EVALUATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) AND HEAVY METAL CONCENTRATION IN SMOKED BEEF OBTAINED FROM BENIN CITY

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, FACULTY OF PHYSICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) HONS DEGREE IN CHEMISTRY.

CERTIFICATION

This is to certify that this project work was carried out by Paschal Chinenye Okonkwo with the matriculation number PSC1908677 under the supervision of Dr A.E. Aiwonegbe in the Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City, Nigeria.

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DEDICATION

This research work is dedicated to God Almighty, who has been my backbone of support and has helped me scale through from the beginning to the successful climax and to my parents who have been there for me financially and otherwise.

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ABSTRACT

Smoked beef is a common food sold on the streets in Nigeria, popularly known as ‘suya’. This project evaluates the levels of sixteen polycyclic aromatic hydrocarbons (PAHs) and five heavy metals in smoked beef, aiming to determine PAH and heavy metal concentrations and compare them with regulatory limits. The study employed analytical techniques including gas chromatography for PAH analysis and atomic absorption spectrometry (AAS) for heavy metal analysis. Samples of smoked beef were collected from various locations around University of Benin axis (Ekosodin, Hall 2 and Maingate) which underwent solvent extraction using a sonic bath, followed by purification with a silica gel column and were analysed via Gas Chromatography/Flame Ionization Detector for their PAH levels. For heavy metals, samples were digested with nitric and perchloric acid and then analyzed using Atomic Absorption Spectrometry. The results revealed the presence of PAHs and various heavy metals, with concentrations varying among samples showed that the PAH levels were well within the regulatory standards while some of the heavy metals were above the regulatory standards. The findings provide valuable insights into the contamination levels of smoked beef and highlight the importance of monitoring and regulating food processing practices to ensure consumer safety.

CHAPTER ONE

INTRODUCTION AND LITERATURE

1.1 Introduction

Smoking is a traditional way of processing or cooking food, especially meat and fish; this involves exposing it to a low heat smoky condition or environment using wood or charcoal, which introduces certain flavours and textures to the meat product. Smoked beef is a common food sold on the streets in Nigeria. It is popularly known as 'suya'.

Suya, a cherished delicacy which originates from Nigeria, is a traditional smoked and spiced meat treat enjoyed throughout West Africa. Made primarily from beef, ram, or chicken, it also features organ meats like kidney, liver, and tripe. Known for its affordability and widespread availability, Suya is celebrated as a unifying dish within Nigerian culture.

Polycyclic aromatic hydrocarbons (PAHs) as the name implies are a class of non-polar, colourless, organic compounds that are found naturally in coal and oil deposits and also produced by the incomplete combustion of organic matter with the simplest and most common PAH being naphtalene. They are a large family of diverse hydrocarbons that contain more than one fused aromatic rings ranging from the two-ring naphthalene and naphthalene derivatives to complex ring structures having up to 10 rings. PAHs with two and three aromatic rings are classified as low molecular weight while the ones with more than 3 ring structures are considered high molecular weight, the solubility of PAH decreases as molecular weight increases (Lamichane *et al*., 2016). Other common examples of PAH include anthracene and biphenyl.

Heavy metals can be defined as elemental metals and metalloids in the periodic table that relatively have a high density of above 5 gcm^{-3} and shows toxicity at ppm level of concentration (Manavi *et al.*, 2019). Some examples are mercury (Hg), astatine (As), lead (Pb), chromium (Cr), zinc (Zn), gold (Au), cadmium (Cd), copper (Cu), iron (Fe), platinum (Pt), manganese (Mn), nickel (Ni), palladium (Pd) etc. These are elements in the periodic table having the characteristics of a metal, an atomic number greater than 20 and also, atomic density above 5 gcm^{-3} (Ali and Khan, 2018).

These metals are dispelled into the environment through natural and human-induced sources or activities such as industrial effluent, exhaust of vehicles, and mining. Heavy metals are non-biodegradable and can remain in the environment for extended periods and also, accumulate in humans, plants and animals when inhaled or absorbed in comparison to organic pollutants (Mahmoud *et al.,* 2023).

1.2 Scope of study

Smoked beef samples used were purchased from three different locations (6.40761, 5.62355; 6.39838, 5.61967; 6.39923, 5.60880) around the University of Benin, Benin City, Edo State, Nigeria. This study focuses on determining the level of all sixteen priority PAHs and five specified heavy metals (Nickel, Cadmium, Chromium, Iron and Lead) in smoked beef samples processed by traditional methods.

1.3 Aim and objective.

This research aims to quantitatively evaluate the level of various polycyclic aromatic hydrocarbons and certain heavy metals present in smoked beef obtained from the University of Benin axis and the objectives are to

1. determine the level of all sixteen PAHs and five stated heavy metals in smoked beef
2. relate the values gotten with regulatory limits.

1.4 Background of study

Smoked beef is consumed in large quantities nationwide, but during the smoking process, PAHs and certain heavy metals can be produced and accumulated in the beef since PAH can be formed by incomplete combustion of organic compounds and occur naturally in coal and heavy metals accumulate on the beef through exhaust from vehicles or the utensil used to smoke the beef directly or other common means. Therefore, the evaluation of PAHs and heavy metals in smoked beef has become a subject of interest for food safety regulators and researchers (EU, 2015).

PAHs have huge health risks such as DNA and cellular damage and also pose a great risk of cancer. Although there's no proven safest way of preparing beef, methods such as smoking, grilling, roasting etc (direct contact with fire) have been found to contain higher levels of carcinogenic compounds such as PAHs (International Agency for Resarch on Cancer, Q&A, 2015).

According to (Rengarajan *et al*., 2015) over 70% of the PAH exposure of non-smokers is associated with food consumption.

However, with all the facts stated above, there is not enough research and information on the level of these toxic compounds especially in emergent countries such as Nigeria where smoked beef is commonly eaten, so there is a need to assess and evaluate the level of PAH in this particular food product.

Drawing from the most recent research, there has been a massive increase in the level of people getting diagnosed with cancer and there are hypotheses claiming that a major cause of the upsurge of new cancer patients might be attributed to smoked beef (Nnaji and Ekwe, 2018). Hence this research work is to try and validate and advance the current facts and claims on PAH in smoked beef. PAH level can be affected by the location of the livestock and the type of wood or charcoal that was used to smoke the beef. Hence the evaluation of the concentration of these compounds in smoked beef is very essential to creating ways that will significantly decrease the already known and potential health dangers to society and develop systems for minimizing and ensuring compliance with the approved level of PAH in smoked beef.

Polycyclic aromatic hydrocarbons (PAHs) raise substantial health concerns due to their known and potential adverse effects on human well-being. The genesis of PAHs transpires during the smoking of beef at heightened temperatures, and exposure to these compounds through smoked beef may escalate the risks of cancer and other chronic health issues. Therefore, a crucial imperative emerges in the evaluation of PAH levels in smoked beef to understand risks and institute countermeasures for minimization.

Insufficient information prevails concerning the levels of PAHs in smoked beef, chemicals that can form during the cooking process, particularly when meat is subjected to intense temperatures. These compounds have been linked to various health risks, including cancer. Evaluating PAH levels in smoked beef becomes pivotal to understanding the health risks associated with this type of food.

The absence of consistent regulations and guidelines for smoked beef products poses a challenge in ensuring their safety. Varied standards and laws across regions create confusion and inconsistency in the market, potentially endangering consumers (Otahen, 2021). Additionally, uncertainties regarding applicable regulations and the availability of reliable techniques for assessing PAHs in smoked beef increase concerns.

The primary step in addressing the issue of PAHs in smoked beef involves determining the extent of the problem, including PAH levels in different types of smoked beef products and factors contributing to their formation. Influential factors such as cooking method, temperature, meat type, and fuel used for cooking must be understood to formulate strategies for reducing PAH levels.

Enhancing the precision and quality of the methodologies used to gauge PAH levels in smoked beef is crucial. Formulating and verifying new scientific and systematic analytical techniques, such as chromatography, and developing standardized procedures for sample preparation and research are necessary for precise and uniform measurements.

Moreover, a knowledge gap between consumers and beef vendors regarding the potential health hazards linked to consuming PAH-contaminated beef poses a challenge. Many individuals may be uninformed about PAHs in smoked beef or might not know how to discern or mitigate PAH levels.

Dependable methods for appraising PAHs in smoked beef necessitate standardization and validation to ensure precision. Despite numerous techniques being devised for this purpose, such as Gas Chromatograohy (GC) and High Performance Liquid Chromatography (HPLC), standardization remains crucial.

In summary, this investigation seeks to comprehensively assess PAH levels in smoked beef, considering factors impacting PAH accumulation. Additionally, this study aims to heighten awareness about potential health risks and advocate for the implementation of regulations and monitoring programs for the safety of this food product. Addressing these issues demands cooperation among researchers, regulators, and the food industry to guarantee the safety and quality of smoked beef.

Heavy metals in smoked beef are of huge concern in regions where it is consumed in large quantities like Nigeria due to the potential health issues associated with heavy metals. Heavy metals such as lead, cadmium, mercury and arsenic are generally considered toxic to humans, others such as iron, copper, zinc, and manganese can be beneficial to the body for normal physical, chemical and biochemical processes (Sarkingobir *et al.,* 2022). However, at a certain level of concentration all heavy metals could be toxic and exposure to these metals may cause adverse health effects. Heavy metals can bioaccumulate in human tissues and cause health risks even when consumed at low levels. For example, cadmium (Cd) exerts toxic effects on the kidney and respiratory systems and also referred to as a human carcinogen (WHO, 2019), copper (Cu) has been previously linked to liver toxicity and neurological problems (Karim, 2018) while zinc (Zn) toxicity may lead to anaemia and sluggishness (Fairweather-Tait and De Sesmaisons, 2018). Lead (Pb) toxicity is the cause of several ailments such as Vitamin-D deficiency and cancer of the skin and lungs, it also affects the human nervous system (Rehman *et al.,* 2017).

Therefore, the need to monitor and constantly assess the concentration level of heavy metals in smoked beef to ensure these metals are within safe and acceptable limits. Also, the desire for a safe environment resulted in the evaluation of heavy metals in smoked beef which might have accumulated on the raw beef, or the live cattle.

1.5 Justification/Relevance of study

Polycyclic aromatic hydrocarbons (PAHs) and heavy metals, acknowledged for their toxicity at certain concentration levels, can induce adverse health outcomes such as cancer, reproductive disorders, and neurological impairments. Research has spotlighted smoked beef products as prominent bearers of elevated levels of these substances, with some of them receiving classification by the International Agency for Research on Cancer (IARC) as carcinogenic to humans.

Consequently, the evaluation of PAHs and heavy metals in smoked beef products emerges as imperative and very essential for ensuring food safety and upholding public health. A regular and standardized monitoring protocol for PAH and heavy metal levels in smoked beef products is requisite, demanding methods of assessment that are both accurate and uniform.

The revelations stemming from this study are poised to wield significant influence in the formulation of global regulations and guidelines for PAHs and heavy metals in smoked beef products, a crucial way of fortifying consumer safety. Policymakers stand to gain crucial insights into policies and regulations aimed at curtailing the concentration of these substances in smoked beef products. This proactive stance not only advances public health objectives but also helps to avert the latent health risks entwined with the consumption of food products containing concerning levels of such substances.

1.6 Literature review

1.6.1. Introduction to beef and nutrients contained

In moderation, beef consumption can positively impact muscle growth and maintenance. However, excessive intake of beef has been associated with an elevated risk of heart disease and cancer (Atli Arnarson, 2023). Beef stands as a pivotal reservoir of both macronutrients and micronutrients, fostering a nutritional profile rich in significance. Among the macronutrients, one encounters water content alongside high-value proteins and fats, encompassing both saturated and unsaturated fatty acids. For the micronutrient, beef and its edibles provide a spectrum featuring haeme iron, zinc, selenium, and an array of vitamins including D, B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine) and B12 (cobalamin) (Chunbao Li, 2017).

Nutrition facts for 100g of beef are :

Calories: 217

Water: 61%

Protein: 26.1 grams

Carbs: 0 grams

Sugar: 0 grams

Fiber: 0 grams

Fat: 11.8 grams

All of these nutrients assume a very important role in orchestrating human physiological functions, and any deficiency within this complex mix of nutrients can lead to health issues. The connection between the various nutrients in beef and overall human health highlights the importance of considering this food as a nutritional powerhouse. Apart from being nutritious, beef has a fascinating history, connecting ancient traditions to today's diverse culinary styles, and capturing changes in society and culture. Whether it's juicy steaks or hearty stews, beef's ability to be used in various dishes is limitless. Exploring beef goes beyond enjoying tasty meals; it reveals a rich culinary story echoing through time and across different cultures.

1.6.2. Why smoked beef

The smoking of meat has become increasingly popular as a food preparation method although this is one of the first food preparation techniques known to man. Beyond its historical roots, smoking also adds a special type of flavour to the meat and also adds very little preservation effect.

1.6.3. Introduction to PAH

Polycyclic aromatic hydrocarbons (PAHs), which contain fused aromatic rings, can be obtained as byproducts from incomplete combustion of fossil fuels, oil spills, and other organic compounds. PAHs, or polycyclic aromatic hydrocarbons, are organic pollutants. They're made up of two or more fused benzene rings arranged in different ways, *(*Blumer *et al.,* 1977). What's interesting is that these compounds don't have other types of atoms or extra parts attached, hence they are kept pure without extra parts, enhancing their simplicity. This straightforward molecular composition might seem simple, but it hides its significant impact on the environment.

Naphthalene stands as the archetypal, simplest PAH in Table 1. Remarkably, its low molecular weight counterparts, featuring 2–3 rings like anthracene, phenanthrene, and phenalene, wield sharp toxicity without inducing carcinogenic effects in the majority of organisms. (Van-Huy Nguyen *et al.,* 2020).

These compounds manifest in the form of colourless, white, or pale yellow-green solids, accompanied by a subtle, pleasant fragrance (Mindjimba, 2020). PAHs, comprising solely hydrogen and carbon, derive their physical and chemical traits from the structural nuances and sizes inherent in each PAH unit (Beran, 2018). Within the environmental regulatory framework, the United States Environmental Protection Agency (USEPA) oversees sixteen priority PAHs, strategically addressing their impact on both human health and ecological well-being. The robust chemical stability, limited water solubility, and pronounced sorption capacity collectively contribute to the remarkable persistence of PAHs within the ecosystem.

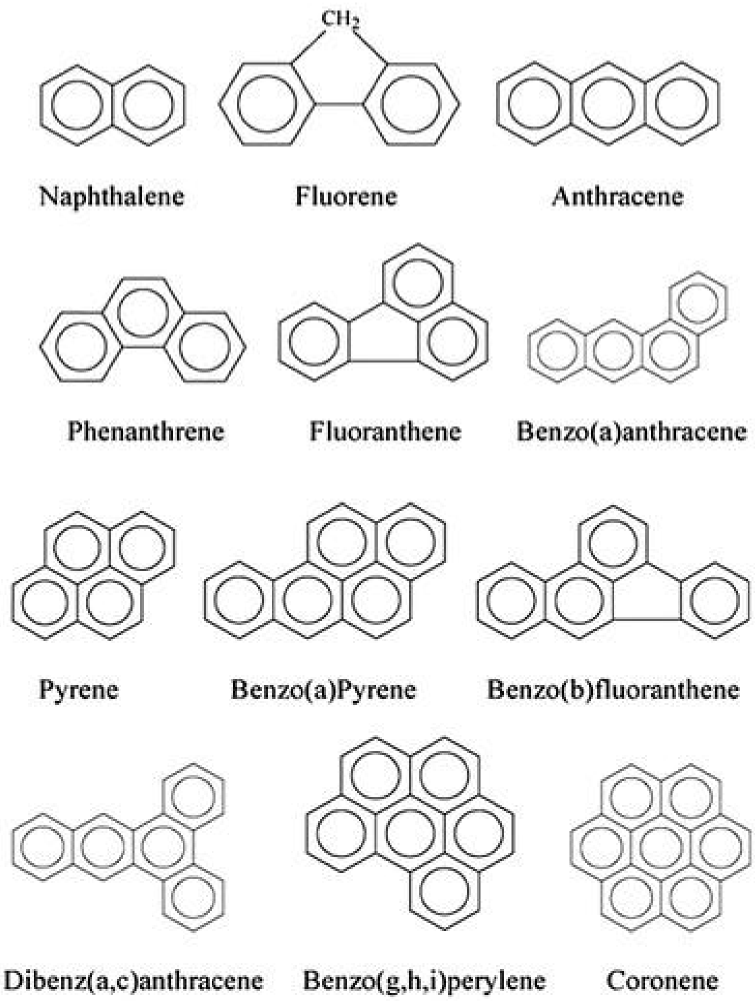


Fig 1.0 Structures of commonly found Polycyclic Aromatic Hydrocarbons

Types of PAHs

1. Pyrogenic Polycyclic Aromatic Hydrocarbons:

Pyrogenic polycyclic aromatic hydrocarbons (PAHs) are born from processes that involve the generation or production of heat. These compounds take shape when organic substances undergo exposure to elevated temperatures in an environment characterized by low oxygen levels. The pyrogenic journey unfolds within a temperature range spanning from about 350°C to a scorching 1200°C or more. These PAHs find their genesis in incomplete combustion processes, ranging from the burning of coal and crude oil to the ignition of petroleum used in motor fuels and trucks.

2.Petrogenic Polycyclic Aromatic Hydrocarbon:

Petrogenic polycyclic aromatic hydrocarbons (PAHs) find their home in petroleum and its by-products, a presence that extends through the realms of storage, transport, utilization, and unintended leakage associated with crude oil and its derivatives (Abdel-Shafy *et al,* 2016). The formation of these petrogenic PAHs is entwined with the intricate processes and maturation stages of crude oil, spanning from oceanic environments to freshwater ecosystems and the aftermath of oil spills. The presence of these compounds holds implications for the well-being of aquatic life, particularly freshwater fish, as they navigate the intricate challenges posed by petrogenic PAHs originating from crude oil.

3. Biological Polycyclic Aromatic Hydrocarbons:

Biogenic polycyclic aromatic hydrocarbons (PAHs) are produced by living organisms such as microorganisms, phytoplankton, algae, and plants through the gradual biological conversion of organic materials (Mojiri *et al,* 2019). In this natural process, these organisms play a role in shaping the composition of biogenic PAHs without the need for artistic metaphors.

Sources of PAHs

The roots of PAH pollution stem from two primary categories: anthropogenic emission sources and natural emission sources (Mojiri *et al.,* 2019). In the realm of natural emissions, events such as volcanic eruptions, natural forest fires, and moorland fires ignited by lightning flashes hold a marginal or less significant role (Mansuer, 2016).

However, the major contributors to PAH pollution lie within the domain of anthropogenic emission sources, neatly categorized into four types: industrial, mobile, domestic, and agricultural. This classification highlights the human-driven activities that play a pivotal role in the proliferation of PAHs in the environment.

PAH emissions, stemming from incomplete combustion, represent a prevalent concern attributed to diverse industrial activities. Sectors such as waste incineration, iron and steel production, aluminium production, cement manufacturing, coal-tar pitch production, dye manufacturing, rubber tyre manufacturing, fungicide and insecticide production, flares/exhaust from refineries, and power production contribute significantly to this source of environmental contamination (Abdel-Shafy and Mansuer, 2016; Gupte *et al.,* 2016; Mojiri *et al.,* 2019). The expansive range of industries implicated underscores the imperative to address incomplete combustion as a key player in the emission dynamics of PAHs.

Mobile emission sources cast a broad net, encompassing the exhaust fumes emanating from vehicles, including aircraft, ships, trains, and both heavyweight and lightweight off-road vehicles.

For domestic emissions, routine household activities emerge as contributors. These include burning garbage, coal coking, wood combustion, cooking on oil or gas burners, and the use of kerosene or wood stoves, alongside various other forms of residential heating (Gupte *et al,* 2016).

Agricultural emission sources hinge on practices such as open biomass burning and the incineration of agricultural waste, particularly when these activities unfold under suboptimal combustion conditions. Notably, high PAH pollution in rural areas primarily stems from domestic and agricultural sources, whereas in urban areas, the interplay involves industrial, mobile, and domestic contributors. The nuanced dynamics of these sources unveil the multifaceted origins of PAH emissions, painting a comprehensive picture of these diverse culprits.

Exposure to PAH

In the present scenario, encountering PAHs is practically inevitable. The avenues through which PAH exposure unfolds primarily include inhalation, ingestion, and dermal contact. Interestingly, exposure can also manifest through a combination of routes, like simultaneous dermal and inhalation exposures from contaminated soil (Rengarajan *et al.,* 2015; Abdel-Shafy and Mansuer, 2016).

Workplaces often serve as hotspots for exposure, involving individuals in settings like coke manufacturing factories and food processing industries. For instance, workers inhaling vehicle exhaust and road dust laden with PAHs, such as traffic police, encounter exposure on the job. However, exposure isn't confined to workplaces; it extends to daily activities such as consuming polluted water, indulging in grilled or smoked food items, and smoking (Suman *et al*., 2016). Remarkably, a single cigarette for smokers results in an intake of 20–40 ng of benzo(a)pyrene

Additionally, up to 70% of PAH exposure for non-smokers can be traced back to their diet. This intricate web of exposure pathways underlines the pervasive nature of PAH encounters in our daily lives. PAHs usually hang out in drinking water at low concentrations. However, there's a notable risk of contamination when you consume it. The global concern for food safety is on the rise, and one specific worry is the residues of PAHs. If these residues surpass the recommended levels, it becomes a serious public health concern. In the realm of low concentrations, the potential impact on our well-being becomes a significant consideration.

Effects of PAH

PAHs, or polycyclic aromatic hydrocarbons, pose significant threats to both the environment and human health. These compounds can lead to a range of health issues, including carcinogenesis, skin problems, respiratory challenges, and impacts on genetic, developmental, and organ systems. PAH pollutants are identified as highly toxic, mutagenic, carcinogenic, and immunotoxicogenic, affecting microorganisms, animals, and humans alike.

Exposure to PAHs can result in both short-term and long-term health effects. Short-term effects encompass eye and skin irritation, nausea, vomiting, and inflammation. On the other hand, long-term exposure may lead to gene mutations, cellular damage, and the development of cancers in the skin, lungs, bladder, and gastrointestinal tract. Additionally, extended exposure to PAHs is suspected to elevate the risk of cell damage through gene mutation. This dual threat of environmental and health concerns calls for heightened awareness and preventive measures in managing PAH exposure.

Extraction and analysis

The process of PAH determination involves several sequential steps, commencing with the solvent extraction of polycyclic aromatic hydrocarbons (PAHs) from sorbents. Subsequent stages encompass the concentration of the extracted material, chromatographic clean-up, eluent concentration, and eventual injection into a gas chromatograph for comprehensive analysis.

A thorough examination reveals that prevalent methods for extracting PAHs from food matrices predominantly employ techniques such as ultrasonic extraction, soxhlet extraction, and mechanical shaking. The subsequent characterization of PAHs in environmental samples relies on a combination of extraction and detection methodologies. Standard analytical procedures entail the extraction and purification of samples using organic solvents, followed by the analysis of PAH content through chromatographic techniques. This systematic approach ensures a meticulous and accurate determination of PAHs in diverse matrices.

1.6.4. Heavy metals

Metals and metalloids of high atomic mass and density are regarded as heavy metals. Heavy metals are toxic elements at low concentrations. There’s no general or unified definition of heavy metals, it is either a toxic or relatively dense metal (Ali and Khan, 2018) but some such as lead and mercury which are both dense and toxic. Generally, lead and mercury are agreed to be heavy metals based on the fact that they are both dense and heavy whereas other heavy metals such as gold, are dense yet not particularly toxic. Some researchers classified these metals as "heavy metals" based on their density, while others excluded them from the list because they do not pose a risk or a major health hazard (Helmenstine, 2021).

Heavy metals are elements that are present and occur naturally on Earth and have the capacity to penetrate the environment through natural processes such as volcanic eruptions, weathering of rocks, erosion of soil, etc. (Hazrat *et al.,* 2018), as well as anthropogenic activities which includes pollutions, industrial operations, agricultural practices, technological applications and mining (Khan, 2023). Examples of heavy metals include lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), gallium (Ga), zinc (Zn), copper (Cu), cobalt (Co), nickel (Ni) etc. Some heavy metals are typically essential and required for life processes when present in low concentrations and they comprise iron, copper, cobalt, zinc etc or relatively harmless such as gold, silver, gallium, etc. but can be toxic in larger amounts or certain compounds and forms (Abdullahi *et al.,* 2022; bobir *et al.,* 2022). Other heavy metals like cadmium, mercury, lead, chromium etc. are toxic at low concentrations and are of no beneficial use to the human system (Balali-Mood *et al.,* 2021).

The high toxicity, contamination, concealment, persistence and biological accumulation of heavy metals in the environment and humans have drawn the attention of many researchers and environmental bodies (Aguilera *et al.,* 2021). The contamination of these metals is a major problem for the environment and humans especially in growing towns and cities of most developing countries primarily due to uncontrolled levels of pollution which comes with an increase in population driven by causative factors like industrial growth and an increase in the numbers of people using petroleum fuels (Sardar *et al.,* 2013). Exposure to these metals via ingestion, inhalation and dermal contact poses an adverse effect on human health. Heavy metal of different elements induces distinct toxicities; therefore, the adverse health effects of these heavy metals are distinctive with the exposure routes of the toxicant and the organs they affect (Mahmoud *et al.,* 2023).

Lead (Pb) is the most important and prevalent toxic heavy metal that can be found in the environment. It is a metal of high toxicity which affects most organs of the body majorly the nervous system (Wani *et al.,* 2015). Lead toxicity affects both adults and children, however, the threat to the health of children is greater than that of adults, due to children's internal and external soft tissues (Wani *et al.,* 2015). Some long-term side effects of lead poisoning include abdominal pain, constipation, kidney dysfunction, damage to the reproductive system etc. (Balali-Mood *et al.,* 2021).

Cadmium (Cd) is a heavy metal of major concern in the environment. Cd with a density of 8.6 gcm^{-3} is a heavy metal that is widely distributed and released into the environment from metallurgical industries, heating systems, trash incinerators, power plants, urban traffic, phosphate fertilizer products and cement work (WHO, 2019). Cadmium can be released from the mineralization of rocks in areas with low artificial pressure. Exposure and accumulation of cadmium negatively affect several organs such as the liver, kidneys, lungs, bones, central nervous system, and respiratory system in the human system (Lar *et al.,* 2015; WHO, 2019).

Chromium (Cr) is an important heavy metal present in the environment at high concentrations and can cause harm. Chromium is considered a serious environmental pollutant due to industrial usage and expansion in particular chemical industries (Usman *et al.,* 2023). The toxicity of chromium to plants and animals depends on its chemical state; Chromium (VI) is highly toxic and mobile while chromium (III) is less toxic (Usman *et al.,* 2023). The potential capability of plants accumulating or stabilizing chromium compounds for bioremediation of chromium pollution has attracted increasing interest in recent years. Chromium exists naturally in the trivalent form chromium (III), other environmental sources of chromium are cement factories (cement contains chromium), wear and tear of asbestos coatings containing chromium, emissions from chromium-based automotive catalytic converters, and cigarette smoke (ATSDR, 2008). Because chromium (III) is poorly absorbed by any route, chromium toxicity is mainly due to chromium in the form of chromium (VI). It can be absorbed through the lungs and gastrointestinal tract, and even to some extent through the skin (ATSDR, 2008). Human exposure to chromium has been found to cause respiratory, cancer, kidney and diseases of the skin etc.

Inhalation is the primary means of nickel (Ni) exposure, its carcinogenic effects extend beyond the development of lung and nasal cancers which also include multiple epigenetic effects (Genchi *et al.,* 2020). Whereas dermatitis, gastrointestinal symptoms, lung fibrosis, and cardiovascular disorders are non-carcinogenic side effects of nickel toxicity (Genchi *et al.,* 2020).

Research has indicated that exposure to heavy metals, regardless of the organs they affect, eventually results in the generation of a reactive oxygen species, which causes oxidative stress (Vyas *et al.,* 2017). This then results in various cancers, neurological conditions, damage to the kidneys, and other abnormalities related to the endocrine system. All the above-listed heavy metals have become more intense and of great concern with an increase in population and activities occurring in the environment which poses a risk to human health.

1.6.5. Atomic Absorption Spectroscopy (AAS)

Spectroscopy is the study of absorption and emission of electromagnetic radiation (EMR) due to the interaction between energy and matter, which depends on the wavelength of the radiation absorbed. Atomic Absorption Spectroscopy (AAS) is one of the spectroscopic and analytical techniques used for the qualitative and quantitative determination of elements majorly metals utilizing the absorption of EMR at a particular wavelength, usually in the ultraviolet or visible region by free atoms in the gaseous state. Atomic Absorption Spectroscopy (AAS) is utilized across many countries, industries and is instrumental in the detection of metals in a sample. As such, this technique is commonly used in pharmacology, archaeology, manufacturing, mining, and forensics etc. (García and Báez, 2012).

1.6.6. Flame Atomic Absorption Spectroscopy (FAAS)

Flame atomic absorption spectroscopy (FAAS), is a popular technique with a long history of usage, which until recently was widely used for the analysis of trace metals. The analyte (sample) usually present in solution, must first be atomized over a flame. The sample solution is drawn through a small tube and transported to the nebulizer, where it is divided into a fine aerosol. The aerosol is then transported to the flame by the carrier gas and separated into individual atoms. FAAS can be used to analyze a large number of trace metals in different sample matrices (Mariella, 2019). The advantages of using FAAS are the accuracy of results, high productivity of samples, low cost of running its operations and high level of technical know-how of the instrument operations is not required (Miiro and Muhammad, 2024).

1.6.7. Components of Atomic Absorption Spectroscopy

Light source

Atomizer

Nebulizer

Monochromator

Multiplier

Readout device/recorder

CHAPTER TWO

MATERIALS AND METHOD

2.1 Material

2.1.1 Apparatus

Mortar and pestle

Beaker

Volumetric flask

Weighing balance

Spatula

Measuring cylinder

Stirrer

Funnel

Filter paper

Sample bottles

Oxygen evaporator

Conical flask

Cotton wool

Retort stand

Dropping pipette

Blow down block

Wash bottle

Digestion tube

Bandelin Sonorex Ultra-sonic bath

Buck Scientific VGP 210 Atomic Absorption Spectrophotometer (AAS).

HP 5890 series II Plus Gas chromatography flame ionization detector

2.1.2 Reagents

All the chemicals and reagents used were of analytical grade and obtained from the school chemical store.

Anhydrous sodium sulphate

Silica Gel

N- Hexane

Nitric acid, HNO_3

Perchloric acid, $HClO_4$

Distilled water

2.2. Methodology

2.2.1. Sample preparation for PAH analysis

The study was carried out across three sites around UNIBEN axis (Hall 2, Ekodosin and Maingate) in Benin City, Edo state. The samples were grounded separately to give six samples which were labelled SA1, SA2, SB1, SB2, SC1 and SC2, to ensure homogeneity.

2.2.2.Extraction process

The extraction method utilized was solvent extraction using a sonic bath. The smoked beef purchased from the three stated locations was subjected to several analyses. To increase the surface area for extraction, the samples were blended into a powdered form. A 5 g portion of the powdered samples was weighed, and approximately 3 g of sodium sulfate was added to absorb any moisture present, after which the samples were shaken. Subsequently, 15 ml of n-hexane was added to the samples, which were then placed in a sonic bath for 15 minutes to facilitate proper extraction of PAHs. The resulting mixture was filtered into small bottles. Furthermore, the solvent mixture was taken to a blow-down block to reduce the solvent level of the samples to 0.5 ml.

Before introducing the solvent samples, the syringe was cleaned with n-hexane to prevent any errors. The pressure of the blow-down block was also verified, as it emitted a gentle stream of oxygen into the samples.

Plate 2.1: Samples in a Sonic bath

2.2.3 Cleanup process

First, set up the retort stand and column. Inside the column, place a small amount of cotton followed by 13 g of silica gel, which acts as the stationary phase, along with a small quantity of sodium sulfate to eliminate moisture molecules.

The solvent samples were then separated using a column packed with 13 g of silica gel and sodium sulfate, with 15 ml of n-hexane used as the eluting solvent to obtain clean solvent samples. These clean solvent samples were then taken to the blow-down block to be dried.

Following drying, the samples underwent analysis for polycyclic aromatic hydrocarbons using Gas Chromatography/Flame Ionization Detector.

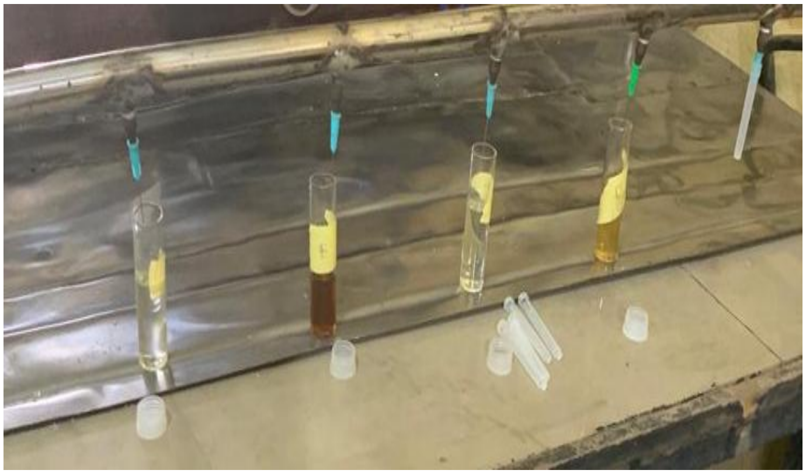


Plate 2.2 Blowdown process

Plate 2.3 Gas Chromatography/Flame Detector.

Table 2.1: Gas Chromatography/ Flame Detector

COLUMN	HP-5, 0.32 mm ID x 0. 5 µm, 30 m
INJECTOR TEMPERATURE	250 ℃
MOBILE PHASE	NITROGEN GAS
DETECTOR TEMPERATURE	350 ℃
INJECTION VOLUME	1 µL
DETECTOR	FID
OVEN PROGRAMME	Oven temperature set at 100 ℃ and held for 1 minute, then ramped to a rate of 4 ℃/min to 200 ℃ and held for 1 minute and finally ramped at 5 ℃/min to 320 ℃. Total run time of 51 minutes

2.2.4 Sample preparation for heavy metals analysis

The study was carried out across three sites around UNIBEN axis (Hall 2, Ekodosin and Maingate) in Benin City, Edo state. The samples were grounded separately to give nine samples which were labelled SA1, SA2, SA3, SB1, SB2, SB3, SC1, SC2 and SC3 to ensure homogeneity.

2.2.5 Digestion process

1 g of each sample was weighed and poured into a digestion flask, then nitric acid and perchloric acid was mixed in a ratio of **3:1** to get an acid mixture and 10 ml of the mixture was poured into each digestion flask containing the 1 g of sample.

1 g of each sample was weighed and poured into a digestion flask, then nitric acid and perchloric acid was mixed in a ratio of **3:1** to get an acid mixture and 10 ml of the mixture was poured into each digestion flask containing the 1 g of sample.

The flasks were placed on a block digester for heat digestion, boiling gently until a white dense fume appeared or a clear solution was obtained.

After allowing the digested sample to cool slightly, 20 ml of distilled water was added. After it cooled, the solution was filtered and transferred into a 100 ml volumetric flask.

Additional distilled water was added to the solution to make up to the 100 ml mark.

The filtered solution was then poured into a clean sample bottle and stored properly for future analysis. The resulting digested sample solutions were then analyzed for Lead (Pb), Copper (Cu), Zinc (Zn), Cadmium (Cd) and Nickel (Ni) concentrations using Atomic Absorption Spectrometry.

CHAPTER THREE

RESULTS AND DISCUSSION

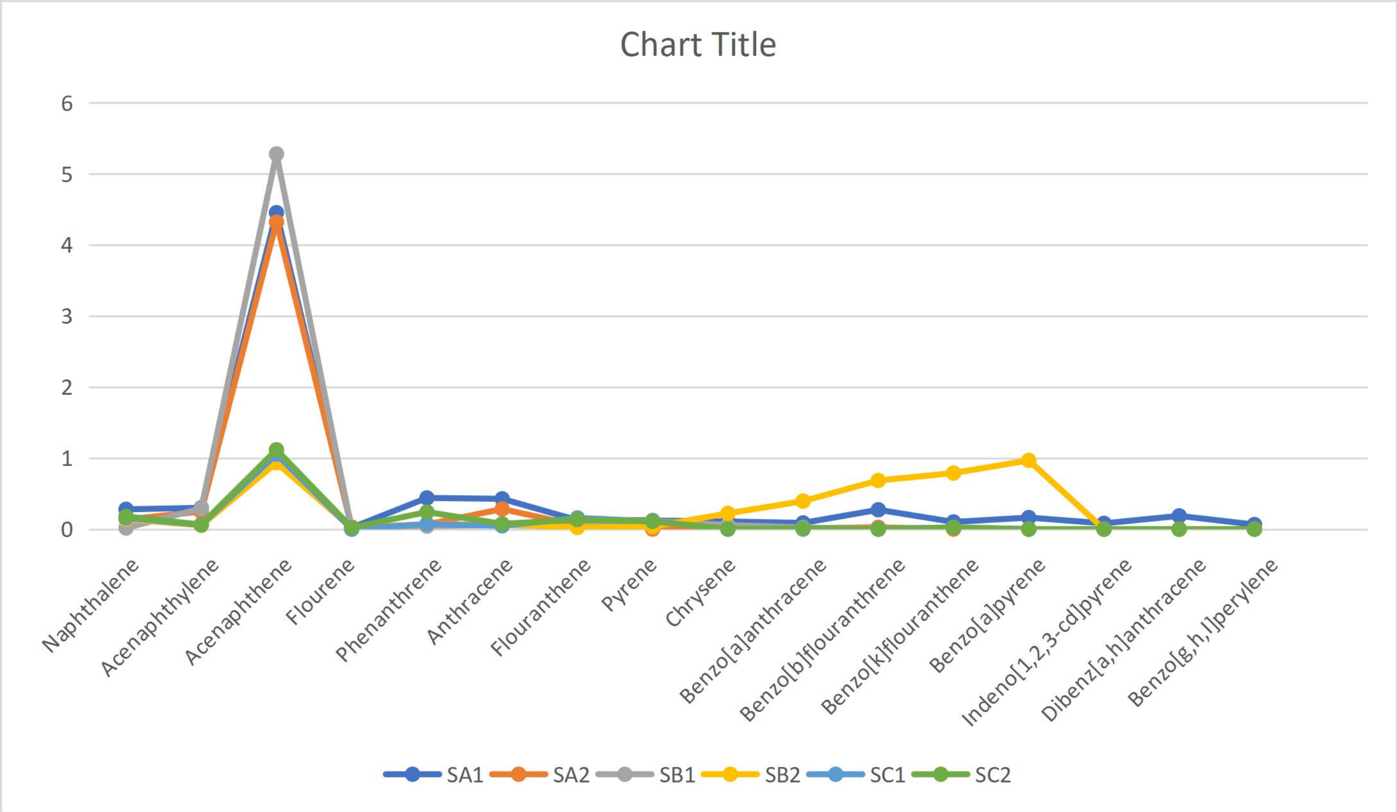
3.1 Results

The purpose of this study was to investigate the levels of Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metals in smoked beef from three locations across Uniben axis, Edo State. The findings from this research are presented in the tables below:

Table 3.1. Levels of PAHs obtained:

			Concentratio	**of PAH**		
			(µg/Kg)			
PAH	**SA1**	**SA2**	**SB1**	**SB2**	**SC1**	**SC2**
Naphthalene	0.27867	0.13543	0.01546	0.13936	0.15693	0.17801
Acenaphthylene	0.29976	0.24739	0.29072	0.05215	0.05978	0.06416
Acenaphthene	4.45351	4.32111	5.27936	0.93412	1.05417	1.11637
Flourene	0.02032	0.02836	<0.00000	0.01631	<0.01000	0.02075
Phenanthrene	0.43883	0.06600	0.04224	0.06769	0.07037	0.24016
Anthracene	0.42623	0.28285	0.05875	0.06457	0.04615	0.07430
Flouranthene	0.1255	0.05162	0.02956	0.02336	0.15728	0.13426
Pyrene	0.12019	<0.01000	0.11263	0.03449	0.11107	0.10931
Chrysene	0.10753	0.04989	0.05818	0.22147	<0.01000	<0.01000
Benzo[a]anthracene	0.08747	0.01147	0.02368	0.39410	<0.01000	0.01129
Benzo[b]flouranthrene	0.27189	0.02545	<0.01000	0.68396	<0.01000	<0.01000
Benzo[k]flouranthene	0.10100	<0.01000	0.02521	0.78881	0.02145	0.02205

Benzo[a]pyrene	0.16236	<0.01000	<0.01000	0.96686	<0.01000	<0.01000
Indeno[1,2,3-cd]pyrene	0.08108	<0.01000	<0.01000	<0.01000	<0.01000	<0.01000
Dibenz[a,h]anthracene	0.18633	<0.01000	<0.01000	<0.01000	<0.01000	<0.01000
Benzo[g,h,I]perylene	0.06404	<0.01000	<0.01000	<0.01000	<0.01000	<0.01000
∑PAH	7.22470	5.21960	5.93580	4.38720	1.67720	1.97070



Tables 3.2 Levels of Heavy Metals obtained

	Concentration	**of metals**	**(mg/L)**		
SAMPLE					
	Nickel (**Ni)**	Cadmium (**Cd)**	Chromium (**Cr**)	Iron (**Fe**)	Lead (**Pb**)
SA1	0.06	0.00	0.12	3.50	0.00
SA2	0.06	0.00	0.21	14.90	0.00
SA3	0.05	0.00	0.24	6.40	0.00
SB1	0.02	0.00	0.09	5.10	0.00
SB2	0.00	0.00	0.15	2.90	0.00
SB3	0.05	0.00	0.19	5.30	0.00
SC1	0.03	0.01	0.18	5.70	0.00
SC2	0.02	0.01	0.09	4.30	0.00
SC3	0.03	0.00	0.14	5.50	0.00

SAMPLE	**Concentration**	**of metals**	**(mg/Kg)**		
	Nickel (**Ni)**	Cadmium (**Cd)**	Chromium (**Cr**)	Iron (**Fe**)	Lead (**Pb**)
SA1	6.0	0.0	12.0	350.0	0.0
SA2	6.0	0.0	21.0	1490.0	0.0
SA3	5.0	0.0	24.0	640.0	0.0
SB1	2.0	0.0	9.0	510.0	0.0
SB2	0.0	0.0	15.0	290.0	0.0
SB3	5.0	0.0	19.0	530.0	0.0
SC1	3.0	1.0	18.0	570.0	0.0
SC2	2.0	1.0	9.0	430.0	0.0
SC3	3.0	0.0	14.0	550.0	0.0
FOA & WHO recommended levels (Mensah *et al* 2009)	67.9	0.2	-	425.5	0.3

3.2 Discussion

Table 3.1 shows the concentrations of various polycyclic aromatic hydrocarbons (PAHs) in smoked beef samples obtained. The following can be observed from the table

Variability in PAH Concentrations: The concentrations of certain PAHs vary significantly across samples. For instance, Acenaphthene shows a wide range of concentrations, from 0.93412 µg/kg in SB2 to 5.27936 µg/kg in SB1, indicating potential differences in smoking methods, duration and beef source.

Presence of Detected and Undetected Compounds: Some PAHs, such as Fluorene and Benzo[a]pyrene, were detected in all samples, while others, like Pyrene and Dibenz[a,h]anthracene, were not detected (below the detection limit) in certain samples.

Overall PAH Content: The sum of PAH concentrations (∑PAH) in each sample varies, reflecting the total PAH contamination level. For instance, sample SA1 has the highest ∑PAH concentration of 7.2247 µg/kg, while sample SC1 has the lowest concentration of 1.6772 µg/kg.

A regulation amendment was done by the COMMISSION REGULATION (EU) regarding the maximum levels of possible carcinogenic polycyclic aromatic hydrocarbons (PAHs) in traditionally smoked meat and smoked meat products ((EU) COMMISSION REGULATION, 2020*)*. The maximum accepted level for these products after the amendment is 5.0 µg/kg for benzo(a)pyrene and 30.0 µg/kg for the sum of benzo(a)pyrene, benz (a)anthracene, benzo(b)fluoranthene and chrysene.

Comparing our results with the regulated levels provided by the EU

Table 3.3 Comparison of benzo(a)pyrene

Sample	Results (µg/kg)	EU levels (µg/kg)
SA1	0.16	5.00
SA2	<0.01	5.00
SB1	<0.01	5.00
SB2	0.97	5.00
SC1	<0.01	5.00
SC2	<0.01	5.00

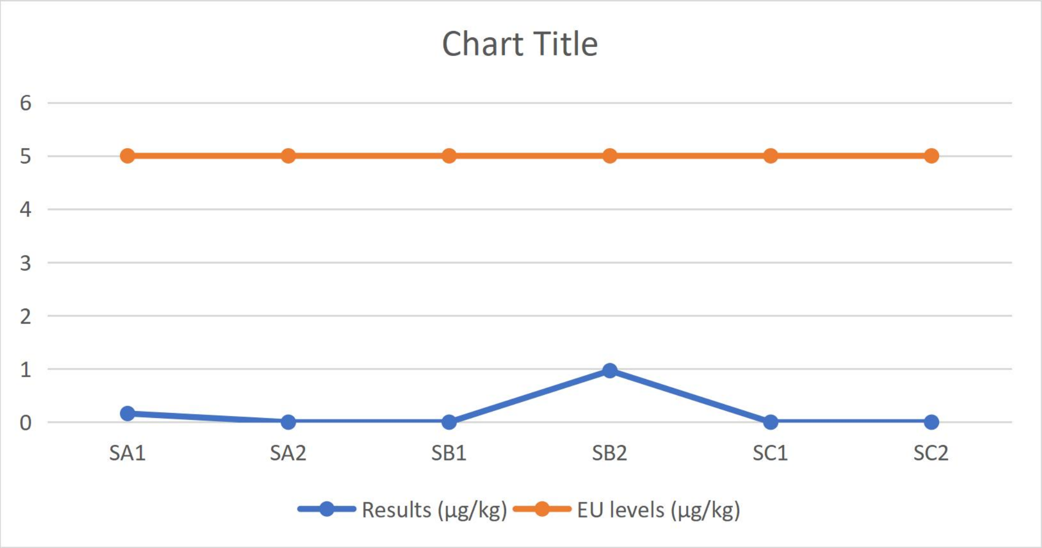
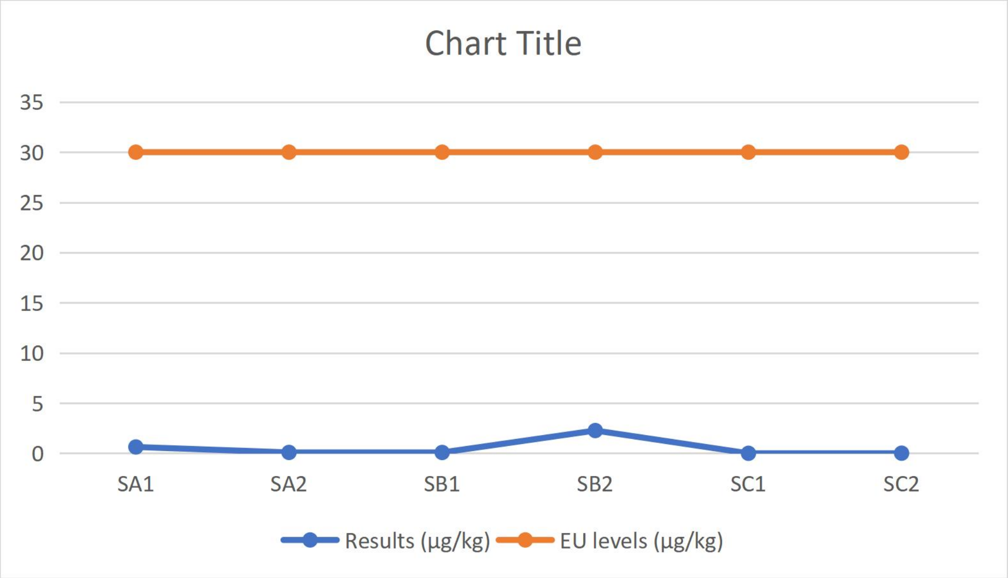


Table 3.4 Comparison of the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene.

Sample	Results (µg/kg)	EU levels (µg/kg)
SA1	0.63	30.00
SA2	0.09	30.00
SB1	0.08	30.00
SB2	2.27	30.00
SC1	<0.01	30.00
SC2	0.01	30.00



When compared with standards to assess health risks the following were observed

Comparison with EU Levels: For example, Benzo[a]pyrene which is considered highly carcinogenic, the concentrations in all samples are compared with the EU maximum level of 5.0 µg/kg. None of the samples exceed this limit, indicating minimal potential health concerns.

Comparison with Sum of Specific PAHs: Additionally, the sum of Benzo[a]pyrene, Benz[a]anthracene, Benzo[b]fluoranthene, and Chrysene concentrations are compared with the EU maximum level of 30.0 µg/kg. None of the samples were observed to exceed this limit, suggesting littile exposure to these PAH compounds.

Some studies have shown strategies to reduce food contamination with PAHs which include

> Marinating meat using ingredients like garlic and onion, which possess antioxidants, can hinder the creation of PAH (Da Paz *et al.,* 2017).
>
> Alternative cooking techniques can decrease the formation of PAHs in cooked foods. These methods involve cooking at lower heat levels and minimizing or eliminating direct food contact with flames. (Banzal and Kim, 2015).
>
> The levels of PAHs were observed to decrease by about 30% in the samples of some fish species that were washed. (Mahugija, 2018; Njale,2018).

Concerning the concentrations of heavy metals;

Table 3.2 presents the concentrations of various heavy metals, including Nickel (Ni), Cadmium (Cd), Chromium (Cr), Iron (Fe), and Lead (Pb), in smoked beef samples collected from previously discussed sources. The following observations were found;

Variability in metal concentrations: The concentrations of heavy metals vary among the samples, indicating differences in the sources of contamination and possibly the geographic locations where the beef was sourced.

Presence of Detected and Undetected Metals: Some samples have metal concentrations below the detection limit, suggesting minimal or absent contamination of those metals in those samples. For example, cadmium and lead concentrations are reported as <0.00 mg/kg in several samples.

Lead Contamination: Notably, lead concentrations are reported as 0.00 mg/kg in all samples, indicating compliance with regulatory limits. Lead is of particular concern due to its toxic effects on neurological development, especially in children.

The measured metal concentrations can be compared with recommended levels established by regulatory authorities or international health organizations. These comparisons help assess the potential health risks associated with consuming smoked beef and inform regulatory decisions to protect public health.

Comparison with FOA & WHO Recommended Levels: The measured concentrations of Nickel, Cadmium, Chromium, Iron, and Lead can be compared with recommended levels provided by organizations such as the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). For example, the FOA & WHO recommended levels for Lead are 0.3 mg/kg, indicating compliance with regulatory limits in the presented samples.

Some food products may help to counteract the effects of these heavy metals due to some properties which they possess including;

Sulfur-rich foods such as cauliflower, broccoli, Brussels sprouts, garlic, and onion can aid in the detoxification process.

Pectin-rich fruits and vegetables like pears, green apples, citrus fruits, cabbage, beets, and carrots contain soluble fibre that enhances the elimination of heavy metals.

Amino acids found in corn, whole grains, spinach, carrots, turnips, plums, grapes, and pomegranates act as natural chelating agents.

Iron-rich foods, including red and organ meats, are crucial to prevent complications from iron deficiency when exposed to lead.

CONCLUSION

This study has therefore shown that the smoked beef samples from around Uniben axis have minimal health risks and do not exceed the EU standard limit for PAHs but are above the limit for some heavy metals.

In conclusion, the measured concentrations of PAHs and heavy metals in smoked beef samples highlight the importance of monitoring and regulating these contaminants to ensure consumer safety and protect public health. Compliance with regulatory standards and guidelines is crucial for minimizing health risks associated with consuming smoked beef products. Continued efforts in quality control and food safety measures are necessary to mitigate the potential adverse effects of PAHs and heavy metals on consumer health.

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