

Full Length Research Paper

Microbiology, heterocyclic amines and polycyclic aromatic hydrocarbons profiles of some grilled, roasted and smoked foods in Lagos and Ogun States, Nigeria

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Dietary intake of polycyclic aromatic hydrocarbons (PAH) and heterocyclic amines (HCA) has posted a great health risk as they have been identified as a most potent human carcinogen. Microbial quality of food is also of concern as they contribute to food poisoning and infection. Sixty food samples comprising roasted yam, plantain, grilled and smoked fish and meat were randomly sampled from Lagos and Ogun State, Nigeria, and the PAHs, HCAs contents and microbial load were determined. Isolates were subjected to antibiogram assay. The pH of the samples ranged between 5.08 and 7.49, titrable acidity was in the range of 0.50 and 1.20. *Staphylococcus*, *Bacillus*, *Micrococcus*, *Proteus*, *Pseudomonas*, *Citrobacter* and *Klebsiella* sp. had been identified. Antibiogram revealed that the isolates were multi-resistant and most resistant to ceftazidime, cloxacillin and tetracycline and more susceptible to ofloxacin. PAHs were detected in some grilled, roasted and smoked samples and with the highest concentrations 314.85 and 139.97 µg/g Dibenzene[a,h]anthracene established in roasted yam and smoked fish samples. Only 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) was detected in grilled fish and meat samples. This study therefore established the presence of chemical and microbial contaminants in some of the food items investigated. It recommended that strict sanitary practices and appropriate cooking methods be enforced during food preparation.

Key words: Antibiogram, carcinogens, hazard analysis and critical control point (HACCP), heterocyclic amines, polycyclic aromatic hydrocarbons, Nigeria.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemically inert, hydrophobic compounds consisting of three or more condensed aromatic rings soluble in organic solvents which are ubiquitous in the environment as a result of incomplete combustion of

organic materials during industrial processing and various human activities (Ishizaki et al., 2010; Wretling et al., 2010). Although widely present in the environment (soil), they can be introduced into the food chain by plants and animals. Various food preparation conditions like drying,

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Table 1. Samples analyzed and their respective codes.

S/N	Samples	Codes
1	Roasted yam	A-E
2	Roasted plantain	F-J
3	Grilled fish	K-N
4	Smoked fish	O
5	Grilled meat	P-S
6	Smoked meat	T

smoking, frying contribute greatly to their introduction into foods (Zelinkova and Wenzl, 2015). Smoked fish has been identified as a major food source of PAHs (Tongo et al., 2016). Polycyclic hydrocarbons and heterocyclic amines have been identified as a most potent human carcinogen (Jamin et al., 2013; Purcaro et al., 2013). Due to their mutagenic and carcinogenic effects, PAHs have been listed on the priority pollutant list of several agencies including the Agency for Toxic Substances and Diseases Register (ASTDR), the International Agency for Research on Cancer (IARC) and the United States Environmental Protection Agency (USEPA) (Yusuf et al., 2015).

Based on their structures, HCAs can be divided into two classes (Turesky and Le, 2011). The first class of HCAs; 2-Amino-3-methylimidazo[4,5-f]quinoline (IQ-) are produced at temperatures below 300°C during heat processing of creatinine/creatine free amino acids mixtures and sugars. The second class, non-IQ-type HCAs is formed by pyrolysis of amino acids and proteins, at temperatures above 300°C (Woziwodzka et al., 2013). Meat and fish products that have been cooked at a temperature of 150°C and more have been identified as potential carriers of mutagenic and/or carcinogenic HCAs (Kizil et al., 2011). The increased consumption of meat has also increased the risk for colorectal cancer (Wada et al., 2017). Colorectal cancer is the third most frequently diagnosed cancer in both men and women with an estimated 51,370 deaths occurring annually (Ollberding et al., 2012). Gibis (2016) reported that trial animals being fed with 0.03 or 0.04% HCAs in their food over a period of 2 years developed tumors after several months with the tumors appearing mainly in the animals' livers or intestines.

The microbial composition of smoked foods is also of concern. Microbial contamination of food is even more complicated than chemical contaminations (Sheen, 2012). Various studies have established the presence of coliforms, *Shigella* sp., *Salmonella* sp., *Staphylococcus* sp. and *Bacillus cereus* in smoked fish (Akinwumi and Adegbehingbe, 2015) and meat (Edema et al., 2008). These organisms are known pathogens; for instance, *Shigella* has been identified as one of the most important agents of diarrhea by World Health Organization (WHO, 2005). Shigellosis has been reported as an

important health problem around the world, occurring predominantly in young children, mainly in developing countries like Nigeria (Partha, 2014). *Salmonella* sp. has also been identified as one of the leading causes of foodborne illnesses around the globe (Faleke et al., 2017). Between the years 2013-2016, of the 24,029 cases of gastrointestinal infections identified by Foodnet, *Salmonella* and *Shigella* had the highest cases besides *Campylobacter* spp. (Marder et al., 2017).

The pH is essential as regulatory agencies may impose certain pH limits on food products; also the pH value of foods contributes to the microbial quality as the pH values of food limits the range of microorganisms which the particular food item may support (Oladipo et al., 2010). This study therefore was carried out to determine safety of commonly consumed pre-cooked food items in Lagos and Ogun States, Nigeria by determining the microbial load and chemical profiles (heterocyclic amines and polycyclic aromatic hydrocarbons contents) which are critical indicators of food safety.

MATERIALS AND METHODS

Sample collection

A total of sixty samples were employed for this study. They included ten samples each of roasted yam, roasted plantain, grilled fish, smoked fish, grilled meat and smoked meat (Table 1). Samples were purchased from food vendors in Lagos (6°27'14.65" N 3°23'40.81" E) and Ogun (7°00'0.00" N 3°34'59.99" E) states, Nigeria. The samples were appropriately labeled and transported in sterile plastic bags and cold chain 4°C to the microbiology laboratory in the Department of Biological Sciences, Covenant University, Ota, Nigeria for analyses.

pH and titrable acidity

A 10 ml aliquot of the sample homogenate in distilled water was measured and titrated against 0.1 mol l⁻¹ NaOH to an endpoint which was determined by pH at 7.0 (Chen and Liu, 2000). The sample homogenates were prepared by weighing twenty-five grams of each sample and homogenized in sterile stomacher using sterile distilled water at a ratio of 1:10. The titratable acidity was expressed as the volume consumed in millilitres of 0.1 mol l⁻¹ NaOH per 100 ml sample. The pH was also determined using a calibrated standard pH meter model PHS-2S, (SHANGHAI JINYKE

REX, CHINA).

Microbial assessment of samples

Coliform test and count

Determination of total coliforms was carried out according to the method of Sangadkit et al. (2012).

Twenty-five grams of each food was homogenized with 225 ml of sterile peptone water (0.1% w/v) for 2 min and then serial 10 fold dilutions were prepared with sterile peptone water. Sample suspension (1 ml) at appropriate dilution was pipetted onto the surface of a Petrifilm™. *Escherichia coli* /Coliform count (EC) plate, the cover film on the plate was slowly applied and incubated at 35°C for 24 h. Plates were examined and read based on the manufacturer's instruction, red colonies surrounded by trapped gas were recorded as coliforms and blue colonies with trapped gas were *E. coli*. Duplicate trials were performed per dilution, while *E. coli* and coliforms were re-confirmed using EMB agar and IMVIC tests. A positive *E. coli* control was included in the experiment for validation (Sangadkit et al., 2012).

Total aerobic plate count (TAPC)

Twenty-five grams of each sample was weighed into sterile plastic McCartney bottles. Samples were homogenized in sterile Stomacher lab blender and 225 ml peptone water for 1:10 dilution. Further, tenfold dilutions of the sample homogenate were made (Sangadkit et al., 2012). Plate count agar was used to determine the total aerobic bacteria by pour plate method. Colonies were counted after 48 h incubation at 37°C

Fungal count

This was carried out following the procedures of the International Commission for the Microbiological Specification for Food (ICMSF, 2002). Tenfold serial dilution for each sample was prepared in 0.1% peptone water and 1% glucose broth and subsequently plated onto standard Saboraud dextrose agar (SDA) for viable fungal counts. The SDA plates were incubated at 27± 2°C for 72 h. The colony forming units (cfu) were counted on plates having between 30 and 300 colonies. The enumerations of the viable fungal count were carried out in duplicate on each sample and the isolated fungi were stored at 4°C for further characterization.

Characterization of microbial isolates

The preliminary identification of the bacterial isolates was based on cultural, morphological, and biochemical test; Gram staining, motility, sugar fermentation, hydrogen sulfide production, urease, catalase and coagulase production, oxidase and bacitracin test, methyl red vogues Proskauer (MRVP), utilization of citrate, glycerol and indole production, starch and aesculin hydrolysis, arginine dihydrolase and gelatin liquefaction. Further identification of bacterial isolates was based on standard bacteriological procedures and employing the Biomerieux® sa API system. Identification of fungal isolates was based on cultural characteristics, morphological and microscopic features with reference to a standard atlas and mycological identification keys (Tsuneo, 2010).

Antimicrobial sensitivity test

The antibiotic sensitivity testing was carried out on Mueller-Hinton

agar, using the disc diffusion method as described by Bauer et al. (1966). Results were interpreted in accordance with the 2002 guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2002). The antibiotics used were Ceftazidime (30 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Erythromycin (5 µg), Cloxacillin (25 µg) Amoxycillin (25 µg), Cotrimoxazole (25 µg), Nitrofurantoin (50 µg), Gentamycin (10 µg), Nalidixic acid (15 µg), Ofloxacin (5 µg), Augmentin (25 µg), Tetracycline (10 µg).

Determination of polycyclic hydrocarbons (PAHs)

This was carried out following the method as described by Adetunde et al. (2012) whereby PAHs in all samples were extracted using solvent extraction by ultra-sonication and the extracts were analyzed for the 16 USEPA PAHs using high-performance liquid chromatography (HPLC) Millenium 2010 HPLC system (Millipore Corp., Milford, MA). The supernatant was decanted following centrifugation of extracts at 2500 rpm for 10 min. The supernatant was cleaned-up using the Whatman nylon filter membrane with further clean-up using the solid phase extraction (SPE) cartridges. The filtered extracts were loaded onto the cartridges after the sorbent of the SPE cartridges was first conditioned with N-hexane. The analytes were eluted with dichloromethane and then blown down to dryness and the extract reconstituted in acetonitrile. The PAHs was quantified using an Agilent 1100 model HPLC system with a vacuum degasser, a temperature controlled column oven, a quaternary pump, and a UV diode array detector. A monomeric type octadecyl silica column, Supelcosil LC PAH 2 cm × 4.6 mm i.d enclosing 5 µm particles at 25 ±1°C ambient temperature on a flow rate 1.0 ml/min was used for the separation of the PAHs. The standard used for quantification was phenanthrene.

Determination of heterocyclic amines (HCAs)

This was carried out using the method of Felton et al. (1994). Briefly, 20 g each of the pulverized samples were homogenized with 60 g 1 M NaOH. Four-gram aliquots of the homogenized mixture were used, for which two were spiked with a mixture of heterocyclic amines in 50 µl methanol. A packet of the Extrelut diatomaceous earth was used to mix each sample thoroughly and poured into an Extrelut 20 column. Following the methods as described by Gross and Grüter (1992), the extractions were made by collecting 40 ml dichloromethane through attached PRS columns and with subsequent washing and extraction onto C18 columns, separating polar and apolar extracts. The eluted polar and apolar mixtures were evaporated to dryness at 50°C, re-dissolved in 50 µl of methanol containing 5 µg/ml caffeine as an internal standard. The optimized HPLC separation by Gross and Grüter (1992) was employed, on a TSK gel ODS80TM column (TosoHaas, Montgomeryville, PA, 250 mm × 4.6 mm I.D.) with a mobile phase of triethylamine phosphate, 0.01M, pH 3.6 (solvent A) and acetonitrile (solvent B). The ternary buffer (pH 3.2), was not employed because it is not necessary for the separation of the heterocyclic amines used in this study. A linear gradient (5-15% B from 0-10 min; 15-25% B from 10-20 min; 25-55% B from 20-30 min) was used. The Millenium 2010 HPLC system (Millipore Corp., Milford, MA) with a 996 photodiode array detector and a Hewlett-Packard 1046A Programmable Fluorescence Detector was used to analyze the samples. The UV absorbance spectra were compared to library spectra acquired from standard solutions to identify chromatographic peaks.

Statistical analysis

Results are presented as mean and standard deviations,

Table 2. Mean pH, titratable acidity and major tentative isolates from samples marketed in Lagos and Ogun States, Nigeria.

Sample	Mean pH \pm SD	pH range	Mean TA \pm SD	TA range	Major tentative isolates
Roasted Yam	6.66 \pm 0.74	5.74 – 7.23	0.90 \pm 0.28	0.65-1.20	<i>S. aureus</i> , <i>Bacillus</i> sp. <i>Micrococci</i> , <i>Pseudomonas</i> sp.
Roasted plantain	5.28 \pm 0.24	5.08 – 5.56	0.64 \pm 0.20	0.50 -0.90	<i>Staphylococcus</i> sp <i>Pseudomonas</i> , <i>Bacillus</i> sp.
Fish samples	6.83 \pm 0.55	6.39 – 7.49	0.73 \pm 0.15	0.55-0.85	<i>Bacillus</i> sp., <i>Pseudomonas</i> sp.; <i>Proteus</i> sp., <i>Staphylococci</i> <i>Citrobacter</i> sp.
Meat samples	7.09 \pm 0.32	6.79 – 7.44	0.65 \pm 0.15	0.50 -0.80	<i>S. aureus</i> ; <i>Bacillus</i> sp., <i>Klebsiella</i> sp. <i>Pseudomonas</i> sp.; <i>Proteus</i> sp.

Key: TA= Titrable acidity, SD- Standard deviation, n= 10.

Table 3. Mean microbial count cfu/g sample marketed in Lagos and Ogun States, Nigeria.

Sample	Total aerobic plate count		Fungal count		Coliform count	
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
Grilled fish	8.39 $\times 10^6 \pm 6.57 \times 10^2$	2.7 $\times 10^3$ - 2.1 $\times 10^6$	1.17 $\times 10^3 \pm 8.26 \times 10^1$	0 - 7.1 $\times 10^4$	-	NG
Smoked fish	9.15 $\times 10^7 \pm 2.43 \times 10^3$	4.9 $\times 10^4$ - 4.0 $\times 10^8$	8.36 $\times 10^5 \pm 1.04 \times 10^1$	0 – 2.3 $\times 10^6$	5.42 $\times 10^6 \pm 4.00 \times 10^2$	0 - 4.8 $\times 10^7$
Grilled meat	5.06 $\times 10^6 \pm 5.66 \times 10^3$	1.8 $\times 10^5$ - 1.3 $\times 10^7$	1.86 $\times 10^4 \pm 1.84 \times 10^1$	0 - 3.7 $\times 10^4$	5.40 $\times 10^6 \pm 3.06 \times 10^2$	0 - 6.0 $\times 10^7$
Smoked meat	2.00 $\times 10^7 \pm 1.41 \times 10^3$	2.2 $\times 10^4$ - 2.0 $\times 10^7$	3.82 $\times 10^5 \pm 2.00 \times 10^1$	0 - 4.7 $\times 10^5$	NG	NG
Roasted plantain	1.62 $\times 10^6 \pm 1.73 \times 10^3$	1.7 $\times 10^4$ - 4.2 $\times 10^6$	2.18 $\times 10^4 \pm 9.38 \times 10^1$	0 - 3.5 $\times 10^4$	NG	NG
Roasted Yam	6.05 $\times 10^3 \pm 7.47 \times 10^1$	2.3 $\times 10^2$ - 7.7 $\times 10^3$	NG	NG	NG	NG

KEY: NG= No growth less than 10.

antibiograms were presented as percentages of susceptibility. One way analysis of variance was employed to compare mean microbial and pH compositions at 0.05% level of significance using SPSS statistics version 20 (SPSS Inc., USA) for the microbial, pH, titratable acidity and PAH compositions.

RESULTS

pH, titratable acidity and identified isolates

The pH was in the range of 5.08- 7.74. The lowest and most acidic pH was obtained from plantain samples while the highest pH was obtained from fish samples. Titratable acidity was between 0.50 and 1.20. Biochemical profiles revealed that the most prominent bacterial isolates were members of the genera *Staphylococcus* and *Bacillus* (Table 2).

Total aerobic plate count, coliform count and fungal count

The total aerobic plate count was observed to be highest in smoked fish and meat samples. In most of the samples, no coliform was detected (Table 3). However, some fish and meat samples had mean coliform counts of $5.42 \times 10^6 \pm 4.00 \times 10^2$ and $5.40 \times 10^6 \pm 3.06 \times 10^2$, respectively. There were no fungal growths in all the yam samples, however, smoked fish and meat samples had

the highest fungal load of $8.36 \times 10^5 \pm 1.04 \times 10^1$ and $3.82 \times 10^5 \pm 2.00 \times 10^1$ (Table 3).

Antibiogram for predominant bacteria isolates to commercial antibiotics

The results of antibiotics sensitivity test obtained from the isolates (most predominant organisms isolated from each sample) revealed that the isolates were multi-resistant to the antibiotics, with *Pseudomonas*, *S. aureus* and *Micrococcus* sp. having the highest rates of multi-resistance. The isolates were most resistant to Ceftazidime and Cloxacillin (90.91% resistance) and to Tetracycline (88.24%). The isolates are, however, 88.24 and 76.47% susceptible to Ofloxacin and Gentamycin respectively (Table 4).

PAHs and HCAs in tested foods in Lagos and Ogun States, Nigeria

Table 4 (Figure 1a and b) showed that of the 16 USEPA PAHs samples, twelve of the PAHs were detected either singly or in combinations in the samples. Acenaphthalene was detected in all the samples, and except for smoked meat samples, Indeno (1,2,3-C, D) perylene was detected in all other samples. Acenaphthene and Benz (ghi) perylene were found present in four of the six sample

Table 4. Antibiogram of predominant isolates from samples to commonly used antibiotics.

Sample	Organism Isolated	CRX	CAZ	CTR	ERY	CXC	AMX	COT	NIT	GEN	NAL	OFL	AUG	TET	%S	%R
Roasted Yam	<i>S. aureus</i>	R	R	R	S	R	S	R	-	S	-	S	R	R	36.36	63.64
	<i>Micrococcus</i>	R	R	S	R	R	R	R	-	R	-	S	S	R	27.27	72.73
	<i>Bacillus</i> sp.	S	S	S	R	S	S	R	-	S	-	S	S	S	81.82	18.18
	<i>Pseudomonas</i> sp.	-	-	-	-	-	R	R	R	R	S	R	S	R	25.00	75.00
Roasted plantain	<i>Pseudomonas</i> sp.	-	-	-	-	-	R	R	R	S	R	R	R	R	12.50	87.50
	<i>Bacillus</i> sp.	R	R	S	S	R	S	R	-	S	-	S	S	R	54.55	45.45
	<i>Staph.</i> sp.	S	R	S	R	R	R	S	-	S	-	S	S	R	54.55	45.45
Fish samples	<i>Proteus</i> sp.	-	-	-	-	-	S	R	S	S	S	S	S	R	75.00	25.00
	<i>S. aureus</i>	R	R	R	R	R	R	S	-	R	-	S	R	R	18.18	81.82
	<i>Pseudomonas</i>	-	-	-	-	-	R	R	R	S	S	S	R	R	37.50	62.50
	<i>Bacillus</i> sp.	R	R	R	R	R	R	S	-	S	-	S	R	R	27.27	72.73
	<i>Micrococcus</i>	R	R	S	R	R	S	R	-	S	-	R	R	R	27.27	72.73
Meat samples	<i>S. aureus</i>	R	R	R	R	R	S	S	-	S	-	S	S	R	45.45	54.55
	<i>Staph.</i> sp.	R	R	R	R	R	S	R	-	R	-	S	R	R	18.18	81.82
	<i>Bacillus</i> sp.	R	R	R	R	R	R	R	-	S	-	S	R	R	18.18	81.82
	<i>Klebsiella</i> sp.	-	-	-	-	-	R	R	S	S	S	S	R	S	62.50	37.50
	<i>Pseudomonas</i> sp.	-	-	-	-	-	R	R	R	R	R	S	R	R	12.50	87.50
% S		18.18	09.09	45.45	18.18	09.09	41.18	23.53	33.33	76.47	50.00	88.24	35.29	11.76	36.33	
%R		81.82	90.91	54.55	81.82	90.91	58.82	76.47	66.67	23.53	50.00	11.76	64.71	88.24		63.67

KEYS: CRX= Cefuroxime; CAZ= Ceftazidime; CTR= Ceftriaxone; ERY= Erythromycin; CXC= Cloxacillin; AMX= Amoxicillin; COT= Cotrimetrazole; NIT=Nitrofurantoin; GEN= Gentamycin; NAL= Nalidixic acid; OFL= Ofloxacin; AUG= Augmentin; TET= Tetracycline; R= Resistance with no zone of inhibition; - = Not tested for i.e. not applicable because it's a Gram positive or Gram negative disk. *Staph.*= *Staphylococcus* (S).

types. The following HCAs content of the samples were analyzed; 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,4-dimethylimidazo[4,5-f]quinolone (MeIQ) and 2-(Diphenyl methoxy)-N,N-dimethylethanolamine Hydrochloride.

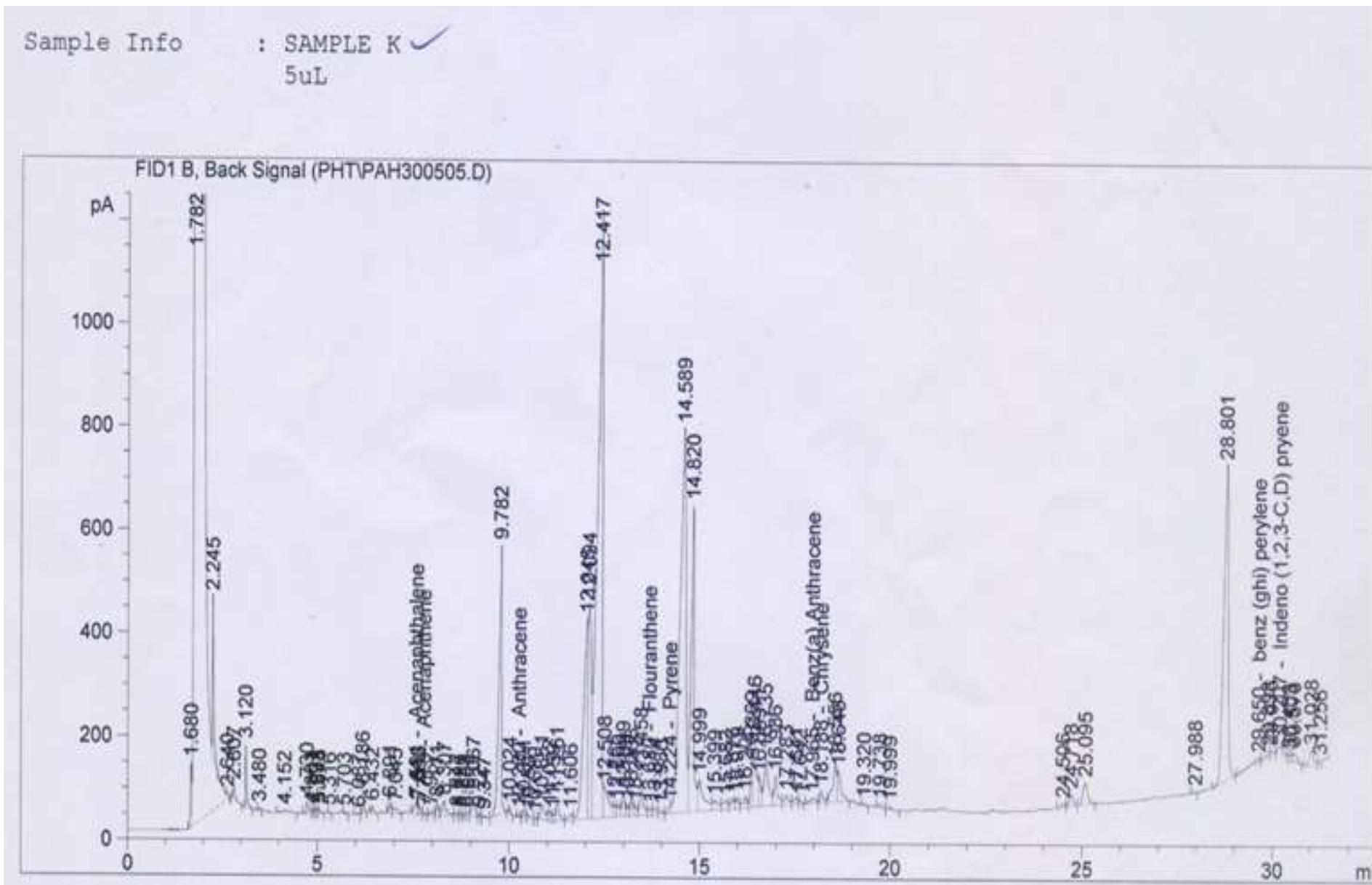
However, only 2-amino-1-methyl-6-

phenylimidazo[4,5-b]pyridine (PhIP) was detected in fish and meat samples(Figure 2).

DISCUSSION

PAHs were detected in all the food samples (Table 5; Figure 1a and b). Of public health concern is the fact that with the exception of Benz (ghi) perylene, all the PAH detected in this study

are listed in the PAH priority list identified by the US EPA, the US Agency for toxic substances and disease registry (ATSDR), and the European food safety authority (EFSA), due to their carcinogenicity or genotoxicity and/or ability to be monitored (EFSA, 2008; ATSDR, 2011; Keith, 2015). PAHs have been implicated in cardiovascular diseases and in developmental impacts including poor fetal growth and neurological development, reduced immune functions, and low



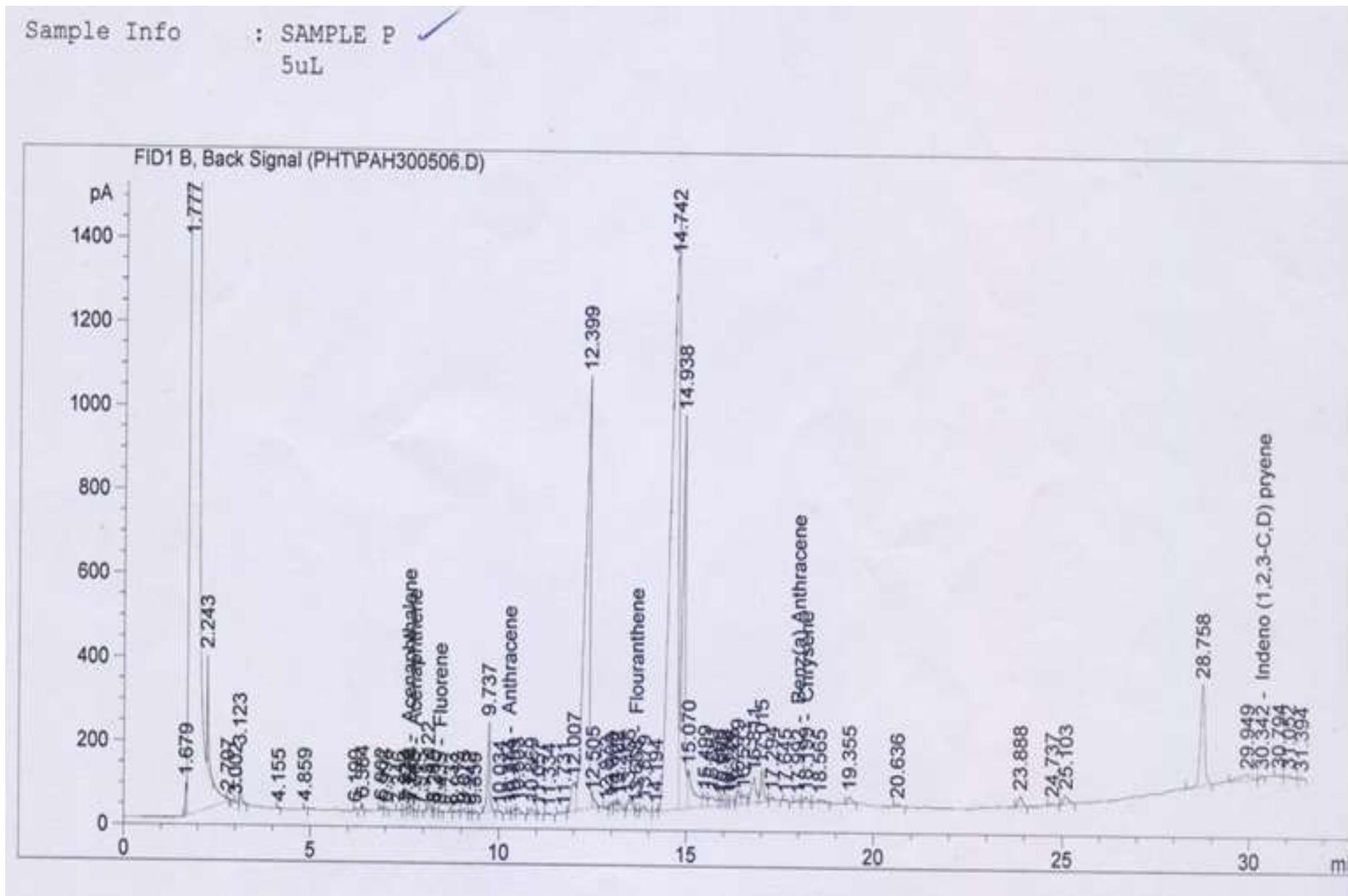


Figure 1b. PAH detected in grilled meat marketed in Lagos and Ogun States, Nigeria.

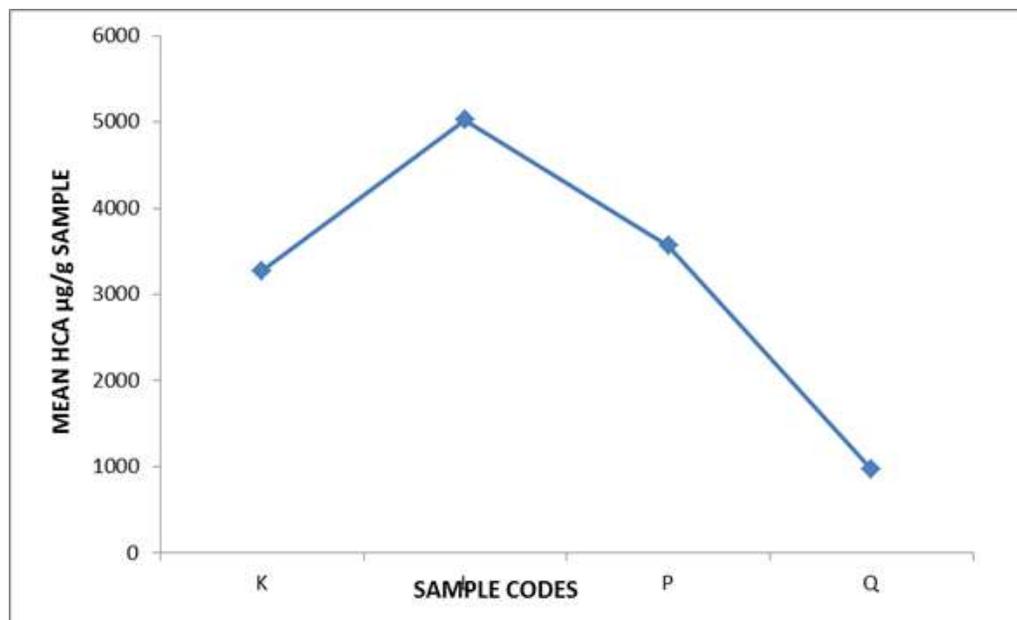


Figure 2. Heterocyclic amines (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in fish and meat samples marketed in Lagos and Ogun States, Nigeria.

Table 5. Mean PAH (µg/g) ±SD detected in some selected food samples marketed in Lagos and Ogun States, Nigeria.

PAH	Grilled Meat	Smoked Meat	Grilled Fish	Smoked Fish	Roasted Yam	Roasted plantain
Acenaphthalene	3.2195±0.22	5.5803±0.61	12.3354±2.80	5.1154±1.11	2.1124±0.08	2.1210±0.01
Acenaphthene	8.8282±1.41	1.0749±0.01	1.3648±0.05	5.5930±0.25	ND	ND
Fluorene	3.0856±0.43	2.5764±0.51	ND	ND	ND	ND
Anthracene	2.5703±1.32	ND	2.3737±1.30	1.4614±1.03	ND	ND
Flouranthene	1.3009±0.55	129.9589±5.33	6.3791±2.01	ND	ND	ND
Pyrene	ND	3.6232±0.02	5.2949±0.66	ND	ND	ND
Benz (a) Anthracene	4.1581±2.00	ND	9.2124±3.12	ND	ND	ND
Chrysene	8.3204±2.11	42.3288±3.22	13.5833±1.34	5.5787±0.81	ND	ND
Dibenz (a,h) Anthracene	ND	ND	ND	139.9682±6.05	314.8474±8.02	35.9105±3.25
Benz (ghi) perylene	ND	ND	20.8664±4.23	68.1131±3.33	45.4627±2.55	23.2140±1.12
Indeno(1,2,3-C,D) pryene	2.8484±0.45	ND	10.6661±1.22	78.7444±5.47	23.9248±3.62	10.2423±1.20
Naphthalene	ND	7.6208±2.24	ND	ND	ND	ND

Key: ND = Not detected, Detection limit= 0.5µg/g, No. of replicates= 2.

intelligence quotient "IQ" (Wormley et al., 2004; Sram et al., 2005; Korashy and El-Kadi, 2006; Winans et al., 2011; Suades-González et al., 2015). PAHs are known to be ubiquitous in the environment and are products of wood and biofuel combustion, their presence in roasted, smoked, and grilled foods could, therefore, be explained. The high temperature and direct exposure to the heat involved in these cooking methods have been reported to increase the production of carcinogenic PAHs within the food (Bansal and Kim, 2015). In all the food samples analyzed, Benzo[a]pyrene (BaP) listed as group 1 carcinogen and identified as the most potent carcinogenic polycyclic aromatic hydrocarbon (ECHA, 2016; IARC, 2017) was not detected. BaP is of significant public health importance. Wretling et al. (2010) reported that the presence of high levels of BaP in smoked meat and fish samples. Previous reports have also established the presence of high concentration of PAHs in roasted food items (Adetunde et al., 2012; Ogbuagu and Ayoade, 2012; Ishizaki et al., 2010). The highest concentration of PAHs was Dibenzene[a,h] Anthracene with a concentration of 314.85 and 139.97 µg/g detected in roasted yam and smoked fish samples (Table 4). This result is in contrast with the results of Martorell et al. (2010) where the least concentration of PAHs was seen in milk, tubers and fruits and the highest concentration in meat and meat products.

The present results also established the presence of HCAs in fish and meat samples. This had previously been reported by Zeng et al. (2018) and Khan et al. (2017). Of the six HCAs, only 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) was detected and in higher concentrations when compared with the daily intake of HCAs in fish and meat as reported by Augustsson *et al.* (1997). This increased concentration of HCA may be due to the cooking methods employed (smoking and grilling). In their study, Oz and Kotan (2016) detected PhIP in 9 of the 30 samples (30%). PhIP has been described as one of the most common HCAs in meats subjected to heat treatment. Szterk (2015) in an analysis of grilled beef, however, reported aminoimidazoazaarenes 4,8-DiMeIQx as the most prevalent. The presence of PhIP in fish and meat samples analyzed calls for concern as it has been established as reasonably carcinogenic (IARC, 1997; Cross and Sinha, 2004; USDHHS, 2016).

This investigation revealed that the food items sampled were in the acidic and neutral pH. The pH of food items most readily contributed to microbial spoilage of the food. As reported Batt (2016), food items around neutral pH and above are most susceptible to microbial spoilage. This could be explained by the diverse microbial species isolated from the fish and meat samples. Sample pH in this study ranged from 5.08- 7.74.

The presence of *Staphylococcus* and *Bacillus* sp. occurred in all the foods sampled. *Bacillus* sp. have previously been isolated from food products with mean

pH values 7.4 ± 0.2 and 7.1 ± 0.1 (Chettri and Tamang, 2015). The presence of *Bacillus* spp. in the meat samples corroborates Batt (2016) where it was reported that *Clostridium*, *Bacillus*, and lactic acid bacteria were among the Gram-positives which spoil meats. *Bacillus* spp. are spore formers hence may withstand food processing and harsh conditions (Chukwu et al., 2016, 2017). The presence of toxigenic species in food has been associated with food poisoning outbreaks (Geng et al., 2017). *Staphylococcus* spp. has also been implicated in staphylococcal foodborne disease caused by ingesting food containing staphylococcal enterotoxins (Wakabayashi et al., 2018). *Staphylococcus* sp. is known to be normal human flora; improper handling of food particularly the post-processing of samples in this study may have contributed to the presence of *Staphylococcus* sp. (Oniciuc et al., 2017). Coliforms were absent in all the samples besides some fish and meat samples. The presence of coliforms could be from the food handlers, food processing environment or from water used for processing, it could be also from the aquatic environment from which the fish was harvested. Faecal coliforms may be introduced into the water bodies through anthropogenic activities like inappropriate disposal of human wastes, sewage discharges or agricultural runoffs (Malham et al., 2014). Rubini et al. (2018) have previously isolated faecal coliforms from sea water. The improper handling of the meat during slaughter and processing may have also contributed to the presence of coliforms. Some coliforms are thermotolerant and are able to withstand the heat treatment during food processing (Paruch and Maehlum, 2012).

The antibiogram revealed that most of the microbial isolates were multi-resistant to the common antimicrobial agents, This poses a serious public health concern because should such a multi-drug resistant organism cause infection, empirical treatment will be difficult (Yah et al., 2007; Jakee et al., 2009). The multi-resistance of isolates could be explained that these organisms may have been subjected to several harsh environmental conditions and exposed to sub-lethal doses of antimicrobial substances, causing them to adapt and mutate into resistant strains (Adegoke et al., 2016). Inappropriate use of antimicrobials, lack of health care personnel with continual health education on antimicrobials and poor quality drugs have also been reported as a major cause of antimicrobial agents resistance specifically in developing countries (Yah et al., 2007; Jakee et al., 2009). Several mechanical, epidemiological and genetic factors may lead to the development of drug resistance (Yah et al., 2007; Bennett, 2008; Canton, 2009; Hung and Kaufman, 2010).

Conclusion

Microbial contaminants, coliforms and chemical

contaminants (PAH's and HCA) were detected in some of the samples marketed in Lagos and Ogun States, Nigeria the quality assessment of food greatly contributed to the overall health of consumers and the incidences of outbreaks of foodborne diseases are largely a product of the microbial quality of food items. Besides this, even more, important is the chemical composition of the food. PAHs and HACs have been identified as human carcinogens. In 2017, National cancer institute reported about 8.2 million cancer-related deaths worldwide. As food items are the most common entry route for PAHs and HCAs, it has, therefore, become necessary to ensure adequate food preparation techniques.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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