ACID VALUE, % FREE FATTY ACID AND PEROXIDE VALUE OF *Elaeis guineensis* (PALM KERNEL) OIL, *Gossypium hirsutum* (COTTON SEED) OIL AND *Glycine max* (SOYA BEAN) OIL

BY

IMAFIDON DIVINE AYOMIDE

BMS2005886

DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF MEDICAL SCIENCE

COLLEGE OF MEDICAL SCIENCE

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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 IMAFIDON DIVINE AYOMIDE (BMS2005886) 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.

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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

The quality of edible oils is a critical factor for consumer health, yet deterioration through hydrolysis and oxidation can lead to rancidity and the formation of toxic compounds. This study aimed to evaluate and compare the quality of commercially available *Glycine max* (Soya Bean Oil), *Elaeis guineensis* (Palm Kernel Oil), and *Gossypium hirsutum* (Cotton Seed Oil) by determining key degradation indices. Oils were extracted from seeds sourced from a local market using the Soxhlet method. The acid value (AV), percentage free fatty acid (%FFA), and peroxide value (PV) were determined in triplicate for each oil sample using standard titrimetric methods (AOCS). The results revealed profound disparities in quality among the oils. Soya Bean Oil exhibited acceptable quality, characterized by a low acid value (3.35 ± 0.03 mg KOH/g), low %FFA (1.68 ± 0.02%), and a moderate peroxide value (13.07 ± 0.92 meq/kg). In stark contrast, Palm Kernel Oil and Cotton Seed Oil were of exceptionally poor quality, displaying extremely high acid values (23.30 ± 3.02 and 26.13 ± 2.75 mg KOH/g, respectively) and alarmingly high peroxide values (99.59 ± 0.80 and 107.49 ± 3.78 meq/kg, respectively). These findings indicate that while the SBO was of acceptable quality, the palm kernel oil and cotton seed oil samples exhibited severe hydrolytic and oxidative rancidity, far exceeding international safety standards and rendering them unsuitable for consumption. This study highlighted a critical public health issue regarding the quality of some oils available in local markets and underscores the urgent need for stricter quality control measures throughout the supply chain to mitigate health risks associated with consuming degraded oils.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Edible oils are essential components of the human diet, supplying a significant portion of dietary energy and serving as ingredients in many foods. Ensuring their quality and stability is therefore vital for nutrition and food safety (Beheshti *et al.,* 2023). Historically, oilseeds have been used for food, medicinal, and fuel purposes, and modern concerns focus on how composition and storage affect oil integrity and consumer health.

Palm oil and palm kernel oil differ in fatty acid profile and stability: palm kernel oil (from the kernel) is rich in short-chain saturated fatty acids such as lauric and myristic acids, while palm oil often contains substantial palmitic and oleic acids, giving it relatively high oxidative stability and making it suitable for frying (Okpe, 2022; Beheshti *et al.,* 2023; Abrante-Pascual *et al.,* 2024). However, the high saturated fat content, notably palmitic acid has been associated with increased cardiovascular disease risk (Beheshti *et al.,* 2023).

Soybean oil is characterized by high polyunsaturated fatty acid content, principally linoleic and linolenic acids, and contains phenolic compounds that may confer health benefits (Beheshti *et al.,* 2023; Swallah *et al.,* 2023). Reported values for crude soybean oil include low free fatty acid levels (around 0.28–0.29%) and peroxide indices between 1.77–1.88 mEq/kg, and diets rich in unsaturated vegetable oils have been shown to influence blood lipid profiles in animal studies (Beheshti *et al.,* 2023; Zahran *et al.,* 2021).

Cottonseed oil typically contains high proportions of linoleic acid and palmitic acid, with reported linoleic ranges of approximately 44.0–59.3% and palmitic ranges around 19.6–27.6%; interesterified variants have shown linoleic content near 56% (Athanasiadis *et al.,* 2024; Gutierrez and Komarnytsky,

2025). The principal pathways of edible oil deterioration are oxidation, producing peroxides and hydroperoxides and hydrolysis, which yields free fatty acids; the rate and extent of these processes depend on factors such as fatty acid composition, oxygen and light exposure, and storage temperature (Loganathan *et al.,* 2022; Geng *et al.,* 2023; Kumar *et al.,* 2025).

Thermally stressed and oxidized oils generate toxic lipid oxidation products (LOPs), including α,β-unsaturated aldehydes like acrolein and 4-HNE, which are cytotoxic, mutagenic, and implicated in cardiovascular and other chronic diseases; animal studies have linked repeated consumption of heated oils to hypertension, endothelial dysfunction, and accelerated atherosclerosis (Grootveld *et al.,* 2020, 2021, 2022; Ruan *et al.,* 2021; Lawaly, 2024). Measuring quality indices such as Acid Value (expressed as mg KOH/g oil), percent free fatty acids, and Peroxide Value (mEq O_2/kg) is therefore essential for assessing lipid integrity and the level of deterioration to protect public health (Jaroslawa and Joanna, 2025; Okpe, 2022; Pashaei and Farhoosh, 2025).

1.2 Justification of the Study

The widespread adulteration and sale of poor-quality edible oils in the market, together with the health risks associated with consuming oxidized or rancid oils, create an urgent need for biochemical evaluation of commonly consumed edible oils. Determining key quality indices such as acid value, percent free fatty acids, and peroxide value, will provide objective evidence of oil freshness, oxidative stability, and the extent of deterioration that may not be apparent to producers, retailers, or consumers.

This study will generate biochemical data that can be used by food industries, regulatory agencies, and consumers to assess and monitor oil quality, strengthen quality-control practices, and guide safer handling and storage. The findings will also contribute to local research on lipid oxidation and its health

implications, inform dietary recommendations, and support biochemical education by providing practical, context-specific examples of oil degradation and its measurement.

1.3 Aim of the Study

The aim of this study was to determine the acid value, percent free fatty acid (% FFA), and peroxide value of palm kernel oil, cottonseed oil, and soybean oil.

CHAPTER TWO

2.0LITERATURE REVIEW

2.1 Overview of Lipids and Edible Oils

2.1.1 Classification of Lipids

Lipids are organic molecules characterized by their hydrophobic nature. They exhibit poor solubility in water but are highly soluble in organic solvents. They are basic components of human biology and nutrition.

They are classified into major categories that perform different cellular functions:

i. **Triglycerides (TGs) or Triacylglycerols (TAGs)**: TGs are the body's primary form of energy storage and serve as essential energy reservoirs (Dakal *et al.,* 2025; Zaharan *et al.,* 2021). They contribute approximately 30–40% of the total energy intake in a standard Western diet and are efficiently digested and absorbed (more than 95% utilized) (Frydrych *et al.,* 2025). In the context of edible oils, fatty acids are predominantly found in the form of triglycerides, which consist of three fatty acids linked to a glycerol backbone (Petersen *et al.,* 2024). Elevated TG levels are linked to significant health risks, including cardiovascular diseases (CVD) and metabolic syndrome (Frydrych *et al.,* 2025).

ii. **Phospholipids**: These are important for maintaining the integrity of cell membranes and also the synthesis of cell membranes, as they form the basic bilayer structure of cell and organelle membranes (Frydrych *et al.,* 2025; Dakal *et al.,* 2025). They are esters of glycerol, phosphoric acid, and fatty acids. They also function as precursors to various signaling molecules like prostaglandins and are involved in lipid transport (Frydrych *et al.,* 2025).

iii. **Sterols (e.g., Cholesterol, Plant Sterols, and Stanols)**: Sterols, such as cholesterol, are vital for maintaining membrane fluidity and for the synthesis of hormones (Zahran *et al.,* 2021; Dakal *et al.,* 2025). Plant sterols and stanols are important in nutrition because they reduce serum low-density lipoprotein (LDL) cholesterol by competing with dietary and biliary cholesterol inhibiting or reducing its absorption in the intestines (Frydrych *et al.,* 2025).

iv. **Sphingolipids**: These lipids are involved in important cellular processes, including apoptosis and cell growth (Zahran *et al.,* 2021). Dysregulation in their metabolism can impair cellular communication (Dakal *et al.,* 2025).

v. **Fatty Acids (FAs)**: FAs are organic substances consisting of a long aliphatic hydrocarbon chain with a carboxyl group at one end (Zhou *et al.,*2020). They are classified based on the presence and number of double bonds:

- Saturated Fatty Acids (SFAs): This class of fatty acids contain no double bonds.
- Monounsaturated Fatty Acids (MUFAs): This class contain a single carbon–carbon double bond.
- Polyunsaturated Fatty Acids (PUFAs): PUFAs contain at least two carbon–carbon double bonds.
- Essential Fatty Acids (EFAs): The human body cannot synthesize certain PUFAs, namely linoleic acid (LA; an Ω-6 PUFA) and α-linolenic acid (ALA; an Ω-3 PUFA), so they must be obtained from the diet and are classified as essential fatty acids (Zhou *et al.,* 2020).

2.1.3 Classification of some Edible Oils

Table 2.1 showing the typical fatty acid composititon of edible oils

Edible Oil	Total Saturated Fatty Acids (SFAs) (%)	Total Monounsaturated Fatty Acids (MUFAs) (%)	Total Polyunsaturated Fatty Acids (PUFAs) (%)	Key Fatty Acids and Characteristics
Olive Oil (OO)	12.5–20.9, 13.8	54.5–80.2, 73.0	4.9–21.2, 10.5	Rich in Oleic Acid (C18:1). Low SFA.
Linseed Oil (Flaxseed)	12.39	20.53 (Oleic acid)	87.61 (Total UFA)	Rich source of α-Linolenic Acid (ALA, 53.11% C18:3).
Soybean Oil (SO)	15.6, 17.45	22.8, 20.16 (Oleic acid)	57.7, 82.54 (Total UFA)	Rich in Linoleic Acid (LA, 61.78%) and α-Linolenic Acid.
Palm Oil (PO)	49.3, 49.7–57.5	37.0, 37.3–40.8	9.3, 9.1–11.0	High Palmitic Acid (C16:0) content. High stability.
Coconut Oil	81.2–94	5–10	1–2.5	Very high SFA content.
Cotton Seed (CSO)	25.9 (or range 27.5-33.7%)	17.8 (Oleic acid range 16.5-27.0%)	51.9 (or range 43.2-54.0% LA)	Rich in Linoleic acid (50.3-55.5%). Low in ALA (0.13-0.4%). High smoke point (215-232ºC). Stability is intermediate (PO>CSO>SFO)

The table above summarizes the typical fatty acid composititon of edible oils discussed in context of saturated versus unsaturated profiles (Zhou *et al.,* 2020; Petersen *et al.,* 2024; Djohan *et al.,* 2022; Zahran *et al.,* 2021)

2.2 Physicochemical Analysis of Oil Quality

The physicochemical analysis of edible oils is important in determining quality, stability, and suitability for domestic usage or consumption and industrial applications (Wazed *et al.,* 2023; Hassan *et al.,* 2022). Oxidation and hydrolysis are two major deterioration reactions that occur during the processing and storage of lipids, which results in changes to chemical, physical, nutritional, and quality characteristics

(Kasaai, 2025). Key analytical indices, especially Acid Value (AV) and Peroxide Value (PV), are widely employed to monitor these deterioration processes.

2.2.1 Acid Value (AV)

The Acid Value is the milligrams of potassium hydroxide (KOH) required to neutralize the free fatty acids in one gram of oil and serves as an indicator of hydrolytic deterioration and acidity (Kabore *et al.,* 2022; Hassan *et al.,* 2022). Low AV denotes good quality and storage stability, while high AV signals hydrolysis or poor handling and can increase with elevated temperature (Wazed *et al.,* 2023). AV is determined by titration of an alcoholic oil solution with standardized KOH (phenolphthalein end-point) using standard methods such as IUPAC and ISO 660. WHO recommends that edible oil AV should not exceed 4 mg/g (Hassan *et al.,* 2022).

2.2.2 Free Fatty Acid

Free fatty acids arise from triglyceride hydrolysis and are expressed as a percentage of a dominant fatty acid; Percentage FFA is closely related to AV (higher AV → higher %FFA) and is measured by titration with NaOH (Wazed *et al.,* 2023; Hassan *et al.,* 2022). High FFA results from poor extraction, damaged seeds, or improper/extended storage (Wazed *et al.,* 2023). For example, crude palm oil is often considered acceptable below ~5% (as palmitic acid) when properly processed (Ugo *et al.,* 2024).

2.2.3 Peroxide Value (PV)

Peroxide Value measures hydroperoxides—the primary products of lipid oxidation—and is reported as milli-equivalents of active oxygen per kilogram (mEq O_2/kg), making it a key early indicator of rancidity and oil stability (Zhang *et al.,* 2021; Abeyrathne *et al.,* 2021). PV increases with exposure to heat, light, oxygen and metal ions, but hydroperoxides are unstable at frying temperatures and decompose into secondary products (Zhang *et al.,* 2021; Wazed *et al.,* 2023). PV is determined by

iodometric titration (KI/acid followed by $Na_2S_2O_3$ titration with starch indicator) and fresh oils typically show PV <10 mEq O_2/kg; Codex limits are ~10 mEq O_2/kg for refined oils and ~15 mEq O_2/kg for virgin oils, while values above ~20–40 mEq O_2/kg indicate rancidity and possible health risks (Kabore *et al.,* 2022; Ugo *et al.,* 2024; Wazed *et al.,* 2023; Zhang *et al.,* 2021).

2.3 Palm kernel Oil (PKO)

Palm kernel oil (PKO) is a crucial component of the global vegetable oil market, distinct from palm oil (PO) due to its unique botanical origin, physicochemical characteristics, and industrial applications.

The oil palm as shown in figure 2.1, scientifically known as *Elaeis guineensis*, yields two different types of oil: palm oil and palm kernel oil (Alhaji *et al.,* 2024). These oils originate from different parts of the oil palm fruit (OPF), which is a drupe found in clusters called fresh fruit bunches (Alhaji *et al.,* 2024).

Figure 2.1. (a) Palm tree and (b) Palm fruit bunch (Source: Alhaji *et al.,*2023)

Botanical Differences in Origin:

- **Palm Oil (PO):** It is extracted from the fleshy pulp known as the mesocarp. The mesocarp is a fibrous matrix. Oil yield from the mesocarp accounts for about 20–22% of the fruit. Crude palm oil (CPO) is typically reddish in color due to high carotenoid content (Alhaji *et al.,* 2024).
- **Palm Kernel Oil (PKO):** It is extracted from the kernel or seed, which is the white kernel flesh or endosperm found within a protective hard shell (endocarp). The kernel yields approximately 10% of the total oil yield from the fruit. PKO obtained from extraction is usually colorless or pale yellow (Alhaji *et al.,* 2024; Okpe, 2022).

2.3.3 Typical fatty acid profile and Physicochemical Characteristics

PKO is classified as a lauric fat because of its high content of lauric acid (Dunford, 2023). It is characterized by a high proportion of saturated fatty acids (SFA), which typically constitutes over 80% of the oil, compared to about 50% for palm oil (PO) (Alhaji *et al.,* 2024).

The composition of PKO is dominated by medium-chain fatty acids (Dunford, 2023).

- **Lauric acid (C 12:0):** This is the major fatty acid, ranging significantly from 29–55%, typically around 48% (Bong *et al.,* 2020). Studies on crude PKO show lauric acid content around 39.18% (Abd Alshafea *et al.,* 2025). Lauric acid imparts sharp melting properties to PKO, meaning it is hard at room temperature but has a low melting point (Okpe, 2022).
- **Myristic acid (C14:0):** Ranges from 6–26% (Dunford, 2023), reported around 16% (Okpe, 2022) or 20.24% in crude PKO (Abd Alshafea *et al.,* 2025).
- **Oleic acid (C18:1):** Ranges from 12–26% (Dunford, 2023), reported around 15% (Okpe, 2022) to 18.82% in crude PKO (Abd Alshafea *et al.,* 2025).

- **Palmitic acid (C16:0):** Typically 4–10% (Dunford, 2023).

PKO is semi-solid at room temperature and possesses a low melting point compared to palm oil (Abd Alshafea *et al.,* 2025).

2.3.4 Reported ranges/behaviors for AV, Percent FFA and PV

The acid value (AV), percent free fatty acid (%FFA), and peroxide value (PV) are critical parameters used to assess the quality, purity, and extent of degradation in PKO.

Acid Value (AV) and Free Fatty Acid (%FFA)

The Acid Value (AV) is the milligrams of potassium hydroxide (KOH) required to neutralize the free acids in one gram of oil; AV (or %FFA) indicates the degree of glyceride hydrolysis (lipase action) and thus oil acidity and quality (Chie-Amadi *et al.,* 2025; Okpe, 2022; Alshafea *et al.,* 2025). High FFA/AV indicates poor quality and susceptibility to degradation, often resulting from prolonged or improper storage and processing (Bong *et al.,* 2020; Abd Alshafea *et al.,* 2025; Alhaji *et al.,* 2024). For some industrial uses (e.g., biodiesel) %FFA > 1% requires pre-treatment to avoid saponification and reduced yields (Bong *et al.,* 2020; Abd Alshafea *et al.,* 2025).

Peroxide Value (PV)

Peroxide Value measures hydroperoxides formed during early lipid oxidation and is reported in mEq O_2/kg; it is a primary indicator of rancidity and oil stability (Okpe, 2022; Abd Alshafea *et al.,* 2025). Rancidity becomes notable when PV is well above 10 mEq O_2/kg; the commonly recommended maximum for edible vegetable oils is ~10 mEq O_2/kg (Chie-Amadi *et al.,* 2025), while Codex Alimentarius permits up to 15 mEq O_2/kg for some fats.

2.4 Cottonseed Oil

Cottonseed oil (CSO) is recognized as one of the world's major sources of edible vegetable oil, derived from the seeds of the cotton plant, predominantly *Gossypium hirsutum* (Ma *et al.,*2021; Kalkan and Makan, 2025). HCottonseed kernels typically contain between 15% and 25% oil and 20% to 25% protein, making cotton a crucial dual-purpose crop (fiber and oil) globally (Zia *et al.,* 2021; Kalkan and Makan, 2025).

2.4.1 Origin, Extraction, and Refinement Overview

Cottonseed (Figure 1.2) is the natural byproduct of cotton (*Gossypium spp.*) cultivation (Kumar *et al.,* 2023; He *et al.,*2023). Oil content in the seeds is variable, influenced by the variety of cotton, environmental factors like weather, and the maturity stage of the seed (Kumar *et al.,* 2023; Zia *et al.,* 2021). The isolation of CSO involves separating triglycerides from the cottonseed, a process that has evolved from conventional to modern, non-conventional techniques aimed at maximizing yield and quality (Kumar *et al.,* 2023).

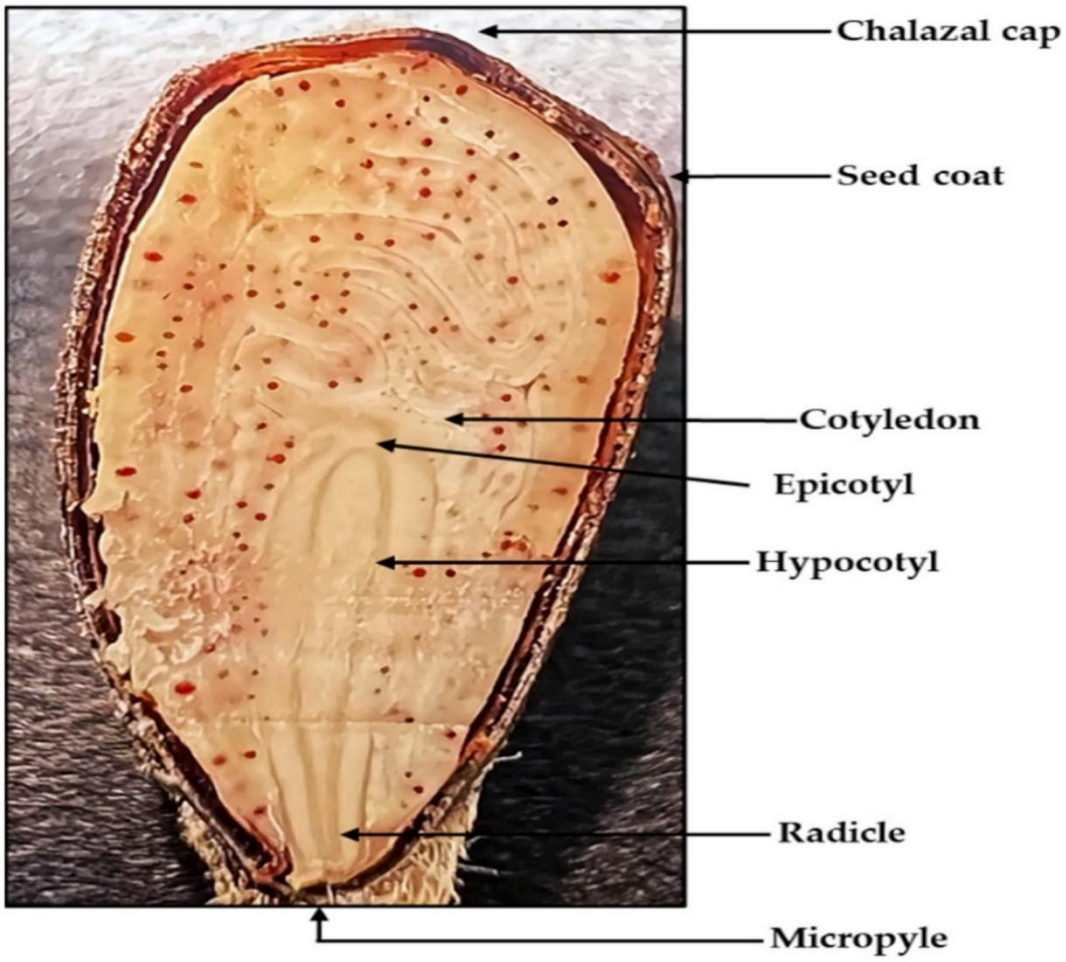

Figure 2.2 Anatomy of Cotton seed (Source: Maeda *et al.,* 2021).

Oil extraction methods include:

1. **Mechanical Extraction (ME):** Uses hydraulic or screw (expeller) pressing. It yields less oil but produces natural, chemical-free crude oil with good aroma and color. Pretreatment includes cleaning/husking (<10% shell), seed softening (steam), and flaking (<0.5 mm) (Kumar *et al.,* 2023; Zia *et al.,* 2021).

2. **Solvent Extraction (SE):** A large-scale industrial method, typically using n-hexane, leaving only 0.5–0.7% residual oil in the cake. It offers high oil recovery and easy solvent evaporation; Soxhlet extraction is common in labs (Kalkan and Maskan, 2025; Kumar *et al.,* 2023).

3. **Non-Conventional (Green) Methods**: Include microwave (MAE), ultrasound (UAE), enzyme-assisted (EAE), and supercritical fluid extraction (SFE) to reduce time and solvent use while preserving bioactives, with optimized UAE systems reporting up to 86.4% oil recovery (Kalkan and Maskan, 2025).

2.4.2 Common Uses

Cottonseed oil (CSO) is widely used in food applications. commercial and home cooking, especially frying and in the manufacture of snacks, baked goods, dressings, margarine, shortening, and similar products due to its mild, nutty flavor and stability (Kumar *et al.,* 2023; Zia *et al.,* 2021).

Its frying suitability stems from a high smoke point (~232°C / 450°F), favorable saturated fat profile, and natural antioxidants (tocopherols) that enhance shelf life and thermal stability (Kalkan and Maskan, 2025; Kumar *et al.,* 2023).

CSO also has several non-food uses, including cosmetics and personal-care formulations, pharmaceuticals, biofuels (e.g., cottonseed oil methyl ester), specialized lubricants, soft soaps, and as a raw material for alkyd resins in paints (Zia *et al.,* 2021; Polaris Market Research, 2024).

2.4.3 Fatty Acid Composition and Stability Profile

The defining quality and nutritional value of CSO, like other plant oils, are heavily dependent on its fatty acid components. CSO is typically categorized within the oleic–linoleic cluster of vegetable oils (Kumar *et al.,* 2023; Zia *et al.,* 2021; Ma *et al.,* 2021).

Table 2.2 Showing Fatty Acid Composition and Stability Profile

Major Fatty Acid Components (Mature Oil)	**Type**	**Typical Relative Content (%)**
Linoleic Acid (C18:2n-6)	Polyunsaturated (PUFA)	51.5–57.62 (The most abundant)
Palmitic Acid (C16:0)	Saturated (SFA)	21.4–25.7(The major SFA)
Oleic Acid (C18:1n-9)	Monounsaturated (MUFA)	15.59–17.8
Stearic Acid (C18:0)	Saturated (SFA)	2.31–3.3
Linolenic Acid (C18:3n-3)	Polyunsaturated (PUFA)	1% (Typically 0.2–0.7)

CSO is frequently characterized by a 2:1 ratio of polyunsaturated fatty acids to saturated fatty acids (Kumar *et al.,* 2023). Due to its inherent profile, particularly the moderate SFA content, CSO is described as a "naturally hydrogenated oil," offering stability for high-temperature applications (Kalkan and Maskan, 2025).

2.5 Soybean Oil

Soybean (*Glycine max L.)* originated in the Eastern Hemisphere, with historical evidence establishing its use as a cultivated food crop in Northeastern China roughly between 1700 and 1100 B.C (Hamza *et al.,* 2024). Legends suggest its use may date back as early as 2500–2300 B.C (Hamza *et al.,* 2024).

Figure 2.3 Showing An image of soybean seeds, the source for soybean oil. (Source: Wang and Komatsu, 2017)

Soybean oil is extracted mainly by mechanical pressing the soybean seeds (Figure 2.3) and solvent extraction, both requiring pretreatment (cleaning, drying, dehulling, grinding, conditioning, and flaking).

Mechanical pressing uses screw or expeller presses, often for small-scale or organic production, but can leave 8–14% residual oil in the meal (Koc *et al.,* 2011; Tellabati and Menon, 2021).

Solvent extraction, the dominant industrial method, uses n-hexane to dissolve oil from soybean flakes, achieving up to 99% extraction efficiency due to hexane's stability and easy recovery (Cravotto *et al.,*

2023). The oil-solvent mixture (*miscella*) is separated, solvent is evaporated and recycled, and the defatted meal is processed into soybean meal (Cravotto *et al.,* 2023).

Emerging methods like supercritical CO_2 and ultrasound-assisted extraction offer high yields (e.g., ~93% with ultrasound) (Herzyk *et al.,* 2024).

2.5.2 Common Uses

Soybean oil is highly versatile, enjoying strong demand across both food and non-food sectors.

i. Food Industry Applications: Soybean oil is a vital and widely consumed edible vegetable oil.

   a. Cooking and Frying: This is the dominant market segment globally, due to its light flavor, high smoke point (reported at 460°F or 210°C), and heart-healthy profile. It is used extensively in households, restaurants, and for deep frying. Refined soybean oil is preferred for direct human consumption and high-temperature applications due to its purity (Gautam *et al.,*2024)..

   b. Health Benefits: Consumers are drawn to its nutritional profile, including low saturated fat content and high levels of polyunsaturated fats and omega-3 fatty acids.

2.5.3 Fatty Acid Composition and Stability Profile

Soybean oil is classified as rich in fatty acids. The major fatty acids found in soybean oil are saturated fatty acids, primarily palmitic acid (C16:0) and stearic acid (C18:0), and unsaturated fatty acids, consisting mainly of oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) (Indiarto *et al.,* 2020; Abdelghany *et al.,* 2019).

Typical average compositions generally reported in include: Palmitic acid (PA, C16:0): ~10%, Stearic acid (SA, C18:0): ~4%, Oleic acid (OA, C18:1): ~18%, Linoleic acid (LA, C18:2): ~55%, Linolenic acid (LNA, C18:3): ~13%.

Soybean oil is highly prone to autoxidation because it is rich in polyunsaturated fatty acids, primarily linoleic (LA) and linolenic (LNA) acids which reduce product stability and cause rancid off-odours (Indiarto *et al.,* 2020; Islam *et al.,* 2023). The many double bonds in these fatty acids greatly increase oxidation susceptibility (fatty acids with two double bonds, like LA, can be ~10–40× easier to oxidize than those with one double bond such as oleic acid), and oxidation is accelerated by free fatty acids, light, heat, and oxygen. Breeding and genetic modification have produced high-oleic, low-linolenic soybean varieties (OA ~35–42%, LNA ~3.77–5.00%) to improve oxidative stability (Indiarto *et al.,* 2020; Knowlton, 2022).

2.6 Health Implications of Oxidized Oils

The consumption of edible oils and fats is integral to the human diet, yet the chemical deterioration resulting from lipid oxidation introduces complex issues concerning nutritional quality and consumer safety (Loganathan *et al.,* 2022; Lenin, 2024). Lipid oxidation products, whether formed during storage, processing, or especially high-heat cooking, pose potential health risks that necessitate strict control and continuous research (Pradeshi, 2020).

2.6.1 Primary and Secondary Oxidation products (hydroperoxides, aldehydes, ketones)

Lipid oxidation, predominantly occurring through autoxidation, is a free-radical chain reaction that converts unsaturated fatty acids into a broad range of compounds (Schaich, 2020). These compounds are broadly classified into primary and secondary oxidation products (Lenin, 2020).

Primary Oxidation Products

The first products formed during lipid oxidation are lipid hydroperoxides (LOOH). These compounds result from oxygen reacting with a double bond in an unsaturated fatty acid, generating an unstable intermediate. LOOH themselves are generally odorless and tasteless and do not immediately alter the food's sensory qualities (Mora *et al.,*2025). However, they serve as crucial markers for the early stages of oxidation and act as precursors to more reactive species.

Secondary Oxidation Products

As hydroperoxides decompose (α-/β-scission), they form more volatile and reactive secondary products—aldehydes, ketones, alcohols, epoxides and polymers—that drive rancidity and off-flavours (Mora *et al.,* 2025; Lenin, 2024). Aldehydes (e.g., 2-alkenals, acrolein, 4-HNE, HHE, MDA, ONE) are especially important because of their reactivity and toxicity; ketones and epoxides (e.g., 9,10-epoxy-stearic acid) and polymeric dimers also accumulate in heated/frying oils and may increase viscosity. (Lenin, 2024; Ying *et al.,* 2025; Bao *et al.,* 2025; Schaich, 2020).

2.6.2 Biological / toxicological effects (oxidative stress, inflammation, nutrient loss)

Oxidized lipids and their carbonyl products promote reactive oxygen species (ROS) formation, driving oxidative stress that underlies cell dysfunction and diseases such as inflammation, cancer and CVD (Bao *et al.,* 2025; Ying *et al.,* 2025). Reactive aldehydes (HNE, HHE, MDA) form protein adducts that impair protein structure/function and are markers of disease and vascular injury linked to atherosclerosis (Ying *et al.,* 2025). Dietary oxidized lipids can also dysregulate lipid mediators and metabolism, inducing intestinal and hepatic inflammation (e.g., oxidized olive oil studies showing epithelial damage, villus

shortening and immune infiltration); specific oxidation products (e.g., 9,10-epoxy-stearic acid) have been proposed as pro-inflammatory lipid mediators. (Bao *et al.,* 2025).

2.6.4 How AV/%FFA and PV relate to health risk assessment and Regulatory Standards

Peroxide Value (PV) measures primary oxidation products (hydroperoxides) and is widely used to assess lipid oxidation, but it is highly temperature-sensitive and unreliable for oils exposed to high heat (e.g., frying) because hydroperoxides decompose rapidly (Pradeshi, 2020; Lenin, 2024; Schaich, 2020). Regulatory limits vary by product: for example, GOED and the European Pharmacopoeia set low PV limits for fish-oil supplements (≈≤5–10 mEq/kg), and food authorities such as Codex and FSSAI apply PV limits around 10 mEq O_2/kg for edible/used frying oils (Pradeshi, 2020; Schaich, 2020).

Acid Value (AV) / percent FFA quantify free fatty acids from hydrolysis and are used in regulatory control as indicators of oil decomposition and rancidity; limits differ by jurisdiction (e.g., Taiwan ≈2.0 mg KOH/g; Codex guidance ≈0.6 mg KOH/g). AV/FFA are simple, practical measures of oil quality but do not capture all toxic oxidation products (Park *et al.,* 2020; Pradeshi, 2020).

Because primary products are unstable at frying temperatures, PV and AV alone can be inadequate for health-risk assessment of thermally stressed oils; regulators therefore often monitor Total Polar Materials (TPM) (typical cutoffs ≈24–25%) and increasingly rely on targeted analysis of secondary oxidation products (aldehydes, epoxides, polymers) to better characterise toxicity and risk. (Schaich, 2020; Pradeshi, 2020).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Apparatus and Equipment

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

Dried seeds of palm kernel (*Elaeis guineensis*), cotton seed (*Gossypium hirsutum*), and soya bean (*Glycine max*) used for this study were acquired locally from New Benin Market, Benin City, Edo State, Nigeria. Upon arrival, the seeds were manually sorted and cleaned to remove any foreign materials, broken seeds, debris, and signs of fungal contamination, ensuring the quality of the starting material (Osei-Amponsah *et al.,* 2020).

The seeds were subjected to specific pre-treatment processes based on their type. The cleaned palm kernels were manually cracked to carefully separate the hard shells from the inner nuts (Okorie *et al.,* 2021). Cotton seeds were delinted to remove residual cotton fibers that could interfere with the extraction process. The soya beans were used directly after the initial cleaning.

Following pre-treatment, the samples from all three sources (palm kernel nuts, delinted cotton seeds, and soya beans) were individually milled into a coarse powder using a heavy-duty electric grinder. This size reduction step was crucial to rupture the cell walls and increase the surface area available for efficient solvent-based oil extraction (Harborne, 1998). The resulting powdered samples were stored separately in airtight, labeled containers and kept in a cool, dry place to prevent moisture absorption and degradation prior to the extraction process (Edeoga *et al.,* 2005).

3.2.2 Oil Extraction

The oil was extracted from the prepared ground samples using the Soxhlet extraction method, a standard procedure for efficient lipid recovery from solid matrices (AOCS Official Method Ba 3-38). Each extraction was performed in triplicate for each seed type to ensure the accuracy and reproducibility of the results (Tiwari *et al.,* 2011).

n-Hexane was selected as the extraction solvent due to its non-polar nature, which makes it highly effective for dissolving oils and fats (lipids), and its low boiling point, which facilitates easy removal from the extracted oil (Sasidharan *et al.,* 2011).

For each extraction, the powdered sample was accurately weighed and placed into a porous cellulose thimble. The thimble was then inserted into the main chamber of a Soxhlet apparatus, which was fitted to a pre-weighed 250 ml round-bottom flask containing 200 ml of n-hexane. The apparatus was equipped with a condenser and heated using a thermostatically controlled heating mantle at 70°C. The

extraction was allowed to run continuously for 6 hours, during which the solvent repeatedly washed over the sample to extract the oil.

After the extraction was complete, the solvent was recovered from the oil-miscella using a rotary evaporator under reduced pressure at 40°C. To remove any final traces of residual solvent, the crude oil was transferred to an oven and dried at 60°C until a constant weight was achieved. The extracted crude Palm Kernel Oil, Cotton Seed Oil, and Soya Bean Oil were then stored in separate, airtight amber glass bottles and kept refrigerated at 4°C until required for acid value, %FFA, and peroxide value analyses (Edeoga *et al.,* 2005).

3.2.3 Determination of Acid Value

The acid value of the oil samples was determined using the titrimetric method as described by the American Oil Chemists' Society (AOCS) Official Method Cd 3d-63, with slight modifications.

Briefly, 5.0 g of the oil sample was weighed into a 250 ml Erlenmeyer flask and dissolved in 50 ml of a neutral solvent mixture (equal parts of ethanol and diethyl ether, neutralized with 0.1 N potassium hydroxide using phenolphthalein as indicator). A few drops of phenolphthalein indicator were added, and the solution was titrated with 0.1 N potassium hydroxide (KOH) while shaking vigorously until a persistent pink color appeared and remained for at least 30 seconds. The acid value was calculated using the following equation:

$$\text{Acid Value (mg KOH/g)} = (V \times N \times 56.1) / W$$

Where V is the volume of KOH used (ml), N is the normality of KOH, 56.1 is the molecular weight of KOH, and W is the weight of the sample (g). All samples were analyzed in triplicates.

3.2.4 Determination of Percentage Free Fatty Acids

The percentage free fatty acids (% FFA) was determined based on the acid value obtained from the previous method, expressed as oleic acid equivalent, following the procedure outlined by the AOCS Official Method Ca 5a-40.

The % FFA was calculated from the acid value using the formula:

% FFA (as oleic acid) = Acid Value × 0.503

Where 0.503 is the conversion factor derived from the molecular weight of oleic acid (282) divided by the equivalent weight for KOH (561). This provides the percentage of free fatty acids relative to the sample weight. Analyses were performed in triplicates for each oil sample.

3.2.5 Determination of Peroxide Value

The peroxide value of the oil samples was measured using the iodometric titration method as per the AOCS Official Method Cd 8-53, with minor adjustments.

Approximately 5.0 g of the oil sample was weighed into a 250 ml Erlenmeyer flask with a stopper. Then, 30 ml of a solvent mixture (3:2 glacial acetic acid:chloroform) was added, followed by 0.5 ml of saturated potassium iodide (KI) solution. The flask was stoppered, shaken gently for 1 minute, and allowed to stand in the dark for 5 minutes. After this, 30 ml of distilled water was added, and the liberated iodine was titrated with 0.01 N sodium thiosulfate ($Na_2S_2O_3$) solution using 1 ml of 1% starch solution as an indicator until the blue color disappeared. A blank titration was performed without the sample.

The peroxide value was calculated as:

Peroxide Value (meq/kg) = [(V_s - V_b) × N × 1000] / W

Where V_s is the volume of $Na_2S_2O_3$ used for the sample (ml), V_b is the volume for the blank (ml), N is the normality of $Na_2S_2O_3$, and W is the weight of the sample (g). All determinations were carried out in triplicates.

3.3 Statistical Analysis

All analytical determinations for acid value, % free fatty acid, and peroxide value were performed in triplicate (n=3). The data obtained from these titrimetric analyses were collated and processed using IBM SPSS Statistics, Version 25. The results were expressed as the mean ± standard deviation (SD) to ensure the precision and reproducibility of the findings. Descriptive statistics were employed to summarize the data, and the final values were presented in tables for effective comparison and interpretation of the quality parameters across the different oil samples.

CHAPTER FOUR

4.0 RESULT

This chapter shows the presentation of results obtained from the study. Where PKO = Palm Kernel Oil, CSO = Cotton Seed Oil, SBO = Soya Bean Oil. All analyses were performed in triplicates.

4.1 Acid Value of Oil Samples

The acid values for the different oil samples were determined and are presented in Table 4.1.

Table 4.1: Acid Value (mg KOH/g) of Various Oil Samples

OIL	SAMPLE A	SAMPLE B	SAMPLE C	MEAN±SEM
SBO	3.413	3.303	3.342	3.35±0.03
PKO	26.27	17.262	26.354	23.30±3.02
CSO	28.875	23.375		26.13±2.75

Table 4.1 presents the acid values (mg KOH/g) for Soya Bean Oil (SBO), Palm Kernel Oil (PKO), and Cotton Seed Oil (CSO). The values for each replicate analysis (Sample A, B, C) are shown, along with the calculated mean ± standard error of the mean (SEM).

4.2 Percentage Free Fatty Acid (%FFA) of Oil Samples

The percentage of free fatty acids, a direct indicator of hydrolytic rancidity, was calculated and is shown in Table 4.2.

Table 4.2: Percentage Free Fatty Acid (as oleic acid) of Various Oil Samples

OIL	SAMPLE A	SAMPLE B	SAMPLE C	MEAN±SEM
SBO	1.717	1.661	1.681	1.68±0.02
PKO	13.227	8.683	13.256	11.72±1.52
CSO	14.524	11.758		13.14±1.38

Table 4.2 shows the percentage of free fatty acids (%FFA), calculated as oleic acid, for Soya Bean Oil, Palm Kernel Oil, and Cotton Seed Oil. The data includes values from the replicate analyses and their corresponding mean ± SEM.

4.3 Peroxide Value of Oil Samples

The peroxide value, which measures the initial stages of oxidative rancidity in the oils, was determined. The results are summarized in Table 4.3.

Table 4.3: Peroxide Value (meq/kg) of Various Oil Samples

OIL	SAMPLE A	SAMPLE B	SAMPLE C	MEAN±SEM
SBO	14.54	13.278	11.382	13.07±0.92
PKO	100	98.039	100.719	99.59±0.80
CSO	101.692	106.195	114.593	107.49±3.78

Table 4.3 details the peroxide values (meq/kg) determined for SBO, PKO, and CSO. The results from the three replicate samples are presented, along with the calculated mean ± SEM for each oil type, indicating the extent of primary oxidation.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study aimed to evaluate and compare the quality of commercially available Soya Bean Oil (SBO), Palm Kernel Oil (PKO), and Cotton Seed Oil (CSO) by determining their acid value, percentage free fatty acid, and peroxide value. The results obtained reveal profound differences in the quality and degradation status among the three oil samples. The findings are discussed below in the context of established quality standards and relevant scientific literature.

The acid value (AV) and the corresponding %FFA are primary indicators of hydrolytic rancidity, reflecting the extent to which triglycerides have been broken down into free fatty acids by moisture or enzymatic action (Kumar *et al.,* 2025). In this study, the soybean oil (SBO) sample showed a low acid value (Table 4.1) and a correspondingly low percentage free fatty acid (Table 4.2). Although the measurement exceeds the values reported for fresh crude soybean oil (0.28–0.29%) by Beheshti et al. (2023), it remains close to the World Health Organization's acceptable limit of 4 mg KOH/g for edible oils (Hassan *et al.,* 2022). These findings indicate that the SBO sample was of acceptable quality with minimal hydrolytic degradation.

In stark contrast, palm-kernel oil (PKO) and cottonseed oil (CSO) exhibited exceptionally high acid values, well above the maximum limit recommended by the World Health Organization and substantially higher than values reported in the literature (Chie-Amadi *et al.,* 2025; Abd-Alshafea *et al.,* 2025). Such elevated acidity indicates extensive hydrolytic cleavage of triglycerides, most likely resulting from prolonged exposure to moisture, heat, or inadequate storage and handling during processing. Such high levels of free fatty acids indicate extensive hydrolytic deterioration. This

degradation could be attributed to several factors, including the use of poor-quality seeds, improper handling, delayed processing which allows for significant lipase enzyme activity, or prolonged and improper storage conditions (Wazed *et al.,* 2023; Alhaji *et al.,* 2024). The high percent FFA renders these PKO and CSO samples unsuitable for direct consumption and suggests they are of very poor quality.

The peroxide value is a critical measure of the initial stages of oxidative rancidity, quantifying the concentration of primary oxidation products (hydroperoxides) formed when unsaturated fatty acids react with oxygen (Okpe, 2022). According to the Codex Alimentarius, the maximum acceptable PV for refined edible oils is 10 meq/kg, and for virgin oils is 15 meq/kg (Kabore *et al.,* 2022). Oils with a PV between 20-40 meq/kg are generally considered rancid (Wazed *et al.,* 2023).

The SBO sample exhibited a peroxide value marginally higher than the 10 meq/kg limit commonly applied to refined oils (Table 4.3), although it remained below concentrations typically associated with rancidity. This modest elevation is likely due to soybean oil's high content of polyunsaturated fatty acids, particularly linoleic acid, which increases its susceptibility to oxidative deterioration (Indiarto *et al.,* 2020).

The peroxide values for palm-kernel oil (PKO) and cottonseed oil (CSO) were alarmingly high, roughly an order of magnitude above the Codex maximum acceptable limit, and thus indicate severe oxidative degradation. This result is especially notable for PKO, which is predominantly composed of saturated fatty acids and would normally be expected to show high oxidative stability (Bong *et al.,* 2020). The very elevated peroxide values imply that the oils were exposed to strong pro-oxidative conditions, for example prolonged heat, light, or oxygen exposure during storage or processing, which exceeded their inherent stability and promoted extensive rancidity.

Synthesizing the results from all three parameters, a clear hierarchy of quality emerges. The Soya Bean Oil sample, despite showing minor signs of primary oxidation, was of acceptable quality, meeting the standards for hydrolytic stability. Conversely, the Palm Kernel Oil and Cotton Seed Oil samples were of extremely poor quality. They failed to meet international standards for both hydrolytic and oxidative rancidity by a significant margin.

The consumption of oils with such high levels of degradation, as seen in the PKO and CSO samples, poses significant health risks. The high concentration of hydroperoxides (indicated by the PV) leads to the formation of toxic secondary oxidation products, such as aldehydes, which are cytotoxic and mutagenic (Grootveld *et al.,* 2021). The ingestion of such oxidized lipids is linked to increased oxidative stress, inflammation, and an elevated risk of cardiovascular diseases (Grootveld, 2022; Ruan *et al.,* 2021). Therefore, the PKO and CSO samples analyzed in this study are not only unpalatable due to rancidity but are also potentially harmful for human consumption.

5.2 CONCLUSION

This study successfully evaluated the quality of oils extracted under controlled laboratory conditions from Soya Bean, Palm Kernel, and Cotton seeds sourced from a local market, revealing significant disparities in their inherent quality. While the oil from Soya Bean seeds was of acceptable quality, characterized by low levels of free fatty acids and moderate peroxide values, the oils produced from the Palm Kernel and Cotton Seed samples were of exceptionally poor quality. These samples exhibited severe hydrolytic and oxidative rancidity that far exceeded international standards, rendering them unsuitable for consumption. This demonstrates a critical issue regarding the quality of raw oilseeds available in the market, which inevitably leads to the production of substandard and potentially unsafe oils. Ultimately, this research underscores an urgent need for stricter quality control measures that begin with the raw seeds and continue throughout the oil production supply chain, as well as for enhanced consumer education to mitigate the potential health risks associated with rancid oils.

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