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Effect of Smoking on Polycyclic Aromatic Hydrocarbons (PAHS) Concentrations in Catfish and Tilapia Muscles

*[1]NNAJI, JC; EKWE, NP

Department of Chemistry, Michael Okpara University of Agriculture, Umudike, P.M.B 7267, Umuahia, Abia State, Nigeria

ABSTRACT: The effects of smoking on proximate composition, energy values and concentrations of polycyclic aromatic hydrocarbons (PAHs) were studied in raw and smoked samples of catfish (*Clarias gariepinus*) and tilapia (*Oreochromis niloticus*). Crude protein was higher in the tilapia sample for both raw and smoked samples. There was significant difference (P<0.05) in the lipid contents of raw and smoked samples of both species. Mean naphthalene concentration was significantly higher (P<0.05) than those of other PAHs analyzed in raw and smoked samples of both species. Mean benzo (a) pyrene (BaP) concentrations and total mean PAH concentrations (∑mPAH) exceeded the EU limits in raw muscle samples. All the PAHs analyzed were detected in the smoked samples. Mean BaP concentrations and total mean PAH exceeded the EU maximum limits (2.0 and 10 µg/kg) in the muscle of smoked fish and fishery products. Total mean concentration of the four indicators of PAH contamination gave the values of 0.018 and 0.050; 0.014 and 0.012 mg/kg for raw and smoked samples of catfish and tilapia respectively. It could be inferred that the smoking process generally increased the mean total PAH levels in the fish samples and there is urgent need for relevant authorities to take appropriate action due to the public health implications of PAH contamination.

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Fish is consumed by a large number of people because of its palatability, flavour and availability (Foran *et al*., 2005). It gives protein improved nutrition because it has high biological value in terms of high protein retention in the body (Anthony and Akinwumi, 1999). It also contains some bioactive compounds with therapeutic properties that are beneficial to human health (Nnaji *et al*., 2010).

Smoke is generated by among others, thermal pyrolysis of hard wood when there is limited access to oxygen. Fish processing by traditional method of smoking enables fish to have stability during storage, increases their appetizing appeal, gives special organoleptic profiles to smoked products, and smoking is also done because of the inactivating effect of smoke (and heat) on enzymes and microorganisms (Chen and Lin, 1997). However, this processing method may have negative impacts on consumer health due to the fact that smoking may lead to the deposition of polycyclic aromatic hydrocarbons (PAHs) on smoked fish.

PAHs are environmental contaminants, originating from incomplete combustion of organic matter (Jira *et al*., 2006; Klimaszewska, 1999). They are formed when complex organic substances are exposed to high temperature or pressure or by the incomplete combustion of woods, coal or oil (Easton *et al*., 2002; Storelli *et al*., 2003; Groova *et al*., 2005; Wretling *et al*., 2010). Food can be contaminated by PAHs that are present in air, soil, or water, or during food processing and cooking. PAHs are also found in water though they are hydrophobic (especially heavy PAHs). It is estimated that nearly 70% of PAHs are consumed with food, including the consumption of smoked fish. Of the several hundreds of PAHs, sixteen have been identified as priority PAHs because they have been considered to be more harmful to man than the others (Andrzej and Zdzislaw, 2005; Anyakora and Herbert, 2005).

MATERIALS AND METHODS

Sampling: Raw samples of two local freshwater species, Catfish (*Clarias gariepinus*), and Nile Tilapia (*Oreochromis niloticus*) were harvested from Michael Okpara University of Agriculture, Umudike (MOUAU) fish pond and were smoked at Ahiaeke market in Umuahia. Triplicate samples of each fish species of similar weights were collected for analysis.

Sample pre-treatment: The standard and total lengths of raw samples were measured with a meter rule while their weights were determined with a balance. Each triplicate sample was divided into two with a stainless steel knife, one half was sent to Ahiaeke Market for smoking, while the other was used for raw sample analysis. The raw samples were stored at -20^{o}C in a refrigerator prior to analysis. The lipid extraction of fish muscle samples was done in the Chemistry Laboratory of Michael Okpara University of Agriculture, Umudike. The extracted solution was

*Corresponding author: judennaji30@gmail.com

then sent to BGI laboratories Ltd, Elelenwo, Port Harcourt where the GC/MS analysis was carried out.

The fish smoking process: Tilapia was descaled and together with catfish was washed with clean tap water. They were subsequently rinsed with distilled water and were brined with 10 % salt solution and placed on wire gauze placed on drum type smoking kiln. Wood served as fuel and a distance of 30 cm was maintained between fish and the flame. Smoking temperature was measured with a mercury-in-glass thermometer and smoking was done for a period of 6 h after which the fish was allowed to cool for 1 h and wrapped in polyethylene bags prior to PAH analysis.

Determination of proximate composition and energy value: Proximate analysis of fish was done with the method of FAO (1994). This includes the determination of moisture, crude fat, crude protein, crude ash, crude fibre, and nitrogen free extracts. The energy value was calculated by using the Atwater general factor system which assigns energy values of 17 kJ/g (4.0 kcal/g) for protein, 37 kJ/g (9.0 kcal/g) for fat and 17 kJ/g (4.0 kcal/g) for carbohydrates and 29 kJ/g (7.0 kcal/g) for alchohols (Scott, 2014). The total combination of ratio is 4:4:9 for protein, carbohydrate (NFE) and lipids. The weight of the fish in grams is obtained and each percentage proximate composition is multiplied by the weight of the fish to get the weight of protein, fat and carbohydrate in g. Then each weight is multiplied by the proper factor in the ratio and results summed to give the total energy value in calories.

Soxhlet extraction method: Homogenized fish muscle sample (10 g) was weighed and mixed thoroughly with 5 g of anhydrous sodium sulphate in a laboratory crucible until a complete homogenate was obtained. The extraction was carried out using a Soxhlet extractor apparatus which consists of a 250 cm^3 round bottomed flask, condenser and an extractor tube, seated in a temperature-controlled heating mantle. The homogenate was carefully transferred into the extraction thimble placed in the extraction chamber of the Soxhlet extraction unit. The extraction was carried out as recommended by USEPA 3540 method, using 150 cm^3 dichloromethane for 16 h (USEPA, 1996). The extract was concentrated to 2 cm^3 using a Fischer brand rotary evaporator in a water bath that was pre-set to a temperature of 35 °C and was stored in an amber bottle and kept in a refrigerator to avoid oxidation of the extract prior to clean up. The same procedure was used for all the fish samples collected.

Sample purification: The extracted samples were purified by passing them through a silica gel column prepared by loading 10 g of activated silica gel (100-200 Mesh) onto a chromatographic column (1cm internal diameter) to 5 cm. This was topped with 1cm of anhydrous Na_2SO_4 was then conditioned with dichloromethane. 2 cm^3 of the concentrated extract was loaded and eluted with 20 cm^3 of dichloromethane. This method is able to remove the very polar lipids off the extract. Prior to analysis with GC/MS, the extracts obtained were preserved in an amber bottle to avoid oxidation.

GC/MS Analysis: An Agilent 7890 Gas Chromatograph equipped with auto sampler connected to an Agilent 5975 MSD mass spectrometric detector was used. 1µl of sample solution was injected in the pulsed spilt less mode onto a 30 mm x 0.25 mm id DB5 MS coated fused silica column with a film thickness of 0.15 µm. Helium was used as the carrier gas and the column head pressure was maintained at 20 psi to give constant flow 1ml/min. Other operating conditions were pre-set, pulse time 0.90 min, purge flow 50 cm^3, purge time 1 min, and injection temperature 300 °C. The column temperature was initially held at 55 ^oC for 0.4 min, increased to 200 ^oC at a rate of 25 ^oC/min, then to 280 ^oC at a rate of 8 ^oC/min and to a final temperature of 300 ^oC at a rate of 25 ^oC/min and held for 2 min at transfer line of 320 ^oC. The mass spectrometer (MS) condition was electron impact positive ion mode. The PAHs identification time was based on retention time since each of the PAHs has its separate retention time in the column. Those with lower retention times were identified first followed by those with longer retention times. The GC/MS was calibrated with calibration standard concentration purchased from Accuu standard, USA. PAHs were identified by comparing the retention times of the peaks with those obtained from standard mixture of PAHs. The standards were supplied by the instrument manufacturers.

Statistical analysis: The PAH analysis was carried out for each sample in triplicate (n = 3). The obtained results were statistically analysed using SPSS (version 20.0) windows software. Mean concentration and standard error of the mean (S.E.M) were calculated for each parameter. The result was subjected to one way ANOVA and the means were compared using Duncan multiple Range test.

RESULTS AND DISCUSSION

Table 1 shows the mean standard and total lengths of fish and the mean weights of fish species used in the study. Mean values for weights and standard lengths were similar ($P>0.05$) but total length of Tilapia was significantly lower ($P<0.05$) than that of catfish.

Table 1 Mean weights and lengths of raw fish samples

Parameter	Catfish (*Clarias gariepinus*)	Nile Tilapia (*Oreochromis niloticus*)
Standard length (cm)	6.15 ±0.27	5.68 ±0.90
Total length (cm)	8.20 ±0.83	6.45 ±0.73
Weight (g)	122.47±1.07	120.95±0.95

Table 2 shows the mean temperatures at which the fish species were smoked and energy values for raw and smoked samples. There were no significant differences (P<0.05) in smoking temperatures and energy values for raw and smoked samples.

Table 2 Mean smoking temperatures and energy values (cal) for each species

Sample	Mean Smoking Temperature (°C)	Mean energy values (cal)	
		Raw	Smoked
Catfish	72.27 ±3.41	416.936±0.075	415.780±0.051
Tilapia	70.39 ±4.59	408.297±0.043	407.587±0.024

Table 3 presents the proximate composition of analyzed fish samples. The result reveal significant differences (P<0.05) in moisture content, crude protein, ash content, crude lipid and crude fibre among the samples. As expected, moisture content was higher in the raw samples compared to their corresponding smoked samples. This observation is due to loss of water during smoking (Salan *et al*., 2006). The highest moisture content was recorded in fresh tilapia sample. Crude protein was also higher in the tilapia sample for both raw and smoked samples. Crude fibre, lipid and ash contents were significantly higher (P<0.05) in smoked muscle samples than in raw samples of both species.

The results of mean concentration for each PAH in raw samples are shown in table 4.

Table 3: Proximate composition of raw and smoked samples

Proximate Analysis	Catfish		Tilapia	
	Raw	Smoked	Raw	Smoked
Moisture content	75.69 ±0.02^{a1}	62.07 ±0.01^{a2}	78.49 ±0.02^{a1}	66.74 ±0.01^{a2}
Crude protein	16.97 ±0.01^{b1}	19.49 ±0.01^{b1}	31.68 ±0.01^{b2}	40.61 ±0.01^{b3}
Crude fibre	0.28 ±0.01^{c1}	1.18 ±0.01^{c2}	0.45 ±0.01^{c1}	1.64 ±0.01^{c2}
Crude lipid	4.90 ±0.01^{d1}	8.77 ±0.00^{d2}	3.15 ±0.01^{d1}	6.92 ±0.01^{d2}
Ash	1.61 ±0.01^{c1}	5.83 ±0.00^{d2}	1.42 ±0.01^{c1}	5.11 ±0.00^{d2}
Nitrogen free extracts (NFE)	76.25 ±0.03^{a1}	64.73 ±0.02^{a2}	63.31 ±0.03^{e2}	45.74 ±0.033^{b3}

Means with different numbers (letters) in the same row (column) are significantly different (P<0.05). Data are presented as Mean ± S.E.M.

Table 4: Mean PAH concentrations (mg/kg) in raw samples

PAHs	Catfish	Tilapia
Naphthalene	63.904 ±0.118^{a1}	39.705 ±0.099^{a2}
Acenaphthylene	0.166 ±0.001^{b1}	0.000 ±0.000
Acenaphthene	0.326 ±0.002^{b1}	0.136 ±0.002^{b2}
Fluorene	0.059 ±0.005^{c1}	0.004 ±0.001^{c2}
Anthracene	0.063 ±0.002^{c1}	0.012 ±0.005^{c2}
Phenanthrene	0.067 ±0.001^{c1}	0.021 ±0.001^{c2}
Fluoranthene	0.017 ±0.001^{d1}	0.004 ±0.001^{c2}
Pyrene	0.004 ±0.002^{d1}	0.002 ±0.000^{c1}
Benz[a]anthracene	0.006 ±0.002^{d1}	0.004 ±0.001^{c1}
Chrysene	0.002 ±0.001^{d1}	0.002 ±0.001^{c1}
Benzo[b]Fluoranthene	0.005 ±0.001^{d1}	0.003 ±0.001^{c1}
Benzo[k]Fluoranthene	0.004 ±0.001^{d1}	0.004 ±0.001^{c1}
Benzo[a]Pyrene	0.005 ±0.002^{d1}	0.005 ±0.001^{c1}
Dibenz[a,h]anthracene	0.001 ±0.001^{d1}	0.000 ±0.000
Indenol[1,2,3-c,d] Pyrene	0.016 ±0.001^{d1}	0.013 ±0.001^{c1}
Benzo[g,h,i]perylene	0.015 ±0.000	0.000 ±0.000
∑Mpah	64.672	39.915
∑PAH4	0.018	0.014

∑mPAH = total mean PAH, ∑PAH4 = sum of the four indicator PAHs. Means with different numbers (letters) in the same row (column) are significantly different (P<0.05). Values are mean ± S.E.M for three replicates, (n = 3)

From the results, it can be seen that naphthalene, acenaphthylene and acenaphthene were predominant in all the samples. Naphthalene concentration was significantly higher (P<0.05) than those of other PAHs analyzed in both species. All the 16 targeted PAHs were detected in all the raw samples except acenaphthylene, dibenz(a,h)anthracene and benzo(g,h,i)perylene which were not detected in Tilapia. Benzo[a]Pyrene (BaP) concentrations were within the range of 1.5 and 10.5 µg kg^{-1} observed in a study of BaP concentrations in four different fish samples from the Niger delta area of Nigeria (Anyakora *et al*., 2008).

Catfish had the highest value of 64.672 mg/kg for total mean PAH (∑mPAH) and sum of PAH4 (∑PAH4) was also higher in catfish. The EU maximum limits for benzo(a)pyrene and total PAHs

in the muscle of smoked fish and fishery products are 2.0 µg/kg and 10 µg/kg respectively (EFSA, 2008). Mean BaP concentrations were above the limit and ∑mPAH values also exceeded the EU limit in both species which is attributed to the high naphthalene concentrations. The high levels of PAH in raw fish muscle can be attributed to the fish rearing process, possibly through the ingestion of PAH contaminated fish feed.

Mean PAH concentrations for each PAH in smoked fish samples are shown in table 5.

Table 5: Mean PAH concentrations (mg/kg) in smoked fish samples

PAHs	Catfish	Tilapia
Naphthalene	68.966 ±0.423^{a1}	41.447 ±0.066^{a2}
Acenaphthylene	0.008 ±0.001^{b1}	0.132 ±0.003^{b2}
Acenaphthene	0.384 ±0.036^{c1}	0.328 ±0.013^{b1}
Fluorene	0.058 ±0.002^{d1}	0.052 ±0.003^{c1}
Anthracene	0.058 ±0.001^{d1}	0.048 ±0.001^{c1}
Phenanthrene	0.060 ±0.001^{d1}	0.049 ±0.003^{c1}
Fluoranthene	0.012 ±0.002^{b1}	0.010 ±0.001^{d1}
Pyrene	0.005 ±0.001^{b1}	0.003 ±0.001^{d1}
Benz[a]anthracene	0.003 ±0.001^{b1}	0.003 ±0.001^{d1}
Chrysene	0.002 ±0.001^{b1}	0.002 ±0.001^{d1}
Benzo[b]Fluoranthene	0.005 ±0.001^{b1}	0.003 ±0.001^{d1}
Benzo[k]Fluoranthene	0.005 ±0.001^{b1}	0.003 ±0.001^{d1}
Benzo[a]Pyrene	0.040 ±0.030^{d1}	0.004 ±0.002^{d2}
Dibenz[a,h]anthracene	0.011 ±0.001^{b1}	0.008 ±0.002^{d1}
Indenol[1,2,3-cd] Pyrene	0.014 ±0.001^{b1}	0.013 ±0.002^{d1}
Benzo[g,h,1]perylene	0.014 ±0.001^{b1}	0.010 ±0.003^{d1}
∑mPAH	69.645	42.115
∑PAH4	0.05	0.012

∑mPAH = total mean PAH, ∑PAH4 = sum of the four indicator PAHs. Means with different numbers (letters) in the same row (column) are significantly different (P<0.05). Values are mean ± S.E.M for three replicates, (n = 3)

All the PAHs analyzed were detected in the smoked samples. Naphthalene concentrations were significantly higher (P<0.05) than the concentrations of other PAHs. Mean BaP concentrations and total mean PAH exceeded the EU maximum limits (2.0 and 10 µg/kg) in the muscle of smoked fish and fishery products. A study of PAH concentrations in fish obtained values of 86.1 and 1026.9 µg/kg dry weight for raw and commercially smoked mudfish (*Clarias gariepinus*) and 104.1 and 611.4 µg/kg dry weight for raw and commercially smoked mackerel (*Scomber scombrus*) (Akpambang *et al*., 2009). These values are less than the values for total PAHs obtained in this study. Another study obtained a BaP concentration of 6.48×10^{-5} and 5.205×10^{-4} mg/kg in freshly and long processed fish samples and these values are lower than results from this study (Ujowundu *et al*., 2014). BaP concentrations ranging from 35.5 to 139 µg/kg dry weight were also found in fish smoked with traditional smoking method (Akpan *et al*., 1994).

Mean chrysene and Benz (b)fluoranthene concentrations were similar (P>0.05) in both raw and smoked samples. Dibenz (a,h) anthracene and benzo(g,h.i)perylene were not detected in raw tilapia sample but were detected in the smoked tilapia sample. Mean chrysene, benzo (b)fluoranthene and indenol(1,2,3-cd)pyrene concentrations did not change, which shows that they were not affected by the smoking process. However, benz(a)anthracene, benzo(a)pyrene and benz(k)fluoranthene were all higher in the raw tilapia sample than in the smoked, which means that the smoking process may have reduced them.

*Conclusion***:** The study revealed that the smoking process increased PAH levels in the Catfish and Tilapia muscles such that mean benzo (a) pyrene concentrations and total mean PAH concentrations exceeded the European Union limits. It is recommended that public health authorities (Abia State Ministry of Health, Federal Ministry of health, National Agency for Food Drugs Administration and Control-NAFDAC etc.) should control and set standards for fish rearing and processing in Abia State and Nigeria due to the associated public health risks.

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