

**FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ACTIVITY OF COTTON SEED  
(*Gossypium spp*), SOYA BEANS (*Glycine max*) AND PALM KERNEL (*Elaeis guineensis*)**

**OIL**

**BY**

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**DEPARTMENT OF MEDICAL BIOCHEMISTRY**

**SCHOOL OF BASIC MEDICAL SCIENCES**

**COLLEGE OF MEDICINE**

**UNIVERSITY OF BENIN**

**BENIN CITY**

**NOVEMBER 2025**

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

**NOVEMBER, 2025**

## CERTIFICATION

We the undersigned thereby certify that Miss Same Efeosedebame HENRY-IJIEBOR, carried out this work in the department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the award of Bachelors of Science Degree (BSc.) in Medical Biochemistry.

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## CERTIFICATION OF PROJECT ON PLAGIARISM

We the undersigned attest and declare that the project undertaken by:

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Titled:

**FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ACTIVITY OF COTTON SEED  
(*Gossypium spp*), SOYA BEANS (*Glycine max*) AND PALM KERNEL (*Elaeis guineensis*)**

Has successfully passed the plagiarism test and does not violate any copyright regulation

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## **DEDICATION**

This work is dedicated to the Most High on the throne and my almighty Father without whom I won't be.

## ACKNOWLEDGEMENT

With deep reverence and sincerity of heart I want to appreciate my source for this , indeed He stands by me and this victory is all for Him. I also want to use this medium to express my deepest honour for my parents Pastor and Pastor (Mrs) Henry-Ijebor my gifts from God. My siblings Pastor J, Barr. P, President Stalwart, Royal mother all the most amazing parts of me, Adesuwa, Mamuzo, Elizabeth, sisters and treasures I can never trade, all my friends in this journey, my family in LUCGMI, CGMI, Pastor Ernest, Mummy Success, with all my wonderful aunts and uncles and many whose names can't be contained here but whose impact fuels this happening now.

Special thanks to my Supervisor Dr Omorowa and to my respectable Head of Department, Dr. Aguebor-Ogie who has been instrumental in my journey in this prestigious department and my project. Thanks for all the corrections, instructions and criticism it proved it's value. May God fully reward you for all you do.

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## ABSTRACT

Antioxidant capacity of edible oils is important for nutrition, shelf-life, and functional uses and play its role in protecting biological systems against oxidative stress caused by free radicals. The antioxidant capacity varied significantly as a result of their fatty acid composition, refining procedure, inherent phyto chemicals and antioxidant. This study evaluated the ferric reducing antioxidant power (FRAP) of three widely consumed edible oils—soya bean oil, cotton seed oil, and palm kernel oil—at differing concentrations (50–250  $\mu\text{g/ml}$ ). The FRAP assay was used to determine the reducing ability of each oil, expressed as percentage FRAP activity. Results obtained showed a clear concentration-dependent increase in antioxidant activity across all samples. Cotton seed oil exhibited the highest mean FRAP value ( $35.20 \pm 12.95\%$ ), followed by soya bean oil ( $24.22 \pm 11.03\%$ ) and palm kernel oil ( $21.89 \pm 11.99\%$ ). Strong positive relationships between concentration and FRAP activity as Pearson correlation analysis revealed ( $r > 0.98$  for all oils), confirming that antioxidant potential increases proportionally with concentration. Although one-way ANOVA indicated no statistically significant difference ( $F = 1.75$ ;  $p > 0.05$ ) among the oils, cotton seed oil (dependent on the extensive refining it was subjected to) consistently demonstrated superior reducing power, suggesting a higher concentration of phenolic and tocopherol compounds. With this we establish the potential of cotton seed oil as a rich natural source of antioxidants and provide comparative insight into the antioxidant strength of common edible oils used in food industry and nutraceutical applications.

## **CHAPTER ONE**

### **1.0 INTRODUCTION**

#### **1.1 Background to the study**

Antioxidants play a critical role in combating oxidative stress, which arises when the production of free radicals exceeds the capacity of the already existing endogenous antioxidant defense systems, leading to lipid peroxidation, damage of cell, the development of conditions such as cancer, cardiovascular disease, diabetes, and accelerated aging. Edible plant oils as important dietary components provides essential fatty acids and fat-soluble vitamins and also contains naturally occurring antioxidant compounds such as, phytosterols, tocopherols, polyphenols and carotenoids. These contribute to the health benefits of oils and their ability to resist oxidative degradation during processing, storage, and culinary applications (Gupta,2018). However, their antioxidant capacities vary significantly based on their fatty acid composition, processing methods, and inherent phytochemicals. Measuring the total antioxidant capacity, for instance through the ‘Ferric Reducing Antioxidant Power’ (FRAP)assay, gives a rounded quantitative method to assess the natural defense mechanisms present in different oils.

#### **1.2 Justification of the study**

Largely, studies focus on foreign commercial brands, leaving a knowledge gap about locally produced varieties used more commonly and what processing does to the bioactive compound retention. As vegetable oil consumption rises in Nigeria and globally, there the need to: quantify the residual natural antioxidant potential (FRAP activity) remaining after processing in these commercially important oils, relate these residual activities with the source-specific

phytochemicals (e.g. isoflavones in soya bean) and provide empirical data to aid food manufacturers to know the relative oxidative stability and potential need for fortification in these common cooking and industrial oils is what justifies the study. (Szydłowska-Czerniak A. 2013). Natural antioxidants leading over synthetic preservatives like BHA and BHT underscores the importance of identifying and quantifying the antioxidant strength of locally produced oils. In regions such as Nigeria, where cotton seed, palm kernel and soybean oils are commonly used, understanding their antioxidant composition can enhance oil processing, storage and utilization in both domestic and industrial contexts (FAO, 2021)

Cottonseed oil, a (*Gossypium* sp.) a byproduct of cotton production is widely used in cooking due to its high smoke point and neutral flavor. Research suggests that its antioxidant activity can be enhanced through minimal processing and preservation of its natural tocopherols.

Soybean (*Glycine max* L.) oil is known for its high polyunsaturated fat content and moderate levels of tocopherols. Its FRAP activity is attributed to its vitamin E content, particularly  $\gamma$ -tocopherol. Soybean oil's numerous health benefits include its potential to reduce cholesterol levels and improve the heart health. However, refining processes can impact its antioxidant potency, highlighting the need for gentle processing methods.

Palm kernel oil, derived from the seeds of oil palm fruits, is rich in saturated fats and contains significant amounts of tocotrienols, a form of vitamin E with potent antioxidant properties. Its FRAP activity is notable due to these compounds, which have been linked to anti-inflammatory and neuroprotective effects. Palm kernel oil's stability and shelf life make it a valuable ingredient in food and cosmetics, but its high saturated fat content warrants moderation in consumption.

### **1.3 Aim/Objective of the study**

The study sets to quantitatively compare the total antioxidant capacity of the three -cottonseed oil, soya bean oil, and palm kernel oil,employing the Objective. (Pulido R. Bravo L. and Saura-Calixto F. ,2000)..

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Understanding antioxidant activity and FRAP assay

Antioxidants combat the issues that arise from uncontrolled generation of free radicals in our body which may lead to various health conditions such as autoimmune diseases, heart and neurodegenerative diseases, cancers, etc. The generation of these free radicals is both endogenous through normal metabolic activities such as mitochondrial respiration, peroxisomal metabolism and immune cell activation as well as exogenously through environmental factors including smoking, pollution, UV-radiation etc. In food science application, especially oil chemistry, these free radicals catalyze lipid oxidation, leading to rancidity, off-flavors, and nutrient degradation, thereby reducing the shelf life and nutritional quality of edible oils.

The term “antioxidant” refers to any molecule that can stabilize or deactivate free radicals before they can attack cells. In order to protect the cells and organ systems of the body against free radical damage man has evolved higher and complex antioxidant systems (enzymatic and nonenzymatic), which work synergistically, and in combination with each other (Pham-Huy et al., 2008). The antioxidants can be endogenous or obtained exogenously (part of a diet or dietary supplements). Antioxidants could be naturally occurring such as phenolic compounds, flavonoids, tocopherols, and carotenoids or synthetic, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Although, increasing awareness of the potential toxicity and instability of synthetic antioxidants has shifted focus toward the natural antioxidants counterparts derived from plant sources (such as edible oils, seeds, and fruits).

Antioxidant activity is a measure of the ability of a substance to inhibit or delay the oxidation of other molecules. In food science, particularly with oils, high antioxidant activity is desirable as it improves oxidative stability, nutrition, shelf-life, and functional uses.

The FRAP (Ferric Reducing Antioxidant Power) assay is a common, simple analytical method for quantifying antioxidant potential. FRAP is particularly valuable for assessing plant oils, whose antioxidant capacity varies due to differences in botanical origin, extraction techniques, chemical composition, and degree of refinement. The FRAP assay does not measure the ability to scavenge free radicals directly (like DPPH or ABTS assays). Instead, it measures the reducing power of the sample—its ability to donate an electron. This reducing power is directly correlated with the concentration of phenolic compounds and other antioxidants (like tocopherols) that act as electron donors.

## **2.2 Limitations of FRAP**

The FRAP assay does not effectively measure compounds that act by hydrogen atom transfer (HAT), a major mechanism that stops radical chain reactions. Thiol-based antioxidants (like glutathione) records unsatisfactory reactions. FRAP runs at non-physiologic pH and a compound's antioxidant potential can change at different pH levels. FRAP is an endpoint assay. It tells you the total reducing power but not how fast the antioxidants react, which is also a critical factor in their effectiveness.

## **2.3 Cotton seed(*Gossypium* sp.) oil**

Cottonseed oil is extracted from the seeds of various species of the cotton plant, primarily *Gossypium hirsutum* and *G. barbadense*. In its crude form, the oil is highly colored (reddish-brown) with a distinct, potent antioxidant known as gossypol which is considered an anti-nutritional factor and is removed during the extensive refining process. This mandatory removal, through alkali treatment and bleaching, results in a bland, light-colored oil that is desirable for frying and salad dressings. The antioxidant capacity measured by FRAP in refined cottonseed oil is largely attributable to the remaining tocopherols (Vitamin E), particularly the alpha and gamma forms, and minute quantities of gossypol derivatives that may survive the refining process. Though significantly lower than the crude oil, the residual reducing power in the refined product is often slightly more robust than other highly refined seed oils. However, cottonseed refining (to remove gossypol and phospholipids) reduces detectable polar antioxidants.

### **2.3.1 Medicinal and Non-medicinal used of cotton seed oil.(*Gossypium* spp)**

a) Therapeutic role in cardiovascular diseases:

Due to greater public awareness of cholesterol and saturated fats, there is a noticeable decline in the consumption of animal fats like butter and lard and a rise in the consumption of vegetable oils (Senger et al., 2017). The fact that CS-O(cotton seed oil) is cholesterol-free, has a high linoleic acid content, and contains a significant amount of polyunsaturated fatty acids are significant characteristics. Among the few seed oils that still have the ability to reduce consumption of saturated fat, CS-O is regarded as a very nutritious and healthful plant-based oil (Mahesar et al., 2017).

b) Wound healing:

Vegetable oils' antioxidant and anti-inflammatory properties make them promising for wound healing. They repair the functions of the skin's lipid barrier, rebuild dermal tissues, and encourage the growth of healthy cells. CS-O's high vitamin E content acts as an antioxidant and offers numerous skin benefits, such as quicker wound healing. (Isaac and Ekpa, 2013).

c) Anti-inflammatory properties

Inflammation cause breakouts, redness, dandruff, and other pertinent issues. Terpenes, various phenols, and fatty acids—particularly linoleic acid—found in CS-O have anti-inflammatory properties and can prevent inflammation (Mueller, 2008).

d) Industry:

Cotton seed oil can also be used in making of various industrial goods, i.e., margarine, soap, rubber, candles, emulsifiers, medications, cosmetics, waterproofing, etc. Livestock: The fact that cotton seed oil is cholesterol-free, high in polyunsaturated fats, and rich in antioxidants like vitamin E prolongs the shelf life of pigs, rabbits, and chickens, among other animals.

Fig 2.1 An image of the cotton seed and its oil extract in a bottle.



### **2.3.2 Soya beans (*Glycine max*) oil**

Soya bean oil, derived from the seeds of the soya bean plant, is the most widely consumed. Soybean seeds are rich sources of health-promoting metabolites such as phenolic acids, isoflavones, and anthocyanins. These polyphenolic compounds are one of the natural antioxidants and have been used as supplements in food formulations on account of their role in preventing many ailments, including cancer, diabetes and has become a dietary choice of many societies around the world, partly because its consumption is associated with a reduced risk of cardiovascular diseases (FAO . 2019)

Soybean seeds are excellent sources of plant-based and high-quality protein and oil. Earlier studies pointed out that the protein and oil contents in soybean seeds may account for 35 to 55% and 13 to 26% on a dry seed weight basis, respectively five fatty acids including two saturated fatty acids (palmitic acid (16:0) and stearic acid (18:0)) and three unsaturated fatty acids (oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3)) are predominantly found in soybean oil and have various health benefits. Soybean oil contains tocopherols (notably  $\gamma$ -tocopherol), phytosterols, and variable phenolics depending on processing and cultivar. These contribute to higher reducing power in many assays. Consequently, the final FRAP activity of refined soya bean oil reflects the low, residual concentration of primarily tocopherols. Soybean oil is commonly used in cooking, food manufacturing, and nutritional products due to its affordability and versatility. However, its high degree of unsaturation makes it susceptible to oxidation, making antioxidant evaluation critical.

### **2.3.3 Medicinal and Non-medicinal uses of soya beans oil (Glycine max)**

#### a) Lowering of Cholesterol and enhancing Cardiovascular Health

According to He et al. (2005), soybeans work on the risk factors for cardiovascular diseases, such as lowering blood or liver triglycerides, lowering low density lipoprotein (LDL) levels, raising high density lipoprotein (HDL) levels and lowering blood pressure.

#### b) Control of Obesity

There are phytoestrogens in it that are suggested to contribute to the prevention of obesity. It has been shown that soybeans play a role in controlling obesity; as a result, they can be incorporated into diets that limit energy intake (Naaz et al., 2003).

#### c) Prevention of Diabetes

The enzyme  $\alpha$ -glucosidase, which is crucial for the digestion of carbohydrates as well as the processing of glycoproteins and glycolipids, is specifically inhibited by genistein.

#### d) The Production of Biodiesel

According to studies, biodiesel made from soybean oil is a potential renewable energy substitute for petroleum diesel because of its high combustion efficiency, low sulfur emissions, and biodegradability (Mohammadi-Moghaddam et al., 2025).

e) Nutrition: Because of its high protein digestibility and balanced amino acid profile, soybean meal—a by-product of oil extraction—represents almost 70% of the worldwide protein feed market and is essential to the nutrition of cattle, poultry, and aquaculture (Belachew et al., 2023; Olafimihan, 2025).

Fig. 2.2 Soya beans and its extracted oil.



#### **2.3.4 Palm Kernel (*Elaeis guineensis*) oil**

Palm kernel oil is extracted from the kernel (seed) of the oil palm fruit, making it chemically distinct from the Red Palm Oil (RPO) derived from the fruit's fleshy mesocarp. PKO has extremely high content of saturated fatty acids (over 80%), predominantly lauric acid, giving it solid physical characteristics at room temperature and making it valuable in confectionery and margarine production. Structurally, this high saturation provides the oil with excellent inherent oxidative stability, meaning it is less prone to rancidity than highly unsaturated oils like soya bean, regardless of antioxidant content. From an antioxidant perspective, PKO is naturally much poorer in strong reducing agents compared to its red palm oil counterpart. The kernels contain relatively lower concentrations of alpha tocopherol and tocotrienols (a form of Vitamin E) and virtually no carotenoids. The subsequent refining steps further strip away from it. In terms of minor antioxidants, palm fruit oil has been extensively researched, whereas kernel oil has received less attention (Sambanthamurthi et al.)

#### **2.3.5 Medicinal and Non-medicinal uses of palm kernel oil**

a) Remedy for convulsion in children:

Alternative and traditional medicine has shown that palm kernel oil is a wonderful solution when it comes to treating convulsion in kids. Its healing properties help combat the effect of epilepsy attack, as well as offering relief (Owoyale and Owolabi, 2014).

b) Prevents aging:

Palm kernel oil is a rich antioxidant agent that contains vitamin E. And vitamin E, coupled with the antioxidant's properties in it, help prevent the signs and symptoms of aging. Observation has

also shown that the use of palm kernel oil does not only prevent fine lines, it helps delay sagging and wrinkled skin (Sen et al., 2010).

c) Control of blood pressure:

Studies have shown that also helps control the free flow of blood from the heart to other organs of the body. And by so doing, it helps in keeping the blood pressure under control, thereby preventing the risk of hypertension (Owoyele and Owolabi, 2014).

d) Tackling body odour:

Owing to its unique strong smell, palm kernel oil helps tackle body odour when it is applied to the skin on a regular basis. It is used for the manufacture of soaps, washing powders, hair creams, personal care products etc. It restores skin elasticity in order to prevent wrinkles It protects the skin from harsh weather conditions. It is a good skin moisturizer (Choo et al., 2018).

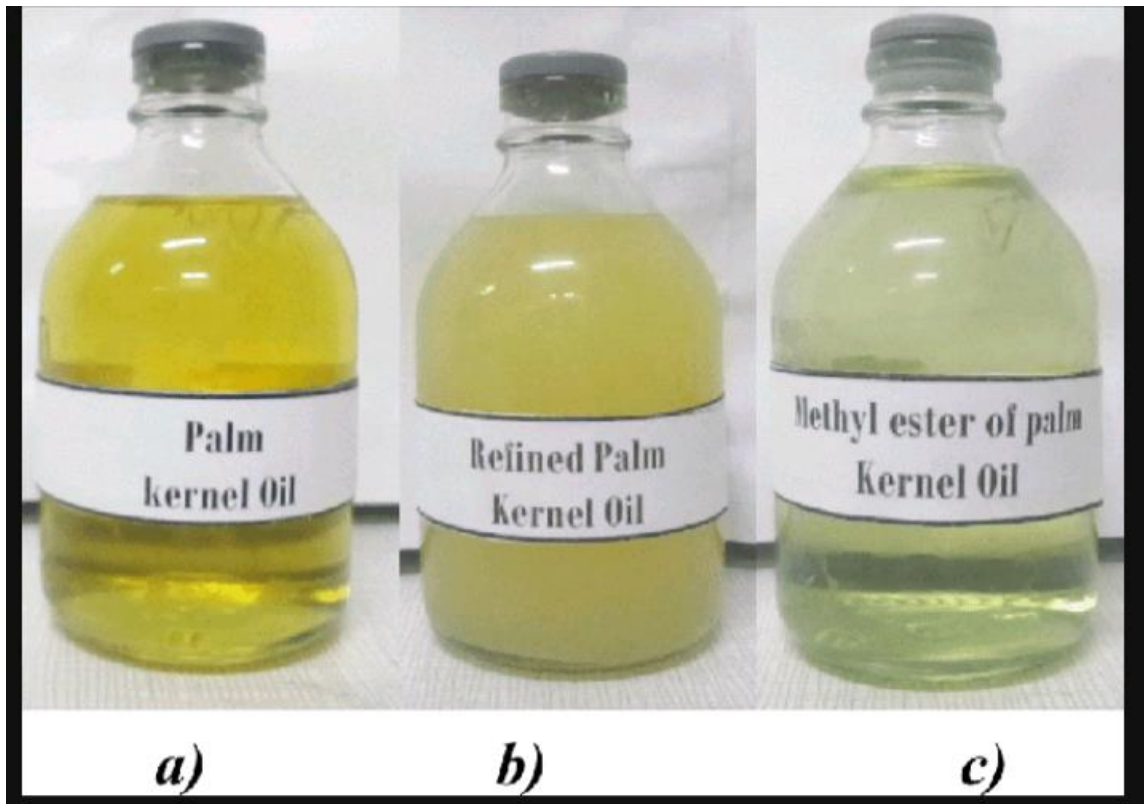
e) Prevents hair loss or breakage:

Black palm kernel oil is used as a hot oil treatment on dry hair and scalp so as to soothe the scalp and moisturize the hair for thickness as well.

Fig2.3 An image of Palm nut broken to show the palm kernel



Fig 2.4 Image of the Palm Kernel oil



## 2.4 Analysis of FRAP activity in selected oils

### Soya Bean Oil (*Glycine max*)

Soya bean oil (SBO), 50 - 150 (High) Due to the synergistic effect of a high concentration of mixed tocopherols (mostly gamma- and delta) and the presence of potent isoflavones, soya bean oil typically demonstrates strong performance in the FRAP assay. (Verma A. K. *et al.* 2017).

The absence of high heat treatment and refining process provides preservation of bioactive compounds of cold pressed soybean oil.

### Cotton Seed Oil (*Gossypium spp.*)

Cottonseed oil (CS-O) has a unique and complex profile that is highly dependent on processing. It contains tocopherols, gossypol (a powerful natural antioxidant in crude oil) Gossypol is largely removed during refining. Crude\_ Very High (often higher than soya bean). Refined\_ (15 - 50) Moderate to Low (Wang Y. *et al.* 2012).

### Palm Kernel Oil (*Elaeis guineensis*)

Over 80% of PKO is saturated fat (mainly lauric acid). Thus, its stability relies almost entirely on its saturated structure, rather than a significant chemical defense mechanism measurable by the FRAP assay. 5 - 20 (Low) Tocopherols, Tocotrienols (trace) Lower FRAP generally due to high saturation (less prone to oxidation) and lower concentration of major antioxidants compared to soya bean oil (sometimes comparable when crude/cold-pressed) and crude cottonseed oil, PKO consistently shows lower total phenolic content and, consequently, lower FRAP values. (Mani S. and Wati L. ,2018).

Cotton seed oil (crude)  $\geq$  soybean > Palm kernel for FRAP/reducing power when comparing refined commercial oils. This ranking reflects higher polar antioxidant levels in soybean and cottonseed versus PKO.

## **2.5 Comparative analysis (factors affecting variability)**

The factors that affect the variability that was observed are;

1. Processing: Refining (degumming, neutralizing, bleaching, deodorizing) removes a significant portion of antioxidants (phenolics, gossypol, some tocopherols). Cold-pressed or virgin oils will always have higher FRAP values.
2. Variety: The specific variety of soya bean, cotton, or palm fruit can lead to different baseline antioxidant levels.
3. Storage: Exposure to light, heat, and oxygen will degrade the antioxidants over time, lowering the FRAP value of an older sample.
4. Extraction Method: Solvent extraction (like with hexane) versus cold-pressing will yield different antioxidant profiles.

Table 2.1 Showing comparative analysis of the oils.

FEATURE	SOYA BEANS OIL	COTTON SEED OIL	PALM KERNEL OIL (PKO)
Primary Antioxidant	Tocopherol (gamma and delta)  Isoflavones	Tocopherols (alpha, gamma)	Tocotrienols,  Tocopherols (alpha)
Unique Compound	Isoflavones (Genistein)	Gossypol (if crude)	Tocotrienols
Expected FRAP Activity	High	Moderate to low	Low
Main Factor	Synergistic effect of tocopherols and isoflavones	Gossypol removal during refining.	Low overall concentration of phenolic compounds

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Materials

##### 3.1.1 Lab equipment, glass wares and consumables

UV-Vis Spectrophotometer (For measuring the absorbance). Set at 593 nm

Analytical Balance (For precise weight of oil samples and chemical reagents). Accurate to 0.0001 g

Vortex Mixer(High-speed mixing for extracting antioxidants from the oi)l.

Centrifuge(To separate the methanolic extract from the oil residue). Capable of 4000 rpm(revolution per minute)

Water Bath/Incubator (Maintaining the optimal reaction temperature). Set at 37°C

Micropipettes(Accurate dispensing of small volumes).

pH Meter(Calibrating the acetate buffer to pH 3.6) Standard laboratory model

Volumetric Flasks (Precise preparation of stock solutions and FRAP reagent).Measuring100 mL, 500 mL, 1L

Cuvettes Sample holders for the spectrophotometer.

Quartz or appropriate plastic

Test Tubes/Centrifuge Tubes:-(For running the assay and performing extractions). 15 mL tubes with caps

Aluminum Foil:- Essential for covering and protecting light-sensitive reagents (TPTZ and Working FRAP reagent).

### **3.1.2 Reagent and chemicals**

All reagents and chemicals used were of HPLC /Analytical grade and they included;

Methanol the solvent for extracting antioxidants from oil samples. TPTZ (2,4,6-Tripyridyl-s-triazine) chromogenic reagent; forms the blue complex upon reduction as it is very light-sensitive; It was dissolved in HCl. Iron (III) Chloride Hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) source of the ferric iron ( $\text{Fe}^{3+}$ ) to be reduced also used to prepare ( $\text{FeCl}_3$ ) stock solution. Glacial Acetic Acid (Component for preparing the low-pH acetate buffer. Highly concentrated acid).Sodium Acetate Trihydrate ( $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ ), for preparing the acetate buffer (pH 3.6) which is the salt component of the buffer system. Hydrochloric Acid (HCl), used to dissolve TPTZ in the stock solution. Iron (II) Sulfate Heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) used to prepare the standard curve, prepared fresh and used for quantitative reporting. Deionized/Distilled Water, used for preparing all aqueous solutions and standards, during this project the purity of it was taken into consideration.

## **3.2 Methods**

### **3.2.1 Sample collection and preparation**

The cotton seed(*Gossypium spp.*), palm fruit(*Elaeis guineensis*) and soya bean (*Glycine max*) seeds were purchased from a local market in Benin metropolis. The cotton seeds were obtained from the bod,it was put in shade and not direct sunlight before oil extraction to preserve the quality of the oil,prevent degradation of heat-sensitive components and ensure uniform drying .

It was oven dried at 40°C for an interval of 1-2 hours, cooled and weighed. The seeds of the soya bean were cleaned manually to remove stones, chaff and other foreign materials and then stored in airtight containers under dry conditions until required for extraction and analysis.

### **3.2.2 Extraction**

The primary challenge with oils is that they are hydrophobic (fats), while the FRAP reagent is aqueous (water-based). They will not mix. Therefore, the method must be a two-stage process:

1. Extraction: Using a solvent (methanol) to pull the polar antioxidants (phenolics, tocopherols) out of the oil.
2. Assay: Testing the antioxidant power of the resulting methanolic extract.

Oil extraction was performed using a mechanical oil press heated to 180 °C to maximize oil yield while maintaining efficient and smooth operation. The prepared seeds were fed gradually and uniformly into the press to ensure consistent pressure and prevent blockages. The expressed crude oil was collected in clean, dry containers immediately after pressing. The oil was then passed through a fine-mesh sieve to remove solid residues, foots and impurities. The filtered oil was transferred to labeled, airtight bottles and stored at room temperature, while the commercial sesame sample was analyzed as obtained.

#### **Part 1: Preparation of Reagents & Standards**

Firstly, all the necessary solutions were prepared. The TPTZ and FRAP reagents are light-sensitive and were as a result kept in foil-wrapped bottles.

Stock Solution 1: 300 mM Acetate Buffer (pH 3.6). For this, Sodium Acetate Trihydrate of 3.1g was dissolved in 800 mL of deionized water. Then, 16.0 mL of Glacial Acetic Acid was added to this. A pH meter was used to adjust the pH to 3.6 (by adding more acid or a base like NaOH when needed). This was transferred to a 1 L volumetric flask and deionized water was added to the 1.0 L mark.

Stock Solution 2: 10 mM TPTZ Solution. Weighing 31.2 mg of TPTZ (2,4,6-tripyridyl-s-triazine), it was dissolved in 10.0 mL of 40 mM HCl (Hydrochloric Acid). Why? The TPTZ will not dissolve in plain water.

Stock Solution 3: 20 mM FeCl<sub>3</sub> Solution. For this I weighed 54.0 mg of Iron (III) Chloride Hexahydrate (FeCl<sub>3</sub>·6H<sub>2</sub>O). Then, proceeded to dissolve in 10.0 mL of deionized water.

Standard Solution: 1000 μM Fe(II) Stock. After weighing 27.8 mg of Iron (II) Sulfate Heptahydrate (FeSO<sub>4</sub>·7H<sub>2</sub>O), it was dissolved in 100.0 mL of deionized water. This solution was prepared fresh on the day of the assay.

#### WORKING FRAP Reagent (Prepared Fresh Daily)

1. The three stock solutions were combined in a 10:1:1 ratio:

10 parts Acetate Buffer

1 part TPTZ Solution

1 part FeCl<sub>3</sub> Solution

2. Mix well and wrap the flask in aluminum foil.

3. Place this reagent in a 37°C water bath to pre-warm it for at least 20 minutes before use.

## Part 2: Antioxidant Extraction from Oil

This was done for each of the three oils (Cotton Seed, Soya Bean, Palm Kernel).

1. Approx. 2.0 g (with the exact weight recorded) of the oil sample was weighed into a 15 mL centrifuge tube.
2. Exactly 10.0 mL of Methanol was added with volumetric pipette to the tube which was tightly covered and placed on a vortex mixer. Mixed at high speed for 3 minutes to create a thorough emulsion, ensuring maximum contact between the oil and the methanol.
3. Centrifuged at 4000 rpm for 10 minutes and then two distinct layers appeared: the oil (bottom or top, depending on density) and the clear methanol layer.
4. Carefully, using a micropipette, the top methanol layer (supernatant) was removed and transferred to a clean, foil-wrapped test tube.
5. This clear liquid is the sample extract containing the antioxidants

### **3.3 Determination of Ferric Reducing Antioxidant Power (FRAP)**

FRAP assay was carried out using The combined method of Siripongvutikorn *et al.* (2024) and Fernando and Dissanayake with slight modifications to suit antioxidant analysis in oil samples. The scavenging activity was obtained by the following formula. The measured absorbance is directly proportional to the total reducing power of the sample.

$$\text{FRAP}(\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A0 is the absorbance of the control

A1 is the absorbance of the extract/standard taken as ascorbic acid.

### 3.4 FRAP assay procedure

#### Principle

The FRAP assay was developed by Benzie and Strain (1996) to measure the ferric reducing power of human plasma. Pulido, Bravo, and Saura-Calixto (2000) adapted this method to quantify the ferric reducing antioxidant power of plant extracts. - Ferric ions ( $\text{Fe}^{3+}$ ) are reduced to ferrous ions ( $\text{Fe}^{2+}$ ) by antioxidants in the sample. The principle is based on the reduction reaction of the ferric ( $\text{Fe}^{3+}$ ) to the ferrous ( $\text{Fe}^{2+}$ )—TPTZ (2,4,6-tripyridyl-s-triazine) complex forming an intense blue-coloured complex/

**Color Change:** The intensity of the blue color is directly proportional to the reducing power of the antioxidants. Measured spectrophotometrically at 593 nm.

#### Reaction

To 2.85 mL of the freshly prepared FRAP working reagent pipette into clean test tubes was added 150  $\mu\text{L}$  of each oil extract or ascorbic acid standard solution. The mixture was vortexed gently to ensure homogeneity and then incubated in the dark for 30 minutes at room temperature to prevent light-induced oxidation. After incubation, absorbance was measured at 593 nm, read after every 10 minutes using a UV–Vis spectrophotometer against a reagent blank. The reagent blank consisted of 2.85 mL of FRAP working reagent mixed with equal measure of 150  $\mu\text{L}$  methanol (the solvent used for extract preparation) instead of the plant extract.

The FRAP activity was expressed as ascorbic acid equivalent (mg) per g of dried seed weight (mg AAE/g).

CONC( $\mu\text{g/ml}$ ) of sample/standard	% FRAP for soya beans oil	%FRAP for cotton seed oil	%FRAP for palm kernel oil
250	38.316	51.317	38.958
200	30.553	42.928	27.874
150	24.300	36.732	20.530
100	18.279	26.211	13.313
50	9.636	18.820	8.755

### 3.5 Instrumental and data analysis.

Table 3.1

Oil Type	Mean FRAP (%)	Range (max–min)	Highest FRAP at conc.	Lowest FRAP at conc.
Soya bean oil	24.217	38.316 – 9.636 = 28.68	38.316 (250 $\mu\text{g/ml}$ )	9.636 (50 $\mu\text{g/ml}$ )
Cotton seed oil	35.602	51.317 – 18.820 = 32.497	51.317 (250 $\mu\text{g/ml}$ )	18.820 (50 $\mu\text{g/ml}$ )
Palm kernel oil	21.886	38.958 – 8.755 = 30.203	38.958 (250 $\mu\text{g/ml}$ )	8.755 (50 $\mu\text{g/ml}$ )

Trend / Pattern Analysis : All three oils showed a dose-dependent increase in FRAP activity — that is, as concentration increases, the FRAP % also increases. What can be determined from this is that the antioxidant activity of the oils is concentration-dependent, which is typical of most plant-derived antioxidant systems.

The mean FRAP activity of cotton seed oil ( $35.60 \pm 12.04$  %) was significantly higher than that of soya bean oil ( $24.22 \pm 10.07$  %) and palm kernel oil ( $21.89 \pm 10.90$  %), indicating that cotton seed oil has a greater ferric-reducing antioxidant capacity ( $p < 0.05$ ).

All oils exhibited concentration-dependent (Dose dependent) activity, confirming that their antioxidant compounds act by electron donation. Cotton seed oil showed the highest antioxidant capacity, likely due to its higher levels of phenolic compounds, tocopherols, and other antioxidants. Palm kernel oil, being more saturated, had the lowest reducing power

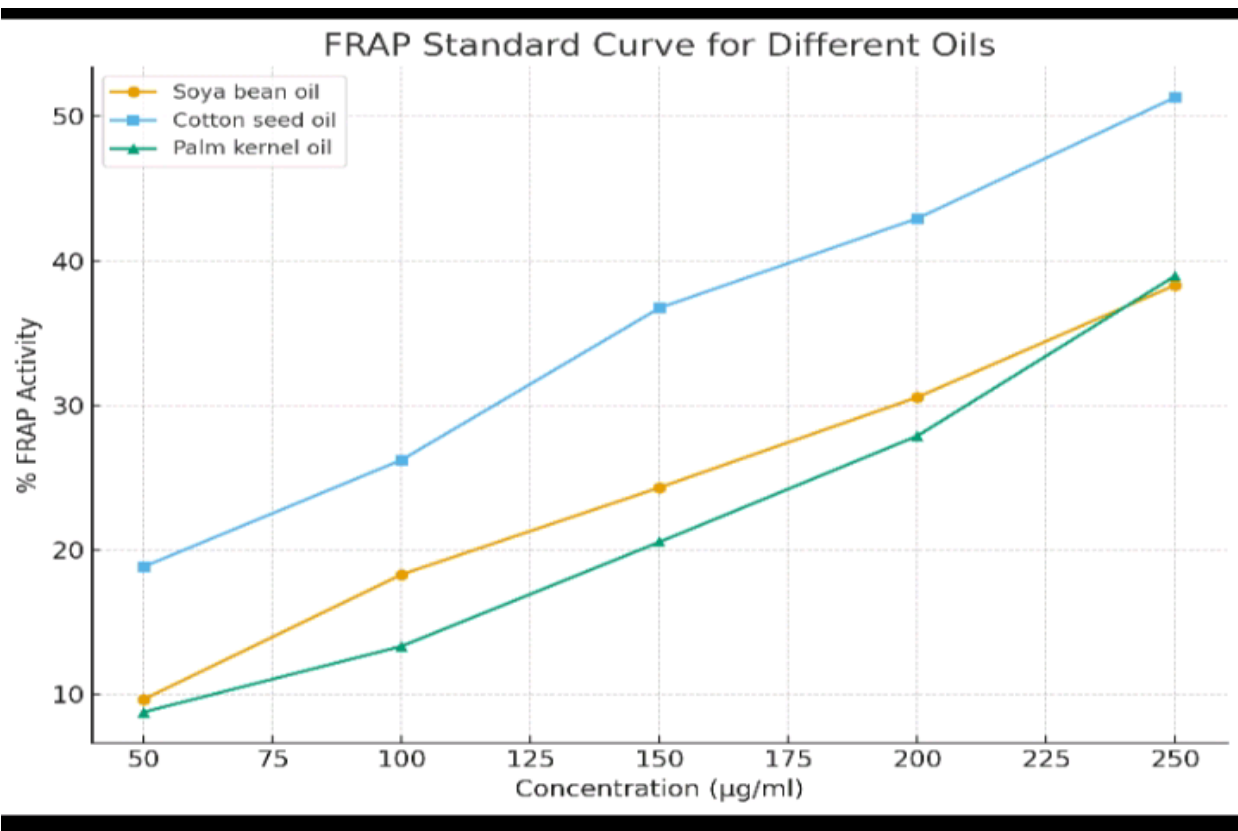


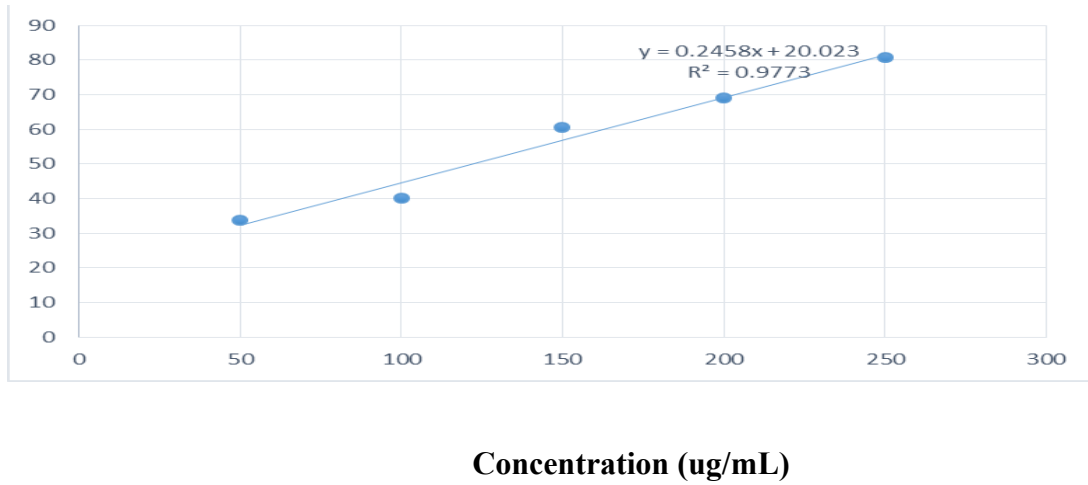
Figure 3.1 A graph plotted with standard curve showing the various oils, their concentration and FRAP activity.

The graph shows a clear concentration-dependent increase in %FRAP activity for all oil samples. Cotton seed oil displayed the highest FRAP values across all concentrations, indicating the greatest ferric reducing ability, followed by soya bean oil and palm kernel oil respectively. This trend confirms that antioxidant activity increases proportionally with sample concentration.

## CHAPTER FOUR

### 4.0 RESULT

#### 4.1: Calibration Curve of Ascorbic Acid



**Figure 4.1: The Curve Calibration of Ascorbic Acid**

#### 4.1 FRAP activity of cotton seed, soya beans and palm kernel oil.

Table 4.1 The Result of FRAP of the various oil samples

Oils	Mean %FRAP	AAE ( $\mu\text{g/mL}$ )
Soyabean oil	24.2168	17.06 $\mu\text{g/mL}$
Cotton seed oil	35.2016	61.75 $\mu\text{g/mL}$
Palm kernel oil	21.8860	7.58 $\mu\text{g/mL}$

### One-Way ANOVA (Between-Oil Comparison)

	Source F	p-value	Decision
Between Oils	1.75	0.215	Not significant (p > 0.05)

Table 4.2

#### Interpretation:

The differences in FRAP means among soya bean, cotton seed, and palm kernel oils are not statistically significant at the 0.05 level, though cotton seed oil shows a higher mean numerically. All oils show an almost perfect positive correlation between concentration and %FRAP activity, meaning antioxidant capacity increases proportionally with concentration.

## CHAPTER FIVE

### 5.0 DISCUSSION AND CONCLUSION

#### 5.1 Discussion

The Ferric Reducing Antioxidant Power (FRAP) assay showed a clear difference in antioxidant capacity among the three refined oils, cotton seed oil, soya bean oil, palm kernel oil following the order: 35.60 > 24.22 > 21.89.

Interpretation and Chemical Justification:

Lowest FRAP Value ( Palm Kernel Oil): Lowest FRAP activity overall. Suggests lower phenolic and unsaturated compound levels, consistent with its more saturated fatty acid composition.

Justification: PKO is a highly saturated oil, primarily composed of lauric, myristic, and palmitic acids. Unlike Crude Palm Oil (CPO) from the fruit's flesh, PKO from the kernel is naturally low in carotenoids (Vitamin A precursors) and lower in tocopherols (Vitamin E).

Furthermore, the industrial refining process (bleaching, deodorization) removed most of the minor heat-sensitive antioxidants, resulting in a very low final FRAP value, consistent with published data that the low natural content of strong antioxidants (carotenoids, some tocopherols/tocotrienols) in the kernel made PKO exhibit the lowest FRAP activity expected using a previous reported method (Mani S. and Wati L. 2018)

Intermediate and Higher FRAP Values (Soya Bean and Cotton Seed Oils): Soya Bean Oil (SBO) and Cotton Seed Oil (CSO) are both polyunsaturated oils with differing antioxidants .Soya

bean oil exhibited moderate FRAP activity, consistently below cotton seed oil but above palm kernel oil. This study has promising potentials based on the results gotten when compared with previous report method (Szydłowska-Czerniak A. 2013)

This reflects a good antioxidant profile, possibly from flavonoids and unsaturated fatty acids. Its primary natural antioxidant is alpha-tocopherol (Vitamin E) which extensive refining severely strips, led to a low but measurable FRAP value, consistent with study previously reported (Lee J. Lee Y. and Choe, E. 2007).

Cotton seed oil exhibited the highest FRAP values across all concentrations. Indicates the strongest reducing power, likely due to higher polyphenolic or tocopherol (vitamin E) contents. Its antioxidant profile includes tocopherols and unique, heat-stable phenolic compounds derived from gossypol. The unrefined cotton seed oil provided the highest antioxidant power because of the gossypol that was retained, unremoved and this was correspondent with data provided by early reported method (Alsaif M.A. 2017).

Although gossypol is toxic and removed, its derivatives can persist in the refined oil, often providing a marginally higher FRAP score than SBO (soy bean oil) comparative with the results mentioned in previous study report (Wang, Y. *et al.* 2012).

## **5.2 Methodological Evaluation and Comparison to Literature**

### Methodological Evaluation

Extraction Method: The methanol/solvent extraction step was necessary and vital because FRAP is an aqueous (water-soluble) assay, the hydrophobic oil phase must first be separated by

partitioning the polar antioxidants (phenolics, tocopherols) into the methanolic extract to accurately measure their capacity.

#### Comparison to Literature:

These results align with the general consensus that highly refined vegetable oils have low FRAP values for instance, (Alves, A. B. *et al.* 2020). This and similar papers provide quantitative data confirming that oils rich in polyunsaturated fatty acids (like Soy and Cottonseed) lose most of their naturally occurring phenolics/tocopherols during refining, resulting in a low FRAP value for the refined product. The minor remaining differences are attributable to oil-specific chemical residues (e.g., gossypol derivatives in CSO) and the degree of saturation.

### **5.3 Conclusion**

In summary, the FRAP activity of the three oils had significant difference based on their intrinsic chemical composition. Soya bean oil showed the intermediate FRAP value due to its rich and diverse profile of tocopherols and isoflavones. Palm kernel oil shows the lowest activity, as it has a much lower concentration of phenolic antioxidants. Cottonseed oil's activity is entirely dependent on processing; while its crude form is a potent antioxidant source (giving the highest FRAP value), the refined oil used commercially will have a FRAP value significantly lower than soya bean oil due to the necessary removal of gossypol. Methodological variability (extraction solvent, refining history, standards) is therefore, the dominant factor producing quantitative differences between studies.

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