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Effect of Application of Seasonings/Spices and Heating/ Processing Methods on the Levels of Polycyclic Aromatic Hydrocarbons and Heavy Metals in Cooked, Fried and Roasted Meats Sold within Enugu Metropolis

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Abstract

Studies were carried out to evaluate the effect of application of seasonings/spices and heating/processing methods on the levels of polycyclic aromatic hydrocarbons and heavy metals in cooked, fried and roasted meats sold within Enugu metropolis using relevant established analytical procedures and instrumentation. Out of the ten polycyclic aromatic hydrocarbons (PAHs) studied, only eight (phenanthrene, anthracene, benzo @anthracene, benzo@ pyrene, acenapthene, pyrene, chrysene and fluorene) were detected in the meat samples. The mean levels of these PAHs in the meat samples were found to be statistically significant at $p < 0.05$. Higher molecular weight polycyclic aromatic hydrocarbons such as benzo @ anthracene, benzo @ pyrene, chryene and pyrene were present at toxic levels in the studied fried and roasted meat samples. Cd, Cu, Zn, Pb, Cr, and Fe were all present in the analyzed meat samples at level within their respective permissible limits. Statistical analysis of the mean levels of the detected PAHs and heavy metals in the meat samples revealed significance at $p < 0.05$. The study shows that application of seasonings and method of heating/processing foods such as meats significantly influences its accumulation of toxic substances such as heavy metals and polycyclic aromatic compounds.

Keywords: Heavy Metals; Roasted Meat (Suya); Pahs; Fried Meat; Cooked Meat; Spices

Introduction

Suya is a spicy meat skewer which is a popular food item in West Africa [1]. The thoroughly sliced meat is marinated in various spices which include peanut cake, salt, vegetable oil and other flavourings and then barbecued [2]. Suya is served with extra additions of dried pepper mixed with spices and sliced onions.

Suya has become a Nigerian dish with different regions claiming the superiority of their recipe and methods of preparations but similar grilled meat recipes are common in West African countries and ingredients use may vary according to personal and regional preference [3]. According to [4], the meat is sliced into continuous sheet, cut into spices and staked on sticks and spiced with groundnut powder, flour, ginger, dried pepper and flavourings such as glutamate. These spices mainly flavour foods including meat. According to [5], a spice is a dried seed, fruit, root, bark or vegetable substance primarily used for flavouring, colouring or preserving food. Many common spices have outstanding antimicrobial effects however; the process can make them a source of food poisoning [5]. Most spices used in meat processing are milled or ground.

Several researchers have shown that heavy metals could be present in spices and the addition of contaminated spices to food may result in the accumulation of these metals in human organs [6]. Admittedly, the extent of contamination of the spices with heavy metals varies from one plant to another.

Among pollutants generated by industry and urbanization, heavy metals and various pathogenic bacteria are the most dangerous because they can cause serious health problems to human population [7]. As a consequence of natural anthropogenic activities heavy metals are present in the environment so that people come in contact with them especially through the consumption of foods [8]. Heavy metals are dangerous because they tend to accumulate in living organism. Some heavy metals are deposited as residues in food, during processing [9]. Contamination with heavy metals is a major threat due to their toxicity, bioaccumulation and bio magnification in the food chain [10].

The largest man made sources include combustion of fossil fuels, fertilizer application, pesticides and industrial processes [11]. Lead, cadmium, arsenic and chromium are known to be entering the meat supply in limited amounts from environmental sources. Some heavy metals (e.g. copper, selenium and zinc) are vital in keeping the human body's metabolism, but they can be toxic at high concentrations. Meat consumption with high lead, cadmium, mercury and arsenic concentrations have been shown to affect the central nervous system, the kidneys and livers, immune systems and intelligent quotient. According to Oladoye and, heavy metals above the permissible levels can affect human health and may result in illness to human fetus, abortion and preterm labour and mental retardation in children.

Adults also may experience high blood pressure, fatigue, kidney and neurological disorder [12]. During the last decades, human population has faced changes in life and food style, which have led to an increase in demand for processed foods. The rise of food production and development of processing technology have increased the chances of foods contamination with various environmental pollutants especially, heavy metals and polycyclic aromatic hydrocarbons [13]. PAHs are fused aromatic fused rings formed from incomplete combustion or pyrolysis of organic materials (such as coal, oil, gas, wood, fossil, fuels and biomass) by a series of complex chemical reaction [14,15]. Food is one source of PAHs [16]. When food particularly meat, meat products and fish is smoked, roasted barbecued or grilled, PAHs are formed and a result of incomplete combustion or thermal decomposition of the organic materials [17] (Figure 1).

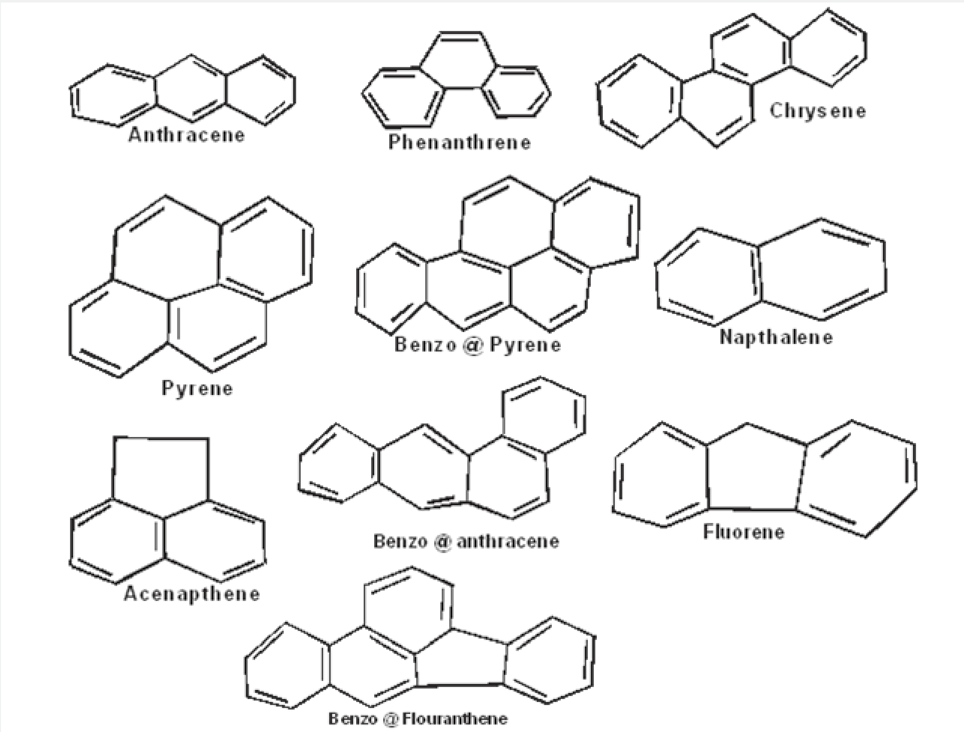

Figure 1.

According to [18] in foods, PAHs may be formed during industrial processing and domestic food preparation such as barbecuing, smoking, drying, roasting, baking, frying and grilling. For the general population, the major routes of exposure to PAHs for non-smokers are from food and inhaled air. The impacts of PAHs exposure are obtained largely at the lungs, breast, oropharynx, genitourinary and gastrointestinal tracts [19]. Several animal and human studies have implicated colon cancer and other forms of cancer to dietary exposure of PAHs [20]. According to [21], cancer is a primary health risk of exposure to PAHs and that PAHs have been linked with cardiovascular disease and poor fetal development. DNA binding and induction mutation are other significant effects in the carcinogenesis of PAHs [22]. The following are examples of PAHs that vary in the number and arrangement of their rings [23]. Human diet and dietary habits have been factored in the etiology of many diseases. Since, cooked, fried and roasted foods especially meats are commonly consumed as a ready to eat snacks by a very large population of the people cutting across all ages and prepared within different kinds of recipes, studies were carried out to assess the effect of application of spices/seasonings and heating/processing methods on the levels of polycyclic aromatic

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hydrocarbons and heavy metals in cooked, roasted (suya) and fried meats sold within Enugu metropolis.

Materials and Methods

Collection and Preparation of Meat Samples

Ten (10) cooked meat (beef) samples with no spice added were prepared by the researchers. Ten (10) samples each of roasted (suya) and fried (beef) meats were respectively purchased from strategic joints/locations within Enugu metropolis. All together 30 meat (fried, cooked and roasted) samples were used for the study. The samples were wrapped in clean labeled paper foils and taken to the laboratory for analysis.

Extraction of the Meat Samples for PAHs Determination

2g each of the homogenized meat samples were thoroughly mixed with anhydrous Na_2SO_4 salt to absorb moisture content and then extracted with 10ml of analytical grade dichloromethane ($CHCl_2$). The dichloromethane extract was cleaned up by passing through a column packed with anhydrous Na_2SO_4 salt. The resulting extract was concentrated on a rotatory evaporator to give an oil residue, which was again dissolved in 1ml CH_2Cl_2 and 1ml was injected into the gas chromatography for analysis. The gas chromatography used was Hewlett packed 589.0 series II complex. The sample components were automatically detected as they emerged from the column (at a constant flow) by the flame ionization detector. The identification of PAHs was based on comparison of the retention times of peaks with those obtained from standard mixture of PAHs. Quantification was based on external calibrations curves prepared from the standard solution of each of the PAHs as determined by [24].

Heavy Metal Determination

Exactly 2.0g of the meat samples were weighed into 250ml beakers, mixed with 20ml of 2:1 $HNO_3/HClO_4$ and heated in a fume cupboard for 5–10 min using hot plate. Completion of digestion was marked by the evolution of white fumes. The digest were allowed to cool, diluted with deionized water and then filtered into 50ml standard volumetric flask and made-up to mark with de ionized water. Calibration standards as well as blanks were prepared at the same time as the samples. All standards were prepared from nutrients (in concentrations of 0.01, 0.2, 0.4, 0.6, 0.8 and 1.0ppm) for Cu, Cd, Zn, Pb, Cr, and Fe and were used to calibrate the spectrometer prior to analysis. The filtrate resulting from wet digestion was subsequently analyzed for heavy metals using Hitachi Z – 5000 flame atomic absorption spectrometer.

Statistical Analysis

The data obtained were presented in mean and standard deviation and subjected to one way analysis of variance (ANOVA) SPSS version 18.0 at 5% level of confidence. PAHs are a group of chemicals that result from incomplete burning of coal, oil, gas, wood, fossil fuels in general and biomass [13]. Studies have shown that most intake of polycyclic aromatic hydrocarbon in foods come from vegetables, barbecued, fried and roasted meats [25]. Table 1 shows that naphthalene was not detected in the analyzed meat samples. The level of phenanthrene was 0.003, 0.007 and 0.010ppm for cooked, fried and roasted meat samples respectively. The order of decrease of phenanthrene was roasted < fried < cooked meat samples as shown in Table 1 and Figure 2. The different methods of preparing the meat samples could have significantly contributed the varying levels of the PAH. The levels of phenanthrene in the meat samples were statistically significant ($p < 0.05$) and above the recommended permissible in the fried and roasted meats samples. The mean levels of anthracene was 0.001ppm for cooked meats, 0.003ppm for fried meats and 0.008ppm for roasted meat (suya) as shown in Table 1. The mean levels of the aromatic compound were statistically significant at $p < 0.05$ and above the recommended permissible limits in the roasted samples.

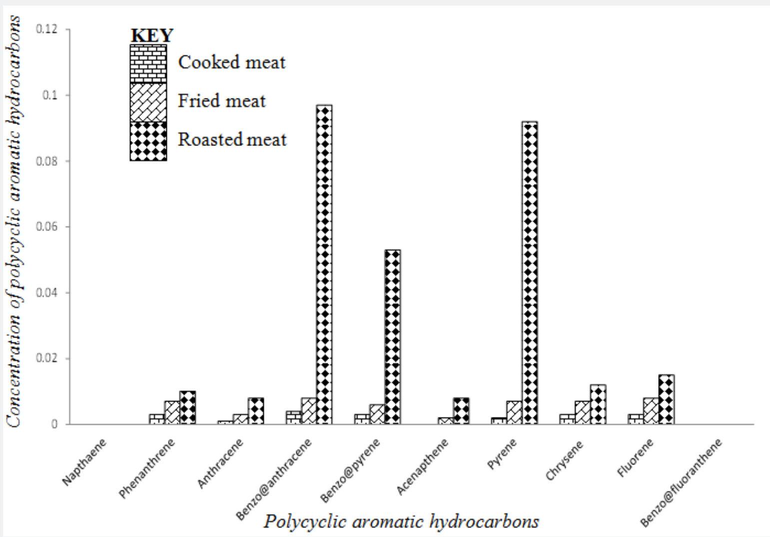

Figure 2: Bar chart representation of the mean levels of the polycyclic aromatic hydrocarbons in the meat samples (ppm).

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Table 1: Mean levels of polycyclic aromatic hydrocarbons in the meat samples (ppm).

PAHs	Cooked Meat (Beef) With no Spice (ppm)	Fried Meat (beef) With Few Spices (ppm)	Roasted Meat (suya) with Many Spices (ppm)	F – Test P Value	WHO STD (ppm)
Napthaene	ND	ND	ND	-	0.005
Phenanthrene	0.003 ± 0.001	0.007 ± 0.001	0.010 ± 0.00	0.03	0.005
Anthracene	0.001± 0.000	0.003± 0.001	0.008 ± 0.001	0.02	0.005
Benzo@anthracene	0.004 ± 0.001	0.008 ± 0.003	0.097 ± 0.004	0.04	0.005
Benzo@pyrene	0.003 ± 0.001	0.006 ± 0.001	0.053 ± 0.002	0.02	0.005
Acenapthene	ND	0.002 ± 0.000	0.008 ± 0.001	0.02	0.005
Pyrene	0.002 ± 0.000	0.007 ± 0.001	0.092 ± 0.002	0.03	0.005
Chrysene	0.003 ± 0.001	0.007 ± 0.002	0.012 ± 0.001	0.01	0.002
Fluorene	0.003 ± 0.001	0.008 ± 0.002	0.015 ± 0.003	0.02	0.005
Benzo@fluoranthene	ND	ND	ND	-	0.005

The order of decrease of anthracene was roasted (suya) < fried (beef) < cooked (beef) meat samples. The results of this study was in agreement with what stated that high amounts of PAHs in processed meats could be due to the duration of the processing, the type of fuel used and the exposure of the samples to smoke generated by the firewood. Hence, roasted (suya) meat samples gave the highest value of anthracene as a result of the longer period of the processing to dry out the fatty components of the meats, the high content of spices in the meats which are mainly of organic origin and the incomplete combustion of the organic material. The results of anthracene in this study were higher than 0.0000162mg/kg reported by in long processed beef samples in Imo State, South Eastern Nigeria. 0.005, 0.008 and 0.097ppm were the values of benzo@anthracene in the cooked, fried and roasted meat samples respectively as shown in Table 1. The aromatic compound decreased as follows in the meat samples; roasted > fried > cooked. The level of benzo@ anthracene in the meat samples were statistically significant ($p < 0.05$). The mean values of benzo@anthracene were above the permissible limits in the fried and roasted meat samples. This should be of a public health concern to consumers of processed meats especially roasted meats, in view of the fact that studies in experimental animals have shown various toxicology effects such as hematological effects reproductive and developmental toxicity and immunological toxicity caused by PAHs. Reports have shown that PAHs with higher molecular weight such as benzo@anthracene, benzo@pyrene and benzo@fluranphene are more carcinogenic than lower molecular weight PAHs such as anthracene, naphthalene, pyrene, acenapthene and phenanthene etc. The result of this study was lower than 1.35mg/g reported [26] in ScomberScombus meats sold in Amassoma town Nigeria. Table 1 shows that the mean levels of benzo@pyrene in the cooked, fried and roasted meat samples were 0.003, 0.006 and 0.053ppm respectively. The concentration of the aromatic compound in the meat samples differed significantly at $p < 0.05$. The mean levels of benzo@pyrene in the fried and roasted meat samples were above the recommended permissible in processed meats (WHO, 2014). This results is consistent with literature findings that the levels of PAHs is usually very high in food preparations such as barbecuing, smoking, drying, roasting, baking and frying.

The order of decrease of benzo@pyrene in the meat samples were; roasted > fried > cooked. According to [27], the formation of PAHs during roasting processes has indicated a possible transformation of low molecular PAHs to high molecular PAHs as the roasting temperature increases. Hence, this transformation may be implicated in the increased concentration of high molecular weight PAHs which are largely carcinogenic (i.ebenzo@ anthracene, chrysene, benzo@fluoroanthene, benzo@pyrene and indeno (1, 2, 3) pyrene in roasted foods benzo@pyrene is regarded as the most potent PAHs. According to Sims, (5,33,36,5), as the living system attempts to degrade benzo@pyrene, it forms reactive metabolises (benzo@pyrene 7,8dihydrodiol which is in part oxidized to 7,8 – diol – 9, 10 epoxide) that bind to DNA causing disruption to the genetic functioning of the affected cells inducing a genotoxic effect. Mutagenic and carcinogenic PAHs such as benzo@pyrene usually have four or more aromatic rings as well as a "bay region" a structural pocket that increases the reactivity of the molecule to the metabolizing enzymes. The results of this study were higher than 0.000788mg/kg reported by in freshly processed beef samples in Owerri, Imo State. Acenapthene was not detected in the cooked meat samples however mean values of 0.002 and 0.008ppm was respectively obtained in the fried and roasted meat samples. The levels of the PAH in the meat samples were statistically significant at $p < 0.05$. The mean levels of acenapthene the roasted meat samples sold within Enugu metropolis was above the recommended permissible limits. The order of decrease of the compound in the meat samples was, roasted <fried. The mean values of acenapthene obtained in this study was lower than 3.84mg/kg reported by [26] in roasted Scomber scombus sold in Amassoma town, Niger-Delta, Nigeria.

According to [28], lower molecular weight PAHs like acenapthene that have bay or bay like region can deregulate gap junction channels, interfacing with intercellular communication

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and also affect nitrogen-activated protein kinases that activate transcription factors involved in cell proliferation. Table 1 shows that the mean levels of pyrene were 0.002, 0.007 and 0.092ppm for cooked, fried and roasted meat samples respectively. The mean levels of the aromatic compound were statistically significant in the analyzed meat samples. The order of decrease of pyrene in the meat samples was roasted > fried > cooked. Only the roasted meat samples have mean levels of pyrene above the recommended limits of WHO for processed meats [29]. The mean levels of pyrene reported in this study were higher than 0.0000337mg/kg reported by in long processed beef samples in Owerri Imo State.

The mean values of chrysene in the cooked, fried and roasted meat samples were 0.003, 0.007 and 0.012ppm respectively. The mean levels of chrysene were statistically significant ($p < 0.05$) in the analyzed meat samples. The levels of chrysene in both the fried and roasted meat samples were above the permissible limits in a processed meat product. Chrysene decreased as follows in the meat samples; roasted > fried > cooked. The higher concentration of chrysene in the roasted (suya) meat samples compared to the other studied meat samples could be as a result of higher fat of the meat and pyrolysis respectively from increased amount of melted fat dropped on the heat source. Hence, the observation of this study corroborates literature findings that roasting especially imparts carcinogenic compounds on foods [30]. The mean levels of flourene were 0.003, 0.08 and 0.015ppm in the cooked, fried and roasted meat samples respectively as shown in Table 1.

The levels of fluorene in the analyzed meat samples were statistically significant at $p < 0.05$. The variation in the levels of the PAH in the meat samples could be due to the different heating processes employed in getting the meat samples ready for consumption. The levels of fluorene in the fried and roasted meat samples were above the WHO recommended permissible limits. The order of decrease of flourene in the meat samples followed the same trend as obtained for the other analyzed PAHs and this were, roasted > fried > cooked. Benzo@fluoranthene was not detected in the all the studied meat samples.

Cadmium: Cadmium is today regarded as the most serious contaminant of the modern age. It is a toxic element to every animal species and for humans as well. It is almost absent in human and animal body at birth however, it accumulates with age [31]. Organs such as the liver, placenta, kidneys, lungs, brain and bones can be affected by cadmium exposure [32]. Table 2 shows that the mean levels of cadmium were 0.08, 0.114 and 0.406ppm in the cooked, fried and roasted meat samples respectively. The metal decreased as follows in the meat samples; roasted > fried > cooked. Cadmium concentrations in the meat samples were statistically significant ($p < 0.05$) and within the recommended permissible limit in edible solid substances [33-39]. The increased spice application in the roasted meat samples could have significantly influenced cadmium levels compared to the other studied meat samples. In addition, the long period it takes to roast the meat samples which increases the amount of wood ashes generated and the possibility of the heavy metals volatizing and penetrating the food samples could have significantly influenced the metal level. The mean levels of cadmium obtained for fried meat samples in this study compared very well with 0.11mg/kg reported by in processed pork meat marketed in Romania [33] obtained lower values of 0.140 mg/kg in meats (suya) in Warri, Delta State than reported in this study.

Copper: The mean level of copper were 3.14, 7.73 and 9.09ppm in the cooked, fried and roasted meat samples respectively as shown in Table 2. The metal decreased in the following order in the meat samples, roasted > fried > cooked as shown in Figure 3. The mean levels of copper in the meat samples were found to be statistically significant and within the recommended permissible limits. The application of spices at varying levels in the meat samples could have significantly influenced the mean level of metal in the meat samples. The result of this study corroborated several literature reports that heavy metals could be present in spices and the addition of contaminated spices to food may result in accumulation of these metals in human organs. Although, it is an essential element of life, copper causes adverse effects on health at acute or chronic intoxication, when introduced in excess in the body, folder or water.

Table 2: Mean levels of heavy metals in the meat samples (ppm).

Metals	Cooked Meat (Beef) with no Spice (ppm)	Fried Meat (Beef) with Few Spices (ppm)	Roasted Meat (suya) with Many Spices (ppm)	F – Test P Value	WHO STD (ppm)
Cd	0.08 ± 0.02	0.114 ± 0.03	0.406 ± 0.06	0.01	0.5
Cu	3.14 ± 0.33	5.72± 0.71	9.09± 0.782	0.02	300
Zn	5.22± 0.64	6.85± 0.52	11.25± 0.93	0.03	350
Pb	0.16± 0.02	0.281± 0.06	0.463± 0.08	0.03	10
Cr	0.171± 0.03	0.55± 0.04	0.94± 0.25	0.01	100
Fe	4.191 ± 0.73	6.04± 0.81	10.26± 0.94	0.03	300

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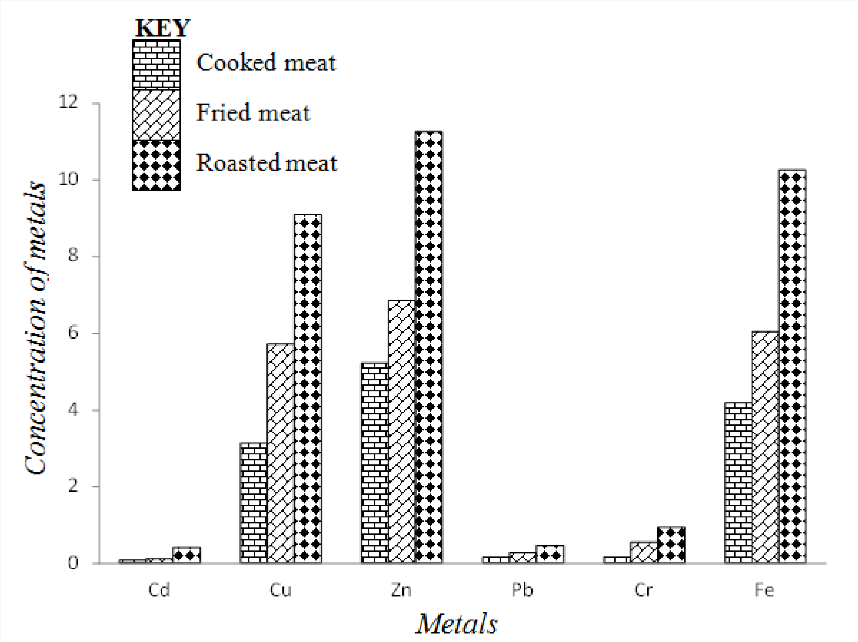

Figure 3: Bar chart representation of the mean levels of heavy metals in the meat samples.

Zinc: Zinc is an essential element in animal and human diet. Too little zinc can cause problems; however, too much zinc is harmful to health (nausea and vomiting, epigastric pain, abdominal cramps and diarrhea) [34]. Zinc supports normal growth and development and is important for protein and DNA synthesis 35. Table 2 shows that the mean levels of zinc in the cooked, fried and roasted meat samples were 5.22, 6.85 and 11.25ppm respectively. The metal decreased in the samples in the following order, roasted > fried > cooked, as shown in Figure 3. The levels of zinc in the meat samples were statistically significant ($p < 0.05$) and within the permissible limits of WHO in edible solid foods. The results obtained for zinc in this study was lower than 76.25mg/kg reportedly [34] in long processed beef samples (suya) sold in Owerri, Imo State.

Lead: Lead is the most recognized toxic environmental pollutant. Toxic levels of lead in man are associated with encephalopathy seizures and mental retardation [35]. Lead is an important pediatric heavy metals poison and it affects bones, brain, blood, kidneys and thyroid gland on exposure [1]. Lead absorption is enhanced by deficiencies of iron, calcium and zinc. The mean levels of the lead in the cooked, fried and roasted meat samples were 0.16, 0.281 and 0.443ppm respectively as shown in Table 2. The mean levels of lead in the meat samples decreased in the following; roasted > fried > cooked. The metal in the meat samples was statistically significant ($p < 0.05$) and within the recommended maximum permissible limits.

Chromium: Food is the major source of exposure to chromium (WHO, 2008), while Cr (III) is essential in human body for improvement of glucose tolerance, excessive intake, especially of the more oxidizing Cr (VI), can harm biological systems Michalski. The health effects from exposure to chromium are dermatitis, skin inflammation, chronic allergic reactant, asthma like condition in lungs and respiratory tract, lung cancer, weak carcinogenic infections. The mean levels of chromium were 0.171, 0.50 and 0.94ppm in the cooked fried and roasted meat samples respectively as shown in Table 2. The order of decrease of the metal in the samples were, roasted > fried > cooked as shown in Figure 3. The mean levels of chromium in the meat samples was found to be statistically significantly and within the established recommended maximum permissible limits. The result of chromium in this study was higher than 0.094mg/g reported by in suya beef sold in Amassoma town, Niger Delta, Nigeria.

Iron: Iron is nutritionally essential for healthy life. Iron is a constituent of red blood cells and function as a primary carrier of oxygen throughout the body. Research has shown that metals such as zinc, iron, copper and cobalt serve as catalyst for enzymatic reactions. The mean levels of iron were; 4.191, 6.04 and 10.26ppm in the cooked, fried and roasted meat samples respectively as shown in Table 2. The levels of the metal differed significantly ($p < 0.05$) in the analyzed meat samples. Obtained a higher mean value of 43.80 mg/kg for iron in long processed fatty beef sold in Owerri state, than what was reported for the metal in this study [36-43].

Conclusion

Eight out of the ten polycyclic aromatic hydrocarbons analyzed were present in the cooked, fried and roasted meat samples. Higher molecular weight polycylic aromatic hydrocarbons such and benzo@anthracene, benzo@pyrene, chrysene and pyrene were detected at toxic levels in the studied

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fried and roasted meat samples. The study revealed that the different methods of heating/processing the meat samples significantly influenced its levels of accumulation of the PAHs. In all the detected PAHs, the roasted meat samples gave the highest values followed by fried meat samples and the least was cooked meat samples. All the studied heavy metals were present in the meat samples at levels within their respective permissible limits for a safe consumable solid food. The study observed that application of seasonings/spices in processing foods such as meats significantly increases the heavy metal bioaccumulation in such food items. Roasted meat (suya) samples consistently gave the highest concentrations for the studied heavy metals while the least was cooked meat samples.

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