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Full Length Research Paper

The techniques of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria

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Food smoking belongs to one of the oldest technologies of food preservation which mankind has used in fish processing. Potential health hazards associated with smoked foods may be caused by carcinogenic components of wood smoke – mainly polycyclic aromatic hydrocarbons (PAHs) and derivatives of PAH. Comparison of the concentration of PAHs in smoked fish samples processed by sawdust, charcoal and firewood were investigated with the aim of determining the process that contributed more concentration of the PAHs to the fish samples. For this study, three species of fishes were investigated: *Arius heude loti* (cat-fish), *Cynoglossus senegalensis* (sole) and Haake (fresh stock fish). The PAHs in the samples were extracted using solvents by ultrasonication and were analysed for the 16 US EPA polycyclic aromatic hydrocarbons using HPLC with a UV DAD detector. The results showed that smoked fish samples that were processed by charcoal gave the lowest level of total PAHs, followed by firewood method, while the sawdust method gave the highest level of total PAHs in the smoked fishes. The level of PAHs in three species of fishes smoked also correlated with the fat content.

Key words: Polycyclic aromatic hydrocarbons (PAHs), smoked fish, fat and oil contents, smoking.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of high lipophilic compounds that comprise a class of chemical compounds known to be potent carcinogens. PAHs are present in the environment; in water, air, soil and traces of these substances have been found in various food products. Food can become contaminated during thermal treatments that occur in processes of food preparation and manufacture (drying and smoking) and cooking (roasting, baking, and frying) (Ishizaki et al., 2010). Most PAHs are chemically inert, hydrophobic, and soluble in organic solvents. PAHs are ubiquitous environmental pollutants, resulting from the incomplete combustion or pyrolysis of organic matter during industrial processing and various human activities They originate from diverse sources such as tobacco smoke, engine exhausts, petroleum distillates, and coal-derived products, with combustion sources predominating (Simko, 2002). Due to their carcinogenic activity, PAHs have been included in the European Union (EU) and the United States Environmental Protection Agency (USEPA) priority pollutant lists. Human exposure to PAHs occurs in three ways, inhalation, dermal contact and consumption of contaminated foods. Diet is the major source of human exposure to PAHs as it accounts for 88 to 98% of such contamination (Farhadian et al., 2011). Processing of food at high temperatures (grilling, roasting, frying and smoking) are major sources generating PAHs. Levels as high as 200 µg/kg have been found for individual PAH in smoked fish and meat samples. For instance, in barbecued meat, 130 µg/kg has been reported whereas the average background values are usually in the range of 0.01 to 1 µg/kg in uncooked foods (Guillén et al., 2009).

Fish is a rich source of lysine suitable for supplementing high carbohydrate diet. It is a good source of thiamin, riboflavin, vitamins A and D, phosphorous,

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calcium and iron. It is high in polyunsaturated fatty acids that are important in lowering blood cholesterol level (Al-Jedah et al., 1999). In Nigeria smoked fish products are the most readily form of fish product for consumption. Out of the total of 194,000 metric tons of dry fish produced in Nigeria, about 61% of it was smoked. One of the greatest problems affecting the fishing industry all over the world is fish spoilage. In high ambient temperature of the tropics, fresh fish have the tendency to spoil within 12 to 20 h (Clucas, 1981). Attempt has been made to reduce fish spoilage to the minimum through improved preservation techniques. At harvest time, fish are usually available in excess of demand. This lead to lower market price and fish spoilage but if storage facilities are provided the surplus of the harvest could be stored and distributed during the off season. Preservation and processing methods explore ways by which spoilage are stopped or slowed down to give product a longer shelf life.

Food smoking belongs to one of the oldest technologies of food preservation which mankind has used in fish processing. Smoking has become a means of offering diversified, high value added products as an additional marketing option for certain fish species where fresh consumption becomes limited (Gómez et al., 2009). Traditional smoking techniques involve treating of pre-salted, whole or filleted fish with wood smoke in which smoke from incomplete wood burning comes into direct contact with the product, this can lead to its contamination with PAHs if the process is not adequately controlled or if very intense smoking procedures are employed (Estaca et al., 2011). The smoke is produced by smouldering wood and shavings or sawdust in the oven, directly below the hanging fish or fillets, laid out on mesh trays.

The actual levels of PAHs in smoked foods depend on several variables in the smoking process, including type of smoke generator, combustion temperature, and degree of smoking (Garcia and Simal, 2005). The composition of the smoke and the conditions of processing affect the sensory quality, shelf life, and wholesomeness of the product. Potential health hazards associated with smoked foods may be caused by carcinogenic components of wood smoke; mainly PAHs, derivatives of PAHs, such as nitro-PAH or oxygenated PAH and to a lesser extent heterocyclic amines (Stołyhwo and Sikorski, 2005). The smoke for smoking of food develops due to the partial burning of wood, predominantly hardwood, softwood and bagasse. Among PAHs, the benzo[a]pyrene (BaP) concentration has received particular attention due to its higher contribution to overall burden of cancer in humans, being used as a marker for the occurrence and effect of carcinogenic PAHs in food (Rey et al., 2009).

PAHs in food samples have been analyzed by high-performance liquid chromatography (HPLC) with ultraviolet (UV) or fluorescence detection (FLD), gas chromatography–mass spectrometry (GC–MS) and GC–MS–MS. Most of these methods, however, require sample preparation steps, such as extraction, concentration, and isolation, to enhance the sensitivity and selectivity of their detection. For example, liquid–liquid extraction with several organic solvents, pressurized liquid extraction gel permeation or open-column chromatography and solid-phase extraction (SPE) have been used as cleanup procedures (Ishizaki et al., 2010). These contemporary analytical procedures make it possible to determine individual PAH in smoked foods at concentrations of the order of 0.1 µg/kg or even 0.01 µg/kg (Stołyhwo and Sikorski, 2005).

This study investigated the use of different types of fish smoking processes on three species of fishes: *Arius heude loti* (cat-fish), *Cynoglossus senegalensis* (sole) and *Haake* (fresh stock fish) in order to monitor the effects of method of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria.

MATERIALS AND METHODS

Fish samples preparation and smoking

Three species of locally consumed fresh fish samples were used in this study and they were bought from a fish-market in Ijora area of Lagos (Nigeria). They were *A. heude loti* also known as cat-fish (called ‘aro’ in Yoruba), *C. Senegalensis* also known as sole (called ‘abo’ in Yoruba), and *Haake* also known as fresh stock fish (called ‘panla’ in Yoruba). They were weighed and their lengths were taken using calibrated weighing balance and ruler. Some of the fishes were smoked while some were fresh homogenised using a blender and dried in an oven for 2 days at low temperature of about 40°C.

The weighed fishes were smoked using three African traditional processes of smoking: firewood, charcoal and sawdust methods. The fishes were smoked for about 8 h at high temperature and a thermometer was used to take the temperature of the smoking process. The smoking process involved placing a piece of cardboard over the fishes to cover the fishes during the process. The piece of cardboard traps the smoke to enable it act directly on the fish samples. The smoked fishes were further dried in an oven at low temperature of 40°C for four days to ensure that the fish samples were properly dried. The smoked dried fishes were homogenised using a mortar and pestle and were stored in a refrigerator at 4°C prior extraction and analysis.

Chemical analysis of fish samples: Fat (oil) and PAHs contents

For the fat content determination, 2 g of each homogenized dried fish samples were used for fat and oil determination. The weighed homogenized fish samples were put into a soxhlet extractor and a mixture of 50 ml chloroform: 50 ml methanol was used to extract the fat content for 2 h from the fish species. The extract was poured into weighed crucibles and allowed to dry in a fume cupboard. The difference in weight between the empty crucible and the extract-contained crucible was taken as the fat content.

For the determination of the PAHs content, 5 g of each type of smoked dried fishes were weighed into amber glass bottles and extracted sequentially by ultrasonication using 25 ml of n-hexane for 1 h. After ultrasonication the supernatant of the extracts were decanted into a vial and 15 ml of fresh solvent was added for

Table 1. Length, weight and fat content of fishes used for this study.

Common name of fish	Scientific name	Length (cm)	Wet weight (g)	Dry weight (g)	Fat content (mg/g)
Cat fish	*Arius heude loti*	36.8-38.5	450 – 525	107.65-139.23	48.935± 0.452
Sole	*Cynoglossus senegalensis*	24.00-26.70	74.49 -80.85	16.06 -19.98	3.429 ±.40
Fresh stock fish	*Haake* sp.	27.00-28.00	89 .08 99.57	19.86 - 21.72	2.337±0.50

Table 2. Temperature of fish smoking processes.

Types of smoking	Temperature of the smoking process (°C)	Observations during the smoking process
Charcoal	250	Least amount of smoke was produced
Firewood	200	Moderate amount of smoke was produced
Sawdust	120	Highest amount of smoke was produced

another 1h of ultrasonication. The process was repeated with another 10 ml of fresh solvent for 1 h. The combined extracts (50 ml) were centrifuged at 2500 rpm for 10 min and the supernatant was decanted (Garcia-Falcon et al., 1996). The supernatant was cleaned-up using the Whatman nylon filter membrane. Further clean-up was done using the solid phase extraction (SPE) cartridges.

The sorbent of the SPE cartridges were first conditioned with n-hexane, after which the filtered extracts were loaded on to the cartridges, the analytes were eluted with dichloromethane. The volume of the dichloromethane was blown down to dryness and extract was reconstituted in 200 µl of acetonitrile. After the solvent extraction of the PAHs from the smoked dried fish samples by ultrasonication, high performance liquid chromatography (HPLC) was used for their separation and analysis. The quantification of PAHs was performed using an Agilent 1100 model HPLC system with a quaternary pump, vacuum degasser, a temperature controlled column oven and a UV diode-array detector. Separation of the PAHs was performed on a monomeric type octadecyl silica column, Supelcosil LC PAH 2 cm × 4.6 mm i.d containing 5 µm particles at ambient temperature (25 ±1°C) at a flow rate 1.0ml/min. Gradient elution using acetonitrile and water was employed (60:40 to 0:100). Peak detection and integration of data was carried out using chemstation software series.

External calibration was carried out using mixed PAH standards. From the chromatogram, the retention times of the standards were used for the identification and quantisation of the individual PAHs. A standard mixture of the USEPA 16 priority PAHs and 2 PAHs derivatives (2000 µg/ml, dichloromethane: benzene): naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene was obtained from SUPELCO, Bellefonte, PA, USA. Appropriate working dilutions of the standard solution with HPLC grade acetonitrile were made. All other solvents used were of high purity analytical grade.

RESULTS AND DISCUSSION

Length weight and fat content of fishes used for this study

The average length of the fresh fish samples studied ranged from 24 to 38.5 cm. The wet weight of the fish samples ranged from 74.49 to 525 g while the dry weight of the fish samples ranged from 16.06 to 139.23 g, with the cat fish having the highest average weight. Of the three species of fishes studied the cat fish had the highest oil content of 48.94 mg/g, while *Haake* had the lowest oil content of 2.34 mg/g. Table 1 shows the sizes, weight and fat content of the fish samples studied.

The fishes were smoked employing the traditional smoking methods with charcoal, firewood and sawdust as the source of fuel. The charcoal had the highest temperature of 250°C, followed by the firewood (200°C) and sawdust (120°C) as presented in Table 2.

The levels of PAH in smoke depends on heat source (coal, wood, gas, etc.), temperature, flame intensity in flame combustion, particulate material generated during combustion,etc. (Muthumbi et al., 2003; Rey et al., 2004; Garcia and Simal, 2005). The combustion temperature during the generation of smoke seems particularly critical and PAHs are formed during incomplete combustion processes, which occur in varying degree whenever wood, coal or oil is burnt (Wretling et al., 2010). PAHs may be formed in three ways: by high temperature (for example, 700°C), pyrolysis of organic materials by low to moderate temperature (for example, 100 to 150°C) and digenesis of organic materials by microorganisms (Neff, 1985). The PAHs studied here can be classified as those from pyrolysis of organic materials at moderately high temperature. Generally it was observed that at high temperatures less smoke were produced and at lower temperatures more smoke were produced during the smoking process.

The four, five and six ring PAHs appear to be more carcinogenic than PAHs with smaller or larger ring systems and highly angular configurations tend to be more carcinogenic than linear ring systems (Neff, 1985). Based on this, the low molecular weight PAHs such as Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene which have two to three

Table 3. PAHs contents found in *Arius heude loti* (Cat fish) by different smoking methods.

PAHs	Sawdust (µg/kg)	Fire wood (µg/kg)	Charcoal (µg/kg)	Oven dried (µg/kg)
Naphthalene	123.8	108.5	ND	ND
Acenaphthylene	320.9	268.6	99.5	27.2
Acenaphthene	513.7	23.9	542.5	ND
Fluorene	ND	801.5	142.9	132.2
Phenanthrene	30.7	ND	ND	ND
Anthracene	187.5	30	35.5	ND
Fluoranthene	329.2	17.9	82.9	68.2
Pyrene	246.8	43.1	ND	ND
Benzo(a)anthracene	ND	ND	ND	20.4
Chrysene	23.1	5.5	ND	8.9
Benzo(b)Fluoranthene	ND	ND	ND	ND
Benzo(k)fluoranthene	18.5	ND	ND	ND
Benzo(a)pyrene	ND	ND	ND	ND
Dibenzo(a,h)anthracene	216.2	19.7	233.2	50.9
Benzo(g,h,i)perylene	ND	ND	ND	ND
Indeno(1,2,3,c)pyrene	47.7	2.2	ND	25
Total PAHs	2058.1	1320.9	1136.5	332.8

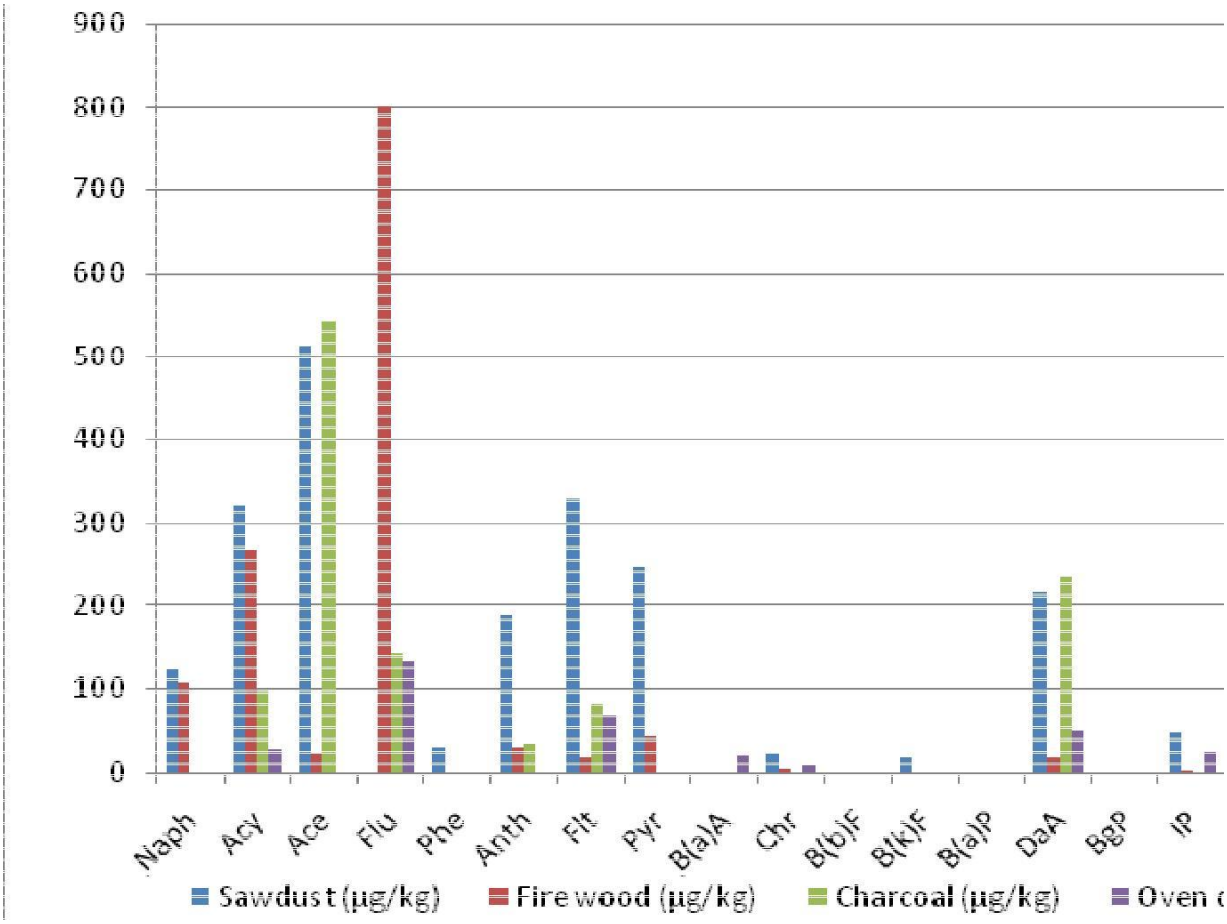
*ND-Not detected.

rings are not regarded as very carcinogenic.

Various smoking treatments were given to the *A. heude loti* as shown in Table 3. The total concentration of the sum of the PAHs in the oven dried method was the least, having a concentration of 332.8 µg/kg. Most of the individual PAHs were not detected in the oven dried fish samples. This could be as a result of the method not involving the use of smoke. Some PAHs were not detected in both oven dried and smoked fishes. Benzo (a) pyrene for example as was not detected in both the oven dried and the smoked fishes. The concentration of total PAHs in the fishes were 2,058.1 µg/kg, 1,320.9 µg/kg, 1,136.5 µg/kg and 332.8 µg/kg for the sawdust smoked, firewood smoked, charcoal smoked and oven dried methods respectively. The level of total PAHs in the smoked fishes was higher than those in the oven dried fishes. The PAHs levels were found to vary with the heat source. This agrees with the findings of some researchers who studied the effects of cooking methods on foods (Muthumbi et al., 2003; Rey et al., 2004). Graph of Table 4 or Table 4 shows the results of the concentration of individual PAHs found in *C. senegalensis* from the various smoking procedures. The oven dried process had most of the individual PAHs as not detected except for acenapthylene, benzo(a)anthracene, chrysene, benzo(a)pyrene and indeno(1,2,3-c,d). However the smoked fishes had higher concentration of the individual and total PAHs. The sawdust smoked *C. senegalensis* had the highest levels of the total PAHs, followed by the fire wood and charcoal. This was consistent with the findings for *A. heude loti* in Table 3 (Graph of Table 3) Benzo (a) pyrene which is considered one of the most toxic and dangerous PAHs was detected in the oven dried (5.6µg/kg) but not in the smoked fishes. Pyrene and benzo[a]pyrene are two of the best characterised PAHs and may be bio transformed in humans and animals to numerous phase 1 metabolites including 1-OH pyrene (1-OH-Pyr) and 3-OH benzo[a]pyrene (3-OH-B[a]P) (Rey-Salgueiro et al., 2009). 3,4-benzopyrene, found in smoked products, serves as an indicator of the possible presence of other polycyclic aromatic hydrocarbons (PAH) and has been used repeatedly as a quantitative index of chemical carcinogens in foods. The level of B (a) P found in the oven dried fish sample was however higher than the European regulatory maximum level for smoked meat and fishes.

Various smoking methods were applied to the *Haake* and the results are presented in Table 5, Graph of Table 5. The oven dried which can be regarded as the blank since smoking was not carried out on it. However the smoked fishes had more PAHs identified and at higher level. Benzo (a) pyrene was not detected in the oven dried and smoked fishes. The total PAHs level in the fishes were 856.2 µg/kg, 780.8 µg/kg, 120.8 µg/kg and 37.9 µg/kg for the sawdust smoked, firewood smoked, charcoal smoked and oven dried respectively. The total PAHs in the smoked *Haake* compared with the oven dried *Haake* were higher.

The values of the total PAHs showed that the *A. heude loti* had highest level of PAHs followed by the *Haake* and C*. senegalensis* this might be related to the oil content in

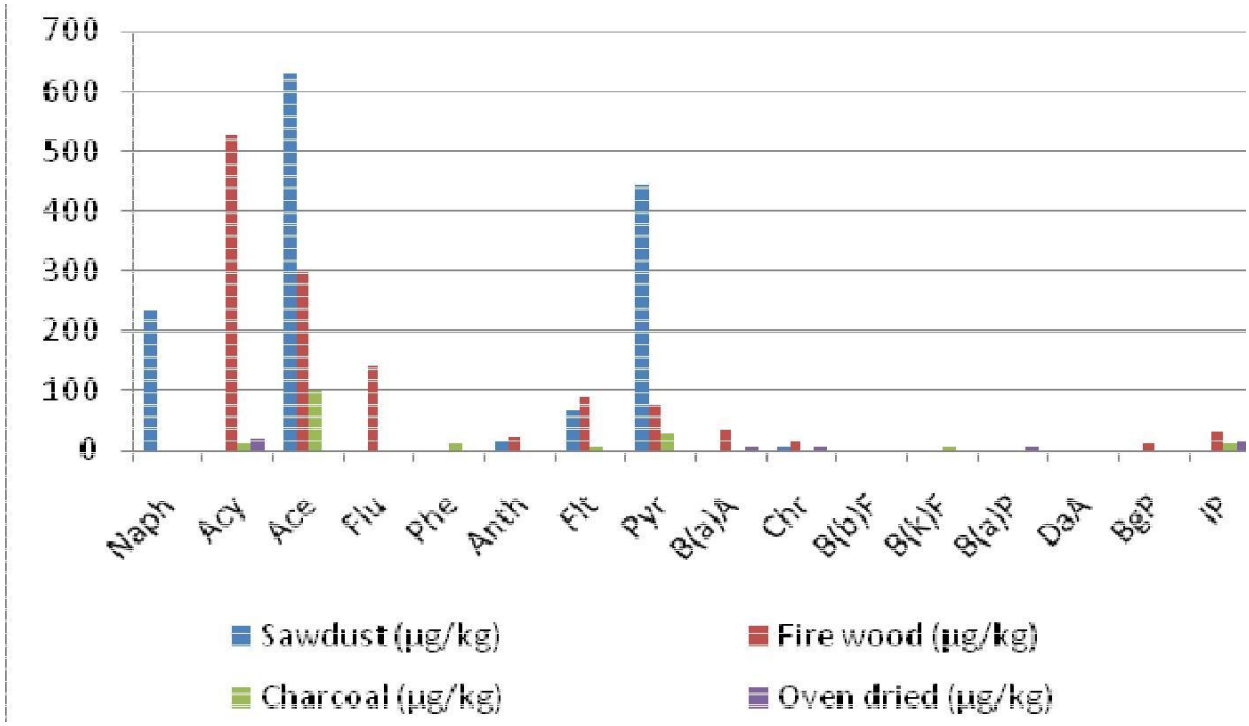


Graph of Table 3. PAHs contents found in *Arius heude loti* (Cat fish) by different smoking methods.

Table 4. PAHs contents found in *Cynoglossus senegalensis* (Sole) by different smoking methods.

PAHs	Sawdust (µg/kg)	Fire wood (µg/kg)	Charcoal (µg/kg)	Oven dried (µg/kg)
Naphthalene	236.0	ND	ND	ND
Acenaphthylene	ND	528.1	12.4	18.3
Acenaphthene	629.7	300.3	98.6	ND
Fluorene	ND	141.7	ND	ND
Phenanthrene	ND	ND	12.0	ND
Anthracene	15.3	21.8	ND	ND
Fluoranthene	68.7	88.5	5.4	ND
Pyrene	441.9	78.3	30.1	ND
Benzo(a)anthracene	ND	36.1	ND	5.3
Chrysene	3.6	17.1	ND	2.5
Benzo(b)Fluoranthene	ND	ND	ND	ND
Benzo(k)fluoranthene	ND	ND	4.9	ND
Benzo(a)pyrene	ND	ND	ND	5.6
Dibenzo(a,h)anthracene	ND	ND	ND	ND
Benzo(g,h,i)perylene	ND	10.9	ND	ND
Indeno(1,2,3-c,d)pyrene	ND	34.7	13.4	14.4
Total PAHs	1395.2	1257.5	176.8	46.1

*ND-Not detected.

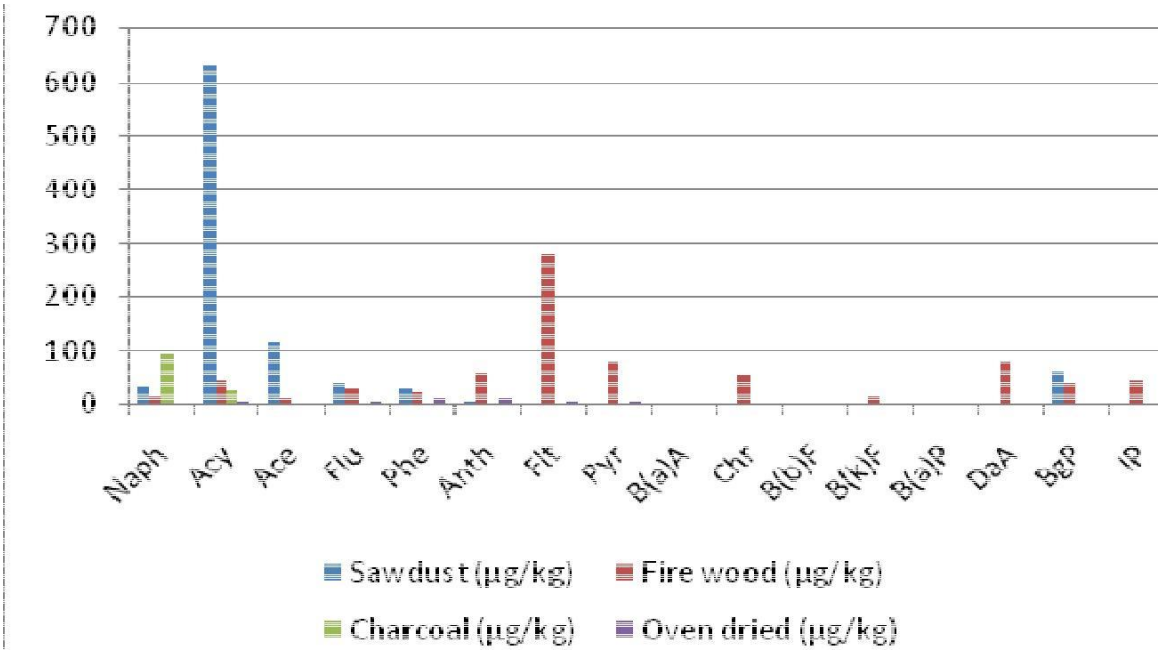


Graph of Table 4. PAHs contents found in *Cynoglossus senegalensis* (Sole) by different smoking methods.

Table 5. PAHs contents found in *Haake* by different smoking methods.

PAHs	Sawdust (µg/kg)	Fire wood (µg/kg)	Charcoal (µg/kg)	Oven dried (µg/kg)
Naphthalene	34.7	14.20	96.30	ND
Acenaphthylene	631.0	42.9	24.5	6.1
Acenaphthene	118.7	11.0	ND	ND
Fluorene	38.7	29	ND	3.2
Phenanthrene	29.1	22.8	ND	12.4
Anthracene	4.0	60.9	ND	11
Fluoranthene	ND	280.6	ND	0.5
Pyrene	ND	81.1	ND	4.7
Benzo(a)anthracene	ND	ND	ND	ND
Chrysene	ND	55.1	ND	ND
Benzo(b)Fluoranthene	ND	ND	ND	ND
Benzo(k)fluoranthene	ND	16	ND	ND
Benzo(a)pyrene	ND	ND	ND	ND
Dibenzo(a,h)anthracene	ND	82.6	ND	ND
Benzo(g,h,i)perylene	64.1	41.5	ND	ND
Indeno(1,2,3,c)pyrene	ND	43.10	ND	ND
Total PAHs	856.2	780.8	120.8	37.9

the fishes. *A. heude loti* had the highest oil content (4.8 mg/g) of the three fishes investigated, followed by *Haake* (3.429 mg/g) and *C senegalensis* (2.337 mg/g). Hence there was generally a correlation between the oil content and total PAHs content in the fish samples. Some authors determined the effects of various processing methods, steaming, roasting, smoking, charcoal grilling, etc. on foods (Garcia et al., 1996; Chen and Lin, 1997; Wu et al., 1997; Mottier et al., 2000; Chen and Chen, 2001; Duedahl-Olesen et al., 2006; Rey et al., 2009). All



Graph of Table 5. PAHs contents found in *Haake* by different smoking methods.

mentioned authors attribute the highest PAH generation during grilling or barbecue through pyrolysis during charbroiling of meat products and either deposition and penetration of smoke components into foods and they found a link between fat foods and PAH levels. The hypothesis is that melted fat from the heated meat drips onto the hot coals and is pyrolyzed, giving rise to PAHs generation, which are then deposited on the meat surface as the smoke rises. Their findings were consistent with the findings from this study. Biological membranes are mostly composed of lipids (oils); majority of organic pollutants are lipophilic. It has been suggested that the larger the lipid content of the biological membrane, the higher is the rate of uptake of pollutants (Hamelink and Spacie, 1977).

Conclusions

Smoking was found to generally increase the PAHs levels with the various smoking methods contributing PAHs to varying degrees. The Sawdust smoked fishes consistently has the highest level of PAHs of all the smoked fished from various methods of processing investigated followed by the fire wood and the charcoal. The oil content of the fish and the temperature of the smoking process were found to affect the PAHs level. In most of the smoked fishes studied, benzo (a) pyrene was not detected except for the oven dried *C. senegalensis* which was found to have a level of 5.6 µg/kg, which exceeds the 5.0 µg/kg maximum level for smoked meat and fish established by the European Commission (Regulation (EC) No 208/2005). The results reveal that the fish samples smoked by the different methods do not constitute a health risk, as the levels of the benzo (a) pyrene are below or lower than maximum levels regulated by the European Commission**.**

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