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Research Article

Impact of Roasting Fuel on Polycyclic Aromatic Hydrocarbons (PAHs) in Roasted Edible Cowhide Meat ("*Kpomo*")

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ABSTRACT

The impact of roasting fuel on the polycyclic aromatic hydrocarbons (PAHs) content of roasted cowhide meat ("*Kpomo*") processed and sold in the vicinity of abattoirs in Bayelsa and Rivers States were investigated. Cowhides are roasted using expired automobile tyres (T), waste plastics (P) and firewood (FW) as fuel. They are thereafter scrape out with knife and washed with water. Processed cowhide meat samples were purchased and transported to the laboratory for analysis. Cowhide scalded with hot water and fur brushed out with knife served as control. PAHs of the samples were analyzed using gas chromatography fitted with flame ionization detector. Analysis of individual PAH content showed that Benzo(b)fluoranthene and Fluoranthene had highest values of 74.68 µg/kg and 28.28 µg/kg in Swale (T) and Igbogene (FW) roasted samples respectively. Concentrations of total PAHs recorded were; Swale (T) 79.25 µg/kg, Tombia (P) 44.03 µg/kg, Igbogene (FW) 41.27 µg/kg, Opolo (FW) 28.75 µg/kg, Rumuokoro (FW) 27.61 µg/kg, and Swale (FW) 22.67 µg/kg. However, PAHs were undetected in Control samples. Kruskal Wallis H Test showed there is significant difference between the concentrations of total PAHs in all the locations and between all the locations and the control. However, there was no significant difference between Opolo and Rumuokoro samples. The presence and high concentrations of polycyclic aromatic hydrocarbons in the roasted edible cowhide meat samples is therefore attributed to the roasting fuels and the distribution is affected by the type of fuel (tyres, plastics and firewood) employed in the roasting procedure. Regular consumption of roasted cowhide could put consumers at high risk of health hazard associated with mutagenic potentials of PAHs.

Keywords: Roasted cowhide meat, tyres, plastics, firewood, polycyclic aromatic hydrocarbons, mutagenic.

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are a class of diverse organic compounds containing two or more fused benzene rings of Carbon and Hydrogen atoms (Kanaly and Harayuma, 2000). Polycyclic Aromatic Hydrocarbons also known as Polynuclear Aromatic Hydrocarbons are a large and diverse group of over a hundred different organic compounds that are found throughout the environment (Vila *et al*., 2001). PAHs are one class of toxic environment pollutant that has accumulated in the environment due to a variety of anthropogenic activities (Albert and Ravendra, 2000). PAHs are derived from the incomplete combustion of organic matters including coal, oil, gas, diesel, tar, wood, garbage or other organic substances such as tobacco and charbroiled meat (Rey-Salguiero *et al.*, 2008). PAHs are further introduced into the environment through volcanoes, forest fires, automobile exhaust, domestic wood burning, thermal decomposition, cigarette smoking and other combustibles (ATSDR, 1995). Subsequently, they are

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found throughout the environment including the air, soil and water (Cerniglia, 1993). PAHs also form directly in food as a result of some heat processes such as barbecuing, charcoal grilling, roasting, frying, smoke drying (Simko, 2002). Polycyclic aromatic hydrocarbons (PAHs) have been the source of much concern because of their toxic potentials. Due to their carcinogenicity, PAHs have been included in the European Union and the Environmental Protection Agency (EPA) priority pollutant list. Several compounds of this group have been shown to be potent carcinogens in experimental animals and they are highly hypothesized to make significant contribution to cancer in humans (European Scientific Committee on Food, 2002).

The World Health Organization emphasizes that PAHs are likely to cause cancer. They are considered probable carcinogens. Therefore, exposure to PAHs should be as low as reasonably achievable (FAO/WHO1991). It seems illogical that PAHs should be carcinogenic because the compounds are non polar and therefore insoluble in blood and aqueous environment of the tissues. However, experimental work indicated that the actual carcinogen is a more polar substance, which can combine with DNA (a high molecular weight compound which contains genetic information) in cells.
Different kinds of activities are carried out in the abattoir during the dressing of meat and fish for human consumption. The use of wood and expired/condemned tyres and other organic fuel sources for roasting cowhide, meat, chicken and fish with coal and wood all contribute to air pollution (Cerniglia, 1992). There is also significant evidence to indicate that such pollutants released to the atmosphere are transported over long distances by atmospheric wind movement (Bakker *et al.*, 2001, Halsall *et al.*, 2001) thus they have effect beyond the immediate environment where they are released. Furthermore, emissions in the smoke used to roast food materials are also known to be imparted into the food. Rose *et al*., 2015 reported that PAHs are formed during domestic and industrial food processing like roasting, toasting, drying, grilling, frying, baking, and barbecuing. Consequently, humans are exposed to PAHs through dietary (smoked foods) and non-dietary sources (inhalation and dermal contact). Comparatively, dietary sources represent the major exposure route. According to Rengarajan *et al*. 2015, over 70% of the PAHs exposure of non-smokers is associated with food consumption. Finally, Roseiro *et al.,* 2011 investigated the influence of different smoking processes on PAHs contamination in chorizo; similarly, they observed that industrially smoked products had a lower level of total PAHs than those traditionally smoked (1703 vs. 3237 µg/kg). Rose *et al.* 2015 evaluated the formation of 15 different PAHs (including the PAH4) in several samples of beef and salmon prepared by various cooking methods, including deep-frying, grilling with coal and wood, roasting in a natural gas oven, and roasting in an electric oven.
Of all the numerous compounds being generated in the course of burning the aforementioned fuel sources, this study investigated and assessed the level of PAHs and heavy metals being imparted to processed cowhide (kpomo) within some designated abattoirs in the Bayelsa and Rivers State.

Materials and Methods

Sampling Location

Cowhide meat samples for this study were collected from four abattoirs in Yenagoa Local Government Area of Bayelsa State and an abattoir in Obio Akpor Local Government Area of Rivers State. Control samples were also collected. The map coordinates Northing (N) and Easting (E) of the abattoir locations is as stated as follows; Igbogene 5^0 2' 17.8188"N and 6^0 24'14.958"E; Tombia, 4^0 57' 17.8092"N; 6^0 20'53.2428"E; Opolo 4^0 56'52.764"N and 6^0 20'3.984"E; Swale 4^0 53'42.9576"N and 6^0 16'39.7164"E; Rumuokoro, 4^0 52' 11.64"N and 7^0 01' 026"E; and the Control 4^0 52' 11.64"N and 7^0 01' 026"E

Cowhide meat Samples: Methods employed for cowhide roasting include roasting with tyre, roasting with firewood while some were roasted with firewood and waste plastics. Thus three samples were collected from each roasting procedure. They are thereafter scrape with knife and washed with water. Different cowhide meat samples were purchased and collected into wide mouthed sterile sample bottles, labeled appropriately and transported on ice packs to the laboratory for analyses. Cowhide scalded with hot water and cow hide brushed out with knife served as control.
Samples from each abattoir were homogenized in a Marlex blender, packaged in medium sized plastic bowls and labeled appropriately for analyses.

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Measurement of Polycyclic Aromatic Hydrocarbons

The Polycyclic Aromatic Hydrocarbon content of samples were determined through the principle of Gas Chromatography by flame ionization detection as sample extracts are being forced through an immobile, inert stationary phase (1,3-dimethyl siloxane) and components of low solubility take a shorter time to be transported through the column while components of higher solubility take a longer elution time leading to the differential mobilities of the fractional components of the polycyclic aromatic hydrocarbons (PAHs). Samples were automatically detected as they emerge from the column (at a constant flow rate) by the FID detector whose response was dependent upon the composition of the respective constituent fractions.
The specification of the Gas Chromatography used is as stated below:

Equipment used: HP 5890 Series II GC, U.S.A.
The operational condition (temperature program) for the GC analysis is stated below:

Injection temperature:
Initial oven temperature = 60^0C
Actual oven temperature = 275^0C
Detection temperature = 300^0C
Capillary column: 30m length, 0.32mm internal diameter
Detector: Flame Ionization Detector

Reagent(s)

PAH Standard for GC calibration: Restek SV Calibration Mix No 5 2,000ug/ml each in
Methylene Chloride, 110 Benner Circle Bellefonte. PA 16823
(b) Dichloromethane (BDH Laboratory reagents), BDH Chemicals Ltd, England
(c) Silical Gel (Bourgoyne & Co. Reagent, Mumbai, India)
(d) Anhydrous Sodium Sulphate (SureChem Products Ltd, Suffolk, England)
Sample preparation and Sample extraction

The different cowhide meat samples (food samples) were each, separately grinded with Marlex blender to obtain a well homogenized sample for extraction. Two grams of each solid matrix sample was weighed into a clean extraction container separately and 10ml of Dichloromethane was added into each and mixed thoroughly by stirring with glass rod and filtered through cotton wool stuffed filter funnel into clean solvent rinsed extraction bottles. This extract was concentrated to 2ml by evaporating on a water bath at 40 ^{0}C.

Sample cleanup/separation

One cm of moderately packed glass wool was placed at the bottom of a 10mm I.D. x 250mm long chromatography column. Slurry of 2g activated silica gel in 10ml dichloromethane was prepared and placed into the chromatography column. To the top of the column was added 0.5cm of anhydrous sodium sulphate. The column was rinsed with additional 10ml of Dichloromethane. The column was pre-eluted with 20ml of Dichloromethane; this was allowed to flow through the column for about 2 minutes until the liquid in the column was just above the anhydrous sodium sulphate layer. Immediately, 1ml of the extracted sample was transferred into the column.

The extraction bottle was rinsed with 1ml of Dichloromethane and added to the receiving end of the column as well. The stop-cork of the column was opened and the eluent was collected into a 10ml graduated cylinder. Just prior to exposure of the anhydrous sodium sulphate layer to air, Dichloromethane was added to the column in 1-2ml increments. Accurately measured volume of 8-10ml of the eluent was collected and labeled 'ALIPHATICS'.

Following the recovery of the aliphatics fraction, the column was eluted with 1:1 mixture of propanol and Dichloromethane in 1-2ml increments. Another accurately measured 8-10ml of the eluent was collected and labeled 'AROMATICS'. The aromatic fraction was concentrated to 1ml for PAHs analysis before being injected into the Gas Chromatograph.

Gas chromatography analysis

The concentrated 'AROMATICS' extracts were transferred into labeled glass vials with Teflon Rubber Crimp cap for GC analysis. One microlitre (1µl) of the concentrated sample was injected by means of an hypodermic syringe through a rubber septum into the column. Separation occurs as the vapour constituent

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partition between the gas and the liquid phases. The constituent aromatic compounds are automatically detected as it emerges from the column (at a constant flow rate) by the Flame Ionization Detector whose response is dependent upon the composition of the vapour, by measuring the detection time. The GC was calibrated by calibration curve method using standard solutions (working concentration of 50, 100, 200 and 1000mg/l PAH mixture by AccuStandards).

GC Operation Condition

Initial oven temperature	=	65^0C
Rate: 25^0C/minute (actual)	=	140^0C
Rate: 10^0C/minute (final)	=	300^0C
Run time	=	44minutes

Chemicals and Reagents Used

All the reagents used for these analyses are of analytical grade and are products of Eagle Scientific Limited, England, BDH Limited, England, Surechem Products Ltd, England, Rieldel-De Haen, Germany, Sigma-Aldrich, Germany, Kermel, China and Burgoyne Burbridges, India.

Results

Mean Concentration of PAHs in Roasted Cowhide samples

The mean values of individual PAHs obtained in roasted cowhide samples are presented in Figures 1a and 1b (PAHs graphed based on concentration values).

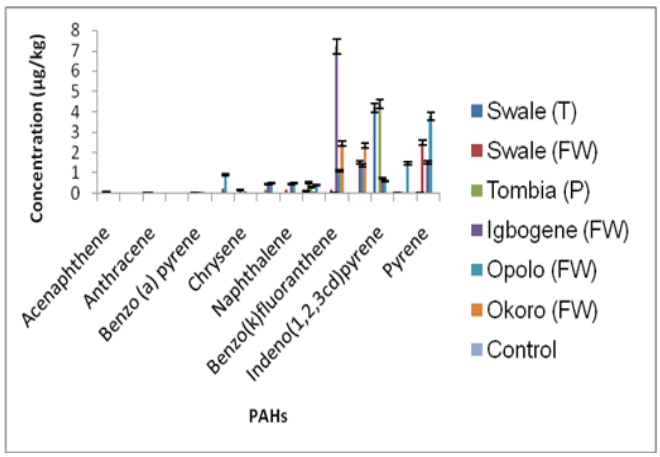

Figure 1a: Concentration of individual PAHs content of roasted cowhide meat

Key: FW = Firewood roasted**;** T = Tyre roasted**;** P = Plastic roasted

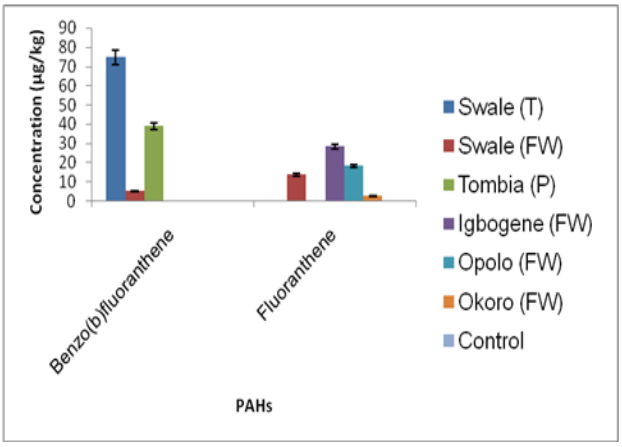

Figure 1b: Concentration of individual PAHs content of roasted cowhide meat

Key: FW = Firewood roasted**;** T = Tyre roasted**;** P = Plastic roasted

Low molecular weight PAH members containing two or three fused benzene rings (naphthalene, acenaphthylene, acenaphthene, anthracene, flourene and phenanthrene) were hardly detected in most samples. Acenaphthene and Acenaphthylene were undetected in most samples except Tombia (P) and Rumuokoro (FW). Trace values of anthracene, flourene, naphthalene and phenanthrene were recorded in samples. The highest value for phenanthrene (1.49 µg/kg) was observed in Opolo (FW) firewood roasted samples. Benzo(b)fluoranthene and Fluoranthene are the individual PAHs with the highest concentration values recorded. Benzo(b)fluoranthene had its highest value ofm74.68 µg/kg recorded in Swale (T) tyre roasted cowhide and Igbogene (FW) firewood roasted cowhide showed highest value of 28.28 µg/kg fluoranthene. Other individual PAHs occurred in varying concentration in the samples. However, the control sample, which is unroasted, recorded zero value of the individual and total PAHs.

Presented in Figure 2 is the concentration of PAH4 content of the roasted edible cowhide. The concept of use of PAH4 consisiting of Benzo(a)pyrene, Benzo(a)anthracene, Benzo(b)flouranthene and Chrysene as marker of carcinogenicity in foods was adopted in 2010 by the European Commission as adviced by of CONTAM (EC, 2009).

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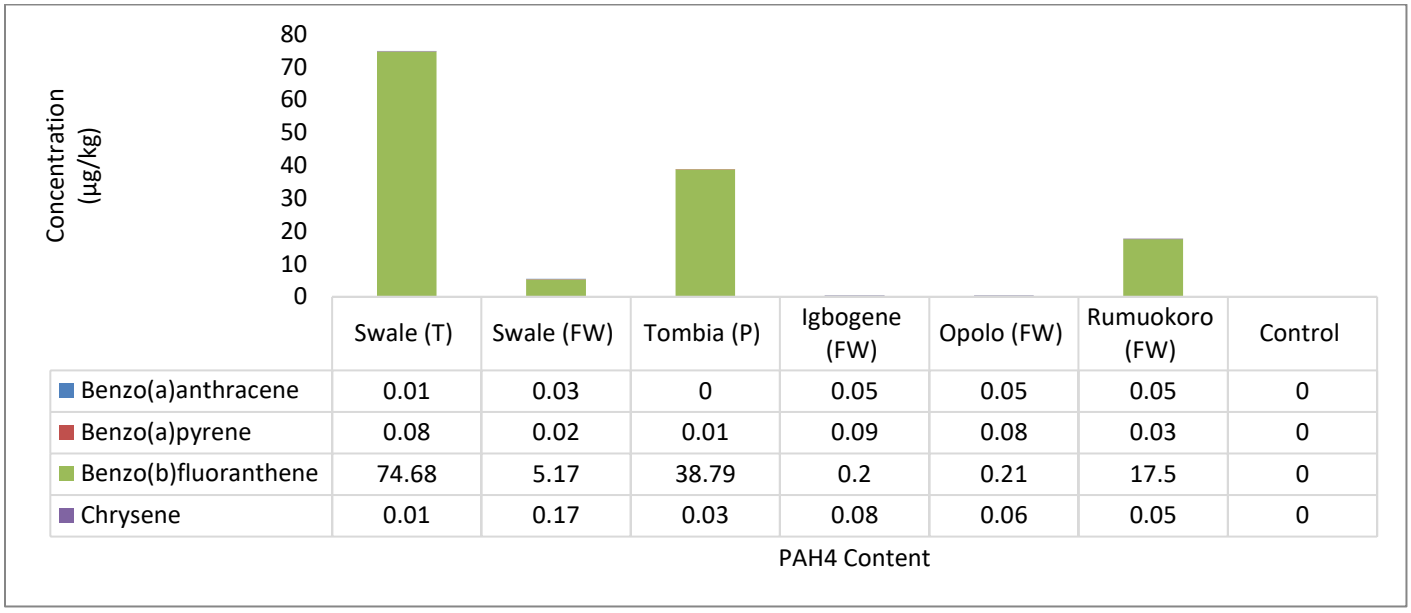

	Swale (T)	Swale (FW)	Tombia (P)	Igbogene (FW)	Opolo (FW)	Rumuokoro (FW)	Control
Benzo(a)anthracene	0.01	0.03	0	0.05	0.05	0.05	0
Benzo(a)pyrene	0.08	0.02	0.01	0.09	0.08	0.03	0
Benzo(b)fluoranthene	74.68	5.17	38.79	0.2	0.21	17.5	0
Chrysene	0.01	0.17	0.03	0.08	0.06	0.05	0

Figure 2: PAH4 content of roasted cowhide meat samples

Key: FW = Firewood roasted**;** T = Tyre roasted**;** P = Plastic roasted

Additionally, the permissible limit of the sum of PAH4 was also amended from 30.0 μg/kg to 12.0 μg/kg as from same date. PAH4 content in analysed samples in tyre roasted sample (74.78 µg/kg), plastic roasted sample (38.38 µg/kg), and firewood roasted sample (17.63 µg/kg) obtained from Swale, Tombia and Rumuokoro abattoirs respectively, exceededed the recommended permissible limits of PAH4 in foods. Other firewood roasted samples obtained from other abattoirs had PAH4 concentration values below the permissible limit. Conversely, PAH4 was undetected in Control (unroasted) samples.

The Concentration of total PAH in the roasted and control cowhide meat samples is shown in Figure 3. The concentration of total PAHs in the roasted cowhide recorded highest value of 79.25 µg/kg in tyre roasted Swale (T) sample, followed by the value of 44.03 µg/kg recorded in plastic roasted Tombia (P) sample. Firewood roasted Igbogene (FW) sample recorded 41.27 µg/kg, Firewood roasted Opolo (FW) samples recorded 28.75 µg/kg, firewood roasted Rumuokoro (FW) sample recorded 27.61 µg/kg while firewood roasted Swale (FW) sample recorded the least value of 22.67 µg/kg. The study revealed that the total PAHs concentration in the unroasted control cowhide sample was zero. Statistical analysis using One Way ANOVA indicated significant difference in the total PAHs concentration of examined samples across all the selected locations and the control sample.

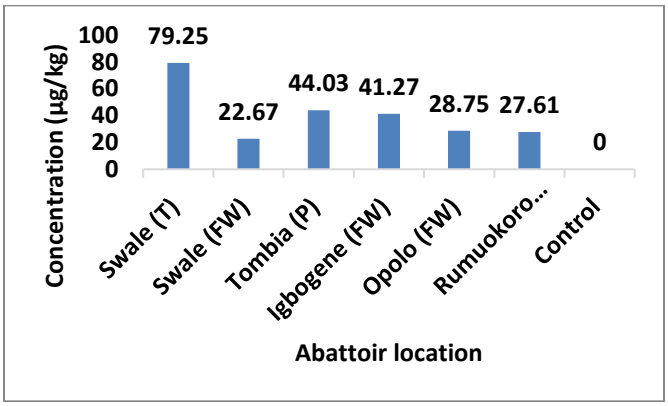

Figure 3: Total PAHs in roasted cowhide meat samples

Key: FW = Firewood roasted**;** T = Tyre roasted**;** P = Plastic roasted

The decreasing order for the total concentration of PAHs in roasted cowhide is:

Swale(T) > Tombia (P) > Igbogene (FW) > Opolo (FW) > Okoro (FW) > Swale (FW) > Control. This thus indicate that tyre roasted cowhide accumulated most PAHs, followed by Plastic roasted and the PAHs in

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firewood roasted cowhide were relatively lower. The result also confirmed the possibility of roasting fuels impacting PAHs unto processed foods.

Discussion

From the results obtained from the sampling locations, it was observed that Benzo(b)fluoranthene and Fluoranthene were high in concentrations with Swale (T) and Igbogene (FW) recording highest value, respectively. Other individual polycyclic aromatic hydrocarbons were also recorded in varying concentrations across the sampled locations. However, Acenaphthene and Acenaphtylene were detected in cowhide obtained from Tombia (P) and Rumuokoro (FW) only. Benzo(a)anthracene, Naphthalene and Phenanthrene was not observed in Tombia (P), Rumuokoro (FW), and Swale (FW), respectively. In the control sample, no individual PAHs were obtained. Control samples were scalded with hot water and the animal fur brushed out with knife. The results indicate that the cowhides were impacted with PAHs during the roasting processes. The obtained highest total PAHs in tyre roasted samples could be attributed to the fact that polycyclic aromatic hydrocarbons (PAH) are incorporated into extender oils for tyres and treads as found in rubber crumbs (Sibeko *et al*., 2020). Plastic material used for roasting as observed in Tombia (P) also documented high concentration of total PAHs impact on the cowhide meat. This corroborates the result of Zhou *et al*., 2015 who recorded that more PAHs were detected from the pyrolysis of plastics than the pyrolysis of biomass in a research to quantify polycyclic aromatic hydrocarbons (PAH) emission from the pyrolysis of different municipal solid waste fractions. Lastly, firewood roasted samples had the least concentration of total PAHs. Consequently, the tyre roasted cowhide is relatively the most toxic of all. However, unroasted but scalded samples had no PAHS which indicated it is the safest processing procedure for edible cowhide meat.

Conclusion and Recommendation

The results indicate that consuming cowhide meat processed with tyre, plastic and firewood will put consumers at high risk of health hazard associated with PAHs bioacummulation and subsequent attendant health risks. To reduce exposure to PAHs, consumers are advised to decrease the consumption of roasted cow hide meat. Furthermore, other methods of processing cowhide such as scalding with hot water and brushing out the animal fur with knife should be encouraged. This will not only reduce the risks on the food being processed but will also reduce the resultant dispersal of numerous pollutants particulates into the air which yet poses human risk via inhalation. Government should regulate the use of these afformentioned materials used in roasting cowhides for meat and also enforce the safer processing procedure for this food. The Government and relevant agencies should enact policies and guidelines that will protect food, the people and the environment.

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