



## ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBON (PAH) IN KOLA NUTS FROM SELECTED MARKETS IN SOUTH WESTERN NIGERIA

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### ABSTRACT

The handling process and outdoor sales of kola nuts in market places where exhausts from incomplete combustion in automobile engines abound are potential routes through which polycyclic aromatic hydrocarbon (PAHs) can contaminate kola nuts which is eaten directly without any treatment or cooking. Five pieces of kola nuts were bought in selected markets in Oyo, Ogun and Osun States. The samples were processed and analyzed for PAHs with Agilent 7890 HP Gas chromatograph. Result show that, samples from Osun State were mostly contaminated with PAHs while those from Oyo State were least contaminated. A greater percentage of the total PAH (90.12%) was contributed by the low molecular weight (3-4 rings). However, the level of PAH contamination in the investigated kola nuts is so low compared with the maximum allowable level in nuts which makes the kola nuts safe for consumption with respect to PAH level.

**KEYWORDS:** *Sterculiaceae*, *Cola nitida*, *Cola acuminata*, chronic toxicity

### INTRODUCTION

The genus *Cola* belongs to the family *Sterculiaceae* (from which the nuts are obtained) is indigenous to tropical Africa and has its greatest diversity in West Africa (Asogwa *et al.*, 2006). Its cultivation is to a large extent in Nigeria but also grown in Ghana, Ivory Coast, Brazil and West Indian Islands (Opeke, 1982). The annual production from these countries alone is in excess of 250,000 tons, while the world production is about 300,000 tons (American Horticultural Society, 2002). Two species *Cola nitida* and *Cola acuminata* are of commercial value (Oladokun, 1982). Nigeria accounts for about 70% of the total world production of kola nuts (Oluokun and Oladokun, 1999). About 90% of the kola nuts produced in Nigeria is consumed within the country while the remaining 10% is exported as sun-dried nuts to other parts of Africa especially neighboring countries in West Africa (Ogutuga, 1975, Akinbode, 1982) where they are used as stimulants or source of colorants for cloth dyeing. *C. acuminata* is frequently used for social and religious ceremonies in Nigeria while *C. nitida* which is referred to as “the true Kola of commerce” has featured in the internal trade of West Africa for a number of centuries (Eijnatten, 1969; Jaiyeola, 2001).

Kola nuts can be exposed to polycyclic aromatic hydrocarbon along the chain of processing to the point of consumption. Polycyclic aromatic hydrocarbon (PAH) compounds are a class of complex organic chemicals which include carbon and hydrogen with a fused ring structure containing at least two benzene rings. They are the most stable form of hydrocarbons having low hydrogen-to-carbon ratio and usually occur in complex mixtures rather than single compounds. These pollutants are mostly formed during incomplete combustion and pyrolysis of fossil fuels or wood and from the release of petroleum products (Manahan, 1994). Agricultural residues (fire woods) are used by 50-75% people in Africa for daily cooking (WHO, 2002). Most of the people in the business of kola nuts processing and preservation are within the category of the 50-75% user of fire woods for

daily cooking. The gaseous, smoke and particulates from the burning of fire woods as well as flames or smoke from cooking stoves could be a source of PAH which can come in contact with the kola under processing and preservation within the homes of the processors. Most markets where kola nuts are sold are characterized with vehicular movements. The engine exhausts which usually cause pollution in the open market places is another possible source of PAH for the kola nuts sold in the open market places. PAHs have been identified as suspected carcinogens. As molecular weight increases, the carcinogenicity of PAHs also increases and acute toxicity decreases. Considering the increasing evidence of the ubiquitous presence of PAHs and health risk associated with their exposure, the present study examines the level and types of PAHs in kola nuts that are being sold in selected markets in South-Western Nigeria with a view to assessing its safety for consumption with respect to PAHs.

### MATERIALS AND METHODS

Five kola nuts were purchased from kola nut sellers at various selected markets in Osun, Ogun and Oyo States in Nigeria. The fresh kola nuts were chopped with knife and sun-dried for five days. The sun-dried samples were later oven-dried for 4 hours at a temperature of 70°C until a constant weight was attained. The samples were pulverized with manual mini grinding machine.

#### Extraction

The extraction method of polycyclic aromatic hydrocarbons profiles in samples was followed by employing the modified methods of ASTM D3328 and ASTM 3415. Three gram (3g) of the pulverized sample was weighed into a 250ml capacity beaker of borosilicate material and 40ml of the ratio 3:1 redistilled hexane: dichloromethane was added. The beaker and its content placed in the sonicator to extract the hydrocarbon for about 2 hours. The organic layer was filtered into a 250ml capacity borosilicate beaker. The extract was dried by passing the filtrate through the funnel containing the anhydrous sodium sulphate. The dried extract was concentrated with a stream of nitrogen gas.

**PAH separation**

The concentrated oil was separated into aliphatic profiles and poly aromatic hydrocarbons profiles by packing the glass column with activated alumina, neutral and activity/grade 1.10ml of the treated alumina was packed into the column and cleaned properly with redistilled hexane. The extract was poured onto the alumina and was allowed to run down with the aid of the redistilled hexane to remove the aliphatic profiles into a pre-cleaned 20ml capacity glass container. The aromatic fraction was recovered by allowing the mixture of hexane and dichloromethane in ratio 3 to 1 and finally removed the most polar PAH by removing the dichloromethane into the pre-cleaned borosilicate beaker. The mixture was concentrated to 1.0ml by stream of nitrogen gas before the gas chromatographic analysis. Agilent 5890 HP was used for the analysis.

**RESULTS**

Determination of the sixteen polycyclic aromatic hydrocarbon (16 PAH) concentration levels in kola nuts from selected markets in Ogun State is presented in Table 1. Result show that, all the 16 PAHs were detected in all the samples analyzed. Out of the PAHs detected, Dibenzo[a,h] anthracene had the least average concentration of  $0.00010 \mu\text{gkg}^{-1}$  with a range of  $0.00003$  to  $0.00019 \mu\text{gkg}^{-1}$  while Phenanthrene had the highest mean concentration of  $0.2868 \mu\text{gkg}^{-1}$  with a range of  $0.00975$  to  $0.65400 \mu\text{gkg}^{-1}$ . The total PAH ranged from

$0.1580$  to  $1.8700 \mu\text{gkg}^{-1}$  with a mean total PAH of  $0.8524 \mu\text{gkg}^{-1}$ . Sample from Ilisan 3 had the least total PAH while sample from Ilisan 3 had the highest total PAH. The ratio between Phenanthrene and Anthracene ranged from  $0.4538$  and  $3.5978$  while the ratio between Fluoranthrene and Pyrene ranged from  $0.9297$  to  $1.2394$  with an average of  $1.1166$ . The sum of low molecular weight PAH in the analyzed samples ranged from  $0.1317$  to  $1.7888 \mu\text{gkg}^{-1}$  with a mean of  $0.8058 \mu\text{gkg}^{-1}$  while the range of the sum of high molecular weight ranged from  $0.0215$  to  $0.0812 \mu\text{gkg}^{-1}$ . Sample from Ilisan 3 had the highest total PAH while Ilisan 2 had the least total PAH.

Determination of the 16 PAH concentration level in kola nuts from selected markets in Osun State is presented in Table 2. All the 16 PAH were detected in all the samples analyzed. Result show that, Dibenzo[a,h]anthracene had the lowest concentration with an average of  $0.00015 \mu\text{gkg}^{-1}$  having a range of  $0.00003$  to  $0.00023 \mu\text{gkg}^{-1}$  while phenanthrene had the highest mean concentration of  $0.2535 \mu\text{gkg}^{-1}$ . Total PAH ranged from  $0.6410$  to  $1.5700 \mu\text{gkg}^{-1}$  with a mean of  $1.1032 \mu\text{gkg}^{-1}$ . Sample from Orafidina 3 had the highest concentration of total PAH while sample from Orafidina 5 had the least total PAH. The ratio between Phenanthrene and Anthracene ranged from  $1.1918$  to  $7.2600$  with a mean of  $2.2340$  while the ratio between Fluorene and Pyrene ranged from  $0.9927$  to  $1.4393$  with a mean of  $1.1643$ .

**TABLE 1:** Polycyclic Aromatic Hydrocarbons in kola nuts from Ogun State

	Ilisan 1	Ilisan 2	Ilisan 3	Mamu 1	Mamu 2	Mean
Naph	0.00033	0.00029	0.00330	0.00012	0.00320	0.00145
Aceny	0.00033	0.00038	0.00058	0.00008	0.00067	0.00041
Acen	0.00730	0.00680	0.09110	0.02620	0.05900	0.03808
Fluo	0.03900	0.02550	0.25400	0.15700	0.23000	0.14110
Phen	0.01130	0.00975	0.72200	0.03700	0.65400	0.28681
Anth	0.02490	0.02710	0.21500	0.07750	0.18500	0.10590
Flua	0.00344	0.00497	0.05930	0.00760	0.04020	0.02310
Pyr	0.000037	0.00401	0.04340	0.00770	0.03790	0.01861
B(a)A	0.037200	0.04200	0.28000	0.00009	0.23700	0.11926
Chry	0.009450	0.01030	0.12200	0.02130	0.10700	0.05401
B(b)F	0.014200	0.01500	0.05040	0.02540	0.04240	0.02948
B(k)F	0.005040	0.00547	0.02200	0.00899	0.01910	0.01212
B(a)P	0.001310	0.00135	0.00545	0.00239	0.00478	0.00306
IP	0.000821	0.00081	0.00279	0.00162	0.00238	0.00168
D(ah)A	0.000030	0.00003	0.00019	0.00004	0.00019	0.00010
B(ghi)A	0.000094	0.00007	0.00037	0.00015	0.00032	0.00020
Phen/Anth	0.4538	3.5978	3.3581	0.4774	3.5351	2.28444
Flua/Pyr	0.9297	1.2394	1.3663	0.9870	1.0607	1.11662
Sum LPAH	0.1365	0.1317	1.7888	0.4214	1.5508	0.80584
Sum HPAH	0.0215	0.0227	0.0812	0.0386	0.0692	0.04664
∑ PAH	0.15800	0.15400	1.87000	0.46000	1.6200	0.8524
% LPAH	86.39	85.26	95.66	91.61	95.73	90.93
% HPAH	13.61	14.74	4.34	8.39	4.27	9.07

Naph- Naphthalene, Acety – Acenaphthalene, Acen – Acenaphthene, Fluo- Fluorene, Phen – Phenanthrene, Anth-Anthracene, Flua-Fluoranthene, Pyr- Pyrene, B(a)A – Benzo[a]anthracene, Chry – Chrysene, B(b)F-Benzo [b] fluoranthene, B(k)F- Benzo[k]fluoranthene, B(a)P- Benzo[a]pyrene, D(ah)A- Diben[a,h]anthracene, B(ghi)P- Benzo[ghi]perylene, IP-indeno[1,2,3,-cd]pyrene; OG 1- OG 5- Sampling location; LPAH- Low molecular PAHs; HPAH- High molecular PAHs.

**TABLE 2:** Polycyclic Aromatic Hydrocarbons in kola nuts from Osun State

	Ekusa1	Ekusa2	Orafid 1	Orafid 2	Orafid 3	Orafid 4	Mean
Naph	0.00114	0.00145	0.00135	0.00143	0.00394	0.00053	0.00164
Aceny	0.00160	0.00084	0.00062	0.00079	0.00079	0.00016	0.00080
Acen	0.00520	0.05400	0.04370	0.05420	0.04600	0.06200	0.04418
Fluo	0.17500	0.28100	0.22600	0.29200	0.28800	0.22200	0.24733
Phen	0.18100	0.29300	0.23400	0.30000	0.45500	0.05810	0.25351
Anth	0.14900	0.23900	0.18900	0.23500	0.23800	0.10800	0.19300
Flua	0.01540	0.03310	0.02740	0.03440	0.03520	0.01360	0.02652
Pyr	0.01420	0.03230	0.01940	0.02390	0.03410	0.01370	0.02293
B(a)A	0.10100	0.16200	0.13800	0.16600	0.22300	0.08540	0.14590
Chry	0.05140	0.08480	0.07130	0.08570	0.17100	0.03370	0.08298
B(b)F	0.04760	0.05570	0.04680	0.05680	0.05080	0.02820	0.04765
B(k)F	0.02650	0.02500	0.02100	0.02520	0.02040	0.01090	0.02150
B(a)P	0.00692	0.00648	0.00528	0.00650	0.00505	0.00281	0.00550
IP	0.00391	0.00324	0.00267	0.00313	0.00266	0.00150	0.00285
D(ah)A	0.00003	0.00022	0.00021	0.00023	0.00013	0.00009	0.00015
B(ghi)A	0.00058	0.00039	0.00034	0.00038	0.00023	0.00049	0.00040
Phen/Anth	1.2148	1.2259	1.2381	1.2766	1.1918	7.2600	2.23453
Flua/Pyr	1.0845	1.0248	1.4124	1.4393	1.0323	0.9927	1.16433
Sum LPAH	0.7425	1.1690	0.9537	1.1978	1.4907	0.5970	1.02512
Sum HPAH	0.0855	0.0910	0.0763	0.0922	0.0793	0.0440	0.07805
∑ PAH	0.8280	1.2600	1.0300	1.2900	1.5700	0.6410	1.10317
% LPAH	89.67	92.78	92.59	92.85	94.95	93.14	92.6633
% HPAH	10.33	7.22	7.41	7.15	5.05	6.86	7.33667

Naph- Naphthalene, Acety – Acenaphthalene, Acen – Acenaphthene, Fluo- Fluorene, Phen – Phenanthrene, Anth-Anthracene, Flua-Fluoranthene, Pyr- Pyrene, B(a)A – Benzo[a]anthracene, Chry – Chrysene, B(b)F-Benzo [b] fluoranthene, B(k)F- Benzo[k]fluoranthene, B(a)P- Benzo[a]pyrene, D(ah)A- Diben[a,h]anthracene, B(ghi)P- Benzo[ghi]perylene, IP-indeno[1,2,3,-cd]pyrene; OS 1- OS 6-sampling location; LPAH- Low molecular PAHs; HPAH- High molecular PAHs. Orafid- Orafidina

Table 3: Polycyclic Aromatic Hydrocarbons in kola nuts from Oyo State

	Oja-Oba1	Beere	Oja-Oba2	Orita-mefa1	Orita-mefa2	
Naph	0.00023	0.0036	0.00047	0.00010	0.00018	0.00092
Aceny	0.00006	0.0007	0.00034	0.00004	0.00005	0.00024
Acen	0.08010	0.1120	0.00400	0.01140	0.00740	0.04298
Fluo	0.22200	0.3230	0.04020	0.11800	0.10600	0.16184
Phen	0.61200	0.9070	0.00772	0.01610	0.01210	0.31098
Anth	0.11800	0.2620	0.00462	0.03490	0.00277	0.08446
Flua	0.00706	0.0570	0.00179	0.00041	0.00366	0.01398
Pyr	0.00722	0.0537	0.00158	0.00390	0.00390	0.01406
B(a)A	0.07990	0.0339	0.02780	0.09940	0.03960	0.05612
Chry	0.01970	0.1510	0.00802	0.01060	0.00991	0.03985
B(b)F	0.02330	0.0598	0.00964	0.04960	0.01530	0.03153
B(k)F	0.00792	0.0267	0.00522	0.00334	0.00336	0.00931
B(a)P	0.00224	0.0069	0.00099	0.00120	0.00141	0.00255
IP	0.00157	0.0033	0.00057	0.00150	0.00090	0.00157
D(ah)A	0.00004	0.0002	0.00004	0.00003	0.00030	0.00012
B(ghi)A	0.00027	0.0004	0.00002	0.00019	0.00010	0.00020
Phen/Anth	5.1900	3.4600	1.6700	3.3200	4.3700	3.60200
Flua/Pyr	0.9800	1.0600	1.1300	1.0500	0.9400	1.03200
Sum LPAH	0.5947	2.2127	0.0965	0.2971	0.2126	0.68272
Sum HPAH	0.0353	0.0973	0.0165	0.0559	0.0214	0.04528
∑ PAH	0.63000	2.3100	0.11300	0.35300	0.23400	0.72800
% LPAH	94.40	95.79	85.40	84.16	90.85	90.12
% HPAH	5.60	4.21	14.60	15.83	9.15	9.89

Naph- Naphthalene, Acety – Acenaphthalene, Acen – Acenaphthene, Fluo- Fluorene, Phen – Phenanthrene, Anth-Anthracene, Flua-Fluoranthene, Pyr- Pyrene, B(a)A – Benzo[a]anthracene, Chry – Chrysene, B(b)F-Benzo [b] fluoranthene, B(k)F- Benzo[k]fluoranthene, B(a)P- Benzo[a]pyrene, D(ah)A- Diben[a,h]anthracene, B(ghi)P- Benzo[ghi]perylene, IP-indeno[1,2,3,-cd]pyrene; IB 1- IB 5- Sampling location; LPAH- Low molecular PAHs; HPAH- High molecular PAHs.

The sum of the low molecular mass PAH ranged from 0.5970 to 1.4907 with a mean of 1.0251 while the sum of the high molecular mass PAH ranged from 0.0440 to 0.0922  $\mu\text{gkg}^{-1}$  with a mean value of 0.0781  $\mu\text{gkg}^{-1}$ . Determination of the 16 PAH in kola nuts purchased from selected markets in Oyo State is presented in Table 3. All the 16 PAH were detected in all the samples analyzed. Among the various PAHs compounds, Dibenz[a,h]anthracene- a five rings compound had the least average concentration of 0.0002  $\mu\text{gkg}^{-1}$  while Phenanthrene had the highest mean concentration of 0.3109  $\mu\text{gkg}^{-1}$ . The total PAH in the analyzed samples ranged from 0.1130 to 2.3100  $\mu\text{gkg}^{-1}$  with a mean value of 0.7280  $\mu\text{gkg}^{-1}$ . The ratio between Phenanthrene and Anthracene ranged from 1.6700 to 5.1900  $\mu\text{gkg}^{-1}$  with an average of 3.6020  $\mu\text{gkg}^{-1}$  while the ratio between Fluorene and Pyrene ranged from 0.9400 to 1.1300  $\mu\text{gkg}^{-1}$  with a mean of 1.0320  $\mu\text{gkg}^{-1}$ . The sum of low molecular PAH ranged from 0.0965 to 2.2127  $\mu\text{gkg}^{-1}$  with a mean of 0.6827  $\mu\text{gkg}^{-1}$  while the total high molecular weight PAH in the analyzed samples ranged from 0.0165 to 0.0973  $\mu\text{gkg}^{-1}$  with a mean of 0.0453  $\mu\text{gkg}^{-1}$ .

## DISCUSSION

Polycyclic aromatic hydrocarbons of the kola nuts samples from all the selected markets across the three States considered in South Western, Nigeria show that, all the samples were contaminated (though at very low level) with all the 16 priority PAH (Tables 1,2 and 3). It was observed from the results that, the 3-4 rings PAHs were predominant in all the samples from the three States. However, Phenanthrene and Fluorene are the most abundant individual PAH compounds in the kola nuts. These compounds are more water soluble than higher molecular weight PAHs and so, may be more susceptible to deposition from polluted air and could also be retained in water vapour. The distribution pattern of the individual PAH in the examined kola nuts was similar to the report of Azza, (2006) who reported Phenanthrene and Fluorene as the most abundant individual PAH in Egyptian vegetables. On average (1.1032  $\mu\text{gkg}^{-1}$ ), samples from Osun State had the highest total PAH among the three States while Ogun had 0.8524  $\mu\text{gkg}^{-1}$  and Oyo 0.7280  $\mu\text{gkg}^{-1}$ . The difference in the total PAH in the studied kola nuts from the three States is an indication of variation in PAH levels which food items could be exposed to. This indicates that, PAH levels in uncooked food largely depends on the origin of the food and can be subjected to regional variation (Azza, 2006). Result suggests that, kola nuts from Osun State were most contaminated with PAH while kola nut from Oyo State were least contaminated. The total PAH range in the individual sample in this work are very much lower than total PAH range reported by so many authors. Azza, (2006) reported a range of 1.22 to 12.63 with a mean of 6.23  $\mu\text{gkg}^{-1}$  in vegetables in Egypt, Gabriela *et al.*, (2008) reported a range of 0.04 to 0.70  $\mu\text{gkg}^{-1}$ . In roasted coffee beans and 0.045 to 0.857  $\mu\text{gkg}^{-1}$  in instant coffee granules. The lower molecular PAHs (LMPAH) (<4 rings) were the dominant hydrocarbons. They usually constitute more than 65% of the total amount of PAHs determined (Baran *et al.*, 2002) and in this study, they constitute up to 90% on average across the board. The contribution of high molecular weight PAH (HPAH) (5-6 rings) was low.

Benzo[b]Fluoranthene and benzo[k]fluoranthene were dominant in all the kola nuts across the board.

Investigation into the source of PAH have been determined using the molecular ratios of some specific hydrocarbons (Lin and Zhu, 2005; Baran *et al.*, 2002 and Azza, 2006). For instance, a ratio of Fluorene to Pyrene concentration (Fluo/Pyr) greater than 1.0 was characteristic of pyrolytic origin whereas, ratio less than 1.0 was characteristic of petroleum hydrocarbons (Banmard *et al.*, 1998). A ratio of Phenanthrene to Anthracene (Phen/Anth) less than 10 suggests combustion sources, while ratio greater than 10 implied petrogenic sources (Yunker *et al.*, 1996; Benlaheen *et al.*, 1997). From the calculation of Phen/Anth and Fluo/Pyr in the analyzed kola nuts, there is an indication that, PAHs in the samples resulted from incomplete combustion products via pyrolytic process from farm waste, burning of fire -woods and stoves for daily cooking and automobiles exhausts in cities and towns where the kola nuts were processed and sold. Furthermore, based on the rule proposed by Fernades *et al.*, (1997) which says that, PAHs with high molecular weight are generated mainly by high temperature combustion while PAHs with lower molecular weight may be derived from fossil fuel combustion, it should therefore, be assumed that, the main source of PAHs in the kola nuts are processes relating to fossil fuel combustion.

## CONCLUSION

This study revealed that, total concentration of PAHs in the investigated kola nuts was highest in samples from Osun State and lowest in samples from Oyo State. Greater percentage of the total PAHs was contributed by the low molecular weight 3-4 rings compounds while the main source of PAH in the investigated kola nuts originated from incomplete combustion processes.

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