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Dependence of PAH Content on Smoking Technique of two Nigerian Fish

Fehintola, Ezekiel Oluwaseun; Omodara, Niyi Basil; and Oyebade, Adeniyi

Department of Chemistry Adeyemi College of Education, Ondo, Nigeria
Corresponding author: Omodara Niyi B. Tel: +2348038083644 E- mail: polycyclobasi@gmail.com

ABSTRACT
Analysis for the presence and concentration of Polycyclic Aromatic Hydrocarbons (PAHs) were carried out on smoked stock and cat fish at deferent time interval in Ondo, Ondo State Nigeria. The proximate analysis was carried out using the method of AOAC. A representative portion equivalent to about 10g sample was taken from each of the pulverized samples and extracted methanol and dichloromethane using ultrasonicator. The extract was separated/purified using column packed with activated silica gel and alumina (3:1) with n-hexane and a 2:1 ratio of dichloromethane and n-hexane the extract was subsequently analysed using gas chromatography (GC/FID). The results showed that the percentage fat content ranges from 1.05 A2 hr – 25.76 D4 hr, protein content ranges from 25.35 C2 hr – 63.33 B4 hr, the moisture contents ranges from 12.67 B4 hr – 63.26 C2 hr while the carbohydrate ranges from 0.28 A2 hr – 15.97 B4 hr. From the GC/FID analysis, the total PAHs concentration was found to be higher in smoked stock fish B2 – B4 hr (1352.23 – 1736.06 mg/kg) than A2 – A4 hr (3.51 – 13.39 mg/kg). The same pattern was observed in the smoked cat fish where the total PAHs concentration generated in D2 – D4 hr (200.11 – 1702.80 mg/kg) were higher than what was obtained for C2 – 4 hr (15.13 – 246.61 mg/kg). The ratios of phe/ anth for the samples ranged from 0.07 – 8.82, which suggests that the PAHs are from combustion source. Similarly, the ratios of BaP/ cry ranged from 0.01 to 6.79 also suggest that the samples are contaminated with PAHs from pyrolytic origin. Furthermore, Fluo/fluo + Pyr ratio which ranges from 0.01 – 1.00 equally suggested a pyrolytic source of the PAHs. Thus, the relatively high concentration of PAHs in the sample may be attributed to the smoking process.
Keyword: concentration, purified, source, smoked, pyrolytic

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are products formed as a result of incomplete combustion of fuels. PAHs forms a class of diverse organic compounds, each of them contain two or more aromatic rings (Marce and Borrull, 2000). Hundreds of different such compounds may be found and released during a variety of combustion and pyrolysis processes and thus the natural and anthropogenic sources of PAHs in the environment are numerous (JECFA, 2005). Traces of PAHs have been detected in many foods, including vegetable oil, fruits, sea food, grilled and roasted meat, smoked fish, tea and coffee: in particular benzo(a)pyrene has been found in these samples at concentration levels between 0.1 and 100 µg/kg (Kayali-Sayadi et al, 1996; Plaza-Bolanos et al, 2010). Food pollution is due to deposition of PAHs from the air or water or results from preservation, drying and cooking procedures (FSA, 2005). Food processing or cooking steps such as roasting, frying and smoking generates PAHs and increase the level of PAHs in the food being cooked (SCF, 2002). Human can be exposed to PAHs through different routes: For the general population, the major routes of exposure are from foods and inhaled air, while in smokers, the contributions from smoking and food may be of a similar magnitude (Gomma et al, 1993, WHO, 1998 and SCF, 2002). The Scientific Committee on food (SCF, 2002), concluded that a number of PAHs are genotoxic carcinogens and recommended that exposure to PAHs should be as low as reasonably achievable. Sixteen substances were identified as a priority due to their potential genotoxicity and/or carcinogenicity in humans. Smoking/grilling, when carried out with traditional methods involving direct

contact with wood combustion fumes, is responsible for high contamination levels with carcinogenic polycyclic aromatic hydrocarbons (PAHs) (Silva et al, 2011). The fish smoking process is not been given the necessary attention even with its implication on the health of the consuming public. This research is therefore carried out to investigate the dependence of polycyclic aromatic hydrocarbon concentration on the smoking technique used for stock and cat fish that are popularly consume in Ondo

RESEARCH METHODS

Sample collection and preparation

Two species of locally consumed fish in Ondo were used in this study and the fresh samples were bought directly from two different local vendors in Ondo, Ondo State Nigeria. The raw fish are: cat-fish and stock fish. The fish were weighed and their lengths taken using calibrated weighing balance and ruler, then gutted and washed thoroughly with clean water and then smoked while some fresh fish were homogenised using a blender and dried in an oven for two days at low temperature of about 40 ^{o}C. The weighed fish were smoked using two processing methods of smoking as: placing about 10 g of the sample over wire gauze that is on burning firewood and charcoal. The fish were smoked for 2 hours, 3 hours, and 4 hours at temperature range of 170 - 180 ^{o}C and a thermometer used to take the temperature of the smoking process. The smoking process involved turning the samples at interval to allow equal distribution of heat. The smoked fish were further dried in an oven at low temperature of 40 ^{o}C for two days to ensure that the fish samples were properly dried. The smoked/dried fish were then homogenized using a 3kv blender, wraps in aluminum foils and stored in a refrigerator at 4 ^{o}C prior extraction.

Proximate Analysis

This analysis involved several repeated analysis of food to determine their nutrient quantity, it estimates moisture content, ash content, crude fibre, crude protein, fat and carbohydrate as described in the official method of the Association of Official Analytical Chemist (AOAC, 2000).

Extraction of raw and processed samples

The samples were homogenized, 5 g of the pulverized sample was weighed into a test tube and extracted sequentially by ultrasonication using 20 ml of methanol for 20 minutes. After ultrasonication, the supernatant of the extract will be decanted into a beaker and 20 ml of fresh solvent added to the same sample in the test tube for another 20 minutes of ultrasonication. The process will be repeated with another fresh solvent for 20 minutes. After this, to the same sample 20ml of dichloromethane will be added followed by ultrasonication for 20 minutes and the supernatant will be decanted to the beaker containing the methanol extract, this will be repeated for two more times. Furthermore, 20 ml of ratio 1:1 mixture of methanol and dichloromethane will be added followed by ultrasonication for 20 minutes, this same step will be repeated as above for two more times and the supernatant decanted into the same beaker. The combined extract (180 ml) will be centrifuged at 2500 rpm for 10 mins and the supernatant will be decanted and cleaned up using the Whatman filter membrane. The extract will be placed in a safe place covered with aluminum foil which is perforated to allow the solvent escape, before the purification/separation is carried out.

Purification of samples

The purification was carried out by using a well packed chromatographic column. 4 g of activated alumina was placed in the chromatographic column and gently tapped for proper settling. Afterwards, 12 g of activated silica gel is added. The column then pre eluted using 20 ml of n-hexane and allowed it to flow through the column until the first drop of liquid in the column was observed. This was immediately followed with a transfer of sample into the column using micro pipette and eluted with 20 ml of n-hexane. The eluate is collected into a sample bottle to evaporate the solvent, this is the aliphatic profile (saturate). To the same sample in the column 20 ml of dichloromethane is added to elute the sample until there is proper separation and the eluate collected into another sample bottle for evaporation into dryness, this is the aromatic profile. The eluate was then reconstituted by dissolving in 1 ml n-hexane and kept in a vial for Gas Chromatography flame ionization detector (GC/FID) analysis.

RESULT AND DISCUSSION

The GC/FID result of smoked stock fish is as contained in table 1. 28 PAHs were found in the fish samples which were smoked employing charcoal and firewood as the fuel at a temperature range of 170 – 180 ^{o}C. From table 1, which shows the results for firewood smoked stock fish (A 2 - 4 hrs) and charcoal smoked stock fish (B 2 – 4 hrs) at various time intervals. Naphthalene was not detectable in A 2hr – 4hr but, was detected in B 2 - 4 samples. Pyrene was not also detectable in A 2hr, 4hr and B 4hr, also, benzo (j) fluoranthene was below detection limit in A 3hr, B 2hr and 3hr. Futhermore, dibenzo (a,i)pyrene was below detection limit in A 3hr and 4hr. It was also noted that the amount of various PAHs detected were higher in the charcoal smoked fish. The benzo(a)pyrene which is a marker for toxicity/carcinogenicity of PAHs decreases from two hour of firewood smoking (A2 hr) to four hour of smoking (A4 hr), while for charcoal smoking, the concentration remain same for two to three hour of smoking and latter increased for four hour smoking period.

The distribution of the total PAHs shows that the total PAH concentration levels in A 2-4hr were lower (13.39, 7.36 and 16.84 mg/kg for 2hr, 3hr, 4hr, respectively) than that of B 2-4hr samples (1352.23, 1388.63 and 1736.06 mg/kg for 2hr, 3hr, 4hr respectively). This could be due to the high fat and protein content obtained for "B" samples compared to the "A" samples (As contained in table 3). Akpan *et al.* (1994) showed that there is strong correlations between fish lipids and PAH compounds; since PAH compounds are stored in fatty fish tissue. Pyrolysis of the fats in the fish generates PAHs that become deposited on the fish. PAH production by cooking over charcoal is a function of both the fat content in the fish and its proximity to the heat source (Phillips, 1999; Kazerouni et al., 2001).

Table 1: Levels of PAHs obtained in firewood and charcoal smoked stock fish at different time of smoking

PAHs	Concentration of PAHs in Samples (mg/kg)						
	Raw	A2	A3	A4	B2	B3	B4
Na	Bdl	Bdl	Bdl	Bdl	0.31	0.17	0.23
Acy	0.04	1.20	0.17	1.18	0.24	0.66	0.19
Ace	0.08	0.58	0.17	0.33	0.15	0.37	0.13
Fl	0.32	0.87	0.46	0.57	1.01	0.70	1.14
An	0.22	1.61	0.34	0.37	0.94	0.30	0.38
Ph	0.08	0.11	0.10	0.25	0.19	0.11	0.19
Flu	0.30	0.83	0.46	0.59	2.14	0.76	1.43
Pyr	0.01	Bdl	0.01	Bdl	0.15	0.07	Bdl
BcA	Bdl	0.02	0.09	0.01	0.02	0.08	0.47
Cry	0.22	0.26	0.27	0.25	0.38	0.25	0.33
BaA	0.12	0.26	0.19	0.22	1.65	0.32	1.72
BeP	0.26	1.12	0.59	0.04	1.01	0.24	2.87
Bbf	0.29	1.38	1.14	0.84	1.95	1.57	3.23
BaP	0.14	1.76	0.42	0.36	0.39	0.39	0.80
Bkf	0.02	0.21	0.19	0.24	0.36	1.37	0.36
Bjf	0.05	0.47	Bdl	1.45	Bdl	Bdl	0.74
7,12DBA	0.18	0.22	0.22	0.17	0.35	2.65	0.25
InP	0.09	0.28	0.28	4.04	2.67	0.69	205.92
3-MCl	0.34	0.86	0.67	0.60	2.48	3.60	0.63
DahA	0.21	0.79	0.62	3.56	Bdl	0.59	3.09
Bghip	0.28	0.51	0.49	0.92	1.25	0.40	4.97
Dalp	0.14	0.41	0.45	0.39	32.88	0.36	0.63
Daip	Bdl	0.29	Bdl	Bdl	0.04	0.10	0.84
Dahp	0.12	0.36	Bdl	0.48	5.44	Bdl	0.68
Total	3.51	13.39	7.36	16.84	1352.23	1388.63	1736.06

Bdl = below detection limit

Control = raw stock fish, A2, 3 and 4 = firewood smoked stock fish for 2, 3 and 4 hrs, B2, 3 and 4= charcoal smoked stock fish for 2, 3 and 4 hrs; Na = Napthalene, Acy = Acenaphthylene, Ace = Acenapthene, Fl = Fluorene, An = Anthracene, Ph = Phenanthrene, Flu = Fluoranthene, Pyr = Pyrene, BaA = Benzo(a)anthracene, BeP = Benzo(e)pyrene, BbF = Benzo(b)fluorene, BaP = Benzo(a)pyrene, BkF = Benzo(k)fluorene, BjF = Benzo(j)fluorene, 7,12-DBaA = 7,12-Dimethylben(a)anthracene, InP = Indeno(1,2,3-cd)Pyrene, 3-MCl = 3- Methyl chlolanthene, D(a,h)A = Diben(a,h)anthracene, BghiP = Benzo(g,h,i)perylene, DalP = Dibenzo(a,l)Pyrene, DaiP = Dibenzo(a,i)Pyrene, DahP = dibezo(a,h)Pyrene

Results for firewood smoked cat fish (C2 – 4 hr) and charcoal smoked cat fish (D2 – 4 hr) is as shown in Table 2. All PAHs were detected except in the samples D 2hr, 3hr and 4hr where benzo (j) fluorathene was not detectable, in addition, naphthalene and pyrene were not detectable in D 4hr. The total concentrations of PAH in firewood smoked stock fish were lower than the concentrations of PAHs for firewood smoked cat fish. This could also be due to the higher content of fat in the firewood smoked cat fish than in stock fish. Although the total concentrations of PAHs in charcoal smoked fish samples were generally higher, the concentrations of PAHs detected in the charcoal smoked cat fish were higher than those detected in charcoal smoked stock fish. All the high molecular weight PAHs except dibenzo (a,i)pyrene and dibenzo (a,h)pyrene were consistently present in much higher amount than other PAHs in all the samples of fish studied. Studies have shown that eating charcoal smoked food may expose one to the same quantity of PAHs as one would receive from smoking 600 sticks of cigarettes (Ziegler, 2000). The findings of this present study agree with Alonge (1988) who reports that PAHs are common and may constitute health hazards in Nigeria. Since, stock fish and cat fish, smoked with firewood and charcoal, are popular delicacies for all classes of people in Nigeria, a precautionary steps need to be taken based on the health implications of the findings of this study.

Table 2: Levels of PAHs obtained in firewood and charcoal smoked cat fish at different time of smoking

PAHs	Concentration of PAHs in Samples (mg/kg)						
	Raw	C 2	C 3	C 4	D 2	D 3	D 4
Na	0.21	0.24	0.39	3.03	0.96	4.49	Bdl
Acy	0.18	0.63	0.17	0.63	1.57	0.16	0.23
Ace	0.14	0.96	0.18	1.48	8.40	0.24	0.14
Fl	0.25	0.25	1.73	0.99	2.67	0.25	0.58
An	0.16	0.45	0.43	0.35	0.17	0.24	0.21
Ph	0.11	0.57	0.23	1.22	1.52	0.24	0.11
Flu	0.47	12.49	5.01	36.14	4.20	10.50	0.59
Pyr	6.25	39.01	23.82	11.53	48.08	19.91	Bdl
BcA	3.39	24.54	6.18	114.38	74.28	28.78	12.77
Cry	0.24	110.12	25.23	0.26	46.41	1.59	0.29
BaA	0.20	4.50	1.99	0.47	0.43	0.23	0.21
Bep	0.22	15.82	3.78	5.07	0.20	0.92	0.24
Bbf	0.31	0.44	1.77	0.63	0.43	0.43	0.99
Bap	0.37	0.40	10.95	0.63	0.36	1.16	1.60
Bkf	0.23	13.50	0.33	0.45	0.19	0.20	0.78
Bjf	0.03	4.47	0.07	1.37	Bdl	Bdl	Bdl
7,12-DBaA	0.21	0.50	1.32	0.18	1.51	0.20	0.25
Inp	0.26	2.25	0.35	0.27	1.44	1.29	0.33
3-MCl	0.35	1.69	0.91	0.35	4.14	0.67	2.88
DahA	0.52	2.15	1.36	0.57	0.83	0.99	2.25
Bghip	0.30	0.66	0.52	0.36	0.47	0.70	1.97
Dalp	0.32	3.03	0.51	0.39	0.36	0.91	0.42
Daip	0.12	6.09	3.37	1.31	0.85	0.02	0.05
Dahp	0.29	1.85	0.62	0.41	0.62	0.30	Bdl
Total	15.13	246.61	91.22	182.48	200.11	1702.80	1657.11

Control = raw cat fish, C2, 3 and 4 = firewood smoked cat fish for 2, 3 and 4 hrs, D2, 3 and 4= charcoal smoked cat fish for 2, 3 and 4 hrs

The proximate analyses of the samples are as contained in table 3. The moisture content of all the samples decreases steadily from 60.31 – 46.67 % in A, 57.83 – 12.67 % in B, 63.25 – 32.72 % in C and 46.91 – 38.01 in D. The crude protein contents of the samples shows a steady increase in all the samples except in D where it shows a steady decrease. The crude protein content increases from 30.44 – 41.37 % in A, 32.00 – 63.33 % in B, 25.35 – 31.19 % in C while the crude protein content decreases from 40.23 – 37.18

% in D. The fat content shows an increase from 1.05 – 2.17 % in A 2hr – 4hr. In B samples, the fat content increases from 1.10 – 6.06 %, also, there is increase in fat content from 5.13 – 20.15 % in C, while the fat content increases from 7.99 – 25.76 % in D.

Table 3: Results of proximate composition of both the stock fish and catfish samples

	Proximate composition					
Samples	**%MC**	**%Ash**	**%CP**	**%Fat**	**%Fibre**	**%CHO**
Raw stock fish	61.08	7.05	30.05	1.48	0.03	0.31
Raw cat fish	63.51	5.64	25.60	5.21	0.00	0.60
A 2	60.34	7.89	30.44	1.05	0.00	0.28
A 3	58.91	7.07	32.17	1.14	0.01	0.70
A 4	46.67	7.69	41.37	2.17	0.02	2.07
B 2	57.83	5.03	32.00	1.10	0.00	4.03
B 3	38.13	3.45	54.27	3.19	0.01	0.93
B 4	12.67	2.68	63.33	6.06	0.00	15.97
C 2	63.26	5.59	25.35	5.13	0.00	0.67
C 3	58.22	6.11	26.43	8.42	0.00	0.82
C 4	32.72	7.69	31.19	20.15	0.00	8.23
D 2	46.91	4.39	40.23	7.99	0.00	0.48
D 3	42.83	4.15	39.00	13.05	0.00	0.97
D 4	38.01	4.20	37.18	25.76	0.00	0.43

The source of PAHs was determined by molecular indices diagnostic ratio of some PAHs. The ratios obtained for stock fish and cat fish smoked at different time intervals, table 4. The ratios of fluoranthene to pyrene, benzo (a) pyrene to chrysene and phenanthrene to anthracene were selected to predict the source of PAHs found in the fish samples. Ratios of fluorathene to pyrene are greater than 1 suggesting that the PAHs found in the smoked fish were of pyrolytic source. Also, ratio of phenanthrene to anthracene less than 10 indicates combustion source and the ratio greater than 10 suggest petrogenic source (Benlachen et al, 1997). The values obtained for these ratios were all less than 10 which suggest that the PAHs detected in all the samples were from combustion and pyrolytic sources. This implies that all PAHs found in the smoked fish were generated due to reactions initiated or aided by smoking temperature.

Table 4: molecular indices of PAHs in the smoked stock and cat fish samples

Sample	**Phe/anth**	**BaP/cry**	**Nap/acen**	**Fluo/fluo + pyr**
A 2	0.07	6.79	-	1.00
A 3	0.30	1.54	-	0.98
A 4	0.67	1.45	-	1.00
B 2	0.21	1.05	2.05	0.87
B 3	0.36	1.54	0.45	0.91
B 4	0.50	2.46	1.73	1.00
C 2	1.26	0.01	0.25	0.01
C 3	0.52	0.43	2.16	0.07
C 4	3.45	2.46	2.05	0.08
D 2	8.82	0.01	0.11	0.06
D 3	0.98	0.73	9.92	0.01
D 4	0.51	2.08	-	1.00

CONCLUSION

From the result of this research, it was evident that the total PAHs generated from both the charcoal smoked stock and cat fish were much more higher than what was obtained from firewood smoked stock and cat fish. This implies that, smoking with firewood will be appropriate for fish smoking vendor and other individual that smokes fish in their homes. Furthermore, the diagnostic ratio indices calculated

showed that the source of PAHs in both the firewood and charcoal smoked fish is from pyrolytic source. Generally, the concentration of PAHs recorded in this research is higher as a result precautionary steps need to be taken when indulging in smoking fish.

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