DPPH SCAVENGING ACTIVITIES OF LOCALLY EXTRACTED *Elaeis guineensis* (PALM KERNEL) OIL

BY

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BMS2101465

DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF MEDICAL SCIENCE

COLLEGE OF MEDICAL SCIENCE

UNIVERSITY OF BENIN

IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCES DEGREE IN MEDICAL BIOCHEMISTRY

NOVEMBER, 2025

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

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CERTIFICATION

We the undersigned hereby certify that UHUNMWANGHO OSASERE FAITH (BMS2101465) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc.) in Medical Biochemistry.

Signed

....

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....

External Examiner **(Date)**

DEDICATION

This project is dedicated to Almighty God, the giver of life who has made it possible to complete my Bachelor of Science Degree (B.Sc.) program in the Department of Medical Biochemistry and my entire family for their tender care and love for me.

ACKNOWLEDGEMENT

My gratitude goes for Almighty God for his grace in all my endeavors, unto him is all the glory. My sincere appreciation goes to my amiable supervisor Dr. N. B. Aguebor-Ogie who doubles as the head of department, alongside other lecturers in the department for their words of wisdom and encouragement.

ABSTRACT

Although *Elaeis guineensis* (Palm kernel) oil is a dietary staple across West Africa, the antioxidant properties of locally processed, unrefined varieties remain under-researched. This study aimed to evaluate the *in vitro* antioxidant capacity of locally extracted palm kernel oil using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The oil was obtained via mechanical pressing and its efficacy was benchmarked against ascorbic acid. The findings revealed a concentration-dependent rise in antioxidant activity. Specifically, inhibition rates grew from 14.13% at the lowest concentration (50 µg/mL) to a peak of 44.15% at 250 µg/mL. The half-maximal inhibitory concentration (IC_{50}) was calculated at 285.77 µg/mL. While the unrefined oil displayed lower scavenging potential than the standard ascorbic acid, the results confirm that locally extracted palm kernel oil retains bioactive compounds, such as tocopherols, capable of reducing oxidative stress. These findings validate the oil's traditional value and suggest it has promise as a functional food ingredient.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Elaeis guineensis oil (Palm kernel oil) is an edible vegetable oil extracted from the kernel (endosperm) of the fruit of the oil palm tree, which is native to West Africa, particularly regions spanning from Angola to Gambia, with Nigeria likely serving as the center of diversity for oil palm germplasm (Okpe, 2022). Palm kernel oil (PKO) differs fundamentally from palm oil, which is extracted from the fruit's fleshy outer pulp. Physically, PKO is semi-solid at ambient temperatures due to its high saturation levels (typically 80-85%) (Sehgal and Sharma, 2020). Chemically, its profile is dominated by lauric acid (approximately 48%), followed by myristic and palmitic acids (Kiin-Kabari *et al.,* 2022). Beyond these fats, the unrefined oil contains vital minor components including squalene, phytosterols, and vitamin E (tocopherols and tocotrienols), all of which contribute to its stability and biochemical characteristics (Nainggolan and Sinaga, 2021).

PKO is one of the most widely consumed vegetable oils in West Africa, especially Nigeria, due to its low cost, high oxidative stability, and cultural significance (Edo *et al.,* 2022). Traditionally, it has been extracted through artisanal methods in smallholder systems, involving manual or semi-mechanized processes such as cracking the nuts, separating the kernels, roasting, grinding, and pressing or boiling to yield the oil. These local extraction techniques contrast with modern industrial methods that use screw presses or solvent extraction for higher efficiency (Ezeoha, 2020). In Nigeria, PKO production occurs alongside palm oil in natural groves, smallholder

farms, and estates, contributing significantly to the economy as over 70% of crude oils are consumed domestically without extensive refining (Ovwigho *et al.,* 2024). Domestically, raw PKO is used for cooking, frying, and as a base in traditional dishes, while its stability at high temperatures makes it ideal for commercial food processin. Beyond food, it finds applications in cosmetics for skin softening and wound healing, and in traditional medicine for promoting neuromotor development, immunity, and anti-inflammatory effects. Industrially, PKO is utilized in soaps, detergents, margarine, ice cream, and biodiesel production.

The health benefits of palm kernel oil (PKO) are largely attributed to its bioactive compounds, especially antioxidants (Baffour-Awuah *et al.,* 2021). Vitamin E isoforms (tocopherols and tocotrienols) function within a biological antioxidant network, scavenging free radicals and protecting cellular membranes from oxidative damage. Phytosterols help lower LDL cholesterol, phenolics exhibit anti-inflammatory effects, phospholipids aid nutrient absorption and brain development, while squalene contributes anticancer and cardioprotective properties (Poudel *et al.,* 2023). Research indicates that PKO can support moderate weight gain, reduce inflammation, and promote wound healing, with potential in mitigating non-communicable diseases such as diabetes, coronary heart disease, and cancer (Imoisi *et al.,* 2021). Moreover, its moderate saturated fat content poses less cholesterol risk than animal fats, and its unsaturated fatty acids (oleic and linoleic acids) may inhibit tumor growth and improve insulin regulation. In West Africa, where Palm kernel oil is a dietary staple, it has long been valued for promoting growth, infant nutrition, and managing oxidative stress-related conditions (Rahim *et al.,* 2023).

Unfortunately, much of the PKO consumed is processed or heated, which can degrade its antioxidants (e.g., vitamin E) and lead to the formation of reactive oxygen species (ROS), potentially exacerbating oxidative stress (Izuddin *et al.,* 2023). Oxidative stress arises from an

imbalance between free radicals and antioxidants, contributing to cellular damage and the pathogenesis of diseases (Sadiq *et al.,* 2023). Natural antioxidants from local sources like unrefined palm kernel oil could offer protective effects by enhancing total antioxidant capacity and modulating lipid profiles, as seen in related studies on red palm oil, which shares some bioactive components with palm kernel oil (Sulaiman *et al.,* 2022).

Given the critical role of antioxidants for preventing lipid peroxidation, maintaining cellular integrity, and addressing chronic diseases, evaluating the DPPH scavenging activities of locally extracted PKO is essential. This could inform dietary recommendations, nutraceutical development, and sustainable use of oil palm resources.

1.2 Aim of the Study

The aim of this study was to determine the *in vitro* DPPH free-radical scavenging (antioxidant) activity of locally extracted *Elaeis guineensis* (palm kernel) oil.

1.3 Justification of the Study

The pathogenesis of various chronic ailments, including neurodegenerative disorders, malignancies, diabetes, and cardiovascular dysfunction is frequently driven by oxidative stress. This condition arises when accumulation of reactive oxygen species (ROS) overwhelms the body's endogenous antioxidant defenses (Sharifi-Rad *et al.,* 2020). Although synthetic antioxidants exist, there is increasing emphasis on exploring natural, safer, and more affordable substitutes (Hassanpour and Doroudi, 2023). Palm kernel oil (PKO), widely produced and consumed in Nigeria, contains bioactive compounds like tocopherols, tocotrienols, phenolics, and squalene that may possess antioxidant properties (Izuddin *et al.,* 2023). However, these

beneficial constituents may be diminished during refining or processing (Ogbeide *et al.,* 2022). Despite its local availability and economic importance, limited studies have evaluated the antioxidant potential of locally extracted, unrefined PKO using reliable assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl), leaving gaps in understanding its biochemical value and health-promoting potential (Ng *et al.,* 2021).

Consequently, this research quantifies the DPPH radical-scavenging potential of palm-kernel oil obtained through local extraction processes and determines how variations in extraction methods shape its antioxidant characteristics.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of the Palm Kernel Oil

Derived from the nut of the *Elaeis guineensis* fruit, Palm Kernel Oil (PKO) is an edible lipid that is distinct from the palm oil extracted from the fruit's fibrous outer flesh (mesocarp). Although both oils originate from the same tree, they possess fundamentally different chemical compositions (Sehgal and Sharma, 2020). Commercially, PKO is highly valued for its significant solid fat content and specific melting behavior, which are essential for various manufacturing processes. However, because unmodified PKO has a relatively low melting point, it is unsuitable for certain applications unless it undergoes hydrogenation (Abel, 2024). For instance, in the confectionery industry, specifically for creating chocolate coatings on ice cream, raw PKO is frequently blended with liquid oils or palm oil to achieve the necessary consistency and texture (Gupta and Singh, 2024).

Broadly speaking, plant-based oilseeds constitute the primary raw materials for numerous industrial sectors. The drive to lower manufacturing expenses has accelerated the search for alternative oil sources that offer future economic viability (Obodai *et al.,* 2022). Utilizing these seed oils for industrial purposes helps reduce the over-reliance on oils that are strictly meant for food consumption. According to Léonce et al. (2021), the future of industrial feedstock lies in the exploration of these alternative resources. Consequently, there is growing interest in specific oil-bearing plants, including non-edible varieties that offer high yields, are easily accessible, and can thrive in nutrient-poor soils. Because these plants often require less intensive agricultural care,

they help lower cultivation costs, making oil yield the primary criterion for selecting seeds for industrial use (Khanal and Shah, 2021).

Figure 2.1: Uncracked Palm Kernel Nuts

2.1.1 Scientific Classification of *Elaeis guineensis*

Table 2.1: Showing Scientific classification of *E. guineensis*

Scientific classification	
Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Clade:	Commelinids
Order:	Arecales
Family:	Arecaceae
Genus:	*Elaeis*
Species:	***E. guineensis***
Binomial name	
Elaeis guineensis	

2.1.2 Distribution

The availability of palm kernels is directly linked to the locations of palm oil processing facilities. On a global scale, plantations in East Asia, specifically Indonesia and Malaysia, dominates the industry. In the African context, South Africa contributes a minor share, accounting for roughly 4% of the continent's total production. However, substantial quantities are produced in West and Central African nations, including Nigeria, Ghana, Cameroon, Liberia, Côte d'Ivoire, Sierra Leone, the Benin Republic, and the Democratic Republic of the Congo. The fatty acid profile of the oil produced in these regions is significant, defining both its nutritional value and its importance as a raw material for industrial applications (Ovwigho *et al.,* 2024).

2.2 Nutritional Profile of Palm Kernel Oil

Much like coconut oil, palm kernel oil (PKO) is characterized by a high concentration of saturated fats, exceeding the saturation levels found in ordinary palm oil derived from the fruit pulp (Ogbeide *et al.,* 2022). The oil is rich in lauric acid, a compound known to impact blood cholesterol. Research indicates that while lauric acid can elevate Low-Density Lipoprotein (LDL) cholesterol, a marker associated with cardiovascular risk, it also significantly boosts High-Density Lipoprotein (HDL) cholesterol (Leonard, 2025). Consequently, the observed increase in total cholesterol is often attributed to a rise in the beneficial HDL fraction rather than LDL alone. Furthermore, PKO is naturally devoid of trans fats and cholesterol (Izuddin *et al.,* 2023). Due to its cost-effectiveness, extended shelf life, and ability to withstand high temperatures without degrading, PKO is a preferred choice for commercial cooking and food processing (Hormenu *et al.,* 2024).

Regarding the kernels themselves, they contain approximately 46-54% oil by dry matter (DM). This high oil content translates to a significant gross energy value of 28.9 MJ/kg DM, making them a viable alternative for high-energy feed ingredients. However, the kernels are not a primary source of protein, which makes up only about 10% of their dry matter content.

Table 2.2 showing the approximate concentration of fatty acids (FAs) in palm kernel oil.

Fatty acid content of palm kernel oil	
Type of fatty acid	**Fraction**
Lauric saturated C12	0.482
Myristic saturated C14	0.162
Palmitic saturated C16	0.084
Capric saturated C10	0.034
Caprylic saturated C8	0.033
Stearic saturated C18	0.025
Oleic monounsaturated C18:1	0.153
Linoleic polyunsaturated C18:2	0.023
Other/Unknown	0.004

Table 2.3 showing chemical composition and nutritional value

Main analysis	Unit	Avg	SD	Min	Max	Nb
Dry matter	% as fed	91.8	1.3	88.2	94.1	14
Crude protein	% DM	9.5	1.5	7.9	13.6	11
Crude fibre	% DM	14.3	2	11.6	17.6	10
NDF	% DM	49.4	2.9	45.6	52.9	6
ADF	% DM	34.8	2.9	31.8	39	6
Lignin	% DM	13.2	3	9.4	16.8	5
Ether extract	% DM	47.9	3.8	43.1	54.2	11
Ash	% DM	2.1	0.4	1.2	2.6	10
Gross energy	MJ/kg DM	28.4				

Minerals	Unit	Avg	SD	Min	Max	Nb
Calcium	g/kg DM	1.1	0.4	0.9	1.7	4
Phosphorus	g/kg DM	3.4	0.3	3.1	3.8	4
Potassium	g/kg DM	3.8		2.9	4.7	2
Magnesium	g/kg DM	1.8		1.7	1.8	2

Amino acids	Unit	Avg	SD	Min	Max	Nb
Arginine	% protein	3.9				1
Histidine	% protein	2.5				1
Lysine	% protein	3.5				1
Methionine	% protein	2.1				1
Threonine	% protein	3				1
Tryptophan	% protein	2.8				1

2.3 Applications of Oil from Palm Kernels

Among the various applications for palm kernel oil are the following:

i. As a food ingredient, palm-kernel oil enhances flavour and supplies a concentrated source of energy.
ii. It is important in the manufacture of confectionery and as a shortening in baked products.
iii. It is used industrially as an emulsifier and lubricant, and serves as a drying base in paints and candles.
iv. Palm-kernel oil is widely used for soap production; soaps made from its lauric-rich fraction are valued for excellent lather, and glycerol is produced as a by-product during saponification.
v. The oil is commonly incorporated into cosmetic formulations.
vi. After hydrogenation, the oil can be converted into margarine.
vii. A dark oil obtained after roasting is used in some traditional medicinal preparations reportedly associated with convulsive effects.
viii. With suitable modification, palm-kernel oil can be processed into an alternative, non-petroleum fuel.
ix. The residue cake from oil extraction, which contains protein as well as fats and carbohydrates, is used in animal feed formulations (Alhaji *et al.,* 2024).

2.4 Operations of the Oil Palm Fruit Processing Unit

Figure 2.2 presents the sequence of unit operations from reception of fresh fruit bunches through to recovery and storage of palm kernels.

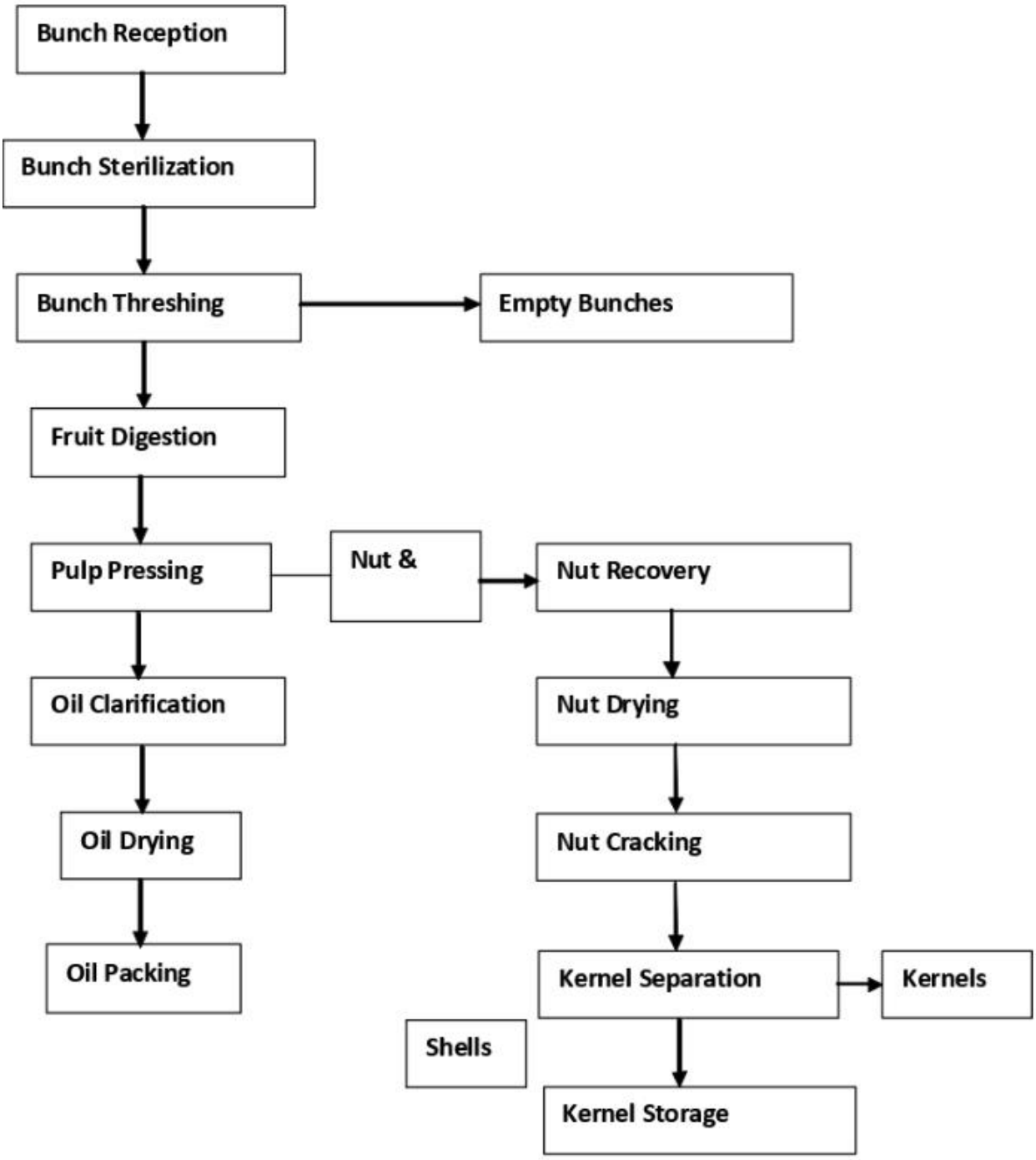


Figure 2.2: Oil palm fruit processing unit operations

After cracking and separating the nuts to recover the kernels, oil recovery is performed by one of several extraction techniques.

2.5 Extraction Methods

Palm kernel oil can be obtained by three principal extraction approaches: traditional (artisanal) methods, chemical (solvent) extraction, and mechanical expression. (Raji *et al.,* 2022).

2.5.1 Traditional (Artisanal) Approaches

i. Conventional roasting method: Oil-bearing cells are ruptured by heating kernels (usually in clay pots over an open fire), and the oil that is produced is collected.
ii. Hand pressing: To remove oil, crushed kernels are encased in cloth and mechanically squeezed (Léonce *et al.,* 2021).
iii. Stone and lever pressing: To extract oil from coated, crushed kernels, basic lever-and-stone mechanisms are used.
iv. Pestle-and-mortar method: Oil drains from an outlet after the kernels are crushed by friction and pressure using a stationary mortar and an animal or human-driven pestle. This is also called the Ghanis method.
v. The conventional aqueous approach involves boiling ground kernels in water, skimming off any oil that separates to the surface, and then heating the mixture to eliminate any remaining moisture (Ogbeide *et al.,* 2022).

2.5.2 Chemical (Solvent) Approach

The crude oil is obtained by treating ground kernels with an organic solvent that dissolves the oil and then removing the solvent, usually by evaporation. Kernel pre-treatment, oil extraction, and solvent recovery phases are typically included in the solvent extraction process. Due to equipment costs and safety concerns, it is less suitable for small operations but effective for high-capacity mills (Osagiede and Nwagu, 2023).

2.5.3 Mechanical Appraoches

Mechanical extraction depends on compressive pressures and typically involves three primary stages: kernel pre-treatment, screw pressing (or alternative mechanical pressing), and oil purification.

These techniques use controlled pressure and friction to extract oil from the solid matrix. They include the following:

2.5.4 Hydraulic Pressing Method

The foundation of this technique is the idea that pressure exerted to a contained body of fluid circulates in all directions without diminishing. Groups of ground kernel which are often in stacked boxes or bags, are compressed by a hydraulic ram or piston, which transmits pressure uniformly so that oil is released through apertures in each container. Horizontal boxes are stacked and squeezed in modern hydraulic presses to extract oil.

2.5.5 Screw Pressing Method

The screw press is a continuous extraction machine featuring a worm shaft rotating inside a perforated barrel. Conditioned kernels are fed into the hopper and pushed forward by the rotating screw. As the space inside the barrel tightens, the friction generates heat and pressure, squeezing the oil out through the perforations while the dry cake is discharged at the end. The heat generated during this process is beneficial as it facilitates the flow of oil. (Osagiede and Nwagu, 2023).

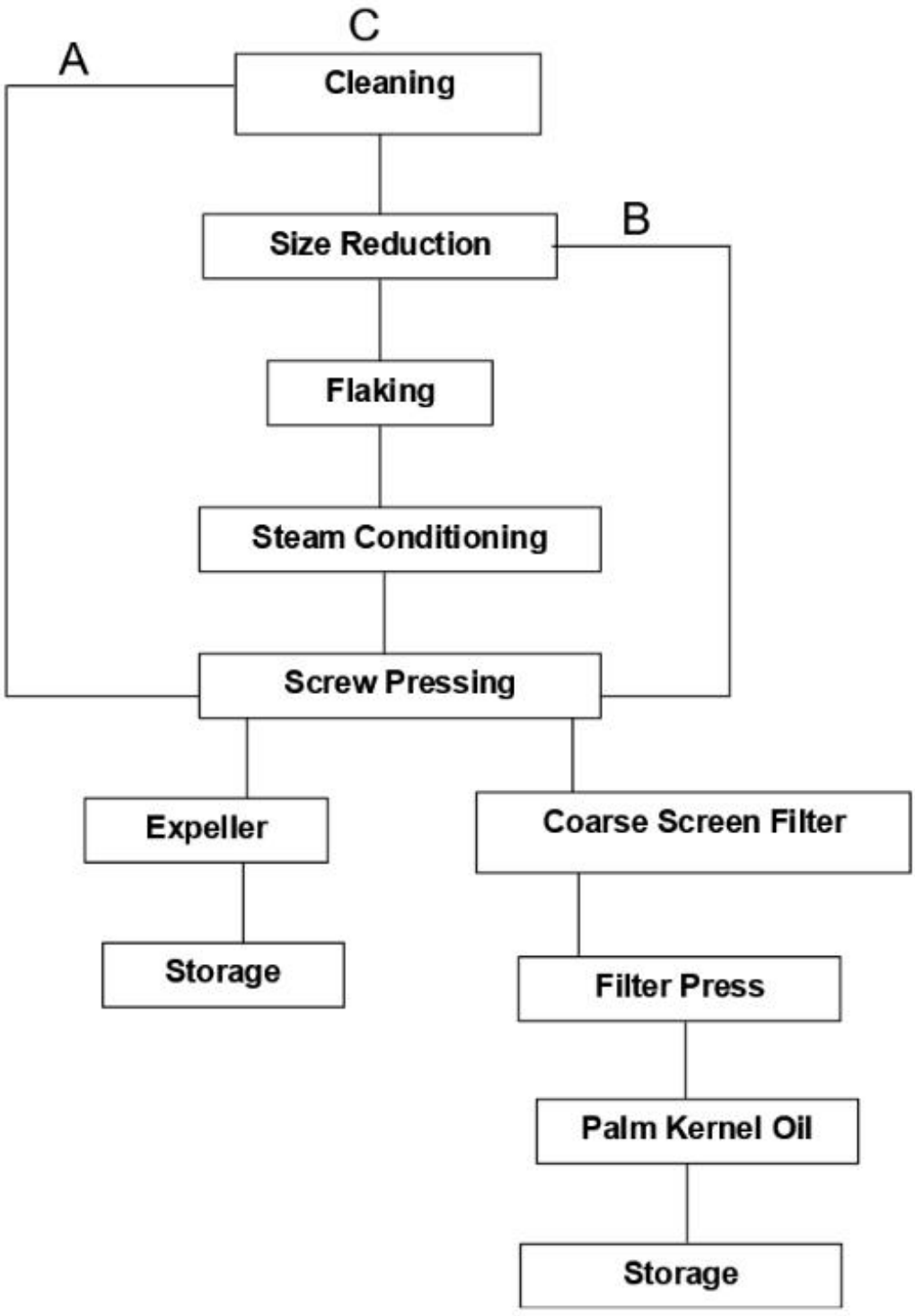


Figure 2.3: Using a screw press to mechanically extract palm kernel oil

2.5.6 Comparative Analysis of Extraction Techniques

Evaluating the various extraction methodologies reveals a clear distinction between traditional and modern approaches. While traditional techniques such as roasting, hot water flotation, and manual pressing are characteristically labor-intensive and yield poor results, modern mechanical and chemical methods offer superior efficiency. However, solvent extraction presents significant barriers for the Nigerian market; the equipment is expensive, and the process carries a high risk of fire and explosion. These factors make it impractical for the small-to-medium scale farmers who dominate the industry in developing nations. Consequently, the mechanical screw press (or expeller) has become the preferred method for commercial PKO extraction in Nigeria (Ogbeide *et al.,* 2022).

Key operational differences between these methods include:

- **Product Quality:** Oil produced via the traditional roasting method is distinctively dark or black in color.
- **Efficiency of Traditional Methods:** Traditional pressure techniques generate insufficient force, resulting in low recovery rates where only 20% to 25% of the available oil is extracted.
- **Efficiency vs. Viability of Chemical Methods:** Solvent extraction is the most efficient method, leaving as little as 1% residual oil in the cake. However, it requires high capital investment, large-scale volume, and specialized technical expertise, making it unsuitable for rural or small-scale urban processors.
- **Mechanical Screw Press Characteristics:** The screw press is a compact unit that is scalable, handling anywhere from 3 to 1,000 tons per day. While the initial purchase price

is high, it requires minimal labor and technical skill to operate. Maintenance is manageable, though the machine consumes a significant amount of power, and high friction can cause rapid wear to the worm shaft and housing components.

2.6 Oxidative Stress, Free Radicals and Biological Significance

Oxidative stress arises from an imbalance between reactive oxygen species (ROS) and antioxidants, leading to cellular damage with profound biological significance in aging, disease pathogenesis, and homeostasis (Sharifi-Rad *et al.,* 2023). Free radicals, unstable molecules with unpaired electrons (e.g., superoxide O2•−, hydroxyl •OH), are generated endogenously via mitochondrial respiration, enzymatic reactions (e.g., NADPH oxidase), and exogenously from pollutants, radiation, and diet. ROS, including non-radicals like hydrogen peroxide (H2O2), play dual roles: at low levels, they facilitate signaling (e.g., in immune response, cell proliferation); at high levels, they induce oxidative damage to lipids (peroxidation), proteins (carbonylation), and DNA (strand breaks), contributing to mutations and apoptosis (Pooja *et al.,* 2025).

Biologically, oxidative stress is implicated in over 100 diseases, including cancer (via oncogene activation), atherosclerosis (LDL oxidation), neurodegenerative disorders (e.g., Alzheimer's via β-amyloid aggregation), diabetes (insulin resistance), and inflammation (NF-κB activation) (Li *et al.,* 2025). In radical-free oxidative stress, non-radical oxidants like hypochlorous acid (HOCl) cause similar damage without electron transfer, as seen in protein oxidation. Environmental factors exacerbate this, e.g., UV radiation increases skin ROS, leading to premature aging. Antioxidants mitigate by neutralizing ROS, but chronic imbalance accelerates aging via telomere shortening and mitochondrial dysfunction (Varesi *et al.,* 2022).

2.6.1 Antioxidants

Antioxidants are compounds that inhibit oxidation by neutralizing free radicals, chelating metals, or quenching singlet oxygen, classified by mechanism (primary/secondary), origin (endogenous/exogenous), and solubility (lipophilic/hydrophilic) (Gulcin, 2020). Mechanisms include hydrogen atom transfer (HAT, e.g., donating H• to radicals), single electron transfer (SET, e.g., reducing radicals), and transition metal chelation (preventing Fenton reactions). Primary antioxidants (chain-breakers) directly scavenge radicals, while secondary (preventive) regenerate primaries or decompose hydroperoxides (Adwas *et al.,* 2019).

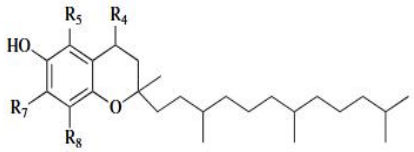
Lipophilic antioxidants (e.g., tocopherols, carotenoids, ubiquinol) act in lipid phases like cell membranes, preventing peroxidation; hydrophilic (e.g., ascorbic acid, glutathione, polyphenols) function in aqueous environments like cytosol (Valgimigli, 2023). Interactions depend on ratios/concentrations; e.g., hydrophilic phenolics synergize with lipophilic tocopherols in emulsions. Electrochemical methods measure both in biphasic systems.

2.6.2 Tocopherols and Tocotrienols

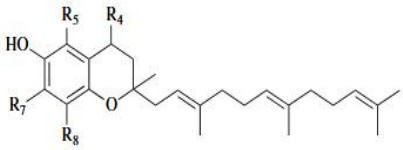
PKO contains low levels of tocopherols and tocotrienols (13-58 ppm α-tocopherol, 21 ppm α-tocotrienol), significantly less than mesocarp palm oil (600-1000 ppm total tocols). α-Tocopherol is higher in Tenera varieties (58.9 mg/100g vs. 44.1 mg/100g in Dura). These vitamin E forms act as antioxidants, protecting against lipid peroxidation and enhancing stability, though refining reduces content (Jain *et al.,* 2022).

The classification of tocopherols and tocotrienols (Tan *et al.,* 2024).

Table 2.4 showing the classification of tocopherols and tocotrienols



Tocopherols



Tocotrienols

Tocopherols	R_4	R_5	R_7	R_8	Tocotrienols	R_4	R_5	R_7	R_8
α-Tocopherol	H	CH_3	CH_3	CH_3	α-Tocotrienol	H	CH_3	CH_3	CH_3
β-Tocopherol	H	CH_3	H	CH_3	β-Tocotrienol	H	CH_3	H	CH_3
γ-Tocopherol	H	H	CH_3	CH_3	γ-Tocotrienol	H	H	CH_3	CH_3
δ-Tocopherol	H	H	H	CH_3	δ-Tocotrienol	H	H	H	CH_3

2.6.3 Phenolics

Phenolic content in palm kernel oil is low (10-94 mg gallic acid equivalents/g), with higher levels in Tenera varieties (94 mg EAG/g vs. 62 mg in Dura). Phenolics contribute to oxidative stability and anti-inflammatory effects, but detailed profiles are sparse in literature; they synergize with other antioxidants for health benefits like reducing oxidative stress (Abdullah and Ramli, 2020).

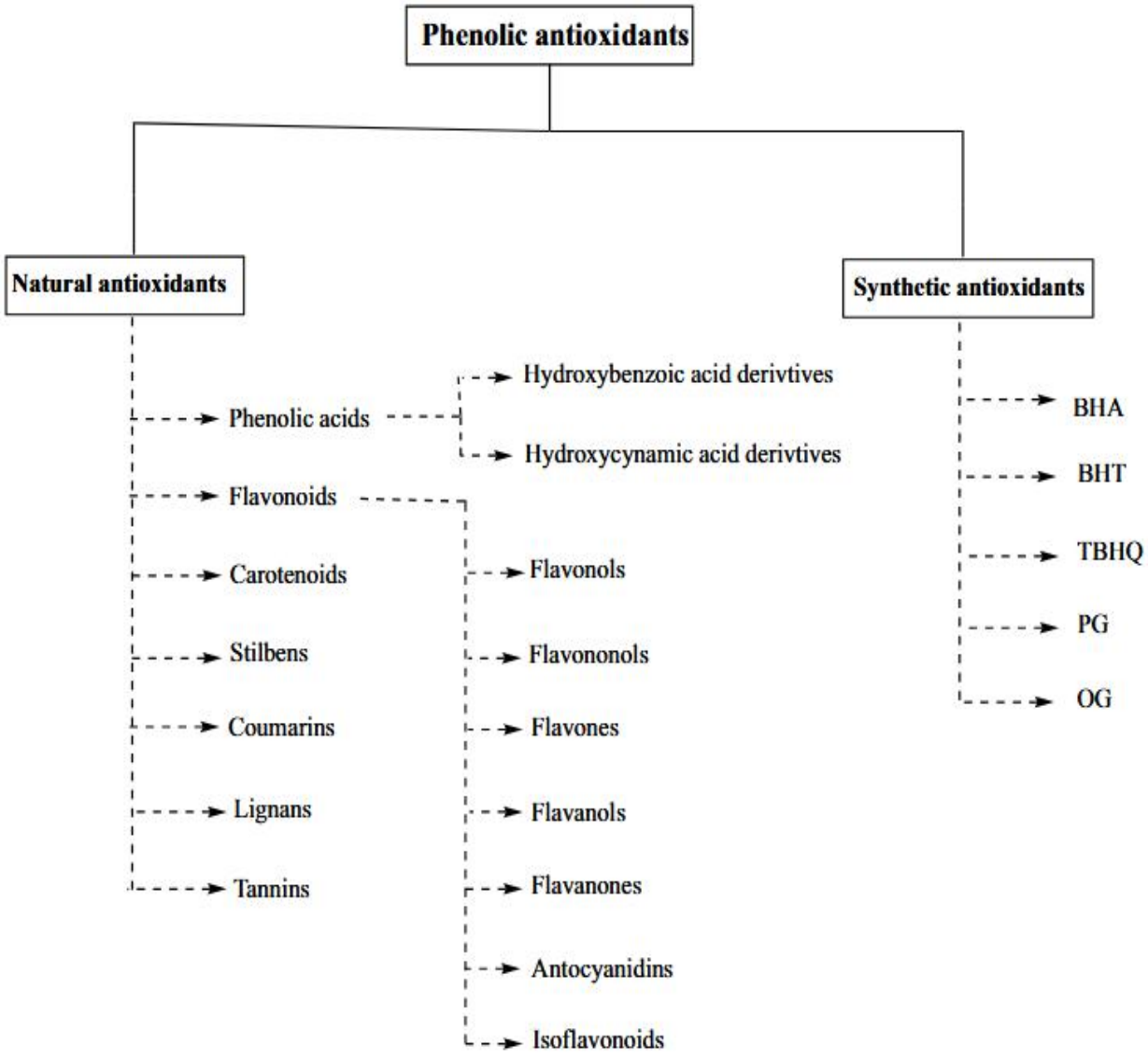


Figure 2.4: Classification of phenolic antioxidants (Sharifi-Rad *et al.,* 2023).

2.6.4 Sterols

Sterols in refined palm kernel oil average 875 mg/kg, with β-sitosterol (68-76%), stigmasterol (11-14%), and campesterol (9-13%) predominant. Total sterols range 350-4980 mg/100g, higher in Tenera (4980 mg/100g). β-Sitosterol exhibits anti-cancer, anti-inflammatory, and cholesterol-lowering properties (Poudel *et al.,* 2023).

Other Components

- Squalene: 200-500 ppm, with anticancer and cardiovascular benefits.
- Phospholipids: 10-50 ppm, aiding nutrient absorption and brain development.
- Carotenoids: <8 mg/kg in refined PKO, low compared to mesocarp oil.
- Triterpene Alcohols: 470 mg/kg.
- Physicochemical Properties: Acid value 3.53-12.22 mg/g, peroxide value 7.3-10 Meq/kg, iodine value 6-45 mg/g, saponification value 187-216 mg KOH/g, moisture 0.03-0.89%, density 0.80-0.91 g/cm^3, refractive index 1.46-1.48. These indicate high stability but potential for hydrolysis.

2.7 Antioxidant Assays

In biochemical research, antioxidant assays serve as fundamental techniques for quantifying the capacity of substances to scavenge free radicals and mitigate oxidative stress (Engwa *et al.,* 2022). These methods typically operate through mechanisms such as hydrogen atom transfer (HAT), single electron transfer (SET), or a combination of both, providing insights into the overall antioxidant capacity of samples like plant extracts, foods, and oils (Gulcin and Alwasel, 2020). Common assays include DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), FRAP (ferric reducing antioxidant power), and ORAC (oxygen radical absorbance capacity) (Munteanu and Apetrei, 2021). Each assay targets specific aspects of antioxidant activity: DPPH focuses on radical scavenging via a decrease in absorbance at 517 nm, offering simplicity and reliability; ABTS evaluates both SET and HAT through decolorization at 734 nm, making it versatile for both hydrophilic and lipophilic compounds; FRAP measures reducing potential by converting Fe^{3+} to Fe^{2+}, emphasizing electron donation

capacity; and ORAC quantifies inhibition of peroxyl radicals using fluorescence decay, simulating physiological radical reactions. These assays are widely applied due to their sensitivity, speed, and ability to handle diverse sample types, though they vary in their relevance to in vivo conditions (Baliyan *et al.,* 2022).

2.8 Radical Scavenging Methods and Their Role in Combating Oxidative Damage

Even with natural antioxidant defenses in organisms, particularly humans, oxidative modifications to key biomolecules such as lipids, proteins, and DNA, can lead to cellular injury, hastening aging and contributing to disease onset (Chaudhary *et al.,* 2023). To mitigate and study these processes, it is crucial to comprehend radical chain reactions within metabolic pathways. These reactions are key drivers of lipid autoxidation and peroxidation, where unstable radicals propagate damage unless interrupted. Agents capable of scavenging radicals play a vital role by neutralizing peroxide radicals, thereby halting chain reactions and enhancing the durability and integrity of products like foods. The primary mechanism for inhibiting lipid oxidation involves direct interaction between antioxidants and radicals, making radical scavenging a cornerstone of antioxidant evaluation (Chaudhary *et al.,* 2023). This approach is a fundamental and routinely employed test in studies assessing antioxidant potential (Gulcin and Alwasel, 2020).

Among the most favored spectrophotometric techniques for measuring antioxidant activity in items such as drinks, edibles, and plant-derived extracts are radical scavenging-based assays like DPPH•, $DMPD^{+}$, $ABTS^{+}$, and O_2^{-}. These methods rely on chromogenic radicals that directly engage with antioxidant molecules, resulting in measurable color changes. Their popularity stems from attributes like high sensitivity, ease of implementation, rapidity, and consistency. Additionally, these assays are adaptable for screening large sample sets, facilitating quick

assessments of antioxidant efficacy in complex matrices (Baliyan *et al.,* 2022; Pooja *et al.,* 2025).

The chemical reactions in these assays typically follow a pattern where antioxidants reduce the radical cations, reversing their formation and leading to decolorization:

· $DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet}$

· $ABTS^{+\bullet} + AH \rightarrow ABTS + A^{\bullet}$

· $DMPD^{+\bullet} + AH \rightarrow DMPD + A^{\bullet}$

Such processes are notably efficient, demanding minimal equipment or costly materials, with sample preparation and analysis often completed in under 30 minutes and requiring little manual effort. Their user-friendly nature allows for rapid, simultaneous testing of multiple samples with high precision (Gulcin and Alwasel, 2020).

2.9 DPPH Radical Scavenging Assay: Principles and Mechanisms

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay is widely recognized as a standard, preliminary method for quantifying the antioxidant potential of biological samples (Gulcin and Alwasel, 2020). Fundamentally, DPPH acts as a "long-lived" or stable nitrogen-centered free radical. Its stability is unique; unlike most free radicals that rapidly pair up (dimerize), the unpaired electron in DPPH is delocalized across the entire molecule. This electron delocalization stabilizes the radical and is responsible for its intense deep violet color, which exhibits a specific absorption maximum at approximately 517 nm in organic solvents (Adam *et al.,* 2021).

The mechanism of this assay relies on a reduction reaction. When the violet DPPH solution reacts with a substance capable of donating a hydrogen atom or an electron (such as the phenolic compounds found in plant extracts), the radical is neutralized to become 1,1-diphenyl-2-picrylhydrazine. Visually, this chemical change is observed as a bleaching effect, where the solution shifts from purple to pale yellow. The degree of color loss is proportional to the concentration and potency of the antioxidants present (Baliyan *et al.,* 2022).

This method is favored for its simplicity, speed, and cost-effectiveness. It does not require complex equipment, only a UV-Vis spectrophotometer, and avoids the need to generate free radicals artificially before testing, unlike the ABTS assay. However, researchers must account for potential interference, as certain compounds (like anthocyanins) may absorb light in the same 500–550 nm range, potentially skewing results (Chaudhary *et al.,* 2023).

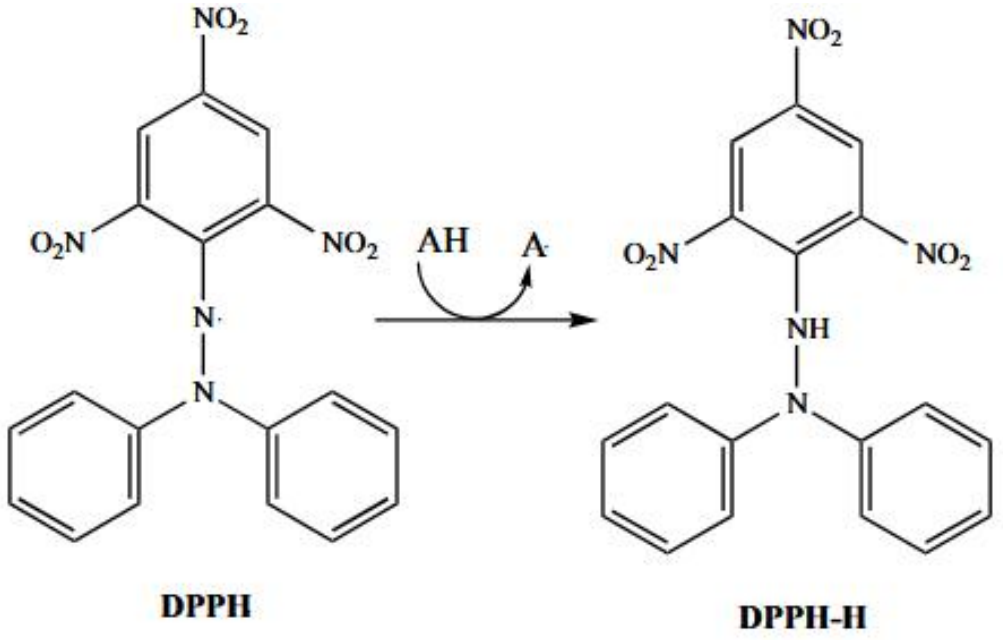


Figure 2.5: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging mechanisms by an antioxidant.

DPPH, a stable free radical, is commonly used to evaluate the scavenging potential of phenols, catechols and anilines; these interactions typically occur via hydrogen-atom transfer (HAT) or sequential proton-loss electron transfer (SPLET).

$ArOH + DPPH^{\cdot} \rightarrow ArOH^{\cdot} + DPPH$ 2(HAT),

$ArOH \leftrightarrows ArO^{-} + H^{+}$ (SPLET),

$ArO^{-} + DPPH^{\cdot} \rightarrow ArO^{\cdot} + DPPH^{-}$,

$DPPH^{-} + H^{+} \rightarrow ArO^{\cdot} + DPPH$ 2.

DPPH scavenging activity is typically assessed in organic solvents (for example, ethanol or methanol) by monitoring the decline in absorbance at 515–528 nm until a stable reading is reached, or by using electron paramagnetic resonance (EPR) spectroscopy. EPR enables direct quantification of the $DPPH^{\cdot}$ radical at sub-micromolar concentrations and offers advantages over conventional spectrophotometry for intensely coloured or turbid samples. Methanol, however, is often avoided as the reaction medium because of its toxic properties (Pooja *et al.,* 2025).

2.10 Scope and Applications of DPPH Radical Scavenging

The DPPH assay stands out as a versatile spectrophotometric technique with broad utility in quantifying antioxidant potential across diverse samples, including pure compounds, beverages, foods, and botanical extracts (Adam *et al.,* 2021). Its appeal lies in its straightforwardness, precision, efficiency, and dependability, positioning it as the go-to method for appraising the radical-neutralizing abilities of substances and plant materials (Gulcin and Alwasel, 2020).

For evaluating oils such as palm kernel oil (PKO), the DPPH assay is particularly advantageous owing to its robustness in organic media, compatibility with fat-soluble antioxidants, affordability, and strong association with phenolic levels. This makes it especially suitable for analyzing radical scavenging in lipid-rich environments, providing reliable insights into the antioxidant profile of unrefined extracts (Pooja *et al.,* 2025).

The assay's compatibility with organic solvents and its applicability to lipophilic antioxidants (e.g., tocopherols and tocotrienols) make it appropriate for unrefined PKO, provided solvent/emulsifier choice and reaction time are carefully optimized. Review and methodological studies further summaries the assay's strengths, limitations and recommended adaptations when applied to hydrophobic matrices (Xiao *et al.,* 2020).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Apparatus and Equipments

The apparatus or equipment used for this study were gotten from the Chemistry laboratory at the University of Benin, and were confirmed to be in good working condition before use. They include: Beakers, spectrophotometer (Jenway 6100, Dunmow, Essex, U.K),water bath(37°C), oven, analytical balance, filter paper (whatman No.1), mortar and pestle.

3.1.2 Chemicals and Reagents

All the chemicals and reagents used in this study were of analytical grade. They include:
Foli-ciocalteu reagent, Sodium carbonate, Vanillian reagent, Ethanol, Sulphuric acid, Aluminum chloride, Sodium acetate, Methanol, Quercetin, Folin-Denis reagent, Tannic acid solution, Acetic acid, Ammonium hydroxide (conc), Ammonium solution

3.2 Methods

3.2.1 Sample Collection and Preparation

3.2.1.1 Sample Collection

Palm nuts were procured from a local market (New Benin Market, Benin City, Edo State, Nigeria). The collected samples were transported to the laboratory for immediate processing.

3.2.2 Extraction of Palm Kernel Oil

The powdered palm kernel samples were extracted using standard phytochemical procedures with minor modifications. Each extraction was performed in triplicate to ensure accuracy and reproducibility. Solvents of different polarities were employed to achieve a wide range of phytochemical recovery. Polar solvents such as 70-80% methanol or ethanol were used to extract phenols, flavonoids, tannins, and alkaloids, while water served as the solvent for more polar compounds like saponins and other water-soluble components.

Extraction was carried out through maceration, where the powdered samples were soaked in the chosen solvent at room temperature for several days with occasional shaking to enhance solvent penetration and compound dissolution. After soaking, the mixtures were filtered using Whatman No. 1 filter paper to separate the extracts from the residues. The filtrates were concentrated by evaporating the solvents at a controlled temperature in an oven to yield the crude extracts). These extracts were then stored in labeled containers and kept until needed for phytochemical evaluation.

3.3 Determination of DPPH Radical Scavenging Activity

The ability of the palm kernel oil to scavenge free radicals was determined using the stable DPPH radical method as described by Siripongvutikorn *et al.* (2024) and Musa *et al.* (2016), with slight modification.

3.3.1 Preparation of Sample Concentrations

A stock solution of the palm kernel oil was prepared by dissolving a in methanol. From this stock solution, serial dilutions were made to obtain various concentrations for the assay (e.g., 250, 200,

150, 100, and 50 µg/mL). A similar set of concentrations was prepared for the standard antioxidant, ascorbic acid.

3.3.2 Procedure

1. Into a set of clean, dry test tubes, 2.0 mL of each diluted sample concentration was placed.
2. A control tube was prepared containing 2.0 mL of methanol instead of the oil extract.
3. To each test tube, 2.0 mL of a freshly prepared methanolic solution of DPPH was added.
4. The tubes were agitated gently to ensure proper mixing of the contents.
5. All the test tubes were then incubated in the dark at room temperature for 30 minutes. The incubation period allows for the scavenging reaction to take place.
6. After 30 minutes, the absorbance of the reaction mixtures was measured at a wavelength of 518 nm using a UV-Vis Spectrophotometer. The discoloration of the DPPH solution (from purple to yellow) indicates the scavenging potential of the oil.

3.3.3 Calculation of Scavenging Activity

The percentage of DPPH radical scavenging activity of the palm kernel oil was calculated using the following equation:

$$\boldsymbol{Scavenging\ activity}\ (\%) = \frac{(\boldsymbol{A_0} - \boldsymbol{A_1})}{\boldsymbol{A_0}} \times \mathbf{100}$$

Where:

- $\mathbf{A_0}$ is the absorbance of the control (containing only methanol and DPPH).
- $\mathbf{A_1}$ is the absorbance of the sample (containing the palm kernel oil and DPPH).

3.4 Statistical Analysis

Data obtained from the spectrophotometric and gravimetric analyses were processed using IBM SPSS Statistics software (Version 21.0.0). Each test was performed in triplicate (n = 3), and the outcomes were reported as mean ± standard deviation (SD) to enhance precision and reliability. Descriptive statistical methods were applied to interpret the data, and the results were clearly presented in tables for easy understanding.

CHAPTER FOUR

4.0 RESULTS

4.1 DPPH Radical Scavenging Activity of Palm Kernel Oil

The antioxidant capacity of the palm kernel oil was assessed by its ability to scavenge the stable DPPH free radical. The assay was conducted across a range of concentrations (50 to 250 µg/mL). The percentage of inhibition for each concentration is summarized in Table 4.1.

Table 4.1: DPPH Radical Scavenging Activity of Palm Kernel Oil (PKO) at Various Concentrations

CONC.(µg/ml) OF SAMPLE/ STANDARD	**SCAVENGING ACTIVITY (%)**
250	44.15
200	39.381
150	26.102
100	20.725
50	14.129

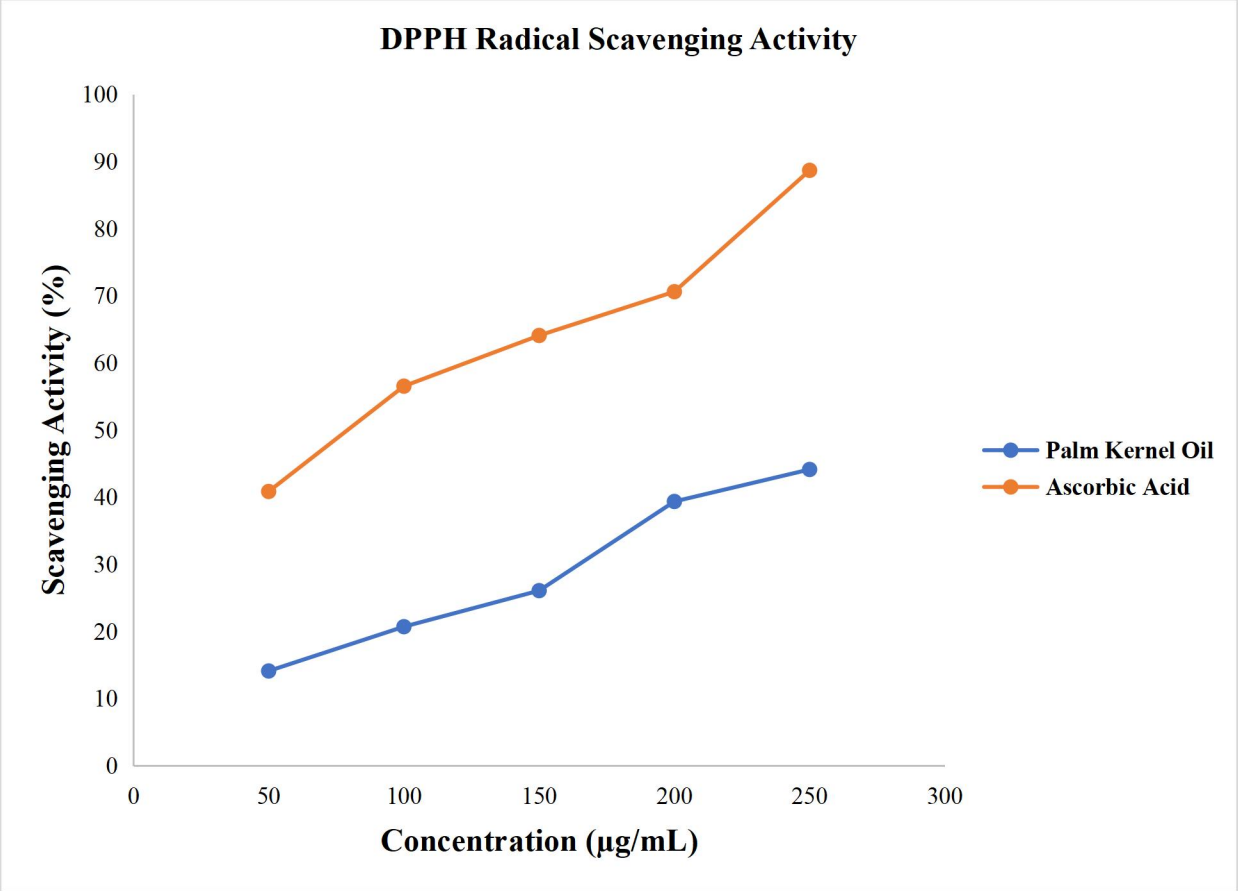


Figure 4.1 shows a comparison of the DPPH radical scavenging activities of palm kernel oil (PKO) and the standard antioxidant, ascorbic acid. Both samples displayed concentration-dependent increases in DPPH radical-scavenging activity, however, ascorbic acid showed greater scavenging potential than palm kernel oil at all tested concentrations.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The observed antioxidant capacity can be attributed to the bioactive components present in the oil. As established in the literature review, unrefined palm kernel oil (PKO) contains lipophilic antioxidants such as vitamin E isoforms (tocopherols and tocotrienols) and phenolic compounds (Nainggolan and Sinaga, 2021; Baffour-Awuah *et al.,* 2021). These molecules can donate a hydrogen atom to the stable DPPH radical, neutralizing it and thereby reducing its absorbance (Gulcin and Alwasel, 2020). The DPPH assay is particularly well-suited for this analysis due to its compatibility with lipid-rich matrices and fat-soluble antioxidants like those found in PKO (Xiao *et al.,* 2020). The mechanical extraction method used in this study, being a low-heat process, likely preserved these thermally sensitive compounds, which might otherwise be degraded during industrial refining or traditional roasting methods (Ogbeide *et al.,* 2022).

While PKO demonstrated antioxidant activity, the comparison with ascorbic acid provides a crucial benchmark for its potency. As shown in Figure 4.1, ascorbic acid demonstrated greater scavenging potential than palm kernel oil at all tested concentrations. For instance, at 250 µg/mL, ascorbic acid achieved nearly 90% inhibition, whereas PKO reached only 44.15%.

This result is expected and scientifically logical. Ascorbic acid is a pure, highly potent antioxidant compound. In contrast, PKO is a complex mixture where the vast majority of its composition consists of fatty acids (primarily lauric, myristic, and palmitic acids), which have no direct radical scavenging activity (Kiin-Kabari *et al.,* 2022). The antioxidant effect of PKO

comes from a very small fraction of minor components. Therefore, the activity of PKO should be interpreted not as weak, but as a moderate effect derived from a natural, unpurified product. This confirms that while PKO is not as powerful as a pure standard, it contains biologically active constituents that contribute to its overall antioxidant profile.

The findings of this study have important implications. They provide scientific validation for the traditional use of unrefined PKO in diets and topical applications where antioxidant effects are beneficial (Rahim *et al.,* 2023). In regions like Nigeria, where PKO is a dietary staple, the consumption of locally extracted, unrefined oil could contribute to the body's overall antioxidant defense system, helping to mitigate oxidative stress implicated in various chronic diseases (Sadiq *et al.,* 2023).

Furthermore, this study supports the idea that processing methods matter. The potential degradation of antioxidants like vitamin E during heating or refining (Izuddin *et al.,* 2023) means that minimally processed PKO, like the one used in this study, likely retains a more beneficial biochemical profile. This highlights the value of artisanal or traditional extraction methods and provides a basis for promoting unrefined PKO as a functional food ingredient.

5.2 CONCLUSION

This study was conducted to evaluate the DPPH radical scavenging activity of locally extracted palm kernel oil. The results demonstrated that palm kernel oil possesses moderate antioxidant properties, though its activity is significantly lower than that of the pure antioxidant standard, ascorbic acid. The scavenging effect is attributed to the presence of bioactive components like tocopherols and phenolics preserved through the mechanical extraction process. In conclusion, this research confirms that locally extracted, unrefined palm kernel oil is a valuable source of natural antioxidants. It provides a scientific basis for its traditional dietary and medicinal uses and highlights its potential as a functional food ingredient for managing oxidative stress-related conditions.

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