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REVIEW ARTICLE

POLYCYCLIC AROMATIC HYDROCARBONS IN FOOD SAMPLES: METHODS OF  
EXTRACTION – A REVIEW

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic pollutants with related to their structures and properties. Strong interest in the field of PAHs is mainly due to the large scale of environmental contamination and the mutagenic and carcinogenic activity of these pollutants which proved in many studies. Foodstuffs can be contaminated by PAHs that are present in air, soil and water or are formed during food processing and cooking. Extraction procedure is a key factor that affects the analysis of PAHs present and it is the most time consuming step during analysis. Choosing the suitable and effective extraction method for the right application requires a consideration of the features of the matrix and of the correct analytes. Suitable methods for extracting PAHs from different food samples have been highlighted in this review; the aim is to provide an overview of current knowledge and information in order to assess the need for further analysis and quantification of these toxic compounds in food.

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds that consist of at least two benzene rings without any substituents. They are ubiquitous pollutants in our environment and their importance appears from their toxicity and carcinogenicity to human. Strong interest in the field of PAHs is mainly due to the large scale of environmental contamination and the mutagenic and carcinogenic activity of these pollutants which proved in many studies. Exposure to PAHs can occur by drinking contaminant, eating foods that have been grilled, and by breathing air contaminated by coal tar or wild fire or even by incense burning (Hiba *et al.*, 2015; Hiba and Nimir, 2015). More than 100 PAHs have been characterized in nature, 16 of which were classified as priority pollutant (ATSDR, 1995). Because of their lipophilic property, PAHs show high affinity for organic matter and their determination requires powerful extraction techniques. This article focuses on the suitable extraction methods of PAHs from different food samples published by different authors, since these are the most time consuming steps during analysis.

Presence of PAHs in food

Foodstuffs can be contaminated by PAHs that are present in air, soil and water or are formed during food processing and

cooking. PAHs have been detected in seafood, smoked foods and broiled meat (Menichini and Bocca, 2003). Number of factors can affect the amount of PAHs in food, those include: time, fuel used, distance from the heat source and drainage of fat, type of cooking (grilling, frying, and roasting) (Phillips, 1999). A comparison of PAHs levels in duck breast steaks, undergoing various processing and cooking treatments for 0.5 hour to 1.5 hours, showed that charcoal grilled samples without skin contained the highest amount of total PAH (320 µg/kg), followed by charcoal grilling with skin (300 µg/kg), smoking (210 µg/kg), roasting (130 µg/kg), steaming (8.6 µg/kg) and liquid smoke flavouring (0.3 µg/kg). The highest amounts of PAHs were observed after smoking of duck breast samples for 3 hours (53 µg/kg) (Chen and Lin, 1997). Different extraction techniques have been used for PAHs analysis such as solid-liquid extraction, soxhlet extraction and sonication, in addition to modern instrumental methods such as pressurized fluid extraction, automatic soxhlet extraction and supercritical fluid extraction (Dariusz and Marzena, 2014).

Extraction of PAHs from Toasted bread and flour

Concentrations of PAHs in flour and baked bread samples were evaluated (Kobayashi *et al.*, 2008; Tuominen *et al.*, 1988). In the extraction procedure, the bread samples were ground, threshed, and stored in amber glass bottles with Teflon-lined caps at -20°C until extraction. The samples were Soxhlet extracted with mixed solvent i.e hexane: dichloromethane (1:1)

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for 16 h followed by clean up procedure prior to analysis (Amal *et al.*, 2010; Dennis *et al.*, 1991).

### Extraction of PAHs from Chocolate candies

Chocolate is one of the main food ingredient and it is favored among most people especially children. The most PAHs pollutant that has been found in chocolate is benzo (a) pyrene (BaP) (Dennis *et al.*, 1991; Lodovici *et al.*, 1995). A high concentration of (BaP) of 10 µg Kg<sup>-1</sup> was reported in a sample of bitter chocolate (Warentest, 2007). In the extraction procedure, Three grams of chocolate candy sample was diluted with 30 mL of de-ionized water and the resulting solution was homogenized by shaking for 12 h. The diluted sample was extracted 3 times with 5 mL n-Hexane, followed by addition of 0.5 mL of ethanol for de-emulsification. The extract obtained was dried under nitrogen flow and re-dissolved in 2 mL of cyclohexane then purified for PAHs analysis (Rupender *et al.*, 2012).

### Extraction of PAHs from tea sample

Tea leaves may accumulate PAHs especially from air (Lin and Zhu, 2004) or during production process of tea leaves, because in some of them combustion gases from burning wood, oil or coal are used to dry the leaves (Lin *et al.*, 2005). To extract PAHs from tea samples, 0.5 g of tea sample were weighted, 15 ml of hexane were added and then the mixture was mixed in a vortex for 20 s. It was sonicated for 30 min at 35 °C, and then centrifuged at 3000 r.p.m for 10 min. The extract was partially evaporated in a rotator evaporator at 55 °C, The extract was evaporated to an approximate volume of 3 ml and filtered through a nylon filter of 0.45 mm. The filtrate was washed three times with 0.75 ml of n-hexane and taken to a thermostat bath at 36 °C with nitrogen and reduced to an approximate 1 ml volume followed by clean up procedure prior for PAHs analysis (Victor *et al.*, 2015; Pincemaille *et al.*, 2014).

### Extraction of PAHs from milk sample

Different investigations of the presence of PAHs have been done in milk and dairy products (Grova *et al.*, 2002; Bulder *et al.*, 2006). Acenaphthene, phenanthrene, fluoranthene, pyrene and chrysene were detected in milk samples, but the more heavy PAHs were not present at the levels above the detection limit of 0.1 ng/g fat (Yabiku *et al.*, 1993). In the extraction procedure, milk samples were stored at - 25 °C and thawed in a water bath at 37 °C for 5 min just prior to the analysis. Two grams of milk sample was saponificated with 4.0 ml of 0.4 M sodium hydroxide in EtOH:H<sub>2</sub>O (9:1, v/v) at 60°C for 30 min. The resultant solution was extracted twice with 2.0 ml of n-hexane, the n-hexane phase was evaporated to dryness and the residue dissolved in 100 µl of acetonitrile was passed through a membrane filter (0.45 µm) and the aliquot was analyzed (Kishikawa *et al.*, 2003; Mohd *et al.*, 2012; Abou-Arab *et al.*, 2014).

### Extraction of PAHs from fish sample

Monitoring of PAHs inputs and their fluxes and fate, particularly in an aquatic environment, have been initiated (Ja'nska' *et al.*, 2006; Baoliang *et al.*, 2004). PAHs concentrations ranging from 0.01-200µg/kg have been reported

by different workers in smoked fish and meat products using different extraction and instrumental methods (Ajai *et al.*, 2010; Wretling *et al.*, 2010). The results also show that the accelerated solvent method was more efficient for the extraction of PAHs from fish species than the Soxhlet and solid-liquid extraction method. In this method, prior to extraction, silica gel was activated by oven-drying for 24 hours at 130°C. Concentrated H<sub>2</sub>SO<sub>4</sub> acid was then added to the silica gel (1:1v/v) and the mixture shaken vigorously. The mixture was then stored at room temperature prior to use. The extraction cell was prepared and then tightly packed with 0.5g of sand and 6.5g of activated silica gel.

The cap of the extraction cell was temporally removed and 50cm<sup>3</sup> of dichloromethane was passed over the column for conditioning. Then the cell was packed with 5g of dried, ground and well homogenized fish sample followed by 0.5g sand and finally with cellulose filter before capping the cell. The cell was placed into the carousel for extraction. 20 cm<sup>3</sup> dichloromethane was then introduced into the extraction cell in the carousel to extract the PAHs in the fish sample. The operating temperature and pressure of the setup was then programmed to 160°C and 2000 psi respectively, and the sample heated by direct contact with the oven. The extraction was achieved by direct contact of the sample with the hot solvent in both static and dynamic modes for 5 minutes. Compressed nitrogen gas was finally used to purge the extract into a collector vial, capped and stored in a refrigerator prior to clean up and analysis (Ajai *et al.*, 2012).

### Extraction of PAHs from meat sample

A high level of PAHs was found in processed food such as roasting, grilling and smoking (Olabemiwo *et al.*, 2011). Grilling meat, fish or other food with intense heat over a direct flame results in fat dripping on the hot fire and yielding flames containing a number of PAHs (Agerstad and Skog, 2005). In the extraction procedure, the sample was grounded in a meat grinder to ensure homogenization. Five grams of the homogenized sample was thoroughly mixed with 10g of anhydrous sodium sulfate in a mortar to absorb moisture and Soxhlet extracted with 50 mL mixture of n-hexane:dichloromethane (3:1v/v) for 10 h, the crude extract was filtered through a layer of anhydrous sodium sulphate. The obtained filtrate was evaporated to near dryness prior for cleanup and analysis process (Ojeyemi *et al.*, 2013).

In the other method of extracting PAHs from meat and fish samples which have been applied by different authors, homogenized samples were hydrolyzed with the solution of potassium hydroxide in ethanol for 2 hours in 40°C, then filtered and extracted with cyclohexane. The cyclohexane solution was washed with water and then with a mix solution of methanol/water (4:1v/v). The cyclohexane liquid extraction with N,N-dimethylformamide/water (9:1v/v) solution was used. Afterwards, repeatedly combined DMF layer extraction with cyclohexane and extract evaporating by rotation evaporator and diluting in cyclohexane. The end stages of analysis were extract purification using a Silica SPE column, evaporating and diluting in phase (acetonitrile) (Mičulis *et al.*, 2011; Marta Obiedzinski, 2007; El Badry, 2010).

## Conclusion

Numerous PAHs are carcinogenic, making their presence in food and environment a health concern. Processing procedure of food is commonly thought to be the major source of contamination by PAHs. Extraction procedure is a key factor that affects the analysis of PAHs present and it is the most time consuming step during analysis. It is performed to release the contaminant from samples and quantitatively transfer them to another medium. Choosing the suitable and effective extraction procedure for the right application requires a consideration of the features of the matrix and of the correct analytes.

Suitable methods for extracting PAHs from different food samples have been highlighted in this review in order to provide information for further analysis and quantification of these toxic compounds in food.

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