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# ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN ALCOHOLIC BEVERAGES CONSUMED IN AWKA, SOUTHEAST NIGERIA

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**ABSTRACT**. The aim of this investigation was to assess the presence of 16 PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene fluoranthene, benz[a]anthracene, pyrene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[ghi]perylene, and indeno[1,2,3-cd]pyrene with symbols Nap, Ace, Ane, Flu, Ant, Phe, Flt, BaA, Pyr, Chr, BbF, BkF, BaP, DahA, BghiP and InP, respectively) in alcoholic beverages consumed in Awka, Southeast Nigeria. The samples used were sourced from international, national, and local-based alcoholic beverages sold in Nigeria, which were analysed for the 16 priority PAHs components using gas chromatography-flame ionization detector (GC-FID) in order to quantify and offer advice to the public on the health implications of consuming these alcoholic beverages. The results show the absence of all the PAHs in all the alcoholic beverages, which could be due to lower temperature and shorter production duration utilized in the production process. This is because during the intense heating of raw materials or additives, PAHs are released. Therefore, the consumption of these alcoholic beverages is safe for consumers and poses no health risk that is detrimental to adults or children. As such, further research into other micropollutants and toxins is advocated to maintain current good manufacturing practices for quality.

**KEY WORDS**: Alcoholic beverages, Food analysis, Public health, PAHs, GC-FID, Southeast Nigeria

## INTRODUCTION

Alcoholic beverages contain between 3 and 50% alcohol by volume of the beverage which include beer, wine, and spirit. Examples of beer are lager, stout, ale, pilsner, etc. and there is white wine, red wine, Malbec, Sherry, Port, Muscat, etc. while spirits include gin, brandy, whisky, vodka, liquors amongst others [1]. In comparison to other ethical and regulatory procedures, the production to packaging process of alcoholic drinks is a set of quality assurance and control standards where contamination does not necessarily affect the flavour, odour, or colour of the beverages. Contaminants such as polycyclic aromatic hydrocarbons, heavy metals, particulate matter (aerosols), microorganisms (viruses, parasites, bacteria, fungi), and gaseous emissions (carbon, sulfur, nitrogen and methane oxides) are nearly impossible to eradicate from the entire process. Because of a variety of metabolic mechanisms, these pollutants have the potential to increase toxicity in humans after consumption [2-4]. The drinking of alcoholic beverages high in polycyclic aromatic hydrocarbons (PAHs) has the potential to cause harmful effects on human health, depending on the rate of exposure and cumulative dosage. A look at the work of Kaminski *et al*. [5] greatly emphasized that PAHs are encountered in different types of beverages such as tea, coffee, beer, soft drink (a drink that contains carbonated water, a sweetener, and a natural or artificial flavour), and fruit juices. For example, Okafor *et al*. [6] found pyrene in beer brewed with *Garcinia kola*, and dos Santos *et al*. [7] found that at least one PAH was detected in all of the beer samples they studied: BbF was the most detected analyte in the samples, and only 9-fluorenone and 9-nitroanthracene were detected in some samples among the PAHs derivatives

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evaluated. Mastanjevi´c *et al*. [8] also reported that BaA was present in all their samples in significant amounts (60 µg/kg in amber and a whopping 737 µg/kg in black malt), which resulted in extremely high PAH4 levels in all the samples.

Polycyclic aromatic hydrocarbons are a group of chemicals composed of two or more fused aromatic rings. They are formed during the incomplete combustion or high-temperature pyrolysis of coal, oil, gas, wood, fossil fuels, garbage, or other substances, such as tobacco and charbroiled meat. The quantity and composition of PAHs produced are closely related to the reaction conditions, temperature, and amount of air and, therefore, may vary considerably. Over 100 PAHs have been identified and occur as complex mixtures, never as individual components [9].

PAHs have recorded many applications in so many industrial productions. Apart from other manifold uses of PAHs, they are mostly used as intermediaries in pharmaceuticals, agricultural products, photographic products, thermosetting plastics, lubricating materials, and other chemical industries [10]. However, they are the largest class of known chemical carcinogens and have been detected in the environment, especially in the air, water, soil, and food through release from volcanoes, forest fires, residential wood burning, cigarette smoke, asphalt roads, coal, coal tar, agricultural burning, municipal, industrial waste incineration, hazardous waste sites and exhaust from automobiles and trucks [11]. Therefore, PAHs are considered ubiquitous in the environment [12, 13].

The American Conference of Governmental Industrial Hygienists (ACGIH) [14] emphasized the impacts of PAHs on human health which depend mainly on the length and route of exposure, the amount or concentration of PAHs one is exposed to, as well as the relative toxicity of the PAHs. The harmful effects that may occur when exposed to PAHs largely depend on the mode of exposure [15].

A variety of other factors which can also have health impacts include subjective factors such as pre-existing health status and age. Occupational exposure to high levels of pollutant mixtures containing PAHs has resulted in symptoms such as eye irritation, nausea, vomiting, diarrhoea, and confusion [16]. Nevertheless, it is not known which components of the mixture were responsible for these effects and other compounds commonly found with PAHs and/or in combination show these symptoms as well.

According to Rascon *et al*. [17], most International monitoring and regulatory agencies such as International Agency for Research on Cancer (IARC), the Environmental Protection Agencies (EPA) and the European Union (EU) have included PAHs on their lists of priority pollutants as they constitute one of the largest groups of contaminants, because of their carcinogenic and mutagenic properties.

In recent years, attention has been drawn to PAHs because several of them are known to be potential human carcinogens and have been implicated in various cancers [18]. Also, some PAHs have been implicated in numerous other toxicological manifestations such as reproductive toxicity, intrauterine growth retardation, learning and intelligence quotient deficit, destruction of oocytes, and inflammation of kidney cells [19, 20]. Experiments on ground and surface water [21, 22], soil [23, 24], aquatic organisms [25, 26], food [27, 28], air [29] and beer [3, 6] amongst others have been conducted due to health concerns about PAHs pollution of these substances and their toxicological concerns. Furthermore, Okafor *et al*. [30] studied heavy metals in alcoholic beverages consumed in Awka, South-East Nigeria and associated carcinogenic and non-carcinogenic health risk assessments recently. Despite these studies, little or no research has been conducted on the assessment of PAH contamination in alcoholic beverages enjoyed in Awka and its environs. As a result, determining and monitoring the levels of the 16 priority EPA PAHs in alcoholic drinks consumed in Awka, southeast Nigeria, is crucial.

## EXPERIMENTAL

*Procurement of materials*

The eight (8) distinct kinds of alcoholic beverages employed in this study (herbal gin, imported red wine, Heineken, imported dry gin, local dry gin, palm wine, Guinness stout, and whiskey) were acquired at some drinking joints along Club Road popularly known as Abakiliki Street in Awka, Anambra State. BDH Chemical Ltd in the United Kingdom and Sigma-Aldrich Chemie GmbH in Germany provided the reagents for the analyses. At 2000 mg/L, a PAH mixture containing equal amounts of naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene in methanol: methylene chloride (1:1) purchased from Supelco (Bellefonte, USA) was used as standard.

*Quality control*

Reagents used for the analyses were of high-quality analytical grade. Detergents and deionized water were used to wash the glassware and sample bottles, which were thereafter soaked overnight with a solution of 10% $HNO_3$ in a 1% HCl solution, followed by rinsing with deionized water.

*Preparation and extraction of PAHs from the samples*

Liquid-liquid extraction of PAHs from the beverages was carried out. The extraction of alcohol samples and their spiked duplicates for PAHs was as described in our previous work [6]. Briefly, 10 g each of the sample was extracted with 1:1 mixture of acetone and methylene chloride spiked with 1 mL of PAH internal standard and shaken thoroughly for proper mixing before placing in an ultrasonic bath. A splitless inlet mode, helium gas was used as the carrier gas, and nitrogen, the makeup gas. The ignition gases were hydrogen and compressed air. A 1 µm sample was injected into the gas chromatograph under the following oven conditions; initial temperature: 60 ºC held for 1 min, ramp rate 1: increased to 210 ºC at 12 ºC/min, ramp rate 2: increased to 320 ºC at 8 ºC/min, final temperature: 320 ºC held for 5 min., total run time: 32.25 min. and detector temperature: 325 ºC. The samples were analysed for the presence of PAHs using the Environmental Protection Agency (EPA) 3510-C Method [31].

*Chromatographic analysis*

The assessment of the sixteen (16) priority EPA PAHs in the alcoholic beverages was determined at Central Laboratory, Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos State, Nigeria using 7890A Agilent Gas Chromatography model, coupled with an HP5 column (30 m × 0.32 mm × 0.25 µm). The procedure for the analysis has been described previously [32, 33]. Identification and quantification of individual PAHs was based on internal calibration standard containing known concentrations of the 16 EPA priority PAHs. The specificity of the 16 PAHs sought in the samples was confirmed by the presence of transition ions (quantifier) as shown by their retention times which corresponded to those of their respective standards.

*Method validation*

The Eurachem Guide recommendation [34] was employed to evaluate performance parameters such as limit of detection (LOD), limit of quantification (LOQ), precision, and recovery. We

adapted and modified the recommendation which had previously been used by dos Santos *et al.* [7]. The analytes' calibration curves were created in triplicate using deionized water (DW) at five concentration levels. For the validation investigation, DW was spiked with PAH solutions at concentration levels of 0.5, 1.0, 2.0, 4.0, and 8.0 g $L^{-1}$. The LOQs and LODs were determined by the examination of ten blanks. By dividing the LOQ by 3.33, the LOD was calculated. At concentrations of 1.0 and 4.0 g/L, intra-day precision was evaluated in three replicates at each concentration. On two separate days, inter-day precision was examined. Three replicates were used to assess recovery at a concentration of 2.0 g/L.

## RESULTS AND DISCUSSION

### *Method validation*

Each analyte's determination coefficients ($R^2$), LODs, and LOQs are listed in Table 1 which was published in our previous study [21]. The $R^2$ values show that the proposed linear models are well-adjusted. According to Christian [35], $R^2$ values larger than 0.995 are acceptable. All PAHs with $R^2$ values between 0.99641 and 0.9983 indicate adequate regression line linearity, good correlation, and hence good instrument calibration. The LODs and LOQs obtained in this investigation were substantially lower than those obtained by Coelho *et al.* [36], with the exception of BghiP, where the authors reported LOD and LOQ of 1.0 and 0.32, respectively, which are marginally lower than those achieved in this work. On the other hand, the LODs and LOQs obtained in this study are consistent with the results obtained by dos Santos *et al*. [7]. All of these details point to the method's sensitivity.

Table 1. Determination coefficient ($R^2$), limit of determination (LOD) and limit of quantification (LOQ) of GC-FID method of PAHs analysis.

| Analyte | $R^2$ | LOD (µg/L) | LOQ (µg/L) |
|---|---|---|---|
| Naphthalene | 0.9891 | 0.0157 | 0.0522 |
| Acenaphthylene | 0.9919 | 0.0083 | 0.0278 |
| Acenaphthene | 0.9983 | 0.0204 | 0.0680 |
| Fluorene | 0.9725 | 0.0211 | 0.0703 |
| Phenanthrene | 0.9967 | 0.0038 | 0.0125 |
| Anthracene | 0.9980 | 0.0056 | 0.0186 |
| Fluoranthene | 0.9860 | 0.0060 | 0.0201 |
| Pyrene | 0.9758 | 0.0130 | 0.0434 |
| Benzo[a]anthracene | 0.9916 | 0.0044 | 0.0147 |
| Chrysene | 0.9641 | 0.0033 | 0.0109 |
| Benzo[b]fluoranthene | 0.9935 | 0.0178 | 0.0592 |
| Benzo[k]fluoranthene | 0.9904 | 0.0244 | 0.0814 |
| Benzo[a]pyrene | 0.9957 | 0.0127 | 0.0423 |
| Indeno[123-c,d]pyrene | 0.9622 | 0.0608 | 0.2025 |
| Dibenzo[a,h]anthracene | 0.9871 | 0.1236 | 0.4115 |
| Benzo[g,h,i]perylene | 0.9833 | 0.1131 | 0.3767 |

Source: Okafor *et al*. [21]

The CV results for intra-day and inter-day accuracy evaluation for concentration levels 2.0 and 8.0 g/L, as well as the percent recovery for level 4.0 g/L, are shown in Table 2. These figures have previously been published [21]. Precision CV values ranged from 5.12 to 18.24 %, indicating that the method was effective. The analyte recovery values in the samples varied from 83.94 to 99.99 %, which is similar to previous research findings of 83.6 to 98.5 percent [36], 80 to 111 percent [37], and 80.10 to 100.30 % [7].

Table 2. Precision and recovery of method GC-FID analysis of PAHs.

| Analyte | Intra-assay | | Inter-assay | | Recovery (%) |
|---|---|---|---|---|---|
| PAHs | 2.00 µg/L | 8.00 µg/L | 2.00 µg/L. | 8.00 µg/L | 40 µg/L |
| Naphthalene | 7.92 | 9.99 | 15.37 | 12.33 | 96.52 |
| Acenaphthylene | 8.33 | 10.01 | 16.41 | 12.34 | 91.47 |
| Acenaphthene | 9.20 | 10.20 | 13.23 | 11.00 | 86.02 |
| Fluorene | 12.52 | 9.12 | 14.66 | 9.70 | 99.99 |
| Phenanthrene | 7.81 | 5.45 | 17.70 | 13.28 | 87.34 |
| Anthracene | 8.00 | 5.12 | 16.15 | 17.17 | 97.12 |
| Fluoranthene | 11.56 | 9.41 | 17.90 | 10.11 | 99.20 |
| Pyrene | 11.56 | 10.40 | 17.90 | 10.11 | 97.56 |
| Benzo[a]anthracene | 12.80 | 13.11 | 13.36 | 13.00 | 98.50 |
| Chrysene | 14.73 | 10.10 | 12.58 | 8.67 | 88.99 |
| Benzo[b]fluoranthene | 10.81 | 9.26 | 18.24 | 16.25 | 95.96 |
| Benzo[k]fluoranthene | 13.47 | 11.00 | 12.50 | 11.20 | 87.91 |
| Benzo[a]pyrene | 10.70 | 7.98 | 15.12 | 15.00 | 91.92 |
| Indeno[123-c,d]pyrene | 10.40 | 6.96 | 15.00 | 12.49 | 94.59 |
| Dibenzo[a,h]anthracene | 9.77 | 8.22 | 13.79 | 12.00 | 83.94 |
| Benzo[g,h,i]perylene | 8.11 | 5.72 | 12.51 | 10.00 | 98.23 |

Source: Okafor *et al.* [21].

*Levels of PAHs in the alcoholic beverages*

For all of the alcoholic beverages, chromatograms (fingerprints) were recorded and displayed in Figures 1 - 8.

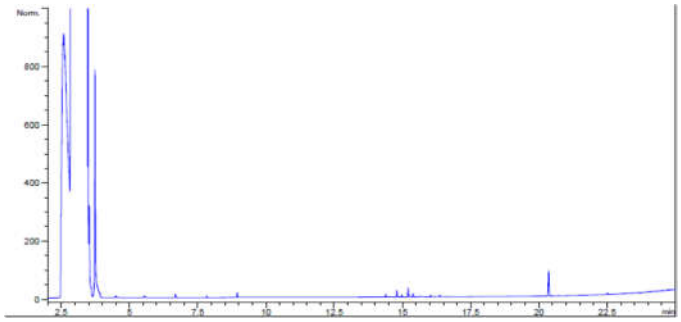

Figure 1. Chromatogram of herbal gin.

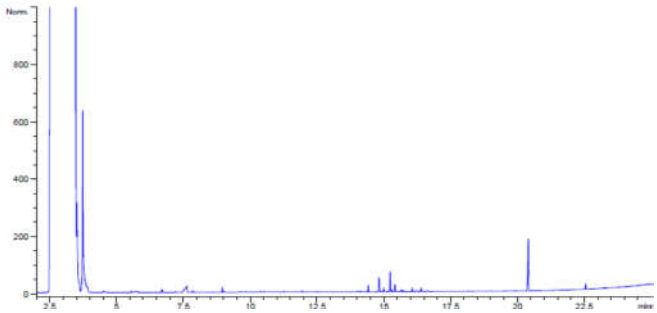

Figure 2. Chromatogram of imported red wine.

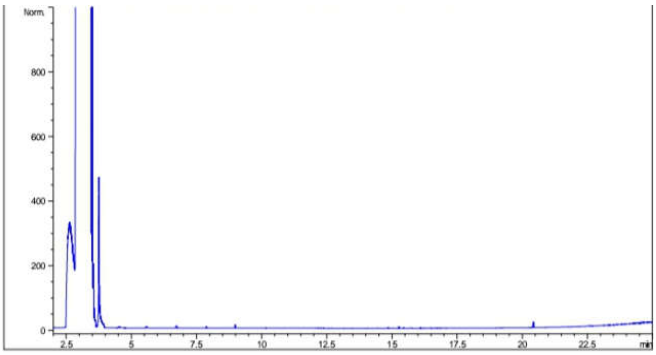


Figure 3. Chromatogram of Heineken.

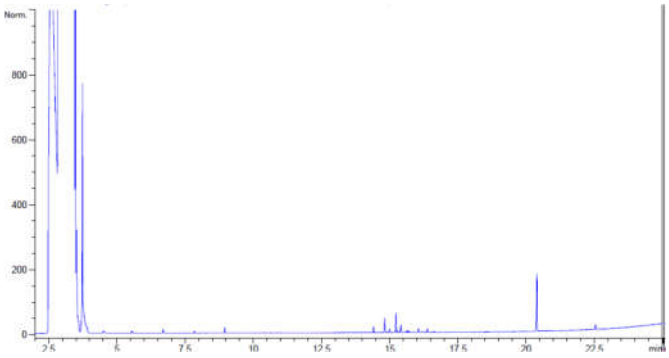


Figure 4. Chromatogram of imported dry gin.

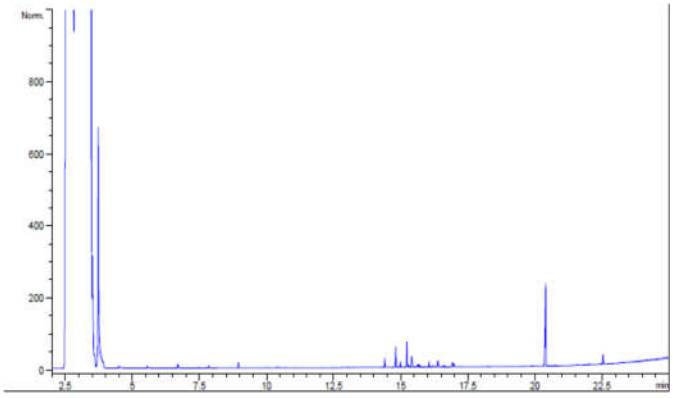


Figure 5. Chromatogram of local dry gin.

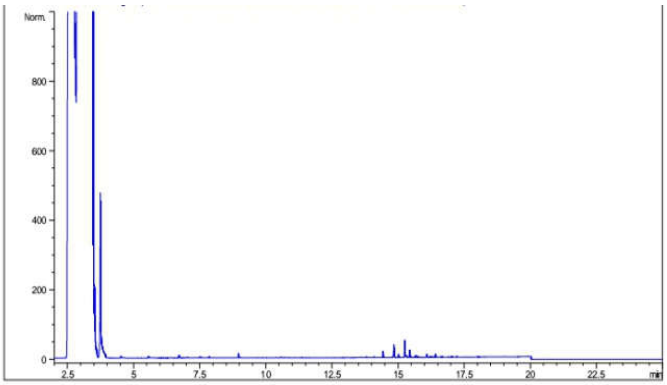


Figure 6. Chromatogram of palm wine.

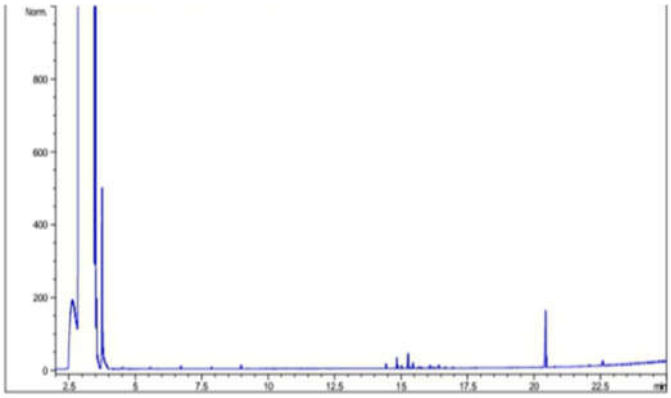


Figure 7. Chromatogram of Guinness Stout.

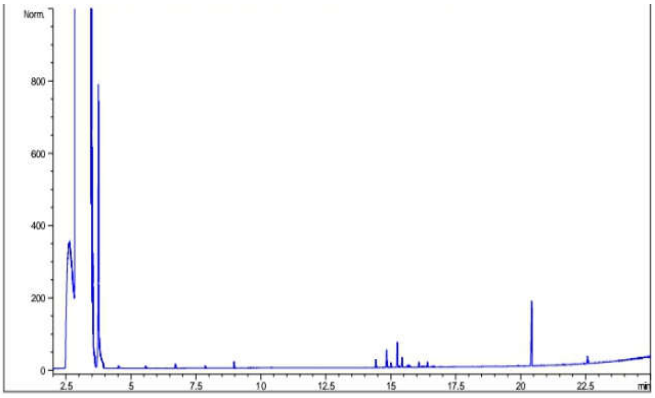


Figure 8. Chromatogram of whisky.

The chromatograms show that none of the 16 US EPA priority PAHs were found in all the beverages. The retention duration of the PAHs corroborates this evidence. The PAHs' retention times in minutes are as follows: naphthalene (6.514), acenaphthylene (9.242), acenaphthene (9.656), fluorene (10.486), phenanthrene (12.228), anthracene (12.305), fluoranthene (14.450), pyrene (14.878), benzo(a)anthracene (17.539), chrysene(17.638), benzo(b)fluoranthene (20.057), benzo(k)fluoranthene (20.116), benzo(a)pyrene (20.799), dibenzo(a,h)anthracene (23.183), indeno(1,2,3-cd)pyrene (23.247), benzo(g,h,i)perylene (23.673). These PAHs had the same retention durations in all samples, showing that PAHs were not present in any of the alcoholic beverages.

Although PAHs were not detected across all the samples analysed, several studies have shown that PAHs are released into beverages from a host of production processes which are direct and indirect kilning, brewing and baking of palm juice and grains such as barley, sorghum, corn, rice, etc [38, 39], as these processes lead to the formation of these PAHs components at higher temperature above 100 ºC dependent on the raw material base [40].

Mastanjevic *et al.* [40] assessment of four brands of malt-based drinks showed that PAHs was detected from preheating finished products from 70 – 250 ºC, as they stated that increasing kilning temperature leads to formation of PAHs in fluorene (Flt), anthracene (Ant), fluoranthene (Flt) and benzo(a)anthracene (BaA), as BaA were ranged from 60.53 – 737.57 µg/kg, was above acceptable concentration and vice versa for Flt, Ant an Flt, which was attributed to heating duration and temperature used during production processes.

Ciemniak *et al*. [41] tested 37 different tea brands in Poland for PAHs, which were brewed with boiling water, and found 16 PAHs ranging from 41.50 to 2910.20 g/kg, with herbal teas > fruit teas > herbal teas > traditional black, green, red, and white teas being the least contaminated, as they stated that different tea compositions can form PAHs at a lower temperature from roasting aromatic plants. It is seen that the case of hops and herbs used in brewing alcohols for medicinal, taste and texture due to intense roasting at high temperature and duration can lead to infusion of PAHs in these alcoholic beverages causing immense health risks [42-45]

Oyekunle *et al.* [45] analysed PAHs in popular soft drinks in Nigeria with plastic bottled ∑16 PAHs ranging between 0.37 – 13.36 µg/mL, while glass bottled ∑16 PAHs ranged between 0.11–13.48 µg/mL that was attributed to production process, as National Institute for Occupational Safety and Health (NIOSH) regulatory limit is 0.1 µg/mL implying that PAHs exposure is possible in adults and children most especially [46].

PAHs is produced in alcoholic beverage from intense heating by fire or high temperature exposure from traditional (70–120 ºC) or industrial (400–750 ºC) heating process [43, 47, 48]. PAHs has the capacity to cause severe eye and dermal irritation, haemolysis, biliousness and vomiting tendencies in children and adults, and chronic exposure can lead to growth development and fertility issues, cancer, silicosis, chronic bronchitis, anaemia, amongst others [49, 50].

Although PAHs was not detected in all the samples in the present work, industrial players should be committed to the prevention of microcontaminants and maintaining high level of quality assurance to serve Nigeria the very best that alcoholic beverage seeks to offer in relation to taste, quality and desire for taste satisfaction.

## CONCLUSION

This study revealed the absence of the 16 priority EPA PAHs in all the investigated alcoholic beverages (palm wine, whisky, Guinness stout, Heineken lager beer, imported dry gin, local dry gin, herbal gin, and imported red wine) consumed in Awka, Southeast Nigeria. Consequently, the consumption of alcoholic drinks is safe for consumers and poses no threat of exposure to polycyclic aromatic hydrocarbons. However, this investigation did not suggest consistent and regular consumption of the investigated alcoholic drinks. The investigation of some other pollutants and contaminants such as heavy metals, straight-chain hydrocarbons, and forever chemicals in alcoholic beverages is recommended.

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