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Characterization of Polycyclic Aromatic Hydrocarbons (PAHs) Present in Smoked Fish from Ghana

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Abstract: The study was conducted to determine the levels of Polycyclic Aromatic Hydrocarbons (PAH) in smoked *Scomba japonicus* sampled from some Ghanaian markets. By way of preparation, smoked fish comes into contact with smoke or extremely high temperature which are potential sources of PAH generation. Levels of 20 individual PAHs including acenaphthene, acenaphtyelene, anthanthrene, anthracene, benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(e)pyrene, benzo(ghi)perylene, benzo(j)fluoranthene, benzo(k)fluoranthene, chrysene, cyclopenta(cd)pyrene, dibenzo(ah)anthracene, fluoranthene, fluorene, indeno(1, 2, 3-cd)pyrene, naphthalene, phenanthrene and pyrene were determined in 34 smoked fish samples using gas chromatographic techniques with flame ionization detector. Benzo(a)pyrene, which is one of the very few PAHs for which a legal limit exists in different types of food matrices inaddition to other high molecular weight PAHs suspected to be carcinogens, were detected in most samples.

Key words: Carcinogens, flame ionization detector, gas chromatography, polycyclic aromatic hydrocarbons, *Scomba japonicus*, smoking

INTRODUCTION

Polycyclic aromatic hydrocarbons or Polynuclear Aromatic Hydrocarbons (PAHs) are ubiquitous environmental contaminants that are formed during the incomplete combustion of carbonaceous materials (Suchanová *et al.*, 2008). PAHs are characterized by such properties as lipophilicity, semi-volatility as well as persistency.

Several of them have been found to induce a number of adverse effects as immunotoxicity, genotoxicity, mutagenicity and carcinogenicity (IARC, 1983; Phillips, 1999; EFSA, 2002).

Grouped in three categories based on their molecular weights and these are low, medium and high. The PAHs belonging to a particular class of molecular weights have similar environmental fates.Low Molecular Weight PAHs (LMW) i.e., those containing two- and three-rings, are those with molecular weight from 152 to l78 g/mol e.g., phenanthrene. The medium (middle) molecular weight (MMW) PAHs, have four-rings and are those with molecular weight of 202 g/mol. This group includes fluoranthene and pyrene. The third class of PAHs based on molecular weights are those of High Molecular Weights (HMW). They contain usually five- to seven-rings and have weights ranging from 228 to 278 g/mol such as benzo(a)pyrene, dibenz(a,h)anthracene and indeno(1, 2, 3-c, d)pyrene (ATSDR, 1995).

The ubiquitous nature of PAHs makes them present as trace contaminants in air, water and soil (Pigini *et al*., 2006). Although air and drinking water may be responsible for some human exposure, the highest PAH intake is typically associated with their occurrence in diet (food) (Suchanová *et al*., 2008). Polynuclear aromatic hydrocarbons are found in substantial quantities in some foods, depending on the mode of cooking, preservation and storage, and are detected in a wide range of matrices; meats, fishes, vegetables and fruits. Those detected in vegetables have been attributed to the deposition of airborne-PAHs from such various sources as vehicular exhaust and those present in fish and mussels, from contaminated waters (Edwards, 1983; Nielsen *et al*., 1996). However, during industrial smoking, heating and drying processes, combustion products come into direct contact with food and PAH contaminations can occur.

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Cereals and vegetable oils (including seed oils and olive residue oils) have been found to be contaminated with these toxicants usually as a result of technological processes like direct fire drying, where the combustion products come into contact with the grain, oil seeds or the oil (Speer *et al*., 1990. SCF, 2008).

Food exposure to carcinogenic PAHs can be said to generally emanate either from their contamination during processing such as from roasting, smoking and charcoal grilling (Chen and Chen, 2005; Chen and Lin, 1997; Mottier *et al*., 2000; Ova and Onara, 1998; Phillips, 1999; Swallow, 1976) or from the external environment due to anthropogenic activities (Larsson, 1984; De Vos *et al*., 1990; Culotta *et al.,* 2002; Gianguzza and Orecchio, 2006). The highest concentrations of PAHs in food are usually found in charcoal grilled/barbecued foods (especially meat and meat products grilled under prolonged and severe conditions), foods smoked by traditional techniques (fish in particular) and other seafoods from polluted waters (Guillen *et al*., 1997; Phillips, 1999).

Food components, such as fats cause PAHs to be generated through either thermal degradation or polymerization and such different thermal processes affect production of polycyclic aromatic hydrocarbons quantitatively (Chen and Line, 1997, Chen and Chen, 2005; Phillips, 1999).

Smoking of food especially fish, is one of the most ancient technologies which has been used for years. It can be defined as the process of penetration of fish by volatiles resulting from thermal destruction of wood (Simko, 2002).

Traditional smoking involves treating of pre-salted, whole, eviscerated or filleted fish with wood smoke. The smoke is produced by smouldering wood and shavings or sawdust in an oven and is placed directly below the hanging fish or fillets that are laid out on mesh trays. The rate of smoke flow and its distribution depend on natural draft as affected by the construction of the kiln and by the weather conditions. However, in modern smoking, automatic smokehouses are used where the smoke is developed in an external generator under controlled conditions of temperature and air access. The smoke circulation is forced and controlled by mechanical equipment (Stolyhwoa and Sikorski, 2005).

In Ghana fish mongers employ the traditional smoking method of using various organic materials such as sugarcane chaff, saw dust, coconut husk, some herbs and some types of paper such as cement bags to generate smoke for fish smoking (Kleter, 2004). The direct exposure of fish to smoke brings about higher concentrations of polycyclic aromatic hydrocarbons in the fish as compared to the indirect methods, where PAHs are partially eliminated by condensation in tars (Roda *et al*., 1999). The highest concentration of polycyclic aromatic hydrocarbons in smoked products is obtained immediately after the smoking is done. The concentration decreases due to decomposition, triggered by light (photo) and interaction with other compounds also present in the environment (Dennis *et al*., 1984; Simko, 1991; Simko *et al*., 1991). However, PAHs also usually penetrate into such smoked products as fish, where they are protected from light and oxygen so after some time, the polycyclic aromatic hydrocarbon concentration in the fish stabilizes at a certain constant level (Simko and Knezo, 1992).

Smoked fish may contribute significantly to the intake of PAHs if such foods form a large part of the usual diet. Owing to the fact that, most homes consume smoked fish as a main protein source in their diet, it is imperative to study the various types and levels of polycyclic aromatic hydrocarbons ingested by the Ghanaian from the consumption of smoked fish.

MATERIALS AND METHODS

The research was conducted in the chemistry department of the Ghana Atomic Energy Commission and the quality control laboratory of the Tema oil Refinery, Ghana. The whole study spanned for six months beginning January 2009.

Fish samples and sampling: Similar sizes (≈ 250 g) of *Scomba japonicus* were purchased from four open markets, Ada, Chorkor, Madina and Winneba and labeled ADM, CKM, MDM and WBM respectively. Samples were taken every month for three months beginning January, 2009. Samples were bought from the fish mongers immediately the samples got to the market in the morning few hours after smoking had been complete. Samples were prepared immediately samples got to the laboratory to avoid ageing.

Reagents: Reagents used in the study comprised the following: n-hexane (95 + % purity, Sigma-Aldrich), dichloromethane and acetone (99.5 + %, BDH, England), Sodium sulfate (Aldrich- Chemie, Germany). A semi-volatile internal standard mixture, product code: ISM-560, Lot number -CB-2411 containing 2000 μg/mL each of analytes: Acenaphthene-d10, Chrysene-d12, Naphthlene-d8, Perylene-d12, and Phenanthrene-d10 in methylene chloride; p-terphenyl, product code: RAH-058, Lot number- NTO1690 with a concentration of 100 mg were all purchased from Ultra Scientific, North Kingstown, USA.

A PAH custom standard of product code CUS-9059 and Lot number CD3298, containing 100μg/mL each of analytes; naphthalene, acenaphthene, acenaphthylene, anthracene, benz(a) anthracene, benzo(a) pyrene,benzo(e) pyrene,bnzo(b)fluoranthene, benzo(j) fluoranthene, benzo(k)fluoranthene, chrysene, benzo(g,h,i)perylene, dibenz(a,h)anthracene, fluoranthene, fluorene, indeno (1, 2, 3-c, d) pyrene, phenanthrene, pyrene, cyclopenta

Fig. 1: Map showing sampling sites

(c,d) pyrene and anthanthrene were purchased from Ultra Scientific, North Kingstown, USA through their accredited agent Industrial Analytical (PTY) Ltd, South Africa.

Sample preparation for the analysis of smoked fish:
Soxhlet extraction: Three smoked fish samples were pooled and homogenized in whole (skin and muscle) without the bones, minced into smaller fillets and blended using a warring blender. Twenty grams (20 g) of the homogenised fish sample was thoroughly mixed with 60 g of anhydrous sodium sulfate in an agate mortar (Wang *et al*., 1999) to absorb moisture. The homogenate was placed into an extraction cellulose thimble (33 * 94 mm), covered with a Whatman filter paper (125 mm diameter) and inserted into a Soxhlet extraction chamber of the Soxhlet extraction unit. Extractions were then carried out with 200 mL of *n*-hexane using EPA 354ºC method (US EPA, 1994) for 8 h. The crude extract obtained was carefully evaporated using a Ribby RE 200B rotary vacuum evaporator at 40ºC, just to dryness. The residue was quantitatively transferred with *n*-hexane onto a 5 mL florisil column for clean up.

Preparation of florisil for clean up: This clean-up step to remove more polar substances was performed using activated florisil (Magnesium silicate) and anhydrous Na_2SO_4. The florisil was heated in an oven at 130ºC overnight (ca. 15 h) and transferred to a 250 mL size beaker and placed in a desiccator. A 0.5 g anhydrous Na_2SO_4 was added to 1.0 g of activated florisil (60-100 mm mesh) on an 8 mL column which was plugged with glass wool. The packed column was filled with 5 mL *n*-hexane for conditioning. The stopcock on the set up was opened to allow the *n*-hexane run out until *n*-hexane just reached the top of the sodium sulfate into a receiving vessel whilst taping gently the top of the column till the florisil settled well in the column. The extract was then transferred onto the column with a disposable Pasteur pipette from an evaporating flask. The crude extract was eluted on the column with the wide opening of the stopcock. Each evaporating flask was immediately rinsed twice with 1 ml portions of *n*-hexane and added to the column by the use of the Pasteur pipette. The eluate was collected into an evaporating flask and rotary evaporated to dryness. The dry eluate was then dissolved in 1 mL *n*-hexane for Gas Chromatographic analysis.

Instrumental analysis: The polycyclic aromatic hydrocarbon analysis carried out was by means of an Agilent Technologies 6890 N network gas chromatograph system, equipped with a Flame Ionization Detector (GC-FID) and operating in a selective split mode. The column used is a SLB5^{TM}- MS fused capillary column (30 m long ×0.25 mm i.d. ×0.25-µm film thickness), coated with a nonpolar stationary phase (HP-5MS, 5% phenyl methyl polysiloxane).

The operation conditions were as follows: The oven temperature was set initially at 60°C (2 min hold), increased to 170°C at 40°C/min. At 170°C, temperature increased at a rate of 10ºC/min to 220ºC and then to 290ºC at a rate of 5ºC/min (10 min hold).

Hydrogen with a purity of 99.999% and air, were used as carrier gas at a constant flow of 30 and 300 mL/min respectively. The inlet temperature was held at 300ºC with a pressure of 231.2 Kpa and a total flow of 208 mL/min. The detector heater was also held at 300ºC with H_2/air flow at 35 and 300 mL/min, respectively. Flow rate was 4 mL/min.

Injections of 4 µL of sample each were performed in the split mode and the split valve was opened after 2 min.

The split ratio was 50:1. Identification of PAHs in the samples was based on comparison of the retention times with those in a standard solution, and quantification on the corresponding areas of the respective chromatograms.

RESULTS AND DISCUSSION

Total concentrations: Figure 2, shows the total of 20 PAHs mean values (concentration) found in smoked fish samples from the various locations as shown if Fig. 1; Ada, chorkor, Winneba and Madina labeled ADM, CKM, WBM and MDM, respectively.

Organic materials such as sugarcane chaff, saw dust, coconut husk are usually used by some fish mongers for smoking while others use herbs and others use some types of papers such as cement paper bags (Kleter, 2004). Location Ada showed the least total PAH concentration. Fish samples from this area are landed and smoked there. Samples are not transported to any long distant place of smoking where there could be possible introduction of external PAHs. People in this area typically use Neem tree for firewood and some type of herb locally known as "*hwadzi*" for their smoking. It is one herb that is believed to give the smoked fish its brown colour. Location Chorkor has a total mean of 20 PAH concentration to be 75.48 µg/kg. This is an area where the local dwellers use sugar cane chaff for smoking fish. Like the people in Ada, the men who land fish at Chorkor are local fisher folks and the fish smoking too is done in the same place. Fish samples from Madina gave a total mean PAH concentration of 175.08 µg/kg. Madina can be described as a landlocked area as such; the inhabitants in this locality get their fresh fish from the main fishing harbour. Unlike Ada and Chorkor, fish mongers in this area use very different types of wood for firewood including Neem tree and sawdust for smoke generation in the fish smoking business.

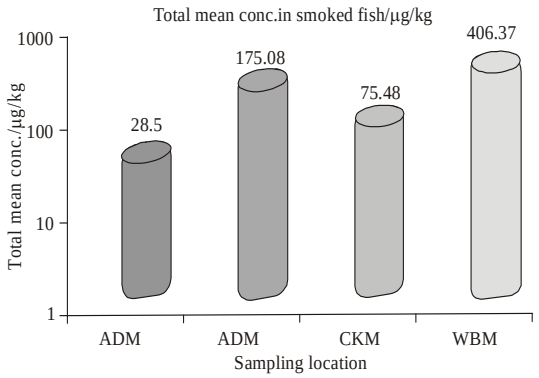


Fig. 2: Total concentrations of mean PAHs in smoked fish samples from four locations

Table 1 shows the total mean concentration of the 20 polycyclic aromatic hydrocarbons analysed in smoked fish samples. The PAHs have been grouped according to molecular weights. The high molecular weights PAHs (228-278 g/mol), include benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(j) fluoranthene, benzo(e)pyrene, benzo(a)pyrene benzo(ghi)perylene, dibenzo(ah) anthracene, Chrysene, cyclopenta(cd)pyrene, indeno(1,2,3-cd)pyrene, and anthranthrene. The medium molecular weights (202 g/mol) are fluoranthene and pyrene and the low molecular weights (152-178 g/mol) include naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene and phenanthrene.

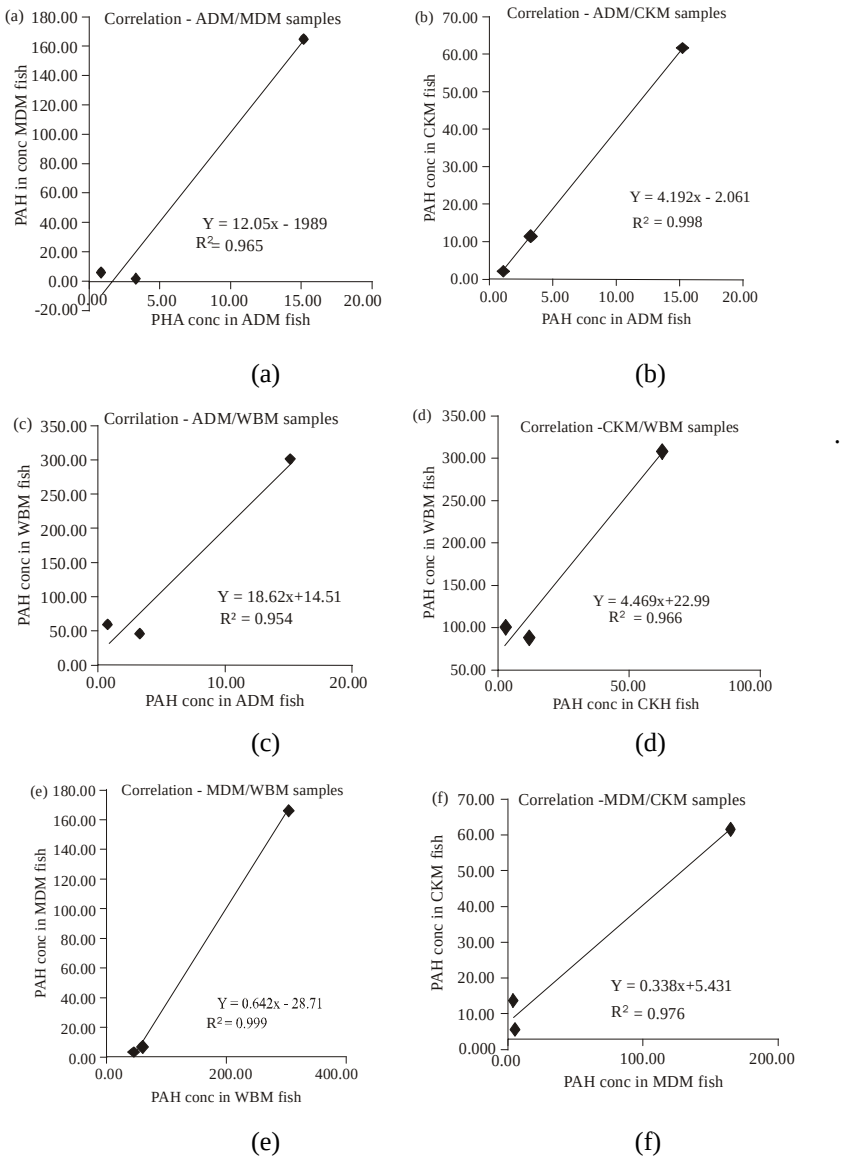
As can be observed from table 1, the total concentration of the high molecular weights (HMW) polycyclic aromatic hydrocarbons were higher than the other molecular weights in all smoked fish samples from the same location. The presence of HMW polycyclic aromatic hydrocarbons are indicative of residues of

Table 1: Total mean concentration of PAHs in smoked fish, grouped by molecular weights (µg/kg)

PAH type	ADM a	ADM b	MDM a	MDM b	MDM c	CKM a	CKM b	CKM c	WBM a	WBM b	WBM c
HMW PAHs	13.81	1.31	16.31	83.37	65.96	27.02	27.10	7.41	131.4	150.2	20.56
MMW PAHs	0.91	-	1.18	3.35	1.85	0.11	2.04	0.54	5.57	50.00	3.52
LMW PAHs	3.32	0.13	2.08	0.58	0.41	1.52	4.87	4.88	17.24	18.15	9.78

Table 2: Some PAH isomer ratios of sampled smoked fish from all locations.

Site/Isomer ratio	Phen/A n	An/ (An+Phe)	Flu/ (Flu+Py)	Nap/Phe	BaA/ (BaA+CH)	Ind/ (Ind+BghiP)
ADM2	3.4	0.23	-	-	-	-
MDM1	0.01	0.99	0.19	6.13	0.96	-
MDM2	-	-	0.03	-	-	-
MDM3	-	-	0.05	-	-	-
CHM1	0.52	0.66	-	-	-	0.74
CHM2	17.16	0.06	0.05	0.03	0.95	-
CHM3	9.71	0.09	0.88	0.06	-	-
WBM1	15.74	0.06	0.97	0.12	0.92	0.78
WBM2	20.01	0.05	-	0.01	1	0.92
WBM3	3.56	0.22	0.3	2.27	0.99	-



Fig, 3: Correlation graphs showing high, medium and low molecular PAH ratios from various locations

previous pyrolytic processes that occurred in the smoking chamber. Pyrolysis of polycyclic aromatic hydrocarbon residues leads to the formation of additional higher molecular weight polycyclic aromatic hydrocarbons and, consequently increases the polycyclic aromatic hydrocarbon concentration in the samples (Guillen and Sopelana, 2004). Most of the carcinogenic polycyclic aromatic hydrocarbons fall within the group of the high molecular weights (EFSA, 2002).

Correlation in smoked fish samples: Based on the molecular weight classification of polycyclic aromatic hydrocarbons, correlation graphs between smoked fish samples from the various markets were done.

Figure 3 shows various graph of correlation of high, medium and low molecular weight polycyclic aromatic hydrocarbons identified in sampled smoked fishes from any two different locations. The locations were Ada, chorkor, Madina, Winneba and labeled as ADM, CKM, MDM and WBM, respectively.

Values obtained were 0.954, 0.966, 0.968, 0.976, 0.998 and 0.999 for the correlation between any two groups of LMW/LMW, MMW/MMW and HMW/HMW PAHs from any two locations. Values are indicative of positive correlation with R^2 values of approximately 1 which is a perfect correlation. These values imply the general distribution of all measured polycyclic aromatic hydrocarbons as being similar regardless of the sampling

location. There are higher concentrations of higher molecular weights PAHs than there are medium and which are also higher than the low molecular weights PAHs.

Table 2 shows the diagnostic isomer ratios of all smoked fish sampled from all four markets. Various possible sources of contamination in sampled smoked fish sold on the open market, is quite difficult to state. Diagnostic isomer ratios which are indices associating different isomer ratios of PAHs to possible sources however have been used in this identification (Dickhut *et al*., 2000; Soclo *et al*., 2000; Yunker *et al*., 2002). Some polycyclic aromatic hydrocarbons have been identified as markers for various sources in urban atmospheres and assigned to possible sources such as petrogenic, fuel combustion, wood and coal combustion or mixed origin.

Comparing results from Table 2 to the diagnostics ratios, it is revealed that most of the values vary from the specified rule of having a particular source. It is therefore concluded that, the sources of the PAHs contamination in smoked fish may be very diverse and are multiple. Source of contamination could either be from vehicular emissions, as most of the traditional kilns are not so far from the roadside or strictly pyrolytic resulting from the polycyclic aromatic hydrocarbon residues of previous pyrolytic processes in the smoking chamber.

The rules have it that, for Flu/(Flu+Py) isomer, if the ratio is approximately one (1), contamination contribution is from petroleum combustion. If less than one (1), it is petrogenic. If it is greater than one (1), is pyrolytic. All values obtained from all locations were less than 1 hence the polycyclic aromatic hydrocarbon source is petrogenic. However, considering the other isomer ratios in the table (Table 2), it is realized that, the major sources of polycyclic aromatic hydrocarbon contamination at each particular location is not conclusive.

CONCLUSION

In this study, freshly smoked fish samples were analysed for polycyclic aromatic hydrocarbon contamination. Twenty (20) PAH types were identified and quantified using GC-FID. Individual polycyclic aromatic hydrocarbon concentrations varied from below the limit of detection to 83.928 μg/kg. The levels in the samples were in decreasing total mean concentration order of Winneba market, Madina market, Chorkor market and Ada market. From the study, sources of polycyclic aromatic hydrocarbon contamination can be said to be multiple and varied.

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