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

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Abstract: Three types of woods were investigated; hard wood HWS Mahogany (Mellicae), soft wood SWS Bamboo (Mycapella) and oil palm wood PWS Elaeis guineensis to smoke African catfish Clarias gariepinus. The Polycyclic Aromatic Hydrocarbons (PAHs) in the experimental fish were extracted using solvents and Ultrasonication and were analyzed for 15 Polycyclic Aromatic Hydrocarbons using high performance liquid chromatography (HPLC) with ultraviolet diode detector. There was no significant difference between the three woods investigated (P > 0.05) in benzo_b fluoranthene and benzo_a pyrene but significant differences (P < 0.05) in PAH occurred between the 3 wood-smoked fish in acenaphthene, fluorine, phenathrene, anthracene, pyrene, dibenzo_ah anthracene, benzo_ghi pyrene, indeno 123c pyrene, fluoranthene with lowest value in PWS. Naphthalene and acenaphthylene had same value in HWS and SWS but were significantly lower in PWS. Conversely, benz_a anthracene showed higher value in HWS compared to SWS and PWS but chrysene displayed higher value in SWS compared to PWS and HWS. It can be concluded that the use of Elaeis guineensis is preferred in smoking of C. gariepinus compared to Bamboo and Mahogany. Since the three woods did not differ in benzo_a pyrene, high value of benz_a anthracene recorded in HWS fell below carcinogenic ranges, hence usage may not impact on human health.

Keywords: Polycyclic Aromatic Hydrocarbons, Smoked Fish, Clarias gariepinus

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of lipophillic compounds comprised of chemical compounds known to be potent carcinogens. PAHs are present in water, air, soil and traces in various food products. Food can become contaminated during thermal treatments that occur in processes of food preparation and manufacturing such as drying, smoking, cooking, roasting, baking and frying [16]. Most PAHs are Ubiquitous environmental pollutants resulting from the incomplete combustion or pyrolysis of organic matter during industrial processing and various human activities [5]. They originate from diverse sources such as tobacco smoke, engine exhausts, petroleum distillates, and coal-derived products, with combustion sources predominating [19]. 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 [11]. Processing of food at high temperatures from 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 whereas the average background values are used in the ranges of 0.01 to 1µg/kg in uncooked foods [14].

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, phosphorus, calcium and iron. It is high in poly unsaturated fatty acids that are important in lowering blood cholesterol level [2]. 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 spoil within 20 hours [9]. Attempt has been made
to reduce fish spoilage through improved preservation techniques.

Fish 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 [13]. Traditional smoking techniques involve treating of whole or filleted fish with smoke from wood and burning that 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 [4]. The smoke is produced by shouldering wood inside an open drum, directly below the hanging fish or hang-out mesh trays.

The level of PAHs in smoked food depends on the smoking process including type of smoke generator, combustion temperature and degree of smoking [1]. 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 [20]. 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 contamination with PAHs if the process is not adequately controlled or if very intense smoking procedures are employed [4]. The smoke is produced by shouldering wood inside an open drum, directly below the hanging fish or hang-out mesh trays.

PAHs in food samples have been analyzed by high performance liquid 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 [3]. 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 [21]. The present study was therefore conducted to investigate the levels of polycyclic aromatic hydrocarbons (PAHs) in hard wood, palm wood and soft wood- smoked fish.

2. Materials and Methods

2.1. 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 07° 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 study.

2.2. Collection and Transportation of Experimental Fish

African catfish, Clarias gariepinus was obtained from a fish farm in Enugu metropolis. The fish was transported to the Departmental Smoking Laboratory where the smoking was carried out. Ethical clearance from the Enugu State University of Science and Technology Committee on Experimental Animal Care was obtained and followed.

2.3. Fish Preparation and Smoking

Three types of woods were investigated; hard wood HWS Mahogany (Mellicae), soft wood SWS Bamboo (Mycapella) and oil palm wood PWS Elaeis guineensis to smoke African catfish Clarias gariepinus. The Polycyclic Aromatic Hydrocarbons (PAHs) in the experimental fish were extracted using solvents and Ultrasonication and were analyzed for 15 Polycyclic Aromatic Hydrocarbons using high performance liquid chromatography (HPLC) with ultraviolet diode detector.

2.4. Chemical Analysis

Each type of smoked dried fish was weighed into a number of glass bottles and extracted sequentially by ultrasonication using 25ml of n-hexane for 1hr. 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 [12] and 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 acetonitrile. Thereafter high performance liquid chromatography (HPLC) was used for separation and analysis. The quantification of PAHs was performed using an Agilent 1100 model HPLC system. Separation of the PAHs was performed on a monomeric type vetadecyl silica column, supereosil Lc PAH 2cm x 4.6mm containing 5µm particles at ambient temperature (25±1°C) and a flow rate of 1.0ml/min. Gradient elution using acetonitrile and water was employed (60:40 to 0.100) with peak detection and integration of data using Chemstation Software Series. External calibration was carried out using mixed PAHs standards from the chromatogram. All solvents used were of high purity analytical grade obtained from Super co, Bellefonte, PA, USA.
2.5. Statistical Analysis

Data collected were subjected to 1 way analysis of variance (ANOVA) using SPSS version 20. Comparisons among means were carried out using Duncan Multiple Range Tests (DMRT) at significance level of (P < 0.05).

3. Results

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

<table>
<thead>
<tr>
<th>Length (cm)</th>
<th>Wet weight (g)</th>
<th>Dry weight (g)</th>
<th>Fat (mg g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.8-38.5</td>
<td>450-525</td>
<td>107.65-139.23</td>
<td>49.735 ± 0.45</td>
</tr>
</tbody>
</table>

The average length (cm), Wet weight (g) Dry weight (g) Fat (mg g$^{-1}$) of fish sample studied is displayed in (Table 1). There was no significant difference between the three woods investigated (P > 0.05) in benzo b fluoranthene and benzo a pyrene (Table 2) but significant differences (P < 0.05) in PAH occurred between the 3 wood-smoked fish in acenaphthene, fluorine, phenathrene, anthracene, pyrene, dibenzo ah anthracene, benzo ghi pyrene, indeno 123c pyrene, fluoranthene with lowest value in PWS. Naphthalene and acenaphthylene had same value in HWS and SWS but were significantly lower in PWS. Conversely, benz_a anthracene showed higher value in HWS compared to SWS and PWS but chrysene displayed higher value in SWS compared to PWS and HWS (Table 2).

Table 2. Mean PAH ±SEM in PWS, HWS and SWS smoked fish.

<table>
<thead>
<tr>
<th>PAH</th>
<th>PWS</th>
<th>HWS</th>
<th>SWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>3.10 ±0.06$^b$</td>
<td>12.43 ±0.98$^a$</td>
<td>12.58 ±0.63$^a$</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>5.25 ±0.48$^b$</td>
<td>28.20 ±0.97$^a$</td>
<td>30.28 ±1.19$^a$</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>7.00 ±0.41$^c$</td>
<td>21.97 ±0.68$^b$</td>
<td>47.00 ±1.47$^a$</td>
</tr>
<tr>
<td>Fluorine</td>
<td>1.23 ±0.14$^c$</td>
<td>7.37 ±0.24$^b$</td>
<td>2.08 ±0.05$^a$</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.07±0.04$^a$</td>
<td>0.23 ±0.05$^a$</td>
<td>0.48 ±0.04$^a$</td>
</tr>
<tr>
<td>Anthracene</td>
<td>2.55 ±0.26$^a$</td>
<td>29.5 ±1.32$^b$</td>
<td>15.75 ±1.31$^b$</td>
</tr>
<tr>
<td>Pyrene</td>
<td>0.20 ±0.07$^a$</td>
<td>4.23 ±0.14$^b$</td>
<td>2.18 ±0.14$^b$</td>
</tr>
<tr>
<td>benz_a anthracene</td>
<td>0.10±0.03$^b$</td>
<td>0.40 ±0.07$^a$</td>
<td>0.23 ±0.02$^a$</td>
</tr>
<tr>
<td>Chrysene</td>
<td>2.58 ±0.25$^b$</td>
<td>5.35 ±0.21$^b$</td>
<td>19.25 ±1.31$^a$</td>
</tr>
<tr>
<td>benzo_b fluoranthene</td>
<td>0.23 ±0.03$^b$</td>
<td>0.57 ±0.04$^b$</td>
<td>0.40 ±0.07$^a$</td>
</tr>
<tr>
<td>benzo_a pyrene</td>
<td>0.10±0.03$^b$</td>
<td>0.23 ±0.10$^b$</td>
<td>0.13 ±0.04$^a$</td>
</tr>
<tr>
<td>dibenzo_ah anthracene</td>
<td>4.13 ±0.42$^b$</td>
<td>18.88 ±0.34$^b$</td>
<td>21.65 ±0.62$^a$</td>
</tr>
<tr>
<td>benzo_ghi pyrene</td>
<td>0.13 ±0.02$^b$</td>
<td>0.55 ±0.02$^b$</td>
<td>0.43 ±0.02$^b$</td>
</tr>
<tr>
<td>indeno_123c pyrene</td>
<td>0.95 ±0.06$^b$</td>
<td>2.35 ±0.16$^b$</td>
<td>4.5 ±0.18$^a$</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>6.25 ±0.62$^b$</td>
<td>18.25 ±3.12$^b$</td>
<td>31.50 ±0.64$^a$</td>
</tr>
</tbody>
</table>

Same letter super script are equal but different letter superscript are not equal at P < 0.05

4. Discussion

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 [17, 18]. Although softwood smoked fish had the highest levels of the total PAHs, 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 pyrene (3-OH-pyrene) [18]. Presence of 3, 4 – benzo pyrene 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 fresh meat or meat drips 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 [12, 6, 21, 7, 8, 10, 18]. 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 [3].

5. Conclusion

It can be concluded that the use of oil palm wood Elaeis guineensis is preferred in smoking of C. gariepinus compared to soft wood of Bamboo and hard wood of Mahogany. The three woods however did not display high value of benz a anthracene since highest recorded in HWS fell below carcinogenic ranges.

References