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Assessment of Polycyclic Aromatic Hydrocarbons (Pahs) in Hardwood and Softwood - Smoked Fish

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Abstract

Two types of wood were investigated; Mahogany (*Mellicae*) and Bamboo (*Mycapella*) to smoke African catfish *(Clarias gariapenus).* The Polycyclic Aromatic Hydrocarbons (PAHs) in the experimental fish were extracted using solvents and Ultrasonication and were analyzed for 16 Polycyclic Aromatic Hydrocarbons using High Performance Liquid Chromatography (HPLC) with ultraviolet diode detector. There was no significant difference between the two woods investigated ($p>0.05$). From the analysis, the result showed that some of the toxic and dangerous PAHs (Benzo (a) Pyrene, Benzo (a) Anthracene, Benzo: (b) Fluoranthene and Benzo (g, h, i) Pyrene.) was Not Detected (ND) in both wood-smoked fish. Mahogany (*Mellicae*) and Bamboo (*Mycapella*) used in smoked fish are not detrimental to humans. The total level of PAHs in Mahogany was 1320.9µg/kg while the total level of PAHs in Bamboo is 2058.1µg/kg. It can be concluded that the use of Bamboo and Mahogany for the purpose of smoking Fish is recommended as both wood do not contain high level of PAHs.

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are a class of high lipophillic 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 manufacturing (drying and smoking) and cooking, roasting, baking and frying [1]. Most PAHs are Ubiquitous environmental pollutants, resulting from the incomplete combustion or pyrolysis and 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 [2]. Due to their carcinogenic activity, PAHs have been included in the European Union and the United States Environmental Protection Agency (USEPA) priority pollutant lists. Human exposure to PAHs accounts for 58 to 98% of such contamination [3]. Processing of food at high temperatures (grilling, smoking, roasting and frying) are major sources of generating PAHs. Levels as high as 200µg/kg have been found for individual PAH in smoked fish and meat samples for instance in barbecued meat, 130µg/kg has been reported where as the average background values are used in the range of 0.01 to 1µg/kg in uncooked foods [4].

Fish is a rich source of lysine suitable for supplementing high carbohydrate diet. It is a good source of thiamin, riboflain vitamins A and D, phosphorus, calcium and iron; it is high in poly unsaturated fatty acids that are important in lowering blood cholesterol level. In Nigeria smoked fish products are the most readily form of fish product for consumption out of the total of 194,000 martinets 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 20 hours [5]. 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 utmost 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 limit [6]. Traditional smoking techniques involve treating of presented, whole or filleted fish with wood and 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 [7]. The smoke produced by shouldering wood inside an open drum, directly below the hanging fish or hang-out mesh trays.

The actual levels of PAHs in smoke food depend on several variables in the smoking process, including type of smoke generator, combustion temperature and degree of smoking [8]. The combustion of the smoke and the condition 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 less extent heterocyclic amines [9]. The smoke for smoking of food develops due to the partial burning of wood, predominantly hard wood, softwood and biogases. Among PAHs, the benzo (a) pyrene (bap) concentration has received particular attention due to its higher contribution to over all burden of cancer in humans, being used as a marker for the occurrence and effect of carcinogenic PAHs in food [10].

PAHs in food samples have been analyzed by High Performance Lipid Chromatography (HPLC) with Ultraviolet (UV) or Fluorescence Detection (FCD), Gas Chromatography – Mass Spectrometry, (GC-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 clear up procedures [11]. These contemporary analytical procedures make it possible to determine individual PAH in smoked foods at concentrations of the order of 0.1µg/kg or even 0.01µg/kg [9]. The present study was therefore conducted to investigate the levels of Polycyclic Aromatic Hydrocarbon (PAHs) in hard wood and soft wood- smoked fish.

Materials and Methods

Experimental site

African Catfish *(Clarias gariepinus)* was smoked in the Research Laboratory of the Department of Animal/Fisheries Science and Management, Faculty of Agriculture and Natural Resources, Enugu State University of Science and Technology (ESUT) Enugu Nigeria (latitude 074° North and 082° South and longitude 068° East and 076° West with annual mean temperature at 30°C) and was sent to Nigeria Institute for Oceanography and Marine Research (NIOMR) Lagos, Nigeria for PAHs Analysis.

Collection and transportation of experimental fish

The table size fish of African catfish, (C*larias gariepinus)* was obtained from a fish farm in Enugu metropolis. The fish was transported using a plastic gallon half filled with water (Open transportation system) to the Departmental Smoking Laboratory where the smoking was carried out.

Fish preparation and smoking

African catfish *(Clarias gariepinus)* was used in this study and were weighed. Their length was taken using a meter rule. The fish were properly washed with brine solution degutted and placed on metal meshed trays. Traditional method of smoking was adopted in this study by using two different medium size drums. Mahogany was used in one of the drums for smoking and the other for the bamboo wood. Fire was obtained by striking matches on the wood. Time interval of 20 minutes was allowed for the fire to stabilize before the meshed trays were placed on the drums. And then fire was reduced to increase quantity of smoke. A piece of cardboard was used to cover each tray because it helped to trap the smoke to enable it act directly on the fish. It took 6 hours for the fish to get dried, then the smoked fishes were homogenized using a mortar and pestle and were stored in a refrigerator at 4°C prior extraction and analysis. During the period of the smoking, the drum using soft wood gave the highest smoke compared to the drum with hardwood.

Chemical analysis of fish

For the determination of PAHs content, 5g of each type of smoked dried fish were weighed into number glass bottles and extracted sequentially by ultrasonication using 25ml of n-hexane for 1hr. after ultrasonication the supernatant of the extracts were decanted into a vial and 15ml of fresh solvent was added for another 1hour of ultrasonication. The process was repeated with another 10ml of fresh solvent for 1hour and the combined extracts (50ml) were centrifuged at 2500 rpm for 10mins and the supernatant was decanted 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 200µl of acetonitrille. After 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, a temperature controlled column oven and a UV diode—array detector. Separation of the PAHs was performed on a monomeric type vetadecyl silica coloum, supereosil Lc PAH 2cm x 4.6mm 1.d containing 5µm 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 PAHs standards from the chromatogram. The reflection times of the standard were used for the identification and quantization of the individual PAHs. A standard mixture of the USEPA 16 priority PAHs and 2 PAHs derivatives (2000µg/ml, dichloromethane: benzene): naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, dibenzo (g, 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.

Statistical analysis

The Studentized t-test was used to analyze data to determine differences between treatment means at probability level of 0.05.

Table 1: Length, Weight and Fat Content of Fish used for this Study.

Common name of the fish	Scientific name	Length (cm)	Wet weight (g)	Dry weight (g)	Fat Content (mg/g)
African fish	*Clarias gariepinus*	36.8-38.5	450-525	107.65 -139.23	49.735 ± 0.45

Results

The results on Length, weight and fat content of fish used in the study are represented in Table 1.

Length, weight and fat content of fish used for this study

The average length of the fresh fish sample studied ranged from 24 to 38.5cm. The wet weight of the fish sample ranged from 1kg-1.5kg per fish. The dry weight of the fish sample ranged from 16.06 to 139.23g, catfish has a large amount of oil content of 48.94mg/g. Table 1 shows the size weight and fat content of the fish sample studied.

The fish were smoked employing the traditional smoking method with fire wood which are; hard wood and softwood as source of the fuel. The hardwood had the highest temperature 250°C, while the softwood is 180°C during the smoking time of fish (Figure 1).

The levels of PAHs in smoke depend on heat source (coal, wood, gas etc). Temperature, flame intensity in flame combustion, particulate material generated during combustion, etc [10,12,13]. 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 [14]. PAHs may be formed in three ways by high

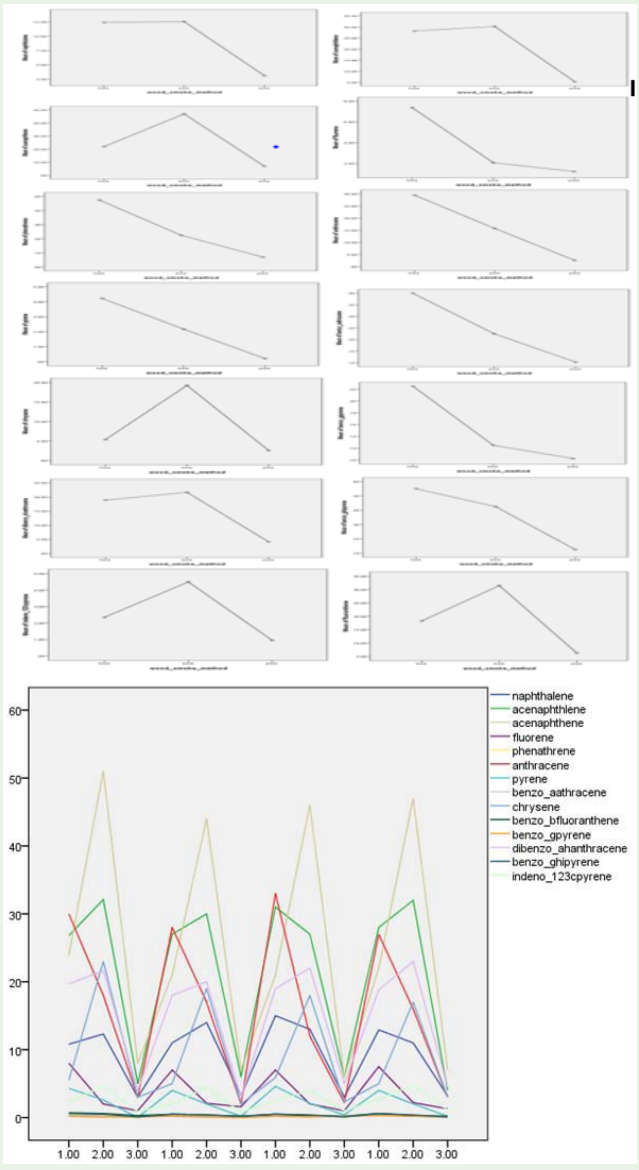

Figure 1: Wood smoke method.

temperature for (example, 70°C) pyrolysis of organic materials by low to moderate temperature (for example, 100 to 150°C) and digenesis of organic materials by micro organisms [15]. The PAHs studied here can be classified as those from pyrolysis of organic materials at moderately high temperature less smoke were produced and at lower temperature 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 longer ring systems and highly angular configurations tend to be more carcinogenic than linear ring system [15]. Based on this, the low molecular weight PAHs such as naphthalene, acenaphthylene, acenaphtene fluorene, phenanthene and anthracene which have two to three rings are not regarded as very carcinogenic.

Discussion

Two smoking treatments were given to the African cat fish. The total concentration of the sum of PAHs in the hardwood was least, having a concentration of 1320.9 µg/kg. Some of the individual PAHs were not detected in both the hardwood smoked fish and softwood smoked fish samples. The concentration of the total PAHs in the fish were 1320.9 µg/kg for hardwood and 2058.1 µg/kg for softwood, but their differences were not statistically significant (P>0.05). The level of total PAHs in the smoked fish varies due to the different heat sources. This agrees with the findings of some researchers who studied the effects of cooking method on foods [10,12]. Figure 1 shows the results of the concentration of individual PAHs found in hardwood and softwood smoked African cat fish. However the softwood smoked fish had the highest levels of the total PAHs but this was not consistent in Benzo (a) pyrene which is considered one of the most toxic and dangerous PAHs. Pyrene and benzo (a) pyrene are two of the best characterized PAHs and may be bio-transformed in humans and animals to numerous phase I metabolites including 1-OH pyrene (1-OH – Pyr) and 3-OH benzo (a) pyrene (3-OH-13 (a) (p) [10]. Three, four – benzo pyrene, found in smoked products, serves as an indicator of the possible presence of other polycyclic aromatic hydrocarbons (PAHs) has been used repeatedly as a quantitative index of chemical carcinogens in foods [16].

The results of softwood and hardwood smoked fish are presented in figure 1. However the softwood smoked fish had more PAHs identified and at higher level when compared to the hardwood smoked fish. The values of the total PAHs showed that the softwood smoked fish had the highest level of PAHs compared to the hardwood smoked fish. Oil content of African cat fish might be related to the level of PAHs in smoked fish. Some authors determined the effects of various processing methods, steaming, roasting, smoking, charcoal griiling, etc. on foods [10,13,17-20]. All mentioned authors attribute the highest PAHs generation during grilling or barbecue through pyrolysis during charcoal broiling of meat products and either deposition and penetration of smoke components into foods and they found a link between that foods and PAHs levels. The hypothesis is that melted fat from the heated fish or meat dips onto the hot wood or coals and is pyrolyzed, giving rise to PAHs generation which are then deposited on the fish surface as the smoke rises. 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 [21].

Conclusion

Smoking was found to generally increase the PAHs levels with the various heat source contributing PAHs to varying degrees. The softwood smoked fish consistently had the highest levels of PAHs compared to the hardwood. The oil of the fish and the temperature of the heat source were found to affect the PAHs level. Benzo (a) pyrene was not detected in both the softwood and hardwood smoked Fish which is dangerous and toxic. This results reveal that the fish smoked with hardwood and softwood do not constitute health risk as the levels of the benzo (a, g, h, i) pyrenes were not detected.

Recommendations

i. It is recommended that hardwood and softwood should be used for smoking fish. This is due to its low levels of polycyclic aromatic hydrocarbons in them.

ii. Traditional method of smoking fish is recommended due to its low level of polycyclic aromatic hydrocarbons and it is not detrimental to fish and human health.

iii. Both hardwood and softwood smoked fish do not have any health risk because the dangerous and toxic PAHs (Benzo (a) Pyrene) are not detected from this research. I recommend that wood smoking should be used alongside charcoal and liquid smoking.

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