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Archives of Applied Science Research, 2015, 7 (4):1-6
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Determination of levels of polycyclic aromatic hydrocarbons on singed cow hide (Punmo) and charcoal grilled meat (Suya)

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ABSTRACT

The aim of this work was to determine the level of polycyclic aromatic hydrocarbon (PAH) in singed cowhides and charcoal grilled meat (suya) commonly consumed in Nigeria. The study area covered major markets in Umuahia. A total number of ten PAH (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene and chrysene) were investigated in singed cowhide and grilled lean meat samples. The PAHs were identified using gas chromatographic fitted with a flame ionization detector. Seven out of ten PAHs in the barbecued charcoal grilled meat (suya) were below the instrument detection limit while the levels of Naphthalene, Acenaphylene and Flourene found in the samples were 9260µg/kg, 9920µg/kg and 2565µg/kg respectively. However only Naphthalene (1.624µg/kg) were found in the singed cow hide. This study confirms the presence of PAHs in barbecued charcoal grilled meat and singed cowhide.

Keyword: Polycyclic Aromatic Hydrocarbon (PAH), Cowhides, Singed cowhides, Carcinogenic, Naphthalene, Benzo [a] pyrene.

INTRODUCTION

Polycyclic aromatic hydrocarbons(PAHs) represents the group of organic compounds consisting of two or more fused aromatic rings that are formed by incomplete combustion or pyrolysis of organic matter and during industrial processes [1].

PAHs are one of the most widespread organic pollutants. In addition to their presence in fossil fuel they are also formed by incomplete combustion of carbon-containing fuels such as wood, coal, diesel, fat, tobacco, and incense [2].

There are different pathways through which humans are exposed to PAHs. Smokers absorb significant amount of these compounds in their system while for non-smokers exposure route may be through food consumption [3].

PAH has been classified as hazardous compounds of environmental concern due to their carcinogenicity and mutagenity[4],[5],[6],[7]. However, the 16 EPA priority PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]flouranthene, benzo-[*a*]pyrene, dibenzo[*a,h*]anthracene, benzo[*g,h,i*] pe-rylene, and indeno[1,2,3-*c,d*]pyrene) is often targeted for measurement in environmental samples[8].

Seven PAHs (benzo[*a*] pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]flu-oranthene, benzo[*k*]fluoranthene, dibenzo [*a*,*h*]an-thracene and indeno[1,2,3-*c*,*d*]pyrene) has been classified by the United States Environmental Protection Agency (USEPA) as Group B2, probable human carcinogens [9],

As measures are presently widely focused on benzo[a]pyrene (BaP), a compound classified as carcinogenic to humans (group 1 of IARC categories), the SCF concluded that BaP could be used as a marker[10].Based on further investigation carried by other member of states, it has been concluded that benzo [a] pyrene is no longer suitable as a sole marker for the occurrence and toxicity of PAH in Foods [8]. A review was made by the European Commission in August 2011 for a new maximum limit for PAHs in food and was published in Regulation (EU) 835/2011 amending (EC), 1881/2006.

The commission recommended 8 specific PAHs (PAH8) which includes BaP, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo-[*ghi*]perylene, chrysene, dibenzo[*a*,*h*]anthracene and indeno[1,2,3-*cd*]pyrene, for which oral carcinogenicity data are available, and/or a subgroup of these, PAH4, including BaP, chrysene, benz[*a*]anthracene and benzo[*b*]fluoranthene, and/ or the sum of four PAHs namely BaP, chrysene, benz[*a*]an-thracene and benzo[*b*]fluoranthene, as more suitable markers for the occurrence and toxicity of PAHs in foods[1]. However, the commission recommendedthe maintenance of separate maximum level for BaP to ensure the comparability of previous and future data [11].

Significant levels of PAH has been found in foods such as oils, fats, smoked meat and smoked meat products,smoked fish and smoked fish products, muscle meat of fish others include smoked, crustaceans, cephalopods, bivalve mollusces and infant foods. The new maximum level for BaP in smoked meat and smoked meat products will be 2.0µg/kg as of September 2014, while until then the level of 5.0µg/kg also defined by previous Regulation 1881/2006 should be applied. As for the sum of PAH4 in the same foodstuffs group, it is set to 30.0µg/kg till September 2014 and 12.0µg/kg after this date [11],[12]. The work aim of this work is to determine the occurrence and levels of PAH in singed cowhide and barbecued charcoal grilled meat in Umuahia, Abia state, Nigeria.

MATERIALS AND METHODS

2.1 Sample Preparation

Raw cow hide (ponmo) and lean beef samples, were obtained from Umuahia main market. The cowhide was smoked using the following heat sources to remove the hairs; wood, tyre, spent engine oil andHot water (control)

The smoked cowhide samples were cleaned with knife, to scrap off burnt hairs and other unwanted materials that might have stocked to the hide while smoking. The dry cleaned cowhide samples were washed with clean water using sponge to remove the remaining dirt that could not be removed by dry cleaning before the samples were allowed to drain out water. The samples were dried in an oven at a temperature of 125^0C for 8 hours. The samples were cooled for 1 hours and grinded using a manual grinder (Sioux valve grinder model #645).

2.1.1 Raw cowhide processed with hot water.

Raw cowhide was soaked in hot water for 2mins to ease the hair removal using knife. It was washed with cold water and allowed to drain out water. The samples were dried on an oven at a temperature of 120^0C for 12 hours and was grinded with s manual grinder (Sioux valve grinder model #645). The cowhide processed with hot water was used as the control.

2.1.2 Charcoal grilled meat (suya)

The lean meat was grilled under open flame using charcoal and the grilling lasted for 10 minutes. It was cooled and dried at temperature for 120^0C for 5 hours. Raw lean meat was oven dried at the same temperature and used as the control.

2.2 Extraction Procedure

2.2.1 Extraction of PAH from the samples

The Soxhlet extractor consisting of the condenser, thimble and receiving flask was thoroughly washed and dried with acetone and Dichloromethane (DCM). Exactly 10g of the sample was packed into the Soxhlet extraction thimble. Sixty milliliter (60ml) of Dichloromethane (DCM) was measured with measuring cylinder was poured into

a 125ml receiving flask. The condenser was connected to refrigerated circulating water bath to achieve low temperature and also to maintain excellent temperature stability for consistent result. The extraction processes was ran for six hours, as the organic solvent vaporized, the receiving flask encountered a lower temperature in the condenser area, and condensed into a hot liquid, from which the analyte of interest was extractedand the rest of the liquid was returned back to the receiving flask. Thus, for each of the extraction process, fresh solvent in the receiving flask was used for the extraction and the extracted analyte left in the receiving flask.

2.2.2 Concentration of the extracts using rotary evaporator
The extracts were concentrated to 2ml using a rotary evaporator and were poured into a clean 2ml GC bottle.

2.3 Gas Chromatography Flame Ionization Detection Analysis

2.3.1 Peak identification/ confirmation by gas chromatography
The identification of Polycyclic Aromatic Hydrocarbons (PAH) was conducted on a gas chromatograph fitted with flame ionization detector, fused to a capillary column Restex 30 meter MXT-1 with Helium carrier at 5psi. The following conditions were maintained in the gas chromatography; Initial oven temperature (100^0C), Hold time (1 minute), Ramp a (47 mm), Final oven temperature (320^0C), Hold time (4 minutes), Injection temperature (200^0C), FID Detector temperature(330^0C), Volume of standard/sample injected (0.8ml), Total time of Analysis (60 minutes).

RESULTS AND DISCUSSION

3.1 PAH Contents in Singed Cowhide Samples

The PAH found in the singed cowhides were Naphthalene (Nph), Acenaphthylene (ACE), Acenaphthene (ACE), Fluorene (FLO), Phenanthrene (PHN), Acethracene (ANT) and Chrysene (CRY). Naphthalene was the most abundant with a concentration of 1624µg/kg. The level of acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene and chrysene found in singed cowhides were below the detection limits. The PAHs contents of the singed cowhides and the instrument detection limits were represented in Table 3.1 and 3.2 respectively.

The presence of PAH in food is significantly due to heat processes such as smoking and grilling. However, environmental pollutants are also considered to be an issue [7]. More*et al*confirmed that some types of edible oil e.g. Olive oil may be contaminated with PAHs due to artificial drying and heating during processing[13]. Processing procedure such as smoking, drying and cooking is commonly thought to be the major source of contamination of PAH [14], [15]. Naphthalene recently considered as possible as carcinogenic for humans by IARC (Group 2A)[16]was present in the singed cowhide studied. Although the level of benzo[a]anthracene was very low in all cases. Naphthalene was the most abundant PAH in almost all the samples.

Table 3.1 Levels of PAH Compounds in Smoked Singed Cowhide Samples (ponmo)

PAH Compounds	Hot Water (Control) Conc. µg/kg	Tyre Conc. µg/kg	Tyre + Spent engine oil Conc. µg/kg	Wood Conc. µg/kg
Naphthalene	1.62µg/kg	< IDL	< IDL	< IDL
Acenaphthylene	< IDL	< IDL	< IDL	< IDL
Acenaphthene	< IDL	< IDL	< IDL	< IDL
Flourene	< IDL	< IDL	< IDL	< IDL
Phenanthrene	< IDL	< IDL	< IDL	< IDL
Anthracene	< IDL	< IDL	< IDL	< IDL
Flouranthene	< IDL	< IDL	< IDL	< IDL
Pyrene	< IDL	< IDL	< IDL	< IDL
Benzo(a)anthracene	< IDL	< IDL	< IDL	< IDL
Chrysene	< IDL	< IDL	< IDL	< IDL

< Means less than, IDL means Instrument detection limit.

The washing of the singed cowhides samples could have contributed to the low values of PAH compounds obtained in study. The concentration of PAH are generally on the surface than in the internal tissues. Consequently, careful washing might have removed an average of up to 50% of the total PAH [10]. Particles bound high molecular mass PAH which remain on the surface are easily washed off whereas low molecular compounds which are in the vapor can penetrate the internal tissues[10]. Therefore are less effectively removed by washing.

3.2 PAH Contents in Charcoal Grilled Meat Samples

Ten types of PAH compounds were investigated in barbecued charcoal grilled meat popularly known as "*suya*". Seven out of the ten had levels below the instrument detection limit while the levels of Naphthalene, Acenaphthylene, and flourene were 9260µg/kg, 9920µg/kg and 2565µg/kg respectively. Naphthalene and flourene found in the barbecued dry meat could be from the wood and oil used as a source of fuel since, most PAH, are formed from incomplete combustion of wood, oil and coal [17]. The quantity of Naphthalene and flourene in the grilled meat were within the permissible limits since PAH levels as high as 200µg/kg has been found in smoked fish and meat [10].

In barbecued meat, 139µg/kg has been reported whereas the average background values are usually in the range 0.01 - 1µg/kg in un-cooked food. In some earlier work done by other researchers, the PAH found in meat were chloratene (9.6µg/kg), pyrene (11.0µg/kg), benzo[a]anthracene (1.0µg/kg), chrysene (3.0µg/kg), benzo[b]fluoranthene (0.8µg/kg), benzo[e]pyrene (0.6µg/kg), benzo[ghi]perylene (1.0µg/kg),dibenz[a,h]anthracene (0.2µg/kg), while in grilled meat, this PAH were found; flouranthene (9.4µg/kg), pyrene (10.6µg/kg), benzo[a]anthracene (1.3µg/kg), benzo[b+j+K]chrolathene (1.7µg/kg), benzo[e]pyrene (0.9µg/kg) and benzo[ghi]pyrene (1.3µg/kg) [18], [19], [20], [13],[21],[22].

Researchers reported charcoal grilled meat without skin containsthe highest amount of total PAH (320 µg/kg), followed by charcoal grilled meat with skin (300 µg/kg), smoking (210 µg/kg).

In our study, PAH was higher in charcoal grilled meat than in singed cowhides. The hides reduced the percentage of PAH that penetrated into the skin. PAH formation during charcoal grilling was shown to be dependent upon the temperature[24],[10] and a number of parameters, time, fuel, distance from the heat and sources and method of fat drainage (grilling, frying and roasting). Cooking resulted in the production of a number of compounds including [10]). Although not precisely known, it is likely that there are several mechanism of PAH formation such as melted fat that undergoes pyrolysis when dripping on to the heat source and pyrolysis of the meat due to the high temperature, [18],[25], [10].

On a quantitative basis, the variation in the data reported in the literature could be attributed in part to the different procedures used to evaluate the presence of PAH, but the main reasons for such variables include; the type of smoke generation, oxygen accessibility, temperature of smoke generation and smoking time.

TABLE 3.2THE DETECTION LIMITS OF PAH BY THE APPLIED GLC CONDITIONS

PAH Compounds	INSTANT PAH IDL (µg/kg)
Naphthalene	0.006915
Acenaphthylene	0.010765
Acenaphthene	0.009855
Flourene	0.0116855
Phenanthrene	0.0116855
Anthracene	0.0116855
Flouranthene	0.2025
Pyrene	0.2365945
Benzo(a)anthracene	0.223918
Chrysene	0.33707

**IDL means Instrument Detection Limits*

Table 3.3 Levels of PAH Compounds in Charcoal Grilled Meat Samples

PAH Compounds	Lean Beef (Control) Conc. µg/kg	Tyre + Spent engine oil Conc. µg/kg
Naphthalene	<IDL	9260
Acenaphthylene	< IDL	9920
Acenaphthene	< IDL	< IDL
Flourene	< IDL	< IDL
Phenanthrene	< IDL	< IDL
Anthracene	< IDL	< IDL
Flouranthene	< IDL	2565
Pyrene	< IDL	< IDL
Benzo(a)anthracene	< IDL	< IDL
Chrysene	< IDL	< IDL

<Means less than, IDL means Instrument detection limit

CONCLUSION

The higher concentration of the PAH content in the charcoal grilled meat (SUYA) and incidence of PAHs collaborates the direct smoking of the meat which results as a source of contamination. The washing and cleaning procedures employed on singed cowhide samples before drying and crushing seem to be effective in reducing the PAHs levels transferred during smoking. The levels of PAHs found in this study may be considered low for singed cowhide (with any type of smoking method) when compared with BaP limit for drinkable water. In this manner there is no apparent risk to the consumers with regards to charcoal grilled meat and singed cowhide.

The result in charcoal grilled meat confirmed the presence of three (3) polycyclic aromatic hydrocarbons due to open-flame charcoal grilling and measures should be taken on the consumption to avoid its carcinogenic effect on humans. Taking into consideration the carcinogenic potential of polycyclic aromatic hydrocarbons (PAHs) any measure directed to the reduction of these contaminants in the environment and in the diet is highly desirable and should be strongly stimulated.

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