QUANTIFICATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) CONCENTRATIONS IN SELECTED COCONUT (*Cocos nucifera*) OIL SAMPLES SOLD IN EDO STATE, NIGERIA: A CHROMATOGRAPHIC ANALYSIS

BY

Moses Tochukwu OKWUADIGBO

BMS2009006

DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF BASIC MEDICAL SCIENCES

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCES (B.SC) IN MEDICAL BIOCHEMISTRY

MARCH, 2025

CERTIFICATION

This is to certify that this project work was carried out by Moses Tochukwu OKWUADIGBO with matriculation number BMS2009006, of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin city, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) degree in Medical Biochemistry.

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(SUPERVISOR)	
EXTERNAL EXAMINER	**DATE**
DR. F. E. OLUMESE	**DATE**
(HEAD OF DEPARTMENT)	

DEDICATION

This work is dedicated to the almighty God, and my father, Mr Samuel Okwuadigbo.

ACKNOWLEDGEMENT

With a heart full of gratitude, I give all glory to the Almighty God for seeing me through this academic journey. His grace, mercy, and kindness has been my source of strength. I appreciate him for life, good health, wisdom, resilience, and the ability to overcome challenges. Every step of this journey has been made possible by his guidance.

Special thanks goes to my Project Supervisor, Dr. B. A. Ogie for his constant contributions, criticism, and corrections throughout this research project and throughout my academic journey. His guidance has been instrumental in shaping the direction and quality of my work. I am also thankful to the head of my department, Dr. F.E Olumese. I sincerely acknowledge your dedication to ensuring that your students have an enabling and conducive environment for academic success.

Words cannot fully express my gratitude to my parents, Mr and Mrs Samuel Okwuadigbo, for their endless love, patience and understanding since my first year in the university. Their sacrifices and prayers have been a great source of strength to me. To Ijeoma Faith Okwuadigbo my elder sister, I cannot express how much I appreciate you for your financial support during my early years in school. To my friend Inenzohi Mary, I appreciate your friendship, encouragement and support. Finally, to all my coursemates, especially MBC cheetahs my class football team, you all have made this journey enjoyable and memorable. May God bless everyone mentioned and thank you all once again.

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ABSTRACT

Coconut (*Cocos nucifera*) is a widely consumed tropical fruit highly valued for its nutritional and therapeutic qualities, with its oil as a major component used in food, cosmetics, and traditional medicine. Recently, polycyclic aromatic hydrocarbon (PAH) contamination of edible oils has led to concerns regarding the safety of coconut oil, especially its processing and storage practices. The aim of this study was to identify the concentrations of PAHs in some coconut oil products available in Edo State, Nigeria, to assess potential contamination and associated health risks. Coconut oil samples were collected from major markets in Edo State and analyzed using gas chromatography-mass spectrometry (GC-MS) following solvent extraction, sample cleanup using silica gel, and rotary evaporation concentration. The analysis showed that PAH levels in all tested coconut oil samples were below the detection limit (<0.05 mg/kg), indicating no significant contamination. This suggests that the processing methods and storage conditions of the sampled oils were sufficient to prevent PAH formation. The findings emphasize the importance of continued monitoring and adherence to best practices in oil extraction, storage, and distribution to maintain product safety and protect consumers who may not have sufficient knowledge.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Edible oils, like other food products, can be contaminated by a range of factors, including the environment, agricultural methods, and processing procedures. Many research on edible oils and fats have discovered pesticide residues, heavy metals, and mycotoxins (Kivevele and Huan, 2015; Tuzimski and Rejczak, 2016; Bhat and Reddy, 2017). These objects could have been polluted by environmental pollutants throughout the processing steps before purifying. Overall, these contaminations have a negative influence on food safety and may pose a significant threat to human health. *Cocos nucifera* (Coconut) is an important tree crop in the world's tropical regions and a major source of edible oil. Coconut oil is widely utilised for its nutritional, medical, and industrial properties, and it is especially popular as a cooking oil in tropical regions. It includes a high concentration of medium-chain triglycerides (MCTs), lauric acid, and antioxidants, making it therapeutically advantageous for antibacterial, anti-inflammatory, and cardiovascular purposes (Konar et al. 2020). Oil is processed using a variety of processes, ranging from traditional hand processing to industrial processing.

Coconut oil is widely marketed in Nigeria and processed using a variety of extraction and processing processes, including cold pressing, solvent extraction, and heat-based approaches (Ng et al. 2021). While coconut oil is prized for its nutritional and therapeutic properties, there have been concerns regarding pollutants introduced during processing and storage (Adejumo et al. 2021). Polycyclic aromatic hydrocarbons (PAHs) are a class of hazardous chemical molecules produced by incomplete combustion of organic materials such as wood, charcoal, and biomass

fuels. Polycyclic aromatic hydrocarbons (PAHs) are a group of over 200 chemical compounds with two or more fused aromatic rings. The principal route of PAH exposure for the general population is inhaling indoor ambient air, consuming PAH-containing food, smoking cigarettes, or breathing smoke from open fireplaces or fossil fuels (Zhang, Cui et al. 2015). Humans are exposed to PAHs from both dietary and non-dietary sources, but the former is regarded the most important (Bansal & Kim, 2015). In addition to industrial operations like heating, drying, and smoking, PAHs can affect food by contamination of the air, water, or soil. Vegetables, fruit, cereals, oils and fats, smoked meat and fish, coffee, and tea are among the foods that contain PAHs (Bansal and Kim, 2015). Environmental pollution, seed drying (particularly with combustion gases), the extraction of solvents, soil burning, packaging, mineral oil residues, and migration from contaminated water or soils are some of the factors that can lead to PAH contamination in oils (Ciecierska & Obiedziński, 2013; Bansal & Kim, 2015). Given that PAHs are carcinogenic and mutagenic, eating food containing them puts one's health at serious danger. Prolonged exposure to PAHs has been linked to chronic diseases like cancer, oxidative stress, and DNA damage. They seriously impact human health by affecting the blood, heart, nerve, and immune systems and are linked to a variety of illnesses, such as lung, oesophagus, and breast cancer (Moorthy et al. 2015; Korsh et al. 2015).

1.2 Justification of the Study

Coconut oil is widely utilised by humans as a cooking oil, medical and cosmetic items, and in other applications, making it an essential part of the diet and commercial activity. However, worries about food safety and contamination have underlined the importance of precautionary measures before eating. One important issue is the possible existence of polycyclic aromatic hydrocarbons (PAHs), a form of hazardous organic contaminant. PAHs have been classed as

carcinogenic and mutagenic, and they have been related to cancer, oxidative stress, and other chronic health problems with prolonged exposure. Studies have revealed that PAH contamination in edible oils offers considerable health hazards, with exposure connected to oxidative stress, carcinogenesis, and metabolic problems, hence assessing PAH concentration levels in coconut oil is vital for evaluating potential health implications.

1.3 Aim of the Study

The aim of this study is to quantify the concentrations of polycyclic aromatic hydrocarbons (PAHs) in selected coconut oil samples sold in Edo State, Nigeria, utilising chromatographic technique to examine contamination levels food safety hazards.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Plant: Cocos nucifera

Cocos nucifera (L.), commonly known as "Coconut," is a significant member of the Arecaceae family, which includes palm trees. It is also known as coconut-of-the-beach, coco-da-bahia, or just coco. The plant's native range includes islands between the Indian and Pacific oceans as well as Southeast Asia, including Malaysia, Indonesia, and the Philippines. It is thought that the coconut palm nut was brought from this location to India and then to East Africa. This plant was discovered at the Cape of Good Hope, and after being brought to West Africa, it expanded to the American continent and other tropical regions of the world.

It Is an arborescent monocotyledonous tree, about 25 m high, with a dense canopy, and it is also known as giant coconut. In the coconut root system, the root is fasciculated. The stem is of unbranched type. At the apex of the stem, a tuft of leaves protects a single apical bud. The pinnate leaves are feather-shaped and have a petiole, rachis, and leaflets. Under ideal conditions, the large adult coconut produces 12-14 inflorescence spikes every year, whereas the adult dwarf coconut can produce 18 spikes in the same period. The axillary inflorescence is composed of globular clusters of female flowers. The plant is monoecious, meaning that it has both male and female reproductive organs.

The coconut fruit is composed of an external epicarp, a mesocarp, and an internal endocarp. The epicarp, the outer skin of the fruit, and the mesocarp, which is thick, fibrous, and brownish when dry, have several commercial uses. The endocarp is the solid, black core. It contains a solid white albumen that varies in thickness according to the age of the fruit and has an oily pulp

consistency, as well as a thick, sweet, and slightly acidic liquid albumen called coconut water. Drupes are large, single-seeded fruits that are produced by coconut palms. The fruit consists of a thin outermost layer (the exocarp or epidermis, 0.1 mm thick), a dense layer of fibrous tissues known as the husk or coir (the mesocarp, 1 to 5 cm thick), and a hard lignified layer or shell (the endocarp, 3 to 6 mm thick) (Niral and Jerard, 2019). The hard endocarp protects the nuts from predators, whereas the fiber mesocarp protects the nuts as they fall from trees (Guerin et al. 2020) and helps them float. A thin brown seed coat layer (the testa) adheres to the endocarp. The testa's solid endosperm spans in thickness from 0.8 to 2.0 cm and weighs between 98 and 553 g fresh weight. The water or liquid endosperm inside ranges in fresh weight from 21 to 449 g depending on variety, management strategies, and environmental circumstances at fruit growth (Beveridge et al. 2022). Coconut is regarded as a functional food and/or nutraceutical, having numerous documented nutritional and therapeutic qualities. Its water has been shown to have antilipemic, hepatoprotective, cardioprotective, and antihypertensive effects (Erukainure and Chukwuma, 2024). Its antidiabetic qualities have been thoroughly described and attributed to the capacity of this plant to decrease blood glucose levels, enhance glucose tolerance, and restore pancreatic shape (Pinto et al. 2015). It has been thought to protect against diabetic retinopathy by modulating antioxidant and anti-inflammatory activities to improve total retina thickness and the thickness of the retinal nuclear layer, as well as increasing the number of neurones within the ganglion cell layer. Coconut water has also been shown to increase insulin production, lower glycosylated haemoglobin levels, promote weight gain, and alter the L-arginine-nitric oxide pathway in diabetic rats (Preetha et al. 2015). These activities have been linked to coconut water’s phytochemical characteristics, which include flavonoids, phytates, oxalates, and alkaloids (Akpro et al. 2019).

2.1.1 General Information

Scientific name: *Cocos nucifera*

Common name(s): Coconut

Family: Arecaceae

Plant type: Tree

Planting month for zone 10 and 11: year round

Origin: Tropical region of Southeast Asia

Availablity: generally available in many areas within its hardiness range

2.1.2 Description

Height: 50 to 100 feet

Spread: 15 to 25 feet

Plant Habit: Tree, arborescent (tree-like), single-trunked

Plant Density: Open to moderate

Growth Rate: Moderate to fast

Texture: Coarse

Leaf Arrangement: Spiral

Leaf Type: Pinnate

Leaf Margin: Entire (smooth)

Leaf Shape: Linear-lanceolate

Leaf Venation: Parallel

Leaf Type and Persistence: Evergreen

Leaf Blade Length: 10–20 feet per frond

Leaf Color: Bright green to yellow-green

Fall Color: No significant color change

Fall Characteristic: Evergreen; old fronds gradually turn brown and drop naturally

2.1.3 Botanical Description of *Cocos nucifera*

Cocos nucifera is an evergreen, arborescent monocot attaining a tall, single-trunked, tree-like habit rather than reduced herbaceous or shrub habit adopted by members of some other genera. Its leaves are spirally arranged at the apex of the trunk long petiolate, and pinnate with very numerous linear leaflets fixed and persistent, its margins entire or only very slightly undulate and venation parallel. The inflorescences are borne in the axils of leaves along the trunk, distinctly monoecious, with unisexual flowers sharply separated in space. The female flowers are borne on the proximal nodes, with the distal nodes carrying males in each inflorescence.

The male inflorescences are densely flowered, bearing several, very small, short-stemmed, and almost globular flowers at each node and these are subtended by diminutive bracts. The anthers of the male flowers are apiculate and lack discs. The flowers have a perianth consisting of four

small, free sepals. Flowers typically bear 4–8 stamens seated on somewhat raised receptacles. These are essentially without distinct filaments, fused at base, anthers dehiscing via longitudinal slits, and often appearing somewhat elongated or wormlike.

Female inflorescences are typically sharper, the racemes or panicles have from one to three, and less often up to five, flowers at a given node in addition the bracts which can become larger and showier. Female flowers are sessile or almost while the calyx consists of three or four minute, united, ovate sepals. The carpel forms an ovary that is often mucilaginous and may be pubescent or papillate on the surface, with each locule usually containing a single ovule.

After fertilization, the ovule develops into the large, fibrous drupe known as the coconut. The seed is ovate to elliptical, its external surface smooth or merely slightly nodular. The abundant whitish endosperm forms both the clear coconut water and the edible meat while the straight embryo has large, flat cotyledons.

There are no known features of atypical female flowers in *Cocos nucifera*. Inflorescence always presents a basal group of female flowers and a distal group of male flowers, never with hermaphrodite flowers.

2.1.4 Taxonomical Classification

The process of naming and grouping organisms according to their similarities and differences is known as taxonomical classification. This classification is handled by the field of biology known as taxonomy, which aids researchers in comprehending the connections among various creatures. In taxonomy, organisms are categorised into groups according to traits they have in common, like their physical attributes, genetic composition, or evolutionary background. All creatures in the same genus, for instance, have a common ancestor.

The taxonomical classification of *Cocos nucifera* is presented as follows:

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Liliopsida

Subclass: Arecidea

Order: Arecales

Family: Arecaceae

Subfamily: Arecoideae

Genus: Cocos

Species: The coconut tree is the only living species of the genus Cocos

Figure 1: ***Cocos nucifera***

2.2 Properties of Coconut Oil

2.2.1 Physical Properties

When heated over 30 °C, coconut oil becomes a colourless liquid. It will solidify at 25°C (Ng et al., 2021). Solidified coconut oil is white in hue. The smoking point of unrefined coconut oil is

170 °C, whereas refined coconut oil is 232 °C (Central F, 2019). Coconut oil has a characteristic coconut scent if it has not been processed, bleached, or deodorised. When coconut oil and water are combined and agitated, they form a white, homogenous combination. Without agitation, coconut oil will be insoluble in water. Coconut oil's density is 924.27 kg/m3 (Nagdeve 2020). An oil's density is determined by its saponification (molecular weight), iodine value (unsaturation), free fatty acid concentration, water content, and temperature. In general, triglycerides have a 10% density difference between their liquid and solid forms. It is commonly known that the specific heat in a solid state does not significantly change with molecular weight. The specific heat of coconut oil will rise in tandem with the iodine value. In the liquid state, the specific heat decreases with iodine value and slightly increases with molecular weight. The flow properties of actual Newtonian liquids are present in coconut oil. As fat crystals are present, non-Newtonian behaviour may happen as coconut oil gets closer to its melting point. According to Ng et al. (2021), viscosity rises with molecular weight but falls with increasing temperature and unsaturation.

2.2.2 Chemical Properties

About 62% of the makeup of coconut oil is made up of medium fatty acids, while 94% is made up of saturated fatty acids (Nagdeve, 2020). Lauric and myristic medium chain acids make up the majority of the saturated triglycerides that make up coconut oil. While C8 (caprylic) and C10 (capric) fatty acids, which comprise the bulk of triglycerides, are both categorised as medium-chain fatty acids, Lauric acid (C12), the main fatty acid in coconut oil, can be classified as either medium-chain or long-chain fatty acid (Ng et al. 2021). Since 95% of medium-chain fatty acids are absorbed directly into the portal vein, but the majority of lauric acid (70–75%) is absorbed with chylomicrons, it operates more like a long-chain fatty acid in terms of digestion (Nagdeve,

2020). Medium-chain fatty acids are more soluble at neutral pH because they are weak electrolytes and highly ionised. This suggests that the difference in solubility that occurs at chain lengths of C:10 and shorter does not involve lauric acid (Eyres et al. 2016). In a 100 g sample, there are 41.84 g of saturated fatty acids that are lauric (C12), 16.65 g of myristic (C14), 8.64 g of palmitic (C16), 6.80 g of caprylic (C8), and 2.52 g of stearic (C18) (Central F, 2019). The amount of moisture in the finished product depends on the method used to extract the coconut oil. For example, compared to coconut oil made from fresh coconut copra, coconut oil made from dried coconut copra has a significantly lower water content. Furthermore, the moisture content of oil extracted by heating methods will be lower than that of oil that was not heated. Due to its slow oxidation and resistance to rancidity, coconut oil has a longer shelf life than other vegetable oils that consumers use (Nagdeve, 2020).

2.3 Uses of the Plant (Medicinal and Non-medicinal Uses)

2.3.1 Medicinal Uses/Benefits of *Cocos nucifera*

Several research have been done to determine the health advantages of *Cocos nucifera*, and the results have shown that it can be used as a natural isotonic drink, an antioxidant and antibacterial agent, a hepatoprotectant, and to treat diabetes. Due to its many bioactive qualities, *Cocos nucifera* is a valuable natural resource for promoting health and preventing disease.

a. **Isotonic Natural Drink:** A study comparing coconut water, an orange beverage, and water among individuals exercising in hot conditions revealed that prior consumption of coconut water led to reduced urine output, suggesting enhanced hydration capacity without causing gastrointestinal discomfort, despite its composition, and also improved subsequent exercise performance (Laitano et al. 2014). Coconut water aids in hydration,

having an effect similar to pure water, as well as allowing a decrease in heart rate during severe activity (Chagas et al. 2017).

b. **Antibacterial Activity:** The antimicrobial qualities of coconut oil have been the subject of numerous investigations. Hovorková et al. (2018) analysed the fatty acid composition of coconut oil and discovered that 42% of it included lauric acid, a functional medium-chain fatty acid (MCFA) having antiviral and antibacterial activities (Apraku et al. 2016). The MCFA has shown membrane-disruptive effect against Gram-positive bacteria, either causing bacterial cell lysis or stopping bacterial growth (Jackman et al., 2020). Hovorková et al. (2018) found that coconut oil had no effect on Lactobacillus species or Gram-positive Bifidobacterium, however it showed mild antibacterial qualities against two tested Gram-negative gut bacteria. Conversely, coconut oil effectively inhibited Staphylococcus aureus at 0.56 mg/mL and Enterococcus cecorum at 1.13–2.25 mg/mL. S. aureus is a common pathogen that affects both humans and animals and can lead to systemic diseases (Tong et al., 2015).

c. **Antioxidant activity:** Coconut water, which typically comprises 5–8% total soluble solids (TSS), is primarily composed of sugars (3–7%). The phenolic compounds in coconut water are salicylic acid and catechin (Mahayothee et al. 2016). Coconut testa contains a variety of flavonoids and phenolic acids that have potent antioxidant qualities. These substances can be substituted for artificial antioxidants in food formulations. According to Appaiah et al. (2014), there are notable amounts of phenolic compounds in the oil extracted from coconut kernels, which includes testa and heat-processed VCO. Among the three varieties of milk, coconut had the highest antioxidant activity, according to tests for total phenol content (TPC), ferric reducing antioxidant power (FRAP), DPPH

radical scavenging activity (DPPH), and oxygen radical absorbance capacity (ORAC) (Alyaquobi et al. 2015). The mean values were 575.15 mg GA/100 g FW, 471.55 mg TE/100 g FW, 68.39 percent, and 784.47 umol TE/100g F.W.

d. **Hepatoprotective effect:** One study found that coconut water lowers the expression of Tnf and Il6 transcripts generated by IL-1β, which in turn lowers hepatocyte inflammation. In addition, it causes primary hepatocytes in mice to express more acute phase proteins (Serpine1 transcript) and antioxidants (HMOX1 protein) (Lakshmanan et al. 2020). These results suggest that coconut water may have a preventative effect via enhancing hepatocytes' antioxidant defences and inflammatory response. These findings are consistent with previous studies that shown coconut water protects hepatocytes from H2O2-mediated oxidative damage and testes from heat-induced damage (S. S. Kumar et al. 2018; Manna et al. 2014).

e. **Effect on Diabetes:** One study found that coconut water lowers the expression of Tnf and Il6 transcripts generated by IL-1β, which in turn lowers hepatocyte inflammation. In addition, it causes primary hepatocytes in mice to express more acute phase proteins (Serpine1 transcript) and antioxidants (HMOX1 protein) (Lakshmanan et al. 2020). These results suggest that coconut water may have a preventative effect via enhancing hepatocytes' antioxidant defences and inflammatory response. These findings are consistent with previous studies that shown coconut water protects hepatocytes from H2O2-mediated oxidative damage and testes from heat-induced damage (S. S. Kumar et al. 2018; Manna et al. 2014).

2.3.2 Non-medicinal Uses of *Cocos nucifera*

In addition to providing food, millions of people rely on *Cocos nucifera* for employment and economic opportunities. The fruit's inherent rich macro and micronutrient profile for human nutrition and health has earned it the title of "wonder fruit." Desiccated coconut, oil, raw kernels, milk, and coconut water are some of the commercially produced goods. The leftover coconut meal from making coconut milk and oil is converted into coconut cake or flour, a high-fiber, high-protein by-product. Glutamin is the primary protein found in coconut meal. According to Khairiyah et al. (2022), dietary fibre can be produced using oil meal (poonac) and coconut residue that is left behind after coconut oil and coconut milk are removed, respectively. As a product with added value, it can be utilised as a low-cost ingredient for animal feed or as a substitute for other food items. However, because it is typically left to decompose, enormous amounts of abandoned coconut residue are hazardous to the environment. Because it contains dietary fibre and protein, *Cocos nucifera* is used in a variety of culinary products. For example, its flour is used in baked goods, snacks, candies, and extruded goods. It can boost immunity, prevent cardiovascular illnesses, and have anti-diabetic and anti-cancer effects (Preetha et al. 2015; Verna et al. 2019). It's interesting to note that, although being gluten-free, it shares nearly all of the nutritional characteristics of wheat flour. Thus, one of the feasible and healthy alternatives for the patients of celiac disease is gluten-free foods using coconut flour (Khairiyah et al. 2022).

2.4 Phytochemicals

Phenols, tannins, leucoanthocyanidins, flavonoids, triterpenes, steroids, and alkaloids were found in *Cocos nucifera* phytochemical analyses. Triterpenes, saponins, and condensed tannins were also recovered from a butanol extract. Because phenolic chemicals in coconut shell extracts can

form a blue complex in alkaline circumstances, the Folin-Ciocalteu technique is frequently employed to determine their presence (Yun et al. 2017). Temperature at extraction has a great influence on the content of phenolics, with increased temperature enhancing their solubility. At 70°C, there was 28.31 mg/100g total phenol content in coconut, while at 28°C, it was 18.85 mg/100g, as tannins have more solubility at higher temperature (Sevindik et al. 2017). More heat, on the other hand, degrades the phenols, making it less bioactive (Ibrahim et al. 2014).

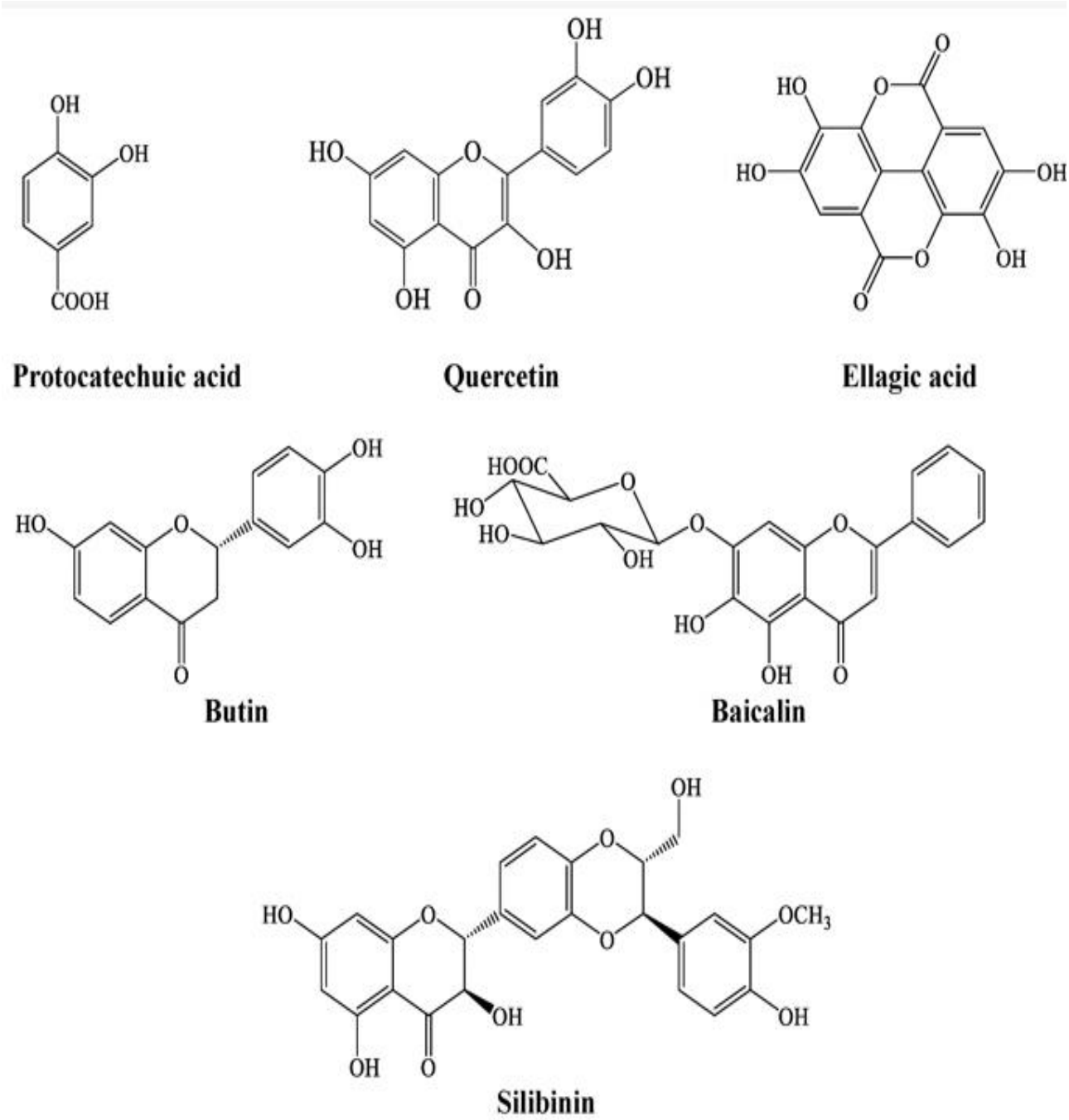


Figure 2: Phenolic Compounds in *Cocos nucifera*

Phytochemical analysis of coconut shell extracts detected the presence of tannins, saponins, and steroids, while alkaloids, triterpenoids, and flavonoids were not detected (Mazaya et al. 2020). Tannins in coconut, detected by its reaction with 1% $FeCl_3$, increased with increased extraction

temperatures and thus darker extracts (Mazaya et al. 2020). Tannins prevent microbial growth by damaging cell walls, binding to microbial adhesins, and interacting with phospholipids leading to cell lysis (Rohaeni, 2016).

Saponins were present in the extracts of coconut shell. Higher temperatures of extraction resulted in higher saponin content. More foam at 70°C compared to 28°C indicated that indeed, higher temperatures resulted in higher concentration of saponins (Mazaya et al. 2020). The bactericidal properties of the saponins are attributed to their ability to disrupt the membranes of bacteria cells. Like detergents, saponins reduce surface tension and permeate the membranes and hence their ability to leak cytoplasm, leading to cell death (Mazaya et al. 2020). This makes saponins effective natural antimicrobial agents.

2.5 Organic Contaminants: Polycyclic Aromatic Hydrocarbons (PAH)

2.5.1 Polycyclic Aromatic Hydrocarbons (PAH)

Polycyclic aromatic hydrocarbons are organic pollutants made up of two or more fused aromatic rings of carbon and hydrogen atoms. They are typically colourless, white, or light yellow solid substances (Abdel-Shafy and Mansour, 2016; Suman et al., 2016). The molecular configurations of aromatic rings in space might be linear, angular, or clustered (Abdel-Shafy and Mansour, 2016). PAHs are classed as light-molecular weight (LMW) or high-molecular weight (HMW) based on the number of aromatic rings contained in the molecules. Depending on their molecular weight, they are discharged as gaseous (LMW PAHs) or particulate (HMW PAHs) (Lee and Vu, 2010). Additionally, PAHs are categorised according to the structure of their rings. According to Gupte et al. (2016), non-alternant PAHs, like fluorene, have both the fusion of six carbon benzene rings and an extra ring with fewer than six carbons, whereas alternative PAHs only have

the fusion of six carbon benzene rings. Because PAHs have concentrated π electrons on aromatic rings, they have a higher biological endurance, making them more resistant to nucleophilic attack. 16 PAHs were identified as priority pollutants by the US Environmental Protection Agency (USEPA) in 1983 because of their toxicity, refractory nature, increased exposure, and greatest concentrations (Zheng et al., 2018; Mojiri et al., 2019). PAHs are characterised by low vapour pressure, low water solubility, and high melting and boiling temperatures, depending on their structure. Higher molecular weight PAHs have a tendency to become more refractory molecules by increasing their lipophilicity and decreasing their water solubility.

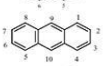
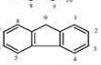
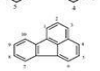
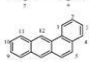
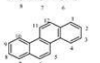
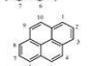
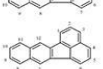
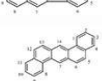
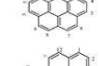
Name	Formula	Structure	Molecular weight (g/mole)	Solubility in water (mg/L)	Phase distribution	Melting point (°C)	Boiling point (°C)	Vapor pressure (mmHg)	Log Kow	Log Koc	Toxicity as per IARC
Naphthalene	$C_{10}H_8$		128.17	31	Gas	80.26	218	0.087	3.29	2.97	2B
Acenaphthene	$C_{12}H_{10}$		154.21	3.8	Gas	95	96	4.47×10^{-3}	3.98	3.66	3
Acenaphthylene	$C_{12}H_8$		152.20	16.1	Gas	92–93	265–275	0.029	4.07	1.40	3
Anthracene	$C_{14}H_{10}$		178.23	0.045	Particle gas	218	340–342	1.75×10^{-6}	4.45	4.15	3
Phenanthrene	$C_{14}H_{10}$		178.23	1.1	Particle gas	100	340	6.8×10^{-4}	4.45	4.15	3
Fluorene	$C_{13}H_{10}$		166.22	1.9	Gas	116–117	295	3.2×10^{-4}	4.18	3.86	3
Fluoranthene	$C_{16}H_{10}$		202.26	0.26	Particle gas	110.8	375	5.0×10^{-6}	4.90	4.58	3
Benzo(a)anthracene	$C_{20}H_{12}$		228.29	0.011	Particle	158	438	2.5×10^{-6}	5.61	5.30	2B
Chrysene	$C_{18}H_{12}$		228.29	0.0015	Particle	254	448	6.4×10^{-9}	5.9	No data	2B
Pyrene	$C_{16}H_{10}$		202.26	0.132	Particle gas	156	393–404	2.5×10^{-6}	4.88	4.58	3
Benzo(a)pyrene	$C_{20}H_{12}$		252.32	0.0038	Particle	179–179.3	495	5.6×10^{-9}	6.06	6.74	1
Benzo(b)fluoranthene	$C_{20}H_{12}$		252.32	0.0015	Particle	168.3	No data	5.0×10^{-7}	6.04	5.74	2B
Benzo(k)fluoranthene	$C_{20}H_{12}$		252.32	0.0008	Particle	215.7	480	9.59×10^{-11}	6.06	5.74	2B
Dibenz(a,h)anthracene	$C_{22}H_{14}$		278.35	0.0005	Particle	262	No data	1×10^{-10}	6.84	6.52	2A
Benzo(g,h,i)perylene	$C_{22}H_{12}$		276.34	0.00026	Particle	273	550	1.03×10^{-10}	6.50	6.20	3
Indeno[1,2,3-cd] pyrene	$C_{22}H_{12}$		276.34	0.062	Particle	163.6	530	10^{-10}–10^{-16}	6.58	6.20	2B

Figure 3: Physicochemical Properties of 16 Polycyclic Aromatic Hydrocarbons

2.5.2 Sources and Route of Exposure to Polycyclic Aromatic Hydrocarbons (PAH) Exposure

Two natural and human sources of PAHs are burning wood and coal (Wu et al. 2014). Dao et al. (2015) state that many of the naturally occurring PAHs are resistant and dangerous. Forest fires, garbage burning, volcanoes, and hydrothermal activity are examples of natural sources of PAH emissions (Li et al. 2015). Both anthropogenic and natural sources, as well as international transport routes, had an impact on the distribution of PAHs worldwide. They are mostly created by burning fossil fuels in waste incinerators, during heating operations, and from vehicle exhaust. They are pervasive environmental pollutants with harmful biological effects, toxicity, carcinogenicity, and mutagenicity. The degree of industrial growth, the mechanism or modes of PAH transport, and the distance between the polluted site and the production source all affect the concentrations of PAHs in the environment. Fossil fuel products, vehicle emissions and refineries, timber, burning biomass, tobacco, wood smoke, and waste all contain high levels of PAHs. Localised loadings of PAHs into the environment are mostly caused by transportation and petroleum refinery activities. Unintentional releases of raw and processed materials as well as the discharge of industrial effluents might result in loadings. Road runoff, surface water, groundwater, soil and sediment, and air all contain PAHs (Hijosa-Valsero, Bécares et al. 2016). PAHs are emitted from the atmosphere to plants, drugs and contaminated foods. They have been observed to occur in soil and sediment levels ranging from 1 mg/kg to more than 300 g/kg at both polluted and uncontaminated sites (Duan, Shen et al. 2015). Inhaling indoor ambient air, eating food containing PAHs, smoking cigarettes, or breathing smoke from open fireplaces are the main ways that the general public is exposed to PAHs. Fossil fuels are used to power our cars,

cook our food, heat our homes, and power our economies (Zhang, Cui et al. 2015). Agriculture crops that contain PAHs in their leaves expose organisms to these pollutants through the food chain (Sun, Wang et al. 2016). Workers' occupational exposure to PAHs includes, for example, coking, bitumen-containing roofing construction, petroleum refinery procedures, and further in coal gasification. Workers including mechanics, street vendors, drivers, miners, and those involved in metalworking operations may also be exposed to PAHs at work through the inhalation of exhaust gases. Food consumption is the main exposure route, while smoking can play a significant role in the case of smokers. Natural and mostly man-made environmental factors, industrial food processing, and some home cooking methods can all contaminate food. Deposition from the sky or deposition and transfer from soil and water are two ways that PAHs might enter the food chain. Inhaling cigarette smoke, industrial air pollutants, vehicle exhaust, toxic waste sites, jet fuel, and smouldering pits, as well as ingestion of barbequed foods are additional ways that people can be exposed. Human beings are also exposed to PAH through inhalation in the air, polluted water that they consume, as well as contaminated foods they ingest. PAHs are routinely examined in the atmosphere for air quality determination, in biological tissue to monitor health impacts, in molluscs and sediments for environmental monitoring, and in food for safety. Particular sources of Benzol(a)pyrene (BaP) environmental pollution and exposure of humans are automobile and industrial exhausts, toxic waste facilities, cigarette smoke, burning biomass, waste combustion, municipal waste incinerators, volcanic emissions, household burning and charcoal broiled and smoked food consumption.

Tobacco smoke, vehicle emissions, medical and e-waste, charcoal, landfills, wildfires, oil spills, and decorative candles are other exposure sources. Environmental contaminants known as PAHs are so pervasive that exposure to them is unavoidable. In both occupational and non-

occupational contexts, it involves ingestion, inhalation, and skin contact. The total absorption dose may be impacted by exposures that concurrently include more than one route, such as skin and airborne exposures.

2.5.3 Toxicity of Polycyclic Aromatic Hydrocarbons (PAH)

The route and length of exposure determine the toxicities of PAHs. Eye and skin irritation, nausea, vomiting, and inflammation are examples of acute consequences; lung, skin, bladder, and gastrointestinal tract malignancies; kidney and liver damage; cataracts; gene mutations; cell damage; and cardiovascular mortality are examples of chronic effects (Garcia et al. 2014). Additionally, PAHs undergo a variety of phase I and II enzyme-based routes, such as the cytochrome P450 peroxidase and aldo-keto reductase pathways, which result in metabolites including radical cations and diol-epoxides. The metabolites cause mutagenic, carcinogenic, immunosuppressive, and teratogenic harm by interacting with proteins and DNA to generate DNA adducts that cause biochemical disturbances and cell damage. These gene alterations are linked to the development of tumours and foetal abnormalities (Grover, 2019). Furthermore, considering that PAHs are a complex mixture of several components with a variety of different structures, they may combine synergistically, making them more dangerous. The gut microbiota regulates the biological action of xenobiotics like PAHs, according to recent research. Through processes like the enterohepatic cycle and the alteration of gene expression by liver enzymes like P450, the host microbiota may intensify the harmful effects of PAHs. The precise processes underlying this microbiota-modulation are still unknown, though (Nogacka et al. 2019). The biotransformation and metabolism of PAHs are intricate.

Multiple enzymes metabolise them through various mechanisms. Among these, PAHs are activated to produce carcinogens by the diol-epoxide, radical cation, and ortho quinone pathways (Sampaio et al. 2021). In particular, BaP is oxidised to dihydrodiol epoxides, such as (+)benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide, by the phase I enzyme cytochrome P1A1/1B1 and the phase II enzyme epoxide hydrolase under the diol-epoxide pathway (Sampaio et al. 2021). The resulting "activated" PAHs exhibit higher reactivities along with improved polarity and electrophilicity. These activated metabolite products (radical cations, diol-epoxides, and o-quinones) can create DNA, RNA, and glutathione adducts and cause mutations, alterations in gene expression, and carcinogenesis if they are not phagocytosed by macrophages and eliminated in urine and faeces (Sampaio et al. 2019).

2.6 Polycyclic Aromatic Hydrocarbons (PAH) Contamination in *Cocos nucifera*

Products from *Cocos nucifera* are essential for the majority of industrial and culinary uses, however some processing techniques inadvertently add contaminants by using polycyclic aromatic hydrocarbons (PAHs), which have been shown to cause cancer. For the safety of consumers, it is essential to understand how these contaminants are added throughout the processing of coconuts. A straightforward method for making coconut oil and other goods is to dry the meat of the coconut, or copra. The coconut material is often exposed to heat and smoke from burning wood and other flammable materials during traditional drying processes. PAHs may condense onto the coconut material as a result of direct contact (Zelinkova and Wenzl, 2015). A review emphasised the possibility of PAH contamination during processing due to artificial drying and heating if caution is not taken, such as through indirect drying methods and proper temperature regulation (Zelinkova and Wenzl, 2015).

Smoking is applied as a method of preservation for coconut products in certain regions. Nevertheless, poor smoking procedures can significantly be a cause of high levels of PAH. Fuel, burning temperature, and exposure time are all the important parameters that take part in PAH formation. The study suggests that direct smoking methods can result in greater PAH contamination in food products (Sampaio et al. 2021).

Other than processing, environmental factors are implicated in PAH contamination. Coconut palm trees cultivated under conditions with increased PAHs in the water, air, or soil can be able to incorporate these compounds and make them be stored in the coconut. In one study, it was noted that food can become contaminated with PAHs either from environmental matrices or during cooking and food preparation (Liu et al. 2024).

Coconut oil can have greater PAH4 than some of the other fats and oils simply because coconut consists of a greater proportion percentage-wise of BaA and Chr that are problematical to purge from coconut oil refineries. In 2008, the EFSA set limits of 2.0 and 10.0 μg/kg for BaP and PAH4 in vegetable oils and fats, respectively, while the limit for PAH4 in coconut oil is 20.0 μg/kg (Sampaio et al. 2022). In Sao Paulo, Brazil, Silva et al. (2018) looked into PAH4 contamination in nontraditional vegetable oils (coconut, safflower, evening primrose, and linseed oils). According to their description, the most common hydrocarbon in these samples is Chr. Furthermore, 96% of the samples contained PAH4, in amounts ranging from 14.99 μg/kg to undetectable. According to the limits permitted by European Regulation 835/2011, 12% and 28% of the samples, respectively, contained unacceptable levels of BaP and PAH4, and the authors explained these undesirable results by citing samples of tainted oils.

2.7 Methods for Detecting and Analyzing PAHs

Several analytical techniques for identifying polycyclic aromatic hydrocarbons (PAHs) in sediment and water are presented in this section. Since PAH contamination in soil and aquatic habitats might have a direct impact on coconut processing and cultivation, it is important to comprehend these techniques. Coconut trees are vulnerable to environmental contaminants, such as PAHs, which build up in water sources and sediments as a result of industrial operations, oil spills, and combustion processes, because they are frequently planted in tropical coastal locations. Through irrigation water, soil absorption, and air deposition during processing techniques like smoking and drying, these pollutants can make their way into the coconut production chain. According to Adeniji et al. (2017), these analytical techniques can be broadly divided into three categories: spectrometric, chromatographic, and immunoassay techniques.

a. **Immunoassay methods:** Due to their tendency to bring significant biases into the final results, immunoassay procedures (EPA 4030 and 4035, Update III), which are mostly accessible as kits, are not very popular. Furthermore, compared to other conventional approaches, the methods' accuracy, precision, and specificity are significantly worse for the majority of aromatic molecules. As a result, their primary function in water and soil analysis is field screening (Adeniji, Okoh, and Okoh 2017). The two most commonly used spectrometries are ultraviolet (UV) and infrared (IR). However, the co-presence of some other chemicals, such as lipids, in the sample matrix makes UV techniques (absorption and fluorescence), which are thought to be sensitive and selective to aromatic compounds like PAHs, more vulnerable to interference. Similarly, IR spectrometric method even though cheap and quick, sees the sample having to undergo obligatory

cleanup step once extracted before going for analysis as well as failing to be so selective (Adeniji et al. 2017).

b. **Liquid Chromatographic methods:** The most often used detectors for measuring PAHs in liquid chromatography, particularly those with high molecular weights, are the UV and fluorescence detectors. The bulk of PAHs, which are in the UV range of 190 to 360 nm, can be found with a UV/visible detector. Particularly when choosing appropriate excitation and emission wavelengths, the fluorescence detector's selectivity and sensitivity for quantifying PAHs in a complex environmental sample are greatly enhanced. The mass spectrometer (MS) is the second useful detector used in liquid chromatography. When dealing with a large number of compounds of interest, it is especially helpful for the characterisation and identification of trace polar components. There have been reports of the full application of LC/MS in the analysis of aromatic chemicals. In order to separate the target compounds from the aqueous mobile phase, the sample must first be introduced into the chromatograph. It then travels through a cutting-edge interface (such as thermospray, electrospray, moving belt, and particle beam) before arriving at the mass spectrometer for identification (Adeniji et al. 2017). However, no one interface is suitable for separating all of the sample's polycyclic aromatic hydrocarbons.

c. **Gas Chromatographic methods:** Organic substances that are non-polar, volatile, and thermally stable can be found and separated using gas chromatographic techniques. Determining some semi-volatile chemicals, such as PAHs, is another use for it. Photoionisation detectors (PID), flame ionisation detectors (FID), Fourier transform-infrared (FT-IR), and mass spectrometers (MS) are detectors that can be employed for analytical characterisation of aromatic chemicals, such as PAHs, in samples by GC

(Gorleku et al. 2014). With a capillary column, the method can provide good resolving power. Because of their low volatilities, propensity to breakdown when exposed to high temperatures, and propensity to preferentially adsorb on the GC inlet and column, PAHs with molecular weights (MW) greater than 300 atomic mass units (amu) are usually challenging to analyse by GC. A packed column gas chromatographic technique is another option. While the solid samples can be extracted using an ultrasonic or Soxhlet extraction device, the approach enables water samples to be neutralised to pH and extracted before analytical analysis. The four sets of PAHs—anthracene and phenanthrene, chrysene and benzo[a]anthracene, benzo[b]fluoranthene and benzo[k]fluoranthene, and dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene—can be successfully separated using capillary columns instead of packed columns, but the packed column in the other methods poses a major obstacle to the proper separation of these compounds. If not, silica gel purification is recommended as an essential part of the procedures, unless the sample matrix is reasonably clean (Adeniji et al. 2017).

2.8 Empirical Review

Numerous articles detailing statistics on the prevalence of PAHs and health risk assessments have been published in recent years, examining whether there may be a danger associated with consuming particular foods. The MOE from eating edible vegetable oils ranged from 2.17 to 4.10 × 105 for adults and 2.86 to 5.38 × 104 for children, according to a study conducted in Iran. Furthermore, the range of the ILCR for adults was 4.17 × 10−6 to 5.20 × 10−6, whereas for children it was 3.17 × 10−5 to 3.94 × 10−5. For their consumption, sunflower, corn, mixed, and frying oils displayed the highest to lowest MOE. On the other hand, frying, mixed, sunflower, and corn oil use had the greatest and lowest ILCRs, respectively. Finally, the scientists came to

the conclusion that, based on the health risk assessment, there was no significant health risk associated with oil consumption for either adults or children (MOE ≥ 1 × 104 and ILCR < 1 × 10−4) (Yousefi et al. 2018).

Linseed and safflower oils, two non-traditional cold-pressed vegetable oils, were examined by Ciecierska and Obiedzinski (2013). They found that the most common individual pollutant was phenanthrene, and the PAH level ranged from 23.41 to 234.30 µg/kg. The range of BaP concentration was 15.74 µg/kg to not detectable. After analysing linseed and borage oils, Roszko et al. found that the PAH4 level was less than 10 µg/kg. The impact of roasting olive fruits on the amount of PAHs in olive oil was assessed by Gharby et al. (2018). They found BbF (0.26–2.92 µg/L), BaA (2.19–31.3 µg/L), Chr (2.44–14.85 µg/L), and BaP (0.51–1.59 µg/L). The levels of PAHs were considerably greater when olives were roasted at high temperatures (around 130 °C), even though they were found in olive oil from unroasted fruits.

Kiralan et al. (2019) compared the effects of chemical refining conditions on the removal of 15 PAHs in another study on olive oil. All of the refining processes (degumming, neutralisation, bleaching, and deodorisation) were analysed by the authors. They evaluated various water and phosphoric acid concentrations during the degumming process, which is a step in the refining process that involves removing phosphatides, or gums, from crude oil. They claim that the application of a solution containing 1% (v/w) water reduced the PAHs level by 82%. Similarly, they found that following the neutralisation process, the overall content of PAHs dropped by 90%. Furthermore, it was shown that raising the activated carbon content in the bleaching stage from 0.3% to 0.9% significantly reduced the levels of PAHs (from 86% to 91%). Lastly, light PAHs were being nearly eliminated by the deodorisation process, and raising the process's

temperature did not successfully lower the total amounts of PAHs. All of these results have consequences for developing new processing procedures for producing high-quality olive pomace oil.

PAH contamination in nontraditional vegetable oils (coconut, safflower, evening primrose, and linseed oils) supplied in São Paulo, Brazil, was assessed by Silva et al. (2018). The most common hydrocarbon found in these samples was Chr. Furthermore, 96% of samples had PAH4 identified, with levels ranging from undetectable to 14.99 μg/kg. The authors attributed the inadequate levels of BaP and PAH4 in 12% and 28% of the samples, respectively, to samples of oil-contaminated oils, in comparison to the permissible values as specified by European Regulation 835/2011.

The production of 15 different PAHs, including PAH4, was evaluated by Rose et al. (2015) in a variety of beef and salmon samples that were cooked using various techniques, including deep-frying, grilling with fire and wood, roasting in an electric oven and roasting in a natural gas oven. The samples of beef and salmon that were grilled using wood and mineral charcoal, respectively, had the highest levels of PAHs. Furthermore, substantial BaP concentration was observed for beef and salmon samples during cooking times longer than 10 and 8 minutes, respectively, suggesting that these animal products are prone to contamination and PAH production. Their elevated lipid levels may be the cause of this vulnerability. In particular, salmon, hake, and tuna have higher lipid contents than other fish, which makes them more susceptible to the generation of PAHs during home cooking.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Apparatus

The apparatus used during for this study were gotten from the Biochemistry laboratory at the University of Benin, and were confirmed to be at experimental standard at the point of use. They include:

1. Beakers (250 mL, 100 mL, 50 mL)
2. Conical flasks
3. Volumetric flasks (25 mL)
4. Graduated cylinders
5. Separatory funnel
6. Filter paper (Whatman No. 1)
7. Syringe filters (0.45 µm, 0.22 µm)
8. Rotary evaporator flask
9. Test tube racks
10. Test tubes
11. Separating funnels with glass or Teflon taps
12. Glass vials (sealed with caps containing Teflon inserts, for sample storage in fridge/freezer)
13. Glass fiber extraction thimbles (e.g., Whatman 37×130mm GF)

14. Glass columns (approx. 340 mm × 6 mm, with a 25 mL reservoir and a tap with PTFE key)
15. 10 mL or 25 mL glass cylinders or vials
16. Glass wool
17. Round bottom flask
18. Amber glass bottles (for PAH sample storage)
19. Autosampler vials (for chromatographic analysis)
20. Capillary columns (for GC-MS/HPLC analysis)
21. Spatula
22. Measuring cylinders
23. Micro pipettes
24. Universal bottles
25. Aluminium foil paper
26. Masking tape
27. Nose masks
28. Neoprene Gloves

3.1.2 Equipment

Below are the equipment utilized in this study:

1. Gas Chromatography-Mass Spectrometry (GC-MS)
2. High-Performance Liquid Chromatography (HPLC)
3. Soxhlet extractor
4. Ultrasonic bath (for sonication-assisted extraction)

5. Rotary evaporator
6. Condenser
7. Heating mantle
8. Muffle furnace
9. Flame Ionization Detector (FID)
10. Nitrogen gas evaporator
11. Analytical balance
12. pH meter
13. Water bath
14. Oven (for drying glassware and samples)
15. Refrigerator (for sample preservation)
16. Fume hood (for handling volatile solvents)
17. Weighing balance (accuracy to ±0.0001 g)

3.1.3 Reagents and Chemicals

All the chemicals and reagents used in this study were of analytical grade. They include;

1. Hexane (Analar grade from Sigma)
2. Acetone (Analar grade)
3. Methanol (Analar grade)
4. Benzene (Analar grade)
5. Anhydrous sodium sulfate (dried overnight at 120°C, stored with silica gel)
6. Aluminium oxide (dried overnight at 120°C, stored in a desiccator over silica gel)
7. Silica gel 60 (70-230 mesh size from Merck, dried at 120°C, stored with silica gel)

8. Fluorene
9. Anthracene
10. Naphthalene
11. 1-Methylnaphthalene
12. 2-Methylnaphthalene
13. 1-Methylphenanthrene
14. Pyrene
15. Phenanthrene
16. Benzo[a]pyrene
17. Chrysene
18. Squalane
19. Decane (C-10)
20. Undecane (C-11)
21. Dodecane (C-12)
22. Tridecane (C-13)
23. Tetradecane (C-14)
24. Pentadecane (C-15)
25. Hexadecane (C-16)
26. Heptadecane (C-17)
27. Octadecane (C-18)
28. Nonadecane (C-19)
29. Eicosane (C-20)
30. Pristane

31. Phytane
32. Heneicosane (C-21)
33. Docosane (C-22)
34. Tricosane (C-23)
35. Tetracosane (C-24)
36. Pentacosane (C-25)
37. Hexacosane (C-26)
38. Heptacosane (C-27)
39. Octacosane (C-28)
40. Nonacosane (C-29)
41. Triacontane (C-30)
42. Dotriacontane (C-32)
43. Tetratriacontane (C-34)
44. Hexatriacontane (C-36)
45. Octatriacontane (C-38)
46. Tetracontane (C-40)

3.2 Methods

3.2.1 Collection

Coconut oil samples were collected from major markets in Edo State to ensure a representative analysis of the products available to consumers. The selected markets included Uselu Market, Oba Market, Oluku Market, New Benin Market, Ring Road Market, Santana Market, Ogida Market, and Ikpoba Hill Market. Samples were purchased from multiple vendors at each market

to account for possible variations in processing methods, storage conditions, and contamination sources. Each sample was carefully transferred into amber glass bottles, sealed, and labeled appropriately. To prevent degradation or external contamination, all samples were stored in a refrigerated environment until further analysis.

3.2.2 Sample Extraction and Purification

The collected coconut oil samples underwent a series of preparation steps to ensure accurate PAH extraction and analysis. These steps included extraction, purification, and concentration to remove impurities and isolate PAHs for chromatographic analysis. A measured quantity of each coconut oil sample was weighed into a clean glass beaker. An appropriate volume of hexane or dichloromethane was added as the extraction solvent. The mixture was subjected to Soxhlet extraction or ultrasonic-assisted extraction to enhance the dissolution of PAHs into the solvent phase. After extraction, the solvent-oil mixture was filtered using glass fiber extraction thimbles to remove solid impurities and undissolved residues. The filtered extract was passed through a separation column containing silica gel or aluminum oxide to remove interfering substances. Anhydrous sodium sulfate was added to eliminate residual moisture in the extract. The purified extract was then collected in a glass container and prepared for concentration. The purified extract was concentrated using a rotary evaporator or nitrogen gas evaporator to reduce solvent volume and increase PAH concentration. The concentrated sample was then transferred into amber glass vials, sealed, and stored in a refrigerated environment until further analysis.

3.3 Instrumental Analysis

Polycyclic aromatic hydrocarbons (PAHs) were detected in the coconut oil samples using Gas Chromatography (GC) with a Flame Ionisation Detector (FID). To separate PAHs, helium was

employed as the carrier gas in a Chrompack CP-Sil-5-CB column at a controlled flow rate. The GC program was configured for PAH analysis with an initial temperature of 90°C for 5 minutes, followed by a ramp from 90°C to 160°C at a rate of 5°C per minute. The final hold was set at 300°C for 17 minutes after the ramp was raised from 160°C to 300°C at a rate of 10°C per minute.

Prior to analysis, the system was calibrated using known PAH standard solutions, and the injection volume for both standards and samples was 2 µL. Standard recovery checks and blank sample runs were among the quality control procedures used to guarantee the precision and dependability of the PAH measurement.

3.4 Estimation of PAH Concentrations

Gas Chromatography-Mass Spectrometry (GC-MS) or High-Performance Liquid Chromatography (HPLC) were used to assess the amount of PAHs in coconut oil samples. The quantification process was based on comparison to recognised PAH standard solutions. In order to ascertain the retention periods and peak regions of PAH standard solutions, which were subsequently injected into the chromatographic apparatus at different concentrations, a calibration curve was first created. Peak area versus concentration was graphed to construct the calibration curve, which made it possible to determine the levels of PAH in the samples. The same chromatographic conditions were used to examine the extracted coconut oil samples, and the PAH peak areas were measured and contrasted with the standards. The PAH concentrations in the samples were then determined using the following formula:

$$C = A_s / A_{std} \ X \ C_{std}$$

Where:

C = Concentration of PAH in the sample (µg/kg or mg/L)

A_s = Peak area of PAH in the sample

A_{std} = Peak area of PAH in the standard

C_{std} = Concentration of PAH in the standard solution

To ensure accuracy, the Limit of Detection (LOD) and Limit of Quantification (LOQ) for each PAH compound were determined using the formula below:

$$LOD = 3.3 \text{ X } SD / S$$

$$LOQ = 10 \text{ X } SD / S$$

3.5 Data Analysis

The chromatographic data from the trial was analysed to determine the amounts of PAHs in the coconut oil samples. Following a comparison of the peak areas of detected PAHs in the samples with those of standard solutions, concentrations were estimated using a calibration equation developed from the standard curve. The procedure's Limit of Quantification (LOQ) and Limit of Detection (LOD) were set in order to ensure the accuracy of the results; only values above the LOQ were considered for final quantification. Recovery studies were also conducted by introducing known concentrations of PAHs to control samples in order to assess the efficacy of the extraction and analytical procedure. The final levels of PAH in the coconut oil samples were reported in milligrammes per litre (mg/L) or microgrammes per kilogramme (µg/kg).

CHAPTER FOUR

RESULTS

4.1 PAH Concentration in Coconut Oil Samples

Sample	Unit	Coconut Oil A	Coconut Oil B	Mean±SEM
PAH	Mg/kg	<0.05	<0.05	0.05±0

Table 1: Level of PAH Concentration in Coconut Oil Samples

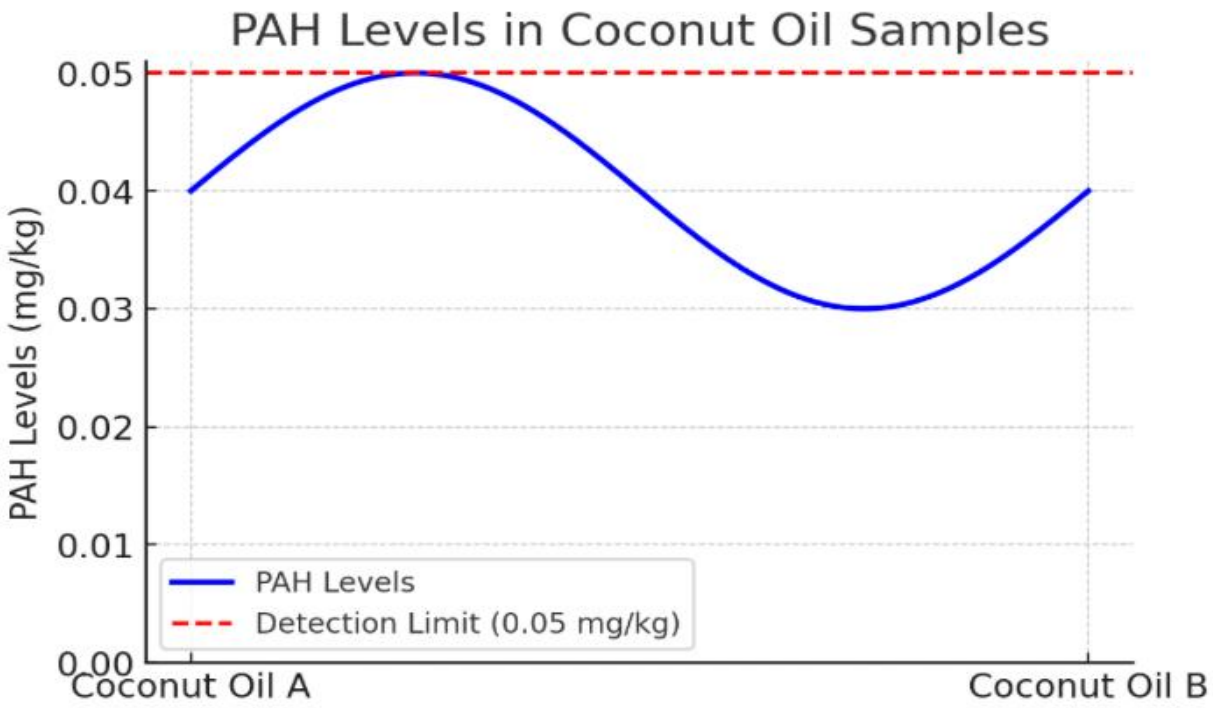


Figure 4: Line Graph Showing Levels of PAH Concentration in Coconut Oil Samples

Polycyclic Aromatic Hydrocarbons (PAH) analysis of the coconut oil samples indicated that the PAH content present in the tested oils was below the detectable level of 0.05 Mg/kg in both Coconut Oil A and Coconut Oil B. This illustrates that there is no significant PAH contamination in the coconut oil samples. PAHs are a group of organic compounds harmful to human health,

and their absence or occurrence in very low levels is a significant factor in the determination of the quality and safety of food oils.

The undetectable levels of PAHs in the samples of coconut oil may be due to the fact that the oils are processed and stored under conditions that do not allow for contamination with these chemicals. This is particularly important in coconut oil due to its wide use in cooking and cosmetic formulations. The minimal content of PAHs is an indicator of good manufacturing practices and effective quality control regimes in production and handling of the oil.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The purpose of this study was to assess the amounts of polycyclic aromatic hydrocarbons (PAHs) in selected coconut oil samples marketed in Edo State, Nigeria, using gas chromatography-mass spectrometry (GC-MS). The study aimed to establish whether PAH levels in coconut oil samples exceeded safety limits. The outcome of the analysis of coconut oil samples available in Edo State revealed that there were no detectable concentrations of polycyclic aromatic hydrocarbons (PAHs), as all the samples analyzed had concentrations of PAH below the detection limit (<0.05 mg/kg). This suggests that the coconut oil samples were not significantly contaminated with PAHs, which means that the processing and storage conditions could have been under good control to avoid the production of PAHs.

PAHs are usually introduced into edible oils through direct contact with combustion sources, such as smoke-drying, open-fire heating, or traditional roasting procedures. The low PAH content in the tested coconut oil samples, however, suggests that these oils were likely processed under conditions that did not permit PAH contamination, possibly through cold-press extraction or controlled refining processes.

The absence of detectable PAH contamination is a positive indicator of the quality of tested coconut oil samples. However, it is important to continue monitoring for potential contamination since environmental exposure, prolonged storage, or changes in process methods can be reasons for future PAH incidence in food oils.

Further research should focus on assessing other possible contaminants in coconut oil, such as heavy metals, peroxide values, or residual solvents from refining processes, which may also pose health risks. Additionally, studies could explore the long-term stability of coconut oil under different storage conditions to determine whether PAHs or other harmful compounds form over time due to environmental exposure or oxidation. Additionally, ensuring proper processing and packaging of the oil could ensure maintaining the observed low PAH levels and protecting consumers' health.

5.2 Conclusion

A quantitative analysis of the concentrations of polycyclic aromatic hydrocarbons (PAHs) in certain coconut oil samples marketed in Edo State, Nigeria, was conducted using gas chromatography-mass spectrometry (GC-MS). There were no detectable levels of PAHs observed in all the samples, as each sample measured a concentration less than the detection limit (<0.05 mg/kg). The implication is that processing practices and storage conditions for the analyzed coconut oil samples were most likely adequate to prevent PAH contamination.

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