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Detecting PAHs in grilled beef and chicken using QuEChERS and GC-MS/UV-Vis.

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Polycyclic aromatic hydrocarbons (PAHs), Grilled meat products, Beef steak, Chicken, Wood/charcoal types, QuEChERS method, Gas Chromatography-Mass Spectrometry (GC- MS), Ultraviolet-Visible (UV-Vis) Spectrophotometry, Food safety, Environmental Pollution, Health risks.

ABSTRACT
Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants that are formed during the incomplete combustion of organic matter, including wood and charcoal used for grilling. Grilled meat products, such as beef steak and chicken, can be contaminated with PAHs, posing a potential health risk to consumers. This study aimed to detect and quantify the levels of PAHs in grilled beef steak and chicken using different wood/charcoal types, employing the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method and Gas Chromatography-Mass Spectrometry (GC-MS) and Ultraviolet-Visible (UV-Vis) spectrophotometry for analysis.Detection and quantification of PAHs in grilled beef steak and chicken.Comparison of PAH levels across different wood/charcoal types, Assessment of the potential health risks associated with PAH contamination in grilled meat products.The UV-Vis analysis revealed varying PAH levels: Beef-Charcoal (1.20 ± 0.10) had the highest concentration, followed by Chicken-Charcoal (1.05 ± 0.08), Beef-Wood (0.85 ± 0.05), and Chicken-Wood (0.70 ± 0.03) at 254 nm. Charcoal-grilled samples showed higher PAH levels.The QuEChERS extraction method yielded satisfactory results, with 80-90% recovery of PAHs from grilled beef and chicken samples.GC-MS analysis detected 12 PAHs in grilled beef and chicken samples, including Naphthalene, Acenaphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz(a)anthracene, Chrysene, and Benzo(b)fluoranthene, indicating varying concentrations of these compounds in the samples.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of environmental pollutants formed during incomplete combustion of organic matter, including wood and charcoal used for grilling (JECFA, 2005). Grilled foods, particularly meat, can be contaminated with PAHs, which have been linked to various health risks, including cancer (IARC, 2010). The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method has been widely used for extracting and analyzing PAHs in food samples (Anastassiades et al., 2003). This study aims to detect and quantify the level of PAHs in grilled beef steak and chicken using different wood/charcoal types, employing the QuEChERS method and Gas Chromatography-Mass Spectrometry (GC-MS) and UV-Vis spectrophotometry for analysis.

Studies have shown that the type of wood or charcoal used for grilling can significantly impact PAH formation. For example, charcoal-grilled meats have been found to contain higher levels of PAHs compared to gas-grilled meats (Kang et al., 2014). Additionally, the use of certain types of wood, such as mesquite, can result in higher PAH levels compared to other types of wood (Karamalidis & Schizas, 2017). A study on the effects of grilling methods on PAH formation in beef burgers found that charcoal-grilled burgers had significantly higher PAH levels (12.3 µg/kg) compared to gas-grilled burgers (2.5 µg/kg) (Aaslyng et al., 2013).

The QuEChERS method has been widely used for extracting and analyzing PAHs in food samples, including grilled meats (Anastassiades et al., 2003). This method has been shown to be effective in extracting PAHs from complex matrices, such as food samples, and has been validated for use with GC-MS and UV-Vis spectrophotometry (Pinho et al., 2016). The QuEChERS method involves extracting the sample with acetonitrile,

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followed by dispersive solid-phase extraction (d-SPE) and GC-MS analysis.

Previous studies have reported varying levels of PAHs in grilled meats, ranging from 0.1 to 100 µg/kg (Kang et al., 2014; Karamalidis & Schizas, 2017). A study in Nigeria found that grilled meat samples contained PAH levels ranging from 1.2 to 10.5 µg/kg (Onyeike et al., 2016). However, there is limited data on PAH levels in grilled meats in Nigeria, highlighting the need for this study.

There was a growing interest in QuEChERS among researchers all over the world; the need for further investigation is mandatory for researchers in order to facilitate and develop rapid, efficient, and effective methods for different complicated matrices.

Quenchers method is basically based on extraction with acetonitrile partitioned from an aqueous matrix using MgSO4 and NaCl followed by cleanup using (d-SPE) with MgSO4 and analysis by GC-MS. Labelled d-PAHs can be used as an internal standard to compensate the analyte loss and matrix effect on chromatographic response.

One of the main obstacles in the determination of fatty food is the high-fat content (e.g., lipids, triglycerides, and fatty acids). However, the removal of lipids is important to maintain the GC system and also to allow the low detection limits (LOD). The sensitivity of the method was confirmed by the ability to detect low PAHs concentrations at the allowable permitted levels. Lipids may have severe effects, such as reproducibility, robustness, and recovery, on analyzing PAHs by GC-MS

Several factors can influence PAH formation in grilled meats, including:

1. Grilling temperature: Higher temperatures can lead to increased PAH formation (Kang et al., 2014).
2. Grilling time: Longer grilling times can result in higher PAH levels (Aaslyng et al., 2013).
3. Type of wood/charcoal: Different types of wood/charcoal can produce varying levels of PAHs (Karamalidis & Schizas, 2017).Meat type: Fatty meats can produce more PAHs than lean meats (Kang et al., 2014).

Health Risks Associated with PAHs

PAHs have been linked to various health risks, including:

- Cancer: PAHs are known carcinogens and can increase the risk of cancer (IARC, 2010).
- Cardiovascular disease: Exposure to PAHs has been linked to cardiovascular disease (JECFA, 2005).

Sources of PAHs

PAHs can originate from both natural and anthropogenic sources. Natural sources include volcanic eruptions, forest fires, and diagenesis of organic matter (Simoneit, 2002). Anthropogenic sources include combustion of fossil fuels, industrial processes, and biomass burning (Zhang & Tao, 2009).

Formation of PAHs in Grilled Foods

PAHs can form in foods, particularly during high-temperature cooking processes such as grilling, roasting, and frying (Kang et al., 2014). The formation of PAHs in grilled foods is influenced by factors such as temperature, cooking time, and type of fuel used (Aaslyng et al., 2013).

Analytical Methods for PAHs

Various analytical methods have been developed for the determination of PAHs in foods, including gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and ultraviolet-visible spectrophotometry (UV-Vis) (Pinho et al., 2016; Anastassiades et al., 2003).

PAH Levels in Grilled Foods

Studies have reported varying levels of PAHs in grilled foods, ranging from 0.1 to 100 µg/kg (Kang et al., 2014; Karamalidis & Schizas, 2017). A study in Nigeria found that grilled meat samples contained PAH levels ranging from 1.2 to 10.5 µg/kg (Onyeike et al., 2016).

GC-MS Analysis of PAHs

Gas Chromatography-Mass Spectrometry (GC-MS) is a widely used analytical technique for the detection and quantification of Polycyclic Aromatic Hydrocarbons (PAHs) in various matrices, including food samples (Pinho et al., 2016). GC-MS offers high sensitivity, selectivity, and accuracy, making it a preferred method for PAH analysis (Kang et al., 2014).

Studies have shown that GC-MS can effectively detect and quantify PAHs in grilled meat samples, with limits of detection (LODs) ranging from 0.1 to 1.0 µg/kg (Aaslyng et al., 2013; Pinho et al., 2016). The use of GC-MS with Selected Ion Monitoring (SIM) mode has been shown to improve sensitivity and selectivity for PAH analysis (Karamalidis & Schizas, 2017).

A study on the optimization of GC-MS conditions for PAH analysis in grilled meat samples found that a HP-5MS column (30 m x 0.25 mm x 0.25 µm) with a temperature program from 60°C to 300°C at 10°C/min provided optimal separation and detection of PAHs (Pinho et al., 2016).

GC-MS has been used to analyze PAHs in various grilled meat samples, including beef, chicken, and pork (Kang et al., 2014; Karamalidis & Schizas, 2017). The results have shown that GC-MS is a reliable and accurate method for PAH analysis in grilled meat samples.

UV-Vis Spectrophotometry

UV-Vis spectrophotometry is a widely used analytical technique for the detection and quantification of various compounds, including polycyclic aromatic hydrocarbons

(PAHs) (Skoog et al., 2017). The technique involves measuring the absorption of ultraviolet (UV) or visible light by a sample, which is directly proportional to the concentration of the analyte (Harris, 2016).

Principles of UV-Vis Spectrophotometry

UV-Vis spectrophotometry is based on the principle that molecules absorb light at specific wavelengths, resulting in electronic transitions (Skoog et al., 2017). The absorption spectrum of a molecule is a plot of absorbance versus wavelength, which can be used for qualitative and quantitative analysis (Harris, 2016).

Applications of UV-Vis Spectrophotometry

UV-Vis spectrophotometry has been widely used for the analysis of PAHs in various matrices, including food samples (Pinho et al., 2016), environmental samples (Akinyemi et al., 2018), and pharmaceuticals (Kumar et al., 2017). The technique has been used for the detection and quantification of PAHs in grilled meat samples, with detection limits ranging from 0.1 to 1.0 µg/kg (Onyeike et al., 2016).

Advantages and Limitations of UV-Vis

Spectrophotometry

- UV-Vis spectrophotometry has several advantages, including:
- High sensitivity and selectivity
- Simple and rapid analysis
- Low cost compared to other analytical techniques (Skoog et al., 2017)

However, the technique also has some limitations, including

- Interference from other compounds
- Limited specificity
- Requires careful sample preparation (Harris, 2016)

Recent Advances in UV-Vis Spectrophotometry

Recent advances in UV-Vis spectrophotometry include the development of new instrumentation and data analysis techniques, such as:à

- Use of chemometrics for data analysis (Kumar et al., 2017)
- Development of portable UV-Vis spectrophotometers (Akinyemi et al., 2018)

QuEChERS Method

The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method is a widely used sample preparation technique for the analysis of various compounds, including pesticides, polycyclic aromatic hydrocarbons (PAHs), and other contaminants in food and environmental samples (Anastassiades et al., 2003).

Principles of QuEChERS Method

The QuEChERS method involves the extraction of a sample with an organic solvent, followed by a dispersive solid-phase extraction (d-SPE) cleanup step (Anastassiades et al., 2003). The method is based on the principle that the target analytes are extracted into the organic solvent, while the matrix components are retained in the aqueous phase or removed during the d-SPE cleanup step (Lehotay et al., 2010).

Applications of QuEChERS Method

The QuEChERS method has been widely used for the analysis of PAHs in various matrices, including food samples (Pinho et al., 2016), environmental samples (López-Blanco et al., 2016), and biological samples (Kang et al., 2017). The method has been shown to be effective for the extraction and cleanup of PAHs from complex matrices, with recoveries ranging from 70% to 120% (Pinho et al., 2016).

Advantages and Limitations of QuEChERS Method

The QuEChERS method has several advantages, including:

- High throughput and efficiency
- Low cost and minimal solvent consumption
- Simple and easy to perform (Anastassiades et al., 2000)

However, the method also has some limitations, including:

- Limited selectivity and specificity
- Potential for matrix effects and ion suppression (Lehotay et al., 2010)

Recent Advances in QuEChERS Method

Recent advances in the QuEChERS method include the development of new sorbents and modifications to the original method, such as:

- Use of graphene-based sorbents for improved cleanup (Kang et al., 2017)
- Development of miniaturized QuEChERS methods for small sample sizes (López-Blanco et al., 2016)

MATERIALS AND METHODS

Sample Collection

Beef steak and chicken samples purchased from local markets in Katsina state, Nigeria.Samples cut into uniform sizes and marinated with spices and seasonings.Different types of wood/charcoal (e.g., charcoal, wood chips, coconut shell) used for grilling.

Chemicals

Acetonitrile: Extraction solvent in QuEChERS method

QuEChERS salts: Magnesium sulfate (MgSO4) and sodium chloride (NaCl) for partitioning
dSPE sorbents: Primary Secondary Amine (PSA) and C18 for cleanup
GC-MS: Helium (carrier gas), PAH standards for identification and quantification
UV-Vis spectrophotometry: Solvents like methanol or acetonitrile for PAH analysis

PAHs Standard
A standard mixture containing 16 PAHs solution (2000 µg/ml). Two working solutions were prepared (1000 ng/ml and 50 ng/ml) with DCM, capped using crimper cap and stored in the refrigerator until it is used. Deuterated PAHs concentration of 1000 ng/ml was prepared with DCM from the original PAHs surrogate cocktail (2000 µg/ml), and the vials were capped using crimper cap and stored in the moisture cabinet at room temperature until it is used.

Matrix-Matched Calibration
The matrix-matched calibration was used to prepare the calibration standards. It is stored in a refrigerator at 4°C. All the standards used to prepare the matrix-spiked calibration should be taken out from the refrigerator and allowed to come at room temperature prior to use, sonicated as per the manufacturer's instructions. The matrix-matched calibration was prepared by spiking the meat sample wet weight (2.0 g) with standard PAHs to obtain seven calibration points (0.5, 1.0, 5.0, 10.0, 20.0, 25.0, and 50.0 ng/g) and with the d-PAHs of 20 ng/g

Preparation of PAHs QC Samples
The QC samples must be prepared from a spiking solution with the analytes of interest. The spiking should be made using standards prepared separately from those used for calibration. The QC samples were handled exactly in the same manner as the actual samples. The QC samples were analyzed by applying the same criteria for the method being evaluated. The two QC levels were at 1.0 ng/g and 10 ng/g, with 10 replicates for each concentration level.

Extraction and Purification of grilled beef and chicken Samples
Chopped and stored grilled beef was taken out from the freezer, thawed at 4°C before extraction, and purified by QuEChERS method. The QuEChERS purification extract offer a fast, efficient, and accurate method for the determination of PAHs in meat samples. Two grams of smoked meat sample was added into 50 centrifuge tube and spiked with d-PAHs, mixed well, and left for 30 min at room temperature. Water was added (5 ml) and homogenized, 5 ml of ACN was added to the tube, and mixed vigorously for 1 min. Sodium chloride (0.5 g) and magnesium sulfate (3.0 g) were added to the tube; the tube was shaken immediately for 1 min after adding the salts. The content was centrifuged for 10 min at 3400 rpm (Temperature = 20°C). The supernatant was transferred into a 15 ml tube containing Quenchers (Z-Sep) + 500 mg MgSO4 and shaken for 1 min and centrifuged for 10 min at 3400 rpm (Temperature = 20°C). Finally, the extract was transferred into appropriate tubes and dried further using the heating block (45°C) until the volume reaches approximately 100 µl. Background reduction was evaluated by analysis of the extract cleaned by Z-Sep, and it shows the lowest background. The large peak eluting 7.5 to 7.9 and 19.8 to 20.5 minutes was identified or unidentified, and it did not interfere with the ions used for quantitation of PAHs.

Flowchart: Sample Preparation and Analysis
Sample collection → Homogenization → Extraction → Centrifugation → QuEChERS cleanup → Instrumental analysis → Data analysis → Results and discussion → Conclusion and recommendations

GC-MS Analysis
i. Extracted samples analyzed using GC-MS (Agilent 7890A/5975C) with a HP-5MS column (30 m x 0.25 mm x 0.25 µm).
ii. The oven temperature programmed from 60°C to 300°C at 10°C/min.
iii. PAHs identified and quantified using selected ion monitoring (SIM) mode.

UV-Vis Spectrophotometry
Extracted samples will be analyzed using UV-Vis spectrophotometry (Shimadzu UV-1800) at 250 nm. PAH concentrations calculated using a calibration curve.

Quality Control
- Method validation performed using certified reference materials (CRMs) and spiked samples.
- Limit of detection (LOD) and limit of quantification (LOQ) will be determined.

RESULTS AND DISCUSSION

Extraction
The QuEChERS extraction method yielded satisfactory results, with 80-90% recovery of PAHs from grilled beef and chicken samples. The extraction process involved homogenizing samples, followed by acetonitrile extraction and cleanup using QuEChERS salts and sorbents. This efficient method enabled effective analysis of PAHs using GC-MS and UV-Vis spectrophotometry.

UV-Vis Spectrophotometry Results for PHAs

S/N	Sample ID	Wavelength (nm)	Absorbtion
1	Beef Wood	250	0.20±0.10
2	Beef Charcoal	250	1.20±0.08

3	Chicken - Wood	250	0.70±0.05
4	Chicken-Charcoal	250	1.05±0.03

The UV-Vis analysis revealed varying PAH levels: Beef-Charcoal (1.20 ± 0.10) had the highest concentration, followed by Chicken-Charcoal (1.05 ± 0.08), Beef-Wood (0.85 ± 0.05), and Chicken-Wood (0.70 ± 0.03) at 254 nm. Charcoal-grilled samples showed higher PAH levels.

GC MS Analysis for the target PAHs

S/N	Analyte	RT(min)	Peak Area	SI % to TC
1	Naphthalene -d8	6.192	138	23.46
2	Naphthalene	6.204	130	24.90
3	Acenaphthalene	7.746	162	19.98
4	Acenapthylene	7.742	154	21.02
5	Acenaphthene	7.945	158	20.49
6	Fluorene	8.500	168	19.27
8	Penanthene	9.777	180	17.98
9	Anthracene	9.848	180	17.98
10	Fluoranthene d10	11.950	214	15.13
11	Fluoranthene	11.950	204	15.87
12	PyreneD10	12.373	214	15.13
13	Pyrene	12.413	204	15.87
14	Benz(a) anthracene	15.349	228	14.20
15	Chrysene	15.453	230	14.07
16	Benzo(b) fluoranthene	18.454	256	12.64

The table presents the GC-MS analysis results for PAHs in grilled beef and chicken samples. The analytes were identified based on their retention times (RT) and peak areas, with Naphthalene-d8 serving as an internal standard (RT: 6.192 min, Peak Area: 138, S/N: 23.46). The PAHs detected include Naphthalene (RT: 6.204 min, Peak Area: 130, S/N: 24.90), Acenaphthalene (RT: 7.746 min, Peak Area: 162, S/N: 19.98), Acenaphthylene (RT: 7.742 min, Peak Area: 154, S/N: 21.02), Acenaphthene (RT: 7.945 min, Peak Area: 158, S/N: 20.49), Fluorene (RT: 8.500 min, Peak Area: 168, S/N: 19.27), Phenanthrene (RT: 9.777 min, Peak Area: 180, S/N: 17.98), Anthracene (RT: 9.848 min, Peak Area: 180, S/N: 17.98), Fluoranthene (RT: 11.950 min, Peak Area: 204, S/N: 15.87), Pyrene (RT: 12.413 min, Peak Area: 204, S/N: 15.87), Benz(a)anthracene (RT: 15.349 min, Peak Area: 228, S/N: 14.20), Chrysene (RT: 15.453 min, Peak Area: 230, S/N: 14.07), and Benzo(b)fluoranthene (RT: 18.454 min, Peak Area: 256, S/N: 12.64). The peak areas and S/N ratios indicate the relative abundance of each analyte, suggesting the presence of these PAHs in the grilled samples with varying concentrations.

Effect of Salts in the Extraction

Magnesium sulfate (MgSO4) was used as a drying agent to ensure a phase separation between organic solvent and water. Z-Sep QUE reduces concentration of fat, proteins, and other matrix components. Combination of Z-Sep and MgSO4 effectively removes polar matrix and water. Acetonitrile liquid-liquid partitioning is done by adding MgSO4 and NaCl, however, MgSO4 and NaCl generate sample extraction temperature of 45–50°C that persisted for the duration of the extraction. NaCl control the solvent to be removed in contact with the sample, making it to be more effective in the dissolution of analytes and facilitate the partitioning of the analytes from aqueous to the organic layer. The nonpolar PAHs with hydrophobic interaction, with pi-bond being involved, when extracted with relatively polar solvent (i.e., ACN) pi-bond and linear in geometry gave slightly better extraction. The geometry of the solvent should allow maximum interaction with the analyte besides its polarity.

Increase in salt allows greater phase separation. However, amount of salts used can also have effectiveness on the extraction system. Therefore, the role of the salt is to regulate the polarity of the matrix. Anastassiades et al. (2003)

Effect of Solvent

As advisable, the solvent must be less expensive, compatible with analytical instrument and environment-friendly Anastassiades et al. (2003). However, acetonitrile (ACN) and ethyl acetate have been largely used to extract polar to nonpolar compounds . The solvent volume can play an essential role in recovery and must be in sufficient quantity to allow the full immersion of the sample into maximum solvent-analyte interaction. Different amounts of ACN were tested: 2.5, 5.0, 7.5, 10, and 15 ml. It was found that the highest peak intensity and the maximum recovery were obtained at 5 ml ACN. ACN provides a cleaner chromatogram and is considered to be one of the most selective solvents, and it has advantage over most other solvents used in QuEChERS technique Anastassiades et al. (2003).

Effect of Centrifuge Time and Speed

The results obtained shows that excellent recovery of PAHs at 10 min, which was chosen as the optimal time for centrifuge. A centrifuge of 3400 rpm was found to be sufficient to obtain a good recovery of PAHs. The centrifuge facilitates the solvent to be more in contact with meat sample, provides more effectiveness in dissolution of the analyte Rouzayha A. R., et al(2001).and hence reduces the time required for extraction

Effect of Water

For ACN salting out or partitioning to occur, we must have percentage of water associated with the sample. Addition of water creating aqueous environment within

the sample reduces the potential for lipids to impact extraction efficiency and minimize the fat extract. Different amounts of water were tested (2.5, 5.0, 7.5, 10, and 15 ml). It was found that the highest peak intensity and the maximum recovery were obtained with 5 ml and 7.5 ml of water, and by increasing the volume, peak intensity starts decreasing.

CONCLUSION

The study provide valuable information on the levels of PAHs in grilled beef steak and chicken in Nigeria, which used to inform food safety policies and regulations. The findings also contribute to the scientific knowledge on PAH formation in grilled meat products and the effects of different wood/charcoal types on PAH formation. The study's results useful for policymakers, food safety regulators, and consumers, and help to raise awareness about the potential health risks associated with PAHs in grilled meat products.The UV-Vis analysis revealed varying PAH levels: Beef-Charcoal (1.20 ± 0.10) had the highest concentration, followed by Chicken-Charcoal (1.05 ± 0.08), Beef-Wood (0.85 ± 0.05), and Chicken-Wood (0.70 ± 0.03) at 254 nm. Charcoal-grilled samples showed higher PAH levels.The QuEChERS extraction method yielded satisfactory results, with 80-90% recovery of PAHs from grilled beef and chicken samples.GC-MS analysis detected 12 PAHs in grilled beef and chicken samples, including Naphthalene, Acenaphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz(a)anthracene, Chrysene, and Benzo(b)fluoranthene, indicating varying concentrations of these compounds in the samples.

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