DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOME SELECTED SMOKED FISH SPECIES IN GWARJIKO AREA OF NIGER STATE, NIGERIA

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Date Received: 13th Nov., 2010; Date Accepted: 25th Dec., 2010

Abstract

Some fish species smoked with the traditional drum-type smoking kilns from Gwarjiko area of Niger state were screened for their polycyclic aromatic hydrocarbons (PAHs) content using GC/MS. The results from the study showed that the PAHs content in different fish species ranged between 0.54-1.98µg/kg and were below the European Union's recommended limit (5µg/kg) for carcinogenicity in smoked meat and fish products. The result also showed that the Chrysichthys auratus species had the highest PAHs profiles of 1.98µg/kg and Tilapia zilli species the lowest (0.54µg/kg). The cumulative PAHs burden in the studied fish species ranged between 14.62-20.70µg/kg. From the findings from this study, it is suggested that smoking method in use is not too appropriate needed to be improved upon or substituted with modern smoking kilns in order to improve on food security in the country.

Key words: Smoking kilns, polycyclic aromatic hydrocarbons, carcinogenicity, Chrysichthys aurtus, and Tilapia zilli

INTRODUCTION

Polycyclic aromatic hydrocarbons are organic compounds consisting of three or more fused benzene rings containing only carbon and hydrogen. They are formed when complex organic substances are exposed to high temperature or pressure or by the incomplete combustion of woods, coal or oil. They can be found in complex mixtures within the environment (Easton et al, 2002; Storelli *et al*, 2003; Grova *et al*, 2005; Wretling, *et a.l* 2010). At ambient temperature, polycyclic aromatic hydrocarbons are solids with low volatility. They are relatively insoluble in water and soluble in many organic solvents and are highly lipophilic. They have low vapour pressure, relatively high melting and boiling points due to their high molecular masses. Most polycyclic aromatic hydrocarbons can be photo-oxidized and degraded to simpler substances (IPCS, 1998). The mutagenic and carcinogenic properties of PAHs have been linked to physico-chemical properties, such as electronegativity, electrophilic potency, dipole moment, intermolecular and subcellular binding, hydrophobicity and others (Dipple, 1976). They can be found existing in the environment both on land, air, water, soil, plants and different food matrix. Smoked foods have been known for several decades to be sources of PAHs, especially benzo(a)pyrene (Chen *et al*, 1996; Mottier *et al*, 2000, Kazerouni, *et al*, 2001; Simko, 2002; Jira, 2004).

In recent years, a rapidly increasing number of chemical compounds have been found to be carcinogenic, that is cancer causing. Indeed, it has been suggested that cancer is primarily an environmental disease. Just as the draining of swamps and the elimination of mosquitoes can be used to control malaria fever, so the elimination from our environment of carcinogens could reduce the incidence of cancer drastically. Among the most potent carcinogens are certain polycyclic aromatic hydrocarbons (IARC 1987). They are wide spread and may as well be an important cause of human cancer. As a result, much of the research into mode of action of carcinogens has been centered on polycyclic aromatic hydrocarbons (Robert and Robert, 1982).

Foods can be contaminated by PAHs from environmental sources, industrial food processing and during home food preparation. Industrial food processing represents the major source of human exposure from diet (Zabik *et al*, 1996; Kannapan *et al*, 2000; Stolyhwo and Skirkoski 2005; Wretling *et al*, 2010). As PAHs represent an important class of

carcinogens, their presence in foods has been intensively studied (Duedahl Olesen, *et al.*, Jira *et al*, 2008, Reinik *et al* 2007. Of the several hundreds of PAHs, sixteen (16) of them have been identified as priority PAHs because they have been considered to be more harmful to man than the others (Chimezie and Hebert, 2006; Wretling *et al*, 2010). Smoking together with drying and salting belongs to the oldest food preservation methods highly suited to the relative primitive conditions of the past, and still much in used in the developing countries (Damazy, 1977).

Smoke contributes to fish preservation by acting as an effective antioxidant, bacteriostatic and bactericidal agent as well as providing a protective film on the surface of the smoked fish (Eyo, 2001). It also deposits on smoked fish some carcinogenic substances like PAHs. Since wood smoking remains the predominant method of preserving meat and fish particularly in Niger state, and smoke condensate have been discovered to be one of the major sources of polycyclic aromatic hydrocarbons (PAHs). This study is aimed at determining the PAHs in some smoked fish species in Gwarjiko area, one of the major smoked fish processing zones of Niger state with a view to evaluating the extent of contamination in order to ascertain whether they are within the acceptable limit of being non-deleterious to health.

MATERIALS AND METHODS

The following instruments and reagents were used for this study. Gas Chromatography (GC) (HP68990 GC Agilent Technology, Palo Alto CA, USA Mass Spectrophotometer (MS) with flame ionization detector (HP5973 Agilent Technology, Palo Alto CA, USA). Capillary columns; 25mm, 30mm, 0.25im, DB5. 5im.

PAH standard mixture (500µg/mL) containing the 16 target PAHs were obtained from NIST, Baltimore, MD.

PAH internal standard mixture containing five isotopically labeled PAHs acenaphthene-$_{d10}$, pyrene-$_{d10}$, chrysene-$_{d12}$. Perylene$_{d12}$ and benzo(ghi)perylene-$_{d12}$ were obtained from LG Prochem, Boras, Sweden. Dichloromethane (pesticides residue grade) were used for this study.

Sample Collection/ pre-treatment

Three commonly consumed fish species the *Clarias gariepinus*, *Tilapia zilli* and *Chrysichthys auratus* species smoked with the traditional drum-type smoking kilns by local fish processors in Gwarjiko area of Niger state were used for this study. These families of fish were selected for this study because they are the most commonly consumed species in the studied area.

Preparation of standard solution

Five standard solutions each containing the 16 target compounds were prepared by diluting 5, 10, 20, 50 and 100 mL of 500µg/mL of each standard PAH with 100 mL of dichloromethane. To all these solution were added 0.5µg each of the five internal standard namely acenaphthene-$_{d10}$, pyrene-$_{d10}$, chrysene-$_{d12}$, Perylene$_{d12}$ and benzo(ghi) perylene-$_{d12}$. These were transferred into a capped and seal vial until ready for analysis.

Extraction procedure

Five gram of the pounded fish sample was weighed and homogenized with 5g of anhydrous sodium sulphate in a laboratory mortar until a completely homogenate was obtained The homogenate was carefully transferred into the extraction thimble placed in the extraction chamber of a soxhlet extraction unit. The soxhlet extraction was carried out for 16 hour using the USEPA 3540 method (USEPA, 1994) with 150ml of dichloromethane. The extract obtained was concentrated to 2ml using a 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 extracted samples were purified by passing them through a silica gel column prepared by loading 10g of activated silica gel onto a chromatographic column (1cm internal diameter) to about 5cm. This was topped with 1cm of anhydrous sodium sulphate. It was then conditioned with dichloromethane. 2ml of the concentrated extract was loaded and eluted with 20ml of dichloromethane. This method was able to remove the very polar lipids off the extract. The extract obtained was preserved in an amber bottle to avoid oxidation of the extract prior to analysis.

Recovery studies

Prior to extraction 0.5µg of each of the five surrogate standards were added to the sample. This is used to monitor unusual matrix effect, gross sample

processing error. These surrogate standards were used to monitor the recovery of different target compounds. The surrogate standard used include acenaphthene-$_{d10}$, pyrene-$_{d10}$, chrysene-$_{d12}$, Perylene$_{d12}$ and benzo(ghi)perylene-$_{d12}$. The compounds they represent are shown in table 4.3. They serve as surrogate for the different sets of target PAH because they have molecular masses and chemical characteristic close to that of the surrogate. That is acenaphthene-$_{d10}$ has similar molecular mass and chemical properties with naphthalene, acenaphtylene, fluorene and acenaphthene which it represents. The surrogate standards were subjected to the same extractions procedure as described above. The surrogate percentage recovery was calculated using the expression:.

$$\% \text{ Recovery} = \frac{\text{Quantity determined}\ \ \text{Quantity determined}}{\text{Quantity added}} \times 100$$

Calibration

A calibration curve was obtained by running several dilutions 5, 10, 20, 50 and 100ml of each standard PAH and 100ml of the appropriate internal standard per ml of dichloromethane. The target PAH compound/internal standard peak height were plotted against the PAH concentration to obtain a linear graph Y= mx + b, with an intercept (b) on y-axis. The concentration of PAH in each sample was calculate using the formula

PAH (µg/kg) =

$$\text{PAH (µg/kg)} = \frac{\text{Response PAH/Response internal standard b} \times \text{mass of added internal standard}}{\text{Sample weight (g)} \times 1000}$$

The limit of detection (LOD) and the limit of Quantification (LOQ) for each PAH were calculated from the standard deviations of results obtained from the analysis of the several dilutions of the standard PAHs. The LOD for individual PAH in the smoked fish samples were calculated as 3 times the standard deviation of the mean and the LOQ as 10 times the standard deviation of the mean

GC/MS analysis

The Agilent 6850 gas chromatographic equipment with auto sampler was connected to an Agilent FID mass selective detector was used. 5µl of sample solution was injected in the pulsed spilt-less mode onto a 30m × 0.25 mm id DB-12 ms coated fused silica column with a film thickness of 0.15µm. Helium was used as the carrier gas and the column head pressure was maintained at 35 psi to give constant flow 1.1ml/min. Other operating conditions were pre-set, pulse time 0.90mins, purge flow 50ml, purge time 1min, injection temperatures, 300°C. The column temperature was initially held at 70°C for 3mins, increased to 160°C at a rate of 20°C/min, then to rate 210°C at a rate of 3°C/min and to a final temperature of 310°C at a rate 5°C/min and held for 10mins, transfer line 320°C. The mass spectrometer (Ms) condition was electron impact positive ion mode respectively. The PAHs identified was dependent on the retention time in the column since each of the PAHs has their separate retention time in the column. Those with low retention time were identified first then followed by those with longer retention time.

RESULTS AND DISCUSSION

The results obtained from the study are shown in tables 1-3 with table 1 being the chromatographic characteristics of the target compounds , table 2 the PAHs profiles in the studied fish species and table 3 the statistical analysis of the obtained results using one way Anova and Tukey's multi range comparison test.

Table 1: Chromatographic characteristics of the target compounds.

PAHs	Retention time (Mins) GC	Major Peak ion m/z	Internal Standard	Percentage recovery (%)	LOD µg/kg	LOQ µg/kg	RSD (%)
Naphthalene	8.93	128	$Acenaphthene_{d10}$	72.30	0.04	0.14	3.87
Acenaphthylene	13.03	152	$Acenaphthene_{d10}$	72.30	0.04	0.12	1.45
Acenaphthene	13.61	154	$Acenaphthene_{d10}$	72.30	0.03	0.11	1.30
Fluorene	15.03	166	$Acenaphthene_{d10}$	72.30	0.02	0.05	4.00
Phenathrene	17.55	178	$Pyrene_{d10}$	89.60	0.03	0.10	5.38
Anthracene	17.72	178	$Pyrene_{d10}$	89.60	0.04	0.14	3.31
Fluoranthene	20.77	202	$Pyrene_{d10}$	89.60	0.04	0.14	2.61
Pyrene	21.41	202	$Pyrene_{d10}$	89.60	0.05	0.15	3.64
Benz(a)anthracene	24.78	228	$Chrysene_{d12}$	67.24	0.06	0.20	3.95
Chrysene	24.99	228	$Chrysene_{d12}$	67.24	0.06	0.21	2.64
Benzo(b)fluoranthene	27.64	252	$Perylene_{d12}$	80.32	0.01	0.35	1.52
Benzo(k)fluoranthene	27.72	252	$Perylene_{d12}$	80.32	0.01	0.42	2.87
Benzo(a)pyrene	28.33	252	$Perylene_{d12}$	80.32	0.01	0.45	3.79
Indeno(1,2,3-cd)pyrene	30.92	276	$Benzo(ghi)perylene_{d12}$	68.26	0.02	0.62	5.54
Dibenz(a,h)anthracene	31.01	276	$Benzo(ghi)perylene_{d12}$	68.26	0.34	1.14	5.79
Benzo(ghi)perylene	31.49	278	$Benzo(ghi)perylene_{d12}$	68.26	0.05	1.55	8.38

Extraction efficiency

To determine the extraction efficiency of the target compounds based on the selected methods used in this study, recovery studies were carried out using five (5) internal standards each serving as a surrogate for the different target compounds (Table1). Acenaphthene$_{d10}$ served as a surrogate for naphthalene, acenaphthylene, acenaphthene and fluorine. These four compounds have same molecular masses (164) close to that of the surrogate. Pyrene$_{d10}$ as surrogate for phenathrene, anthracene, fluoranthene and pyrene. The molecular mass and structures of these compounds are also similar to that of pyrene. Chrysene$_{d12}$ was used as surrogate for benzanthracene and chrysene. Perylene$_{d12}$ was used as surrogate for benzo(b)fluoranthene, benzo(k) fluoranthene and benzo(a)pyrene. Benzo(ghi)perylene$_{d12}$ was used as surrogate for benzo(ghi)perylene, indeno-1,2,3-(cd)pyrene and dibenz(a,h) anthracene respectively. One common feature existing among these compounds and the surrogate is the possession of 5-6 aromatic rings.

The percentage recoveries obtained by the use of these standards are also shown in Table 1. According to the European commission (2005), PAHs recovery of 50-120% is an indication that a procedure adopted for PAHs analysis is an acceptable procedure. From the table, the efficiency of extraction method ranged from 52.24-69.25%. The GC conditions were set to give a baseline separation of the target compounds in a reasonable time of less than 35minutes. The retention time of the instrument was within the limit reported by other workers of not more than 32 minutes (Andrzej and Zdzislaw, 2005). The PAHs were determined using the procedure described above. The retention time increases with increasing molecular masses, with lower molecular weight compounds having lower retention time than the higher molecular weight compounds (table 1). The retention time ranged from 8.93-31.49 minutes respectively.

Chromatographic characteristics

Table1 also shows the chromatographic characteristics of target compounds using with retention time ranging between 8.93- 31.49 minutes. The limit of detection (LOD), the point at which analysis is just feasible ranged between 0.01-0.34 µg/kg. The limit of quantification (LOQ), the point at which results can be reported with a high degree of confidence ranged between 0.10-1.55µg/kg. The relative standard deviation (RSD) ranged between 1.30-8.38%. The internal standard used for the target compounds, which have similar characteristics with the target compounds are also shown in table1.

Table 2: PAHs Profiles in different smoked

PAHs/ Fish Species	X	Y	Z
Naphthalene	0.65	1.35	0.80
Acenaphthylene	1.34	0.86	1.96
Acenaphthene	1.78	0.55	1.79
Fluorene	1.30	0.56	1.54
Phenathrene	0.59	0.93	1.25
Anthracene	1.88	1.30	0.70
Fluoranthene	1.20	0.65	1.35
Pyrene	1.34	nd	0.70
Benz(a)anthracene	0.85	0.85	nd
Chrysene	1.62	0.54	1.26
Benzo(b)fluoranthene	1.60	1.60	1.40
Benzo(k)fluoranthene	0.60	1.20	0.86
Benzo(a)pyrene	1.20	1.15	1.98
Indeno(1,2,3-cd)pyrene	1.25	0.63	1.63
Dibenz(a,h)anthracene	1.75	1.26	1.57
Benzo(ghi)perylene	0.91	1.19	1.91
Cumulative PAHs	19.86	14.62	20.70

nd = Not detected

X = *Clarias gariepinus* Y= *Tilapia zilli* Z= *Chrysichthys auratus*

From the result in (Table 2) the PAHs content ranged between 0.59-1.88µg/kg in *Clarias gariepinus* with cumulative PAHs burden of 19.86µg/kg. The PAHs content of the *Tilapia zilli* species ranged between 0.54-1.60µg/kg with cumulative PAHs burden of 14.62µg/kg. The PAHs content in *Chrysichthys auratus* ranged from 0.70-1.98µg/kg with cumulative PAHs burden of 20.70 µg/kg Benzo(a)pyrene the index PAH of contamination ranged between 1.15-1.98µg/kg in all the samples, with *Chrysichthys auratus* having the highest of 1.98µg/kg. The result fell below the recommended value of 5µg/kg by the European Union for smoked meats and fish. The result compared favourably with that reported by Karl and Leinemann, (1996) of 0.5-2.4µg/kg using traditional smoking kilns. Steinig and Meyer (1976) also reported similar concentrations of between 0.3-3.9µg/kg in smoked fish using traditional smoking kilns. The other PAHs values of fish species within the area fell within the limit reported by Karl and Leinemann (1996) and Simko (2002) of between 0.1-10µg/kg. Generally, the variations in the PAHs content in all the samples can be attributed to the species, age and physiological compositions of the different fish species and also the conditions and parameters of smoking used. Also the low PAHs profiles values could be attributed to the long time exposure of the fish species before analysis, since they were not collected on the same day they were smoked neither were the analyzed immediately on sampling. This is in line with Stolyhwo and Sikorski (2005) finding that in the presence of light, PAHs are susceptible to photo- degradation and oxidation and that the susceptibilities of individual PAHs are different and their half life of degradation being from a few hours to several days. Simko (1991), also reported that immediately after smoking, that the internal layer of smoked fish contained 10.60 µg/kg benzo(a)pyrene and after seven days storage decreased to 1.30µg/kg. Therefore, the PAHs content in fish depend on the properties of the fish, method and parameters of smoking, composition of the smoke and the duration of exposure to atmospheric conditions.

Table 3: Statistical analysis of PAHs Profiles in different smoked Fish species (M ± SEM) (µg/kg)

PAHs/Fish species	X	Y	Z
Naphthalene	0.65 ± 0.01^{a}	1.35 ± 0.01^{c}	0.80 ± 0.04^{b}
Acenaphthylene	1.34 ± 0.03^{b}	0.86 ± 0.04^{a}	1.96 ± 0.06^{c}
Acenaphthene	1.78 ± 0.04^{b}	0.55 ± 0.01^{a}	1.79 ± 0.01^{b}
Fluorene	1.30 ± 0.03^{b}	$0.56\ 0.04^{a}$	1.54 ± 0.03^{c}
Phenathrene	0.59 ± 0.04^{a}	0.93 ± 0.04^{b}	1.25 ± 0.01^{c}
Anthracene	1.88 ± 0.01^{c}	1.30 ± 0.01^{b}	0.70 ± 0.03^{a}
Fluoranthene	1.20 ± 0.03^{b}	0.65 ± 0.04^{a}	1.35 ± 0.04^{b}
Pyrene	1.34 ± 0.06^{c}	0^{a}	0.70 ± 0.04^{b}
Benz(a)anthracene	0.85 ± 0.04^{b}	0.85 ± 0.06^{b}	0^{a}
Chrysene	1.62 ± 0.04^{c}	0.54 ± 0.03^{a}	1.26 ± 0.06^{b}
Benzo(b)fluoranthene	1.60 ± 0.03^{b}	1.60 ± 0.03^{b}	1.40 ± 0.03^{a}
Benzo(k)fluoranthene	0.60 ± 0.03^{a}	1.20 ± 0.06^{c}	0.86 ± 0.04^{b}
Benzo(a)pyrene	1.20 ± 0.01^{a}	1.15 ± 0.04^{a}	1.98 ± 0.06^{b}
Indeno(1,2,3-cd)pyrene	1.25 ± 0.03^{b}	0.63 ± 0.04^{a}	1.63 ± 0.01^{c}
Dibenz(a,h)anthracene	1.75 ± 0.03^{c}	1.26 ± 0.03^{a}	1.57 ± 0.04^{b}
Benzo(ghi)perylene	0.91 ± 0.01^{a}	1.19 ± 0.04^{b}	1.91 ± 0.01^{c}

Those with different superscripts are significantly different from each other ($P<0.05$), where c>b>a. X = *Clarias gariepinus*, Y= *Tilapia zilli*,, Z= *Chrysichthys auratus*

To validate the obtained result, it was subjected to statistical analysis using one way Anova, at 95 % confidence level (Table 3), From the result of statistical analysis, it is obvious that there are significant differences between the PAHs content in the different fish species represented by different superscripts letters across rows $P<0.05$. There are variations in naphthalene content with *Tilapia zilli species* having the highest value and *Clarias gariepinus* species the lowest. Also, there are significant differences in all other parameters with highest value in acenaphtylene, fluorene, phenathrene, indeno(1,2,3cd)pyrene and benzo(ghi)perylene in *Chrysichthys auratus* species and higher anthracene , pyrene, chrysene and dibenz(a,h)anthracene content in *Clarias gariepinus* species. Generally, *Tillapia zilli* has the lowest value of all the parameters. The variations in the PAHs content of the fish species could be attributed to their different physiological compositions, age and their feeding habits

CONCLUSIONS

Smoked fish constitute a significant part of human diet, because of their desirable sensory properties, high nutritional values and abundant fatty acid. From our study, it was observed that the traditional drum-type smoking kiln was in use for postharvest preservation of fish, where the fish are hung directly above smoldering woods. In such smoking kilns, it is difficult to control the combustion temperature which is often high to enhance the formation of PAHs in smoked fish. Since from our findings, it was discovered that the smoking methods in use impart some traces of PAHs on smoked fish and this could have some cumulative carcinogenic effect with time, we therefore in line with European Union (2005), recommend that smoking process be adequately controlled by suppressing the smoking temperature, reducing the dripping of fats from the fish into the smoking woods which will thereby reduce the negative effect of smoke on fish.

Also, frantic effort should be made by government in improving the electricity generation in the country especially in the fishing zones of the country in order to reduce the usage of traditional smoking methods for their postharvest preservation of fish. They should also come up with modern smoking kilns which will not allow direct transmission of smokes from woods into the fish being smoked.

ACKNOWLEDGEMENTS

My special thanks goes to the management and laboratory staff of the following laboratories, International Energy Services and Jaros Inspection laboratory, all in Portharcourt for giving me the opportunity to work in their laboratories and for their wonderful assistance in putting me through the usage of their various equipment. My profound gratitude also goes to Mr. Sunny for his hospitality and care while I was in Portharcourt for this work.

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