QUANTIFICATION OF SMOKE CONTRIBUTED PAHs IN ROASTED COWHIDE (PONMO) FROM NORTHERN NIGERIA

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Abstract: Cowhides were collected from processors before and after exposure to smoke generated from burning wood. Dried cowhide samples (roasted and hot water processed) were extracted using 3:1 ratio of dichloromethane: hexane in a sonicator and the extract cleaned up in an alumina packed column with the same solvent mixtures then analyzed with gas chromatograph fitted with flame ionization detector. The study revealed that the total PAH concentrations in the non roasted cowhide was 2.1602 μgkg^{-1}while total concentrations in the roasted samples was 2.7370 μgkg^{-1}. The % PAH contamination levels from smoke in the smoked cowhide are in the range of 0-94. The study further revealed that the PAH4 (Benzo[a]pyrene, benz[a]anthracene, chrysene and benzo[b]fluoranthene) had no contaminations ofbenz[a]anthracene and chrysene contributed from smoke.However, benzo[b]fluoranthene and benzo[a]pyrene concentrations (µg/kg) from smoke relative to other environmental sources were lower; 0.0005, 0.0052 and 0.0087, 0.6222, respectively. The mean difference of the 16 USEPA listed PAHs in the roasted and non roasted samples were not significant statistically ($p>0.05$) except fluoranthene ($p<0.05$). This shows that smoke contributed fluoranthene significantly to the roasted cowhide. Generally, benzo[a]pyrene and sum of PAH4 concentrations were below the Food Standards Agency limits of 2 μgkg^{-1} and 12 μgkg^{-1}, respectively.

Keywords: Benzo[a]pyrene, cowhide, polycyclic aromatic hydrocarbons, PAH4, roasting

Introduction

In Nigeria, the skin of animals attached to the meat or the skin alone is often consumed as delicacies in meals. One skin often consumed is the cow skin or cowhide. Cowhides are commonly referred to as *Ponmo* (Yoruba), Kanda (Northerners), *Akpupoanu* (Igbos), *Ano* (Igala) and *Ohian* (Edo) (Tijani and Ajayi, 2016). Consumption of '*Ponmo'* is nolonger seen as a poor man's food in Nigeria where it was associated with the poor and uneducated Yoruba in south western Nigeria (Adeyeye and Aye, 2015). It is also consumed by the rich nowadays.

Cowhides are often processed by skinning, dehairing, washing, boiling, cutting, soaking and cooking (Joseph, 2006). Removal of hairs from cowhides maybe processed by flame fuelled by firewood and spent engine oils thus contributing toxic organic compounds such as polycyclic aromatic hydrocarbons (PAHs), dioxins, furan and benzene (Okiei *et al*., 2009). Also heavy metals like Fe, Mn, Cu, Pb, Zn and Cd as well as crude protein, ether extract and ash content have also been determined in cowhide processed either by fermentation or singeing with wood, discarded motor bike tire or liquefied petroleum gas (Akwetey *et al*., 2013).

Polycyclic aromatic hydrocarbons result from incomplete combustion of materials in the environment such as garbage, petroleum products, coal, meat and tobacco (Lau *et al*., 2010). The United States Environmental Protection Agency (USEPA, 1993) had classified benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene as carcinogenic PAHs. Cancer refers to a medical condition which is characterized by the uncontrolled growth and spread of abnormal cells in a part of the body leading to formation of malignant tissues (American Cancer Society, 2016). This means that cowhides exposed to smoke from any source could be contaminated with PAHs and hence poses threat to human health.Actually, cancer has been adduced to be a major public health problem in the United States and many other parts of the world (Siegel *et al*., 2015). Pollution indices help in the analysis and conveyance of environmental information to decision makers, managers, technicians and the public (Caeiro *et al*., 2005).

Polycyclic aromatic hydrocarbons have been reportedly determined in smoked bush meat (Abdul *et al*., 2014) and also in roasted plantains, yam and fish (Ogbuagu and Ayoade, 2012). Furthermore, levels of polycyclic aromatic hydrocarbons have been determined on singed cowhides (Ponmo) and charcoal grilled meat (Suya) (Ogbonna and Nwaocha, 2015). However, the PAHs determined represent the entire PAHs from all sources. It is therefore necessary to assess the level of PAH contamination from the smoke in smoke exposed foods. Reports of quantification of PAHs from smoke in smoked foods had not been available from literature to the best of our knowledge. It is against this background that the study was conducted to quantify the PAHs contributed from the smoke in cowhides processed by roasting in parts of Northern Nigeria.

Materials and Methods

Study site

The locations from where samples were collected include Makurdi (Benue State), Lokoja (Kogi State), Lafia (Nassarawa State), Jalingo and Wukari town (Taraba State) as shown in Fig. 1 below;

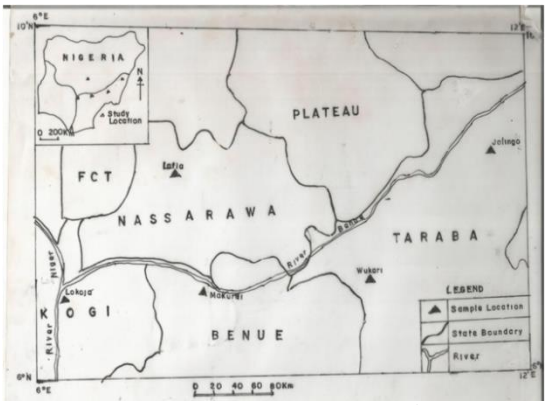

Fig. 1: Map showing sample locations in parts of northern Nigeria

Benue State, a state in the north central region of Nigeria has an area of 34,059 km^2 with a population of about 4,253,641 based on the 2006 census (FRN, 2010). Benue State whose capital is Makurdi is a rich agricultural region where some crops that are grown include potatoes, cassava, soyabean, guinea corn, yams, rice and groundnuts.

Kogi State also in north central Nigeria covers a total area of 29,833 km^2. The estimated population in 2005 is 3,595,789. The capital is Lokoja with a population of 196,643 (FRN, 2010). The common farm produce in the state include coffee, cocoa, palm oil, cashews, ground nuts, maize, cassava, yam, rice and melon while the mineral resources include coal, limestone, iron, petroleum and tin. The State has the largest iron and steel industry, the Ajaokuta steel company limited. Nassarawa State which is also located in north central Nigeria has an area of 27,117 km^2 with a population of 2,040,097 and its capital is Lafia. It is bounded by Kaduna in the north, Benue and Kogi in the south and Plateau and Taraba in the east. The major economic activities include production of salt. Yam, cassava and egusi are produced and sold as cash crops.

Taraba State has a total area of 54,473 km^2 and is located at 8 ^{o}00'N 10^{o}30'E co-ordinates. The estimated population as at 2005 is 2,688,944. Jalingo is the capital of Taraba State which is named after the Taraba River. It is surrounded in the west by Plateau and Benue States and the Cameroon on the east. The main features in the state include the Mambilla plateau and the rivers Benue, Donga, Taraba and Ibi being the main rivers in the State. Crops that are produced in commercial quantities include maize, rice, sorghum, millet, cassava and yam. Wukari, a local government in Taraba lies on the co-ordinates 7^{o}51'N 9^{o}47'E or 7.850^{o}N 9.783^{o}E covering an area of 4,308 km^2 and having a population of 241,546 based on the 2006 census.

Sample collection and preparation

The cowhides were collected randomly from the processors before and after roasting. Fresh cowhides were purchased from butchers from which parts of a whole were preparedby placing in hot water (control) in a clean bowl for about 30 min and a clean knife used to scrape the hair off whilethe other part were roasted by processors using wood then washed with sponge and later rinsed with clean water. All the samples were placed in an oven (Genlab number: Mino/18) at 60^{o}C and allowed to dry until constant weights were attained. They were pulverized in a mortar with pestle and blended in a blender then put in a non transparent container prior to extraction.

Extraction of polycyclic aromatic hydrocarbons from the samples

Recovery experiment was carried out by spiking 3 g of the pulverized samples with 1ppm of the four deuterated PAHs namely acenaphthene-d_{12}, phenanthrene-d_{10}, chrysene-d_{10} and perylene-d_{12}. The modified method 3550C was employed for the extraction (USEPA, 2007). The samples were extracted by weighing 3.0 g of the blended samples into a 250 ml capacity beaker and 50 ml of ratio 3:1 (75:25 ml) redistilled hexane-dichloromethane mixture added. The beaker and its content were placed in a sonicator to extract the hydrocarbons for 30 min. The organic layer was filtered into the 250 ml capacity beaker and the extract dried by passing the filtrate through a funnel containing anhydrous sodium sulphate. The extract was then concentrated with a stream of nitrogen gas.

Extract clean up

Neutral alumina packed into a column up to 10cm was washed with redistilled hexane then the extract was poured onto the alumina and allowed to run down with the aid of redistilled hexane to elute the aliphatic profiles into a pre-cleaned 20 mL glass container. The aromatic fraction was eluted with a 3:1 mixture of hexane and dichloromethane to recover the non polar PAH fractions but the most polar PAHs were recovered by eluting with dichloromethane into the pre-cleaned beaker. The mixture was concentrated to 1 mL by a stream of nitrogen gas then analyzed with gas chromatograph (HP6890) coupled to a flame ionization detector.

Working condition for the Gas chromatograph

Model: HP6890; Column: HP-1; Column length/column internal diameter/Column film: 30m, 0.25 µm, 0.25 µm; Split ratio 20:1; Injection temperature: 250^{o}C; Detector temperature: 320^{o}C; Detector: Flame ionization detector; Initial temperature: 60^{o}C for 5 min; First rate: 15^{o}C/min for 14 min and maintained for 3 min; Second rate: 10^{o}C/min for 5 min and maintained for 4 min; Mobile phase or carrier: Nitrogen; Nitrogen column pressure: 30 psi; Hydrogen pressure: 28 psi; Compressed air pressure: 32 psi.

Determination of % PAH contamination level from smoke in roasted cowhide

The % PAH contamination level (PCL) from smoke in roasted cowhide was developed from the study to measure the extent of contamination of each PAH. This was calculated using

$$\%PCL = \frac{Css - Cns}{Css} \times 100$$

C_{ss} =Concentration of PAH in smoked samples (µg/kg)

C_{ns} =Concentration of PAH in non smoked samples (µg/kg)

Results and discussions

The recoveries of PAHs were in the range of 95.5-98.3 %. It has been reported by scientific opinion on food chain contaminants as requested for by European Union that recovery should be between 50 % - 120 % (EFSA, 2008).The recoveries in the present study using ultrasonication method to extract the samples were within this range therefore ultrasonication and 3:1 ratio of hexane and dichloromethane as solvent mixture is a highly efficient process. Furthermore, instrument calibration was done using pure PAH standard mixtures in the concentration ranges of 0.2 - 10 µg/l. The linearity of the calibrations gave chromatograms of PAHs whose retention times when compared with that of the samples guided the identification of the individual PAHs in the samples.

PAH concentrations (µg/kg) in the non roasted cowhide samples (control) are presented in Table 1. The result revealed that phenanthrene had the most concentrated PAH with mean values of 0.6817±0.1429 while naphthalene (0.0027±0.0005) had the least concentration in the cowhide. The concentrations of the other PAHs in decreasing order were benzo[a]pyrene (0.6222±0.1636) > pyrene (0.2474±0.0331) > chrysene (0.1757±0.2688) > benz[a]anthracene (0.1256±0.0150) > benzo[g,h,i]perylene (0.0756±0.0201) > dibenz[a,h]anthracene (0.0727±0.0130) > anthracene (0.0629±0.0104) > benzo[k]fluoranthene (0.0245±0.0315) > acenaphthene (0.0144±0.0164) > fluorene (0.0060±0.0049) > benzo[b]fluoranthene (0.0052±0.0007) > Indeno [1, 2, 3-cd] pyrene (0.0038±0.0012). The totalmean PAHs concentration is 2.1602 µg/kg.

Table 1: PAH concentrations (µg/kg) in non roasted cowhide

PAH	Wukari	Jalingo	Lafia	Lokoja	Makurdi	Mean	SD
Naphthalene	0.0021	0.0026	0.0025	0.0030	0.0035	0.0027	0.0005
Acenaphthylene	0.0231	0.0248	0.0285	0.0213	0.0229	0.0241	0.0027
Acenaphthene	0.0027	0.0024	0.0023	0.0306	0.0340	0.0144	0.0164
Fluorene	0.0028	0.0023	0.0022	0.0109	0.0119	0.0060	0.0049
Phenanthrene	0.7875	0.8332	0.7255	0.5192	0.5430	0.6817	0.1429
Anthracene	0.0550	0.0560	0.0552	0.0721	0.0762	0.0629	0.0104
Fluoranthene	0.0171	0.0018	0.0160	0.0206	0.0222	0.0157	0.0081
Pyrene	0.2273	0.2403	0.2073	0.2763	0.2858	0.2474	0.0331
Benzo[a]anthracene	0.1246	0.1240	0.1022	0.1353	0.1414	0.1256	0.0150
Chrysene	0.0591	0.0524	0.0474	0.0632	0.6564	0.1757	0.2688
Benzo[b]fluoranthene	0.0050	0.0055	0.0041	0.0057	0.0059	0.0052	0.0007
Benzo[k]fluoranthene	0.0093	0.0096	0.0808	0.0111	0.0115	0.0245	0.0315
Benzo[a]pyrene	0.5170	0.4364	0.5710	0.7796	0.8068	0.6222	0.1636
Indeno[1,2,3-cd]pyrene	0.0021	0.0047	0.0028	0.0046	0.0047	0.0038	0.0012
Dibenz[a,h]anthracene	0.0619	0.0693	0.0598	0.0841	0.0885	0.0727	0.0130
Benzo[g,h,i]perylene	0.0585	0.0591	0.0657	0.0990	0.0959	0.0756	0.0201
Total PAH						**2.1602**	

Table 2: Mean PAH concentrations (µg/kg) in roasted cowhide (n=15)

PAH	Wukari*	Jalingo*	Lafia*	Lokoja*	Makurdi*	Mean**	SD
Naphthalene	0.0030	0.0013	0.0013	0.0041	0.0031	0.0026	0.0012
Acenaphthylene	0.0086	0.0002	1.9482	0.0093	0.0133	0.3959	0.8678
Acenaphthene	0.0299	0.0100	0.0117	0.0377	0.0154	0.0209	0.0012
Fluorene	0.0114	0.0045	0.0040	0.0178	0.0073	0.0090	0.0057
Phenanthrene	2.0713	0.1078	0.5381	0.8005	0.6761	0.8388	0.7368
Anthracene	0.0780	0.0709	0.0273	0.0913	0.0287	0.0592	0.0294
Fluoranthene	0.0325	0.0514	0.0200	0.0288	0.0560	0.0377	0.0153
Pyrene	0.3167	0.4614	0.1136	0.4862	0.1632	0.3082	0.1689
Benz[a]Anthracene	0.1554	0.1510	0.0057	0.2090	0.0411	0.1124	0.0854
Chrysene	0.0728	0.0729	0.0244	0.0855	0.0370	0.0585	0.0263
Benzo[b]fluoranthene	0.0064	0.0056	0.0028	0.0081	0.0056	0.0057	0.0196
Benzo[k]fluoranthene	0.0125	0.0088	0.0052	0.0189	0.0131	0.0117	0.0051
Benzo[a]Pyrene	0.8720	0.5635	0.3942	1.1647	0.5515	0.7092	0.3079
Indeno[1,2,3-cd]Pyrene	0.0051	0.0023	0.0025	0.0068	0.0067	0.0047	0.0022
Dibenz[a,h]Anthracene	0.0943	0.0057	0.3673	0.0462	0.0099	0.1047	0.1511
Benzo[g,h,i]Perylene	0.1031	0.0051	0.0622	0.1095	0.091	0.0578	0.0497
Total						**2.7370**	

*****Mean values for triplicate determinations; ******Mean PAH values for all sample points (n=15)

The PAHs concentrations (µg/kg) in the roasted cowhide samples are presented in Table 2. Phenanthrene (0.8388±0.7368) is the most concentrated while indeno[1,2,3-cd]pyrene (0.0047±0.0022), the least concentrated. The other PAHs in decreasing order are benzo[a]pyrene (0.7092±0.3079); acenaphthylene (0.3959±0.8678); pyrene (0.3082±0.1689); benz[a]anthracene (0.1124±0.0854); dibenz[a,h]anthracene (0.1047±0.1511); chrysene (0.0585±0.0263); benzo[g,h,i]perylene (0.0578±0.0497); fluoranthene (0.0377±0.0153); acenaphthene (0.0209±0.0012); benzo[k]fluoranthene (0.0117±0.0051); fluorene (0.0090±0.0057); benzo[b]fluoranthene (0.0057±0.0196) and naphthalene (0.0026±0.0012). The total mean concentration of PAHs in the cowhide sample is 2.7370 µg/kg.

The PAH concentrations (µg/kg) contributed from smoke in the roasted cowhide samples are presented in Table 3. Their concentrations in decreasing order include 0.3718 (acenaphthylene); 0.1571 (phenanthrene); 0.0608 (pyrene); 0.0220 (fluoranthene); 0.0087 (benzo[a]pyrene); 0.0065 (acenaphthene); 0.0032 (dibenz[a,h]anthracene); 0.0030 (fluorene); 0.0009 (indeno[1,2,3-cd]pyrene) ; 0.0005 (benzo[b]fluoranthene).

Furthermore, some PAHs had more concentrations contributed from the smoke to the roasted cowhide when compared with their concentrations in the control samples (hot water processed or non–roasted) which represent concentrations from other environmental sources as shown in Fig. 2. These PAHs include; acenaphthylene (0.3718, 0.0241) and fluoranthene (0.0220, 0.0157). However there were other PAHs that contaminated the sample from smoke but their concentrations was less than those from the environmental sources. The PAHs are acenaphthene (0.0065, 0.0144); fluorene (0.0030, 0.0060); phenanthrene (0.1571, 0.6817); pyrene (0.0608, 0.2474); benzo[b]fluoranthene (0.0005, 0.0052); benzo[a]pyrene (0.0087, 0.6222); indeno[1,2,3-cd]pyrene (0.0009, 0.0038) and dibenz[a.h]anthracene (0.0032, 0.0727). The concentrations of the PAH4 (Benzo[a]pyrene, benzo[a]anthracene, chrysene and benzo[b] fluoranthene) from smoke in the roasted cowhide relative to their concentrations from other environmental sources revealed that benz[a]anthracene and chrysene had no contaminations from the smoke while benzo[b]fluoranthene

and benzo[a]pyrene concentrations (µg/kg) were low relative to other sources of contamination; 0.0005, 0.0052 and 0.0087, 0.6222, respectively.

Table 3: PAH contaminations from smoke in roasted cowhide (RCH) samples

PAH	PAH Conc. in RCH (µg/kg)	PAH Conc. in NRCH (µg/kg)	PAH Conc. in Smoke (µg/kg)	% PCL
Naphthalene	0.0026	0.0027	0.0000	0
Acenaphthylene	0.3959	0.0241	0.3718	94
Acenaphthene	0.0209	0.0144	0.0065	31
Fluorene	0.0090	0.0060	0.0030	33
Phenanthrene	0.8388	0.6817	0.1571	19
Anthracene	0.0592	0.0629	0.0000	0
Fluoranthene	0.0377	0.0157	0.0220	58
Pyrene	0.3082	0.2474	0.0608	20
Benz[a]Anthracene	0.1124	0.1256	0.0000	0
Chrysene	0.0585	0.1757	0.0000	0
Benzo[b]fluoranthene	0.0057	0.0052	0.0005	09
Benzo[k]fluoranthene	0.0117	0.0245	0.0000	0
Benzo[a]Pyrene	0.7092	0.6222	0.0087	12
Indeno[1,2,3-cd]Pyrene	0.0047	0.0038	0.0009	19
Dibenz[a,h]Anthracene	0.1047	0.0727	0.0032	31
Benzo[g,h,i]Perylene	0.0578	0.0756	0.0000	0

RCH-Roasted cowhide; **NRCH**-Non roasted cowhide

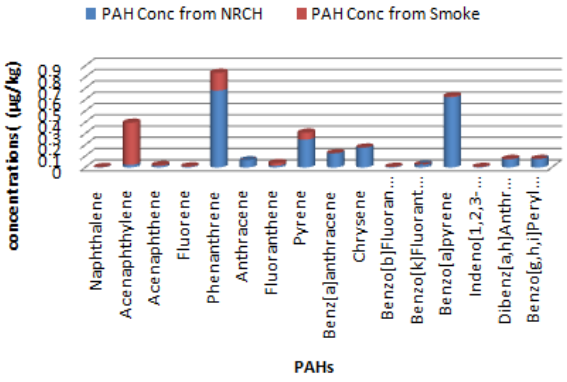

Fig 2: Comparison of PAH concentration from smoke in roasted cowhide and Non–roasted cowhide

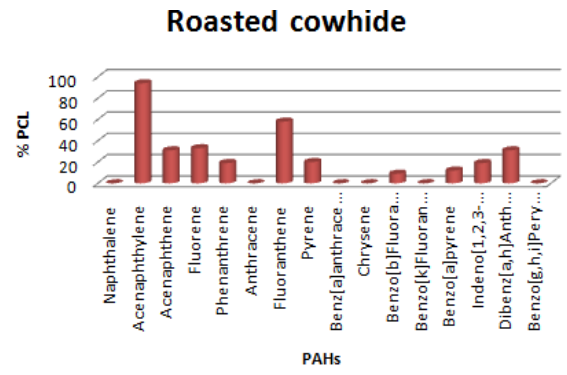

Fig 3: % PAH Contamination level from smoke in roasted cowhide

The % contamination levels from smoke in the roasted cowhide are indicated in Fig. 3. The contamination levels of acenaphthylene (94) and fluoranthene (58) was more than 50 %; while the contamination levels of acenaphthene (31), fluorene (33), phenanthrene (19), pyrene (20), benzo[b]fluoranthene (09), benzo[a]pyrene (12), indeno[1,2,3-cd]pyrene (19) and dibenz[a,h]anthracene (31) from the smoke were however less than 50 % in the roasted cowhide.

Generally, the PAHs found in the control samples may have passed into it from subcutaneous fats since cowhides have been shown to be attached to subcutaneous fats which are usually scraped off (Joseph, 2006). Furthermore other sources from the environment such as the soil and water which the cow skin had contact with may have contributed the PAHs. Erema and Adaobi (2013) corroborated this view that PAHs enter the body by different routes such as the air breathed, by drinking water or through skin in contact with soil. The reason for these variation in contaminations of the PAHs from the smoke is not clear but generally, data reported in literature on quantitative basis regarding smoked foods have been shown to be highly variable (USEPA, 1993).

The low contaminations of the PAHs from the smoke may suggest that they could have been trapped in the pores of the cowhide after the roasting which was followed with sun drying, soaking in water and thorough washing with sponge. Furthermore, the presence of the PAHs likenaphthalene, anthracene, benz[a]anthracene, chrysene, benzo[k]fluoranthene and benzo[g,h,i]perylene which did not contaminate the roasted cowhide from the smoke indicates that the lower concentrations of these PAHs recorded in the roasted cowhide than the non roasted samples suggests that environmental sources contributed concentrations of these PAHs which were degraded due to high temperatures associated with the roasting activity. Generally, benzo[a]pyrene and the sum of PAH4 concentrations in the roasted cowhide was 0.7092 µg/kg and 0.8858 µg/kg, respectively which is far lower than the recommended limits of 2 µg/kg and 12 µg/kg (Food Standard Agency, 2012).

The total PAHs content of both the non roasted (hot water processed) and the roasted samples were not significantly different ($p>0.05$) however, fluoranthene was significantly higher in the roasted samples than the non roasted ones indicating that fluoranthene contamination in the roasted cowhide was significantly associated with the roasting process.

Conclusion

The study revealed that though smoke contributed PAHs that may be responsible for carcinogenicity; other sources from the environment could also be a major contributor of some of these PAHs. Furthermore the % PAH contamination levels from smoke in the roasted cowhide indicated that most of the PAHs were either absent or below 50 %. This suggests that sundrying, washing with sponge and clean water employed by processors is a very important process which brings down the contamination levels due to smoke to a minimum therefore the roasted cowhides may not be as harmful as speculated except it is not properly washed. In addition, the data generated for this study will constitute a baseline for more studies to be conducted out on the PAH contamination of smoke in cowhides especially in northern Nigeria.

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