NISEB Journal Vol. 16, No. 2, June, 2016 1595-6938/2016
Printed in Nigeria (2016) Society for Experimental Biology of Nigeria
http://www.nisebjournal.org

Levels of polyaromatic hydrocarbons (PAH) in smoked dry catfish (*Clariasgariepinus*) using GC-MS

Pessu, P.O., Atanda S.A., Ihionu,G.C. and Nwanade, C.F.
Nigerian Stored Products Research Institute 32/34 BarikisuIyede Street off University of Lagos Road Yaba PMB12543 Lagos Nigeria.

Abstract
Polycyclic aromatic hydrocarbons (PAH) are widespread environmental contaminants representing an important group of carcinogens that have been detected in smoked fish. A research work was carried out to determine the levels of polyaromatic hydrocarbon (PAH) in smoked, dry catfish (Clariasgariepinus) using a combination of hot wet smoking and drying at 110^{o}C for 2 hours 45 minutes and drying at 60^{o}C for 18 hours respectively. The PAH in the smoked dry catfish were extracted, cleaned and quantified using gas chromatography/ mass spectrometer (GC-MS). The result showed that the total PAH content in the smoked dry catfish was 1.70 µg/kg. The value was well below the safe limit of 5µg/kg recommended by the European Union for smoked fish. The combination of hot wet smoking and drying is hereby recommended for processing of catfish, other fisheries and livestock products due to the low PAH content obtained. This would further reduce the risk associated with the consumption of smoked products containing PAH which has hitherto lead to rejection of smoked products from Nigeria in international markets. This in turn would enhance value addition, improve the marketability of the product for both local and international markets and reduce the incidence of cancer among consumers of smoked foods.
Keywords: polyaromatic hydrocarbons, smoking kiln, multipurpose dryer, carcinogenic, GC-MS

Introduction
Fish has long being recognized as a valuable source of cheap, affordable high quality protein and other nutrients needed in human diet especially that are required for body growth and maintenance(1). The African catfish, *Clariasgariepinus*, is easilycultured in Nigeria and is of great economic interest. It has enjoyed wide acceptability in most parts of the country because of its unique taste, flavour and good texture (2). An estimate of 40% post- harvest loss of total fish landings have been reported in Nigeria. This is due to its high perishable nature largely due to handling, prevailing high ambient temperature, among other factors, leading to increase in bacterial and enzymatic actions coupled with inadequate infrastructure for post-harvest processing technology (processing, preservation and marketing) (3, 4). A number of processing techniques are in operation in Nigeria. These include chilling, freezing, salting, canning, drying and smoking. However, smoking is the most popular method of fish processing. It was reported that smoking involves heat application to remove water, inhibit bacterial and enzymatic action on fish. It imparts aroma, taste and colour on processed fish (5, 6). Traditionally, fish is smoked in pits or on raised smoking "tables" where the control of heat is difficult and at times impossible. Traditional direct smoking process involves the generation of smoke in the same chamber where the product is processed, exposes it to excessive and uncontrolled smoke containing mostly PAH (7).PAHs are ubiquitous environmental pollutants, resulting from the incomplete combustion or pyrolysis of organic matter during industrial processing and various human activities. They originate from diverse sources such as tobacco smoke, engine exhausts, petroleum distillates, coal-derived products, with combustion sources predominating and processing of food at high temperatures (grilling, roasting, frying and smoking). They are a class of high lipophilic, hydrophobic compounds soluble in organic solvents. Most PAHs are known to be potent carcinogens and based on this; PAHs have been included in the European Union (EU) and the United States Environmental Protection Agency (USEPA) priority pollutant lists (8, 9). In 2005, the European Commission (EC) introduced Benzo [α] pyreneBaP (as a marker of the occurrence and carcinogenic potency of the entire class of carcinogenic and genotoxic PAHs) at maximum levels of 5 µg kg $^{-1}$ in smoked fish and meat [10]. The limit is to be further reduced to 2.5µg kg $^{-1}$in coming years. Up till now no maximum allowable levels for PAHs and/or BaP have been established by the government of Nigeria.BaP concentrations ranging from 11.1 to 66.9 and from 35.5 to 139 µg kg $^{-1}$ dry weight, respectively in Nigerian traditionally smoked fish have been reported (11, 12). Also, values of total petroleum hydrocarbon (TPH) serving as a precursor for the presence of PAH in smoked fish using Nigerian Stored Products Research Institute (NSPRI) smoking kiln and other smoked fish obtained from some markets in Lagos State have been reported. The results showed TPH in NSPRI kiln smoked fish was not detected while smoked fish from Mushin, Bariga, Egbeda, Makoko have TPH values of 0.0007 mg/kg, 0.0005 mg/kg, 0.0026 mg/kg and 0.0003 mg/kg respectively (13).Based on this incidence of high PAH reported in smoked fish in Nigeria, this study was aimed at processing catfish using a combination of smoking and

Corresponding Author's Email: abimbola91@yahoo.com

drying procedure with modified (NSPRI) fabricated kiln and dryer to produce smoked catfish with a low PAH content.

Materials and method

Fish processing

Fifty live catfish (*Clariasgariepinus)* (20 kg) were obtained from a fish farm in AbuleIjesha-Yaba, Lagos State and transported using NSPRI fish box in ice to the institute's processing centre. They were stunned, cleaned, filleted, trimmed and were spiced using combination of ginger, garlic, salt, onion.

Smoking equipment

The modified smoking kiln is an all metal structure with insulation in between the metal sheet layers with a dimension of 60cm * 60cm * 120cm. It has an opening (vent) on each side of the combustion chamber to allow in air for combustion; and a fan powered with a solar battery to aid combustion and proper circulation of air within the kiln.The combustion chamber is 41cm from the base of the kiln. There are four tray layers in the smoking chamber at heights 56cm, 71.5cm, 87.5cm and 102cm respectively.There is a chimney at the top to allow warm humid air escape from the smoking chamber.The kiln is fitted with four rollers at the base to allow for easy transportation.The modified smoking kiln smokes faster and has a better fuel efficiency than the initial design (Figure 1).

Multipurpose dryer

The multipurpose dryer is a rectangular structure consisting of two major compartments; the heating chamber and the drying chamber. The drying chamber houses trays used for various agricultural produce. The two compartments are separated by a heat exchanger plate constructed of flat steel plate. The heat source is kerosene stove placed under the heating point which was regulated to give clear blue flame (Figure 2).

Smoking and drying procedure

The smoking chamber of the kiln was preheated with red hot charcoal for 30 minutes placed in the fuel tray at the base of the smoking kiln before loading the fish and also the perforated sheet surface was greased with groundnut oil to prevent the fish from sticking unto the sheet. The spiced fish were arranged on the perforated sheet and turned repeatedly to enhance even smoking. The fish were smoked for 2 hours 45 minutes with smoking temperature ranged between 70^{o}C and 100^{o}C (hot smoking). The smoked fish were later removed and transferred into a multipurpose dryer that have been prepared for drying. The smoked catfish were spread on trays and left to dry for a period of 18 hours at a temperature between 45-55^{o}C for proper drying.

Figure 1: NSPRI Smoking Kiln

Figure 2: NSPRI Multipurpose Drier

Sampling

After the smoking and drying process, 200 g of smoked dry catfish was taken from all the parts. The sample was cooled at 4 ^{o}C and then sent to the laboratory. Afterwards it was homogenatedin laboratory using sample mill.

Reagents and standards

Cyclohexane (*ECD* tested), N,N-dimethylformamide, methanol (HPLC grade), and sodium chloride (ACS) were purchased from Acros, ethanol – from J.T. Baker, sodium sulphate (ACS) – from Fluka, potassium hydroxide – from Avsista. PAH reference standards mixture (500µg/mL) containing the 16 target PAHs obtained from NIST Baltimore, MD while Anthracene-D10-Ant-10, deuterated benzo[a]pyrene-d_{12}, (Sigma-Aldrich) and benzo[g,h,i]perylene (LGC standard) were used as internal standards.

Equipment

MilliQ filter system was used to produce double distilledwater (Millipore, Bedford, MA, USA),
Gas chromatography (HP 68990GC Agilent Technology, Palo, Alto CA, USA) mass spectrophotometer with flame ionization detector (HP 5973 and silica solid phase extraction (SPE) tubes (500 mg) from Phenomenex was used for this study.

Extraction and quantification of PAHs

All the glassware was carefully washed and rinsed with distilled solvent (acetone and hexane) before use. The extraction and quantification of PAHs from smoked dry catfish were carried out with slight modification [8].

Stock solutions

Stock solutions containing 2 µg/mL of PAH's standard were dissolved in toluene while internal standards consisting of Chrysene, Anthracene-D10-Ant-10, deuterated benzo[a]pyrene-d_{12}
(2 µg/mL) were dissolved in toluene and benzo[g,h,i]perylene (200µg/mL) was dissolved in cyclohexane. They were all stored at 4°C, in volumetric flask (with glass stoppers) wrapped in aluminum foil to avoid possible light degradation.

Saponification and extraction

5 g of homogenized sample was fortified with labelled internal standards and hydrolyzed with the solution of methanolic potassium hydroxide in a Duran bottle. A microwave assisted extraction was carried out at 120 °C for 20 minutes in an ultrasonic bath with n-hexane. The n-hexane solution was washed twice with water in equal volume (50 mL), afterwards – with a mix of methanol and double distilled water, then re-extracted with N, N-dimethylformamide/water (9:1) blend, and repeatedly extracted from it with n-hexane.

Clean-up

The silica- SPE- cartridge was conditioned with 3 mL of hexane after which the extract was applied to the cartridge and eluted into evaporator glass tube with 5 mL of hexane, concentrated by evaporation under a stream of nitrogen and reconstituted with cyclohexane and internal standards.

Chromatographic conditions

Helium was used as the carrier gas and the column head pressure was maintained at 35 psi to give constant flow 1.2mL/min. The column temperature was initially held at 70^{o}C for 3minutes, increased to160^{o}C at a rate of 20^{o}C/min, then to 210^{o}C at a rate of 3^{o}C/min and to a final temperature of 310^{o}C at a rate of 5^{o}C/min and held for 10minutes and transfer line of 320^{o}C.Injection volume was 10 µL while the mobile phase (acetonitrile-water) gradient was 80% acetonitrile +20% water with a flow rate of 1.2mL/min. Other operating conditions were pre-set: pulse time 0.90 minute, purge flow 50 cm^{3}, purge time 1minute and injection temperature 300^{o}C.

Results

Identification of PAHs

The measured peak areas are the SRM chromatograms representing the detection of any of the 16 PAHs is indicated by the mass per charge ratio (m/z) in relation to the retention times as shown in Figure 3. The individual and total PAH concentrations are shown in the Table 1.

Quantification

Calibration by internal standardization is employed in quantification of PAH's. It requires the determination of response factor R_f.

Response factor Rf= A_{St} * C [IS]/A[IS] *C[St]…….. (i)

R_f = response factor as determined by the analysis of standards (PAH and internal standard)

ASt= area of the PAH peak in the calibration standard

A[IS] = area of the internal standard in the calibration standard

C[St] = PAH concentration for the calibration standard solution

C [IS] = internal standard concentration for the calibration standard solution

Calculation for absolute amount that was extracted from the sample

$X_{PAH} = A_{PAH} * X_{[IS]} / A_{[IS]S} * R_f$ .....(ii)

X_{PAH} = absolute amount of PAH that was extracted

A_{PAH}= area of PAH peak of the sample

$A_{[IS]S}$= area of internal standard peak of the sample

$X_{[IS]}$= absolute amount of internal standard added to the sample

The concentration of PAH in the sample

C (µg/kg) = X_{PAH}/ m……..(iii)

C (µg/kg) = concentration of PAH in the sample

m (g) = mass of the sample

Table 1: Chromatographic properties of the test priority compounds

Retention Time (min)	Major Peak Ion m/z	Area (pA*s)	Amount/Area	Mean concentration (µg/kg)	Group	Name
7.978	128	316.722495	8.0128e-9	2.53786e-6	1	Naphthalene
8.801	152	52.44293	1.54321e-8	8.09304e-7	1	Acenaphthylene
9.852	154	32.52925	4.27350e-8	1.39014e-6	1	Acenaphthene
11.353	166	122.32583	1.98413e-6	2.42710e-4	1	Fluorene
12.934	178	67.57877	7.04225e-3	4.75907e-1	1	Phenanthrene
14.738	178	35.25928	9,08100e-3	3.20190e-1	1	Anthracene
16.038	202	131.55203	3.25436e-3	4.28118e-1	1	Fluoranthene
16.916	202	43.58090	4.80767e-3	2.09523e-1	1	Pyrene
18.055	228	276.03128	1.61129e-4	4.44767e-2	1	Benzo (α) anthracene
19.404	228	178.92691	4.75186e-4	8.50235e-2	1	Chrysene
20.601	252	96.74863	9.51131e-4	9.20206e-2	1	Benzo (α) fluoranthene
21.674	252	205.23669	6.27888e-5	1.28866e-2	1	Benzo (k) fluoranthene.
22.789	252	126.27307	1.02862e-4	1.29887e-2	1	Benzo (α) pyrene
24.094	276	220.18590	1.02862e-5	2.26488e-3	1	Indeno (1,2,3-cd) pyrene
25.698	276	142.85168	1.14937e-4	1.64190e-2	1	Dibenzo (a, h) anthracene
27.815	278	52.04700	3.68186e-5	1.91630e-3 1.70198	1	Benzo (g, h, i) pyrene

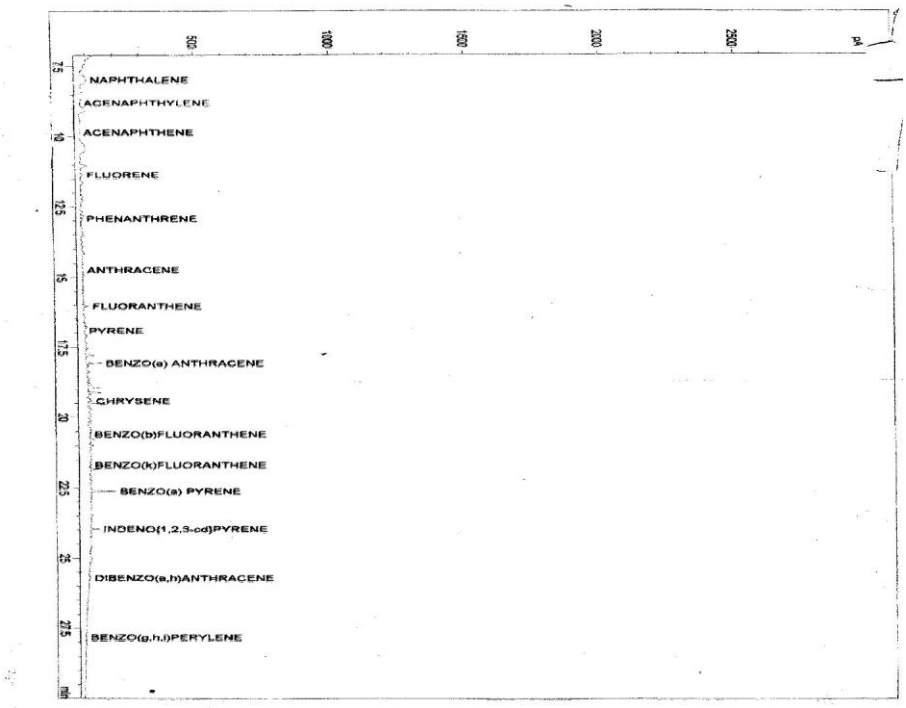

Figure 3: Mass per charge ratio (m/z) in relation to the retention times

Discussion

In this study, the amounts of the low molecular weight PAHs such as naphthalene, fluorine, pyrene, fluoranthene and anthracene were found to be slightly lower than the high molecular weight PAHs such as benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene in smoked dry catfish sample (Table 1). The retention time increases with increasing m/z ratio of the target compounds. The retention time obtained was within the limit reported by other workers of not more than 32 minutes (14, 15).Benzo [α] pyrene has the least concentration of while benzo [α] fluoranthene has the highest concentration.Levels of PAHs in the raw catfish were insignificant as they were under the limit of detection (<0.1 µg kg^{-1}). The main amount of PAH in smoked foods comes from wood smoke. Cold- and hot-smoked fish contain much more PAH than raw fish, depending

on the properties of the fish, method and parameters of smoking, composition of the smoke, and exposure to the smoke.The variation of PAH contents of smoked fish depend on the type of smoking oven (automatic smoking oven or traditional smoking oven). The density of smoke was regularly controlledwith fan powered with a solar battery to aid combustion and proper circulation of air throughout the smoking process. Wood smoke contains appreciable amounts of carcinogenic PAHs, which are the main cause of concern regarding its toxicity. However, according to a research, the combustion of charcoal, being an already pyrolized material, gives a relatively clean smoke (16) and accordingly, lower PAH levels in the catfish sample coupled with the drying in the multipurpose dryer. The results obtained from this study were significantly lower than those reported byother authors for samples from the Nigerian market (12, 17).

Conclusion and Recommendations

Based on the ubiquitous nature of PAH in the environment, the incidence of contamination of catfish cannot be excluded however from this study the total amount of PAHs found in catfish smoked and dried using NSPRI smoking kiln and dryer have shown that the commodity is safe for consumption. BaP amount as a marker of toxicity was below the limit of 5 μg kg^{-1}set by the European Commission in 2005. In agreement with previous works, it has been demonstrated that, the use of controlled charcoal smoke instead of smoke from burning wood can help in lowering the final PAH load at levels as 2 μg kg^{-1}. It is highly recommended that regulatory bodies in Nigeria should enforce the use of this process to obtain safe traditionally smoked foodstuffs within the stipulated legal limits.

References

1. Adeyemi OT, Osilesi OO, Onajobi F, Adebawo OO, Oyedemi SO, Afolayan A J: Stability study of smoked fish, Horse Mackerel (*Trachurustrachurus*) by different methods and storage at room temperature. *African Journal of Biochemistry Research*, 7 (6): 98-106, 2013.

2. Osibona, A. O., Kusemiju, K., Akande, G. R: Proximate composition and fatty acids profile of the African Catfish *Clariasgariepinus*acta SATECH 3(1) :20-25, 2006

3. Akande GR: "Post-harvest processing in Fisheries". A paper presented at training for officers of UNDP assisted programme on artisanal fisheries development, Ogun State at Federal College of Fisheries and Marine Technology, Lagos. 1- 20, 1996.

4. Saliu JK: Effect of smoking and frozen storage on the nutrient composition of some African fish. *Adv. Nat. Appl. Sci.* 2(1): 16-20, 2008.

5. Eyo AA: Fish processing technology in the tropics. University of Ibadan Press, Ibadan, Nigeria. pp. 165-168, (2000)

6. OlleyJ, Doe PE,Heruwati ES: The influence of drying and smoking on the nutritional properties of fish. In Burth JR (**eds**); An Introd. overview in fish smoking and drying. Elsevier, London, pp. 1- 14, 1988.

7. AkpambangVOE, Purcaro G, Lajide L, AmooIA, Conte LS, Moret S: Determination of polycyclic aromatic hydrocarbons (PAHs) in commonly consumed Nigerian smoked/grilled fish and meat "*Food Additives and Contaminants*. 1096-1103, 2011.

8. Stumpe-Vīksna,I Morozovs, A: Levels of Benzo(a)pyrene (BaP) in fish smoked according to different procedures *LLU Raksti* 21 (315) :24-29, 2008.

9. Šimko, P: Determination of polycyclic aromatic hydrocarbons in smoked meat product and smoke flavouring food additives. *Journal of chromatography B*, 770: 3-18, 2002

10. BasakS, Şengör GF, Karakoç, FT: The Detection of Potential Carcinogenic PAH Using HPLC Procedure inTwo Different Smoked Fish, Case Study: Istanbul/Turkey *Turkish Journal of Fisheries and Aquatic Sciences* 10: 351-355, 2010.

11. Afolabi OA: Quality changes in Nigeria Traditionally Processed Freshwater species. *J. Food Technol.* 18:17-29, 1984

12. Akpan V, Lodovici M, Dolara P: Polycyclic aromatic hydrocarbons in fresh and smoked fish samples from three Nigerian cities. *Bull. Environ. Contam. Toxicol.* 53:246- 253, 1994

13. Atanda, SA, Pessu PO, Kemabonta KA, AgodaS,Isong IU, Borisade TJ, Usanga, OE, Olatunde IG, Adeniran TR, Abiose OR: Comparative assessment of the levels of total petroleum hydrocarbon (TPH) n local and Nigerian Stored Products Research Institute (NSPRI) kiln smoked fish.*Journal of Chemical Society of Nigeria.* 2:102-106, 2011.

14. Andrzej S, Zdzislaw ES: Polycyclic aromatic hydrocarbons in smoked fish: critical review. *J. of Food Chemistry*,91: 303-311, 2005.

15. Ajai AI, Suleiman, MAT, Dauda BEN, Sadiku SOE, Aberuagba F: Effect of extraction methods on the polycyclic aromatic hydrocarbons content of smoked catfish species in Niger State of Nigeria. *Jordan Journal of Biological Sciences*, 5: 71 – 80, 2012.

16. Larsson, BK, Sahlberg, GP, Eriksson AT, Busk LÅ: Polycyclic aromatic hydrocarbons in grilled food. *J. Agric. Food Chem.* 31:867-873, 1983.

17. Anyakora C, Arbabi M, Coker: A screen for benzo(a)pyrene in fish samples from crude oil polluted environments. *Am. J. Environ. Sci.* 4:145-150, 2008