



Comparative Studies of Polycyclic Aromatic Hydrocarbons in Raw and Charcoal Roasted Beef Consumed Within Port Harcourt City and Environs

Dibofori-Orji A. N.*, ThankGod P., Ali S. T.

Department of Chemistry, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria

Abstract Analysis of raw and roasted beef (suya), sold and consumed in Iwofe, Trans Amadi and Port Harcourt city were screened for the presence of 16 polycyclic aromatic hydrocarbons (PAHs). Column chromatography, packed with anhydrous Na₂SO₄ and silica gel were used for PAH extraction with dichloromethane as the eluting solvent. The identification and concentration of PAHs were carried out by gas chromatography GC/FID with the aid of 16 PAH standards. Appreciable mean amount of fluoranthene (4.2×10^{-3} mg/kg) were found present in raw beef; while a mean of 1.2×10^{-2} mg/kg of fluoranthene was detected in roasted beef (suya). In addition to these, other members of the PAH family were detected at varying amounts. Polycyclic aromatic hydrocarbon has been related to roasting food with charcoal or wood and raw beef depending on the dietary origin and handling of the animal. In view of this, it is necessary to occasionally carry out analysis on roasted and raw beef to investigate the amount of PAHs present.

Keyword: Roasted beef, benzo(a)Pyrene, Gas Chromatograph, Polycyclic aromatic hydrocarbons (PAHs) Carcinogenic

Introduction

The term polycyclic aromatic hydrocarbons (PAHs) also known as the poly nuclear aromatic hydrocarbons refers to a ubiquitous group of several hundred chemically-related, environmentally persistent organic compounds having various structures and varied toxicity. Most PAHs are formed by a process of thermal decomposition (pyrolysis) and subsequent recombination (pyrosynthesis) of organic molecules [1]. Several analyses showed that large amount of food intake of PAHs comes from vegetables and cooked meats [2]. Exposure of PAHs includes breathing in PAHs in tobacco smoke, auto emissions or industrial exhausts, consumption of charcoal broiled food and contaminated water. Human exposure to PAHs is over 90% linked to food and it can enter food through water and air [3-4]. They can also be absorbed through the skin when in contact. The impacts of PAHs exposure are observed largely at the breast, lungs, genitourinary, oropharynx and gastrointestinal tracts [5]. Several animal and human studies have implicated colon cancer and other forms of cancer to dietary exposure to PAHs [6]. Continual exposure to high levels of pollutant mixtures containing PAHs results in symptoms such as eye irritation, nausea, vomiting, diarrhea, and confusion [7]. Some PAHs are recognized as mutagenic and carcinogenic. In mammalian cells PAHs go through metabolic activation to diol epoxides that bind covalently to cellular macromolecules, such as DNA, leading to errors in DNA replication and mutations that can initiate carcinogenesis [8].

Toxicity of PAHs tends to increase with the number of rings. For instance, PAHs containing four fused rings, such as Chrysene and Benzo(a)anthracene, are weakly carcinogenic. Five or six-fused ring polycyclic hydrocarbons, such as benzo(b)fluoranthene, benzo(a-) pyrene, and indeno(1,2,3-c,d)pyrene are very powerful carcinogens. Researchers



have it that high consumption of fried or barbecued food (meats) was related with increased risks of colorectal cancer [9].

Roasted beef (Suya) are commonly sold and consumed as ready-to-eat food by large population in Port Harcourt, Nigeria. The “suya” meat is prepared from boneless beef. The meat is sliced into continuous sheets, cut into pieces and staked on sticks and spiced with groundnut oil, salt, ground nut powder/flour, ginger, dried pepper and flavourings such as monosodium glutamate. The sticks are then arranged round on wire gauze placed over an open charcoal fire [10].

The aim of this study was to report the concentration and distribution of PAHs in roasted ready –to –eat beef (Suya) and raw beef consumed by people of Port Harcourt, Nigeria and to assess possible human health risk associated with consumption.

Materials and Methods

Sample Collection

Nine (9) samples of roasted beef (suya) and raw beef were respectively collected from three locations; Iwofe, Trans Amadi and Port Harcourt city in Rivers State Nigeria.

All collected samples were stored in stainless steel containers and taken to the laboratory for preparation and treatment

Extraction for Gas Chromatography Analysis

2 g of each sample were weighed into a clean extraction container and 10 ml of dichloromethane was added, thoroughly mixed and allowed to settle. The mixture was carefully filtered into clean solvent and rinsed into extraction bottle. The extract was concentrated to 2 ml and then transferred for cleanup and separation (This involved further purification of the extract prior to gas chromatographic analysis). To achieve this, 1 cm of moderately packed glass wool was placed at the bottom of 10 mm ID × 250 mm long chromatographic column. Slurry of 2 g activated silica in 10 ml dichloromethane was prepared and placed into the chromatographic column. To the top of the column, 0.5 cm of sodium sulfite was added.

The column was rinsed with additional 10 ml of dichloromethane. The column was pre-eluted with 20 ml of dichloromethane which was allowed to flow at a certain rate until the liquid in the column was just above the sulfite layer. Immediately, 1 ml of the extracted sample was transferred into the column. The extraction bottle was rinsed with 1 ml of dichloromethane and added to the column as well. The eluant was collected with a 10 ml graduated cylinder and labelled ‘Aliphatic’. The concentrated aliphatic fractions were transferred into labeled glass vials with rubber crimps caps for GC analysis. 1µl of sample in a vial was injected into the injection port of the preheated GC to prevent condensation of sample. The GC temperature was kept high enough to effect vaporization and separation of sample components which were captured as different peaks at the readout. By means of PAH standards, the identity and concentration of the sample components were known.

Results and Discussion

Table 1: Total Mean Concentration (mg/kg) of PAHs in Raw beef samples

PAHs	IWOFE	TRANS AMADI	PH CITY
Naphthalene	6.8×10^{-5}	3.4×10^{-5}	9.5×10^{-5}
2-methyl Naphthalene	2.0×10^{-5}	1.4×10^{-4}	8.2×10^{-6}
Acenaphthylene	3.1×10^{-5}	5.8×10^{-5}	2.3×10^{-4}
Acenaphthene	9.9×10^{-6}	7.7×10^{-5}	7.5×10^{-5}
Flourene	3.9×10^{-6}	4.0×10^{-5}	3.1×10^{-5}
Phenanthrene	6.6×10^{-5}	1.5×10^{-4}	9.5×10^{-5}
Anthracene	3.9×10^{-5}	4.6×10^{-5}	7.4×10^{-5}
Fluoranthene	1.1×10^{-3}	1.1×10^{-3}	4.2×10^{-3}
Pyrene	5.3×10^{-4}	3.3×10^{-4}	5.5×10^{-4}



Benz(a)anthracene	8.9×10^{-5}	1.1×10^{-3}	3.6×10^{-5}
Chrysene	1.0×10^{-4}	3.2×10^{-5}	2.4×10^{-5}
Benzo(b)fluoranthene	1.2×10^{-3}	1.4×10^{-4}	3.3×10^{-4}
Benzo(k)fluoranthene	1.4×10^{-4}	2.9×10^{-5}	1.8×10^{-4}
Benzo(a)pyrene	1.3×10^{-4}	6.9×10^{-5}	4.2×10^{-5}
Indeno(1,2,3-cd)pyrene	5.7×10^{-5}	1.4×10^{-5}	2.8×10^{-5}
Dibenz(a,h)anthracene	8.1×10^{-5}	1.6×10^{-5}	1.1×10^{-4}
TOTAL	3.7×10^{-3}	3.3×10^{-3}	6.0×10^{-3}

The results of PAHs concentration levels determined in raw beef collected from Iwofe, Trans Amadi and Port Harcourt city as sampling sites are shown in Table 1 respectively. In the study, Naphthalene reads highest in PH city as 9.5×10^{-5} , 2-methyl Naphthalene reads highest in TRANS AMADI as 1.4×10^{-4} , Acenaphthylene reads highest in PH CITY as 2.3×10^{-4} , Acenaphthene reads highest in TRANS AMADI as 7.7×10^{-5} , Fluorene reads highest in TRANS AMADI as 4.0×10^{-5} , Phenanthrene reads highest in TRANS AMADI as 1.5×10^{-4} , Anthracene reads highest in PH CITY as 7.4×10^{-5} , Fluoranthene reads highest in PH CITY as 4.2×10^{-3} , Pyrene reads highest in PH CITY as 5.5×10^{-4} , Benz(a)anthracene reads highest in TRANS AMADI as 1.1×10^{-3} , Chrysene reads highest in IWOFE as 1.0×10^{-4} , Benzo(b)fluoranthene reads highest in IWOFE as 1.2×10^{-3} , Benzo(k)fluoranthene reads highest in PH CITY as 1.8×10^{-4} , Benzo(a)pyrene reads highest in IWOFE as 1.3×10^{-4} , Indeno(1,2,3-cd)pyrene reads highest in IWOFE as 5.7×10^{-5} , Dibenz(a,h)anthracene reads highest in PH CITY as 1.1×10^{-4} . In general, Port Harcourt city showed highest mean concentration of PAHs as (4.2×10^{-3} mg/kg) for fluoranthene and lowest mean concentration of PAHs as (3.9×10^{-6} mg/kg) for Fluorene in Iwofe. From the Table, Iwofe has total mean concentration of (3.7×10^{-3} mg/kg), Trans Amadi has total mean concentration of (3.3×10^{-3} mg/kg) and Port Harcourt city has total mean value of (6.0×10^{-3} mg/kg).

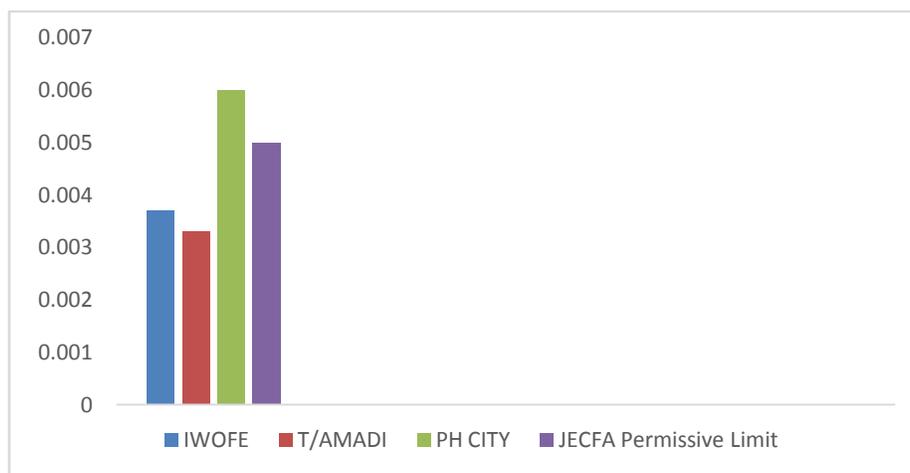


Figure 1: Total Mean Concentration of PAH (mg/kg) in Raw Beef

Table 2: Total Mean Concentration of PAHs (mg/kg) in Roasted Beef (Suya)

PAHs	IWOFE	TRANS AMADI	PH CITY
Naphthalene	1.0×10^{-3}	1.1×10^{-3}	1.0×10^{-4}
2-methyl naphthalene	2.6×10^{-5}	4.3×10^{-6}	6.2×10^{-5}
Acenaphthylene	2.7×10^{-5}	5.3×10^{-5}	4.3×10^{-5}
Acenaphthene	1.3×10^{-4}	7.2×10^{-6}	8.9×10^{-6}
Fluorene	3.5×10^{-3}	8.7×10^{-3}	4.6×10^{-4}
Phenanthrene	4.2×10^{-5}	1.4×10^{-4}	2.6×10^{-4}
Anthracene	6.5×10^{-5}	1.0×10^{-4}	7.6×10^{-5}
Fluoranthene	2.7×10^{-3}	3.0×10^{-3}	1.2×10^{-2}



Pyrene	3.0×10^{-4}	2.3×10^{-4}	4.4×10^{-3}
Benz(a)anthracene	5.4×10^{-4}	8.0×10^{-6}	4.6×10^{-4}
Chrysene	1.2×10^{-3}	1.4×10^{-6}	9.4×10^{-4}
Benzo(b)fluoranthene	1.1×10^{-4}	2.9×10^{-5}	6.1×10^{-3}
Benzo(k)fluoranthene	2.8×10^{-5}	3.8×10^{-5}	4.9×10^{-4}
Benzo(a)pyrene	2.7×10^{-5}	3.8×10^{-6}	7.8×10^{-6}
Indeno(1,2,3-cd)pyrene	1.0×10^{-6}	2.6×10^{-6}	7.3×10^{-6}
Dibenz(a,h)anthracene	2.4×10^{-3}	1.6×10^{-5}	6.8×10^{-6}
Total	1.2×10^{-2}	1.3×10^{-2}	2.5×10^{-2}

The results of PAHs concentration levels determined in roasted beef collected from Iwofe, Trans Amadi and Port Harcourt city as sampling site are shown in Tables 2 respectively. Naphthalene reads highest in Trans Amadi as 1.1×10^{-3} , 2-methyl naphthalene reads highest in PH CITY as 6.2×10^{-5} , Acenaphthylene reads highest in Trans Amadi as 5.3×10^{-5} , Acenaphthene reads highest in Iwofe as 1.3×10^{-4} , Fluorene reads highest in Trans Amadi as 8.7×10^{-3} , Phenanthrene reads highest in PH City as 2.6×10^{-4} , Anthracene reads highest in Trans Amadi as 1.0×10^{-4} , Fluoranthene reads highest in PH City as 1.2×10^{-2} , Pyrene reads highest in PH City as 4.4×10^{-3} , Benz(a)anthracene reads highest in Iwofe as 5.4×10^{-4} , Chrysene reads highest in Iwofe as 1.2×10^{-3} , Benzo(b)fluoranthene reads highest in PH City as 6.1×10^{-3} , Benzo(k)fluoranthene reads highest in PH City as 4.9×10^{-4} , Benzo(a)pyrene reads highest in Iwofe as 2.7×10^{-5} , Indeno(1,2,3-cd)pyrene reads highest in PH City as 7.3×10^{-6} , Dibenz(a,h)anthracene reads highest in Iwofe as 2.4×10^{-3} . The result for all the mean concentration of PAHs reveals Fluoranthene with the highest mean concentration of (1.2×10^{-2} mg/kg) in PH City while Indeno(1,2,3-cd)pyrene has the lowest mean concentration of (1.0×10^{-6} mg/kg) in Iwofe. The total mean value for roasted beef (Suya) is (1.2×10^{-2} mg/kg) in Iwofe, (1.3×10^{-2} mg/kg) in Trans Amadi and (2.5×10^{-2} mg/kg) in Port Harcourt city. This report is far lower than reported literature (14.83 mg/kg) and slightly lower (0.04 mg/kg) for fresh processed beef and (0.07 mg/kg) for long processed beef but this result has mean value above JECFA [11] permissible limit of (0.005 mg/kg).

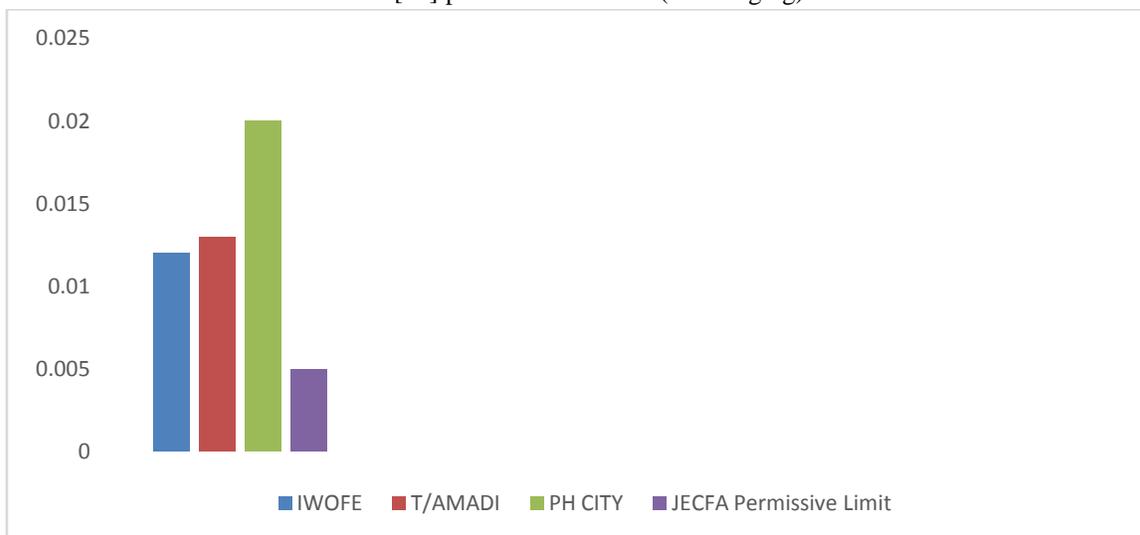


Figure 2: Total Mean Concentration of PAH (mg/kg) in Roasted Beef (Suya)

This research reveals the presence of PAHs in both raw beef and roasted beef (Suya), stating that PAHs are more concentrated in roasted beef (Suya). Generally, the samples showed readings of PAH at different levels and because these substances are known to be carcinogenic in the human body, it creates a cause for alarm in consuming such beef products. The presence of PAHs may result from burning of tyres, roasting with charcoal or wood [12-13], industrial emissions, cigarette smoking etc. The result also reveals that Port Harcourt city is more contaminated with these PAHs than Trans Amadi and Iwofe. This could be as a result of air movement circulating pollutants within the city of Port Harcourt.



Studies have revealed that eating a charcoal-broiled food may expose one to the same quantity of PAHs as one would receive from smoking 600 cigarettes [14]. Epidemiological studies carried out by Bababunmi *et al* [15], Fritz and Soos [16], Emerole [17] and Kazerouni *et al.*, [18] indicated a statistical correlation between the increased occurrence of cancer of the intestinal tract and frequent intake of roasted food. This result corroborates with Alonge [19] who reported that PAHs are common and may constitute health hazards in Nigeria. The roasted and raw beef as presently consumed by the people of Iwofe, Trans Amadi and Port Harcourt city may therefore create high health risk.

Conclusion

This study reveals higher concentration of PAHs in the charcoal grilled meat (Suya) than in the raw beef. This suggests that pollution into the environment as a result of industrial activities release PAHs but more PAHs are released during roasting, with charcoal or wood. Since the metabolites of PAHs target cellular macromolecules such as DNA and lead to mutations and carcinogenesis causing serious adverse health problems to living organisms, we recommend increased awareness on the toxicity and risk of PAHs because high consumption may be injurious to health.

Acknowledgement

The researchers acknowledge the staff and Management of International Energy Services for making their laboratory facilities available to make this study a success.

References

1. Thamaraiselvan, R., Peramaiyan, R., Natarajan, N., Boopathy, L., Palaniswami, R. & Ikuo, N. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine*, 5(3), 182-189.
2. Glen, M.R. (1995). Activated carbon applications in the food and pharmaceutical industries; CRC Press 125.
3. Garcia-Falcon, M.S., Perez-Lamela, M. & Simal-Gandara, J. (2004). Comparison of strategies for extraction of high molecular weight polycyclic aromatic hydrocarbons from drinking waters. *Journal of Agricultural and Food Chemistry*, 52(23), 6897-6903.
4. Rey-Salgueiro, L., García-Falcón, M.S., Martínez-Carballo, E. & Simal-Gándara, J. (2008). Effects of a chemical company fire on the occurrence of polycyclic aromatic hydrocarbons in plant foods. *Journal of Agricultural and Food Chemistry*, 108(1), 347-353.
5. Goldman, R. (2003). Shields PG. Food Mutagens. *Journal of Nutrition*, 133:965S-973S.
6. Giovannucci, E., Rimm, E.B., Stampfer, M.J., Colditz, G.A., Ascherio, A. & Willet, W.C. (1994). Intake of fat meat and fiber in relation to risk of colon cancer in men. *Journal of Cancer Research*. 54:2390-2397.
7. Bolling, A.K., Pagels, J., Yttri, K.E., Barregard, L., Sallsten, G. & Schwarze, P.E. (2009). Health effects of residential wood smoke particles: the importance of combustion conditions and physicochemical particle properties. *Journal of Particle Fibre Toxicology*, 6: 20.
8. Badry, N.E. (2010). Effect of household cooking methods and some food additives on polycyclic aromatic hydrocarbons (PAHs) formation in chicken meat. *Journal of World Applied Sciences*, 9(9), 963-974.
9. Cross, A.J., Ferrucci, L.M. & Risch, A. (2010). A large prospective study of meat consumption and colorectal cancer risk: An investigation of potential mechanisms underlying this association. *Journal Cancer Research*, 70(6), 2406-2414.
10. Inyang CU, Igyor MA, Uma EN (2005). Bacterial quality of a smoked meat product ("Suya"). *Nig. Food J.* 23:239-242.
11. JECFA (2005). *Summary and conclusions of the sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives*. Food and Agriculture Organization (FAO), Rome, World Health Organization (WHO), Geneva.



12. Dibofori-Orji, A.N. & Braide S.A. (2011). Trace Metals and Polycyclic Aromatic Hydrocarbons (PAHs) levels in Cattle Skin {Kanda} Processed with Burning Tyres. *International Journal of Applied Environmental Sciences*, 6(2), 133-141.
13. Dibofori-Orji, A. N. & Etori, O. S. (2013). Suspended Particulate Matter (SPM) and Trace Metals Emission from the Combustion of Tyres in a Nigeria Abattoir. *Chemistry Material Research*, 3(13), 21-26.
14. Ziegler, R.G. (2000). Persons at high risk of cancer. *Journal of Wall Street*, 14:10-12.
15. 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: Bartsch H, Armstrong N(ed) Host factors in carcinogenesis, IARC Scientific Publication. 39:395-403.
16. Fritz, W. & Soos, K. (1980). Smoked Food and Cancer. *Journal of American Society of Nutrition*, 29:57-64.
17. Emerole, G.O. (1980). Carcinogenic PAHs in some Nigerian Foods. *Journal Environmental Contamination Toxicology*, 2, 641-646.
18. Kazerouni, N., Sinha, R., Hsu, C.H., Greenberg, A. & Rothman, N. (2001). Analysis of 200 foods Items for benzo[a]pyrene and estimation of its intake in an epidemiologic study. *Journal of Food Chemical Toxicology*, 39, 423-436.
19. Alonge, D.O. (1988). Carcinogenic polycyclic aromatic hydrocarbons (PAH) determined in Nigerian Kundi (smoke-dried meat). *Journal of Science Food Agriculture*, 43:167-172.

