

Proximate Analysis and Polycyclic Aromatic Hydrocarbon Levels in Some Selected Raw Food Stuffs in Aroje and Owode, Nigeria.

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ABSTRACT

Some studies have shown that accumulation of polycyclic aromatic hydrocarbons (PAHs) in soil has the potential of contaminating the food chain. PAHs are complex organic compounds, many of which have been implicated for many health challenges. Information on the levels of PAHs in raw food in Nigeria is very scanty. Thus, an investigation into the levels of PAHs in some raw foods is of great importance from medical and environmental point of view. This study examined some proximate analysis parameters and levels of PAHs in raw cassava, yam, tomatoes, pineapple and maize and levels of PAHs in soil where these raw foodstuffs were collected. The samples were collected from two agrarian towns: Aroje and Owode in Oyo and Osun states, Nigeria. The proximate analysis was carried out according to the methods of Association of Official Agricultural Chemists (AOAC), while PAHs were determined using Gas chromatography-flame ionization detector (GC-FID). The results of proximate analysis for ash, moisture, protein, fat, fibre and carbohydrate ranged from 0.31-1.22%, 13.10-93.48%, 0.41-9.00%, 0.27-3.10%, 3.38-73.1% and 1.26-4.10% respectively. The concentration of total PAHs in the food samples are: Cassava (0.19571 µg/Kg), Yam (0.15536 µg /kg), Tomatoes (0.02350 µg /kg), Pineapple (0.00753 µg/kg) and maize (0.13718 µg/kg) for Aroje and Cassava (0.20958 µg/kg), Yam (0.16951 µg/kg), Tomatoes (0.02408 µg/kg), Pineapple (0.00752 µg/kg) and Maize (0.13734 µg/kg) for Owode. The average concentrations of total PAH in Aroje and Owode soils are 4.33583 and 4.37730 µg/kg respectively. The source diagnostic indices calculated showed that the PAHs in the samples were from pyrolytic source and there exists a correlation between some PAHs and total PAHs in the soil where the foodstuffs were cultivated.

Key words: Proximate Analysis, PAHs, Foods, Gas Chromatograph,

1 INTRODUCTION

Organic Compounds consisting two or more benzenoid group are known as polycyclic aromatic hydrocarbons (PAHs) (Marce and Borrull, 2000). The PAHs containing two, three and four benzene rings are known as light PAHs (L-PAHs) while those containing more than four benzene rings are known as heavy PAHs (H-PAHs). H-PAHs are more stable and toxic than L-PAHs (ATSDR 1995). PAHs are ubiquitous pollutants in our environment. Hence, their presence in the environment is attracting global attention on daily basis because of their alleged carcinogenic effects and other health challenges (Martinez *et al.*, 2004). Some of these (PAHs) have been demonstrated to be mutagenic and carcinogenic for humans (Menzie *et al.*, 1992), while those PAHs that are considered to be less toxic may even increase the carcinogenicity of other PAHs (Phillips 1999; Martienz *et al.*, 2004). Sixteen of these PAHs that are considered as priority by the American Environmental Protection Agency (EPA) are; naphthalene, acenaphthylene, acenaphthene, fluorine, anthracene, phenanthrene, benzo (a) anthracene, fluoranthene, chrysene, pyrene, benzo (k) fluoranthrene, benzo (b) fluoranthene, benzo (a) pyrene, dibenzo (a,h) anthracene, dibenzo (b,c) fluoranthene and benzo (ghi) perylene (Marce and Borrull, 2000). A very high number of the PAHs have been established to be the products of incomplete combustion of wood, oil, coal and garbage (Toth and Blass 1972; Adetunde *et al.*, 2012). Thus, man can be exposed to PAHs through the inhalation of smoke from combustion of the biomass. However, studies have shown that diet is the main source through which man is exposed to PAHs, with grains and vegetables being the major dietary sources. Goman *et al* (1993), in their study showed that both processed and unprocessed foodstuff contained high levels of PAHs.

Plants can be exposed to PAHs through the soil in which they are grown. Soil system according to Wilds and Jones (1995), is an important repository for atmospheric PAHs. PAHs from atmosphere are deposited in soil system, and they can reside there for more than 20 years (Wild *et al.*, 1990). Accumulation of such PAHs deposit can lead to contamination of food chains (Kipopoulou *et al.*, 1999; Samsøe-Petersen *et al.*, 2002). Invariably, PAHs found in fresh foods can be partly accounted for by the concentration of PAHs in the soil where such foods are grown. This study was aimed at quantifying the PAHs in some selected raw foodstuffs obtained from

two agrarian towns in Oyo and Osun States, Nigeria. The levels of PAHs in soil in the immediate environment where these food stuffs were purchased were also determined.

2 MATERIALS AND METHODS

2.1 Sampling

Samples of Yam, Cassava, Maize, Tomato, and Pineapple were purchased directly from farmers at two locations Aroje, Ogbomoso (Oyo State) and Owode, Ede (Osun State), both in the South Western part of Nigeria. Similarly, the soil samples were collected from nearby farmlands around the two towns. Aroje is situated in Ogbomoso, Oyo State, Nigeria with geographical coordinate of 8° 2' 0" North, 4° 11' 0" East. While, Owode is situated in Ede, Osun State, Nigeria with coordinate of 7° 37' 0" North, 4° 27' 0" East. For the raw yam and cassava, peeling was done for each using a properly cleaned kitchen knife. About 10 g of each sample was cut to small pieces and placed into a 1 liter-cup ken wood kw10 blender and 30 mL of water added. The samples were then pulverized for 5 mins. For tomato and pineapple, the liquid juice was squeezed into beakers and their concentrated juice covered and kept for analysis. The soil samples were collected from the sites where the food items samples are being cultivated, using a hand corer with a surface area of 72.54 cm. Particles smaller than 1mm were removed from sample by sieving.

2.2 Proximate analysis

Moisture content was determined by oven method at 105 °C. Ash content was determined at 550 °C, lipid and fibre were also determined according to the procedures of AOAC (2000). Crude nitrogen was determined based on the Kjeldhal procedure and crude protein value obtained by multiplying the nitrogen value by a factor of 6.25. The carbohydrate was estimated by difference as follows:

Carbohydrate = 100-(% ash + % crude protein + % lipid + % fibre)

Energy (Kcal) = (% carbohydrate × 4) + (% Crude protein × 4) + (% lipid × 9). (Hassan *et al*, 2008)

2.3 Extraction and clean-up of samples

The samples were each pulverized to ensure homogenization. 5 g of the pulverized sample was thoroughly mixed with 10g of anhydrous sodium sulphate in a mortar (Wang *et al.*, 1999) to absorb moisture. The homogenate was placed into an extraction thimble and wrapped with a Whatman filter paper (125 mm diameter). This was then inserted into a Soxhlet extraction chamber of the Soxhlet extraction unit. Extractions were then carried out with 50 mL mixture of redistilled n-hexane and dichloromethane in the ratio 3:1 for effective recovery. Subsequently, the crude extract was filtered through a layer of anhydrous sodium sulphate. The obtained filtrate was evaporated to near dryness. The clean-up was carried out using activated silica gel and anhydrous sodium sulphate. The column was prepared by loading an activated silica gel (12g) onto a chromatographic column (id=1cm). About 1 gm of anhydrous sodium sulphate was added to the top of the silica gel in the column. After conditioning the column with 20ml hexane the sample was applied and eluted with 200ml mixture of dichloromethane: hexane (3:1). The eluate was collected into an evaporating flask evaporated to near dryness. The eluate was then dissolved in 1ml n-hexane for Gas Chromatographic analysis.

2.4 Instrumentation

Gas Chromatography model GC17 (Shimadzu, Japan) with a DB-1 fused silica capillary column (30 m X 0.25 mm, 0.25 µm film thickness) was used. The injector temperature was 275 °C, flame ionization detector temperature was 300 °C and the column temperature programmed as follows: The temperature program was from 40 °C and increased to 140 °C at the rate of 20 °C min⁻¹. Thereafter, ramped to 290 °C at the rate of 10 °C min⁻¹ and held at this temperature for 12 min.

3 RESULTS AND DISCUSSION

3.1 Proximate analysis results

The results of proximate analyses are as shown in Tables 1 and 3. The moisture content for all the samples was high except for maize which was relatively low. The high moisture content will provide an enabling environment for the activities of micro organisms and this can give the samples a storage disadvantage (Laden *et al.*, 1997). The fat contents recorded for all the samples were low except for maize (which was fairly high), so the samples can be recommended as a weight reducing diet since low fat food reduces cholesterol and obesity (Gordon and Kessel, 2002). All samples have low protein content except for maize with comparatively

appreciable protein. In spite of the low protein contents of these samples; they can still serve as a source of protein considering the level of protein deficiency in the society.

The fibre contents of all the samples were relatively low but moderate. It has been established that, foods that contained fibre caused the expansion of the inside walls of the colon thereby easing passage of waste, making them effective anti-constipation agents. Fibre also lowers cholesterol level in the blood and reduces the risk of various cancers. Also, there is emphasis on keeping fibre intake low in the nutrition of infants and weaning children because high fibre levels in weaning diet can cause irritation of the gut mucosa (Bello, *et al.*, 2008), Maize could be considered as potential source of carbohydrate when compared to other samples as it has the highest percentage of carbohydrate followed by cassava, which is in agreement with the findings of Adewusi *et al.* 1995. It is also observed that there is no marked difference in the proximate compositions of samples from both locations (Tables 1 and 3).

3.2 PAHs distributions in the samples

The PAHs contents and their distributions in the foodstuffs analyzed are as shown in Figures 1 and 2. The sixteen priorities PAHs (EU) were found in all the samples analyzed in this study except tomato, where Naphthalene was not detected. The total concentration of PAHs in the samples collected from Aroje are 0.19571, 0.15536, 0.02350, 0.00754 and 0.13718 $\mu\text{g}/\text{kg}$ for cassava, yam, tomatoes, pineapple, and maize respectively and 0.20958, 0.16951, 0.02408, 0.00752 and 0.13734 $\mu\text{g}/\text{kg}$ for cassava, yam, tomato, pineapple and maize samples collected from Owode. The average total concentration of PAHs in each sample is within the acceptable limit. Although the total PAHs concentration mean found in each sample was below the maximum limit, there is need to be cautious, as bioaccumulation may raise the level above the maximum limit, in due course of time. More importantly, the presence of some high molecular PAHs at measurable quantity called for extra vigilant. The average total concentration of PAHs in each sample was relatively less than concentration of PAHs in the soil samples (Table 5).

The average total concentration of all PAHs in Aroje soil is 4.335838 $\mu\text{g}/\text{kg}$ and 4.37730 $\mu\text{g}/\text{kg}$ for Owode soil. Sources of the PAHs in the soil can be traced to emission from vehicles and bush burning (Olajire *et al.*, 2007). PAHs deposited in soil have capacity to reside in soil for more than 20 years without being degraded (Wild *et al.*, 1995). Thus, continuous deposit of PAHs may lead to accumulation, and accumulated PAHs may cause contamination of the food chain (Kipopoulou *et al.*, 1999). The PAH source diagnostic indices were calculated and presented in (Tables 2 and 4). The values of these ratios are frequently used to distinguish between petrogenic and pyrogenic sources of PAHs. The phenanthrene/anthracene ratio of the samples ranged from 0.0524 - 1.293 for sample collected at Aroje and 0.1626 - 1.2734 for sample collected at Owode. The values of this ratio in the food samples studied were less than one, meaning that the source of PAHs in the food sample was pyrolytic in nature. Similarly the Phenanthrene/ Anthracene ratios in the soil samples from Aroje and Owode were greater than 1 but less than 10 which also indicated Pyrolytic source. The value of naphthalene/acenaphthene ratio ranged from 0.0000 - 0.0049 in food samples from Aroje and 0.0000 - 0.0049 in food samples from Owode, which also an indication of pyrolytic source. These ratios were not calculated for pineapple as naphthalene was not detected in this sample.

The value for fluoanthrene/fluoanthrene + pyrene ranges from 0.8042 - 1.0680 Aroje samples and 1.0000 - 1.0694 Owode samples. Benzo(a)anthracene/benzo(a)anthracene + chrysene value ranged from 1.0000 - 1.9940 and 1.0001 - 1.9940 for Aroje and Owode samples respectively. The value of ratio of Indeno(1,2,3-cd)pyrene/Indeno(1,2,3-cd)pyrene + Benzo(g,h,i)perylene ranged from 0.0000 - 1.0020. Thus, all the values of source diagnostic indices calculated for the samples investigated indicated that the PAHs in them were from pyrolytic source such as combustion of grass and wood. In view of the evidence of the source of PAHs in the food samples and soil, the correlation coefficient matrixes were calculated using SPSS software package. This was done to ascertain the level of correlation between the polycyclic aromatic hydrocarbons in the raw food samples and the soil in the two sampling locations. As shown in table 6 there was correlation ($P < 0.05$) between Acenaphthene, Fluorene, Phenanthrene, Fluoranthene, Benzo(a)anthracene, and Benzo(k)fluoranthene and the average total PAHs in the food samples. Also, it can be clearly seen from Table 7, that a correlation exists between all PAHs detected in the food samples and average total PAHs in the soil samples. This implies that PAHs found in the food samples were drawn from the soils where they were grown.

Table 1: Proximate Compositions of the Food Samples from Aroje

Sample	Proximate Parameter (%)						Energy value (Kcal per100g)
	Ash	Moisture	Protein	Fat	Carbohydrate	Fibre	
Cassava	1.0±0.15	59.20±0.25	1.20±0.01	0.30±0.13	36.51±0.22	1.80±0.14	153.54
Yam	1.20±0.06	64.90±0.01	2.00±0.03	0.30±0.06	27.50±0.04	4.10±0.03	120.70
Tomato	0.64±0.10	93.36±0.55	1.09±0.15	0.27±0.56	3.38±0.01	1.26±0.06	20.31
Pineapple	0.31±0.55	85.40±0.06	0.41±0.55	0.44±0.03	12.04±0.06	1.40±0.10	53.76
Maize	0.10±0.10	13.10±0.15	9.00±0.04	3.10±0.10	72.20±0.01	1.50±0.02	352.7

Values are average of 3 readings

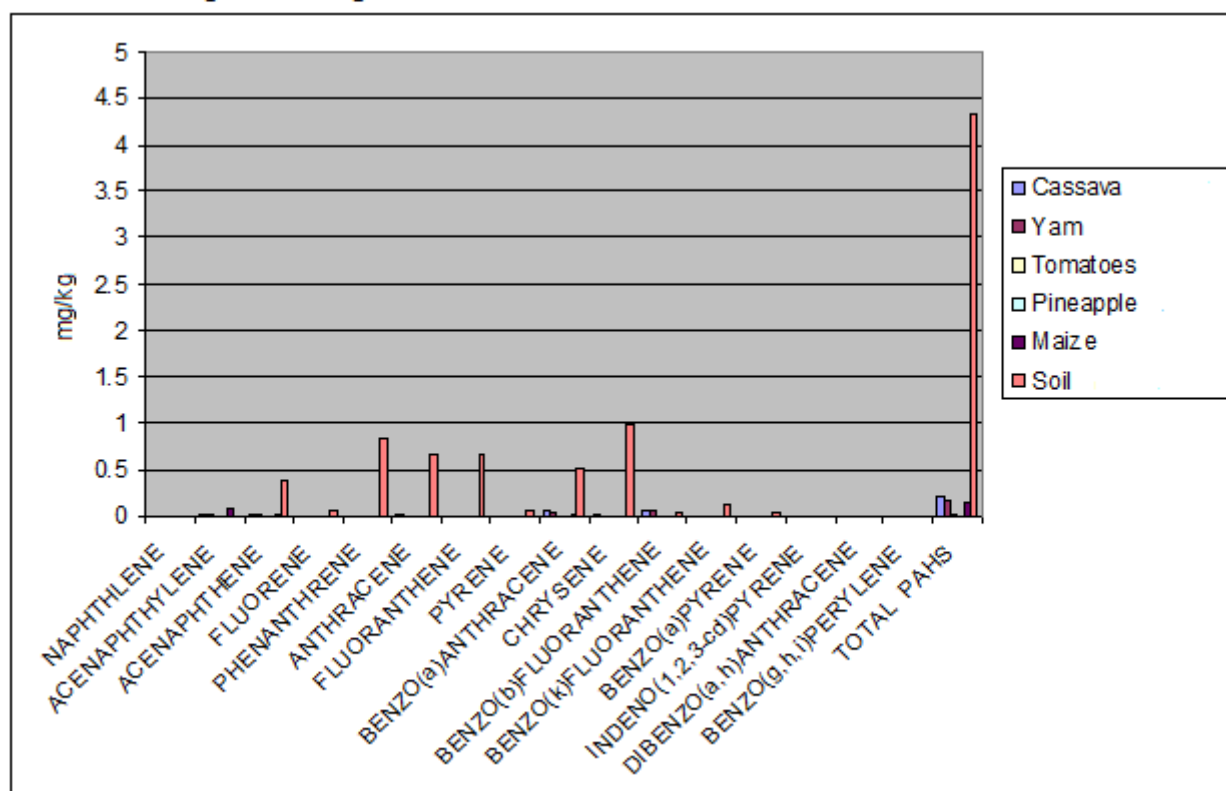


Figure 1: Distribution of PAHs in the Food Samples from Aroje

Table 2: Molecular indices of PAHS in the Food Stuffs from Aroje

Sample	Diagnostic Ratio					
	Phe/An	BaP/Chr	Na/Acy+Pyr	Fl/Fl +Pyr	BaA/BaA+Chr	InP/InP+BghiP
Cassava	0.2785	0.01308	0.0000	0.8042	1.0100	0.0000
Yam	0.2775	0.1321	0.0000	1.0000	1.0010	0.0000
Tomatoes	0.0154	0.02482	0.0000	1.0000	1.0020	0.0000
Pineapple	0.0152	0.0000	-	1.0000	1.0000	1.0000
Maize	0.2875	0.0077	0.0009	1.0000	1.0050	1.0000
Soil	1.2930	0.0299	0.0049	1.0680	1.9940	1.0020

*Acronyms are as defined on table 6

Table 3: Proximate Composition of the Food Stuffs from Owode

Sample	Proximate Parameter (%)						Energy value (Kcal per 100g)
	Ash	Moisture	Protein	Fat	Carbohydrate	Fibre	
Cassava	1.10±0.11	59.50±0.30	1.12±0.01	0.32±0.15	36.53±0.22	1.55±0.12	153.48
Yam	1.22±0.05	64.85±0.02	2.02±0.02	0.30±0.01	27.65±0.05	4.00±0.25	121.38
Tomato	0.61±0.11	93.48±0.52	1.04±0.13	0.28±0.55	3.46±0.01	1.20±0.05	20.52
Pineapple	0.38±0.50	84.75±0.05	0.48±0.45	0.46±0.03	12.50±0.05	1.45±0.11	56.06
Maize	1.11±0.01	13.30±0.02	8.05±0.05	3.06±0.15	73.10±0.02	1.50±0.25	352.14

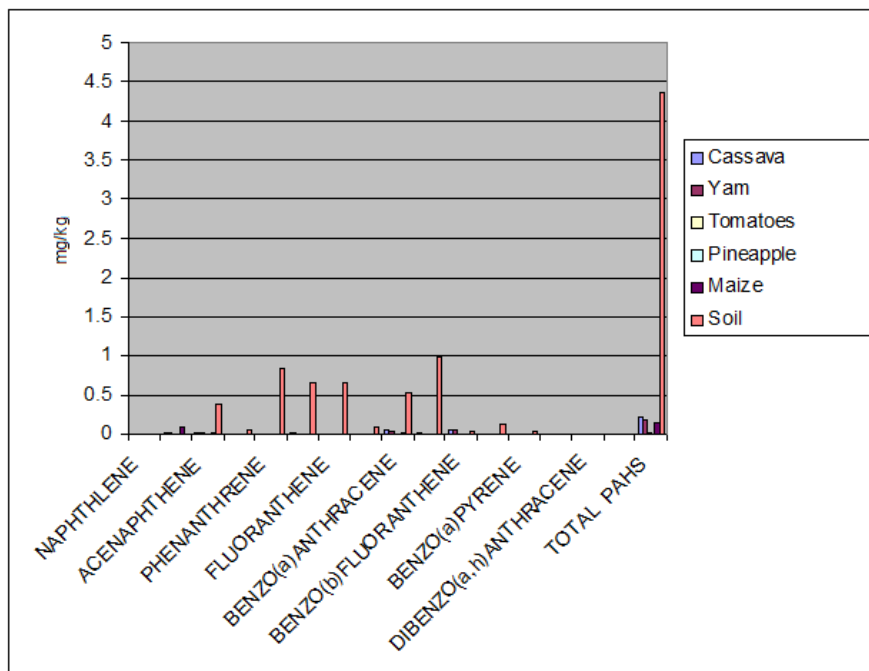


Figure 2: Distribution of PAHs in the Food Stuffs from Owode

Table 4: Molecular indices of PAHS in the Food Stuffs from Owode

Sample	Diagnostic Ratio					
	Phe/An	BaP/Chr	Na/Acy+Pyr	Fl/Fl +Pyr	BaA/BaA+Chr	InP/InP+BghiP
Cassava	0.1626	0.0137	0.0000	1.0001	1.0102	0.0000
Yam	0.3154	0.0132	0.0000	1.0001	1.0099	0.0000
Tomato	0.0156	0.0255	0.0000	1.0001	1.0016	0.0000
Pineapple	0.0149	0.0000	-	1.0000	1.0001	0.0000
Maize	0.2888	0.0079	0.0014	1.0001	1.0050	1.0000
Soil	1.2734	0.0301	0.0049	1.0694	1.9940	1.0020

*Acronyms are as defined on table 6

Table 5: Distribution of PAHs in the Soil Samples from Aroje and Owode

Name of PAH	Concentration of PAHs ($\mu\text{g/Kg}$)	
	Aroje soil	Owode soil
Naphthalene	0.00180	0.00184
Acenaphthylene	0.00429	0.00439
Acenaphthene	0.36621	0.36622
Fluorene	0.05523	0.05525
Phenathrene	0.83946	0.83951
Anthracene	0.64929	0.65929
Fluoranthene	0.64502	0.65503
Pyrene	0.06831	0.06941
Benzo(a)Anthracene	0.52318	0.53319
Chrysene	0.99431	0.99432
Benzo (b) Fluoranthene	0.03611	0.03612
Benzo (k) Fluoranthene	0.11717	0.11718
Benzo(a)Pyrene	0.02977	0.02988
Indeno(1,2,3-cd) Pyrene	0.00106	0.00107
Dibenzo (a,h) Anthracene	0.00277	0.00278
Benzo (g,h,i) Perylene	0.00180	0.00182
Total PAHs	4.33583	4.37730

Table 6: correlation coefficient matrix for individual PAHs and the total PAHs in soil(n = 5) of Aroje

	Na	Acy	Ace	Fl	Ph	An	Flu	Pyr	BaA	Chr	Bbf	Bkf	Bap	Inp	Dah A	Bgh ip	total
Na	1																
Acy	.963	1															
Ace	.101	.494	1														
Fl	-.031	.423	.980	1													
Ph	.000	.424	.996	.987	1												
An	-.644	.136	.760	.874	.795	1											
Flu	-.209	.287	.967	.986	.985	.866	1										
Pyr	.074	.168	.022	.111	.041	.259	.085	1									
BaA	-.214	.287	.963	.984	.982	.867	.999	.124	1								
Chr	.092	.387	.721	.737	.707	.666	.681	-.427	.653	1							
Bbf	-.580	-.131	.796	.823	.841	.770	.904	-.057	.902	.523	1						
Bkf	-.442	.142	.900*	.954	.932	.927	.981	.151	.982	.637	.929	1					
BaP	-.571	.008	.847	.907	.889	.909	.955	.116	.956	.591	.962	.991	1				
Inp	1.000	.958	.226	.157	.150	-.081	.008	.206	.010	.194	-.408	-.128	-.261	1			
DahA	-.084	.236	.747	.751	.744	.661	.732	-.512	.704	.973	.658	.694	.673	.014	1		
Bghip	1.000	.958	.226	.157	.150	-.081	.008	.206	.010	.194	-.408	-.128	-.261	1.000	.014	1	
TOT A	.084	.489	.997	.992	.996	.804	.975	.069	.972	.727	.796	.919	.865	.223	.744	.223	1

Correlation is significant at the 0.05 level, n=5, df= 4, df= degree of freedom

Na = Naphthalene, Acy= Acenaphthylene, Ace= Acenaphthene, Fl = Flourene, Ph= Phenanthrene, An= Anthracene, Flu=Fluoranthene, Pyr= Pyrene, BaA= Benzo(a)anthracene, Chr= Chrysene, BbF= Benzo(b)Fluoranthene, BkF= Benzo (k) Fluoranthene, BaP= Benzo (a) Pyrene, InP= Indeno (1,2,3-cd) Pyrene, DahA= Dibenzo(a, h) anthracene, Bghip= Benzo(g,h,i)Perylene

Table 7: correlation coefficient matrix for individual PAHs and the total PAHs in soil (n = 5) of Owoode

	Na	Acy	Ace	Fl	Ph	An	Flu	Pyr	BaA	Chr	Bbf	Bkf	Bap	Inp	DahA	Bghip	total
Na	1																
Acy	-.374	1															
Ace	.998	-.273	1														
Fl	.995	-.248	.998	1													
Ph	1.000	-.298	.998	.992	1												
An	1.000	-.298	.998	.993	1.000	1											
Flu	1.000	-.299	.998	.992	1.000	1.000	1										
Pyr	1.000	-.299	.998	.992	1.000	1.000	1.000	1									
BaA	.996	-.269	.999	1.000	.995	.995	.994	.994	1								
Chr	1.000	-.296	.998	.993	1.000	1.000	1.000	1.000	.995	1							
Bbf	.024	-.150	.165	.214	.114	.121	.113	.113	.207	.116	1						
Bkf	1.000	-.299	.998	.992	1.000	1.000	1.000	1.000	.994	1.000	.114	1					
Bap	1.000	-.299	.998	.992	1.000	1.000	1.000	1.000	.995	1.000	.116	1.000	1				
Inp	1.000	-.299	.998	.992	1.000	1.000	1.000	1.000	.994	1.000	.112	1.000	1.000	1			
DahA	1.000	-.297	.998	.992	1.000	1.000	1.000	1.000	.995	1.000	.117	1.000	1.000	1.000	1		
Bghip	1.000	-.299	.998	.992	1.000	1.000	1.000	1.000	.994	1.000	.112	1.000	1.000	1.000	1.000	1	
TOTAL	.999	-.279	1.000	.997	.999	.999	.999	.999	.998	.999	.149	.999	.999	.999	.999	.999	1

Correlation is significant at the 0.05 level, n=5, df= 4, df= degree of freedom

Na = Naphthalene, Acy= Acenaphthylene, Ace= Acenaphthene, Fl = Flourene, Ph= Phenanthrene, Anth= Anthracene, Flu=Fluoranthene, Pyr= Pyrene, BaA= Benzo(a)anthracene, Chr= Chrysene, BbF= Benzo(b)Fluoranthene, BkF= Benzo (k) Fluoranthene, BaP= Benzo (a) Pyrene, InP= Indeno (1,2,3-cd) Pyrene, DahA= Dibenzo(a, h) anthracene, Bghip= Benzo(g,h,i)Perylene

CONCLUSION

The results obtained from this study showed that all the samples contained high moisture content which could pose storage disadvantage on the samples. Three of the samples i.e. maize, cassava and yam can serve as a good source of carbohydrate. It is also observed that the total PAHs concentration in each of the samples analysed is moderate and cannot pose any problem due to consumption. The source diagnostic indices calculated showed that the PAHs in the samples were from pyrolytic source; this implies that there was uptake of PAHs from soil on which they were grown. This is further supported by the result of correlation analysis, which shows that there exists a correlation between some PAHs and total PAHs in soil in the vicinity of places where the foodstuffs were cultivated. The baseline data for the PAHs concentrations in these food samples can be used as a standard for the comparison of effect of processing methods on the level of polycyclic aromatic hydrocarbons in these foods.

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