Levels of Polycyclic Aromatic Hydrocarbons (PAH4) in Some Popular Tea Brands in Nigeria

Benson, N.U.[1,*], Fred-Ahmadu, O.H.[1], Olugbuyıro, J.A.O.[1], Adedapo A.E.[1], Anake, W.U.[1], And Olajıre, A.A.[2]

[1]Analytical and Environmental Chemistry Unit, Department of Chemistry, Covenant University, Ota, Nigeria.

[2]Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

*corresponding author:

E-mail: nsikak.benson@covenantuniversity.edu.ng

Abstract The occurrence of 16 USEPA priority polycyclic aromatic hydrocarbons (PAHs) in twenty-three (23) popular green, black and herbal tea brands sold in Nigeria were investigated with focus on four PAHs (PAH4), classified by the European Food Safety Authority (EFSA) as suitable carcinogenic and mutagenic indicators: benz[a]anthracene (B[a]A), chrysene (CHR), benzo[b]fluoranthene (B[b]F) and benzo[a]pyrene (B[a]P). PAHs congeners were analyzed using an Agilent 7890A gas chromatograph (GC) with 7683B auto-sampler equipped with flame ionization detector (FID), with percentage recovery higher than 90.24%. The degree of contamination concentration expressed as sum of sixteen priority PAHs ($\sum_{16}$PAHs) ranged between 1.63±0.33–75.53±6.07, 4.71±0.23–79.61±7.02, and 12.52±0.15–26.89±0.68 µg/kg, for green, herbal and black tea, respectively. The PAH4 levels ranged from 1.28 – 44.57, 4.34 – 11.20, and 0.76 – 34.82 µg/kg, in green, black and herbal tea products, respectively. The highest PAH4 contribution relative to $\sum_{16}$PAHs was 71.37% in Twinings Pure Green Tea, 48.76% in Top Tea Regular (black tea), and 85.53% in Kidney Flush Herbal Tea. The estimated BaP-equivalent carcinogenicity (BaP-TEQ) and mutagenicity (BaP-MEQ) risks indicate highest TEQ in Joint Care Herbal Tea, while Typhoo Pure Green Tea had the lowest BaP-TEQ (0.16) and BaP-MEQ (0.43). Benzo[a]pyrene played a significant role in the carcinogenicity and mutagenicity potentials.

Keywords: Polycyclic aromatic hydrocarbons, PAH, Beverages, Carcinogens, Food contaminant, Mutagens

1. Introduction

Polycyclic aromatic hydrocarbon (PAHs) commonly refers to a large class of organic compounds, which are closely fused of two or more aromatic rings which obeyed the order of arrangement in either linear, angular or cluster. PAHs are usually formed from incomplete biomass combustion processes such as food or pyrolysis. The well-known polycyclic aromatic hydrocarbon is benzo[a]pyrene (B[a]P), a five-member ringed compound (Qiao, Zhang, Luo, & Chen, 2014; Choi, Harrison, Komulainen, & Delgado-Saborit, 2010). PAHs are normally categorized into light and heavy fractions on the basis of the number of fused aromatics. The light PAHs contains not more than four (4) member fused aromatic rings while more than four (4) member fused aromatic rings are called heavy PAHs (Arababi, Nasseri, & Anyakora, 2009; Olatunji, Fatoki, Opelu, & Ximba, 2014). PAHs are considered of high priority worldwide based on their carcinogenic and mutagenic effects by health and environmental administrators (IARC, 2010). Several studies have been conducted on PAHs since they represent an important class of known carcinogens (Yusuf *et al*., 2015; Benson, Essien, Asuquo, & Eritobor, 2014; Oluseyi, Olayinka, Alo, & Smith, 2011).

Due to the high carcinogenic potential posed by B[a]P and its pervasiveness in environmental samples, the Scientific Committee on Food recommended B[a]P as a suitable marker for PAHs occurrence and carcinogenic effects in food (EC, 2002, 2016; Semanováa, Skláršováb, Šimonc, & Šimkob, 2016). Consequently, legislative maximum limits were set for B[a]P in foods by the European Commission. In 2008, based on the review of scientific findings and data, European Food Safety Agency, EFSA CONTAM (Contaminants in Food chain) panel reported that B[a]P alone was not suitable as marker for PAHs presence in food, but PAH4 (the sum of four PAH compounds namely benzo[a]pyrene, benzo[a]anthracene, chrysene, and benzo[b]fluoranthene), and PAH8 (the sum of benz[a]anthracene, chrysene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) were proposed as a more suitable marker for carcinogenic characteristics in food, with PAH8 not providing much added value compared to PAH4 (EFSA, 2008; López-Jiménez, Ballesteros-Gómez, & Rubio, 2014).

All over the world, humans are widely exposed to PAHs through contaminated dietary products and inhalation of polluted air. Tea (*Camellia sinensis*) is a dietary beverage commonly consumed by humans the world over. It comes in different forms and brands, and is generally classed as green, black, herbal, oolong, fruit, chamomile, and mint teas. Many health benefits including antioxidative, anticarcinogenic, and antimutagenic effects are associated

with tea consumption due to the presence of flavonoids, theanine, and epigallocatechin gallate (EGCG) (Da Silva Pinto, 2013; Jain, Manghani, Kohli, Nigam, & Rani, 2013). Despite these beneficial potentials, PAHs occurrence has been reported in many food items: tea (Fred-Ahmadu & Benson, 2017; Iwegbue *et al*., 2015; Kayli-Sayadi, Rubio-Barroso, Cuesta-Jimenez, & Polo-Díez, 1998; Khiadani *et al*., 2013; Li *et al*., 2011; López-Jiménez, Ballesteros-Gómez, & Rubio, 2014; Orecchio, Ciotti, & Cullotta, 2009; Pincemaille, Schummer, Heinen, & Moris, 2014; Schulz, Fritz, & Ruthenschrör, 2014; Shi *et al*., 2016), coffee brew (Londoño, Reynoso, & Resnik, 2015), fruits and vegetables (Camargo & Toledo, 2003), cereals (Orecchio & Papuzza, 2009), fish (Nwaichi & Ntorgbo, 2016), and canned sardines (Benson, Anake, Adedapo, Fred-Ahmadu, & Eke, 2017). Several authors have reported the occurrence of PAH4 at concentrations that are higher than authorized PAHs thresholds as prescribed in the regulation 835/2011/EC (6 µg/kg for BaP and 35 µg/kg for the sum of PAH4 in bivalve molluscs: Mate teas (184.6-1615 µg/kg) (Ziegenhals, Huebschmann, Speer, & Jira, 2008), black tea (9.0 to 44.6 µg/kg) (Ziegenhals, Huebschmann, Speer, & Jira, 2008); 6.4-700 µg/kg Drabova *et al*., 2012), 4.9 to 103.6 µg/kg (Sclemitz & Pfannhauser, 1997), 21.6-65.8 µg/kg (Ishizki, Saito, Hanioka, Narimatsu, & Kataoka, 2010).

PAH4 has proven to be a better marker for assessing the carcinogenicity of PAHs in foods. The aim of this study was to determine the occurrence and degree of PAH4 (BaA, Chr, BbF and BaP) contamination in green, black and herbal tea brands commercially marketed and widely consumed in Nigeria.

2. Materials and Method

2.1 Samples

Eleven green teas, eight herbal teas, and four black tea brand samples were purchased from local retail outlets in Lagos and Ogun States, Nigeria. The selected tea samples were either imported or locally produced branded. Samples were oven dried and crushed mechanically in the laboratory with mortar and pestle into powder, and later persevered in well-labeled containers.

2.2 Chemicals and Equipment

A stock solution of PAH mix in dichloromethane:benzene (1:1) at certified concentration of 2.0 mg/mL, purchased from Accustandard (AccuStandard No. Z-014G-R) (New Haven, CT, United States) served as the standard reference solution for calibration and internal standard. The n-hexane used was a GC grade and of highest purity (≥96.0%) purchased from Merck (KGaA, Germany). Silica gel (600-200 mesh) was purchased from Loba Chemie (India) while anhydrous sodium sulphate used was sourced from Sigma-Aldrich (UK). Helium and nitrogen gases with 99.9999% purity were purchased from Foshan Huate Gas Coy Ltd. (China). Deionised water was used all through the bench work. A stock solution containing each PAH mix was prepared in n-hexane, and stored in amber bottles and preserved in the refrigerator at 4^{o}C for a maximum of 28 days. The working solution were obtained using serial dilution with n-hexane to give six calibration standard PAH solutions containing 5, 10, 20, 30, 40, and 50 µg/L of stock solution. Calibration curves for all analyzed PAH standards (n=6) had values of residual standard deviations (RSD) that ranged between 77.02 and 100.60%, demonstrating good repeatability for the analytical method. Triplicate determinations were made on all extracted tea samples. Equipment used in this study included hot air oven (Uniscope SM9053, Surgifriend Medicals, England), Stuart heat-stir (US152) Hot plate, fume cupboard, Stuart orbital shaker SSL1, Centrifuge, Langford Sonomatic 1400 Ultrasonic Bath (UK), Vortex - J.P. Selecta (Barcelona, Spain) and Thermo-Scientific MaxQ 4000 Bench-top orbital shaker (USA).

2.3 Sample extraction, clean up and analysis

The extraction method used is reported by Londoño, Reynoso, & Resnik (2015). 0.5 g of tea sample was weighed on an analytical balance and 15.0 mL of n-hexane was added. The mixture was taken to vortex (J.P. Selecta, Barcelona, Spain) for 20 seconds then to sonicator (Langford Sonomatic 1400 Ultrasonic Bath, United Kingdom) for 20 minutes at 60^{0}C. After the sonication, the mixture was centrifuged at 3,000 rpm for 10 minutes. The supernatant was decanted into a 100 mL flask. Sonication and centrifuging was repeated twice with 10.0 mL of n-hexane and total supernatant volume was approximately 35 mL. The extract was then taken to the water bath (Uniscope SM801A Laboratory Water Bath, Surgifriend Medicals, England) at 55^{0}C for evaporation to about 3.0 mL. This was filtered through filter paper and collected in glass tubes; the 100 mL flask was washed three times with 0.75 mL of n-hexane and filter paper was washed with 1 mL of n-hexane resulting in a total volume of approximately 6.25 mL, which was evaporated in the water bath at 36^{0}C to a volume of approximately 2.0 mL.

The silica gel (60-200 mesh) was activated at 130^{0}C overnight in a hot air oven and cooled in a desiccator at room temperature. 3.0 g of the silica gel was weighed and packed into a clean column plugged with cotton wool and set up on a retort stand. 1g of anhydrous Na_2SO_4 was added to the top of the silica gel, then 5.0 mL -10.0 mL of n-hexane was used to condition the column. The sample was added when the solvent was about 2.0 mL in the column. The colour band was monitored and gradually eluted with n-hexane until it was collected into a 100 mL conical flask. The collected eluent was then concentrated to about 2.0 mL.

The following sixteen priority PAHs were determined: acenaphthene (ACN), acenaphthylene (ACY), anthracene (ANT), benzo[a]anthracene (B[a]A), benzo[a]pyrene (B[a]P), benzo[b]fluoranthene, (B[b]F), benzo[g,h,i]perylene (B[ghi]P), dibenzo[a,h]anthracene (D[ah]A), fluoranthene (FLA), benzo[k]fluoranthene (B[k]F), chrysene (CHR), indeno[1,2,3cd]pyrene (I[cd]P), phenanthrene (PHE), naphthalene (NAP), fluorene (FLR), and pyrene (PYR). PAHs were analyzed using an Agilent 7890A with an auto-sampler Agilent 7683B, coupled to flame ionization detector (FID). The GC is equipped with an HP-5 column (19091J-413) (30 m x 0.32 mm x 0.25 µm) from Agilent (USA). The carrier gas used was helium

maintained at a flow rate of 4.84 ml/min. The oven temperature program is as follows: 0.4 min at 50°C, to 195°C at 20°C/min, hold 3.0 min, to 250°C at 8°C/min, hold 5.0 min, to 290°C at 5°C/min, hold 1.0 min.

3. Results and Discussion

3.1 PAH4 concentration

The results of PAH4 in green, black and herbal tea brands are presented in Table 1, 2 & 3. Benzo[a]pyrene was detected in all tea samples except Typhoo Pure Green Tea. The concentration of B[a]P ranged from 1.12±0.15 to 15.9±0.31 µg/kg in green tea brands considered in this study. The B[a]P levels in herbal and black tea ranged from 0.76±00 to 28.35±0.35 µg/kg, and 2.04±0.15 to 6.22±0.03 µg/kg, respectively. B[a]A was found in six out of eleven samples of the investigated green tea brands, and in four out of eight herbal teas, and in three out of four black tea samples. The concentration of B[a]A ranged between 2.04±0.25 and 9.31±1.39 µg/kg in green tea samples. In the herbal tea brands, the concentration of B[a]A ranged from 1.10±0.05 to 10.22±0.41 µg/kg, and in the black tea, the levels of B[a]A ranged from 0.82±0.41 to 2.11±0.07 µg/kg. On the average, the concentration of B[a]A was found to be relatively lower in the black tea samples than the herbal teas. Comparatively, it was observed that the concentrations of B[a]A are higher in green tea samples. Chrysene was detected in seven out of eleven green tea samples with concentrations that ranged from 0.83±0.23 to 26.02±2.46 µg/kg. CHR was found in four out of eight samples for herbal tea, and in all black tea samples. The concentrations of CHR ranged from 2.53±0.31 to 20.21±0.20 µg/kg, and 0.89±0.59 to 3.65±0.27 µg/kg for herbal and black tea samples, respectively.

Table 1. Concentratıons (mean ± standard devıatıon, n=3) of PAH4 congeners ın branded green tea samples

Analyte	TPG	HGT	GBG	SBG	LBG	LGL	LGR	LGJ	LGS	BGT	TWG
B[a]A	ND	3.97±0.04	2.04±0.25	5.25±0.17	4.29±0.31	ND	ND	ND	9.31±1.39	4.47±0.21	ND
CHR	ND	6.1±0.3	3.24±0.08	8.43±0.28	12.92±0.46	ND	ND	ND	26.02±2.46	6.96±0.61	0.83±0.23
B[b]F	ND	10.26±0.28	5.15±0.11	14.36±0.13	6.35±0.26	ND	ND	ND	0.42±0.11	ND	ND
B[a]P	ND	1.22±0.30	1.12±0.15	10.05±8.19	2.36±0.14	1.28± 0.41	2.83±0.38	4.8±0.15	8.82±0.12	3.73±0.31	15.9±0.31
∑PAH4	0	21.56	11.56	38.09	25.93	1.28	2.83	4.80	44.57	15.16	16.73
$\sum_{16}$PAHs	1.63	42.43	23.72	58.74	43.24	3.87	5.59	6.76	73.53	28.61	23.45
$\frac{\%\ \sum PAH4}{\sum_{16}PAHs}$	0	50.80	48.67	64.85	59.95	33.03	50.70	71.04	60.62	52.98	71.37

Table 2. Concentratıons (mean ± standard devıatıon, n=3) of PAH4 congeners ın branded black tea samples

Analyte	LYL	TTG	TTL	TTR
B[a]A	0.82±0.41	2.03±0.68	2.11±0.07	ND
CHR	1.48±0.14	2.94±0.04	3.65±0.27	0.89±0.59
B[b]F	ND	ND	ND	ND
B[a]P	2.04±0.15	6.22±0.03	2.75±0.30	5.21±0.26
∑PAH4	4.34	11.20	8.52	6.10
$\sum_{16}$PAHs	14.28	26.89	19.16	12.52
$\frac{\%\ \sum PAH4}{\sum_{16}PAHs}$	30.39	41.64	44.46	48.76

BaA=benzo[a]anthracene, BaP=benzo[a]pyrene, BbF=benzo[b]fluoranthene, CHR=chrysene, ND=Below LOD

Table 3. Concentratıons (mean ± standard devıatıon, n=3) of PAH4 congeners ın branded herbal tea samples

Analyte	NLF	TBN	MHT	SSH	AHT	JCT	KFT	ACT
B[a]A	ND	4.24±0.14	ND	10.22±0.41	1.20±0.03	1.10±0.05	ND	ND
CHR	ND	6.89±0.11	ND	20.21±0.20	2.53±0.31	5.37±1.42	ND	ND
B[b]F	ND	ND	ND	1.97±1.6	ND	ND	ND	ND
B[a]P	6.56±0.17	11.07±0.49	0.76±0.00	1.91±0.26	8.48±0.16	28.35±0.35	4.03±0.19	8.4±0.10
∑PAH4	6.56	22.21	0.76	34.32	12.21	34.82	4.03	8.40
$\sum_{16}$PAHs	7.92	37.03	5.03	79.61	25.90	50.21	4.71	14.81
$\frac{\%\ \sum PAH4}{\sum_{16}PAHs}$	82.85	59.97	15.20	43.11	47.15	69.35	85.53	56.74

Table 4. Comparison of results of study with similar studies

	Min – Max, µg/kg					
	B[a]A	CHR	B[b]F	B[a]P	∑PAH4	
Green Tea	0.7-74.4	29.6	0.15-66.6	0.4-61.3	8.0-355.9	[24]
	15.7	–	25.3	3.1	73.7	[31]
	16.3-23.5	24.9-47.6	–	7.4-9.7	59.4-101.2	[34]
	–	–	10.8-20.4	Nd-23.0	–	[20]
	0.7-28.3	2.9-42.4	0.7-23.9	0.2-17.9	4.5-102.3	[30]
	1.8-40.4	6.7-61.5	2.2-33.4	1.6-32.6	12.3-167.9	[29]
	2.04-9.31	**0.83-26.02**	**0.42-14.36**	**1.12-15.9**	**1.28-44.57**	**This study**
Black Tea	0.2-62.8	2.5-109.1	0.1-67.6	0.2-92.5	4.1-332.0	[24]
	0.7-31.9	2.0-45.4	1.9-22.0	0.4-5.9	5.0-1.3.7	[31]
	–	–	–	9.4	–	[20]
	175.0	241.0	37.6	39.7	811.6	[35]
	1.4-196.1	3.9-229.0	0.9-123.2	0.2-151.7	7.4-699.4	[30]
	1.3-13.1	3.4-18.1	1.5-8.1	0.8-14.1	9.0-44.6	[29]
	4.3-44.5	5.5-51.7	5.2-35.7	5.3-73.2	21.9-205.1	[32]
	–	–	210.4	1574.1	3569.0	[33]
	0.82-2.11	**0.89-3.65**	–	**2.04-6.22**	**4.34-11.2**	**This study**

The level of B[b]F in green tea samples ranged between 0.42±0.11 and 14.36±0.13 µg/kg, and was detected in 5 out of 11 samples. B[b]F was not detected in in the black and herbal tea samples except in Sahul Slim Herbal Tea, where the measured concentration was found to be 1.97±1.6 µg/kg. Generally, the concentrations of B[a]A, B[a]P, B[b]F, and CHR in the green and black tea samples when compared with similar studies from other countries indicated enhanced levels of the carcinogenic PAHs (Table 3).

Londoño, Reynoso, & Resnik (2015) has reported concentration of B[a]P and B[b]F in green tea that ranged from 0.4 to 61.3 µg/kg, and 0.15 to 66.6 µg/kg, respectively. The measured concentrations of CHR and B[a]P in green tea samples as reported by [30], ranged from 2.9 to 42.4, and 0.2 to 17.9 µg/kg, respectively. Also, Ishizaki, Saito, Hanioka, Narimatsu, & Kataoka (2010) has also reported concentrations ranging from 4.3 to 44.5 µg/kg, and 5.3 to 73.2 µg/kg for B[a]A and B[a]P, respectively, in black tea samples. However, the levels of B[a]P measured in the present study were similar to concentrations reported by Grover, Singh, & Pal, 2013) (Table 4).

The PAH4 levels ranged from 1.28 – 44.57, 4.34 – 11.20, and 0.76 – 34.82 µg/kg, in green, black and herbal tea products, respectively. The mean concentrations of $\sum_{16}$PAHs in green tea samples varied between 1.63±0.33 and 75.53±6.07 µg/kg in Twinings Pure Green Tea and Lloyd Sense Green Tea samples, respectively. Concentrations of $\sum_{16}$PAHs in herbal and black tea samples varied from 4.71±0.23 to 79.61±7.02 µg/kg, and 12.52±0.15 and 26.89±0.68 µg/kg, respectively. The results however indicated that the lowest mean $\sum_{16}$PAHs was obtained for the Twinings Pure Green Tea samples. The highest PAH4 contribution relative to $\sum_{16}$PAHs was 71.37% in Twinings Pure Green Tea, 48.76% in Top Tea Regular (black tea), and 85.53% in Kidney Flush Herbal Tea.

A comparatively lower concentration of PAH4 has been reported in black tea samples: 9.0 - 44.6 µg/kg, (Ziegenhals, Huebschmann, Speer, & Jira, 2008), 4.9-103.6 µg/kg, (Schlemitz & Pfannhauser, 1997), 21.6 - 65.8 µg/kg, (Ishizaki, Saito, Hanioka, Narimatsu, & Kataoka, 2010), and 6.4 - 700 µg/kg (Drabova *et al*., 2012). It is generally observed that the concentrations of sum of PAH4 were higher when compared with the threshold values stipulated under the Regulation 835/2011/EC for other foodstuffs (EC, 2006).

3.2 Carcinogenic and Mutagenic Assessments

The computed BaP-equivalent carcinogenicity and mutagenicity representing potential cancer and mutation effects evaluation are shown in Table 5.

Table 5. BaP-equıvalent carcınogenıcıty and mutagenıcıty rısks classıfied by tea type

		Mean	Min.	Max.	Range
Black tea	BaP-TEQ	5.02	3.04	7.82	3.04-7.82
	BaP-MEQ	7.09	4.74	10.45	4.74-10.45
Green tea	BaP-TEQ	6.37	0.16	16.58	0.16-16.58
	BaP-MEQ	8.56	0.47	19.82	0.47-19.82
Herbal tea	BaP-TEQ	9.92	1.19	28.69	1.19-28.69
	BaP-MEQ	11.72	2.02	29.04	2.02-29.04

TEF: toxic equivalency factors for cancer potency relative to BaP

MEF: mutagenic potency factor relative to BaP

The most potent carcinogenic PAH congeners are benzo[a]pyrene and dibenz(g,h,i)perylene. However, benzo[a]pyrene was observed in all investigated tea samples except Typhoo Pure Green Tea, while

dibenz[g,h,i]perylene was not detected in any of the herbal, black or green tea samples. Therefore, it can be ascertained that benzo[a]pyrene's contribution to the overall carcinogenicity and mutagenicity of the studied samples were quite significant. The aggregate measure of carcinogenicity or toxicity (BaP-TEQ), with respect to the listed contributing congeners shows that Sahul Slim Herbal Tea recorded the highest value of 145.18 mg/kg, which is indicative of its high carcinogenic risk while Kidney Flush Tea had the lowest value of 3.61 mg/kg. The mutagenic equivalency quotient (BaP-MEQ) values on the other hand show that Joint Care Herbal Tea and Typhoo Pure Green Tea had the highest and lowest mutagenic risk quotients with 29.04 and 0.47 mg/kg, respectively (Table 4). Generally, the BaP-TEQ values are higher than the BaP-MEQ values for each sample except for Heladiv Green Tea, which has slightly higher value for BaP-MEQ. Thus the health risk might be higher by considering the carcinogenic PAH potential rather than the mutagenic potential. Correlatively speaking, it has been observed that a weak but positive correlation ($r = 0.46$, $p<0.05$) exists between BaP-TEQ and BaP-MEQ.

4. Conclusion

The occurrence of polycyclic aromatic hydrocarbons (PAHs) in 23 imported and locally produced green, herbal, and black tea samples is described in this study through the assessment of PAH4 (sum of benzo[a]anthracene, benzo(b)fluoranthene, benzo(a)pyrene, and chrysene) presented by the European Food Safety Agency as indicators for the occurrence of PAHs in food products. Enhanced concentrations of B[a]A, B[a]P, B[b]P, and CHR are detected in most tea brands, and largely exceeded the limits stipulated in the Regulation 835/2011/EC for other foodstuffs.

Acknowledgement

We thank Elder Philips Adeleye and Mr. Ogunleye Moses of the Central Instrumentation Laboratory, Covenant University, Nigeria, and Olalekan A. Oyedeji for their assistance. The authors acknowledge the financial assistance provided by Covenant University, Nigeria.

References

Arababi M., Nasseri S. and Anyakora C. (2009), Biodegradation of Polycyclic Aromatic Hydrocarbon in Petroleum contaminated soils, *Iranian Journal of Chemistry and Chemical Engineering*, **28**, 53-59

Benson N.U., Anake W.U., Adedapo A.E., Fred-Ahmadu O.H. and Eke K.P. (2017), Polycyclic aromatic hydrocarbons in imported Sardinops sagax: Levels and health risk assessments through dietary exposure in Nigeria, *Journal of Food Composition and Analysis*, **57**, 109-116, doi:10.1016/j.jfca.2016.12.024

Fred-Ahmadu, O.H. and Benson, N.U. (2017), Polycyclic aromatic hydrocarbons (PAHs) occurrence and toxicity in *Camellia sinensis* and herbal Tea. *Polycyclic Aromatic Compounds*, http://doi.org/10.1080/10406638.2017.1335216

Benson N.U., Essien J.P., Asuquo F.E. and Eritobor A.L. (2014), Occurrence and distribution of polycyclic aromatic hydrocarbons in surface microlayer and subsurface seawater of Lagos Lagoon, Nigeria, *Environmental Monitoring and Assessment*, **186**, 5519-5529, doi:10.1007/s10661-014-3800-z.

Camargo M.C. and Toledo M.C. (2003), Polycyclic aromatic hydrocarbons in Brazilian vegetables and fruits, *Food Control*, **14**, 49-53.

Choi H., Harrison R., Komulainen H. and Delgado-Saborit J.M. (2010), Polycyclic aromatic hydrocarbons. In: World Health Organization, Regional Office for Europe, eds. Selected Pollutants, *WHO Guidelines for Indoor Air Quality*. Geneva: World Health Organization, 289–325.

Da Silva Pinto M. (2013), Tea: a new perspective on health benefits, *Food Research International*, **53**, 558-567.

Drabova L., Pulkrabova J., Kalachova K., Tomaniova M., Kocourek V. and Hajslova J. (2012), Rapid determination of polycyclic aromatic hydrocarbons (PAHs) in tea using two-dimensional gas chromatography coupled with time of flight mass spectrometry, *Talanta*, **100**, 207-216.

EFSA (2008), Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on polycyclic aromatic hydrocarbons in food (2008), *The EFSA Journal*, **724**, 1–114.

European Commission (2002), Decision No 2002/658/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, *Official Journal of the European Union*, **221**,8.

European Commission (2006), Regulation No 1881/2006 setting maximum levels of certain contaminants in foodstuffs, *Official Journal of the European Union*, **364**, 5.

Fiedler H., Cheung C.K. and Wong M.H. (2002), PCDD/PCDF, Chlorinated pesticides and PAHs in Chinese teas, *Chemosphere*, **46**, 1429-1433.

Grover I.S., Singh S. and Pal B. (2013), Priority PAHs in orthodox black tea during manufacturing process, *Environmental Monitoring and Assessment*, **185**, 6291–6294.

IARC (International Agency for Research on Cancer). (2010), Monographs on the evaluation of carcinogenic risks to humans. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures, Lyon: IARC (International Agency for Research on Cancer), http://monographs.iarc.fr/ENG/ Monographs/vol92/mono92.pdf [Accessed 14.01.13].

Ishizaki A., Saito K., Hanioka N., Narimatsu S. and Kataoka H. (2010), Determination of polycyclic aromatic hydrocarbons in food samples by automated on-line in-tube solid-phase microextraction coupled with high-performance liquid chromatography-fluorescence detection, *Journal of Chromatography A*, **1217**, 5555-5563.

Iwegbue C.M.A., Agadaga H., Bassey F.I., Overah L.C., Tesi G.O. and Nwajei G.E. (2015), Concentrations and profiles of polycyclic aromatic hydrocarbons in some commercial brands of tea-, coffee-, and cocoa-based food drinks in Nigeria, *International Journal of Food Properties*, **18**, 2124-2133.

Jain A., Manghani C., Kohli S., Nigam D. and Rani V. (2013), Tea and human health: The dark shadow, *Toxicology Letters*, **220**, 82–87.

Kayali-Sayadi M.N., Rubio-Barroso S., Cuesta-Jimenez M.P. and Polo-Díez L.M. (1998), Rapid determination of polycyclic aromatic hydrocarbons in tea infusion samples by high-performance liquid chromatography and fluorimetric detection based on solid-phase extraction, *Analyst*, **123**, 2145–2148.

Khiadani M., Amin M.M., Beik F.M., Ebrahimi A., Farhadkhani M. and Mohammadi-Moghadam F. (2013), Determination of polycyclic aromatic hydrocarbons concentration in eight

brands of black tea which are used more in Iran, *International Journal of Environmental Health and Engineering*, **2**, 1–5.

Li X.Y., Li N., Luo H.D., Lin L.R., Zou Z.X., Jia, Y.X. *et al*. (2011), A novel synchronous fluorescence spectroscopic approach for the rapid determination of three polycyclic aromatic hydrocarbons in tea with simple microwave-assisted pretreatment of sample, *Journal of Agricultural and Food Chemistry*, **59**, 5899-5905.

Lin D., Tu Y. and Zhu L. (2005), Concentrations and health risk of polycyclic aromatic hydrocarbons in tea, *Food and Chemical Toxicology*, **43**, 41-48.

Londoño V.A.G., Reynoso C.M. and Resnik S.L. (2015), Polycyclic aromatic hydrocarbons (PAHs) survey on tea (Camellia sinensis) commercialized in Argentina, *Food Control*, **50**, 31-37.

López-Jiménez F.J., Ballesteros-Gómez A. and Rubio S. (2014), Determination of polycyclic aromatic hydrocarbons (PAH4) in food by vesicular supramolecular solvent-based microextraction and LC–fluorescence detection, *Food Chemistry*, **143**, 341–347.

Nwaichi E. and Ntorgbo S. (2016), Assessment of PAHs levels in some fish and seafood from different coastal waters in the Niger Delta, *Toxicology Reports*, **3**, 167–172.

Olatunji O.S., Fatoki O.S., Opeolu B.O. and Ximba B.J. (2014), Determination of Polycyclic Aromatic Hydrocarbon (PAHs) in processed meat products using gas chromatography-flame ionization detector, *Journal of Food Chemistry*, **156**, 296-300.

Oluseyi T., Olayinka K., Alo B. and Smith R. (2011), Comparison of extraction and clean-up techniques for the determination of polycyclic aromatic hydrocarbons in contaminated soil samples, African *Journal of Environmental Science and Technology*, **5**, 482-493.

Orecchio S, Ciotti V. and Cullotta L. (2009), Polycyclic aromatic hydrocarbons (PAHs) in coffee brew samples: Analytical method by GC–MS, profile, levels and sources, *Food and Chemical Toxicology*, **47**, 819–826.

Orecchio S. and Papuzza V. (2009), Levels, fingerprint and daily intake of polycyclic aromatic hydrocarbons (PAHs) in bread baked using wood as fuel, *Journal of Hazardous Materials*, **164**, 876–883.

Pincemaille J., Schummer C., Heinen E. and Moris G. (2014), Determination of polycyclic aromatic hydrocarbons in smoked and –smoked black teas and tea infusions, *Food Chemistry*, **1450**, 807-813.

Qiao J., Zhang C.D., Luo S.M. and Chen W. (2014), Bioremediation of highly contaminated oilfield soil: bioaugmentation for enhancing aromatic compounds removal, *Frontiers of Environmental Science & Engineering*, **8**, 293–304.

Schlemitz S. and Pfannhauser W. (1997), Supercritical fluid extraction of mononitrated polycyclic aromatic hydrocarbons from tea-correlation with the PAH concentration, *Zeitschrift für Lebensmitteluntersuchung und -Forschung A*, **205**, 305-310.

Schulz C., Fritz H. and Ruthenschrör A. (2014), Occurrence of 15 + 1 EU priority polycyclic aromatic hydrocarbons (PAH) in various types of tea (Camellia sinensis) and herbal infusions, *Food Addit. Contam,*, **31**, 1723–1735.

Semanováa J., Skláršováb B., Šimonc P. and Šimkob P. (2016), Elimination of polycyclic aromatic hydrocarbons from smoked sausages by migration into polyethylene packaging, *Journal of Food Chemistry*, **201**, 1–6.

Shi Y., Wu H., Wang C., Guo X., Du J. and Du L. (2016), Determination of polycyclic aromatic hydrocarbons in coffee and tea samples by magnetic solid-phase extraction coupled with HPLC–FLD, *Food Chemistry*, **199**, 75-80.

Yusuf K., Ezechukwu L., Fakoya K., Akintola S., Agboola J. and Omoleye T. (2015), Influence of fish smoking methods on polycyclic aromatic hydrocarbons content and possible risks to human health, African *Journal of Food Science*, **9**, 126-135.

Ziegenhals K., Huebschmann H., Speer J. and Jira W. (2008), Fast GCHRMS to quantify the EU priority PAH DFS, *Journal of Separation Science*, **31**, 1779-1786.