OPEN ACCESS

Scholars International Journal of Chemistry and Material Sciences

Abbreviated Key Title: Sch Int J Chem Mater Sci
ISSN 2616-8669 (Print) |ISSN 2617-6556 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://saudijournals.com/sijcms/

Original Research Article

Proximate Analysis and Quantification of Polycyclic Aromatic Hydrocarbons in Some Smoked and Roasted Food Items

Omodara Niyi Basil*, Fehintola Ezekiel Oluwaseun and Ojo Babatunde Moses

Department of Chemistry, Adeyemi College of Education, Ondo, Ondo State, Nigeria

DOI: 10.36348/sijcms.2019.v02i08.001 | **Received:** 08.10.2019 | **Accepted:** 15.10.2019 | **Published:** 20.12.2019

***Corresponding author:** Omodara Niyi B

Abstract

Analysis for the presence and concentration of sixteen Polycyclic Aromatic Hydrocarbons (PAHs) were carried out in roasted plantain, roasted meat and smoked fish in Ondo, Ondo State Nigeria. The proximate analysis was carried out using the method of AOAC. A representative portion of about 5 g of sample was taken from the homogenized sample and extracted with ultrasonicator using three solvent systems: methanol, methanol: dichloromethane (1:1v/v) and dichloromethane. The aromatic fraction was subsequently analyzed with Gas Chromatography (GC/FID). The results showed that the percentage fat content ranges from 4.32 % roasted plantain to 19.27 % roasted meat, protein content ranges from 6.07 % roasted plantain to 44.86 % smoked fish, the moisture contents ranges from 19.39 % smoked fish to 58.31 % roasted plantain while the carbohydrate ranges from 8.89 % roasted meat to 22.09 % roasted plantain. From the result of GC/FID analysis, sixteen PAHs found in the samples ranged from 0.99 – 0.10 µg/kg, 0.01 – 0.73 µg/kg and 0.00 – 0.72 µg/kg for roasted plantain, roasted meat and smoked fish respectively. The sum of all PAHs concentration found in the samples was 6.16 µg/kg for roasted plantain, 6.22 µg/kg for roasted meat and 4.97 µg/kg. The ratios of phenathrene to anthracene ranged from 1.21 in fish to 3.66 in meat, which suggest that the PAHs are from pyrogenic source. Similarly, the ratios of Flouranthene to Pyrene which ranged from 1.01 in plantain to 3.76 in fish also suggest pyrogenic source due to combustion and the benzo (a) anthracene to chrysene ranged from 0.03 in meat to 0.51 in plantain which equally suggested pyrogenic source. Thus, the relatively high concentration of PAHs in the roasted plantain and meat may be attributed to the smoking process.

Keywords: Eluants, extraction, gas chromatography, pyrolytic.

INTRODUCTION

Polycyclic Aromatic hydrocarbon (PAHs) are hazardous organic chemicals consisting of two or more benzenoid group [1]. Those containing up to four benzene rings are known as light PAHs (L-PAHs) and those containing more than four benzene rings are known as heavy PAHs (H-PAHs). H-PAHs are more stable and toxic than L-PAHs [2]. They are ubiquitous pollutants in the environment. The presence of PAHs in the environment is of global concern because of their carcinogenicity and other health related challenges [3]. In fact, some have been demonstrated to be mutagenic and carcinogenic for humans [4]. Those PAHs that are considered to be less toxic may even increase the carcinogenicity of other PAHs [5, 3]. Sixteen of the PAHs that are considered as priority by the American Environmental Protection Agency (AEPA) are; naphthalene, acenaphthylene, acenaphthene, fluorine, anthracene, phenanthrene, fluoranthene, chrysene, benzo (a) anthracene, pyrene, benzo (k) fluoranthrene, benzo (b) fluoranthene, benzo (a) pyrene, dibenzo (a,h) anthracene, dibenzo (b,c) fluoranthene and benzo (g, h, i) perylene [1].

A very high number of the PAHs have been established to be the products of incomplete combustion of wood, oil, coal and garbage [6]. Thus, man can be exposed to PAHs through the inhalation of smoke from combustion of this biomass. However, studies have shown that diet is the main source through which man is exposed to PAHs, with grains and vegetables being the major dietary sources [7]. Goman *et al.*, [8], in their study showed that the highest levels of PAHs were found in foodstuff both processed and unprocessed. As PAHs are ubiquitous in the environment, it is not surprising that they are present in almost all food. For example, it has been reported that cereals were found to contain PAHs at levels of 6 - 14 µg/kg, fats and oils at 8 - 11 µg/kg and seafood at 7 - 8 µg /kg respectively [7,

9]. However, a high level of PAHs is not usually observed in raw food [10]. Food processing or cooking steps such as roasting, grilling, barbecuing and smoking generate PAHs and increase the level of PAHs in the food being cooked [11]. Charred food of almost any composition contains PAHs [5] while only very low level of PAHs was detected when food was cooked by some cooking steps such as steaming. In some studies, cereals were found to be the main dietary source of PAHs, accounting for some 27 to 35% of total dietary exposure, a result probably due to the high amount of consumption [11]. Although barbecued food only contributed a smaller part of the PAHs intake, people with a diet rich in roasted, barbecued or grilled, smoked food may have significant intake of PAHs [11].

The consumption of traditionally smoked and roasted foods could be responsible for the higher incidence of primary liver and stomach cancer in Nigeria compared with that in Europe and in the USA [12]. In south western part of Nigeria today, roasted plantain, roasted meat and roasted corns are some processed foods that are often consumed by people. Also, smoked fish and meat are popular delicacies among all classes of people. These foods are very often consumed and are suspected of containing contaminants such as PAHs that are harmful to human health [13]. Even though, different researcher has worked on the concentrations of PAHs in some foods in the western world. However, PAHs in food has not been fully studied in Nigeria. Therefore, this research was carried out to determine the proximate composition and quantify the concentration of PAHs in some roasted and smoked food samples.

MATERIALS AND METHODS

MATERIALS

The equipment, reagents and wares materials used in this study were of Analytical grade and food standards. They were obtained from Pascal Scientific and Laboratory stores Akure and Multi-Environmental Management Consultancy Ltd., Ikorodu, Lagos, while the wet Chemistry was carried out in the Department of Chemistry Adeyemi College of Education Ondo.

Sampling

Samples of Plantain and fish samples were bought directly from farmers in Ondo while the cow meat was purchased from abattoir in Ondo.

Preparation

The fresh fish samples Atlantic mackerel (*Scomber scombrus)* were thawed, scaled, eviscerated and washed in water. These were then placed over wire gauze on open charcoal fire. The cow meat was prepared from boneless beef. The meat was cut into pieces and staked on sticks and spiced with groundnut oil, salt, dried pepper and flavourings such as monosodium glutamate. The sticks are then arranged round on wire gauze placed over an open charcoal fire [14].

Roasted plantain was prepared by firstly washing fairly riped plantain in water and peeled. The raw pulps are placed on wire gauze that was over an open charcoal fire to roast until they were slightly brown.

Proximate Analysis

The proximate analysis involved several repeated analysis of the samples to determine their moisture content, ash content, crude fiber, crude protein, fat and carbohydrate, using standard methods as described in the official method of the Association of Official Analytical Chemist [15].

Extraction of Samples

Each of the samples was pulverized to ensure homogenization. 10 g of the pulverized sample was weighed into a test tube and extracted sequentially by ultrasonication for 20 minutes using 20 ml of methanol. After ultrasonication, the supernatant of the extract was decanted into a beaker and 20 ml of fresh solvent added for another 20 minutes of ultrasonication. The process was repeated with another fresh solvent for 20 minutes. After this, to the same sample 20ml of methanol and dichloromethane ratio 1: 1 was added followed by ultrasonication for 20 minutes and the supernatant was decanted to the beaker containing the methanol extract, this was repeated twice. Furthermore, 20 ml of dichloromethane was added followed by 20 minutes of ultrasonication. This step was repeated for two more times and the supernatant decanted into the same beaker. The combined extract (180 ml) was centrifuged at 2500 rpm for 10 mins and the supernatant decanted and cleaned up using whatman filter membrane. The extract was placed in a safe place covered with perforated aluminium foil to allow the solvent escape, before the separation/clean up.

Clean-up of Samples

The clean-up was carried out by using a packed chromatographic column. Accurately weighed activated alumina (4g) was placed into the chromatographic column. Afterwards, 12g of activated silica gel was added to the top of the alumina in the column. The column was pre eluted using 20 ml of n-hexane which was allowed to flow through the column until the first drop of liquid in the column was observed. Fractions in the extract were eluted sequentially as follows: Saturate fraction, eluted with 20 ml of n-hexane, eluate collected into sample bottles and was evaporated to near dryness. Polycyclic aromatic hydrocarbons fraction, eluted with 20 ml mixture of n-hexane: dichloromethane (3:2), eluate also collected into sample bottled and evaporated to near dryness. The collected aliphatic fraction was not subjected to further analysis while the polycyclic aromatic fraction was reconstituted by dissolving in 1 ml n-hexane and kept in refrigerator for GC/FID

analysis. The procedures were repeated for all the samples.

GC Analysis of PAHs

The polycyclic aromatic hydrocarbon analysis was carried out using gas chromatograph system. The system consists of a Hewlett Packard Model 5890 gas chromatography (GC) equipped with a flame ionization detector (FID) and a data processor (Hewlett Packard, Wilmington, DE, USA) .The Column used was HP-1932530, a non-polar, fused-silica capillary column (30 m length × 25 µm inner diameter × 0.25 µm film thickness). The oven temperature was set initially at 60 °C (5 min hold), increased to 250 °C at 15°C/min for 14 min. and a final ramp to 320 °C at 10 ºC/min (4 min hold). Nitrogen gas was used as the carrier gas at a flow rate of 1 mL/min at a pressure of 30 psi. The injector temperature was set at 250 °C, injection volume was 1 mL (splitless) and the detector temperature was set at 320 °C. Verification of peaks was carried out based on retention times compared to those of external PAHs standards.

RESULTS AND DISCUSSION

From the result of proximate analysis of the samples as shown in Figure-1, the moisture content ranges from 19.39 % smoked fish to 58.31 % roasted plantain, the fat content ranges from 4.32 % roasted plantain to 19.27 % roasted meat. For the protein content it ranges from 6.07 % roasted plantain to 44.86 % smoked fish, crude fibre ranges from 0.20 % smoked fish to 0.42 % roasted plantain while for carbohydrate it ranges from 8.89 % roasted meat to 22.09 % roasted plantain.

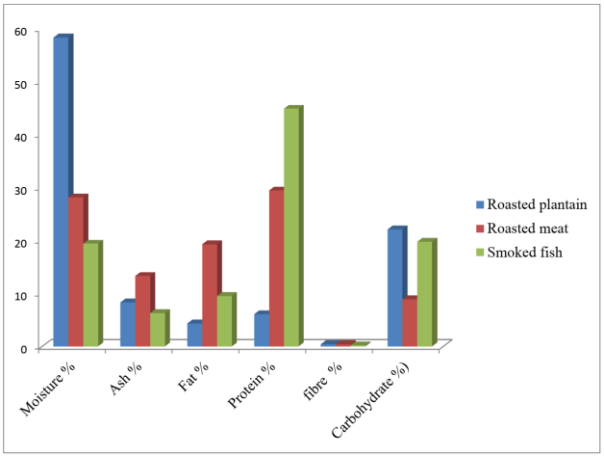

Fig-1: Proximate composition of the samples

The concentrations of various PAHs present in the food samples is shown in Table 1. All the 16 targeted PAHs were detected in reasonably quantity in all the samples except in the roasted meat where Dibenzo(a,h) anthracene was below detection limit.

The concentration of PAH in the roasted plantain ranges between Fluorathene 0.10 µg/kg and Dibenzo(a,h) anthracene 0.99 µg/kg; while in roasted meat, it ranges from Dibenzo(a,h) anthracene 0.01 µk/kg to Benso (a)anthracene 0.73 µg/kg, the concentration of PAHs in smoked fish ranges from Dibenzo (a,h) anthracene which is below detection limit to Chrysene 0.72 µg/kg. As can be seen from Table1, the average total PAH level of roasted plantain is 6.16 µg/kg that of roasted meat is 6.22 µg/kg while for the smoked fish the average total PAH is 4.97 µg/kg. The total PAHs concentration for roasted meat was the highest (3.30 µg/kg), this could be ascribed to high fat content of the meat (19.27%) compared to that of fish (9.51%) and plantain (4.32%). Akpan *et al*., [16] reported that strong correlation exists between lipids and PAH compounds; since PAH compounds are stored in fatty tissue. Although, protein content has also been linked to the PAHs concentration in food samples, this is at variance with the result of this present study as roasted plantain with protein content of (6.07%) has higher concentration of PAHs (2.15 µg/kg) than the smoked fish with the highest protein content of 44.86 % but has a total PAHs of 1.23 µg/kg. This may be due to the longer time of smoking during which there must have been oxidation of PAHs. PAHs with maximum concentrations detected in roasted plantain was Dibenzo(a,h) anthracene (0.99 µg/kg) while benzo(a) anthrancene (0.73 µg/kg) was detected as maximum concentration in roasted meat, for smoked fish PAH with the maximum concentration detected was Chrysene (0.72 µg/kg).

In this study, the sum of the average amounts of the low molecular weight PAHs (those containing 2 to 4 aromatic rings) such as naphthalene, acenaphthene, and pyrene, were found to be lower 2.59 µg/kg than the high molecular weight PAHs 3.62 µg/kg , those having 4 to 6 aromatic rings such as benzo(a)anthracene, benzo(a)pyrene [BaP], indeno (1,2,3,cd) pyrene in roasted plantain sample. But, the low molecular weight PAHs were found to be higher 3.33 µg/kg in roasted meat and 2.70 µg/kg in smoked fish than the higher molecular weight PAHs 2.89 µg/kg and 2.26 µg/kg in roasted meat and smoked fish respectively. Similar results were obtained by Glenn [17], who worked on food snacks in Amassoma, Niger Delta, Nigeria and Borokovocova *et al*., [18], who determine the levels of PAHs in samples of the food basket of the Czech Republic.

Table-1: Distribution of PAHs in the samples

PAHs	Roasted plantain (µg/kg)	Roasted meat (µg/kg)	Smoked fish (µg/kg)
Naphthalene	0.52	0.59	0.37
Acenaphthylene	0.34	0.55	0.01
Acenaphthane	0.57	0.18	0.42
Fluorene	0.27	0.38	0.59
Phenanthrene	0.46	0.51	0.40
Anthracene	0.14	0.14	0.36
Fluorathene	0.20	0.67	0.43
Pyrene	0.18	0.62	0.12
Benzo(a)anthracene	0.58	0.73	0.28
Chrysene	0.15	0.19	0.72
Benzo(b)fluoranthene	0.54	0.26	0.49
Benzo(k)fluoranthene	0.46	0.46	0.22
Benzo(a)pyrene	0.49	0.52	0.32
Indeno(1,2,3-cd)pyrene	0.26	0.29	0.13
Dibenzo(a,h)anthracene	0.99	0.01	Bdl
Benzo(g,h,i)perylene	0.12	0.41	0.12
Total PAHs	6.16	6.22	4.97

Reports from previous publications have revealed that PAHs with higher molecular weight (HMW) are more carcinogenic than the lower molecular weight (LMW) PAHs [20, 12]. In this study, the HMW carcinogenic PAHs constitute about 53% of the total PAHs in the roasted plantain, 46 % in roasted meat and 41% in smoked fish.

The source of PAHs detected in a sample can further be determined by molecular ratios of some PAHs and ratios of fluoranthene to pyrene and phenanthrene to anthracene were selected to verify the sources of the PAHs detected in the samples. Ratio of fluoranthrene to pyrene (Flu/Pyr) greater than one (Flu/Pyr>1) is attributed to pyrolytic source while Flu/Pyr<1 is attributed to petroleum hydrocarbon source [14]. Similarly, ratio of phenanthrane to anthracene (Ph/An) less than ten (Ph/An<10) indicates combustion source and Ph/An>10 is attributed to petrogenic source [21]. This suggests that the PAHs detected from the samples originated from the roasting/ smoking process.

Table-2: Molecular indices of PAHs in the samples

PAH ratio	Sample			Value of ratio	Indication
	Roasted plantain	Roasted meat	Smoked fish		
Ph/An	3.39	3.66	1.21	< 10 > 10	Pyrogenic Petrogenic
Flu/Flu + Py	1.01	1.10	3.76	<0.4 ≥0.4-0.5 >0.5	Petrogenic Pyrogenic Biomas coal combustion
BaA/BaA + Chr	0.51	0.03	0.41	>0.2 0.2-0.35 >0.35	Petrogenic Pyrogenic Biomas coal combustion

Studies have shown that eating a charcoal-broiled food may expose one to the same quantity of PAHs as one would receive from smoking 600 cigarettes [22]. Epidemiological studies carried out by Bababunmi *et al*., [23] and Kazerouni *et al*., [24] indicated a statistical correlation between the increased occurrence of cancer of the intestinal tract and frequent intake of roasted food. The results of this study also corroborated by Alonge [12] who reported that PAHs are common and may constitute health hazards in Nigeria. The roasted plantain, meat and smoked fish are commonly consumed by the people Nigeria may therefore create high health risk.

CONCLUSION

PAHs concentrations in roasted plantain, roasted meat and smoked fish obtained from Ondo, in Western Nigeria have been successfully analysed in this study. It could be concluded from the results of analysis carried out that the samples were contaminated by different concentrations of individual PAHs traceable to roasting/ smoking process. The concentrations detected were lower than recommended values but, since there is possible health risk associated with PAHs, it is important to monitor the presence and concentrations of PAHs in these samples. In addition, the Contribution of the type of wood used in smoking and the length of time of smoking to the quality and quantity of PAHs in smoked fish needs to be investigated. On this basis, it is recommended that adequate attention should be paid to the consumption of roasted and smoked food items.

REFERENCES

1. Marcé, R. M., & Borrull, F. (2000). Solid-phase extraction of polycyclic aromatic

compounds. *Journal of chromatography A*, *885*(1-2), 273-290.
2. Agency for Toxic Substances and Disease Registry (ATSDR). (1995). Toxicological Profile for Polyaromatic hydrocarbons-Update US Department of Health and Human Services, Atlanta, GA: 150-155.
3. Martinez, O., Salmeron, J., Guillen, M. D., & Casas, C. (2007). Textural and physicochemical changes in salmon (Salmo salar) treated with commercial liquid smoke flavourings. *Food Chemistry*, *100*(2), 498-503.
4. Menzie, C. A., Potocki, B. B., & Santodonato, J. (1992). Exposure to carcinogenic PAHs in the environment. *Environmental science & technology*, *26*(7), 1278-1284.
5. Visciano, P., Perugini, M., Amorena, M., & Ianieri, A. (2006). Polycyclic aromatic hydrocarbons in fresh and cold-smoked Atlantic salmon fillets. *Journal of food protection*, *69*(5), 1134-1138.
6. Ortmann, A. C., Anders, J., Shelton, N., Gong, L., Moss, A. G., & Condon, R. H. (2012). Dispersed oil disrupts microbial pathways in pelagic food webs. *PLoS One*, *7*(7), e42548.
7. Food Standard Agency of UK (FSA). (2002). PAHs in the UK Diet: 2000 Total Diet Study Samples. Food Survey Information Sheet No.31/02. UK: FSA; 2002. Available from: URL: http://www.food.gov.uk/science/surveillance/fsis-2002/31pah
8. Gomaa, E. A., Gray, J. I., Rabie, S., Lopez- Bote, C., & Booren, A. M. (1993). Polycyclic aromatic hydrocarbons in smoked food products and commerical liquid smoke flavourings. *Food Additives & Contaminants*, *10*(5), 503-521.
9. Falco, R. N. (2003). *U.S. Patent Application No. 29/166,972*.
10. WHO. (1998). Guidelines for drinking-water quality, 2nd ed: Addendum to Vol. 2 Health criteria and other supporting information. Geneva: WHO; Available from: http://www.who.int/water_sanitation_health/dwq/en/2edaddvol2a.pdf
11. Scientific Committee on Foods of EC (SCF). (2002). Opinion of the Scientific Committee on Food in the risk to human health of PAHs in food (expressed on 4th December, 2002). Available from: http://ec.europa.eu/food/fs/sc/scf/out153en.pdf
12. Alonge, D. O. (1988). Carcinogenic polycyclic aromatic hydrocarbons (PAH) determined in Nigerian kundi (smoke- dried meat). *Journal of the Science of Food and Agriculture*, *43*(2), 167-172.
13. Guillén, M. D., Sopelana, P., & Partearroyo, M. A. (2000). Determination of polycyclic aromatic hydrocarbons in commercial liquid smoke flavorings of different compositions by gas chromatography− mass spectrometry. *Journal of agricultural and food chemistry*, *48*(2), 126-131.
14. Kwon, K. K., Lee, H. S., Yang, S. H., & Kim, S. J. (2005). Kordiimonas gwangyangensis gen. nov., sp. nov., a marine bacterium isolated from marine sediments that forms a distinct phyletic lineage (Kordiimonadales ord. nov.) in the 'Alphaproteobacteria'. *International journal of systematic and evolutionary microbiology*, *55*(5), 2033-2037.
15. AOAC. (2002). O fficial methods of Analysis of the Association of official Analytical Chemist 17th Edition. Arington, Virginial U.S.A.
16. Akpan, V., Lodovici, M., & Dolara, P. (1994). Polycyclic aromatic hydrocarbons in fresh and smoked fish samples from three Nigerian cities. *Bulletin of environmental contamination and toxicology*, *53*(2), 246-253.
17. Roy, G. M. (1994). *Activated carbon applications in the food and pharmaceutical industries*. CRC Press. 125.
18. Borovikova, E. Y., & Kurazhkovskaya, V. S. (2005). Infrared spectra and factor group analysis of vesuvianites in OH region. *Vibrational spectroscopy*, *39*(1), 95-98.
19. Basak, S., Şengör, G. F., & Karakoç, F. T. (2010). The detection of potential carcinogenic PAH using HPLC procedure in two different smoked fish, case study: Istanbul/Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, *10*(3), 351-355.
20. Basak, S., Şengör, G. F., & Karakoç, F. T. (2010). The detection of potential carcinogenic PAH using HPLC procedure in two different smoked fish, case study: Istanbul/Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, *10*(3), 351-355.
21. Benlahcen, K. T., Chaoui, A., Budzinski, H., Bellocq, J., & Garrigues, P. H. (1997). Distribution and sources of polycyclic aromatic hydrocarbons in some Mediterranean coastal sediments. *Marine Pollution Bulletin*, *34*(5), 298-305.
22. Ziegler, R. G. (2000). Persons at high risks of cancer. *Wall Str. J*, *14*, 10-12.
23. Bababunmi, E. A., Emerole, G. O., Uwaifo, A. O., Thabrew, M. I. (1982). The role of aflatoxins and other aromatic hydrocarbons in human carcinogenesis. In: Host factors in carcinogenesis, eds. Bartsch H, Armstrong N. *IARC Scientific Publication*, 39:395-403.
24. Kazerouni, N., Sinha, R., Hsu, C. H., Greenberg, A., & Rothman, N. (2001). Analysis of 200 food items for benzo[a]pyrene and estimation of its intake in an epidemiologic study. *Food Chem. Toxicol*, 39, 423-436.