Health risk assessment and source identification of Polycyclic Aromatic Hydrocarbons (PAHs) in commercially available singed cowhide within the Greater Accra Region, Ghana

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Abstract

This study ascertains the sources and potential carcinogenic threats of polycyclic aromatic hydrocarbons (PAHs) in singed cowhide. The objective was to assess the sources of PAHs and the health threats singed cowhide possess to the consuming public. A total of fifty-four (54) cowhide samples from selected markets within the Greater-Accra Region of Ghana were analyzed using Agilent GC 6890N, MS5975B Series gas chromatography in a splitless mode. The study shows that, singed cowhide within the Greater Accra Region is dominated by positive genotoxicity PAHs classified as carcinogens (1) and possible carcinogens (2B) as well as positive and questionable genotoxic PAHs that are not classifiable (3). The [B(a)P]eq and PEC results suggest that consumption of singed cowhide at the rate of 25.2 g/day poses potential adverse health effects such as cancer, mutations and birth defects in terms of B(a)P to humans. Results further show that, the HQ/HI < 1, thus, there is no concern for potential human health risks caused by exposure to non-carcinogenic PAHs in singed cowhide. However, the carcinogenic toxic equivalent (TEQs) values for both adults and children were greater than the screening values and therefore, there is concern for potential human health risks caused by exposure to carcinogenic PAHs in singed cowhide. Source assessment of PAHs in singed cowhide shows that, PAH sources in singed cowhide is predominantly from pyrolitic rather than petrogenic origins. Thus, the PAHs in singed cowhide within the Greater Accra Region originate primarily from incomplete combustion and of petroleum origin due to singeing.

Keywords: Health risk assessment, source identification, PAH, singed cowhide, Greater- Accra, Ghana

Introduction

Animal skin or hide is known for its nutritive composition and a delicacy widely consumed as a meat source. In Africa, cowhide is the most consumed animal skin (Akwetey et al, 2013; Appiah, 2016). Different communities in Africa have different indigenous names for processed cowhide. For instance, it is called 'Wele' in Ghana and 'Ponmo' in Nigeria (Akwetey et al., 2013). The raw hide is derived from singeing- a process by which hair on the skin of slaughtered livestock such as cattle, cow or goat is removed in open fire (Appiah, 2016). Singeing is widespread in Africa because the process evokes acceptable flavours in the meat that conforms to consumer taste besides, preserving the carcass hide for consumption (Appiah, 2016). Singeing was traditionally done using fire wood. However, in recent times, firewood has become relatively scarce especially in urban centres resulting in the use of unregulated processing techniques including; the use of unsorted garbage containing plastics and other organic materials, discarded engine oil and car tyres as sources of fuel (Eremong, 2011; Okafo et al., 2012; Akwetey et al., 2013; Dada et al., 2018). These sources of fuel have however, been known to potentially contain carcinogenic compounds such as dioxins, furans and polycyclic aromatic hydrocarbons (PAHs) leading to the exposure of processors and consumers to potential human health risks (USFA, 1999; Eremong, 2011; Akwetey et al., 2013). According to Appiah, (2016), these toxins have the propensity to accumulate in human tissues resulting to cancer. Potential known human health effects associated with singeing of cowhide include; short-term tissue irritation (such as skin, respiratory, eyes and gastrointestinal), decreased fertility, developmental neurological effects and renal toxicity amongst butchers exposed to relatively high levels of PAHs (Hill, 2015). Exposure assessment has been used to determine whether humans are in contact with a potentially hazardous chemical and, if so, to what extent, by what route, through what media and for how long (WHO, 2021). PAHs are made up of large category of different organic compounds containing two or more aromatic rings and occur generally in complex mixtures made up of hundreds of compounds and are ubiquitous in the environment (Menichini, 2003; Loai et al., 2018; Dan et al., 2020). As a group, PAHs are among the most common contaminants at waste sites and are considered as products of pyrolytic or petrogenic reactions involving organic materials which end up as pollutants in the environment (USEPA, 1993). PAHs are produced by anthropogenic activities and natural occurrences including carbon-containing materials such as oil, wood, garbage through combustion and pyrolysis processes (Hamidi et al., 2016). According to Hamidi et al., (2016), significant sources of PAHs in foodstuff are through food processing (i.e. smoking, grilling and roasting) since these processes allow direct contact between food and combustion products. Several factors affecting PAH formation has been identified. These include: the distance of food from the heat source (Nawrot et al., 1999; Knize et al., 1999), fat content of the food (WHO, 1998), duration of cooking (WHO, 1998), temperature used (WHO, 1998), whether melted fat is allowed to drop onto the heat source (Nawrot et al., 1999) and, type of fuel used (SCF, 2002).

The United States National Academy of Science in 1972 published a list of strongly carcinogenic PAHs and also identified seven common PAH compounds as probable human (group B2) carcinogens" in 1993 (Tay et al., 2013). The Scientific Committee on Food (SCF) in 2002, proposed the use of Benzo[a] pyrene [B(a)P] as a marker for the occurrence of carcinogenicity and genotoxicity subsequent to the concerns regarding the potential human health risk with respect to PAHs in food. In view of this, the European Commission (EC) and the European Food Safety Authority (EFSA) established a guideline for [B(a)P] in smoked meat products in 2005. Subsequently, [B(a)P] maximum level of 5.0 μg/kg in smoked meat products was set for the European Union (EC, 2006). However, further studies on the toxicity and occurrence of PAHs in food products acknowledged the possible contribution from other higher-molecular-weight (5-6 rings) PAHs to toxicity. It was therefore concluded that, [B(a)P] was no longer suitable to be solely used as a marker for the occurrence and toxicity of PAH in foods (EFSA, 2008, Joon-Goo et al., 2019). In furtherance to its mandate to ensure the safety of meat products, EFSA identified a group of 4 PAHs (PAH4) and a group of 8 PAHs (PAH8) as superior indicators of carcinogenicity based on data relating to the occurrence and toxicity of PAHs. Subsequently, it was concluded that, in determining carcinogenicity, the use of PAH8 presented a more meticulous approach compared to the use of PAH4' (Commission Regulation (EU) No 835/2011 of 19 August 2011). For the purposes of this study, the list as presented by the United States National Academy of Science is considered as PAH3, while, USEPA, (1993) "probable human (group B2) carcinogens" is considered as PAH7 (Table 1). The 16 PAHs regarded as 'priority pollutants' to be controlled in the United States and extensively monitored by the research community previously (Hamidi, et al., 2016) is the main focus of this study.

Detailed assessment of the likely sources of PAH in singed cowhide has the potential of informing polices on safe processing methods to be adopted within the framework of public health safety (Hill, 2015). Source identification of PAHs have previously been widely conducted using key isomeric ratios (Nkpaa et al., 2013; Tay et al., 2013; Adeniji, et al., 2019).

Unlike in the developing countries, the strict regulation enforcement in the meat industry by developed countries such as Europe and the United States of America ensures the safety of consumers of meat products. The Environmental Health Department and the Veterinary Services Department of the various Municipals, Ministries, Departments and Agencies (MMDA's) are mandated to ensure safe, healthy and hygienic meat and

TABLE 1
List of PAHs considered as carcinogenic

PAH Compound	*PAH3*	*PAH4*	*PAH7*	*PAH8*
Naphthalene				
Acenaphthalene				
Acenapthene				
Fluorene				
Phenanthrene				
Anthracene				
Fluoranthene				
Pyrene				
Benzo[a]Anthracene		×	×	×
Chrysene		×	×	×
Benzo[b]Fluoranthene	×	×	×	×
Benzo[k]Fluoranthene			×	×
Benzo[a]pyrene	×	×	×	×
Indeno[1,2,3-c,d]pyrene			×	×
Dibenzo[a,h]anthracene	×		×	×
Benzo[g,h,i]perylene				×

PAH3- US National Academy of Science strongly carcinogenic PAH compounds (1972)
PAH4- European Food Safety Authority (EFSA) superior indicators of carcinogenicity (2008)
PAH7- USEPA identified seven common PAH compounds as ‘‘probable human (Group B2) carcinogens (1993)
PAH8- European Food Safety Authority (EFSA) superior indicators of carcinogenicity

meat products in Ghana. Nevertheless, these agencies are not ensuring the strict regulation enforcement in the meat industry, thereby allowing unhygienic meat processing methods in the industry. This negligence on the part of regulatory agencies expose the consuming public to potential health implications in terms of carcinogenic potencies. According to Appiah (2016), the Food and drugs Authority (FDA) in Ghana reported in 2010 that, the total number of out-patients reporting to the health facilities with food borne diseases in Ghana in 2009 was about 20,000 per year, with an annual death rate estimated at 6,500 and total cost to the economy estimated at US$ 69 million. It is therefore imperative for the government of Ghana through the regulatory agencies to protect the health of consumers of singed cowhide and other smoked meat products by ensuring the strict regulation enforcement in the meat industry. One of the ways to achieve this, is through the assessment and documentation of the levels, sources and potential human health risks of PAHs and other toxic residues in meat products.

This paper thus, seeks to identify and characterize the sources of PAHs in commercially available singed cowhide within the Greater Accra Region of Ghana as well as its potential health implications in terms of carcinogenic potencies on the health of the consuming public.

Materials and Methods

Sampling and preservation

A total of fifty-four (54) samples of singed cowhide were purchased from seven selected markets in the Greater Accra Region. The markets are Mallam Atta, Accra Central, Agbogbloshie, Odawna, Ashaiman, Tema and Tema-Newtown. Sampling was carried out between February 2018 and August 2019. Except at Odawna market where only 3 vendors were located at the time of sampling, four vendors were located in each market and singed cowhide were bought from these vendors within the selected markets. The samples were wrapped in aluminum foils with sample identification codes. Thereafter, the samples were transported to the organic laboratory of the Environmental Chemistry and Sanitation Engineering Division (ECSED) of the Council for Scientific and Industrial Research-Water Research Institute (CSIR-WRI) in an ice-chest containing ice-blocks. The samples were. stored in a refrigerator at a temperature of -19°C until analyzed.

Standards and Reagents

Dichloromethane (DCM), iso-octane, acetone and hexane were of High-performance liquid chromatography (HPLC) grade solvent. beta, β-Ginaphthyl (β, β-BN) and 3, 6-Dimethylphenanthrene (3, 6-DMP) were used as internal standards. A PAH standard mixture (Reference material 1491) for the analysis of 16 different PAHs was purchased from the National Institute of Standards and Technology (NIST), Norway. Solvents and chemicals were obtained from British Drug House (BDH) Laboratory Supplies, England.

Sample Extraction

The sample extraction process was performed using Soxhlet as described in the USEPA Method 3540C. The method was used to extract the semi-volatile and non-volatile PAHs from the singed cowhide matrix. Each singed cowhide sample was cut into pieces and homogenized. About 10g of each singed cowhide sample was weighed in labelled aluminum foils. 20 g of anhydrous sodium sulphate (Na_2SO_4) was weighed and added to each singed cowhide sample and thoroughly mixed. Each mixture was then poured into a cellulose extraction thimble. Using a micro syringe, 100 µL of the internal standard of concentration 100 µg/mL was added to each sample mixture. 120 mL of DCM was used for a 6 hrs extraction of semi-volatile and non-volatile PAHs. The resultant extract was then evaporated to 1ml under a gentle steam of nitrogen (N_2).

Sample Clean-up

The USEPA Method 3630C as a Standard Method -Silica Gel Column Chromatography Clean-up was used. A slurry of 10g activated silica gel in 10 ml DCM was prepared and transferred into a chromatographic column (measuring 13 mm ID x 30 mm long) with fritz at the bottom which was pre-conditioned with 10 ml hexane. About 1g of Na_2SO_4 was added to the silica gel and washed with 10 ml hexane. The sample extract was then transferred onto the column and eluted with 20 ml hexane for aliphatic fractions into a separate test tube. About 20 ml DCM was eluted into another test tube and one drop of iso-octane was added. The eluents were then combined and concentrated to 1ml over a gentle stream of N_2 gas. The final extracts were transferred into a 2 ml sample vial for injection into the gas chromatography- mass spectrum (GC-MS) for PAHs analysis.

Calibration

Six (6) different calibration solutions of 0.5, 1.0, 2.0, 5.0, 10.0 and 15.0 µg/L were prepared by dilution from standard reference material (SRM)1491 for data quality control and quality assurance (QC/QA). A mixture of internal standard was spiked into each of the six calibration solutions. A 1 µL was then injected into the gas chromatogram (GC). The calibration solutions were thereafter, run in both SCAN (where the instrument acquires a continuous range of ion fragmentation data to detect all possible PAH compounds within the sample) and Selected Ion Monitoring (SIM) (where the mass spectrometer allows the detection of specific compounds with very high sensitivity) modes. Each of the 16 PAH compounds were then identified from the mass spectrometer (MS) library and used for calibration.

GC-MS condition

Agilent GC 6890N, MS 5975B inert XL EI/CI MSD Series gas chromatography fitted with a 7683B Series automatic sampler was used for the analysis. The carrier gas was helium maintained at a constant flow rate of 2 mL/min. The chromatographic separation was performed using a 30-m HP5-MS [a

(5%-phenyl)-methylpolysiloxane phase with very low bleed characteristics that is ideal for GC/MS] fussed silica capillary column with dimensions 30 m long, 0.25 mm ID and 0.25 µm film thickness. The temperature programing was: Initial temperature: 80^{o}C for 2 min, final temperature: 1:280^{o}C, Rate: 10^{o}C/min; final time: 1:0 min; final temperature: 2: 300^{o}C; Rate: 1: 3^{o}C/min; final time: 2:2 min and the total run time was 34.86 minutes. Using a 10 µL syringe, 1 µL of each final extract was injected in splitless mode using a 7683B Series automatic sampler with an injector port temperature of 280^{o}C. Samples were run in both SCAN and SIM modes. The limits of detection for this study was 0.001µg/kg for individual PAHs in biological material.

Recovery efficiencies

Recovery studies were conducted using the same experimental procedure for final extracts, and a solution containing known PAH concentrations. The method shows a total recovery efficiency of PAHs ranging from 78.2 to 102.4 % and a mean of 97.5 %. The Mean R.S.D (%) was 13 % and the value of potential error for PAHs determination was appraised to be 15 %. Concurrently, procedural blanks were also run together with the samples for quality assurance and quality control.

Data analysis

Descriptive statistics was applied on the PAH data generated for this study using Excel 2013 Inc. Human health risk assessments were conducted using Toxic Equivalent Factors (TEF) and Potency Equivalent Concentration (PEC) as per the USEPA guideline and the cancer and non-cancer health risks methods. Molecular ratios of Chrysene/ Benzo[a] Anthracene [Chr/B(a)A], Anthracene/ (Anthracene + Phenanthrene) [Ant/(Ant + PA], Fluoranthene/ (Fluoranthene + Pyrene) [Flu/ (Flu + Pyr], Benzo[a]Anthracene/ (Benzo[a] Anthracene + Chrysene) [B(a)A/(B(a)A + Chr], Indeno{1,2,3-c,d}pyrene/ (Indeno{1,2,3-c,d}pyrene + Benzo{g,h,i}perylene) [In(123-cd)P/(In 123-cd)P+B(ghi)P], Benzo[a] Anthracene/228 [B(a)A/228], Benzo[a] Anthracene / (Benzo[a]Anthracene + Chrysene) [B(a)A/(B(a)A + Chr)], Chrysene / Benzo[a] Anthracene [Chr/B(a)A], Fluoranthene/ Pyrene [Flu/Pyr] and lower molecular weight- PAH/ higher molecular weight- PAH [LMW-PAH/ HMW-PAH] were used to identify the sources of PAHs. To determine the distribution of each source principal component analysis (PCA) was employed by extracting the eigenvalues for dimensionality reduction. Loading scores > 0.3 were considered significant.

Human health risk assessment

Human health risk assessment has been widely conducted by calculating the possibility of any severe health effects coming from the exposure of an individual to carcinogenic and/or non-carcinogenic substances over a particular period of time (Adeniji et al., 2019). In this study, health risks that may arise when an individual or a population is exposed to the 16 priority PAHs in singed cowhide was estimated using the US EPA standard models (USEPA, 1989; USEPA, 1993 and USEPA, 2000).

Toxic Equivalent Factors (TEF) and Potency Equivalent Concentration (PEC) assessment in B(a)P

The USEPA guideline have been previously used to describe the carcinogenic risk from exposure to PAH in meat products (Cheung et al., 2007). The guideline employed the SCF proposal to use B(a)P as a maker for the occurrence of carcinogenicity and genotoxicity of PAH in food (Scientific Committee on Food, 2002; Abdulazeez, 2017). The method typically, computes the total sum of the carcinogenic health risk utilizing the product of the individual PAH concentrations and their toxic equivalent factors (TEFs) (Cheung et al., 2007). TEF is a derivative of the cancer potencies of the individual PAH compounds in relation to the cancer potency of B(a)P (Cheung et al., 2007). The individual PAHs and their TEF values relative to the cancer potency of B(a)P are presented in Table 2 (Nisbet et al., 1992). According to Nisbet et al., (1992), the product of the individual PAH concentration and its corresponding TEF value results in a

TABLE 2

PAHs and their toxic equivalency factors (TEF) relative to the cancer potency of B(a)P

PAH compound	TEF
B(a)P	1
Nap	0.001
AcPy	0.001
Acp	0.001
Fl	0.001
PA	0.001
Ant	0.01
Flu	0.001
Pyr	0.001
B(a)A	0.1
Chr	0.01
B (b)F	0.1
B(k)F	0.01
In(1,2,3-cd)P	0.01
DB(ah)A	5

(After Nisbet et al. 1992)

B(a)P equivalent concentration [i.e (B(a)Peq] for each PAH compound as presented in Eqn1:

$$B(\mathrm{a})\mathrm{Peq} = \mathrm{C}_{\mathbf{PAH}} \ \ \mathrm{X\ TEF} \quad 1$$

where, C_{PAH} is the concentration of individual PAH compound.

The carcinogenic potency equivalent concentration (PEC) is computed by summing up the individual B(a)Peq as presented in Eqn 2 (Nisbet et al. (1992):

$$\mathbf{PEC = (\textstyle\sum B(a)Peq)} \qquad 2$$

A screening value (SV) which indicates the threshold for potential adverse health effects in food was later developed by Russell et al., (1997). To determine the potential adverse health effects in food, the computed PEC values should be compared with the computed screening value for a particular food. If, PEC < SV, then the long-term consumption of that food is not associated with potential adverse health effects, while, a PEC > SV connotes a potential adverse health effect associated with the long-term consumption (Russell et al., 1997). The computation of screening value in food is as presented in Eqn 3 (Russell et al., (1997):

$$\mathbf{SV = [(RL/SF) \times BW]/CR} \qquad 3$$

where, SV denotes the screening value (μg/g) i.e the threshold concentration of total PAHs in singed cowhide that is of potential public health concern, RL denotes the acceptable maximum risk level (unitless), SF denotes the USEPA (1993) oral slope factor (μg/g day^{-1}) for PAHs, which is used to estimate an upper-bound probability of an individual developing cancer as a result of a lifetime exposure (i.e 70 years) to carcinogenic PAHs and has a value of 7.30 (μg/g day^{-1}) (USEPA, 1993), CR denotes the consumption rate in g/day, BW denotes the average body weight (kg) set at 70kg for adults (Jiang et al., 2005). The meat consumption rate was set at 0.0252 kg/day from the annual per capita meat consumption of 9.2 kg for Ghana (FAO, 2003; ASNS, 2003). RL denotes the acceptable maximum risk level set at 10^{-5} (unitless) (USEPA, 1993) such that, the maximum risk would be one additional cancer death per 100000 persons, if an adult who weighs 70kg consumed 25.2 g/day of meat, with the same measured concentrations of PAHs for a lifetime (i.e 70 years) (Nyarko et al., 2011). In order to estimate the minimum threshold for consumer protection from carcinogenic effects of PAHs likely to be detected in singed cowhide for safety reasons, a consumption rate was set at 1g/day (Nyarko et al., 2011).

Carcinogenic and non-carcinogenic assessment of PAHs

Human health risk assessment studies have been conducted by calculating the possibility of any severe health effects as a result of the exposure of an individual to carcinogenic and/or non-carcinogenic substances over a particular period of time (US EPA, 2001; US EPA, 2009; Adeniji et al., 2019). In this study, health risks that may arise when an individual or a population is exposed to the 16 priority PAHs in singed cowhide was estimated using Dan et al., (2020). The carcinogenic and non-carcinogenic health risks associated with PAHs in singed cowhide were assessed via the Estimated Chronic Daily Intake (ECDI) and Hazard Quotient (HQ) for children and adults were evaluated (Dan et al, 2020). The estimated daily intake for polycyclic aromatic hydrocarbons in singed cowhide was computed using Eqn 4 (Dan et al., 2020):

$$\mathrm{ECDI} = \frac{MI\ x\ MC}{BW} \qquad 4$$

Where, MI is the Estimated quantity of singed cowhide consumed (g/person/day). Values for the estimated quantities of singed cowhide used for computing the ECDI (both children and adults) are those reported by Dan et al., (2020) and obtained through Food Frequency Questionnaire (FFQ) method; MC is the mean concentration of each PAH in singed cowhide as analyzed in mg/kg; BW is the average body weight of each group (children and adults) in kg.

According to Tay (2019), the hazard quotient (HQ) is a numeric estimation of the toxicity potential in a system posed by a single element within a single route of exposure. The Hazard Quotient of individual polycyclic aromatic hydrocarbons was computed using Eqn 5 (Dan et al., 2020):

$$HQ = \frac{Ef\ X\ EDtotal\ X\ EDI}{RfDo\ X\ BW\ X\ AT} X\ 10e^{-3} \qquad 5$$

Where, Ef is Exposure frequency; EDtotal is Exposure duration, total (70 years); RfDo is Oral reference dose in mg/kg/day; BW is the average body weight for adults and children; AT is the averaging time for non-carcinogens (365 days/year EDtotal) and its estimated daily intake.

The Hazard Index (HI) was obtained by summing the HQ of each contaminants and used to determine the total risk from possible multiple contaminated pathways as presented in Eqn 6 (Dan et al., 2020):

$$\mathrm{HI} = \sum_{i=1}^{n} \mathrm{HQi} \qquad 6$$

To elicit the carcinogenic health risk, the strength of individual polycyclic aromatic hydrocarbon in singed cowhide was further assessed using carcinogenic Toxic Equivalents (TEQs). This approach was employed to directly assess the carcinogenicity of PAH contamination in singed cowhide (Tongo et al., 2016). TEQs are evaluated as the sum of the product of the concentration of individual polycyclic aromatic hydrocarbons congeners and their toxicity equivalency factor as presented in Eqn 7 (Dan et al., 2020):

$$\text{Carcinogenic Toxic Equivalents (TEQs)} = \sum \mathrm{PAHi\ X\ TEFi} \qquad 7$$

Where, ∑PAHi is the concentration of individual PAH measured in the singed cowhide samples and TEFi is the toxicity equivalency factor of PAHs. Carcinogenic toxic equivalents were computed for only 6 out of the 16 priority PAHs regarded as potential carcinogens by the United States Environmental Protection Agency.

To ascertain the threshold concentrations in singed cowhide, the screening value of PAHs were computed as in Eqn 8 (Dan et al., 2020):

$$\text{Screening Value (carcinogenic)} = \frac{\left(\frac{\mathrm{RL}}{\mathrm{SF}}\right)\mathrm{X\ BW}}{\mathrm{IR}} \qquad 8$$

Where, RL is the maximum acceptable risk level; SF is the cancer slope factor; BW is the body weight for children and adults; IR is the ingestion singed cowhide rate. The value and connotation of each parameter as described in Eqns 4-8 are presented in Table 3.

Results and Discussions

The PAHs with the highest concentration in the singed cowhide was Indeno (1,2,3-cd) pyrene (561.0 µg/kg) whereas, the lowest PAH was naphthalene (48.0 µg/kg). The dominance pattern of PAHs in singed cowhide was in the

TABLE 3

Parameters and values used for the evaluation of carcinogenic risk through consumption of singed cowhide

Parameters	values	Reference
Body weight	15-child 70-adults	USEPA (1989); USEPA (2000)
Estimated quantity (g/person/day)	22.31-child 17.11-adults	Dan et.al. (2020)
Exposure frequency (Ef)	250 days/year	Oregon Department of Environmental Quality (2010)
Exposure duration (years)	6- child 30-adults	Grzetic et al. 2008; Dan et.al. (2020)
Maximum acceptable risk level (RL)	10^{-5}	Dan et.al. (2020)
Oral reference dose of PAHs (mg/kg/day)		Values reported by Tongo et al (2016)
Average time for non-carcinogens (day/year)	365 days/year	Dan et.al. (2020)
Toxic Equivalent Factor (TEFi)		Tongo et al (2016)
Cancer Slope Factor (CSF) (ingestion)		Values reported by USEPA (1993)

(After, Dan et.al. 2020)

order of: In(1,2,3-cd) P > Flu > Ant > PA> Pyr > Chr > B(a)A > B (b)F > B(a)P > AcPy > Fl > B(ghi)P > B(k)F > Nap > Acp > DB(ah)A (Fig 1). The result of this study is inconsistent with a previous study by Chung et al, (2011) which showed that highest PAHs in smoked meat products was phenanthrene (18.18 µg/kg) and dibenzo (a,h) anthracene (0.89 µg/kg) as the lowest PAH.

The total concentration of PAHs in singed cowhide ranged from 29.0 to 2377.0 µg/kg and a mean value of 708.7 µg/kg (Table 4), as depicted pcitorially in Fig 2. The results suggest that during smoking, the cowhides shares direct

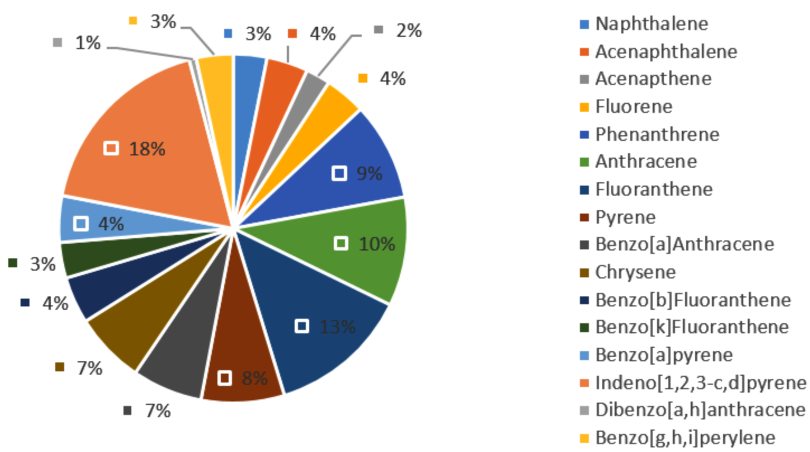

Figure 1 PAH dominance in commercially available singed cowhide within the Greater Accra Region

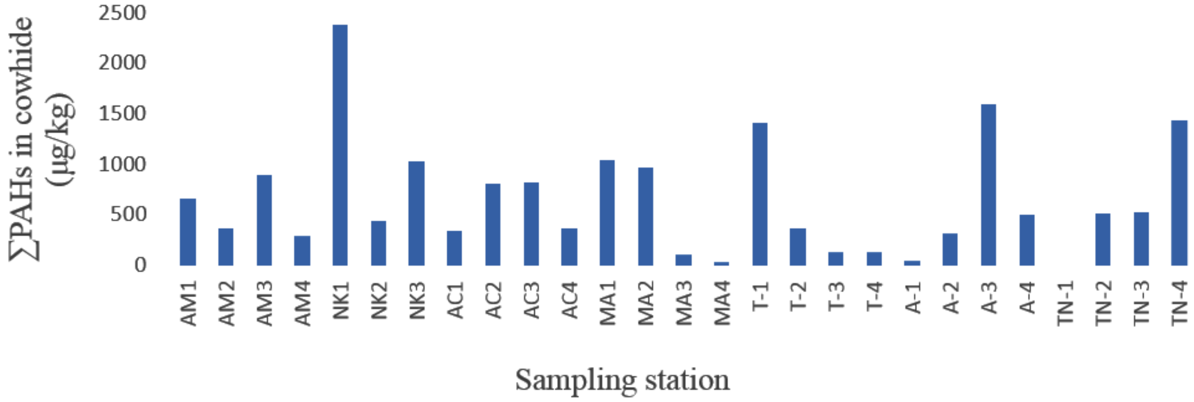

Figure 2 ∑PAHs in singed cowhide from selected markets in the Greater Accra Region

contact with the extremely high temperatures, and the extended smoking period coupled with the petroleum/ organic materials used as heat sources resulting in significantly higher accumulation of PAHs. According to Chen & Lin (1997), when meat is in direct contact with a heat source, PAHs are generated through pyrolysis of fats in the meat. The melted fat from the meat, drips onto the heat source generating PAHs. Consequently, the generated PAHs are deposited on the meat surface as the smoke rises. Abdulazeez (2017), also postulated that, the direct contact of smoked meat with flame generates PAHs that is deposited on its surface through pyrolysis of drippings from the meat, even if not in direct contact, fat dripping onto the flame generates PAH compounds that are carried back onto the surface of the meat. A previous study on the level of total PAHs in duck meat showed that levels as high as 130 and 320μg/kg were detected in roasted and charcoal grilled duck meat respectively while, a level as low as 8.6μg/kg was detected in steamed duck meat (Chen & Lin 1997). Chen & Lin (1997) and Abdulazeez (2017), are consistent with the dripping of fat onto the flame during singeing of cowhide thereby generating PAH compounds that are carried back onto the surface of the meat. The nutritional value of raw cowhide is approximately 56.5 - 69.2 % protein, 1.2 -

TABLE 4
Statistical summary of PAH content (μg/kg) in singed cowhide purchased from the Tema-Ashaiman metropolis in the Greater Accra Region

PAH Compound	Abbreviation	Sampling Station											
		T-1	T-2	T-3	T-4	A-1	A-2	A-3	A-4	TN-1	TN-2	TN-3	TN-4
Naphthalene	Nap	0.051	ND	ND	ND	ND	ND	0.051	ND	ND	ND	ND	0.051
Acenaphthalene	AcPy	0.068	ND	0.07	ND	ND	0.103	0.065	ND	ND	ND	ND	0.067
Acenapthene	Acp	ND	ND	0.066	ND	ND	ND	ND	ND	ND	ND	ND	0.064
Fluorene	Fl	0.077	ND	0.076	ND	ND	ND	0.074	ND	ND	ND	ND	0.075
Phenanthrene	PA	0.146	0.087	0.174	ND	ND	ND	0.112	0.085	ND	0.083	0.088	0.113
Anthracene	Ant	0.162	0.093	0.192	ND	ND	ND	0.122	0.091	ND	0.089	0.093	0.123
Fluoranthene	Flu	0.093	0.121	0.111	0.067	0.110	ND	0.131	0.123	ND	0.270	0.272	0.115
Pyrene	Pyr	0.106	0.068	0.127	0.067	0.067	ND	0.099	0.070	ND	0.070	0.073	0.093
Benzo[a]Anthracene	B(a)A	0.123	ND	0.120	ND	ND	0.120	0.121	ND	ND	ND	ND	0.118
Chrysene	Chr	0.098	ND	0.089	ND	ND	0.091	0.095	ND	ND	ND	ND	0.091
Benzo[b]Fluoranthene	B(b)F	0.129	ND	0.119	ND	ND	ND	0.131	ND	ND	ND	ND	0.108
Benzo[k]Fluoranthene	B(k)F	0.111	ND	0.106	ND	ND	ND	0.112	ND	ND	ND	ND	0.126
Benzo[a]pyrene	B(a)P	0.128	ND	ND	ND	0.120	ND	0.125	ND	ND	ND	ND	ND
Indeno[1,2,3-c,d]pyrene	In(123-cd)P	ND	ND	ND	ND	0.133	ND	0.286	0.135	ND	ND	ND	0.168
Dibenzo[a,h]anthracene	DB(ah)A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[g,h,i]perylene	B(ghi)P	0.114	ND	ND	ND	ND	ND	0.116	ND	ND	ND	ND	0.116
∑ PAHs		**1.41**	**0.37**	**1.25**	**0.13**	**0.43**	**0.31**	**1.59**	**0.50**	-	**0.51**	**0.53**	**1.43**
∑ 7c-PAHs		**0.70**	-	**0.43**	-	**0.12**	**0.21**	**0.70**	-	-	-	-	**0.56**
PAH3		**0.26**	-	**0.12**	-	-	-	**0.26**	-	-	-	-	**0.11**
PAH4		**0.48**	-	**0.33**	-	**0.12**	**0.21**	**0.47**	-	-	-	-	**0.32**
PAH8		**0.70**		**0.43**		**0.25**	**0.21**	**0.99**	**0.14**				**0.73**
PAH dominance in wele (%)													
LMW-PAHs (2–4 Ri ngs)		**50**	**100**	**65.3**	**100**	**41.2**	**32.8**	**38.0**	**73.3**	-	**100**	**100**	**30**
HMW-PAHs (5–6 Ri ngs)		**50**	**0**	**34.7**	**0**	**58.8**	**67.2**	**62.0**	**26.7**	-	**0**	**0**	**70**

∑PAHs - sum of PAHs; PAH7- USEPA (1993) probable human (Group B2) carcinogens; PAH3- US National Academy of Science (1972) strongly carcinogenic PAH compounds;PAH4- EFSA group of 4 PAHs (PAH4) as superior indicators of carcinogenicity; PAH8- group of 8 PAHs as superior indicators of carcinogenicity; ND- not detected,T1-T4: Tema Market; A1-A4: Ashaiman market; TN1-TN4: Tema Newtown market

TABLE 4 *cont.*

Statistical summary of PAH content (μg/kg) in singed cowhide purchased from the Accra metropolis in the Greater Accra Region

PAH Compound	Abbreviation	Sampling Station														
		AM1	AM2	AM3	AM4	NK1	NK2	NK3	AC1	AC2	AC3	AC4	MA1	MA2	MA3	MA4
Naphthalene	Nap	ND	0.048	ND	0.048	0.063	ND	0.049	ND	0.05	ND	ND	0.058	0.05	0.056	0.053
Acenaphthalene	AcPy	ND	0.061	ND	ND	0.078	ND	ND	ND	0.089	0.055	ND	0.063	ND	0.055	ND
Acenapthene	Acp	ND	ND	ND	ND	0.086	ND	ND	ND	0.096	0.084	ND	0.062	ND	ND	ND
Fluorene	Fl	ND	ND	ND	ND	0.086	ND	ND	0.069	0.072	0.09	0.068	0.076	ND	ND	ND
Phenanthrene	PA	ND	0.088	0.09	0.083	0.191	ND	0.096	ND	0.062	0.069	ND	0.09	0.088	0.086	ND
Anthracene	Ant	ND	0.095	0.097	0.088	0.202	ND	0.103	0.079	0.122	ND	ND	0.097	0.098	0.093	ND
Fluoranthene	Flu	0.1	ND	0.131	ND	0.123	0.134	0.17	ND	ND	0.12	0.108	0.121	ND	0.115	0.095
Pyrene	Pyr	ND	0.072	0.075	0.068	0.137	0.067	0.071	ND	ND	ND	ND	0.072	0.081	0.07	ND
Benzo[a]Anthracene	B(a)A	ND	ND	ND	ND	0.101	ND	0.085	ND	ND	0.255	0.183	ND	0.088	ND	ND
Chrysene	Chr	ND	ND	ND	ND	0.134	ND	0.113	0.188	0.311	ND	ND	ND	0.117	ND	ND
Benzo[b]Fluoranthene	B(b)F	ND	ND	ND	ND	0.126	ND	0.117	ND	ND	0.144	ND	ND	ND	ND	ND
Benzo[k]Fluoranthene	B(k)F	ND	ND	ND	ND	0.108	ND	0.102	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[a]pyrene	B(a)P	ND	ND	0.121	ND	0.129	ND	0.121	ND	ND	ND	ND	ND	0.126	ND	ND
Indeno[1,2,3-c,d]pyrene	In(123-cd)P	0.558	ND	0.261	ND	0.561	0.234	ND	ND	ND	ND	ND	0.291	0.321	0.515	0.142
Dibenzo[a,h]anthracene	DB(ah)A	ND	ND	ND	ND	0.132	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[g,h,i]perylene	B(ghi)P	ND	ND	0.112	ND	0.12	ND	ND	ND	ND	ND	ND	0.111	ND	ND	ND
∑ PAHs	**0.66**	**0.36**	**0.89**	**0.29**	**2.38**	**0.44**	**1.03**	**0.34**	**0.80**	**0.82**	**0.36**	**1.04**	**0.97**	**0.99**	**0.29**	
∑ 7c-PAHs	**0.10**	-	**0.12**	-	**0.85**	-	**0.54**	**0.19**	**0.31**	**0.40**	**0.18**	**0.11**	**0.24**	-	-	
PAH3		-	**0.07**	**0.08**	**0.07**	**0.38**	**0.07**	**0.29**	**0.19**	**0.31**	-	-	**0.07**	**0.20**	**0.07**	-
PAH4		**0.10**	**0.07**	**0.21**	**0.07**	**0.39**	**0.20**	**0.35**	**0.19**	**0.31**	**0.12**	**0.11**	**0.19**	**0.20**	**0.19**	**0.10**
PAH8		**0.56**	-	**0.49**	-	**1.41**	**0.23**	**0.54**	**0.19**	**0.31**	**0.40**	**0.18**	**0.40**	**0.65**	**0.52**	**0.14**
PAH dominance in Wele (%)																
LMW-PAHs (2–4 Ri ngs)		**15**	**100**	**44.4**	**100**	**44.7**	**46.2**	**46**	**44**	**61.2**	**85.5**	**100**	**61.4**	**41.8**	**48**	**51**
HMW-PAHs (5–6 Ri ngs)		**85**	**0**	**55.6**	**0**	**55.3**	**53.8**	**54**	**56**	**83.2**	**14.5**	**0**	**38.6**	**58.2**	**52**	**49**

∑PAHs- sum of PAHs; PAH7- USEPA (1993) probable human (Group B2) carcinogens; PAH3- US National Academy of Science (1972) strongly carcinogenic PAH compounds; PAH4- EFSA group of 4 PAHs (PAH4) as superior indicators of carcinogenicity; PAH8- group of 8 PAHs as superior indicators of carcinogenicity; ND- not detected AM1-AM4: Agbogbloshie market; NK1-NK3: Kwame Kkrumah market; ACI-AC4: Accra central market; MA1-MA4: Malata marke

3.6 % fat and 1.3 - 1.9 % ash which is all lost during singeing (Eremong, 2011). The use of prolong heating and high temperature which produces smoke during the singeing of cowhide contributes to loss of proteins, micronutrients and unsaturated fatty acids (Nwabugo, 2016). Additionally, a possible mechanism for the formation of PAHs in cowhide during singeing is the incomplete combustion of discarded engine oil and petroleum/ organic materials used as sources of fuel which generates PAHs that are brought onto the surface of the cowhide and are adsorbed.

Similar high total PAHs (∑PAHs) value of 2609.8 μg/kg in meat sausages was reported by Santos et al., (2011). Santos et al., (2011), attributed the high ∑PAHs content of the meat sausages to the extended period of smoking. However, the ∑PAHs concentration obtained in this study was significantly higher than those in smoked meat products (0.6 – 4.9µg/kg) by Chung et al., 2011.

The [B(a)P] concentrations in singed cowhide from the Greater Accra Region ranged from 120.0 to 129.0 µg/kg with a mean of 124.0 µg/kg. This suggests the significantly high levels of [B(a)P] than the acceptable EU levels of 5.0 µg/kg in smoked meat products in singed cowhide. The high [B(a)P] levels in singed cowhide could be the result of direct contact between the cowhide and the petroleum/organic materials during singeing since B[a]P is the most commonly formed PAH in processes involving incomplete combustion of organic materials (Abdulazeez, 2017). [B(a)P] has a positive genotoxicity and is listed as Group 1 carcinogen by the International Agency for Research on Cancer (Table 2) (Singh et al., 2016). [B(a)P] is therefore the only known HMW-PAH with positive genotoxicity properties and a definite carcinogenic PAH (Karishma et al., 2018). The [B(a)P] results suggest that, consumption of commercially available singed cowhide in the Ghanaian market has a long-term positive genotoxicity and cancerous tendencies on the Ghanaian public. Owing to the strict artisanal procedures associated with the processing of traditional meat products in European countries, the B(a)P levels in singed cowhide shows a significant deviation from previous studies from the Southern (Falcó et al., 2003; Fontcuberta et al., 2006; Purcaro et al., 2009) and Northern (Duedahl-Olesen et al., 2006; Reinik et al., 2007; Andrée et al., 2010) European Countries where a [B(a)P] content lower than 5.0 µg/kg is strictly ensured in meat products. Fig 3 presents the comparison of [B(a)P] content in singed cowhide to PAH3, PAH4, PAH7and PAH8. The results show that the PAH3 levels in singed cowhide more than doubled that of [B(a)P] except in sample AM3 where, the PAH3 content was reduced by almost half while, the PAH7 levels more than quadrupled that of [B(a)P]. Using the identified group of 4 PAHs (PAH4), and group of 8 PAHs (PAH8) as superior indicators of carcinogenicity based on data relating to occurrence and toxicity by the EFSA, the levels of PAH4 and PAH8 were compared to the levels of B(a)P in singed cowhide (Fig 3). Consistent with the results from the comparison of B(a)P content in singed cowhide to PAH3 and PAH7. PAH4 more than doubled compared to B(a)P whilst, the PAH8 levels more than quadrupled, compared to B(a)P suggesting that, indeed [B(a)P] is not suitable to be solely used as a marker for the occurrence and toxicity of PAH in food. In determining carcinogenicity in food, the use of PAH8 presents a more meticulous approach compared to the use of the other markers for the occurrence and toxicity of PAH in food. Manda et al., (2012), reported that, the mean PAH4 in smoked meat available in Abobo market in Abidjan, Côte d'Ivoire was 57.23 µg/kg which is about 4-times lower than the mean PAH4 (228.9 µg/kg) in singed cowhide from the Ghanaian markets within

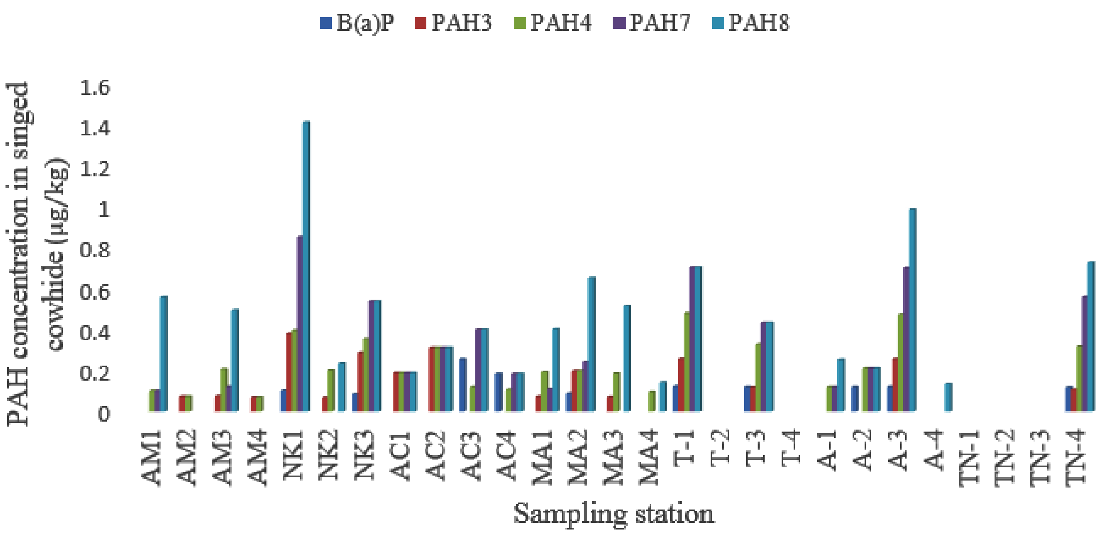

Figure 3 Comparison of [B(a)P] content in singed cowhide to PAH3, PAH4, PAH7 and PAH8 contents

the Greater Accra Region. Literature indicates that, the maximum threshold for PAH4 in smoked meat products is 30 µg/kg (1/9/ 2012 to 31/08/2014) and 12 µg/kg in Commission Regulation (EC) No 1881/2006 amended by Commission Regulation (EU) No 835/2011. This study records a PAH4 mean value of 228.9 µg/kg and therefore suggests about 8-times the maximum threshold established by (1/9/ 2012 to 31/08/2014) Commission Regulation (EC) and about 19-times the maximum threshold established by Commission Regulation (EC) No 1881/2006 amended by Commission Regulation (EU) No 835/2011. Thus the PAH4 results from this study also suggests that the materials and methods generally used in singeing for commercial consumption are unacceptable.

Carcinogenicity and mutagenicity classification
Using the International Agency for Research on Cancer (IARC, 2007) classification of carcinogenicity and mutagenicity (Table 5), the singed cowhide within the Greater Accra Region is dominated by positive genotoxicity PAHs classified as carcinogens (1) and possible carcinogens (2B) as well as positive and questionable genotoxic PAHs that are not classifiable (3). The [B(a)P] equivalent concentrations [B(a)Peq] ranged 120 – 129 µg/g. This suggests that the [B(a)P]eq in all singed cowhide from the Greater Accra Region were higher than the screening value (SV) of 7.77×10^{-13} µg/g. A B(a)P carcinogenic potency equivalent (PEC) value of 870 µg/g was also greater than the SV of 7.77×10^{-13} µg/g. The [B(a)P]eq and PEC results suggest that consumption of singed cowhide at the rate of 25.2 g/day poses potential adverse health effects such as cancer, mutations and birth defects in terms of B(a)P to humans.

Human health risk assessment of PAHs

Estimated Chronic Daily Intake (ECDI) of PAHs through ingestion of singed cowhide:
The estimated chronic daily intake of PAHs through consumption of singed cowhide is

TABLE 5
Genotoxicity of PAH compounds and their IARC classification

PAH Compound	Genotoxicity	IARC classification
Acenaphthene	Questionable	Yet to be assessed
Acenaphthylene	Questionable	Yet to be assessed
Anthracene	Negative	3
Benz(a)anthracene	Positive	2B
Benzo(b)fluoranthene	Positive	2B
Benzo(k)fluoranthene	Positive	2B
Benzo(g,h,i)perylene	Positive	3
Benzo(a)pyrene	Positive	1
Chrysene	Positive	2B
Dibenz(a,h)anthracene	Positive	2A
Fluoranthene	Positive	3
Flourene	Negative	3
Indeno(1,2,3-cd)pyrene	Positive	2B
Phenanthrene	Questionable	3
Pyrene	Questionable	3
Naphthalene	Positive	2B

(After Singh et al., 2016)

1 – Carcinogenic

2A – Probably carcinogenic

2B – Possibly carcinogenic

3 – Not classifiable

presented in Table 6. The ECDI ranged 13.1 – 56.8, with a mean and standard deviation value of 28.6 ± 9.9 for adults. The order of decreasing pattern of ECDI for carcinogenic PAHs in singed cowhide was: Indeno (1,2,3-cd)P > Chr > DB(ah)A > B(a)A > B (b)F > B(a)P > B(ghi)P > Flu = Ant > B(k)F > PA > Pyr > Acp = Fl > AcPy > Nap for adults. The ECDI varied with a range of 77.8 – 337.8, with a mean and standard deviation value of 170.5 ± 59.0 for children. The order of decreasing pattern of ECDI for carcinogenic PAHs in singed cowhide was: Indeno (1,2,3-cd)P > Chr > DB(ah)A > B(a)A > B (b)F > B(a)P > B(ghi)P > Ant > Flu > B(k)F > PA > Pyr > Acp = Fl > AcPy > Nap for children. This suggests that, the estimated chronic daily intake was highest in Indeno (1, 2, 3-cd) pyrene, which is classified as a probable carcinogen (2A) and lowest in Naphthalene which is classified as a possible carcinogen (2B) according to the IARC classification for both adults and children populations.

Hazard quotient (HQ) (non-carcinogenic risk) and hazard indices (HI) of PAHs through ingestion of singed cowhide:

The results of hazard quotient (HQ) of PAHs through consumption of singed cowhide is presented in Table 7. The HQ ranged 9.05×10^{-5} – 0.485, with a mean and standard deviation value of 0.303 ± 0.19 and 1.79 – 13.45, with a mean value and standard deviation of 8.87 ± 4.64 for adults and children respectively. The hazard index (HI) for adults and children were 1.94 and 55.76 respectively. According to Tay (2019), the PAH pollution index is defined by HQ/HI. Where, HQ/HI < 1, there is no concern for potential human health risks caused by exposure to non-carcinogenic elements and where, HQ/HI >1, there may be a concern for potential human health risks caused by exposure to non-carcinogenic elements. The HQ/HI ranged 4.65×10^{-5} – 0.249, with a mean value and standard deviation of 0.156 ± 0.09 and 0.032 – 0.241, with a mean value and standard deviation of 0.159 ± 0.08 (Table 7) for adults and children respectively. This suggest that, all the HQ/HI < 1, thus, there is no concern for potential human health risks caused by exposure to non-carcinogenic PAHs in singed cowhide.

Carcinogenic toxic equivalents (TEQs) and screening values (SV) for PAHs through ingestion of singed cowhide

According to Dan et al., (2020), the carcinogenic health of a consuming public is assessed through the evaluation of TEQs, while, screening value is the threshold concentration of contaminant in edible tissue of potential public concern. Where the TEQs > SV, there is a potential health concern and if, TEQs < SV, there is no potential health concern (Dan et al, 2020). The TEQs values for PAH in singed cowhide ranged 1.7836 – 8918 while, the screening value for PAH through the consumption of singed cowhide were 0.001 and 0.0002 for adults and children respectively. The TEQs for the individual PAHs were all greater than the computed SVs for both adults and children. Based on (Ijeoma et al., 2015), the assessed PAHs in singed cowhide were of potential carcinogenic risk to both adults and children since they are known to cause cancer, mutations and birth defects in humans and, therefore of potential health concern to the consuming public.

Source identification of PAH in singed cowhide within the Greater Accra Region

In this study, the dominance of 2-4 ringed PAHs which were regarded as lower molecular weight PAHs (LMW-PAHs) ranged 15 – 100% while, the dominance of 5-6 ringed PAHs regarded as the higher molecular weight PAHs (HMW-PAHs) ranged 0 - 85% of the total PAHs in singed cowhide (Table 2). Fig 4 presents the dominance of LMW-PAHs and HMW-PAHs in singed cowhide from the Greater Accra Region. This suggest that, LMW-PAHs dominates singed cowhide from the Greater Accra Region. Studies have shown that, LMW -PAHs are considered to be acutely toxic and non-carcinogenic due to their high water solubility compared to HMW-PAHs which are more lipophilic (Tay et al., 2013).

LMW-PAHs are found mostly in the large atmospheric particles which deposit faster and are commonly from sources related to high-temperature processes (Adeniji et al., 2019).

Diagnostic ratios have been used to distinguish the sources of PAH in different environmental media depending on their physical and chemical properties and stability against photolysis

TABLE 6

Estimated Chronic Daily Intake (ECDI), Hazard Quotient (HQ), Hazard Index (HI), Carcinogenic Toxic Equivalents (TEQs) and Screening Value (SV) of PAHs in singed cowhide

PAH Compound	Abreviation	Mean (mg/kg)	ECDI		HQ		SV		
			Adult	Children	Adult	Children	TEQs	Adults	Children
Naphthalene	Nap	52.3	13.1	77.8	0.447773973	12.43736986	1.7836	0.0010918	0.000234
Acenaphthalene	AcPy	70.4	17.6	104.8	NA	NA	1.7836	0.0010918	0.000234
Acenapthene	Acp	76.3	19.1	113.5	0.217751142	6.048255708	1.7836	0.0010918	0.000234
Fluorene	Fl	76.3	19.1	113.5	0.326626712	9.072383562	1.7836	0.0010918	0.000234
Phenanthrene	PA	101.7	25.4	151.3	NA	NA	1.7836	0.0010918	0.000234
Anthracene	Ant	113.3	28.3	168.6	9.05188E-05	1.796244749	17.836	0.0010918	0.000234
Fluoranthene	Flu	113.2	28.3	168.4	0.484589041	13.45994521	1.7836	0.0010918	0.000234
Pyrene	Pyr	81.7	20.4	121.6	0.466324201	12.952621	1.7836	0.0010918	0.000234
Benzo[a]Anthracene	B(a)A	131.4	32.9	195.5	NA	NA	178.36	0.0010918	0.000234
Chrysene	Chr	133	33.3	197.9	NA	NA	17.836	0.0010918	0.000234
Benzo[b]Fluoranthene	B(b)F	124.9	31.2	185.9	NA	NA	178.36	0.0010918	0.000234
Benzo[k]Fluoranthene	B(k)F	110.8	27.7	164.9	NA	NA	1783.6	0.0010918	0.000234
Benzo[a]pyrene	B(a)P	124.3	31.1	185.0	NA	NA	178.36	0.0010918	0.000234
Indeno[1,2,3-c,d]pyrene	In(123-cd)P	227	56.8	337.8	NA	NA	178.36	0.0010918	0.000234
Dibenzo[a,h]anthracene	DB(ah)A	132	33.0	196.4	NA	NA	8918	0.0010918	0.000234
Benzo[g,h,i]perylene	B(ghi)P	115	28.8	171.1	NA	NA	17.836	0.0010918	0.000234
		∑PAHi = 1783.6			HI(Adults) =1.9432	HI(Children)=55.7668			

NA- Not available
ECDI- Estimated Chronic Daily Intake
HQ- Hazard Quotient
HI- Hazard Index
∑PAHi-Summation of individual PAHs
TEQs-Carcinogenic Toxic Equivalents
SV-Screening Value

TABLE 7
HQ/HI values for PAHs in singed cowhide

PAH Compound	Abbreviation	HQ/HI	
		Adults	Children
Naphthalene	Nap	0.2304	0.2230
Acenaphthalene	AcPy	NA	NA
Acenapthene	Acp	0.1121	0.1085
Fluorene	Fl	0.1681	0.1627
Phenanthrene	PA	NA	NA
Anthracene	Ant	4.65×10^{-5}	0.0322
Fluoranthene	Flu	0.2494	0.2414
Pyrene	Pyr	0.2399	0.2323
Benzo[a]Anthracene	B(a)A	NA	NA
Chrysene	Chr	NA	NA
Benzo[b]Fluoranthene	B(b)F	NA	NA
Benzo[k]Fluoranthene	B(k)F	NA	NA
Benzo[a]pyrene	B(a)P	NA	NA
Indeno[1,2,3-c,d]pyrene	In(123-cd)P	NA	NA
Dibenzo[a,h]anthracene	DB(ah)A	NA	NA
Benzo[g,h,i]perylene	B(ghi)P	NA	NA

NA- Not available

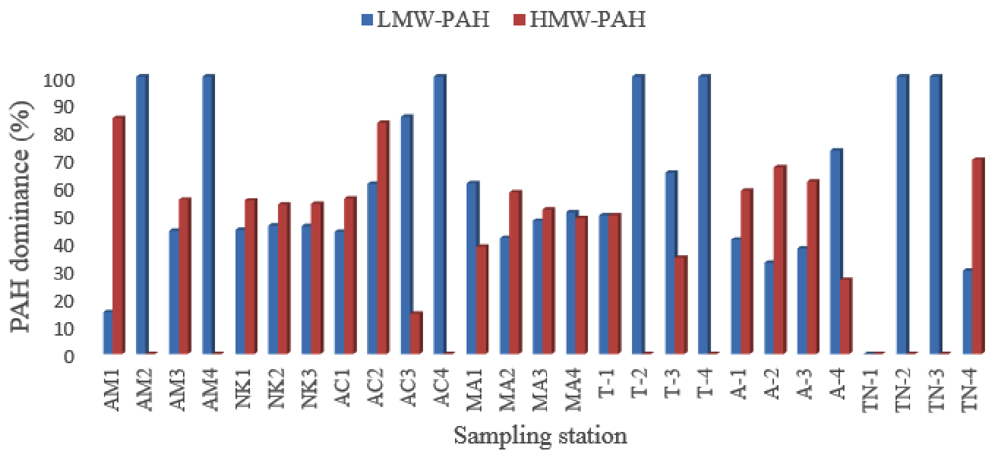

Figure 4 Dominance of LMW-PAHs and HMW-PAHs in singed cowhide from the Greater Accra Region

(Yunker et al., 2002). Ten molecular diagnostic ratios have been used as indicators that has the potential to distinguish between petrogenic and pyrolitic sources of PAHs in singed cowhide (Table 8). These ratios aided in characterizing contributions from pyrolitic (incomplete combustion of car tyres, and unsorted refuse) and petrogenic (unburnt discarded engine oil and other organic materials) origins of PAHs in singed cowhide (Jamhari et al., 2014). The key isomeric ratios and their associated sources used for source identification of PAH in singed cowhide has been listed in Table 8. In all, five of the ratios; Chr/B(a)A, Ant/(Ant + PA), Flu/(flu + Pyr), B(a)A/(B(a)A + Chr) and In(123-cd)P/(In 123-cd)P +B(ghi)P shows accumulation of PAHs in singed cowhide from pyrolytic sources, two of the ratios; B(a)A/228 and B(a)A/(B(a) A + Chr) shows accumulation of PAHs in singed cowhide from petroleum sources while, three of the ratios; Chr/B(a)A, Flu/Pyr and LMW-PAH/HMW-PAH shows mixed (i.e both pyrolytic and petrogenic) sources. The results show that the PAH sources in singed cowhide is predominantly from pyrolitic than petrogenic origins. The pyrolytic sources of PAHs in singed cowhide may be the result of contamination from the burning of carbon-containing materials such as car tyres and unsorted garbage through combustion and pyrolysis processes. The

TABLE 8
Source identification of PAH in singed cowhide from the Greater Accra Region, Ghana
(After Adeniji et al., 2019)

	Source		This study		
	Petrogenic	**Pyrolitic**	**Min**	**Max**	**PAH Source**
PA/Ant	> 15	< 10	0.5082	0.9455	Pyrolytic
Chr/B(a)A	< 0.4	> 0.9	0.7417	1.3295	petrogenic/pyrolitic
Ant/(Ant + PA)	< 0.1	> 0.1	0.5140	0.6630	Pyrolytic
Flu/Pyr	< 1.0	> 1.0	0.8740	2.3944	petrogenic/pyrolitic
Flu/(Flu + Pyr)	< 0.4	> 0.4	0.4664	0.7054	Pyrolytic
Ant/178	< 0.1	> 0.1	0.0004	0.0011	Petrogenic
B(a)A/228	< 0.2	0.2 - 0.35	0.0004	0.0011	Petrogenic
B(a)A/(B(a)A + Chr)	< 0.2	> 0.35	0.4293	0.5742	Pyrolytic
In(123-cd)P/(In 123-cd)P +B(ghi)P	< 0.2	> 0.2	0.6997	0.8238	Pyrolytic
LMW-PAH/HMW-PAH	> 1.0	< 1.0	0.4881	5.8966	petrogenic/pyrolitic

potential human health effects associated with the singeing of cowhide may include; the short-term tissue irritation such as skin, respiratory, eyes and gastrointestinal and decreased fertility, developmental neurological effects and renal toxicity amongst the butchers exposed to relatively high levels of PAHs.

Fig 5 a-c presents the results of source ratios of PAHs in singed cowhide. In Fig 5a, source ratios that plot in field I show ratios of Flu/

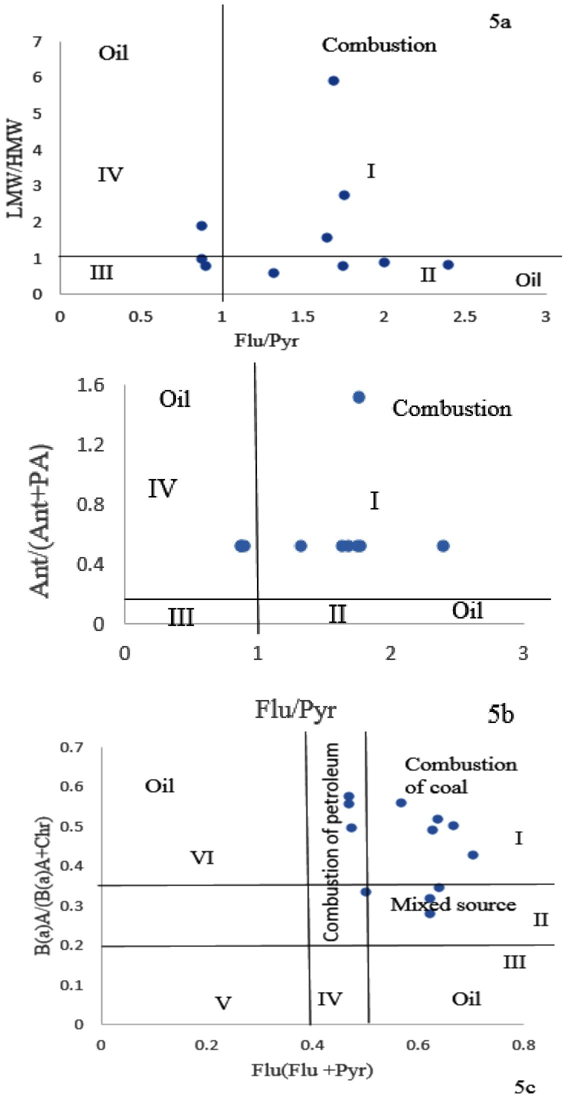

Figure 5 a-c: Diagnosis ratios analysis of: (a)- Flu/Pyr vs LMW/HMW-PAH; (b)- Flu/Pyr vs Ant /(Ant + PA); (c)- Flu (Flu+Pyr) vs B(a)A/B(a)A+Chr)

Pyr > 1 and LMW/HMW-PAH > 1 and depicts combustion sources; source ratios that plot in field II show ratios of Flu/Pyr < 1 and LMW/HMW-PAH < 1 and depicts oil sources; while, source ratios that plot in field III show ratios of Flu/Pyr < 1 and LMW/HMW-PAH < 1 and depicts a mixed source of oil and combustion sources and, source ratios plot in field IV show ratios of Flu/Pyr > 1 and LMW/HMW-PAH < 1 and depicts oil sources. In Fig 5b, source ratios that plot in field I show ratios of Flu/Pyr > 1 and Ant/ (Ant+PA) > 0.1 and depicts combustion sources; while, source ratios that plot in field IV show ratios of Flu/Pyr < 1 and Ant/ (Ant+PA) < 0.1 and depicts oil sources. According to Jamhari et al., (2014), these are contributions from incomplete combustion of car tyres, and unsorted garbage- pyrolytic sources and unburnt discarded engine oil and other organic materials - petrogenic source. In Fig 5c, source ratios that plot in field I show ratios of Flu/(Flu +Pyr) > 0.4 and B(a)A/[B(a)A+Chr] > 0.35 and depicts sources typical of combustion of petroleum, while, source ratios that plot in field I show ratios of Flu/(Flu +Pyr) > 0.5 and 0.2 < B(a)A/B(a)A+Chr) > 0.35 and depicts mixed sources and, source ratios that plot in field IV show ratios of 0.4 < Flu/(Flu +Pyr) > 0.5 and 0.2 < B(a)A/[B(a)A+Chr] > 0.35 and depicts sources typical of combustion of petroleum. The results of the different diagnostic ratios show that, the principal sources of PAHs in singed cowhide are significantly related to contributions from incomplete combustion of car tyres, unsorted garbage, unburnt discarded engine oil and other organic materials and therefore, pyrolytic and petrogenic in origin.

Principal Component Analysis (PCA)

PCA is a powerful tool used in statistics to reduce large data into simple meaningful data and allows easy visualization of differences and similarities between data sets (Li et al., 2019). The PCA results from this study were characterized by four principal components (Table 9) that accounted for 89.56% of the total variance with eigenvalues >1. PC1 accounted for 29.42% of the total variance and has significant positive loadings for chrysene, acenaphthalene, benzo(a)anthracene, anthracene, fluorene, benzo(b)fluoranthene and benzo(k)fluoranthene characteristic of pyrolitic fingerprint. These are generally the result of the incomplete combustion of car tyres, and unsorted garbage

TABLE 9

Rotated component matrix of the principal components

PAH	PC1	PC2	PC3	PC4
Chrysene	0.959	-	-	-
Acenaphthalene	0.865	-	-	-
Benzo (a) Anthracene	0.834	0.446	-	-
Anthracene	0.721	-	0.476	0.339
Benzo (g,h,i) Perylene	0.343	0.895	-	-
Naphthalene	0.346	0.893	-	-
Indeno (1,2,3- cd) Pyrene	-	0.812	-	-
Benzo (k) Fluoranthene	0.528	0.696	-	0.389
Benzo (b) Fluoranthene	0.595	0.658	-	-
Fluorene	0.572	0.653	-	0.373
Benzo (a) Pyrene	-	0.596	-	-
Fluoranthene	-	-	0.840	-
Pyrene	-	0.401	0.780	-
Phenanthrene	0.408	-	0.758	0.349
Acenaphthene	-	-	-	0.927
Dibenzo (a,h) Anthracene	0.423	-	-	0.669
Explained variance (%)	**29.42**	**28.87**	**16.00**	**15.27**

Rotation method: Varimax with Kaiser Normalization

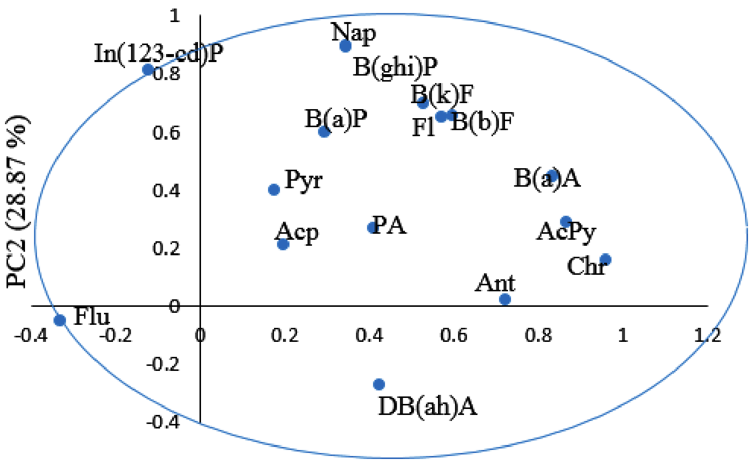

Figure 6 Loadings and score plot for PC1 and PC2

(Li et al., 2019). PC2 accounted for 28.87% of the total variance and has significant positive loadings for naphthalene, benzo(ghi) perylene, indeno(1,2,3-cd)pyrene, fluorene, benzo(b) fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene. These PAHs are predominantly characteristic of incomplete combustion of petroleum products and are attributable to pyrolitic sources such as contributions from incomplete combustion of car tyres, and unsorted garbage. PC3 accounted for 16.0% of the total variance and has positive loadings for fluoranthene, pyrene and phenanthrene which reflect sources attributable to petrogenic sources such as unburnt discarded engine oil and other organic materials. PC4 accounted for 15.27% of the total variance and has positive loadings for acenaphthene and dibenzo(ah) anthracene. This suggest sources attributable to mixed (pyrolitc and petrogenic). Results of the PAH source distribution show that the principal sources of all PAHs were petrogenic such as unburnt discarded engine oil and pyrolytic inputs such as incomplete combustion of car tyres, and unsorted refuse, with pyrolitic sources of PAHs as more predominant and other organic materials.

Fig 6 presents the loadings and score plots for PC1 and PC2 which together explains nearly 58.30 % of the total variance. The grouping and source distribution between the PAHs show that chrysene, acenaphthalene, benzo(a) anthracene, anthracene, benzo(b)fluoranthene benzo(k)fluoranthene, naphthalene, benzo(ghi) perylene, benzo(a)pyrene, fluorene, pyrene phenanthrene, and acenaphthene were observed in the first quadrant and have been shown to group together indicating their close relations, suggesting similar PAH sources. Dibenzo(ah) anthracene, fluoranthene, and indeno(1,2,3-cd) pyrene were observed in the second, third and fourth quadrant respectively suggesting their independence in terms of their source inputs in singed cowhide.

Conclusions and Recommendations

This study shows PAH4 concentrations of approximately 8-times the maximum threshold established by (1/9/ 2012 to 31/08/2014) Commission Regulation (EC) and approximately 19-times the maximum threshold established by Commission Regulation (EC) No 1881/2006 amended by Commission Regulation (EU) No 835/2011. The study also shows that, the singed cowhide within the Greater Accra Region is dominated by positive genotoxicity PAHs classified as carcinogens (1) and possible carcinogens (2B) as well as positive and questionable genotoxic PAHs that are not classifiable (3). The [B(a)P] eq and PEC results suggest that consumption of singed cowhide at the rate of 25.2 g/day poses potential adverse health effects such as cancer, mutations and birth defects in terms of B(a)P to humans. Results further show that, the HQ/ HI < 1, thus, there is no concern for potential human health risks caused by exposure to non-carcinogenic PAHs in singed cowhide. However, the carcinogenic toxic equivalent (TEQs) values for both adults and children were greater than the screening values therefore, there is concern for potential human health risks caused by exposure to carcinogenic PAHs in singed cowhide. Source assessment of PAHs in singed cowhide using diagnostic ratios and principal components analysis shows that, the principal sources of the PAHs were petrogenic such as unburnt discarded engine oil and pyrolytic inputs such as incomplete combustion of car tyres, and unsorted, with pyrolitic sources as more predominant refuse and other organic materials. Thus, the PAHs in singed cowhide within the Greater Accra Region originate primarily from incomplete combustion and of petroleum origin due to singeing. Thus, the mandated regulatory agencies such as the Environmental Health Department and the Veterinary Services Department of the various Municipals, Ministries, Departments and Agencies (MMDA's) should ensure strict measures for safe, healthy and hygienic meat and meat products in Ghana.

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