	_	9400000 (1880)				_	0.1 (0.1)	_	1 (1)
22	**PHE**	<0.015 (0.01)	4.5	2		1.3 (2.6)		2.93 (58.6)	0.006	_		0.17 (0.25)	2.6	9700000 (1940)				400	0.1 (0.1)	50	5 (5)
23	**PYR**	<0.015 (0.01)	5	3.1		1.2 (2.4)		1 (20)	_	1.4		0.3 (0.45)	0.6	10000000 (2000)				680	0.2 (0.2)	40	1 (1)

[a]PAH2

[b]PAH4 + PAH2

[c]PAH8 + PAH4

Table 5A. Basic information on DLs for PAHs in plant- and animal-based foods analyzed by the HPLC method (Refer to Table 5B for the detailed information with the matching reference ID number)

Ref	Food	DM[a]	IV[b]	Ext[c]	Place of study	
Plant-based						
	Cereals					
1	Bread	FLD	20	Solvent + GPC	Poland	Ciecierska and Obiedzinski, 2013
	Oils					
2	Vegetable oils	FLD	500	SFE	Spain	Zougagh et al., 2004
3	Vegetable oils	FLD	20	LLE + SPE	Portugal	Teixeira et al., 2007
4	Olive oil	FLD	10.1-20	Vortexing + SPE	Riyadh	Bogusz et al., 2004
5	Vegetable oils	UV-Vis	50	SPFN	Turkey	Dost & Ideli, 2012
6	Vegetable oils	FLD	20	Solvent	India	Pandey et al., 2004
7	Vegetable oils	FLD	50	Solvent	Spain	Yusty and Devina, 2005
	Beverages					
8	Milk	FLD	20	SPFN	Japan	Kishikawa et al., 2003

9	Heated milk	FLD	100	Solvent + SPE	Italy	Naccari et al., 2011
10	Sugarcane juice	FLD	20	Solvent + CC	Brazil	Tfouni et al., 2009
11	Cane sugar	FLD	30	Homogenizer + CC	Brazil	Tfouni et al., 2007
Meat-based						
12	Pork meat	FLD	20-500	SPE	Poland	Janoszka 2011
13	Charcoal-grilled beef and chicken	FLD	20	SPE	Malaysia	Farhadian et al., 2011
14	Grilled meat, beef, kebab, and chicken	FLD	20	SPE	Malaysia	Farhadian et al., 2012
15	Salmon, pork, steak	UHPLC-MS-MS	20	SPE	Italy	Gosetti et al., 2011
16	Mussels, shrimp, mackerel, squid	FLD	20	SPFN	Gulf of Naples, Italy	Perugini et al., 2007
17	Fish, shrimp,	FLD	20	MAE	China	Zhang et al., 2010

	crab					
18	Pelagic fish	FLD	NA	MWAE	Atlantic ocean	Ramalhosa et al., 2012
19	Mussels	FLD	20	Solvent extraction + Sea Pak Cart	Campania coast, Italy	Serpe et al., 2010
20	Fish	FLD	15	QuEChERS	Portugal	Ramalhosa et al., 2009

DM[a]: Detection method; IV[b]: Injection volume; and Ext[c]: Extraction method

Table 5B. Comparison of detection limits (LODs) between different PAHs (in both concentration and mass terms) using HPLC

Order	PAH	Reference ID																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
		ng/g (pg)	ng/g (ng)	ng/g (pg)	ng/g (pg)	ng/mL (pg)	ng/g (pg)	ng/mL (pg)	ng/g (pg)	ng/g (pg)	ng/g (pg)	ng/g (pg)	ng/col (pg)	ng/g (pg)	pg/g (pg)	ng/mL (pg)	ng/g (pg)	ng/mL (pg)	ng/g	ng/g (pg)	ng/mL (pg)
		Plant-based foods											Meat-based foods								
1	BAPa	0.12 (1)	_	_	1 (10)	0.46 (23)	1 (20)	0.27 (13.5)	0.001 (0)	0.08 (8)	0.005 (0	0.1 (3	0.0005 (0.5	0.03 (	30 (0.	0.02 (0.4)	0.1 (2	0.004 (0.08	_	0.2 (4	0.17 (2.5)

		2.4)							.02)		.1)	)	)	0.6)	6)		)	)		)	
2	**CH Ra**	0.15(3)	_	_	_	_	0.3(6)	_	0.029(0.58)	0.09(9)	_	_	_	_	_	_	0.16(3)	0.0088(0.16)	_	0.8(16)	0.21(3)
3	**BA Ab**	0.05(1)	14(7)	_	_	0.61(30.5)	_	0.39(19.5)	0.002(0.04)	0.11(11)	0.009(0.18)	0.03(0.9)	0.0005(0.5)	_	_	_	0.12(2.4)	0.03(0.6)	_	0.2(4)	0.09(1.3)
4	**BB Fb**	0.	16	_	_	0.31	_	0.47	0.0	_	0.0	0.	0.00	0.	10	_	0.	0.04	_	0.	0.23

		1 (2)	(8)			(15.5)		(23.5)	11 (0.22)		06 (0.12)	01 (0.3)	05 (0.5)	01 (0.2)	(0.2)		05 (1)	2 (0.84)		8 (16)	(3.4)
5	**BK Fc**	0.1 (2)	_	0.004 (0.08)	_	0.47 (23.5)	_	0.07 (3.5)	0.001 (0.02)	0.006 (6)	0.004 (0.08)	0.01 (0.3)	0.00005 (0.5)	_	_	0.76 (15)	0.01 (0.2)	0.0004 (0.08)	0.04	0.2 (4)	0.13 (1.9)
6	**B(ghi) Pc**	0.15 (3	_	_	_	_	0.1 (2)	0.67 (33.5)	0.003 (0.6)	0.001 (1)	_	_	0.001 (1)	_	_	0.14 (2.8)	0.16 (3	0.02 (0.4)	_	_	0.09 (1.3)

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		)														)				
7	**DB (ah)Ac**	0.13(2.6)	_	_	_	_	_	0.46(23)	0.007(0.14)	_	_	0.17(5)	0.001(1)	_	_	0.33(6.6)	0.06(1.2)	0.013(0.26)	_	0.2(4)	0.35(5)
8	**Ipc**	0.28(5.6)	_	0.09(1.8)	_	_	_	_	0.014(0.28)	_	_	_	_	_	_	_	0.21(4)	_	_	_	0.13(1.9)
9	**BAF**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
10	**CP**	0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_

	cdP	.08(1.6)																		
11	**DBaeP**	0.29(5.8)	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
12	**DBahP**	0.16(	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_

		3.2)																			
13	**DBaiP**	0.13(2.6)	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
14	**DBalP**	0.3(6)	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0.46 (6.9)
15	**MCH**	0.0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0.2	_

		7 (1.4)																		(4)	
16	**ACP**	–	–			–	4 (80)		–	–			–	–		0.26 (5.2)		–	–		0.86 (13)
17	**ACY**	–	–			–	–		–	–			–	–		–		–	8.15		–
18	**ANT**	0.07 (1.	12 (6)			1.15 (57.5)	0.1 (2)		0.004 (0.08)	0.11 (11)			–	–		0.06 (1.2)		0.017 (0.34)	–		0.46 (7)

		4)																		
19	**FA**	_	_			0.67 (33.5)	_		_	_			0.002 (2)	0.02 (0.4)		0.29 (5.8)		0.004 (0.08)	_		0.1 (1.5)
20	**FLR**	0.13 (2.6)	_			0.7 (35)	_		0.011 (0.22)	_			_	_		_		0.003 (0.06)	_		0.12 (1.8)
21	**NPH**	_	_			_	_		_	_			_	_		_		_	_		1.4

																					(21)
22	**PHE**	0.06 (1.2)	_			0.38 (19)	0.1 (2)		0.03 (0.6)	0.091 (9)			_	_		0.1 (2)		0.07 (1.4)	_		0.03 (4.5)
23	**PYR**	0.08 (1.6)	16 (8)			0.26 (13)	2 (40)		0.008 (0.16)	0.2 (20)			_	_		0.26 (5.2)		0.14 (2.8)	_		0.01 (1.5)

[a]PAH2

[b]PAH4 + PAH2

[c]PAH8 + PAH4

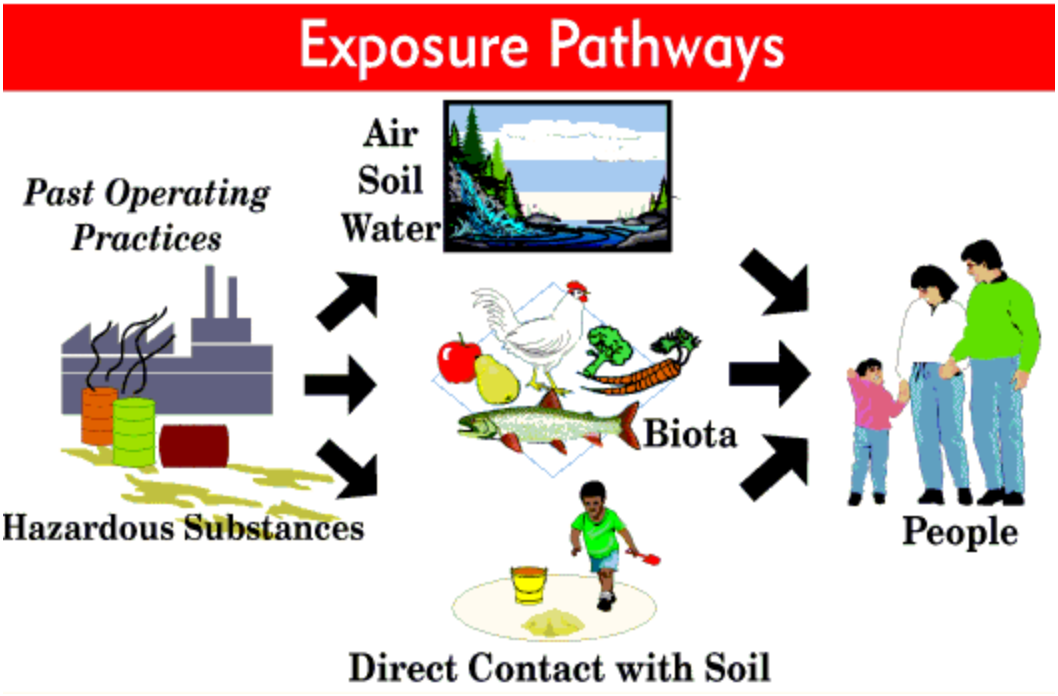

Figure 1. The channels of exposure of humans to PAHs (ATSDR-Report on toxic substances, 2011)

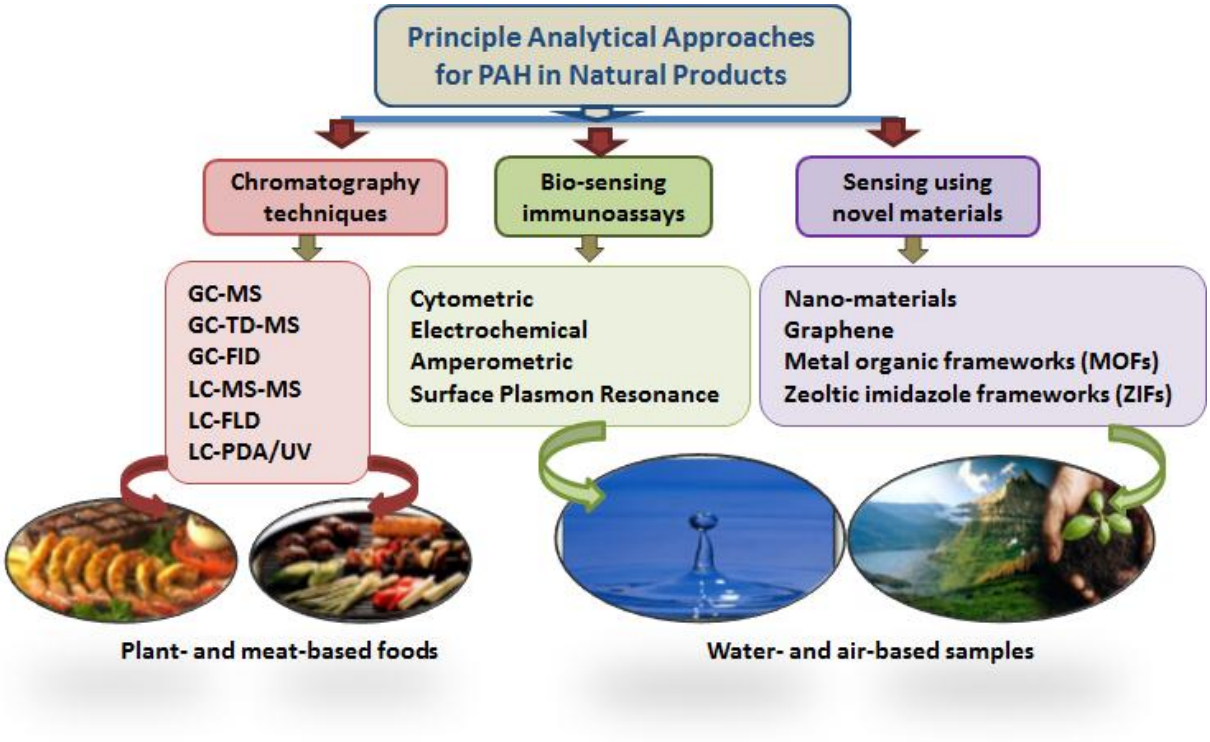

Figure 2. Classification of analytical approaches for PAH contained in various food samples