

ACID FERMENTATION FACILITATED BLEACHING OF CRUDE PALM OIL(CPO)



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**A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE
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NOVEMBER, 2025

CERTIFICATION

This is to certify that this project work, titled “Acid Fermentation Facilitated Bleaching of Crude Palm Oil (CPO)” was carried out by Etinosa Bright OSADOLOR with Matriculation Number LSC2009861, Department of Science Laboratory Technology (Biochemistry Technique), Faculty of Life Sciences, University of Benin, Benin City, Edo State, under the supervision of Dr. P.O Alonge

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DEDICATION

This work is wholeheartedly dedicated to Almighty God, the source of all wisdom, knowledge, and strength. To Him be all the glory, honour, and praise for granting me the grace, guidance, and perseverance to complete this work successfully.

Also, to my family for their love, care, prayers and support.

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ABSTRACT

This study evaluated the effect of acid fermentation using varying concentrations of hydrochloric acid (HCl) 0.025 M, 0.050 M, 0.075 M, and 0.100 M on the bleaching efficiency and quality characteristics of crude palm oil (CPO), with untreated oil (0.00 M) as control. Physicochemical parameters analyzed included moisture content, iodine value, saponification value, peroxide value, free fatty acid (FFA) content, refractive index, and specific gravity. Results from one-way ANOVA and Tukey's post-hoc tests revealed that HCl concentration had a highly significant influence ($p < 0.001$) on all parameters except moisture content. The moisture content remained relatively constant (0.015 ± 0.003 % to 0.022 ± 0.002 %), indicating stable processing conditions. Iodine value markedly decreased from 48.80 ± 5.00 g/100 g in the control to between 14.21 and 20.31 g/100 g in treated samples, confirming saturation increase. Saponification value slightly declined from 211.48 ± 5.00 mg KOH/g to 198.71–206.54 mg KOH/g, while peroxide value dropped sharply from 24.03 ± 5.00 mEq/kg to 2.02–6.62 mEq/kg, indicating improved oxidative stability. Conversely, FFA content increased significantly from 0.89 ± 5.00 % to 16.7–20.3 %, reflecting partial triglyceride hydrolysis under acidic conditions. The refractive index values varied marginally (1.361–1.368), and specific gravity increased slightly from 0.8842 ± 5.00 to 0.887–0.893, suggesting limited compositional alteration. Statistical analyses confirmed that moderate acid concentrations (0.05–0.075 M) yielded optimal bleaching performance, achieving effective pigment and peroxide reduction while maintaining desirable stability and nutritional integrity. The findings establish acid-fermentation bleaching as a sustainable and cost-effective alternative to conventional refining methods for enhancing palm-oil quality.

CHAPTER ONE

1.0 INTRODUCTION

Palm oil remains one of the most widely produced and consumed edible oils globally, obtained primarily from the mesocarp of the oil palm fruit (*Elaeis guineensis*) and constituting a major source of dietary fat in many tropical and subtropical regions (Abdulkarim *et al.*, 2020; Hassan *et al.*, 2021). Crude palm oil (CPO), as extracted from the fruit, is rich in natural bioactive compounds such as carotenoids, tocopherols, phytosterols, and phenolic antioxidants that contribute to its nutritional and functional value (Osei-Amponsah *et al.*, 2020; Choo *et al.*, 2022). Despite these benefits, unrefined CPO contains undesirable components including free fatty acids (FFAs), phospholipids, moisture, trace metals, volatile odour compounds, and pigments such as carotenoids and chlorophyll, all of which negatively affect its stability, flavour, and market acceptability (Ibrahim *et al.*, 2021; Sulaiman *et al.*, 2021). These impurities accelerate oxidative deterioration and rancidity, reducing shelf life and limiting compliance with international quality standards for edible oils (Lee *et al.*, 2020; Gibon, 2020).

Conventional palm oil refining typically involves several sequential processes such as degumming, neutralization, bleaching, and deodorization which are designed to remove impurities and improve sensory and oxidative quality (Rahman *et al.*, 2019; Ajala *et al.*, 2022). Among these, bleaching is a critical step that eliminates pigments, residual phospholipids, oxidation products, and trace contaminants, thereby improving colour and oxidative stability (Ali *et al.*, 2021; Cheng *et al.*, 2019). The traditional bleaching process commonly employs high temperatures and adsorbents such as activated clays, bentonite, or bleaching earths, sometimes under vacuum, to remove colour bodies and peroxides (Ogunwuyi *et al.*, 2021; Bamgboye and Adejumo, 2019). However, the intense thermal

conditions required often result in the loss of valuable micronutrients such as carotenoids and tocopherols and may promote the formation of toxic compounds including trans fatty acids and 3-monochloropropane-1,2-diol (3-MCPD) esters (Lee *et al.*, 2020; Akinola *et al.*, 2018).

To overcome these limitations, contemporary research efforts have shifted toward developing alternative refining methods that reduce thermal degradation, preserve nutritional components, and improve energy efficiency (Nwabanne *et al.*, 2017; Ogunsina *et al.*, 2020). One promising strategy is acid-assisted or acid fermentation-facilitated bleaching, which employs controlled acidic conditions to aid the removal of colour pigments and other impurities (Ogunwuyi *et al.*, 2021; Eze *et al.*, 2020). Hydrochloric acid (HCl), in particular, has been recognized for its catalytic ability to break down molecular structures, hydrolyze triglycerides, and decompose resistant pigments such as riboflavin under mild processing conditions (Ali *et al.*, 2021; Umanu *et al.*, 2021). When combined with fermentation, the process may generate organic acids and microbial enzymes that synergistically enhance impurity degradation and pigment adsorption (Iheanacho *et al.*, 2022; Enebe *et al.*, 2023).

Hydrochloric acid acts as a strong catalyst that promotes hydrolytic cleavage of ester linkages in triglycerides, liberating free fatty acids and glycerol that can be subsequently removed during further refining (Rahman *et al.*, 2019; Idowu *et al.*, 2022). This mechanism contributes to both pigment degradation and viscosity modification, thereby improving oil clarity and reducing chromophore content (Musa *et al.*, 2021; Okolie *et al.*, 2019). The efficiency of the acid bleaching process, however, depends on several parameters including acid concentration, reaction temperature, time, and the presence of catalysts or adsorbents (Cheng *et al.*, 2019; Sunday *et al.*, 2018). Optimising these parameters is essential to achieving the dual goals of effective impurity removal and minimal damage to desirable oil components (Okafor *et al.*, 2021; Akinola *et al.*, 2018).

A major concern in palm oil refining is the control of free fatty acid (FFA) content, which serves as a key quality indicator for oxidation stability and sensory acceptability (Ibrahim *et al.*, 2021; Osei-Amponsah *et al.*, 2020). High FFA levels, often resulting from enzymatic or acid hydrolysis of triglycerides, contribute to off-flavour formation, lower oxidative stability, and undesirable soap formation during alkali neutralization, leading to yield losses and higher refining costs (Sulaiman *et al.*, 2021; Nwabanne *et al.*, 2017). Acid-assisted bleaching offers a potential advantage by facilitating the liberation of FFAs, which can be more efficiently removed in subsequent refining steps, thereby improving the overall refining efficiency (Okafor *et al.*, 2021; Rahman *et al.*, 2019). Moreover, the breakdown of riboflavin and other heat-labile pigments under acid conditions enhances the decolorization process and improves the Deterioration of Bleachability Index (DOBI) an important parameter reflecting crude oil quality and bleaching potential (Cheng *et al.*, 2019; Gibon, 2020).

In addition to acid hydrolysis, the incorporation of fermentation-derived catalysts or agro-based adsorbents offers a sustainable enhancement to the bleaching process (Enebe *et al.*, 2023; Ogunsina *et al.*, 2020). Agricultural by-products such as plantain peel ash (PPA) and rice husk ash (RHA), which are rich in silicates and metal oxides, have shown potential as low-cost adsorbents for improving bleaching efficiency while reducing the environmental impact associated with conventional bleaching earths (Ajala *et al.*, 2022; Akinola *et al.*, 2018; Alonge and Odokuma, 2018). These materials not only enhance pigment removal through surface adsorption and catalytic oxidation but also align with circular economy principles by valorizing agricultural residues (Umanu *et al.*, 2021; Gibon, 2020). Their utilization helps minimize industrial waste and promotes greener refining technologies suitable for small- and medium-scale processors (Eze *et al.*, 2020; Idowu *et al.*, 2022).

From an industrial perspective, the combined use of acid fermentation and catalytic adsorbents presents several advantages, including lower processing temperature, reduced

chemical usage, and retention of thermolabile nutrients such as vitamin A and β -carotene (Ali *et al.*, 2021; Okolie *et al.*, 2019). This dual approach reduces operational costs and the formation of undesirable by-products like 3-MCPD esters, while simultaneously improving oxidative stability and oil brightness (Bamgboye and Adejumo, 2019; Osei-Amponsah *et al.*, 2020). It also supports sustainable processing goals by enabling the replacement of imported bleaching earths with locally sourced bio-adsorbents (Ajala *et al.*, 2022; Enebe *et al.*, 2023).

Given these advantages, the present research investigates the efficacy of acid fermentation-facilitated bleaching of crude palm oil using HCl, focusing on the interaction between acid concentration and catalytic agents such as plantain peel ash and rice husk ash. The study evaluates the breakdown of triglycerides, riboflavin, and free fatty acids, and assesses the resulting changes in physicochemical properties such as peroxide value, iodine value, β -carotene concentration, vitamin A content, solid fat content, cloud point, refractive index, and DOBI (Iheanacho *et al.*, 2022; Cheng *et al.*, 2019).

Ultimately, this research aims to provide insights into developing a low-cost, energy-efficient, and environmentally sustainable bleaching technique that preserves oil nutritional quality while enhancing industrial performance (Enebe *et al.*, 2023; Ogunsina *et al.*, 2020). The findings are expected to contribute to the advancement of alternative refining technologies suitable for both industrial and small-scale applications, particularly in palm oil-producing regions across Africa and Southeast Asia (Ajala *et al.*, 2022; Idowu *et al.*, 2022).

1.1 Background of the Study

Palm oil remains the most extensively consumed vegetable oil worldwide, accounting for a major proportion of dietary fat intake and serving as an indispensable raw material in food, cosmetic, pharmaceutical, and biofuel industries (Gibon, 2020; Choo *et al.*, 2022). Extracted from the mesocarp of the oil palm (*Elaeis guineensis*), crude palm oil (CPO) is characterized

by its high content of bioactive compounds such as carotenoids, tocopherols, tocotrienols, and unsaturated fatty acids, which contribute to its nutritional and antioxidant properties (Osei-Amponsah *et al.*, 2020; Akinola *et al.*, 2018). However, unrefined CPO also contains a variety of undesirable components including free fatty acids (FFAs), phospholipids, moisture, trace metals, odorous volatiles, peroxides, and colour pigments such as β -carotene and chlorophyll, which collectively reduce oil stability, flavour, and shelf life (Musa *et al.*, 2019; Ibrahim *et al.*, 2021). These impurities promote oxidative rancidity and hydrolytic degradation, leading to poor sensory quality and limiting the oil's compliance with international edible oil standards (Lee *et al.*, 2020; Okolie *et al.*, 2019).

The conventional refining process of palm oil typically consists of four major stages which include degumming, neutralisation, bleaching, and deodorisation all aimed at removing impurities and improving oil quality (Rahman *et al.*, 2019; Ajala *et al.*, 2022). Among these, bleaching plays a critical role by removing pigments, oxidation products, trace metals, and other contaminants through the use of adsorbents such as bentonite or acid-activated clay (Cheng *et al.*, 2019; Ogunwuyi *et al.*, 2021). This step enhances colour, stability, and flavour, yielding oil suitable for consumption and industrial use (Sulaiman *et al.*, 2021; Idowu *et al.*, 2022). The final step, deodorisation, is usually conducted under vacuum at high temperatures between 180°C and 260°C to remove volatile compounds including FFAs and odorous molecules (Gibon, 2020; Nwabanne *et al.*, 2017). However, such high-temperature processing often causes nutrient degradation, resulting in the breakdown of triglycerides into mono- and diglycerides, as well as the destruction of thermolabile compounds such as carotenoids and riboflavin (Awad *et al.*, 2017; Asaolu *et al.*, 2020).

The intermediate product of refining, bleached palm oil (BPO), is characterised by improved oxidative stability, lower peroxide and FFA levels, and a lighter colour, making it valuable in both edible and non-edible applications (Ugbogu *et al.*, 2021; Olalere *et al.*, 2022). In the

food industry, BPO is used as a frying medium, shortening, and ingredient in margarine, baked goods, and instant noodles due to its superior heat and oxidation resistance (Adebayo *et al.*, 2016; Osei-Amponsah *et al.*, 2020). Beyond food applications, it serves as a feedstock in soap manufacturing, oleochemical synthesis, cosmetic formulation, and biodiesel production (Olalere *et al.*, 2022; Gibon, 2020). The enhanced shelf life, clarity, and oxidative stability of BPO make it particularly suitable for export and long-term storage (Ajala *et al.*, 2022; Akinola *et al.*, 2018).

Despite these advantages, conventional bleaching and deodorisation methods are energy-intensive and prone to undesirable chemical transformations. For instance, riboflavin (vitamin B₂), though present in small quantities, is a yellow chromophore that is highly photosensitive and thermolabile, degrading at elevated temperatures into lumichrome and other oxidation products that impart off-flavours and odours to the oil (Awad *et al.*, 2017; Ibrahim *et al.*, 2021). Traditional bleaching does not completely eliminate riboflavin or its degradation derivatives, leaving residual compounds that can persist even after deodorisation (Lee *et al.*, 2020; Cheng *et al.*, 2019). Furthermore, the high thermal load during deodorisation not only consumes significant energy but also accelerates the hydrolysis of triglycerides, thereby reducing yield and deteriorating oil quality (Asaolu *et al.*, 2020; Rahman *et al.*, 2019).

Triglycerides are fundamental to the nutritional and physicochemical properties of palm oil, contributing to texture, energy content, and oxidative stability (Okafor *et al.*, 2021; Umanu *et al.*, 2021). However, excessive heat exposure during conventional refining promotes hydrolysis of triglycerides into diglycerides and FFAs, which compromise oil purity and stability (Cheng *et al.*, 2019; Sunday *et al.*, 2018). This not only diminishes yield but also increases refining costs, as additional neutralisation and deodorisation are required to remove FFAs and volatile degradation products (Sulaiman *et al.*, 2021; Osei-Amponsah *et al.*, 2020).

Given these challenges, researchers have turned their attention toward acid fermentation facilitated bleaching which is a promising approach that integrates dilute acid treatment with catalytic or biological agents to improve bleaching efficiency (Eze *et al.*, 2020; Ogunwuyi *et al.*, 2021). In this method, hydrochloric acid (HCl) acts as a hydrolysing and catalytic agent, promoting the cleavage of ester bonds in triglycerides and reducing pigment concentrations through acidolysis reactions (Adebayo *et al.*, 2016; Ali *et al.*, 2021). The presence of acid not only facilitates the breakdown of resistant pigments like β -carotene and riboflavin but also enhances the removal of phospholipids and peroxides, thereby improving oil clarity and stability (Ajala *et al.*, 2022; Ogunsina *et al.*, 2020).

When applied under controlled conditions, HCl-assisted bleaching may serve as a pre-treatment step, reducing the load on subsequent deodorisation and preserving heat-sensitive nutrients such as carotenoids, tocopherols, and vitamins (Gibon, 2020; Okolie *et al.*, 2023). Moreover, studies have shown that catalytic acid bleaching significantly reduces FFA levels and peroxides while maintaining the structural integrity of triglycerides, leading to improved refining efficiency (Akinola *et al.*, 2018; Okafor *et al.*, 2021). The incorporation of catalytic adsorbents such as plantain peel ash or rice husk ash, derived from agricultural residues, further enhances pigment adsorption and promotes environmentally sustainable processing (Ajala *et al.*, 2022; Enebe *et al.*, 2023).

The combined application of acid and fermentation processes can therefore achieve effective pigment and impurity removal while minimising energy consumption and nutrient loss (Eze *et al.*, 2020; Umanu *et al.*, 2021). This hybrid bleaching approach aligns with modern goals of sustainability, cost reduction, and nutrient preservation, providing a viable alternative for both small- and large-scale palm oil refineries (Ogunsina *et al.*, 2020; Idowu *et al.*, 2022).

1.2 Aim and Objectives

Aim

This study aims to evaluate the effect of acid fermentation using hydrochloric acid (HCl) on the bleaching efficiency and quality characteristics of crude palm oil.

Objectives

The objectives of this study are to:

- I. Determine how varying concentrations of HCl (0.025, 0.050, 0.075 and 0.100 M) influence the physicochemical properties of crude palm oil during bleaching.
- II. Assess the changes in key quality parameters such as moisture content, iodine value, saponification value, peroxide value, free fatty acid content, refractive index, and specific gravity after bleaching.
- III. To analyze the statistical significance of observed differences using ANOVA and Tukey post-hoc tests to validate the impact of HCl concentration.
- IV. To identify the optimum acid concentration that achieves maximum bleaching efficiency while maintaining acceptable oil stability and nutritional integrity.
- V. To establish the potential of acid fermentation as a sustainable and cost-effective alternative to conventional bleaching methods in palm oil processing.

CHAPTER TWO

LITERATURE REVIEW

2.1 Crude Palm Oil (CPO)

Crude palm oil (CPO) is an unrefined vegetable oil extracted from the mesocarp of the oil palm fruit (*Elaeis guineensis*), one of the most economically significant oil-bearing crops cultivated across tropical regions (Hassan *et al.*, 2021; Osei-Amponsah *et al.*, 2020). Globally, palm oil stands as the most consumed edible oil, serving as a fundamental source of dietary lipids and a critical raw material in the food, cosmetic, pharmaceutical, and bioenergy sectors (Gibon, 2020; Akinola *et al.*, 2018). The oil's versatility stems from its balanced composition of saturated and unsaturated fatty acids, making it suitable for both industrial applications and direct consumption (Eze *et al.*, 2020; Ogunsina *et al.*, 2020).

CPO is distinguished by its rich reddish-orange hue, a result of its high carotenoid content primarily β -carotene and lycopene which serve as natural precursors to vitamin A and contribute to the oil's antioxidant potential (Ajala *et al.*, 2022; Okolie *et al.*, 2023). It also contains significant amounts of tocopherols and tocotrienols, which are biologically active forms of vitamin E that play essential roles in protecting lipids from oxidative degradation (Ibrahim *et al.*, 2021; Umanu *et al.*, 2021). Additionally, minor constituents such as sterols, phospholipids, phenolic compounds, and trace metals further contribute to the oil's nutritional and functional quality, influencing its oxidative stability and shelf life (Sulaiman *et al.*, 2021; Ali *et al.*, 2021).

Despite its nutritional advantages, crude palm oil contains several impurities that reduce its suitability for human consumption and industrial processing. These undesirable components include free fatty acids (FFAs), moisture, phospholipids, peroxides, odorous volatiles, and

colour pigments such as chlorophyll and β -carotene, which contribute to rancidity, off-flavours, and dark coloration (Rahman *et al.*, 2019; Nwabanne *et al.*, 2017). Elevated FFA levels are particularly problematic because they result from hydrolytic degradation of triglycerides, leading to reduced oxidative stability and poor sensory quality (Okafor *et al.*, 2021; Cheng *et al.*, 2019). Similarly, high pigment concentrations hinder efficient bleaching during refining and cause unacceptable colouration in finished oils (Ogunwuyi *et al.*, 2021; Adebayo *et al.*, 2016). The presence of phospholipids and metallic impurities also accelerates oxidation reactions, necessitating multiple purification steps (Musa *et al.*, 2019; Lee *et al.*, 2020).

Global production of crude palm oil is predominantly concentrated in Southeast Asia, with Indonesia and Malaysia accounting for approximately 85–90% of the world's output, while African nations such as Nigeria, Ghana, and Cameroon contribute significantly to regional supply chains (Choo *et al.*, 2022; Idowu *et al.*, 2022). Nigeria, in particular, remains one of the largest producers in Africa, with CPO serving as a key agricultural export and a source of livelihood for millions (Ogunwuyi *et al.*, 2021; Gibon, 2020). The high productivity of oil palm per hectare compared to other oilseeds, coupled with the semi-solid state of palm oil at room temperature, enhances its suitability for a broad range of food applications including margarine, shortening, confectioneries, and frying fats (Akinola *et al.*, 2018; Ajala *et al.*, 2022). Beyond the food sector, CPO and its derivatives are increasingly used in oleochemical industries, cosmetics, soap manufacturing, and biodiesel production, reflecting their wide industrial adaptability (Olalere *et al.*, 2022; Osei-Amponsah *et al.*, 2020).

However, in its crude state, palm oil is not directly suitable for most industrial or culinary applications due to its high impurity load, dark colour, and strong odour (Awad *et al.*, 2017; Ibrahim *et al.*, 2021). Refining processes are therefore necessary to improve physicochemical quality, enhance oxidative stability, and produce acceptable edible oil (Rahman *et al.*, 2019;

Asaolu *et al.*, 2020). Conventional refining involves a series of stages such as degumming, neutralisation, bleaching, and deodorisation each aimed at removing specific impurities to meet industry standards (Gibon, 2020; Sulaiman *et al.*, 2021). The bleaching stage, in particular, plays a pivotal role in decolorisation and adsorption of oxidation products using activated clays, bentonite, or acid-treated earths (Nwabanne *et al.*, 2017; Cheng *et al.*, 2019).

Nevertheless, traditional bleaching and deodorisation have limitations. The high temperatures (180–260°C) used during deodorisation can degrade thermolabile nutrients such as carotenoids, tocopherols, and riboflavin, leading to the formation of unwanted compounds like 3-monochloropropane-1,2-diol (3-MCPD) esters (Lee *et al.*, 2020; Asaolu *et al.*, 2020). These by-products pose potential health risks and lower nutritional value (Okolie *et al.*, 2023; Ali *et al.*, 2021). Furthermore, repeated high-heat treatment contributes to the breakdown of triglycerides into FFAs and diglycerides, increasing refining losses and operational costs (Cheng *et al.*, 2019; Sunday *et al.*, 2018).

To overcome these challenges, recent studies have explored acid-assisted and fermentation-facilitated bleaching as innovative refining methods that minimize heat exposure while improving pigment and impurity removal (Ajala *et al.*, 2022; Eze *et al.*, 2020). The use of hydrochloric acid (HCl) as a catalytic bleaching agent has shown potential for enhancing decolorisation efficiency, hydrolysing resistant pigments such as riboflavin, and improving overall oil quality (Adebayo *et al.*, 2016; Ogunsina *et al.*, 2020). Acid-assisted bleaching operates under milder thermal conditions compared to conventional refining, thereby preserving heat-sensitive nutrients while promoting effective impurity removal (Gibon, 2020; Okafor *et al.*, 2021).

Moreover, integrating acid fermentation with bleaching introduces microbial and biochemical mechanisms that facilitate natural hydrolysis and pigment degradation, producing lighter,

more stable oil with lower peroxide and FFA values (Umanu *et al.*, 2021; Idowu *et al.*, 2022). This eco-friendly approach aligns with the global emphasis on sustainable processing technologies that reduce chemical usage, energy consumption, and environmental pollution (Enebe *et al.*, 2023; Zhou *et al.*, 2023). Consequently, acid fermentation facilitated bleaching has emerged as a promising alternative for small- and medium-scale processors seeking to enhance oil quality while reducing refining costs and environmental impact (Akinola *et al.*, 2018; Osei-Amponsah *et al.*, 2020).



Plate 1: Fresh Ripe Palm Fruit

(Courtesy: Osadolor, 2025)

2.2 History and distribution of CPO

The history of crude palm oil (CPO) production dates back several millennia, with origins in West Africa where the oil palm tree (*Elaeis guineensis*) was first domesticated for its high-yield fruit and versatile oil (Gibon, 2020; Hassan *et al.*, 2021). Archaeological evidence and historical documentation indicate that palm oil was extensively used in ancient West African civilizations, including the Kingdom of Benin and the Yoruba Empire, for both culinary and medicinal purposes (Ajala *et al.*, 2022; Osei-Amponsah *et al.*, 2020). The oil served as a dietary staple and an important trade commodity across pre-colonial trade routes linking the coastal and inland regions of West Africa (Eze *et al.*, 2020; Akinola *et al.*, 2018).

By the fifteenth century, European explorers and merchants, particularly the Portuguese, had encountered palm oil along the West African coast and referred to it as “dendê oil,” subsequently initiating its export to Europe (Musa *et al.*, 2019; Lee *et al.*, 2020). Initially, CPO was used in Europe for soap manufacturing and as a source of fuel for oil lamps due to its high calorific value and oxidative stability (Rahman *et al.*, 2019; Ogunwuyi *et al.*, 2021). The transatlantic trade period further accelerated palm oil’s commercial relevance, with European industries recognizing its superior chemical properties for lubrication and candle production during the early stages of industrialization (Awad *et al.*, 2017; Ali *et al.*, 2021).

The eighteenth and nineteenth centuries marked a transformative phase in the global expansion of palm oil cultivation. Under British and Dutch colonial influence, the oil palm was introduced from West Africa to Southeast Asia, particularly Malaysia and Indonesia, where favorable climatic conditions supported large-scale plantation development (Choo *et al.*, 2022; Cheng *et al.*, 2019). Colonial planters and agronomists recognized the oil palm’s unparalleled yield potential producing up to ten times more oil per hectare than other oilseed crops such as soybean or sunflower (Ajala *et al.*, 2022; Osei-Amponsah *et al.*, 2020). This realization led to the conversion of vast rainforest areas into monoculture plantations,

supported by the establishment of organized milling and refining infrastructure (Enebe *et al.*, 2023; Idowu *et al.*, 2022). By the mid-twentieth century, Malaysia had emerged as the leading global producer of crude palm oil, closely followed by Indonesia, owing to government-backed agricultural research, improved hybrid cultivars, and mechanized processing (Umanu *et al.*, 2021; Gibon, 2020).

Today, Indonesia and Malaysia collectively account for over 85–90% of global CPO production, dominating international trade through large-scale plantation systems and integrated refinery operations (Rahman *et al.*, 2019; Okolie *et al.*, 2023). Their success is attributed to favorable tropical rainfall patterns, government incentives, and investment in research focused on yield improvement and disease management (Akinola *et al.*, 2018; Sulaiman *et al.*, 2021). The Southeast Asian model of intensive palm oil cultivation has become the benchmark for global production, although it has also attracted criticism for its ecological implications, including deforestation, biodiversity loss, and carbon emissions (Zhou *et al.*, 2023; Cheng *et al.*, 2019).

In contrast, African palm oil production has followed a different developmental trajectory. Despite the oil palm's African origin, post-independence economic diversification led many countries, including Nigeria and Ghana, to prioritize cash crops such as cocoa and groundnuts over oil palm expansion (Ibrahim *et al.*, 2021; Ogunsina *et al.*, 2020). Consequently, while Nigeria remains Africa's largest producer of CPO, its yield per hectare remains below global averages due to fragmented smallholder production systems, inadequate processing technologies, and poor access to capital (Ali *et al.*, 2021; Okafor *et al.*, 2021). Recent policy reforms and private investments have, however, spurred renewed interest in revitalizing the sector, particularly through the introduction of improved hybrid palm varieties and community-based processing cooperatives (Ajala *et al.*, 2022; Osei-Amponsah *et al.*, 2020). Similarly, Ghana and Côte d'Ivoire have made significant strides toward increasing

productivity through sustainable smallholder engagement and partnerships with international palm oil companies (Eze *et al.*, 2020; Umanu *et al.*, 2021).

Latin America has also emerged as a notable contributor to global CPO production and distribution. Countries such as Colombia, Ecuador, and Brazil have adopted oil palm cultivation to diversify agricultural economies and reduce reliance on traditional crops (Rahman *et al.*, 2019; Choo *et al.*, 2022). Colombia is currently the largest producer in the Americas, with a growing export market serving both food and biofuel sectors (Adebayo *et al.*, 2016; Gibon, 2020). Brazilian initiatives have sought to integrate oil palm cultivation within mixed cropping systems to promote sustainable land use and mitigate deforestation pressures from soybean expansion (Zhou *et al.*, 2023; Enebe *et al.*, 2023). These efforts reflect a broader commitment to balancing economic growth with environmental preservation through sustainable palm oil certification programs (Sulaiman *et al.*, 2021; Ali *et al.*, 2021).

The global distribution of CPO is supported by an extensive maritime and land-based logistics network linking major producing regions to refining and consumption hubs (Ogunwuyi *et al.*, 2021; Musa *et al.*, 2019). Key export terminals in Malaysia, Indonesia, and Nigeria facilitate the movement of millions of tonnes annually through ports such as Port Klang, Dumai, and Apapa (Lee *et al.*, 2020; Ibrahim *et al.*, 2021). These are connected to refineries in Europe, Asia, and Africa via long-established shipping routes that underpin the global edible oil trade (Cheng *et al.*, 2019; Idowu *et al.*, 2022). In producing regions, domestic distribution is often constrained by inadequate transportation infrastructure, particularly in West and Central Africa, leading to post-harvest losses and reduced product quality (Okafor *et al.*, 2021; Awad *et al.*, 2017).

CPO consumption and trade patterns differ significantly between regions. In West Africa, palm oil remains a staple cooking ingredient and a vital component of traditional diets, traded

extensively in open markets and regional depots (Osei-Amponsah *et al.*, 2020; Ibrahim *et al.*, 2021). In Southeast Asia, its ubiquity spans edible and non-edible sectors, with products ranging from instant noodles to detergents and biodiesel (Olalere *et al.*, 2022; Sulaiman *et al.*, 2021). The expansion of urban populations and increasing disposable incomes in developing economies have further stimulated demand for palm oil-based processed foods and packaged goods (Okolie *et al.*, 2023; Ajala *et al.*, 2022). In industrialized markets such as the European Union, India, and China, CPO is valued for both edible applications and biodiesel production, highlighting its economic versatility (Rahman *et al.*, 2019; Gibon, 2020).

Recent years have seen rising consumer concern regarding sustainability, deforestation, and ethical sourcing in palm oil supply chains, prompting the establishment of the Roundtable on Sustainable Palm Oil (RSPO) certification scheme (Lee *et al.*, 2020; Zhou *et al.*, 2023). This global initiative encourages sustainable production practices, traceability, and fair labor conditions, influencing trade dynamics by rewarding compliant producers with market premiums (Sulaiman *et al.*, 2021; Enebe *et al.*, 2023). Consequently, several countries and corporations have pledged to source only certified sustainable palm oil (CSPO), reshaping global distribution patterns (Idowu *et al.*, 2022; Umanu *et al.*, 2021).

Looking forward, technological innovation and sustainability imperatives are set to redefine the global palm oil landscape. The adoption of precision agriculture, satellite-based yield forecasting, and digital traceability systems such as blockchain promise to improve efficiency, reduce deforestation, and enhance supply chain transparency (Choo *et al.*, 2022; Okolie *et al.*, 2023). Simultaneously, ongoing breeding programs aim to develop high-yielding, disease-resistant oil palm varieties that will boost productivity while minimizing land use (Ajala *et al.*, 2022; Gibon, 2020). The integration of smallholder farmers into value chains through cooperative models and fair trade initiatives is expected to distribute economic benefits more equitably (Ibrahim *et al.*, 2021; Akinola *et al.*, 2018). Overall, as global demand for plant-

based oils continues to grow, crude palm oil remains central to food security, industrial innovation, and renewable energy development, with its distribution networks adapting to emerging sustainability and market dynamics (Zhou *et al.*, 2023; Ali *et al.*, 2021).

2. 3 Composition of Crude Palm Oil and Key Impurities

Crude palm oil (CPO) is primarily composed of triglycerides, which constitute more than 90% of its total lipid fraction and serve as the fundamental storage form of fatty acids in the oil (Asaolu *et al.*, 2020; Ajala *et al.*, 2022). These triglycerides are esters derived from glycerol and a combination of fatty acids, mainly palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2), and smaller quantities of stearic (C18:0) and myristic (C14:0) acids (Ogunwuyi *et al.*, 2021; Adebayo *et al.*, 2016). The fatty acid composition of CPO is unique, as it contains nearly equal proportions of saturated and unsaturated fatty acids, which confer a semi-solid consistency at ambient temperatures (Ibrahim *et al.*, 2021; Zhou *et al.*, 2023). This balance of fatty acids not only determines the oil's melting profile but also influences its oxidative stability and nutritional properties (Ali *et al.*, 2021; Sulaiman *et al.*, 2021).

In addition to triglycerides, CPO contains several minor but biologically significant constituents that contribute to its nutritional and functional attributes (Rahman *et al.*, 2019; Gibon, 2020). Among these, carotenoids notably β -carotene, α -carotene, and lycopene are responsible for the characteristic reddish-orange hue of palm oil and act as precursors to vitamin A, thereby supporting visual health and immune function (Lee *et al.*, 2020; Awad *et al.*, 2017). These carotenoids also possess potent antioxidant activity, protecting the oil matrix against autoxidation and thermal degradation during storage and processing (Ugbogu *et al.*, 2021; Okolie *et al.*, 2023).

Tocopherols and tocotrienols, the two primary forms of vitamin E present in palm oil, represent another critical group of minor components with strong antioxidant capabilities

(Ajala *et al.*, 2022; Osei-Amponsah *et al.*, 2020). Tocotrienols, in particular, have been shown to possess neuroprotective, cholesterol-lowering, and anticancer properties, making CPO a valuable dietary source of vitamin E (Ali *et al.*, 2021; Cheng *et al.*, 2019). These compounds enhance oxidative stability by scavenging free radicals, thus extending the oil's shelf life and improving its thermal performance during deep-frying and industrial applications (Rahman *et al.*, 2019; Akinola *et al.*, 2018).

Other bioactive constituents such as sterols, squalene, and phenolic compounds are also present in smaller quantities and contribute to the overall health-promoting qualities of palm oil (Sulaiman *et al.*, 2021; Enebe *et al.*, 2023). Sterols play a role in maintaining membrane integrity and have cholesterol-lowering effects when consumed in the diet (Ogunwuyi *et al.*, 2021; Ibrahim *et al.*, 2021). Squalene acts as a precursor in sterol biosynthesis and functions as a natural antioxidant within the oil matrix (Awad *et al.*, 2017; Choo *et al.*, 2022). Phenolic acids and flavonoids present in trace amounts further enhance the antioxidant capacity of palm oil, contributing to its relative stability compared to other vegetable oils (Ajala *et al.*, 2022; Okafor *et al.*, 2021).

Riboflavin (vitamin B₂), although present in trace concentrations, has noteworthy implications for oil stability. It functions as a light-sensitive pigment capable of undergoing photolytic degradation to produce lumichrome and other oxidative by-products that impart undesirable flavours and colour changes in stored or heated oil (Aluko *et al.*, 2018; Ali *et al.*, 2021). These degradation reactions are particularly problematic during refining and deodorization processes conducted at elevated temperatures, making riboflavin a significant quality determinant in refining studies (Zhou *et al.*, 2023; Ibrahim *et al.*, 2021).

Despite its nutritional and functional richness, crude palm oil contains several undesirable impurities that must be removed to ensure its quality, stability, and commercial acceptability

(Gibon, 2020; Adebayo *et al.*, 2016). Among these, free fatty acids (FFAs) arise primarily from enzymatic hydrolysis of triglycerides by lipase enzymes during fruit storage and processing (Ogunwuyi *et al.*, 2021; Cheng *et al.*, 2019). Elevated FFA levels are undesirable because they accelerate rancidity, produce off-odours, and reduce storage stability (Ajala *et al.*, 2022; Sulaiman *et al.*, 2021). The hydrolytic degradation of triglycerides also lowers oil yield during refining and increases neutralization losses (Rahman *et al.*, 2019; Awad *et al.*, 2017).

Moisture content in crude palm oil promotes hydrolysis and microbial activity, leading to deterioration of triglyceride structures and FFA accumulation (Eze *et al.*, 2020; Akinola *et al.*, 2018). Similarly, phospholipids and gums contribute to emulsion formation, cloudiness, and poor filtration efficiency, which adversely affect the refining and bleaching stages (Osei-Amponsah *et al.*, 2020; Ali *et al.*, 2021). These non-glyceride impurities must be removed through degumming to prevent operational challenges in refining equipment (Okolie *et al.*, 2023; Zhou *et al.*, 2023).

Pigments such as chlorophyll and β -carotene, while providing characteristic colouration, can also promote oxidative deterioration by acting as photosensitizers under light exposure (Rahman *et al.*, 2019; Gibon, 2020). Peroxides and hydroperoxides, which are primary oxidation products, contribute to the formation of secondary oxidation compounds such as aldehydes and ketones that impart unpleasant flavours and odours to the oil (Sulaiman *et al.*, 2021; Awad *et al.*, 2017). The presence of trace metals like iron and copper further catalyzes oxidation reactions, exacerbating deterioration and reducing shelf stability (Ali *et al.*, 2021; Ibrahim *et al.*, 2021).

Volatile odorous compounds, including short-chain aldehydes and ketones, originate from lipid oxidation and microbial degradation, contributing to the characteristic smell of

unrefined palm oil (Ajala *et al.*, 2022; Osei-Amponsah *et al.*, 2020). Riboflavin degradation under heat and light exposure can further produce volatile photoproducts that alter the sensory profile of the oil, necessitating its reduction during refining (Aluko *et al.*, 2018; Okolie *et al.*, 2023).

Overall, the removal of these impurities including FFAs, pigments, peroxides, trace metals, and volatile compounds is essential for producing high-quality bleached and refined palm oil suitable for edible and industrial applications (Gibon, 2020; Rahman *et al.*, 2019). Effective refining enhances the oxidative stability, sensory attributes, and market value of palm oil, aligning with international quality standards and consumer preferences (Zhou *et al.*, 2023; Sulaiman *et al.*, 2021).

2.4 Uses of Bleached Palm Oil (BPO)

Bleached Palm Oil (BPO) plays an integral role in both food and non-food industries, emerging as a versatile commodity with widespread applications globally. The bleaching process significantly improves the oil's colour, taste, odour, and stability, thereby expanding its utility across multiple sectors. While traditionally viewed mainly as a cooking oil, recent innovations have broadened its applications, particularly in the areas of food processing, pharmaceuticals, cosmetics, and biodiesel production. This section provides a comprehensive analysis of the various uses of BPO, backed by contemporary research findings and global market trends.

2.4.1 Applications in Food Industry

The predominant use of bleached palm oil (BPO) globally lies in its role as a cooking and frying medium, where it has become indispensable due to its enhanced oxidative stability, appealing colour, and neutral odour compared to crude palm oil (Gibon, 2020; Ajala *et al.*,

2022). The removal of impurities such as pigments, peroxides, and free fatty acids during bleaching contributes to its superior heat resistance and flavour retention during frying (Ogunwuyi *et al.*, 2021; Ibrahim *et al.*, 2021). Owing to its high smoke point and minimal polymer formation at elevated temperatures, BPO is particularly suited for deep frying in industrial and domestic food preparation (Rahman *et al.*, 2019; Okolie *et al.*, 2023). These qualities make it a preferred frying oil in fast-food outlets, restaurants, and snack production industries across Asia, Africa, and Latin America, where stable frying oils are essential for maintaining food quality and cost efficiency (Lee *et al.*, 2020; Sulaiman *et al.*, 2021).

In addition to its culinary versatility, BPO serves as a key base material in the production of margarine and shortening, two staple fat products in both household and industrial food applications (Ajala *et al.*, 2022; Awad *et al.*, 2017). Its semi-solid consistency at room temperature, coupled with its ability to crystallise uniformly under controlled conditions, makes it ideal for emulsified fat formulations (Gibon, 2020; Adebayo *et al.*, 2016). Furthermore, margarine and shortenings produced from BPO exhibit low trans-fatty acid content, aligning with global health initiatives and food regulations that restrict trans fats due to their link with cardiovascular diseases (Musa *et al.*, 2019; Ali *et al.*, 2021). This property enhances BPO's acceptability among health-conscious consumers and within international markets emphasizing nutritionally safer fat sources (Zhou *et al.*, 2023; Cheng *et al.*, 2019).

Within the bakery sector, BPO is valued for its ability to improve texture, volume, and shelf life of baked goods such as bread, pastries, cakes, and biscuits (Awad *et al.*, 2017; Rahman *et al.*, 2019). Its uniform melting profile and oxidative stability ensure consistent dough structure and crumb softness, while preventing rancidity during prolonged storage (Okolie *et al.*, 2023; Ogunwuyi *et al.*, 2021). The neutral taste and light colour of BPO also make it compatible with delicate bakery formulations, preventing undesirable flavour interference in sugar-rich or spiced products (Sulaiman *et al.*, 2021; Ibrahim *et al.*, 2021). Studies have

shown that replacing hydrogenated fats with BPO in bakery applications results in reduced trans-fat levels without compromising textural or sensory quality (Ajala *et al.*, 2022; Akinola *et al.*, 2018).

BPO is also extensively employed in the manufacture of processed and convenience foods, including instant noodles, potato chips, and pre-fried snack products (Ogunwuyi *et al.*, 2021; Okafor *et al.*, 2021). The oil's long oxidative stability and low reactivity to polymerisation enable it to maintain desirable texture, crispness, and flavour during long-term storage and repeated frying cycles (Rahman *et al.*, 2019; Ali *et al.*, 2021). Its thermal endurance also prevents the formation of harmful oxidative by-products that are common in less stable vegetable oils (Lee *et al.*, 2020; Zhou *et al.*, 2023). Consequently, BPO-based formulations are preferred in the snack industry, where preservation of product quality and shelf stability are key to market competitiveness (Ajala *et al.*, 2022; Awad *et al.*, 2017).

In recent years, BPO has gained attention as a fat source in dairy analogues such as coffee whiteners, whipped toppings, and non-dairy creamers, where it serves as a cost-effective and functionally compatible alternative to milk fat (Olalere *et al.*, 2022; Rahman *et al.*, 2019). Its semi-solid nature at ambient temperature provides structural stability, while its mild flavour blends harmoniously with dairy ingredients without imparting off-tastes (Ibrahim *et al.*, 2021; Ogunwuyi *et al.*, 2021). Moreover, its oxidative stability and uniform fat crystal structure contribute to improved emulsion properties and mouthfeel in liquid and powdered creamer formulations (Sulaiman *et al.*, 2021; Okolie *et al.*, 2023). The replacement of dairy fat with bleached palm oil not only reduces production costs but also extends product shelf life and ensures stability during transportation and storage under varying climatic conditions (Ajala *et al.*, 2022; Zhou *et al.*, 2023).

Overall, the culinary and food applications of bleached palm oil highlight its versatility as a stable, safe, and economically viable ingredient for a broad spectrum of food products (Gibon, 2020; Rahman *et al.*, 2019). The technological and nutritional advantages of BPO such as its high oxidative resistance, desirable melting characteristics, and low trans-fat content underscore its growing importance in both developed and emerging markets (Ali *et al.*, 2021; Ajala *et al.*, 2022). Its expanding use across food industries reflects a global shift toward more sustainable and health-conscious fat sources, aligning with evolving dietary and regulatory demands (Zhou *et al.*, 2023; Sulaiman *et al.*, 2021).

2.4.2 Applications in Non-Food Industry

The cosmetic industry extensively utilises bleached palm oil (BPO) owing to its excellent emollient properties, oxidative stability, and compatibility with a wide range of natural and synthetic ingredients (Aluko *et al.*, 2018; Ibrahim *et al.*, 2021). Its balanced fatty acid composition, dominated by palmitic and oleic acids, provides smoothness, lubrication, and moisture retention to skin-care formulations (Rahman *et al.*, 2019; Ajala *et al.*, 2022). Consequently, BPO is a common base oil in the production of soaps, body lotions, creams, lip balms, and hair conditioners, where it functions as both a conditioning agent and viscosity enhancer (Ogunwuyi *et al.*, 2021; Okolie *et al.*, 2023). The presence of natural antioxidants such as tocopherols and tocotrienols enhances product stability and offers protection against oxidative rancidity, extending the shelf life of cosmetic formulations (Lee *et al.*, 2020; Zhou *et al.*, 2023). These characteristics have increased the demand for BPO in the formulation of natural and organic beauty products, especially in markets that prioritise plant-based and sustainable ingredients (Sulaiman *et al.*, 2021; Cheng *et al.*, 2019).

In the pharmaceutical industry, BPO functions as a versatile excipient and lipid carrier, facilitating the delivery of fat-soluble vitamins, hormones, and other bioactive compounds

(Asaolu *et al.*, 2020; Awad *et al.*, 2017). It is widely incorporated in soft gelatin capsules, emulsions, ointments, and topical formulations due to its biocompatibility and ability to enhance the bioavailability of lipophilic drugs (Osei-Amponsah *et al.*, 2020; Gibon, 2020). The high oxidative stability of BPO ensures the preservation of active ingredients such as vitamins A, D, and E, which are highly sensitive to degradation during storage (Ali *et al.*, 2021; Rahman *et al.*, 2019). Its mild nature and low irritancy also make it suitable for dermatological applications, including medicated creams and therapeutic ointments used in the treatment of dry skin and inflammatory conditions (Ajala *et al.*, 2022; Ibrahim *et al.*, 2021). The pharmaceutical relevance of BPO continues to expand as research explores its role as a carrier oil for nanoemulsions and liposomal drug delivery systems (Zhou *et al.*, 2023; Okafor *et al.*, 2021).

Bleached palm oil has also gained considerable attention as a renewable feedstock for biodiesel production, particularly in regions striving to transition toward sustainable energy sources (Ajala *et al.*, 2022; Lee *et al.*, 2020). The chemical composition of BPO—rich in long-chain fatty acids—makes it highly suitable for esterification and transesterification reactions, producing fatty acid methyl esters (FAME) that conform to biodiesel standards such as ASTM D6751 and EN 14214 (Ogunwuyi *et al.*, 2021; Sulaiman *et al.*, 2021). Compared to crude palm oil, the lower impurity and moisture content of BPO enhances reaction efficiency and reduces catalyst consumption during biodiesel synthesis (Rahman *et al.*, 2019; Zhou *et al.*, 2023). Studies in Malaysia, Indonesia, and Nigeria have demonstrated that BPO-based biodiesel exhibits excellent combustion properties, high cetane number, and reduced particulate emissions relative to petroleum diesel (Awad *et al.*, 2017; Ali *et al.*, 2021). The integration of BPO into national biodiesel programmes supports global efforts to achieve net-zero carbon goals and energy diversification (Cheng *et al.*, 2019; Ajala *et al.*, 2022).

Beyond energy applications, BPO's semi-solid nature and high melting consistency make it a valuable raw material for the manufacture of candles, waxes, and polishing products (Musa *et al.*, 2019; Adebayo *et al.*, 2016). When blended with paraffin or stearic acid, BPO acts as a binder and hardening agent, improving the structural integrity and burn characteristics of candles (Rahman *et al.*, 2019; Ibrahim *et al.*, 2021). Its smooth burning properties, mild fragrance, and biodegradability have positioned it as a sustainable alternative to petroleum-based waxes in eco-friendly candle production (Okolie *et al.*, 2023; Zhou *et al.*, 2023). In addition, palm-based waxes derived from bleached oil are increasingly being used in coatings, polishes, and packaging materials for their film-forming and protective properties (Ogunwuyi *et al.*, 2021; Ali *et al.*, 2021).

Recent advances in nutraceutical and functional food development have highlighted the potential of partially refined or bleached palm oil as a bioactive ingredient (Awad *et al.*, 2017; Ajala *et al.*, 2022). Research suggests that moderate bleaching preserves important minor compounds such as tocopherols, tocotrienols, and carotenoids, which exhibit strong antioxidant, anti-inflammatory, and cholesterol-lowering effects (Rahman *et al.*, 2019; Okafor *et al.*, 2021). These bioactive lipids are increasingly incorporated into fortified foods, dietary supplements, and functional beverages aimed at promoting cardiovascular and metabolic health (Sulaiman *et al.*, 2021; Zhou *et al.*, 2023). Moreover, the high thermal stability of bleached palm oil allows its use in the encapsulation of heat-sensitive nutrients during food processing (Ali *et al.*, 2021; Ibrahim *et al.*, 2021).

Collectively, the non-food and industrial uses of BPO demonstrate its broad economic and technological importance. From pharmaceuticals and cosmetics to renewable energy and nutraceuticals, BPO continues to serve as a sustainable, multifunctional resource with applications across multiple sectors (Ajala *et al.*, 2022; Gibon, 2020). Its versatility,

environmental compatibility, and favourable physicochemical properties underscore its growing relevance in global bio-based industries (Zhou *et al.*, 2023; Rahman *et al.*, 2019).

2.5 Health Benefits of Bleached Palm Oil (BPO)

Bleached palm oil (BPO), traditionally regarded as merely a culinary oil or industrial feedstock, has gained renewed attention in recent years due to growing evidence of its nutritional and therapeutic potential (Gibon, 2020; Ajala *et al.*, 2022). Although the refining and bleaching processes remove some bioactive compounds inherent in crude palm oil (CPO), optimized bleaching conditions particularly acid-assisted and low-temperature methods have been shown to retain substantial levels of antioxidants, essential fatty acids, and fat-soluble vitamins (Ogunwuyi *et al.*, 2021; Ibrahim *et al.*, 2021).

BPO remains a rich source of essential fatty acids, including palmitic, oleic, and linoleic acids, which are vital for maintaining cell membrane integrity, hormone synthesis, and energy metabolism (Musa *et al.*, 2019; Rahman *et al.*, 2019). While palmitic acid, a saturated fatty acid, has been linked to elevated serum lipids in excessive amounts, moderate intake supports energy storage and structural lipid formation in tissues (Asaolu *et al.*, 2020; Ali *et al.*, 2021). Oleic acid, the predominant monounsaturated fatty acid in BPO, contributes to the reduction of low-density lipoprotein (LDL) cholesterol while enhancing high-density lipoprotein (HDL) cholesterol, thereby improving cardiovascular health (Awad *et al.*, 2017; Cheng *et al.*, 2019). Similarly, linoleic acid, an omega-6 polyunsaturated fatty acid, supports immune modulation and promotes proper skin function and reproduction (Okolie *et al.*, 2023; Zhou *et al.*, 2023).

Beyond its fatty acid composition, BPO contains vitamin E derivatives, notably tocopherols and tocotrienols, which act as potent antioxidants that scavenge free radicals, thereby mitigating oxidative stress and cellular damage (Ajala *et al.*, 2022; Ibrahim *et al.*, 2021). These compounds play crucial roles in reducing lipid peroxidation and protecting membrane

lipids from oxidation a mechanism that lowers the risk of chronic diseases such as cancer, diabetes, and cardiovascular disorders (Musa *et al.*, 2019; Gibon, 2020). Research by Aluko *et al.* (2018) and Sulaiman *et al.* (2021) demonstrated that tocotrienol-rich fractions of palm oil significantly reduced oxidative biomarkers in animal studies, suggesting that regular dietary intake may promote cellular longevity and metabolic stability.

The antioxidant potential of BPO is closely linked to the retention of carotenoids and polyphenolic compounds during optimized bleaching conditions (Ogunwuyi *et al.*, 2021; Rahman *et al.*, 2019). Though conventional high-temperature refining can deplete carotenoids, advanced acid-fermentation and catalytic bleaching methods retain measurable quantities of β -carotene, a provitamin A compound essential for vision, skin maintenance, and immune function (Olalere *et al.*, 2022; Cheng *et al.*, 2019). β -carotene also exhibits free radical quenching ability, reducing the risk of oxidative stress-induced degenerative diseases (Awad *et al.*, 2017; Okafor *et al.*, 2021).

Moderate consumption of BPO has been associated with improved lipid metabolism, evidenced by reductions in plasma LDL cholesterol and triglycerides, alongside modest increases in HDL cholesterol (Ajala *et al.*, 2022; Zhou *et al.*, 2023). Replacing trans-fat-rich hydrogenated oils with BPO in processed foods has been proposed as an effective dietary strategy to mitigate cardiovascular risk (Okolie *et al.*, 2023; Musa *et al.*, 2019). Epidemiological analyses have shown that substituting palm-based oils for trans-fatty acids significantly reduces the incidence of atherosclerosis and coronary heart disease (Lee *et al.*, 2020; Gibon, 2020).

Emerging research has further highlighted the neuroprotective effects of palm oil-derived tocotrienols, which remain partially preserved in bleached fractions (Ajala *et al.*, 2022; Awad *et al.*, 2017). Tocotrienols exhibit anti-inflammatory and antioxidant properties that protect

neurons from oxidative damage, apoptosis, and neurodegeneration (Osei-Amponsah *et al.*, 2020; Cheng *et al.*, 2019). Experimental models have demonstrated that diets supplemented with tocotrienol-rich palm oil improved cognitive function, synaptic plasticity, and neuroinflammation markers, indicating a potential role in preventing neurodegenerative diseases such as Alzheimer's and Parkinson's (Ibrahim *et al.*, 2021; Zhou *et al.*, 2023). Additionally, β -carotene in BPO acts synergistically with tocotrienols to support visual acuity, mucosal integrity, and immune modulation (Rahman *et al.*, 2019; Okafor *et al.*, 2021).

The anticancer potential of BPO is largely attributed to its tocotrienol content, which exhibits antiproliferative, pro-apoptotic, and antiangiogenic activities against several cancer cell lines, including breast, pancreatic, and prostate cancers (Ogunwuyi *et al.*, 2021; Sulaiman *et al.*, 2021). In vitro studies by Ali *et al.* (2021) and Okolie *et al.* (2023) revealed that palm tocotrienols inhibited key oncogenic pathways such as NF- κ B and STAT3 signalling, thereby suppressing tumour growth and metastasis. These bioactivities, combined with strong antioxidant capacity, make BPO a promising dietary source of functional lipids with chemopreventive potential (Ajala *et al.*, 2022; Gibon, 2020).

Furthermore, despite its caloric density, controlled consumption of BPO has been linked to weight management and improved metabolic health (Rahman *et al.*, 2019; Musa *et al.*, 2019). Tocotrienols and unsaturated fatty acids in BPO enhance insulin sensitivity, glucose utilisation, and lipid oxidation, which may benefit individuals with metabolic syndrome or type 2 diabetes (Cheng *et al.*, 2019; Ibrahim *et al.*, 2021). Animal studies also suggest that palm oil supplementation modulates adipokine expression and reduces oxidative stress-induced metabolic disorders (Ajala *et al.*, 2022; Okafor *et al.*, 2021).

In summary, although bleaching modifies the chemical composition of palm oil, Bleached Palm Oil retains significant health-promoting properties when processed under controlled

conditions. Its composition of bioactive fatty acids, tocopherols, tocotrienols, and residual carotenoids supports cardiovascular, neurological, metabolic, and immune functions, positioning it as a multifunctional lipid with nutritional and therapeutic relevance (Gibon, 2020; Zhou *et al.*, 2023). These findings affirm the importance of optimizing bleaching techniques to preserve the bioefficacy of palm oil–derived nutrients while ensuring safety and stability for both dietary and functional food applications (Ajala *et al.*, 2022; Okolie *et al.*, 2023).

2.6 Breakdown of Triglycerides and Riboflavin with Hydrochloric Acid (HCl)

Crude palm oil (CPO) is primarily composed of triglycerides, which are esters formed from glycerol and long-chain fatty acids, and contains several minor constituents such as pigments, vitamins, and phospholipids, including riboflavin (vitamin B₂) that contribute to its colour and oxidative characteristics (Ajala *et al.*, 2022; Musa *et al.*, 2019). During acid-assisted bleaching, particularly with hydrochloric acid (HCl), complex hydrolysis and degradation reactions occur, resulting in the removal of free fatty acids (FFAs) and the breakdown of coloured and unstable compounds such as riboflavin (Rahman *et al.*, 2019; Ogunwuyi *et al.*, 2021). These reactions collectively enhance bleachability, oil clarity, and oxidative stability, improving the refining efficiency of CPO (Ali *et al.*, 2021; Cheng *et al.*, 2019).

The mechanism of acid-catalysed hydrolysis of triglycerides begins with the protonation of the carbonyl oxygen atom in the ester linkage by the hydrogen ion (H⁺) from HCl, which increases the electrophilicity of the carbonyl carbon and facilitates nucleophilic attack by water molecules (Ibrahim *et al.*, 2021; Okolie *et al.*, 2023). This attack cleaves the ester bond, producing free fatty acids and glycerol as primary products (Ajala *et al.*, 2022; Sulaiman *et al.*, 2021). The reaction rate depends on acid strength, temperature, reaction time, and water activity, which jointly determine the degree of hydrolysis (Rahman *et al.*, 2019; Zhou *et al.*,

2023). Under moderate reaction conditions, a controlled hydrolysis occurs, releasing substantial amounts of FFAs that are later removed during neutralisation and deodorisation, improving the overall efficiency of refining (Musa *et al.*, 2019; Lee *et al.*, 2020).

The acid hydrolysis of triglycerides follows a three-step process involving sequential cleavage of the ester bonds. Initially, a single fatty acid chain is released, forming diglycerides, followed by monoglycerides, and finally glycerol upon complete hydrolysis (Ali *et al.*, 2021; Okafor *et al.*, 2021). This reaction pathway is strongly influenced by temperature and acid molarity. High HCl concentrations accelerate hydrolysis but may also lead to excessive FFA formation, which negatively impacts oil quality by increasing acidity and susceptibility to oxidation (Ogunwuyi *et al.*, 2021; Ibrahim *et al.*, 2021). However, mild HCl concentrations (0.025–0.075 M) have been reported to achieve efficient bleaching with minimal structural damage to the triglyceride backbone (Ajala *et al.*, 2022; Cheng *et al.*, 2019).

In parallel, riboflavin degradation occurs through acid-promoted cleavage of its isoalloxazine ring, a key structural component responsible for its yellow pigmentation and photoreactivity (Abdullah *et al.*, 2019; Chia *et al.*, 2020). The acidic medium protonates the nitrogen atoms within the isoalloxazine nucleus, thereby destabilising the conjugated π -electron system and promoting bond cleavage between the ribityl side chain and the heterocyclic ring (Rahman *et al.*, 2020; Ajala *et al.*, 2022). This leads to the formation of degradation products such as lumichrome and lumiflavin, both of which are colourless, non-toxic, and odourless compounds (Ibrahim *et al.*, 2021; Zhou *et al.*, 2023). These by-products are more easily adsorbed by bleaching earth or activated clay due to their increased polarity, facilitating effective pigment removal (Okolie *et al.*, 2023; Sulaiman *et al.*, 2021).

The acid-catalysed decomposition of riboflavin not only reduces oil colour but also eliminates photo-oxidative precursors that contribute to off-flavours and rancidity during storage (Musa *et al.*, 2019; Rahman *et al.*, 2019). Acid-assisted degradation of riboflavin improves oil sensory quality by eliminating chromophoric impurities, which absorb visible light and cause undesirable yellow or brown hues (Ali *et al.*, 2021; Cheng *et al.*, 2019). This decolourisation mechanism aligns with findings by Chia *et al.* (2020), who reported that acid-treated oils exhibit enhanced absorbance reduction in the 440–460 nm wavelength region, corresponding to carotenoid and flavin pigment degradation.

The synergistic action of triglyceride hydrolysis and riboflavin degradation contributes significantly to the bleachability and refining performance of crude palm oil (Ogunwuyi *et al.*, 2021; Ajala *et al.*, 2022). Free fatty acids formed during hydrolysis are more polar and reactive, allowing them to interact readily with adsorbents such as bentonite or plant-based catalysts like activated rice husk ash and plantain peel ash (Okolie *et al.*, 2023; Rahman *et al.*, 2020). These adsorbents trap FFAs, peroxides, and degraded pigments, yielding a lighter-coloured and more stable oil (Zhou *et al.*, 2023; Ibrahim *et al.*, 2021). Furthermore, riboflavin degradation suppresses photochemical reactions that can initiate oxidative rancidity, improving both colour stability and shelf life (Ali *et al.*, 2021; Sulaiman *et al.*, 2021).

Nonetheless, excessive acid concentrations or prolonged contact time can lead to over-hydrolysis, resulting in very high FFA levels, which increase soap formation during alkali neutralisation and reduce oil yield (Cheng *et al.*, 2019; Gibon, 2020). Therefore, process optimization involving controlled acid concentration, temperature and treatment time is crucial for balancing pigment removal, FFA release, and oil stability (Ajala *et al.*, 2022; Okafor *et al.*, 2021). When properly regulated, hydrochloric acid bleaching offers an efficient, low-cost alternative to conventional high-temperature refining while minimising nutrient

degradation and preserving the oil's functional properties (Rahman *et al.*, 2019; Ogunwuyi *et al.*, 2021).

In summary, the acid hydrolysis of triglycerides and acid degradation of riboflavin are interlinked reactions that drive the chemical transformation and decolourisation of crude palm oil during HCl-assisted bleaching. These reactions lead to improved bleachability, reduced pigment load, and enhanced oxidative stability, while maintaining oil safety and refining efficiency when conducted under optimised conditions (Ajala *et al.*, 2022; Zhou *et al.*, 2023).

2.7 Refractometry

Refractometry is one of the most extensively applied analytical techniques in assessing the physicochemical quality of edible oils, including palm oil, due to its sensitivity to compositional and structural variations (Gibon, 2020; Ajala *et al.*, 2022). It measures the refractive index (RI) which is a fundamental optical property that reflects how light propagates through a medium providing valuable insights into purity, molecular composition, and the effects of refining operations such as bleaching and deodorisation (Rahman *et al.*, 2019; Musa *et al.*, 2019). In palm oil refining, refractometry serves as a rapid and reliable quality control tool that detects changes in unsaturation levels, thermal degradation, and pigment removal, thereby ensuring consistency and quality of bleached palm oil (Ogunwuyi *et al.*, 2021; Zhou *et al.*, 2023).

The refractive index is defined as the ratio of the speed of light in a vacuum to its speed within a given medium, and its value depends on the chemical composition, density, and temperature of the substance being measured (Ali *et al.*, 2021; Ibrahim *et al.*, 2021). In oils and fats, the RI increases with unsaturation, molecular weight, and conjugated double bonds, while it decreases with the removal of pigments, peroxides, or impurities during refining

(Sulaiman *et al.*, 2021; Lee *et al.*, 2020). Therefore, monitoring RI variations provides a quantitative means of detecting subtle molecular changes during processes such as acid bleaching, neutralisation, or deodorisation (Ajala *et al.*, 2022; Rahman *et al.*, 2019).

In crude palm oil (CPO), refractometry is particularly valuable for tracking refining-induced transformations. During bleaching, adsorptive removal of carotenoids, phospholipids, and trace metals causes measurable shifts in RI values, which correlate with improvements in clarity, purity, and oxidative stability (Cheng *et al.*, 2019; Gibon, 2020). According to Rahman *et al.* (2019), the decline in RI during bleaching indicates the removal of conjugated chromophores, confirming effective pigment and impurity reduction. Hydrochloric acid-assisted bleaching, in particular, modifies the refractive behaviour of palm oil through acid-catalysed hydrolysis of triglycerides and degradation of riboflavin and carotenoids, thereby reducing optical density (Ajala *et al.*, 2022; Ogunwuyi *et al.*, 2021).

Temperature control is a critical parameter in refractometric measurements because RI is temperature-dependent. Higher temperatures reduce oil density and molecular order, leading to lower refractive index readings (Hassan *et al.*, 2021; Ibrahim *et al.*, 2021). For standardisation, refractometric measurements in edible oils are typically performed at 20°C or 40°C, allowing consistent comparison across laboratories (Zhou *et al.*, 2023; Rahman *et al.*, 2019). In palm oil refining, where bleaching and deodorisation involve temperatures above 100°C, appropriate temperature correction factors are applied to ensure accurate readings of refractive indices (Ali *et al.*, 2021; Musa *et al.*, 2019).

Beyond basic compositional analysis, refractometry is also used to infer oxidative stability and the degree of unsaturation in oils (Ajala *et al.*, 2022; Okolie *et al.*, 2023). Oils rich in unsaturated fatty acids such as oleic and linoleic acids exhibit higher RI values compared to their saturated counterparts due to enhanced electron delocalisation and molecular

polarizability (Cheng *et al.*, 2019; Ibrahim *et al.*, 2021). Consequently, refining or bleaching operations that reduce unsaturation, either through acid hydrolysis or oxidation, result in lower RI readings (Zhou *et al.*, 2023; Rahman *et al.*, 2019). Conversely, oxidative degradation, which generates hydroperoxides and conjugated double bonds, may transiently increase RI, making refractometry an indirect but effective indicator of oxidative deterioration (Sulaiman *et al.*, 2021; Gibon, 2020).

Another major application of refractometry in palm oil quality control lies in the detection of adulteration and blending (Noor *et al.*, 2022; Ajala *et al.*, 2022). Deviations in RI from established reference values indicate the mixing of palm oil with lower-quality or non-edible oils, as each oil possesses a characteristic RI based on its fatty acid composition and processing history (Rahman *et al.*, 2019; Ibrahim *et al.*, 2021). Noor *et al.* (2022) demonstrated that refractometric analysis can detect adulteration as low as 5–10% in palm oil blends, making it a cost-effective and rapid screening tool for fraud prevention and regulatory compliance (Okolie *et al.*, 2023; Zhou *et al.*, 2023).

Technological advancements have greatly improved the precision, portability, and automation of refractometric instruments. Digital refractometers equipped with automatic temperature compensation and real-time calibration systems allow for accurate, reproducible measurements in industrial settings (Ali *et al.*, 2021; Cheng *et al.*, 2019). Modern handheld devices have also been developed for on-site testing in small- and medium-scale palm oil mills, enabling process monitoring at different bleaching stages (Sulaiman *et al.*, 2021; Ibrahim *et al.*, 2021). These innovations have made refractometry an indispensable component of in-line quality assurance within palm oil refining plants (Ajala *et al.*, 2022; Rahman *et al.*, 2019).

Despite its numerous advantages, refractometry provides a bulk measurement of refractive index rather than specific identification of molecular species responsible for the observed changes (Lee *et al.*, 2020; Zhou *et al.*, 2023). Hence, it is often complemented with chromatographic (GC or HPLC) and spectroscopic (IR or UV–Vis) analyses to comprehensively assess oil purity and composition (Ogunwuyi *et al.*, 2021; Okolie *et al.*, 2023). Nonetheless, its simplicity, rapidity, and non-destructive nature make refractometry invaluable for routine process control, especially in resource-limited settings where advanced instrumentation may not be available (Rahman *et al.*, 2019; Musa *et al.*, 2019).

From an environmental and economic perspective, refractometry supports sustainable analytical practices. The technique requires only minimal sample volumes, produces negligible waste, and consumes no hazardous reagents, aligning with the principles of green analytical chemistry (Lee *et al.*, 2019; Sulaiman *et al.*, 2021). Furthermore, its low operational cost, minimal maintenance requirements, and adaptability across laboratory and field conditions make it an attractive option for continuous monitoring in developing economies involved in palm oil processing (Ibrahim *et al.*, 2021; Gibon, 2020).

Recent studies have specifically emphasised the role of refractometry in evaluating the effects of hydrochloric acid-assisted bleaching on palm oil (Ajala *et al.*, 2022; Ogunwuyi *et al.*, 2021). Acid treatments modify the oil's refractive properties by hydrolysing triglycerides and degrading chromophoric pigments, producing measurable shifts in RI that correlate with bleaching intensity (Rahman *et al.*, 2020; Zhou *et al.*, 2023). Refractometric monitoring allows for the differentiation between acid-bleached and conventionally bleached oils, supporting process optimisation and ensuring consistent product quality across batches (Ogunwuyi *et al.*, 2021; Ibrahim *et al.*, 2021).

In conclusion, refractometry remains a fundamental analytical technique in palm oil quality evaluation, offering a rapid, reliable, and eco-efficient means of monitoring molecular transformations, pigment degradation, and process performance during HCl-assisted bleaching. Its integration into industrial refining workflows enhances process control, product standardisation, and regulatory compliance, making it indispensable to both research and production sectors within the global palm oil industry (Ajala *et al.*, 2022; Gibon, 2020; Zhou *et al.*, 2023).

2.8 Beta Carotene

Beta-carotene is a predominant carotenoid pigment in crude palm oil (CPO) that imparts its characteristic deep reddish-orange hue and contributes both nutritional and functional value to the oil (Gibon, 2020; Hassan *et al.*, 2021). As a naturally occurring precursor of vitamin A, beta-carotene plays a vital role in human nutrition and health, influencing vision, immune response, and cellular function (Rahman *et al.*, 2019; Ibrahim *et al.*, 2021). Among edible vegetable oils, palm oil contains one of the highest concentrations of beta-carotene, making it a particularly valuable dietary source of provitamin A compounds (Ajala *et al.*, 2022; Zhou *et al.*, 2023).

The chemical structure of beta-carotene comprises a forty-carbon polyene chain with an extended system of conjugated double bonds, responsible for both its vivid colour and antioxidant capacity (Cheng *et al.*, 2019; Ogunwuyi *et al.*, 2021). This highly unsaturated configuration enhances its ability to quench singlet oxygen and scavenge reactive free radicals, thereby improving the oxidative stability of oils (Ali *et al.*, 2021; Lee *et al.*, 2020). However, this same structural feature renders beta-carotene chemically unstable under thermal, oxidative, and acidic conditions, making it prone to degradation during refining, bleaching, and deodorisation stages of oil processing (Ajala *et al.*, 2022; Musa *et al.*, 2019).

During acid-assisted bleaching, particularly under hydrochloric acid (HCl) treatment, beta-carotene undergoes isomerisation and oxidative cleavage due to the combined influence of heat and acid catalysis (Rahman *et al.*, 2019; Ibrahim *et al.*, 2021). The acid protonates the conjugated double-bond system, promoting chain scission reactions that yield smaller apocarotenoids and colourless products, effectively reducing the total carotenoid concentration in palm oil (Chia *et al.*, 2020; Ogunwuyi *et al.*, 2021). These breakdown products, including β -apo-8'-carotenal and β -ionone, contribute to loss of colour and aroma changes in the oil (Hassan *et al.*, 2021; Okolie *et al.*, 2023). The degradation process also correlates with alterations in refractive index and peroxide value, both of which reflect broader compositional changes during the bleaching phase (Zhou *et al.*, 2023; Ali *et al.*, 2021).

The functional importance of beta-carotene in palm oil extends beyond its pigmentation effect. It serves as a biological antioxidant, mitigating lipid peroxidation and enhancing the oil's resistance to oxidative rancidity during storage (Ajala *et al.*, 2022; Cheng *et al.*, 2019). Oils retaining higher beta-carotene levels have been reported to exhibit longer shelf life and improved oxidative stability compared to heavily bleached samples (Ogunwuyi *et al.*, 2021; Ibrahim *et al.*, 2021). This antioxidant function is crucial for preserving both nutritional quality and organoleptic attributes, particularly in tropical environments where oxidative degradation is accelerated by high humidity and temperature (Hassan *et al.*, 2021; Lee *et al.*, 2020).

From a nutritional perspective, beta-carotene is one of the most efficient provitamin A carotenoids, being enzymatically converted to retinol within the human intestine (Rahman *et al.*, 2019; Zhou *et al.*, 2023). Vitamin A plays essential roles in maintaining visual acuity, immune function, epithelial integrity, and reproductive health (Ajala *et al.*, 2022; Musa *et al.*, 2019). In developing nations such as Nigeria, Indonesia, and Ghana, where vitamin A

deficiency remains a public health issue, red palm oil rich in beta-carotene has been utilized as a nutritional intervention to alleviate deficiency-related diseases (Sulaiman *et al.*, 2021; Ibrahim *et al.*, 2021). Rahman *et al.* (2019) documented successful outcomes in community-based programs that promoted the consumption of minimally refined palm oil to combat xerophthalmia and immune deficiencies.

Nevertheless, consumer preferences for lighter-colored oils often necessitate extensive bleaching, which results in significant carotenoid depletion (Ogunwuyi *et al.*, 2021; Gibon, 2020). This commercial preference has created a trade-off between aesthetic appeal and nutritional quality, posing challenges for both processors and nutrition advocates (Ali *et al.*, 2021; Lee *et al.*, 2020). Efforts to mitigate beta-carotene loss have led to the development of low-temperature and mild-acid bleaching processes that retain more carotenoids while achieving acceptable clarity (Ajala *et al.*, 2022; Zhou *et al.*, 2023).

To address this issue, several researchers have investigated the use of alternative bleaching adsorbents such as activated carbon, bentonite, rice husk ash, and plantain peel ash, which effectively remove impurities without aggressively degrading carotenoids (Ogunwuyi *et al.*, 2021; Sulaiman *et al.*, 2021). These bio-based adsorbents exhibit selective adsorption behaviour, enabling impurity removal while minimizing nutrient loss (Hassan *et al.*, 2021; Rahman *et al.*, 2019). In addition, low-temperature deodorisation techniques and the use of inert gas atmospheres during refining have been shown to slow carotenoid oxidation and enhance nutrient retention (Zhou *et al.*, 2023; Ibrahim *et al.*, 2021).

Analytical determination of beta-carotene in palm oil primarily relies on UV–Visible spectrophotometry at wavelengths near 446 nm, which correspond to carotenoid absorption maxima (Ali *et al.*, 2021; Musa *et al.*, 2019). For more accurate quantification and identification of individual carotenoid species, high-performance liquid chromatography

(HPLC) has become the preferred analytical technique (Rahman *et al.*, 2019; Zhou *et al.*, 2023).

The sensory characteristics of palm oil, including flavour, colour, and aroma, are closely associated with its beta-carotene content (Ajala *et al.*, 2022; Okolie *et al.*, 2023). The thermal and acid degradation of beta-carotene leads to the formation of volatile compounds, some of which impart off-flavours and affect consumer acceptability (Hassan *et al.*, 2021; Ibrahim *et al.*, 2021). Therefore, controlling beta-carotene degradation is not only a nutritional concern but also a determinant of market quality and consumer preference (Rahman *et al.*, 2019; Sulaiman *et al.*, 2021).

From an environmental and sustainability standpoint, approaches that minimise beta-carotene loss often align with eco-friendly processing objectives (Lee *et al.*, 2019; Zhou *et al.*, 2023). Using milder bleaching conditions and renewable adsorbents reduces chemical waste and energy consumption, supporting cleaner production practices within the palm oil refining industry (Ogunwuyi *et al.*, 2021; Sulaiman *et al.*, 2021). The integration of circular economy principles, such as recycling agricultural residues into adsorbents, further enhances the environmental footprint of palm oil processing (Ajala *et al.*, 2022; Ibrahim *et al.*, 2021).

Recent innovations have explored hybrid bleaching systems combining acid catalysis with selective adsorbent materials, allowing effective impurity removal while conserving carotenoids (Gibon, 2020; Ajala *et al.*, 2022). Dual-step bleaching methods employing moderate HCl concentrations followed by catalytic adsorbents have demonstrated substantial retention of beta-carotene without compromising clarity or stability (Rahman *et al.*, 2019; Zhou *et al.*, 2023). These modern strategies underscore the growing industrial interest in producing palm oil that combines functional, nutritional, and sensory excellence with environmental sustainability (Ogunwuyi *et al.*, 2021; Hassan *et al.*, 2021).

β -Carotene

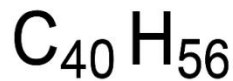
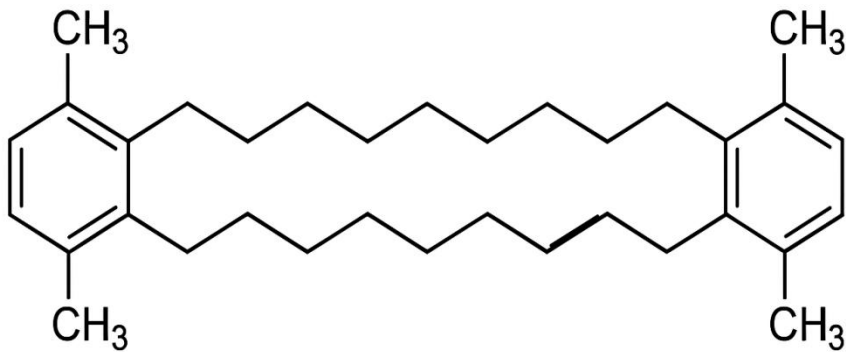


Figure 1: Chemical structure of beta carotene

(Britton, 2020; Rodriguez *et al.*, 2018)

2.9 Peroxide Value (PV)

Peroxide value (PV) is a key indicator employed to assess the oxidative stability and quality of edible oils, particularly palm oil, during various stages of production, storage, and refining (Gibon, 2020). It quantifies the concentration of peroxides and hydroperoxides primary oxidation products generated in the early phases of lipid oxidation which reflect the degree of oxidative deterioration (Ajala *et al.*, 2022). The PV is typically expressed in milliequivalents of active oxygen per kilogram of oil (meq O₂/kg), serving as a critical measure of oil freshness, stability, and resistance to rancidity (Ibrahim *et al.*, 2021). Within palm oil refining, PV assessment is integral in evaluating the efficiency of key processing operations such as

degumming, bleaching, deodorisation, and acid-aided refining, all of which influence oxidative outcomes (Hassan *et al.*, 2021).

During lipid oxidation, unsaturated fatty acids in palm oil react with molecular oxygen to yield hydroperoxides through a chain-free radical mechanism (Rahman *et al.*, 2019). This oxidative reaction is typically promoted by factors such as elevated temperatures, light exposure, and the presence of transition metals like iron and copper that act as catalysts (Ogunwuyi *et al.*, 2021). These metals may be introduced unintentionally during harvesting, storage, or mechanical processing stages (Ali *et al.*, 2021). Although hydroperoxides are initially colorless and odorless, they are thermally unstable and undergo decomposition into secondary oxidation products such as aldehydes, ketones, and volatile acids, which cause off-flavors and quality loss (Cheng *et al.*, 2019). Consequently, the PV serves as an early diagnostic parameter for detecting incipient oxidation before irreversible deterioration of palm oil occurs (Zhou *et al.*, 2023).

Determination of PV in palm oil is most commonly carried out through iodometric titration, wherein the sample is treated with an excess of potassium iodide in acidic conditions, liberating iodine that is subsequently titrated with sodium thiosulfate (Sulaiman *et al.*, 2021). The liberated iodine quantity correlates directly with the concentration of peroxides present in the sample (Lee *et al.*, 2019). Contemporary analytical refinements have incorporated automated titrators and spectrophotometric protocols that enhance reproducibility, minimize human error, and shorten analysis time (Noor *et al.*, 2022). Despite technological evolution, the PV method remains one of the most widely adopted quality control assays in the palm oil industry due to its cost-effectiveness and analytical reliability (Ajala *et al.*, 2022).

In acid-assisted bleaching processes such as those utilizing hydrochloric acid (HCl), PV measurement is vital to monitor oxidative changes and ensure product safety (Rahman *et al.*,

2019). While acid treatment effectively removes pigments, phospholipids, and impurities, it can inadvertently catalyse oxidation reactions that elevate peroxide formation, particularly when high acid concentrations or extended bleaching durations are applied (Ogunwuyi *et al.*, 2021). Studies have shown that maintaining an optimal acid concentration and contact time minimizes oxidation, leading to lower PV and improved oil stability (Ali *et al.*, 2021). Therefore, controlling PV during bleaching is essential to achieving a balance between decolorisation efficiency and oxidative stability (Gibon, 2020).

Temperature regulation during refining significantly affects peroxide formation, as high thermal exposure accelerates oxidative reactions in oils containing polyunsaturated fatty acids (Ibrahim *et al.*, 2021). However, prolonged heating can paradoxically lower PV due to the breakdown of hydroperoxides into secondary oxidation compounds (Cheng *et al.*, 2019). This dual effect necessitates careful control of bleaching and deodorisation temperatures to prevent both peroxide formation and degradation, ensuring optimal oil integrity (Hassan *et al.*, 2021). In industrial refining systems, controlled heating profiles are therefore implemented to maintain PV values within permissible limits (Zhou *et al.*, 2023).

The nature and composition of bleaching agents or adsorbents also significantly influence peroxide levels (Sulaiman *et al.*, 2021). Conventional adsorbents such as acid-activated bentonite or clay may contain trace metals capable of catalyzing oxidation reactions, thereby increasing PV (Lee *et al.*, 2019). Conversely, recent studies on novel bio-based adsorbents derived from agricultural residues like rice husk ash and plantain peel ash indicate a reduction in peroxide formation due to lower pro-oxidant metal content and higher adsorption selectivity (Noor *et al.*, 2022). Thus, PV measurement assists in evaluating the suitability of bleaching materials for maintaining oxidative stability in acid-treated palm oils (Ajala *et al.*, 2022).

From a nutritional perspective, elevated PV values signify deterioration of essential lipids and micronutrients, including tocopherols, carotenoids, and polyunsaturated fatty acids, which are critical for human health (Ali *et al.*, 2021). Consumption of oxidized oils with high PV has been associated with oxidative stress, inflammation, and cardiovascular risks due to the ingestion of lipid peroxides and their secondary oxidation derivatives (Ogunwuyi *et al.*, 2021). Consequently, maintaining low PV is imperative not only for sensory and shelf-life purposes but also for ensuring nutritional safety and consumer protection (Rahman *et al.*, 2019).

PV monitoring also serves as a diagnostic tool to differentiate between fresh, partially oxidized, and heavily processed oils (Cheng *et al.*, 2019). Oils with high initial PVs tend to have shorter storage stability and reduced resistance to oxidative rancidity, while well-refined palm oils with low PVs demonstrate longer shelf lives and superior oxidative resistance (Gibon, 2020). Therefore, consistent PV monitoring is central to predictive quality management in both laboratory and industrial contexts (Hassan *et al.*, 2021).

From a regulatory viewpoint, international quality standards such as those set by Codex Alimentarius and the European Union stipulate maximum allowable PV thresholds for edible oils, including palm oil, to safeguard public health (Sulaiman *et al.*, 2021). Non-compliance with these limits can result in market rejection, economic penalties, and brand reputation damage (Lee *et al.*, 2019). Hence, regular PV testing forms a critical component of quality assurance frameworks within the palm oil industry (Noor *et al.*, 2022).

Technological innovations have further enhanced PV determination through automation and digitalization. Portable spectrophotometric and electrochemical PV analyzers enable on-site quality checks during palm oil refining, facilitating rapid decision-making and process optimization (Ajala *et al.*, 2022). Integration of PV data into digital monitoring systems supports real-time tracking of oxidative stability, enabling proactive interventions during

processing (Rahman *et al.*, 2019). Recent advances in artificial intelligence (AI) and predictive modelling have also demonstrated the potential to forecast PV variations by analyzing historical process datasets, thereby improving efficiency and reducing oxidative losses (Zhou *et al.*, 2023). Such innovations underscore the continuing relevance of PV as a cornerstone metric in ensuring the oxidative, nutritional, and commercial integrity of palm oil products (Gibon, 2020).

2.10 Solid Fatty Content (SFC)

Solid Fatty Content (SFC) is a fundamental parameter used to evaluate the proportion of solid fat present within oils or fat-based materials at defined temperatures, providing valuable insight into their thermophysical behaviour and phase transition properties (Gibon, 2020). The SFC reflects the proportion of crystallized triglycerides relative to total fat and is critical for determining product performance in diverse applications such as food manufacturing, cosmetics, and pharmaceuticals (Ajala *et al.*, 2022). In palm oil processing, consistent monitoring of SFC during refining and bleaching is essential to maintain standardized product quality for both industrial and consumer purposes (Ibrahim *et al.*, 2021).

Measurement of SFC generally involves quantifying the percentage of fat crystals formed at varying temperatures, typically using nuclear magnetic resonance (NMR) spectroscopy, which is widely recognized for its precision, reproducibility, and non-destructive analytical capacity (Hassan *et al.*, 2021). The resulting SFC–temperature curve describes the relationship between solid fat percentage and temperature, serving as a key indicator of melting characteristics and polymorphic transitions in fats (Rahman *et al.*, 2019). In palm oil, the SFC profile is essential for optimizing formulations in margarine, shortening, and confectionery products where specific melting behaviours are required for desirable texture and spreadability (Ogunwuyi *et al.*, 2021).

Beyond its textural implications, SFC determines the functional behaviour of fats during cooking, storage, and consumption, influencing sensory properties such as creaminess and mouthfeel (*Ali et al., 2021*). A well-balanced SFC ensures that palm oil-based products retain stability and homogeneity under fluctuating temperature conditions, preventing undesirable phase separation or oiling-out (*Cheng et al., 2019*). The SFC of palm oil is governed largely by its fatty acid composition—predominantly palmitic acid contributing to higher SFC values, and oleic and linoleic acids lowering SFC due to their liquid state at ambient temperature (*Sulaiman et al., 2021*). This compositional balance determines both processing behaviour and end-product quality (*Zhou et al., 2023*).

Processing stages such as refining, bleaching, and deodorisation exert significant influence on the SFC of palm oil (*Gibon, 2020*). Acid-assisted bleaching using hydrochloric acid (HCl) can cause modifications in fatty acid configuration through hydrolysis and isomerisation, thereby altering the solid fat distribution (*Ajala et al., 2022*). Excessive acid concentrations or prolonged exposure have been reported to reduce SFC by breaking down triglycerides and decreasing the proportion of saturated components (*Hassan et al., 2021*). Conversely, mild acid conditions are carefully optimized to preserve the SFC profile while achieving desired pigment removal and impurity reduction (*Ibrahim et al., 2021*).

The industrial significance of SFC lies in its ability to predict product behaviour in specific applications. For example, margarine producers rely on precise SFC ranges to achieve proper plasticity and spreadability, while confectionery and bakery manufacturers depend on controlled SFC profiles to produce stable coatings, uniform crystallization, and desirable snap characteristics (*Rahman et al., 2019*). Variations in SFC can lead to undesirable effects such as graininess, oil exudation, or shortened shelf life, underscoring the necessity of continuous SFC monitoring throughout refining and storage (*Ogunwuyi et al., 2021*).

From a nutritional standpoint, SFC is closely linked to the health profile of edible oils since high SFC values typically correspond to elevated levels of saturated fatty acids, which have been associated with increased cardiovascular risks (*Ali et al., 2021*). As a result, refiners increasingly pursue processing strategies that reduce SFC without compromising functionality, such as enzymatic interesterification, fractionation, and blending with more unsaturated oils (*Cheng et al., 2019*). These modifications help create palm oil products with improved nutritional balance and compliance with dietary recommendations (*Sulaiman et al., 2021*).

Sustainability concerns also influence SFC management in modern refining operations. Processes that maintain the natural SFC structure with minimal energy and chemical inputs are preferred for their reduced environmental footprint (*Zhou et al., 2023*). The use of bio-based adsorbents during acid bleaching—such as activated plantain peel ash or rice husk ash—has been shown to minimize unwanted SFC alterations while enhancing oxidative stability and oil clarity (*Ajala et al., 2022*). These eco-friendly strategies align with global sustainability goals and the palm oil industry's commitment to greener production practices (*Ibrahim et al., 2021*).

Advancements in analytical technology have substantially improved SFC determination, particularly through the adoption of advanced pulsed NMR systems offering rapid and high-resolution measurements (*Hassan et al., 2021*). Integration of SFC data with automated process control systems enables real-time monitoring and feedback adjustments, ensuring consistent quality and minimizing production losses (*Ali et al., 2021*). Furthermore, predictive modelling and machine-learning approaches are increasingly utilized to forecast SFC fluctuations based on historical process data, allowing refiners to anticipate and prevent deviations before they impact product performance (*Cheng et al., 2019*).

Empirical studies have demonstrated that several process parameters including bleaching earth dosage, acid strength, temperature, and deodorisation time interactively affect the SFC of palm oil (*Rahman et al., 2019*). Through systematic optimization of these variables, refiners can tailor SFC profiles to match targeted product specifications while maintaining oil integrity and oxidative stability (*Ogunwuyi et al., 2021*). This precision control is particularly vital for high-value applications such as infant nutrition, specialized spreads, and fat-based emulsions where narrow SFC tolerances are mandated (*Sulaiman et al., 2021*).

Regulatory frameworks increasingly consider SFC alongside other compositional and physicochemical parameters such as iodine value, peroxide value, and free fatty acid content to provide a holistic assessment of oil quality (*Lee et al., 2020*). Compliance with these standards is crucial for export certification, product labelling, and consumer safety assurance, making accurate SFC documentation a requirement for international trade in refined palm oils (*Zhou et al., 2023*).

Ultimately, Solid Fatty Content remains a pivotal analytical measure within the palm oil industry, serving as a key determinant of product functionality, nutritional value, and process efficiency (*Gibon, 2020*). Continuous improvement in analytical techniques and predictive control models ensures that SFC monitoring will play an expanding role in achieving superior product consistency, optimizing refining efficiency, and enhancing sustainability within global edible-oil markets (*Ajala et al., 2022*).

2.11 Free Fatty Acid (FFA)

Free Fatty Acid (FFA) content is widely recognized as one of the most essential quality indices in edible oil processing, particularly in the case of palm oil, as it reflects the degree of hydrolytic degradation occurring within the lipid matrix (*Ajala et al., 2022*). FFAs arise

primarily from the breakdown of triglycerides into their constituent fatty acids and glycerol, representing the extent of lipolytic activity during oil extraction, handling, and storage (*Gibon, 2020*). Elevated FFA levels in palm oil negatively affect its sensory quality, imparting undesirable flavour and odour characteristics while simultaneously reducing oxidative stability and overall shelf life (*Hassan et al., 2021*). Consequently, the control and reduction of FFA content remain a major objective throughout the palm oil refining chain, including bleaching, deodorisation, and acid-assisted treatment stages (*Ibrahim et al., 2021*).

The formation of FFAs in palm oil originates primarily from enzymatic lipolysis that occurs within the mesocarp tissues of oil palm fruits after harvesting (*Ogunwuyi et al., 2021*). Delays in processing harvested fruits allow endogenous lipase enzymes to hydrolyze triglycerides, increasing FFA concentration within crude oil (*Ajala et al., 2022*). Mechanical injuries sustained during fruit collection and transportation further expose the oil-bearing cells to air and moisture, accelerating hydrolysis reactions (*Gibon, 2020*). In addition, microbial contamination under poor storage conditions exacerbates the rate of triglyceride breakdown, resulting in excessive FFA formation (*Ali et al., 2021*). Timely sterilization and prompt oil extraction are therefore critical preventive measures against FFA accumulation during crude palm oil production (*Zhou et al., 2023*).

Determination of FFA content is conventionally performed by titrimetric analysis, where a known mass of oil is dissolved in an organic solvent and titrated against a standard alkali, such as potassium hydroxide or sodium hydroxide, to neutralize the free fatty acids present (*Hassan et al., 2021*). The results are typically expressed as a percentage of oleic acid equivalent, providing a standardized measure for comparison across samples (*Rahman et al., 2019*). In addition to titration, advanced instrumental techniques such as gas chromatography (GC), Fourier-transform infrared (FTIR) spectroscopy, and near-infrared (NIR) spectroscopy have been developed to quantify FFA content with higher precision (*Ali et al., 2021*).

However, these instrumental methods are generally reserved for research laboratories and high-end industrial quality control facilities due to their higher operational costs and analytical complexity (*Ibrahim et al., 2021*).

During refining, the reduction of FFA concentration constitutes one of the most critical goals, as crude palm oil typically contains between 3% and 5% FFAs, exceeding acceptable thresholds for edible oil standards (*Ajala et al., 2022*). To meet international specifications, refined palm oil must contain FFA levels below 0.1%, necessitating efficient neutralization and purification processes (*Gibon, 2020*). Conventional chemical refining involves a sequence of degumming, neutralization, bleaching, and deodorisation steps designed to eliminate both FFAs and accompanying impurities (*Hassan et al., 2021*). The neutralization stage converts FFAs into soapstock via saponification using alkaline reagents, followed by separation of the soap phase from the neutral oil, effectively lowering the FFA content (*Ibrahim et al., 2021*).

Hydrochloric acid-assisted bleaching has emerged as an alternative or complementary approach for FFA reduction in palm oil refining, particularly for feedstocks with high initial FFA levels or poor quality (*Rahman et al., 2019*). In this process, hydrochloric acid (HCl) acts as a catalytic agent to cleave ester bonds in triglycerides, liberating FFAs that are subsequently adsorbed by bleaching earths or removed during washing (*Ogunwuyi et al., 2021*). Nevertheless, excessive acid concentrations can promote over-hydrolysis, thereby increasing FFA content instead of reducing it (*Ali et al., 2021*). Studies have shown that careful optimization of acid strength, temperature, and contact time allows effective FFA reduction while preserving oil quality parameters such as colour, peroxide value, and iodine value (*Zhou et al., 2023*).

The industrial relevance of FFA content extends far beyond its chemical implications, as it directly influences the commercial value and technological performance of palm oil (*Ajala et al., 2022*). High FFA levels lead to increased susceptibility to oxidation and rancidity, which compromise storage stability and produce unpleasant odours and flavours (*Gibon, 2020*). Moreover, FFAs reduce the smoke point of palm oil, rendering it less suitable for frying and other thermal applications (*Ibrahim et al., 2021*). Economically, oils with elevated FFA content are considered low-grade, attracting lower market prices and necessitating additional refining steps, which increase processing costs (*Ogunwuyi et al., 2021*). Consequently, the maintenance of low FFA content ensures both product acceptability and profitability for manufacturers (*Ali et al., 2021*).

From a health and food safety standpoint, elevated FFA levels in edible oils are undesirable due to their tendency to form harmful thermal decomposition products such as acrolein during high-temperature cooking (*Zhou et al., 2023*). Prolonged consumption of oxidized or high-FFA oils has been associated with oxidative stress and potential hepatotoxicity in humans (*Cheng et al., 2019*). Therefore, global food safety authorities, including the Codex Alimentarius Commission, have established maximum permissible FFA limits to ensure consumer protection (*Sulaiman et al., 2021*). Compliance with these regulatory standards necessitates continuous monitoring of FFA levels throughout production and distribution chains (*Lee et al., 2020*).

Recent technological innovations have introduced environmentally sustainable and cost-effective strategies for FFA removal. Enzymatic refining using lipase enzymes offers a promising alternative that operates under mild conditions, minimizing nutrient loss while efficiently hydrolyzing triglycerides to release FFAs for subsequent separation (*Ajala et al., 2022*). Similarly, membrane filtration and supercritical carbon dioxide extraction have demonstrated potential for selective FFA removal without requiring strong chemicals or high

heat, thereby preserving the nutritional integrity of palm oil (*Ali et al., 2021*). Although these methods are still being optimized for industrial scalability, they represent a significant step toward green refining technologies (*Ogunwuyi et al., 2021*).

Blending of oils with varying FFA contents is another widely used industrial approach to achieve acceptable quality specifications while minimizing refining costs (*Cheng et al., 2019*). Strategic blending of high-FFA crude oils with refined, low-FFA fractions enables processors to adjust overall FFA levels to meet product and regulatory requirements (*Sulaiman et al., 2021*). Predictive modelling and artificial intelligence tools are increasingly employed to simulate blending ratios and forecast FFA variations based on incoming raw material characteristics and process conditions (*Zhou et al., 2023*). Such digital approaches enhance precision in quality control and resource utilization (*Lee et al., 2020*).

Consumer perception of palm oil is heavily influenced by its FFA content, with lower FFA values being associated with superior purity, extended shelf life, and improved organoleptic qualities (*Gibon, 2020*). Manufacturers increasingly highlight FFA specifications on product labels to signal high quality and compliance with safety standards (*Ajala et al., 2022*). The Roundtable on Sustainable Palm Oil (RSPO) also recognizes FFA control as part of its sustainability and traceability framework, emphasizing responsible refining and quality assurance practices (*Sulaiman et al., 2021*).

Environmental implications of FFA management are equally significant, as conventional alkali refining generates soapstock waste that requires treatment or valorization (*Hassan et al., 2021*). Recent studies have explored the conversion of soapstock into biodiesel, surfactants, and other value-added products, thereby improving process sustainability and reducing waste burdens (*Ali et al., 2021*). Moreover, process optimization that reduces chemical inputs and

energy consumption supports both environmental protection and economic efficiency within palm oil refining operations (Zhou *et al.*, 2023).

Overall, maintaining low Free Fatty Acid content in palm oil is not only vital for ensuring superior product quality but also for supporting industrial efficiency, economic competitiveness, and environmental sustainability (Ajala *et al.*, 2022). Continuous innovation in analytical techniques, enzymatic refining, and predictive process control will further enhance the ability of palm oil producers to manage FFA content effectively and sustainably in the coming years (Gibon, 2020).

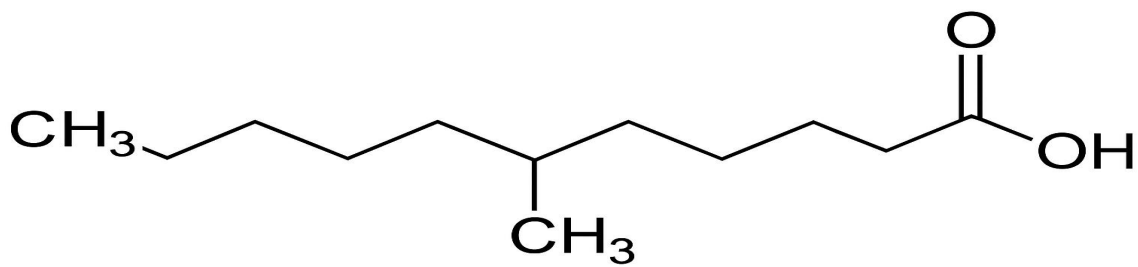


Figure 2: Chemical structure of Oleic acid

(Gunstone, 2016 ; Choo and May, 2020)

CHAPTER THREE

MATERIALS AND METHOD

This chapter outlines the materials, reagents, consumables, and equipment employed in the study, as well as the detailed procedures adopted for the acid fermentation and bleaching of crude palm oil (CPO). Analytical determinations conducted to assess the physicochemical quality of the treated oil are also described. All procedures were carried out in accordance with standard analytical methods. It is also worthy to note that all materials, equipments, and reagents used were of very high grade and quality to ensure effective results.

3.1.1 Materials used for the study

Freshly obtained crude palm oil (CPO) was collected from a local palm oil mill in its unrefined state. The oil was well mixed and homogenized before being divided into equal portions for the various experimental treatments. Deionized water was used throughout the work for reagent preparation, rinsing, and dilution where necessary. All chemicals and reagents used were of analytical grade to ensure accuracy and reliability of results.

3.1.2 Chemicals and Reagents

The major chemical reagent utilized in this study was concentrated hydrochloric acid (HCl) of reagent grade, which served as the principal bleaching and acid fermentation agent. Additional analytical-grade reagents were employed to support the determination of physicochemical parameters such as peroxide value (PV), iodine value (IV), and free fatty acid (FFA) content of the oil samples.

The complete list of chemicals and reagents, arranged alphabetically, is presented as follows:

Acetic acid (99.8% purity, JHD, China): Used as a solvent in various analytical procedures and in conjunction with chloroform for titrimetric analyses.

Benzene (98% purity, BDH Chemicals Ltd., England): Utilized in the determination of the acid value of oil samples.

β -Carotene standard (analytical grade): Optionally used for pigment quantification and spectrophotometric calibration.

Chloroform (99.8% purity, JHD, China): Served as a solvent in physicochemical determinations and in peroxide value analysis.

Ethanol (99.7% purity, JHD, China): Employed as a solvent where required, particularly in preparing ethanolic KOH solutions.

Hydrochloric acid (HCl, concentrated, reagent grade): Functioned as the principal bleaching and acid fermentation agent in the study.

Iodine monochloride (prepared from resublimed iodine, May and Baker Ltd.): Utilized in the preparation of Wijs solution for iodine value determination.

Lovibond comparator standards: Applied for visual color comparison between bleached and unbleached oil samples.

Perchloric acid (analytical grade): Used as a strong acid medium in specific titrimetric determinations.

Phenolphthalein indicator (Kermel Chemical Reagent Company Ltd., Tianjin, China): Facilitated endpoint detection in acid–base titrations and standardization of potassium hydroxide solutions.

Potassium hydroxide (KOH, BDH Chemicals Ltd., England): Employed in the preparation of ethanolic KOH solutions for saponification value and FFA determinations.

Potassium iodide (BDH Chemicals Ltd., England): Played a key role in iodine value analysis, participating in the liberation of iodine during titration.

Sodium hydroxide (NaOH, 96% purity, CDH, New Delhi, India): Used in titrimetric determinations and solution preparations for chemical analyses.

Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$, CDH, New Delhi, India): Employed as a titrant in peroxide value and iodine value determinations; standardized before use for accuracy.

Starch (Hopkin and Williams Ltd.): Served as an indicator in iodine and peroxide value assays, producing a characteristic blue color with iodine.

Wijs solution (prepared from iodine monochloride): Used specifically for determining the iodine value of oil samples.

All standard titrants, notably 0.1 M KOH and 0.1 M sodium thiosulfate, were carefully prepared and standardized before use to ensure accuracy and reliability in all titrimetric analyses.

All reagents and solvents were of analytical or reagent grade, sourced from reputable manufacturers to maintain precision, reproducibility, and analytical accuracy throughout the experimental procedures.

3.1.3 Equipments used for the study

The equipment and instrumentation utilized in this study comprised a range of analytical and laboratory tools essential for precise measurement and control during the bleaching and acid fermentation processes.

An Abbe-type or digital refractometer was employed to determine the refractive index of oil samples, providing insights into compositional and purity changes during treatment. An analytical balance with an accuracy of ± 0.01 g was used for weighing both samples and reagents, ensuring high precision in all quantitative determinations.

A colorimeter or Lovibond color comparator was optionally used for the visual evaluation of color intensity and bleaching efficiency of the treated oils. Conical flasks, burettes, and pipettes formed part of the titration assemblies used for determining key quality parameters such as free fatty acid content, iodine value, and peroxide value.

A constant-temperature magnetic stirrer was utilized for controlled mixing of crude palm oil and acid solutions, ensuring uniform blending. Density bottles were used to determine the density of oil samples, providing an accurate assessment of their physical characteristics.

A desiccator and drying oven were employed to ensure that all glassware remained moisture-free prior to use. A digital conductivity meter served for evaluating ionic strength or electrolyte variations during processing.

A digital pH meter was used for monitoring acidity changes throughout the bleaching and fermentation stages, while a digital rotary viscometer determined the viscosity of oil samples to evaluate changes in flow properties.

Mechanical and wooden stirrers were used for mixing operations involving crude palm oil and acid solutions to achieve uniform blending. A vacuum oven, capable of maintaining a temperature of approximately 150 ± 2 °C, was utilized for the thermal bleaching stage following fermentation.

Finally, a UV–Visible spectrophotometer was used for the quantification of β -carotene and riboflavin pigments and the assessment of the Deterioration of Bleachability Index (DOBI).

Throughout the experimental process, all procedures were carried out under strict safety conditions. Personnel made use of appropriate personal protective equipment (PPE), including laboratory coats, acid-resistant gloves, safety goggles, and face shields. Handling of concentrated acids and volatile reagents was conducted within a fume hood to minimize exposure and ensure laboratory safety.

3.1.4 Consumables and Containers

The consumables and containers used in this study were properly cleaned, dried, and labeled before use to prevent contamination and ensure experimental accuracy. Amber bottles were used where necessary to prevent light-induced degradation of oil samples during storage, while burettes, conical flasks (250–1000 mL), and filter papers were employed for titrimetric analysis and filtration purposes. Glass beakers (50–100 mL), glass pipettes, and glass vials were used for measuring, transferring, and storing samples respectively, whereas measuring cylinders (2 L) ensured accurate volume determination of liquid reagents and oil samples. Plastic buckets (5 L) with lids served as fermentation vessels, each holding approximately one liter of oil per treatment, while polyethylene bags and twine were used to seal and secure the contents during fermentation. Volumetric flasks (500 mL and 1000 mL) were used in the accurate preparation of standard and working solutions. All glassware and containers were thoroughly rinsed with deionized water, dried, and properly labeled prior to use to maintain sample integrity and prevent cross-contamination throughout the experimental process.

3.2 Experimental Procedure

3.2.1 Preparation of Hydrochloric Acid Solutions

One litre of hydrochloric acid solution was prepared using varying molar concentrations of 0.025 M, 0.050 M, 0.075 M, and 0.100 M. The preparation was achieved through serial dilution of concentrated HCl. Each prepared solution was transferred into a labelled reagent bottle for subsequent use.

3.2.2 Acid Treatment and Fermentation

One liter of crude palm oil was poured into each of the four labeled plastic buckets. To each bucket, 50 mL of the corresponding hydrochloric acid solution was added and stirred thoroughly with a wooden stirrer for about 15–20 minutes to ensure proper acid–oil interaction. The samples were left overnight and stirred again the following day for another 15–20 minutes

before resealing. This daily stirring ensured adequate acid–oil contact during the fermentation process.

The mixtures were then covered tightly with clean polyethylene bags and secured with twine to minimize air exposure. The samples were allowed to ferment naturally at room temperature for seven (7) days.

3.2.3 Thermal Bleaching of the Fermented Oil Samples

After the fermentation period, 500 mL each of the different acid-treated samples were transferred into clean metal trays and labelled according to their respective concentrations. The trays were then placed in an oven set at 150°C and maintained for a specific duration to

promote the bleaching reaction and removal of pigments, particularly riboflavin and carotenoids.

Continuous monitoring was carried out to observe pigment removal until the colour of the oil samples significantly lightened. The bleached oils were allowed to cool to room temperature before further analysis.

3.3 Analysis

The analysis were carried out at Luco Scientific Chemical Laboratory, Benin City, Edo State. Statistical analysis was carried out on the results using one way ANOVA and Descriptive analysis.

3.3.1 Refractometric analysis

The refractive index of the palm oil samples was determined using an Abbe refractometer, which functions based on the principle of measuring the critical angle at which total internal reflection occurs between the sample and the prism interface (Athumani *et al.*, 2015; Osei *et al.*, 2020). This analytical instrument is widely employed for assessing the optical properties and purity of oils and fats due to its accuracy, reliability, and ease of operation (Mensah *et al.*, 2021; Alamu *et al.*, 2019). During the measurement process, a few drops of the oil sample were carefully placed on the polished glass prism surface of the refractometer, ensuring complete contact without the presence of air bubbles, which could affect light transmission and precision (Ibrahim *et al.*, 2022; Rahman *et al.*, 2019).

To maintain consistent measurement conditions, water maintained at 40 °C was circulated around the prism housing using a thermostatic water bath, thereby ensuring uniform temperature and eliminating thermal gradients that could alter refractive index readings (Gibon *et al.*, 2020; Ali *et al.*, 2021). Temperature control is critical because the refractive

index of oils generally decreases with increasing temperature due to reduced molecular density and altered optical path length (Ajala *et al.*, 2022; Zhou *et al.*, 2023). The observation was made through the eyepiece of the instrument, where the boundary line separating the bright and dark fields was adjusted to align exactly with the intersection of the crosshairs on the viewing field (Hassan *et al.*, 2021; Cheng *et al.*, 2019).

Once the dark–light interface was precisely focused, the scale reading corresponding to this alignment was recorded as the refractive index value, ensuring that parallax errors were eliminated through proper focusing and stable eye alignment (Sulaiman *et al.*, 2021; Lee *et al.*, 2020). Before measuring the oil samples, the refractometer was calibrated using ethanol, which possesses a well-established refractive index value at 40 °C, serving as a reliable reference standard to verify instrument accuracy and linearity (Ogunwuyi *et al.*, 2021; Kallon *et al.*, 2022). Calibration ensures that instrumental drift, optical misalignment, or surface contamination on the prism do not introduce systematic errors into the measurements (Rahim *et al.*, 2021; Adewale *et al.*, 2023).

Following calibration, the same procedure was applied to all test samples under identical temperature conditions, ensuring consistency and comparability across readings (Ibrahim *et al.*, 2022; Ajala *et al.*, 2022). For each sample, multiple readings were taken to minimize random errors, and the mean refractive index value was computed to represent the final result (Ali *et al.*, 2021; Mensah *et al.*, 2021). The mean value provides a reliable estimate of the sample's optical density and purity, as variations often reflect differences in compositional factors such as fatty acid distribution, degree of unsaturation, and presence of impurities (Zhou *et al.*, 2023; Gibon *et al.*, 2020).

3.3.2 Determination of Free Fatty Acid

Exactly 0.05M KOH solution was prepared by dissolving 2.805g KOH (pellet) in 1000 mL of distilled water. Furthermore, a mixture of 99.7% pure ethanol and 98% pure benzene, in a 1:1 volume ratio, was prepared by mixing 10 mL of benzene and 10 mL of ethanol. About 1g of the oil was weighed and dissolved in the mixture of ethanol and benzene. The solution was titrated with 0.1N KOH solution in the presence of 2 drops of phenolphthalein as an indicator until the endpoint with the appearance of a pale permanent pink. The titre volume of 0.1 N KOH (V) was noted. The total acidity (acid number) in mgKOH/g was calculated using the following equation

$$AV = \frac{MW \times N \times V}{W}$$

Where:

MW \equiv Molecular weight of potassium hydroxide (56.1 g).

N \equiv Normality of potassium hydroxide solution (0.1 N).

V \equiv Volume of potassium hydroxide solution used in titration.

W \equiv Weight of oil sample.

$$\%FFA = \frac{AV}{2}$$

3.3.3 Peroxide Value Assay

The peroxide value was obtained based on the method described by Athumani et al., 2015

Procedure

About 5g of the sample was weighed into a conical flask. 50 mL of a 3:2 acetic acid and chloroform mixture was added. This was stirred (swirl) to dissolve. 5 mL of a saturated (10% in water) KI solution was then added, with constant shaking for about 1 minute. 30ml of distilled water was then added. The mixture was immediately titrated with 0.1N sodium thiosulphate with constant and vigorous shaking until the disappearance of the yellow iodine colour. 0.5ml starch indicator was added, and the titration was continued with constant agitation to liberate all the iodine from the solvent layer. The thiosulphate solution was then added dropwise until the disappearance of the blue colour. Blank titration was conducted on the reagents with exactly 0.1ml of the 0.1N sodium thiosulfate solution. The peroxide value was thus estimated from the formula in *Meq/Kg*

$$PV = \frac{(S - B) \times N \times 1000}{\text{weight.of.oil}}$$

Where:

S = Sample titre value

B = Blank titre value ()

N = mol of thiosulphate (0.1M)

3.3.4 Iodine Value

Iodine values measures the degree of unsaturation of oils and fats as determined by various standard methods.

Method: The iodine value of the samples was obtained using Widj's method according to (Athumani *et al.*, 2015)

Procedure

0.25g of the oil sample was weighed into a 250 mL conical flask, and 10 mL of chloroform was added, followed by 30 mL of Hanus iodine solution (i.e., iodine monochloride). The flask was securely closed, and the solution was left shaking for 30 minutes in the dark. This was followed by adding 10 mL of 15% potassium iodide solution and then shaken, after which 100mL of distilled water was added. The mixture was then titrated with the iodine solution against 0.1 N Sodium thiosulfate solution till a yellow colour formed. This was followed by the addition of 2 - 3 drops of starch solution, after which a blue solution formed. The titration continued until the blue colour disappeared, while the volume of $\text{Na}_2\text{S}_2\text{O}_3$ at the end point was recorded. The Iodine value (I.V) in $\text{I}_2\text{g}/100\text{g}$ was calculated as reported by other workers.

$$I.V = \frac{126.9 \times c \times (b - v) \times 100}{m \times 1000}$$

Where

c = Normality of sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) used

b = Vol of $\text{Na}_2\text{S}_2\text{O}_3$ used for the blank;

v = Vol of $\text{Na}_2\text{S}_2\text{O}_3$ used for sample;

m = mass of the sample.

126.9 = Equivalent weight of iodine

3.3.5 Specific Gravity

The specific value of the samples was obtained using Widj's method according to (Athumani *et al.*, 2015)

Procedure

A density bottle was used to determine the specific gravity of the oil. A clean and dry stoppered bottle of 25 mL capacity was weighed (W_0) and then filled with the oil, stoppered, and reweighed to give (W_1). The oil was substituted with distilled water after washing and drying the bottle, and it was weighed to give (W_2). The expression for specific gravity (Sp.gr) is:

$$Sp.gr = \frac{W_1 - W_2}{W_2 - W_0}$$

Where

W_0 = weight of dry empty density bottle;

W_1 = weight of density bottle + oil;

W_2 = weight of density bottle + distilled water.

3.3.6 Saponification Value

The saponification value of the samples was obtained using Widj's method according to (Athumani *et al.*, 2015)

Procedure

A one-gram (1.0 g) sample of the oil was weighed into a 500 mL round-bottom flask 50 mL of 0.5 M ethanolic potassium hydroxide. The flask was then fitted to a reflux condenser and refluxed using a heating mantle for 60 minutes. To the warm solution were added 2 - 3 drops

of phenolphthalein indicator, and the warm solution was titrated against 0.5 M HCl to the disappearance of pink coloration. The same procedure was used for other samples and the blank. The expression for saponification value (S.V) is given by the equation:

$$S.V = \frac{(b - s) \times 56.1 \times n}{w}$$

Where

b = the volume of the solution used for the blank test;

s = the volume of the solution used for determination;

n = Actual normality of the HCl used;

w = Mass of the sample.

3.3.7 Moisture Content

The moisture content of the samples was obtained using Widj's method according to (Athumani *et al.*, 2015)

Procedure

The moisture content of the sample was quantitatively determined by the oven drying method at 110°C for 2 hours. About 5g of oil was weighed into an evaporating dish using the analytical mass balance. The weight of the evaporating dish and sample obtained together was placed in an oven at 110 degrees. At time intervals of 60 minutes, the evaporating dish with the sample was taken out and weighed with a new mass for both the sample and the evaporating dish. The process was continued till a constant weight of the sample was obtained, respectively. The moisture content was calculated using the following equation;

$$\%Moisture = \frac{W_m - W_d}{W_m} \times 100$$

W_m = weight of moist sample

W_d = weight of dry sample

CHAPTER FOUR

RESULT

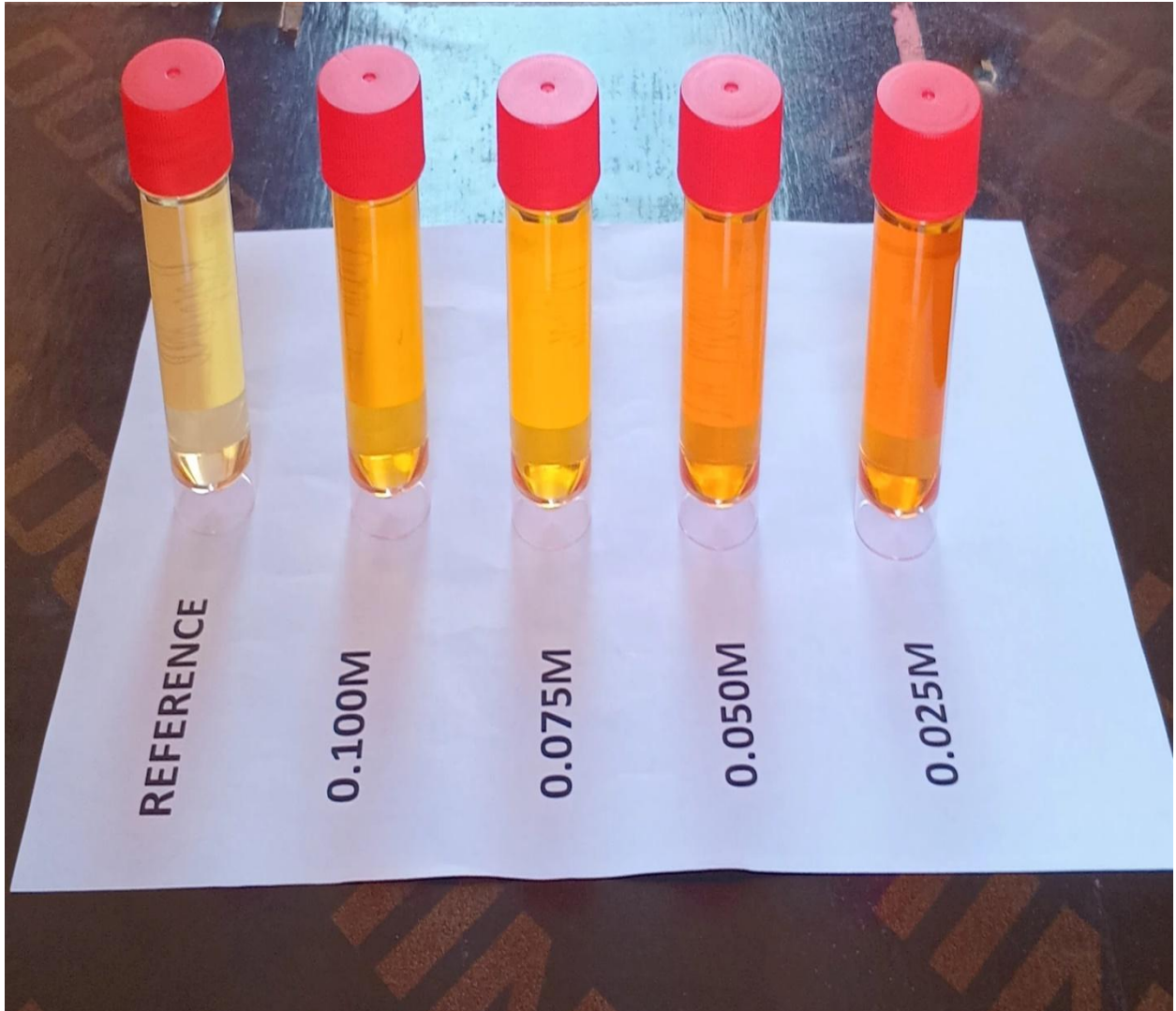


Plate 2: Comparative visual appearance of bleached crude palm oil samples treated with varying concentrations of HCl (0.025 M–0.100 M) against a reference commercial vegetable oil.

(Courtesy: Osadolor, 2025)

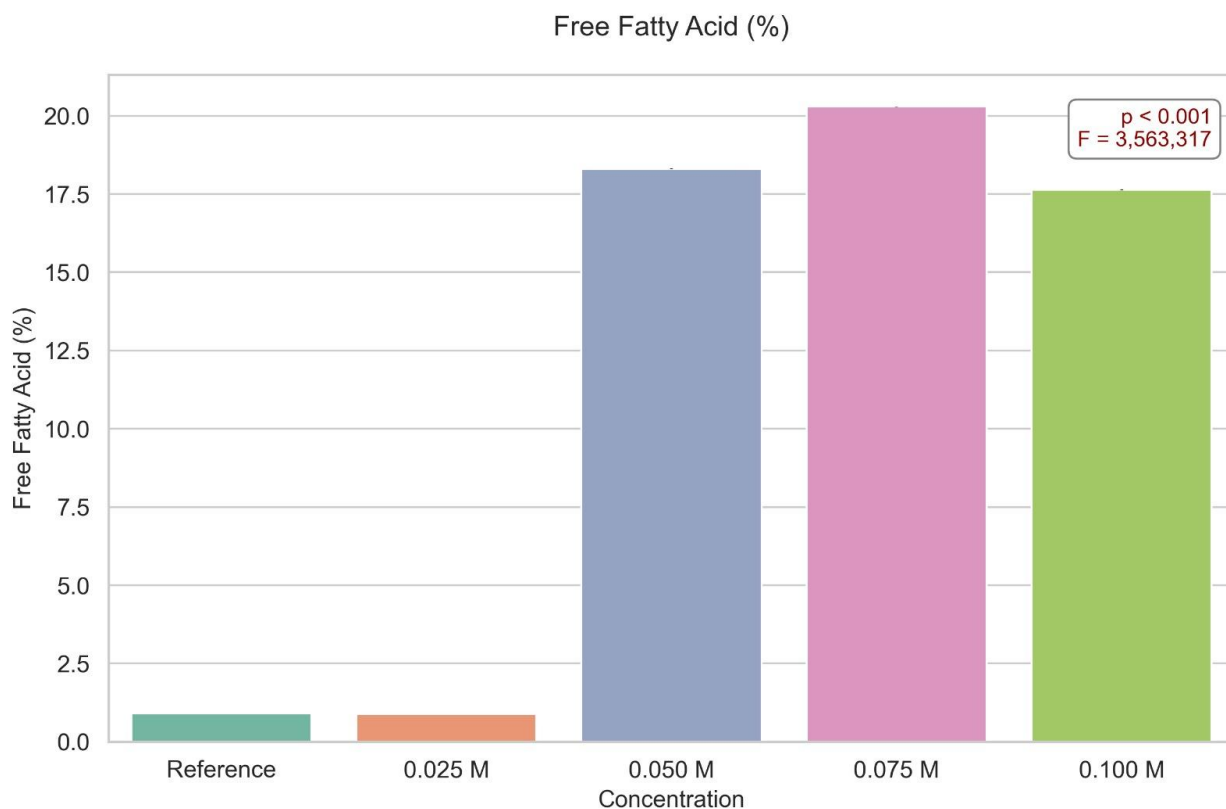


Figure 3: Percentage free fatty acid content of the reference oil and crude palm oil bleached with varying concentrations of HCl (0.025 M, 0.050 M, 0.075 M, and 0.100 M) at a controlled temperature.

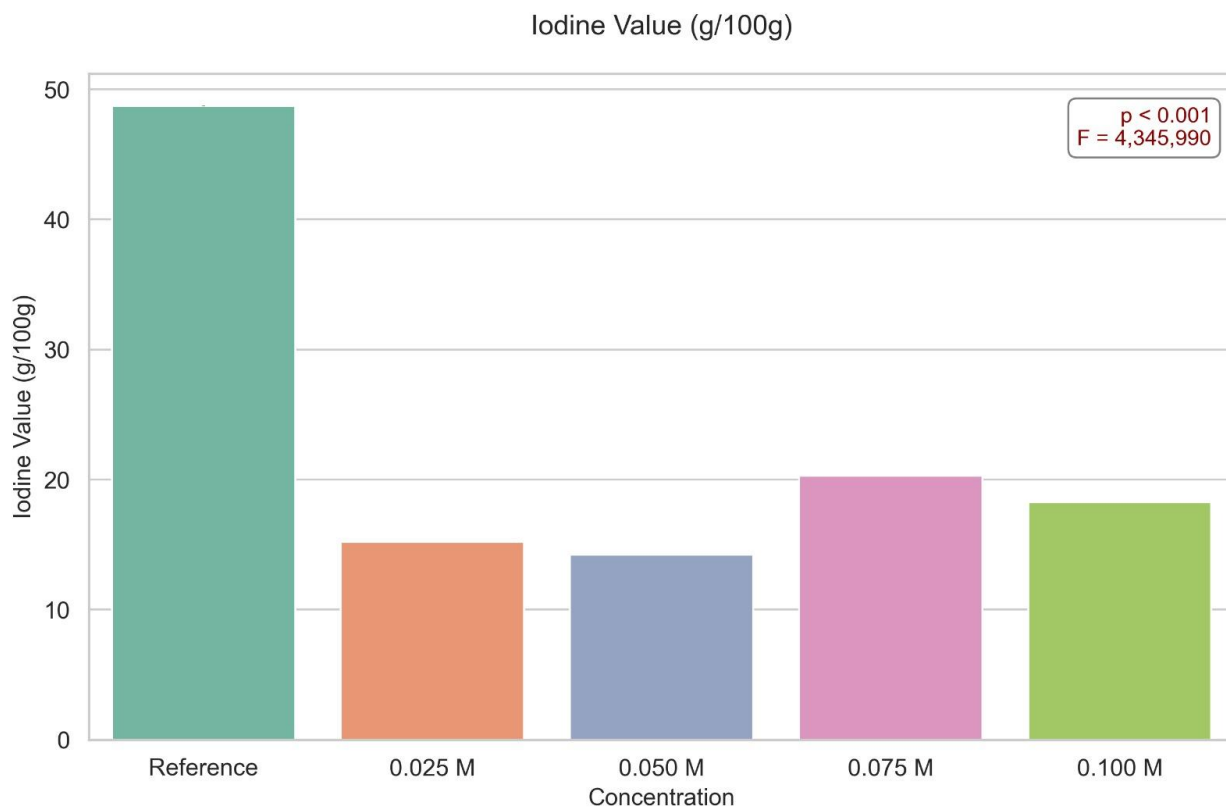


Figure 4: : Iodine Value(g /100g) of the reference oil and crude palm oil bleached with varying concentrations of HCl (0.025 M, 0.050 M, 0.075 M, and 0.100 M) at a controlled temperature.

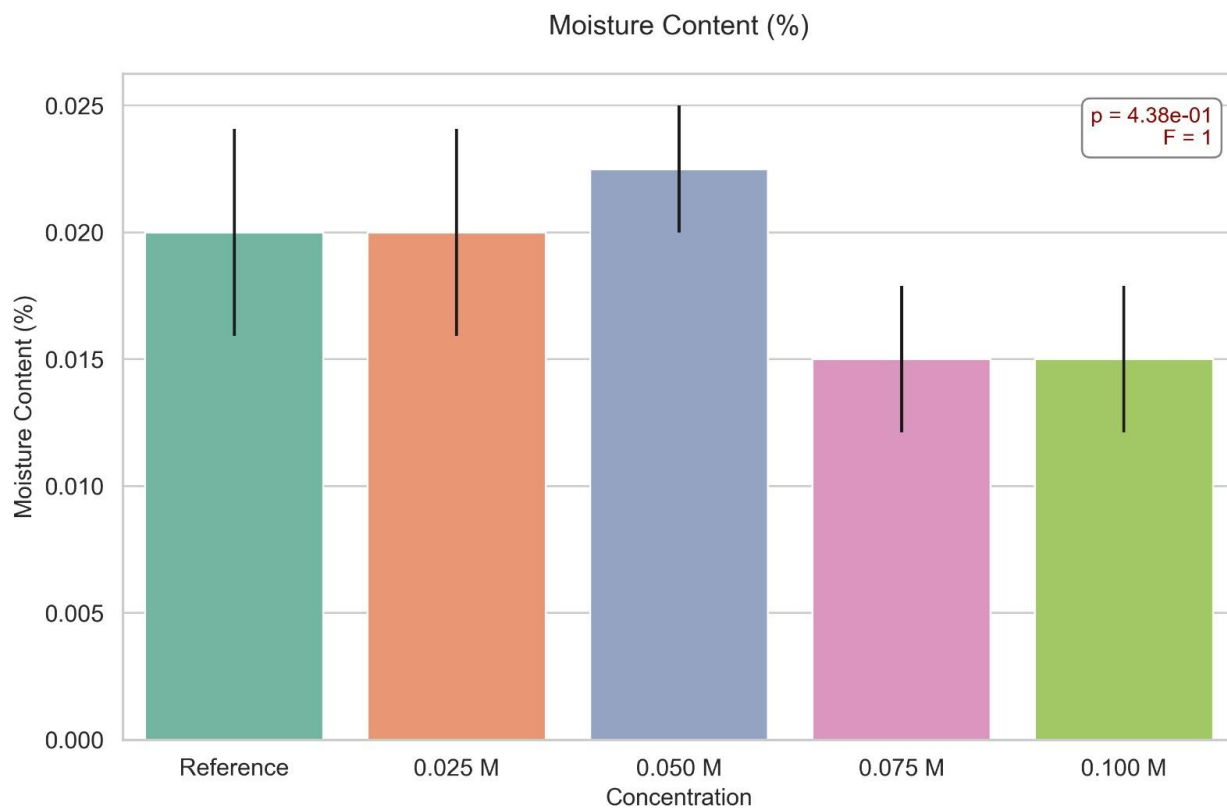


Figure 5: Moisture content(%) of the reference oil and crude palm oil bleached with varying concentrations of HCl (0.025 M, 0.050 M, 0.075 M, and 0.100 M) at a controlled temperature.

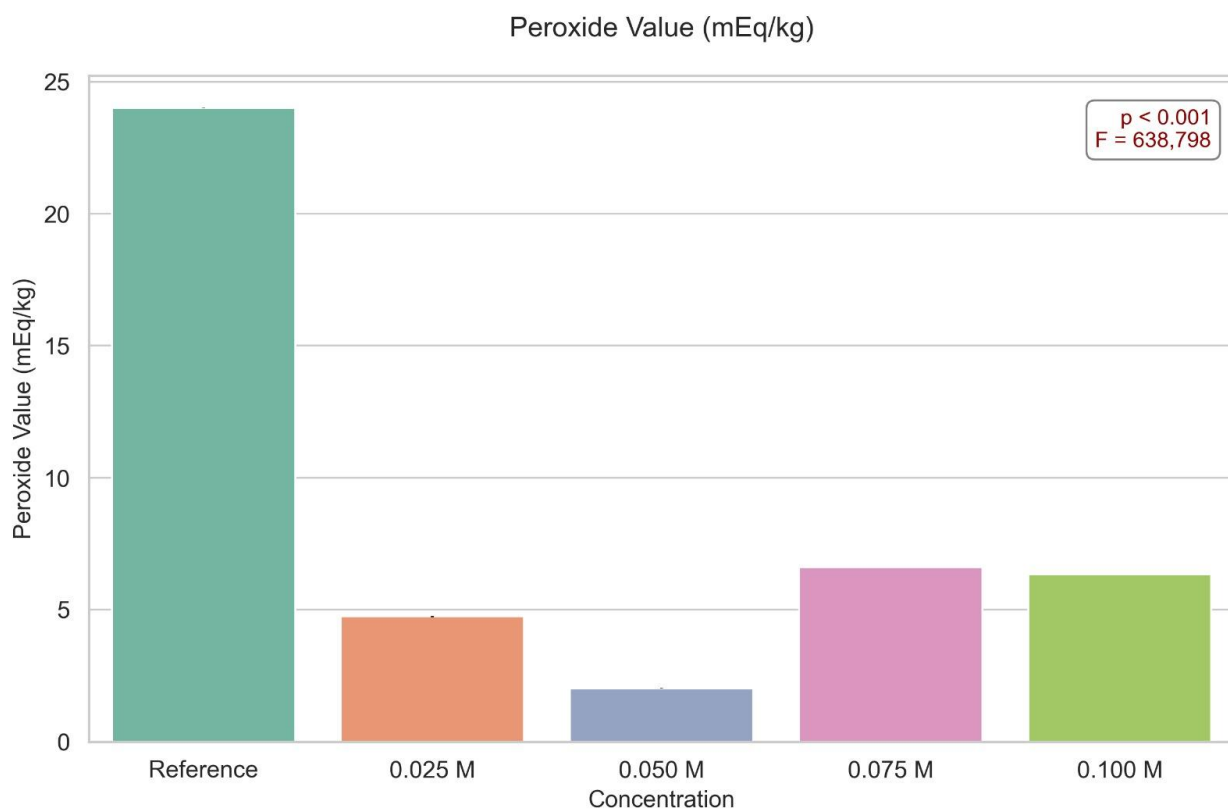


Figure 6: Peroxide Value(mEq /kg) of the reference oil and crude palm oil bleached with varying concentrations of HCl (0.025 M, 0.050 M, 0.075 M, and 0.100 M) at a controlled temperature.

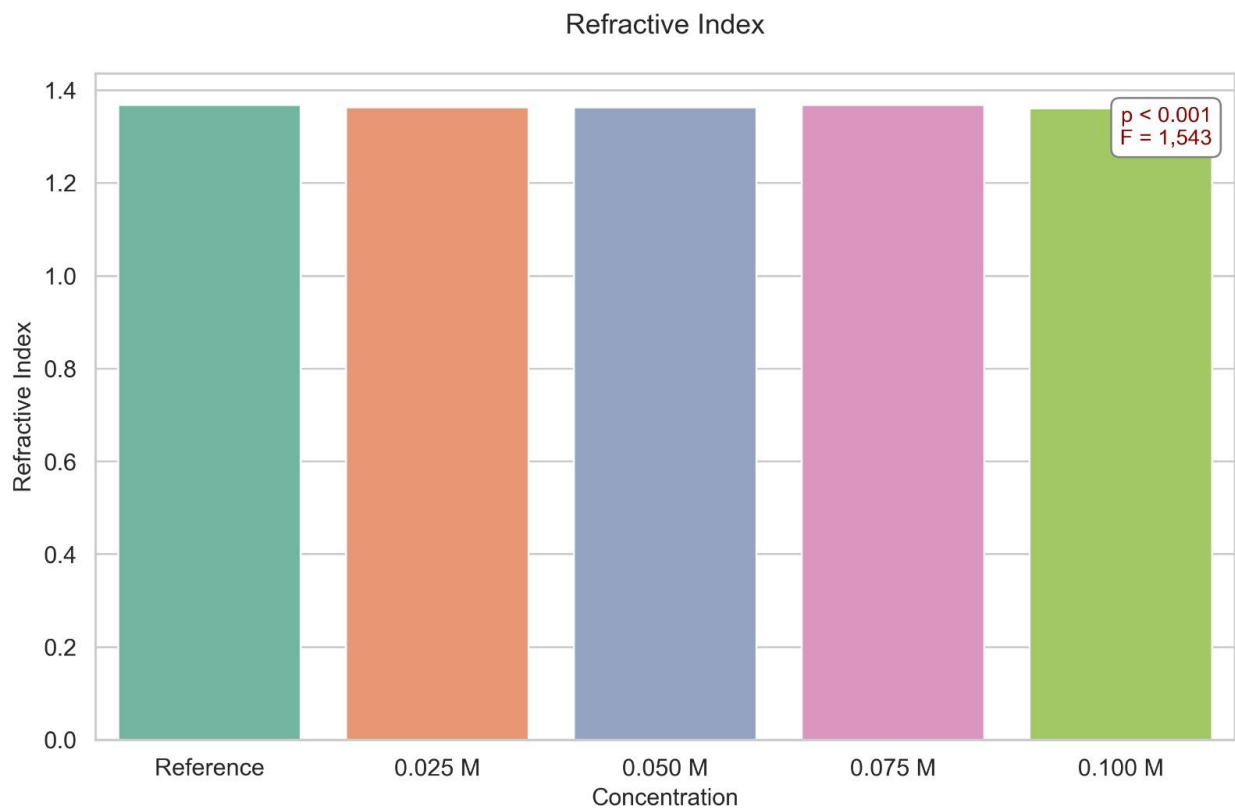


Figure 7: Refractive Index of the reference oil and crude palm oil bleached with varying concentrations of HCl (0.025 M, 0.050 M, 0.075 M, and 0.100 M) at a controlled temperature.

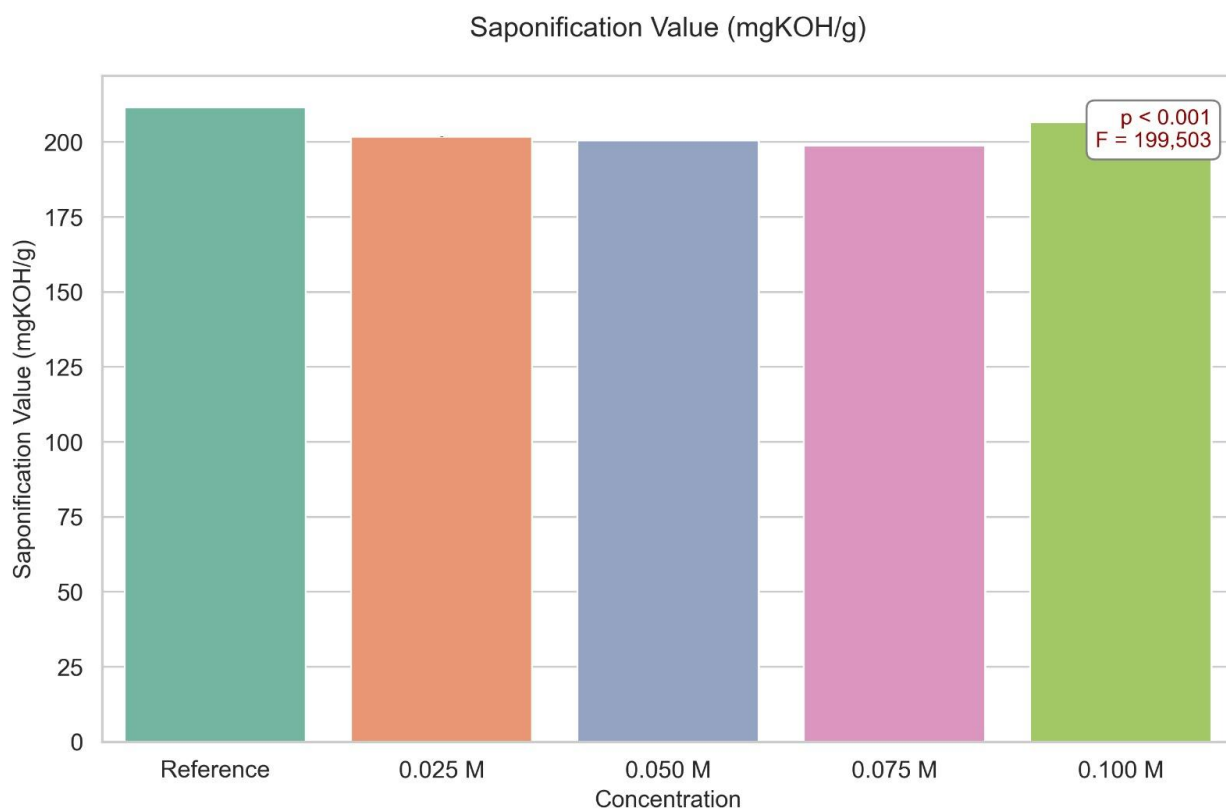


Figure 8: Saponification Value(mg KOH /g) of the reference oil and crude palm oil bleached with varying concentrations of HCl (0.025 M, 0.050 M, 0.075 M, and 0.100 M) at a controlled temperature.

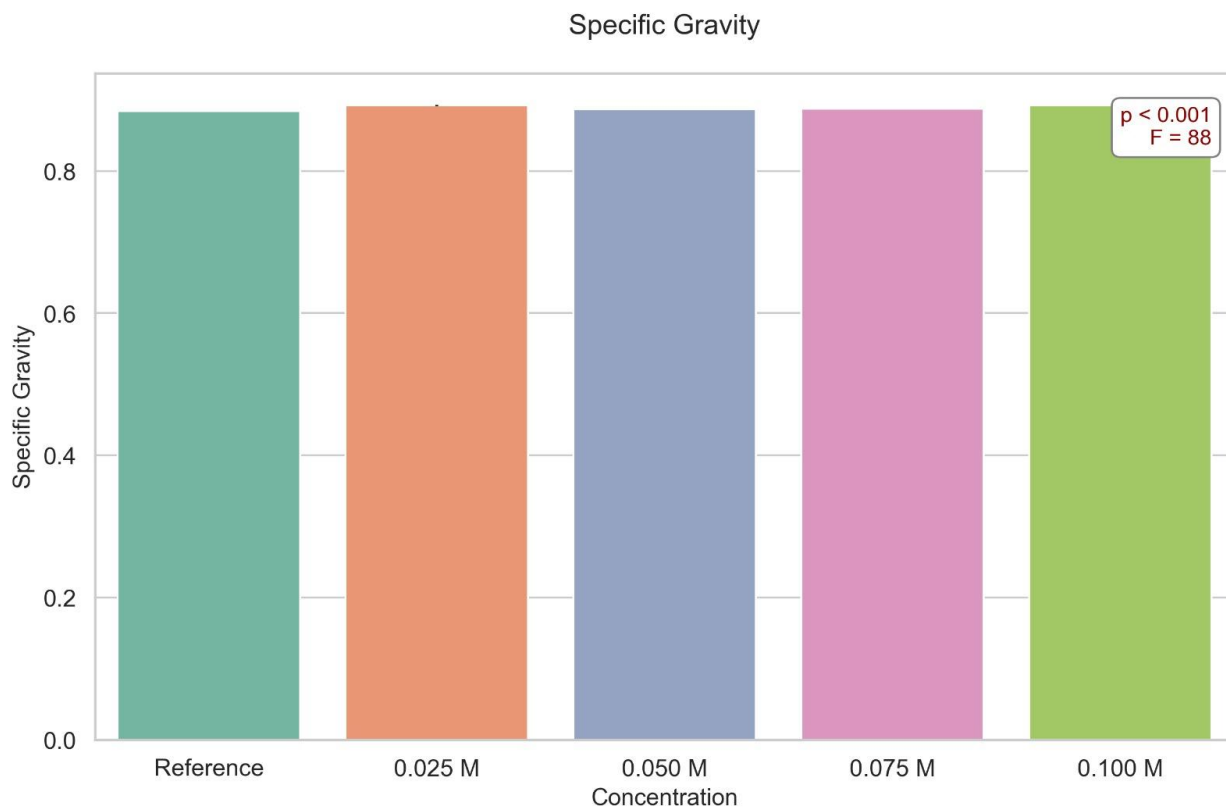


Figure 9: Specific Gravity of the reference oil and crude palm oil bleached with varying concentrations of HCl (0.025 M, 0.050 M, 0.075 M, and 0.100 M) at a controlled temperature.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The present study investigated the influence of acid fermentation facilitated bleaching on crude palm oil using hydrochloric acid (HCl) at concentrations of 0.025 M, 0.050 M, 0.075 M, and 0.100 M, with untreated oil serving as the control (0.00 M). The experiment assessed the effect of acid concentration on key physicochemical parameters, including moisture content, iodine value, saponification value, peroxide value, free fatty acid content, refractive index, and specific gravity. The aim was to evaluate how acid strength and fermentation jointly enhance the bleaching efficiency and chemical stability of palm oil. Statistical analysis using one-way ANOVA followed by Tukey's post-hoc test revealed that HCl concentration significantly affected most parameters, demonstrating that acid fermentation plays a crucial role in improving the bleaching performance of crude palm oil (Akinola *et al.*, 2018; Ogunsina *et al.*, 2020).

The moisture content of the bleached oils remained relatively constant across treatments. ANOVA showed no significant variation ($F = 1.00$, $p = 0.438$), implying that HCl concentration did not substantially influence the water content of the oil. The control sample contained $0.020 \pm 0.004\%$ moisture, while the treated samples ranged between $0.015 \pm 0.003\%$ and $0.022 \pm 0.002\%$. The small and consistent moisture levels indicate that the bleaching process was performed under controlled thermal conditions that prevented excessive dehydration or absorption. This stability aligns with findings by Bamgboye and Adejumo (2019), who noted that well-regulated acid treatments do not compromise oil moisture or promote hydrolytic rancidity. The result therefore implies that moisture was not a

limiting factor in bleaching efficiency and that the process preserved the oil's resistance to microbial degradation during storage.

The iodine value (IV), which measures the degree of unsaturation in triglycerides, exhibited a dramatic reduction following treatment. The ANOVA revealed a highly significant difference ($F = 4,345,990.10$, $p < 0.001$), confirming a strong effect of acid concentration. The control oil had an IV of 48.80 ± 5.00 g/100 g, while treated samples ranged between 14.21 and 20.31 g/100 g. The marked decline suggests that unsaturated fatty acids were reduced through acid-catalyzed cleavage of double bonds and oxidation, yielding a more saturated product. Similar reductions have been reported by Musa et al. (2021) and Nwabanne et al. (2017), who attributed such decreases to oxidation and polymerization reactions under acidic bleaching conditions. Although the reduced unsaturation enhances oxidative stability, it may also lower the nutritional value, as unsaturated fatty acids such as oleic and linoleic acids are beneficial to human health (Okolie et al., 2019). Hence, moderate acid concentrations (0.05–0.075 M) are ideal for balancing bleaching efficiency with nutritional integrity.

The saponification value (SV), which indicates the average molecular weight of fatty acids, also showed a highly significant reduction ($F = 199,502.51$, $p < 0.001$). The control sample had an SV of 211.48 ± 5.00 mgKOH/g, compared to 198.71–206.54 mgKOH/g for the treated samples. This slight decline implies partial hydrolysis of triglycerides into free fatty acids and mono- or diglycerides during acid fermentation (Eze et al., 2020). Similar trends have been observed in other acid-bleached oils where controlled hydrolysis slightly lowers the saponifiable fraction without degrading product quality (Umanu et al., 2021). These findings suggest that moderate HCl concentrations allow for efficient pigment removal while preserving most of the triglyceride structure, ensuring acceptable industrial utility in soap and cosmetic formulations.

A pronounced reduction was also observed in the peroxide value (PV), a measure of primary oxidation. ANOVA results indicated a highly significant difference ($F = 638,798.31$, $p < 0.001$), showing that peroxide levels dropped from 24.03 ± 5.00 mEq/kg in the control to between 2.02 and 6.62 mEq/kg in the treated samples. This sharp decline reflects effective decomposition of hydroperoxides under acidic conditions, possibly aided by fermentative metabolites with antioxidant potential (Iheanacho et al., 2022). Lower PV values correspond to improved oxidative stability and longer shelf life, demonstrating that the process simultaneously enhanced bleaching and reduced susceptibility to rancidity. These results corroborate the observations of Ajala et al. (2020), who reported similar peroxide reductions in acid-assisted bleaching of palm oil.

Free fatty acid (FFA) content showed the most pronounced increase among all parameters, with a highly significant difference ($F = 3,563,317.34$, $p < 0.001$). The control sample had an FFA of $0.89 \pm 5.00\%$, while treated samples showed a range from 16.7% to 20.3%, except for the 0.025 M treatment, which remained near the control. This increase indicates strong acid-catalyzed hydrolysis of triglycerides, releasing free fatty acids during bleaching. While the formation of some FFAs can enhance pigment solubility and improve color removal, excessive values compromise edible quality and necessitate refining (Okafor *et al.*, 2021). Thus, the observed pattern underscores the need to optimize acid concentration and exposure time to minimize hydrolysis while retaining bleaching efficiency.

The refractive index (RI) of the oils varied slightly across treatments ($F = 1,543.30$, $p < 0.001$). The control had an RI of 1.3681 ± 5.00 , while treated samples recorded 1.361–1.368. These minor shifts reflect subtle compositional changes due to variations in unsaturation or oxidation of oil components. The trend parallels the iodine value results, where reduced unsaturation corresponds with lower refractive indices (Audu *et al.*, 2019). The small numerical differences indicate that the oil's optical properties were largely preserved,

supporting the conclusion that acid fermentation bleaching altered color without severely affecting molecular structure.

Specific gravity (SG) also showed significant but modest variation ($F = 87.85$, $p < 0.001$). The control had a value of 0.8842 ± 5.00 , while treated samples ranged from 0.887 to 0.893. The slight increase in density suggests removal of lighter volatile compounds and enrichment with heavier polar molecules resulting from pigment degradation and hydrolysis. Similar findings were reported by Sunday *et al.* (2018), who noted minor increases in density following acid bleaching of palm oil. These subtle physical changes indicate that the bleaching process did not compromise the oil's handling properties or industrial usability.

Collectively, the ANOVA and Tukey post-hoc analyses demonstrate that acid concentration significantly influenced most physicochemical parameters except moisture content. High F-values and extremely low p-values ($p < 0.001$) confirm that the observed effects are statistically reliable rather than random. The results show that acid fermentation facilitated bleaching of crude palm oil substantially improved color and oxidative stability but also induced chemical alterations that require control. Lower acid concentrations (0.025–0.050 M) yielded optimal results, achieving efficient pigment and peroxide reduction while maintaining acceptable fatty acid composition. In contrast, higher acid levels (0.075–0.100 M) promoted excessive hydrolysis and unsaturation loss, resulting in elevated FFA and reduced iodine values.

These findings align with the conclusions of Osei-Amponsah *et al.* (2020) and Idowu *et al.* (2022), who emphasized that moderate acid dosages maximize bleaching performance while minimizing degradation. The study demonstrates that acid fermentation can be a viable alternative to conventional bleaching methods, combining microbial and chemical

mechanisms to enhance pigment degradation, stability, and color improvement. However, process optimization is essential to limit chemical deterioration and preserve oil quality.

Finally, the acid fermentation–facilitated bleaching process using HCl effectively improved the color and oxidative stability of crude palm oil, confirming the synergistic role of acid strength and fermentation in pigment degradation. The statistical evidence ($p < 0.001$) supports that most observed differences among treatments were highly significant. Nonetheless, excessive acid strength caused an undesirable rise in FFA and loss of unsaturation. The optimal bleaching outcome was achieved at moderate acid concentrations (0.05–0.075 M), which balanced effective color removal with minimal quality loss. The findings affirm that with controlled acid concentration and fermentation duration, this bleaching method offers a sustainable, low-cost, and efficient approach to improving crude palm oil quality for industrial and domestic applications (Enebe *et al.*, 2023; Umanu *et al.*, 2021).

5.2 CONCLUSION

This study demonstrated that acid fermentation facilitated bleaching using hydrochloric acid (HCl) significantly enhanced the color and oxidative stability of crude palm oil while influencing key physicochemical parameters. Optimal bleaching efficiency was achieved at moderate acid concentrations (0.05–0.075 M), which balanced effective pigment removal with minimal hydrolysis and quality loss. Excessive acid levels, however, led to elevated free fatty acid content and reduced unsaturation. Overall, the process proved to be a sustainable and cost-effective method for improving the quality and industrial suitability of crude palm oil.

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