ALKALI- FERMENTION FACILITATED METHOD FOR BLEACHING CRUDE PALM OIL(CPO)

BY

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LSC2010027

(BIOCHEMISTRY TECHNIQUES)

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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A PROJECT WORK TO BE SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCE, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA. IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELORS OF SCIENCE DEGREE (B.SC) IN SCIENCE LABORATORY TECHNOLOGY (BIOCHEMISTRY TECHNIQUES).

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this Project work, titled "Alkali Fermentation Facilitated Bleaching of Crude Palm Oil (CPO)" was carried out by Solomon Osahon IDAHOSA with Matriculation Number, LSC2010027 in the Department of Science Laboratory Technology, Faculty of Life Science, University of Benin, Benin City, Edo State.

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DEDICATION

This work is dedicated to God Almighty, whose grace, wisdom, and strength sustained me throughout the course of this study. His unfailing love, divine guidance, and endless mercy gave me the courage to overcome every challenge and complete this research successfully.

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TABLE OF CONTENTS

LIST OF FIGURES

LISTS OF PLATES

LISTS OF TABLES

ABSTRACT

This study investigated the bleaching of crude palm oil (CPO) using an alkali-fermention facilitated process as a sustainable alternative to conventional clay bleaching process. Crude palm oil samples were treated with sodium hydroxide (NaOH) at varying concentrations of 0.025 M, 0.050 M, and 0.075 M, followed by a controlled fermentation period. This study examined the bleaching efficiency and quality improvement of crude palm oil (CPO) using an alkali-fermention facilitated process across varying sodium hydroxide (NaOH) concentrations (0.025 M, 0.050 M, and 0.075 M). Physicochemical analysis of crude palm oil (CPO) bleached using an alkali-fermention facilitated process revealed distinct variations across sodium hydroxide (NaOH) concentrations of 0.025 M, 0.050 M, and 0.075 M compared with a reference sample. The pH ranged from 7.00 ± 0.01 – 7.00 ± 0.01 across treatments, showing negligible variation, suggesting stable acidity and effective neutralization during alkali fermentation. Sugar content values were consistent across treatments, ranging from 0.006 ± 0.004 mg/L for 0.025 M, 0.050 M, and 0.075 M NaOH, indicating minimal fermentable carbohydrate alteration during the bleaching process. The Beta-carotene concentration, used as a bleaching efficiency indicator, declined sharply from 200.94 ± 0.10 mg/kg (0.025 M) and 198.09 ± 0.06 mg/kg (0.050 M) to 122.62 ± 0.03 mg/kg (0.075 M), demonstrating a significant pigment degradation and enhanced color lightening at higher alkali concentration. Viscosity increased progressively from 31.30 ± 0.07 mPa·s (0.025 M) to 33.70 ± 0.07 mPa·s (0.075 M) compared with 37.90 ± 0.07 mPa·s in the reference sample, indicating reduced impurity load and improved oil flow characteristics after bleaching. Protein content remained relatively constant across all treatments **(**0.006 ± 0.004 %**)**, suggesting limited influence of alkali concentration on nitrogenous material denaturation. The acid value decreased from 36.62 ± 0.01 mg KOH/g (0.025 M) to 33.71 ± 0.01 mg KOH/g (0.075 M) relative to 1.57 ± 0.01 mg KOH/g in the reference oil, indicating significant hydrolysis and removal of free fatty acids under alkali conditions. Cholesterol content ranged narrowly between 0.012 ± 0.004 mg/kg (0.025 M)**,** 0.014 ± 0.005 mg/kg (0.050 M), and 0.014 ± 0.005 mg/kg (0.075 M), showing minor but consistent sterol reduction across treatments. Overall, the 0.050 M NaOH concentration achieved optimal bleaching, balancing pigment removal and oil stability while minimizing nutrient loss. The findings confirm that the alkali-fermention facilitated bleaching process offers an effective, eco-friendly, and sustainable alternative to conventional refining methods

CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION

The global demand for high-quality edible oils continues to grow, driven by population expansion, industrialization, and rising health awareness. Among the various vegetable oils, palm oil (Elaeis guineensis) remains the most widely produced and consumed, owing to its high yield per hectare, oxidative stability, and versatile industrial applications (Corley and Tinker, 2015). However, crude palm oil (CPO) in its unrefined form contains numerous impurities such as free fatty acids (FFAs), carotenoids, chlorophylls, phospholipids, trace metals, and oxidative by-products that impair its physicochemical integrity, consumer acceptability, and storage stability (Gibon, V. *et al.*, 2007).

Refining is therefore essential to render palm oil palatable, shelf-stable, and nutritionally safe. The refining process comprises degumming, neutralization, bleaching, and deodorization (AOCS, 2017). Among these, bleaching plays a critical role in removing colour pigments and oxidative impurities. Traditionally, acid-activated clays are employed for bleaching due to their large surface area and high adsorption capacity (Latip *et al.* 2013). However, their use presents environmental and economic drawbacks, including non-renewability, high cost, and co-removal of beneficial micronutrients such as tocopherols and phytosterols (Sundram *et al.*, 2003).

To address these challenges, attention has shifted toward bio-based and environmentally sustainable alternatives. One promising approach is the alkali-fermention facilitated bleaching process, which utilizes bio-adsorbents derived from renewable agro-wastes to improve pigment removal and reduce environmental impact (Ofori-Boateng and Lee, 2013).

This study, therefore, focuses on evaluating the physicochemical impact and bleaching efficiency of alkali-fermention facilitated processes on crude palm oil, with the goal of establishing an eco-friendly and cost-effective alternative to conventional methods. Parameters such as acid value, β-carotene, viscosity, protein, sugar, cholesterol, and pH were analyzed to determine the optimal alkali concentration for maximum bleaching efficiency and minimal nutrient loss. The work contributes to sustainable industrial biochemistry by integrating green process engineering principles into edible oil refining (Mukherjee and Mitra, 2009).

1.1 **BACKGROUND OF STUDY**

From a biochemical perspective, the presence of these impurities has implications for both lipid metabolism and nutritional quality. For instance, high FFA content increases oil acidity, leading to rancidity, while residual phospholipids and metals catalyze oxidative degradation (Gibon *et al.*, 2007). Moreover, the high concentration of β-carotene, which imparts the deep reddish-orange colour to CPO, though nutritionally valuable as a provitamin A, is often aesthetically undesirable in food-grade oil and needs to be reduced for commercial acceptance (Tanumihardjo, 2002).

Consequently, refining of palm oil is indispensable to render it palatable, shelf-stable, and safe for consumption. The refining sequence traditionally comprises degumming, neutralization, bleaching, and deodorization, each with distinct biochemical roles (American Oil Chemists' Society, 2017). Among these, bleaching is particularly significant, as it targets both the chromophoric compounds (carotenoids and chlorophylls) and a portion of oxidative and thermal degradation products (Latip *et al.*, 2013).

Historically, the adsorption bleaching method has relied heavily on acid-activated clays or natural earths due to their high porosity and surface charge. However, these materials are non-

renewable, generate significant adsorbent-oil waste, and contribute to environmental disposal concerns. Additionally, they sometimes result in the co-removal of beneficial micronutrients such as tocopherols and phytosterols, which possess antioxidant and health-promoting properties (Qureshi *et al.*, 2000).

In the pursuit of green chemistry approaches and cost-effective refining alternatives, attention is shifting towards biodegradable and renewable bio-adsorbents derived from agro-wastes rich in polysaccharides. These materials, when subjected to alkali treatment or alkali fermentation, exhibit enhanced adsorption capacity due to the development of functional binding groups (hydroxyl, carboxyl, phenolic), increased porosity, and elevated surface reactivity (Zakaria *et al.*, 2017). The alkali-facilitated process not only modifies surface chemistry of adsorbents but also supports microbial fermentation, which can further degrade residual contaminants and improve adsorbent specificity (Ofori-Boateng and Lee, 2013).

Moreover, this study aligns with the principles of industrial biochemistry, wherein bioresource utilization and process optimization are paramount. The use of alkali-fermention facilitated processes signifies a shift towards integrated biochemical engineering solutions that refine edible oils more sustainably and potentially recover valuable minor compounds, promoting a circular bioeconomy within the palm oil industry (Mba *et al.*, 2015; Corley and Tinker, 2015).

Therefore, this research seeks to systematically evaluate the efficacy of alkali-treated polysaccharide adsorbents, elucidate the mechanisms of pigment and impurity removal, and contribute to the ongoing discourse on environmentally responsible palm oil processing.

Palm oil has emerged as a cornerstone of global vegetable oil consumption, owing to its high yield, functional versatility, and relative economic viability. Crude palm oil (CPO), extracted

primarily through mechanical and thermal processing of the mesocarp of the oil palm (Elaeis guineensis), contains a rich blend of triacylglycerols, carotenoids, tocopherols, and other minor components that confer both nutritional and commercial value. However, alongside these desirable constituents, CPO also harbours biochemical impurities such as free fatty acids (FFA), phospholipids, chlorophylls, trace metals, and oxidation by-products, which necessitate a multi-stage refining process for oil stabilization, purification, and standardization (Gibon *et al.*, 2007).

The refining of edible oils, including palm oil, typically involves the sequential processes of degumming, neutralization (alkali refining), bleaching, and deodorization. Each step is strategically designed to remove specific classes of impurities, thereby enhancing the oil's sensory properties, shelf-life, and overall marketability. Among these, the bleaching step is of particular interest to industrial biochemists due to its dual function of colour reduction and adsorptive removal of pro-oxidants and degradation products (Che Man and Tan, 1999).

Traditionally, bleaching is accomplished using acid-activated clays, which possess a large surface area and high ion-exchange capacity. These clays adsorb not only pigments such as Beta-carotene and chlorophyll, but also peroxides, residual soaps, and trace transition metals (such as Fe^{2+}, Cu^{2+}) that catalyze lipid oxidation. While effective, the use of such materials presents challenges: Environmental burden due to spent adsorbent disposal, Oil retention within the adsorbent matrix leading to product loss, High cost of regeneration and procurement of imported bleaching earths and non-selective adsorption that may strip away valuable nutraceutical components such as tocotrienols and phytosterols.

In response to these limitations, recent research has focused on the development of bio-based bleaching agents, particularly those derived from natural polysaccharide-rich agricultural wastes,

including plantain peels, cassava starch residues, and palm oil mill effluent biomass (Akinola *et al.*, 2019; Ofori-Boateng and Lee, 2013). These materials are biodegradable, locally sourced, and economically feasible, aligning with the global shift toward sustainable refining practices.

Polysaccharides, by their biochemical nature, offer functional groups (–OH, –COOH, –NH_2) capable of interacting with oil impurities via adsorption, ion exchange, and hydrogen bonding mechanisms. However, their native structural rigidity and low surface area often limit their adsorptive performance. To address this, chemical activation through alkali treatment (commonly using NaOH or KOH) is employed to enhance surface area, pore volume, and introduce reactive functional moieties, transforming them into more effective adsorbents (Zakaria *et al.*, 2017).

The concept of alkali-facilitated bleaching further intersects with microbial fermentation in some approaches, where alkali-preconditioned materials undergo controlled fermentation to release biosurfactants, chelating agents, or enzymes capable of breaking down oil-soluble complexes. While fermentation is more commonly studied in oil extraction and degumming phases, the biochemical activation of adsorbents via alkali and/or fermentation opens promising avenues for multi-functional bleaching systems.

This background establishes the rationale for investigating the effectiveness of alkali-treated polysaccharide materials in bleaching crude palm oil, as well as understanding the biochemical implications of such treatments on the physicochemical properties of the refined oil such as acid value, saponification value, oxidative stability, and carotene content.

1.2 AIM AND OBJECTIVES OF THE STUDY

1.2.1 Aim of the Study

The aim of this study is to investigate the physicochemical impact and bleaching efficiency of alkali-facilitated fermented process on crude palm oil, with a focus on determining their suitability as sustainable alternatives to conventional bleaching agents.

1.2.2 Objectives of the Study

The objectives of the study are to:

I. Assess the initial physicochemical properties of crude palm oil before treatment.

II. Evaluate the effectiveness of the alkali-facilitated fermented technique in bleaching.

III. Improving oil quality, and compare its performance with conventional bleaching earths in terms of decolorization, nutrient retention, and overall oil quality.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF PALM OIL

Researchers have described palm oil as one of the most important edible vegetable oils in the world, obtained from the mesocarp the reddish, fleshy middle layer of the fruit of *Elaeis guineensis* (African oil palm) and *Elaeis oleifera* (South American oil palm). It has been reported that these oil palms historically played significant roles in the diets, medicinal practices, and local economies of West African communities. Over time, the cultivation of oil palm was said to have expanded across tropical regions due to its high oil yield, adaptability, and economic importance. Studies by Mukherjee and Mitra (2009) indicated that Indonesia and Malaysia currently account for over 85% of global palm oil production as a result of large-scale commercial cultivation supported by favourable climatic conditions.

Corley and Tinker (2015) stated that palm oil is among the most efficient oil-producing crops, capable of yielding up to ten times more oil per hectare compared to crops such as soybean and sunflower. This high productivity was reported to have positioned palm oil as a cornerstone of the global edible oil industry and an essential raw material in the cosmetic, pharmaceutical, and biofuel sectors.

From biochemical and industrial perspectives, researchers explained that palm oil consists mainly of triacylglycerols (TAGs), which are responsible for its semi-solid consistency at room temperature. The oil's distinctive reddish-orange colour was attributed to its high content of carotenoids, particularly Beta-carotene and α-carotene, both of which function as provitamin A

compounds. Sundram *et al.* (2003) reported that the oil contains approximately equal proportions of saturated (~50%) and unsaturated (~50%) fatty acids, which allows it to be fractionated naturally into palm olein (liquid) and palm stearin (solid). These fractions were said to have wide applications in the production of frying oils, margarine, baking fats, soaps, and biodiesel.

It was further reported that palm oil is rich in palmitic acid (C16:0) and oleic acid (C18:1), whereas palm kernel oil extracted from the seed of the same fruit contains higher levels of lauric and myristic acids. This composition makes palm kernel oil chemically and functionally similar to coconut oil, a property that enables its extensive use in confectionery, detergents, and personal care products.

According to Solomons and Bulux (1997) and Tanumihardjo (2002), palm oil has been recognized as a nutritionally functional fat because it contains abundant natural antioxidants such as tocopherols and tocotrienols (forms of vitamin E), phytosterols, squalene, and coenzyme Q10 (ubiquinone). These compounds were reported to contribute to antioxidant defense mechanisms, anti-inflammatory activities, and cholesterol-lowering effects. Red palm oil (RPO), which is minimally processed and retains its carotenoid content, was said to have been effectively utilized in vitamin A deficiency intervention programmes, particularly among children and pregnant women in low-income tropical regions.

However, researchers such as Gibon (2007) observed that crude palm oil (CPO) in its unrefined state contains undesirable impurities including free fatty acids (FFAs), moisture, phospholipids, trace metals, and chlorophyll which promote oxidative rancidity and reduce shelf life. It was therefore noted that refining processes are essential to ensure the oil's stability, palatability, and safety for human consumption. Refining stages such as degumming, neutralization, bleaching,

and deodorization were reported to be necessary for removing these impurities while preserving key nutritional components.

In recent studies, it was also reported that the sustainability of palm oil production has become a major concern due to its links with deforestation, biodiversity loss, and greenhouse gas emissions. Consequently, the Roundtable on Sustainable Palm Oil (RSPO) was established to promote sustainable cultivation, traceability, and environmental responsibility within the palm oil supply chain. RSPO (2023) reported that certified sustainable palm oil represents an increasing portion of the global market as the industry strives to balance productivity with ecological sustainability.

Furthermore, Mba *et al.* (2015) stated that palm oil has gained significant attention in the fields of green chemistry and biotechnology. Its fatty acid derivatives were reported to serve as renewable feedstocks for the production of biodegradable lubricants, eco-friendly surfactants, bioplastics, and other oleochemical products. These innovations have been said to highlight the versatility of palm oil as an industrial bioresource, extending its relevance well beyond the food industry.

Plate 2.1 (a) Matured Oil Palm (b)Fresh Palm Oil Fruits Bunch

(Source: May *et al.*, 2024).

2.2 PHYSICAL, CHEMICAL, AND BIOCHEMICAL PROPERTIES OF PALM OIL

Researchers reported that palm oil exhibited a complex and unique combination of physical, chemical, and biochemical properties which determined its behaviour during processing, storage, and consumption. These properties were said to influence its industrial functionality, nutritional significance, and health implications. It was noted that understanding these characteristics was crucial in food technology, biochemical studies, and oil refining processes, and that they were routinely analyzed using standardized laboratory instruments and protocols (Gibon *et al.*, 2007).

From a physicochemical perspective, crude palm oil (CPO) was described as being semi-solid at room temperature due to its nearly equal distribution of saturated and unsaturated fatty acids. This property was reported to allow its natural fractionation into liquid (olein) and solid (stearin) components without chemical hydrogenation, thereby making it a healthier option compared to trans-fat-containing oils. The relatively high levels of palmitic acid (C16:0) and oleic acid (C18:1) were said to contribute significantly to the oil's texture and oxidative stability. The intense red-orange coloration of crude palm oil was attributed to its carotenoid content, though this colour was observed to diminish considerably during refining unless special methods were used to retain the pigments, as seen in red palm oil.

According to several biochemical studies, palm oil was not merely a lipid-based source of energy (approximately 9 kcal/g) but also functioned as a carrier of bioactive compounds such as tocopherols, tocotrienols (vitamin E variants), phytosterols, carotenoids, and minor quantities of polyphenols. These compounds were reported to contribute to the oil's antioxidant, anti-inflammatory, and cholesterol-lowering effects. Analytical evaluations of such biochemical

properties were said to employ gas chromatography for fatty acid profiling, iodine value determination for assessing unsaturation levels, acid-base titration for estimating free fatty acid content, and UV–Vis spectrophotometry for determining pigment and oxidation levels.

Palm oil's multifunctional properties and oxidative stability were reported to make it highly suitable for applications such as deep-frying, bakery formulations, cosmetics, and biofuel production. Moreover, its natural balance between saturated and unsaturated fatty acids was said to render it less prone to oxidative degradation than many polyunsaturated oils, thereby extending its shelf life and maintaining its flavour stability.

2.2.1 Physical Properties of Palm Oil

It was reported that the physical characteristics of palm oil determined its appearance, handling, and suitability for both food and non-food use. These characteristics were said to depend largely on the fatty acid composition, processing history, and temperature of the oil.

I. Melting Point: Palm oil was observed to melt over a temperature range of 30°C to 40°C, depending on its ratio of saturated to unsaturated fatty acids. This semi-solid nature at ambient temperature was said to eliminate the need for hydrogenation in food manufacturing, thereby preventing the formation of harmful trans fats.

II. Colour: Crude palm oil was described as possessing a distinct reddish-orange colour attributed to its high carotenoid content, primarily α- and Beta-carotene. This colour was recognized as an indicator of freshness and vitamin A content, although it was generally removed during refining unless the aim was to produce red palm oil.

III. Density: Choo *et al.* (2009) reported that the density of crude palm oil typically ranged from 0.891 to 0.899 g/cm³ at 40°C. This property was regarded as important for packaging, formulation, and storage considerations.

IV. Refractive Index (RI): The refractive index of crude palm oil was found to fall between 1.456 and 1.459 at 40°C. Researchers indicated that this value was useful for confirming oil identity and detecting adulteration.

V. Viscosity: The viscosity of palm oil was observed to vary with temperature and was found to influence its flow behaviour during processing. An increase in viscosity at lower temperatures was described as a characteristic feature of palm oil and could serve as an indicator of early oxidation or triglyceride polymerization.

2.2.2 Chemical Properties of Palm Oil

The chemical composition of palm oil was reported to be dominated by triacylglycerols (TAGs), which constituted about 95% of its total lipids. The remaining fraction contained minor lipids such as mono- and diglycerides, free fatty acids (FFAs), and unsaponifiable compounds like sterols, pigments, and hydrocarbons. These chemical constituents were said to determine the oil's reactivity, nutritional value, shelf stability, and industrial applicability.

Researchers explained that a key aspect of palm oil's chemical behaviour lies in its fatty acid composition. Palmitic acid (C16:0), constituting about 44%, was described as the major saturated fatty acid responsible for its semi-solid texture and oxidative stability. Oleic acid (C18:1), making up approximately 39%, was identified as a monounsaturated fatty acid that enhanced fluidity and contributed to cholesterol regulation. Linoleic acid (C18:2), representing about 10%, was classified as an essential polyunsaturated omega-6 fatty acid required in human nutrition but

susceptible to oxidation. Stearic acid (C18:0), which comprised about 4–5%, was noted to have neutral effects on blood cholesterol and to contribute to the solid fraction of the oil.

These fatty acids were reported to be esterified to glycerol molecules to form TAGs, and their specific distribution and structural arrangement were said to influence the oil's melting characteristics, digestibility, and stability.

Researchers identified several standard chemical indices for evaluating palm oil quality:

I. Acid Value (AV): Reported as an indicator of free fatty acid concentration in the oil, measured in mg KOH/g. A high acid value was said to signify hydrolytic activity or microbial contamination. For crude palm oil, (Gibon *et al.* 2007) suggested that an AV below 5 mg KOH/g was generally acceptable.

II. Saponification Value (SV): The saponification value, which ranged between 195 and 205 mg KOH/g, represented the amount of potassium hydroxide required to saponify one gram of oil. Higher SV values were said to correspond to shorter fatty acid chain lengths.

III. Iodine Value (IV): This value measured the degree of unsaturation in the oil. Palm oil was reported to have an IV of 50–55 g I_2/100 g, placing it in the moderate category compared to highly unsaturated oils such as sunflower and soybean oils. Higher IV values were said to indicate a greater tendency toward oxidation.

IV. Peroxide Value (PV): The peroxide value, which indicated the extent of primary oxidation, was found to be below 10 meq/kg in freshly processed palm oil. Higher PV values were interpreted as signs of oxidative deterioration, especially during overheating or poor storage conditions (Sundram *et al.*, 2003).

Due to its moderate unsaturation, palm oil was observed to maintain a good balance between oxidative stability and functionality. It was reported to resist rancidity better than many polyunsaturated oils while avoiding the health risks associated with hydrogenated or fully saturated fats.

Researchers also explained that palm oil's chemical properties made it well-suited for diverse industrial and culinary applications, such as deep-frying, margarine production, soap manufacturing, and biodiesel formulation.

Analytical studies were said to employ methods such as titration (for acid and saponification values), gas chromatography (for fatty acid profiling), and iodometric or spectrophotometric techniques (for peroxide and iodine values). The information obtained from these analyses was regarded as critical for refining optimization and quality control.

Analytical Techniques: The biochemical properties of palm oil were further evaluated using advanced methods, including:

I. High-Performance Liquid Chromatography (HPLC) for tocopherols and tocotrienols,
II. UV–Visible spectrophotometry for carotenoid quantification, and
III. Gas Chromatography–Mass Spectrometry (GC–MS) for sterol and Coenzyme Q10 analyses.

2.3 MAJOR COMPONENTS OF PALM OIL

Researchers reported that palm oil was predominantly composed of lipid molecules, particularly glycerides, which played vital roles in determining its nutritional quality, oxidative stability, and industrial versatility. These glycerides were said to include triglycerides (TAGs), diglycerides (DAGs), and monoglycerides (MAGs), all of which were identified as esters formed between

fatty acids and glycerol molecules. The proportions and structural configurations of these lipid molecules were noted to influence the physical state, energy density, digestibility, and overall suitability of palm oil for various food and non-food applications.

From both biochemical and food science perspectives, it was observed that these components were routinely analyzed to evaluate the quality and purity of palm oil. Analytical methods such as Gas Chromatography (GC), Thin-Layer Chromatography (TLC), and High-Performance Liquid Chromatography (HPLC) were reported to be commonly employed for this purpose (Chong *et al.*, 2015).

2.3.1 Triglycerides

Triglycerides (TAGs) were identified as the dominant lipid class in crude palm oil, accounting for approximately 95% of the total lipid content (Sundram *et al.*, 2003). Each triglyceride molecule was described as consisting of a glycerol backbone esterified with three fatty acid chains, which varied in carbon chain length and degree of saturation.

Researchers reported that the most abundant fatty acids present in palm oil triglycerides included:
I. Palmitic acid (C16:0) – about 44%,

II. Oleic acid (C18:1) – about 39%,

III. Linoleic acid (C18:2) – about 10%,

IV. Stearic acid (C18:0) – approximately 4–5%, and

V. Myristic acid (C14:0) – occurring only in trace amounts.

This particular fatty acid composition was said to contribute to the semi-solid consistency of palm oil at room temperature as well as its high thermal and oxidative stability. The balance

between saturated and unsaturated fatty acids in its triglyceride structure was reported to facilitate the fractionation of palm oil into palm olein (liquid) and palm stearin (solid), both of which were noted to have wide industrial and culinary applications, including use in frying oils, margarines, and bakery fats.

From a nutritional perspective, triglycerides in palm oil were said to provide approximately 9 kcal per gram, making the oil an energy-dense food source. It was further reported that palm oil triglycerides were efficiently metabolized by the human digestive system, and that their molecular structure influenced factors such as texture (mouthfeel), shelf-life, and lipid absorption efficiency. Berger (2005) emphasized that the absence of trans fats and the high oxidative resistance of palm oil triglycerides made them a safer and healthier alternative to partially hydrogenated oils used in food processing.

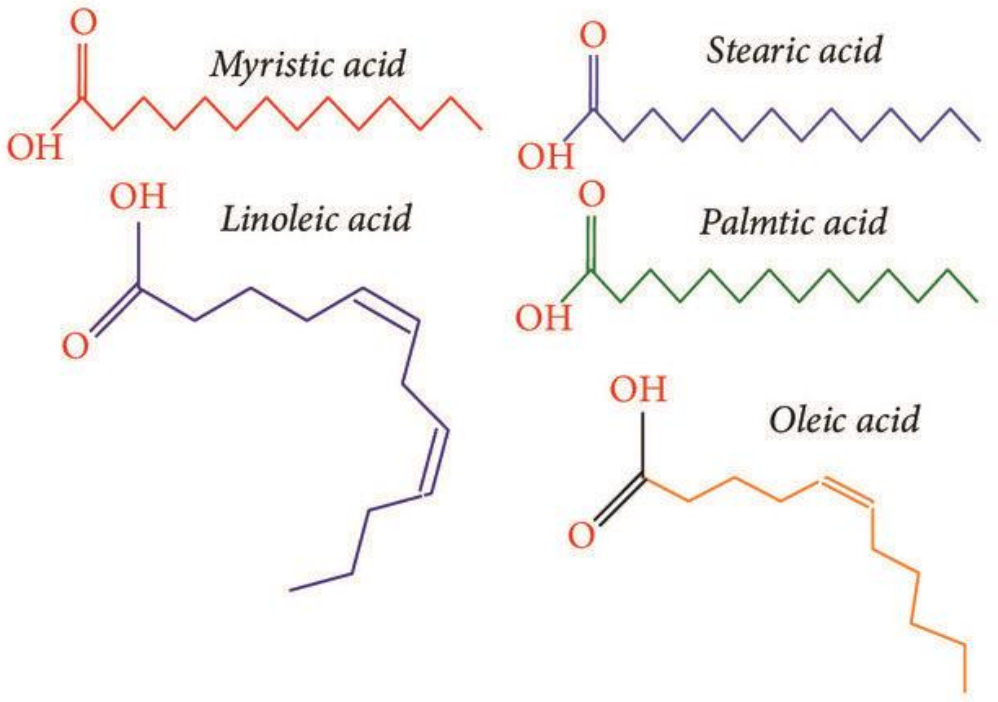

Fig. 2.1 Structures of the Triglycerides present in Palm oil (Sadrolhosseini A.R. *et al.*, 2017).

2.3.2 Mono- and Diglycerides

Researchers reported that monoacylglycerols (MAGs) and diacylglycerols (DAGs) were present in palm oil in relatively small quantities typically less than 5% of the total lipid content but were recognized as important components due to their biochemical and industrial functions. It was explained that diacylglycerols consisted of two fatty acid chains attached to a glycerol molecule, while monoacylglycerols contained only one fatty acid chain esterified to glycerol.

These compounds were said to be formed naturally during enzymatic hydrolysis of triglycerides or could arise under unfavourable processing or storage conditions, such as microbial contamination or the activity of lipase enzymes. Researchers observed that the accumulation of

MAGs and DAGs was often taken as an indication of lipid degradation, which could adversely affect the oxidative stability, flavour, and shelf life of palm oil.

However, Choo, Y. M., *et al.* (2009) reported that, when present in controlled amounts, mono- and diglycerides served as valuable natural emulsifiers. They were said to play significant roles in the production of margarine, ice cream, and various processed foods where emulsification and texture stability were essential. From a biochemical standpoint, these partial glycerides were identified as intermediates in lipid metabolism, and elevated concentrations were reported to indicate the onset of hydrolytic rancidity in palm oil.

It was also stated that close monitoring of MAG and DAG levels during the refining process especially in the degumming and neutralization stages was critical for maintaining overall oil quality. Analytical techniques such as Gas Chromatography with Flame Ionization Detection (GC–FID), Thin-Layer Chromatography (TLC), and High-Performance Liquid Chromatography (HPLC) were commonly reported to be used for their quantification and structural identification.

In addition, Gunstone (2011) noted that purified mono- and diglycerides obtained from palm oil were widely utilized in food, pharmaceutical, and cosmetic industries because of their surfactant and stabilizing properties. These compounds were said to enhance the consistency, texture, and shelf stability of various formulations, thereby extending the industrial relevance of palm oil beyond its conventional uses as an edible fat.

2.4 MINOR COMPONENTS OF PALM OIL

Researchers reported that although the minor components of palm oil were present in much smaller quantities compared to triglycerides, they played crucial roles in determining the oil's nutritional value, oxidative stability, and industrial functionality. These compounds were said to

include various pigments, sterols, antioxidants, unsaponifiable matter, and other bioactive molecules that contribute significantly to the oil's quality and health-promoting properties.

It was explained that in the fields of biochemistry, food science, and nutraceutical research, these minor constituents attracted considerable interest due to their roles in disease prevention, product stability, and shelf-life enhancement. According to Chong *et al.* (2015), modern analytical techniques such as UV–Visible spectrophotometry, Gas Chromatography–Mass Spectrometry (GC–MS), and High-Performance Liquid Chromatography (HPLC) were widely used for their identification and quantification.

Researchers further stated that understanding the concentration and function of these minor components was important during refining, as excessive removal during processing could lead to a loss of nutritional value, while insufficient removal might result in spoilage, discolouration, or off-flavours in the final product.

2.4.1 Coenzyme Q10, Chlorophyll, Phosphatides, Alcohols, and Steroids

It was reported that chlorophyll is a natural green pigment found in crude palm oil, which, although beneficial in plants, acted as a pro-oxidant when exposed to light. This compound was said to accelerate oxidative rancidity, thereby making its removal during the bleaching stage of refining essential (Latip *et al.*, 2013).

Researchers also identified phosphatides mainly phospholipids such as phosphatidylcholine and phosphatidylethanolamine as key components that could cause cloudiness, foaming, and emulsion instability in palm oil. These were said to be removed effectively during degumming to ensure oil clarity and improve oxidative stability.

Coenzyme Q10 (ubiquinone) was described as a lipophilic antioxidant involved in mitochondrial electron transport and ATP synthesis. Although present in small amounts, it was reported to contribute to cardiovascular and cellular health (Aggarwal *et al.*, 2007). Researchers noted that its retention during minimal processing enhanced the nutraceutical value of palm oil.

Steroid alcohols and other unsaponifiable compounds, including methyl sterols and triterpenes, were said to possess biological properties such as hormonal, anti-inflammatory, and metabolic regulatory effects. These compounds formed part of the unsaponifiable fraction of palm oil, which is resistant to alkaline hydrolysis.

2.4.2 Carotenes (Provitamin A Precursors)

Palm oil was reported to be one of the richest natural dietary sources of α- and Beta-carotenes, which account for up to 90% of its total carotenoid content. These compounds were identified as precursors to vitamin A and were recognized for their antioxidant activity, which enables them to neutralize free radicals and singlet oxygen species.

Researchers stated that the characteristic red-orange colour of crude palm oil was due to its high carotene content. However, it was observed that these pigments were thermally sensitive and were largely destroyed during high-temperature refining. To preserve them, red palm oil (RPO) was produced through gentle refining methods, allowing its use in vitamin A deficiency intervention programmes, especially in vulnerable populations such as children and pregnant women (Solomons and Bulux, 1997).

2.4.3 Squalene

Squalene was described as a triterpene hydrocarbon found in small quantities within palm oil. It was reported to act as a biochemical precursor in cholesterol biosynthesis and was associated

with antioxidant, anti-aging, and skin-regenerative properties (Huang *et al.*, 2009). Researchers observed that while squalene was more abundant in olive and shark liver oils, its presence in palm oil contributed to its growing use in nutraceuticals and dermatological products.

2.4.4 Vitamin E (Tocopherols and Tocotrienols)

Researchers stated that palm oil contains a balanced mixture of tocopherols (with saturated side chains) and tocotrienols (with unsaturated side chains), both of which constitute the vitamin E complex. These compounds were said to exhibit strong antioxidant activity and biological importance.

They were reported to stabilize lipid membranes, inhibit lipid peroxidation, and provide protective effects against oxidative damage. Studies such as those of Sundram *et al.* (2003) indicated that vitamin E in palm oil exhibited anti-cancer, neuroprotective, and cardioprotective properties, while also reducing inflammation and potentially improving lipid metabolism.

Among the tocotrienols, γ- and δ-forms were identified as particularly potent, and recent research has focused on their possible roles in stroke prevention, cholesterol regulation, and cancer inhibition.

2.4.5 Flavonoids and Polyphenols

Flavonoids and polyphenols were reported to be present in trace quantities in palm oil and were noted for their strong antioxidant and anti-inflammatory properties. These compounds were said to help neutralize reactive oxygen species, protect cellular components, and potentially inhibit free radical-induced DNA damage (Ng *et al.*, 2011).

It was noted that these compounds are heat-sensitive and may be lost during prolonged or high-temperature processing. Consequently, researchers recommended minimal refining techniques to retain their beneficial effects in the oil.

2.4.6 Phytosterols and Phytostanols

Researchers reported that phytosterols and phytostanols plant-derived sterols structurally similar to cholesterol were found in palm oil at concentrations of about 200–300 mg/kg. These compounds were said to competitively inhibit the absorption of dietary cholesterol in the intestines, resulting in reduced low-density lipoprotein (LDL), cholesterol levels and improved cardiovascular health (Jones *et al.*, 1999).

In addition to their cholesterol-lowering effects, these sterols were reported to have anti-inflammatory and immunomodulatory properties. Their physiological benefits have made them valuable components in functional foods, particularly sterol-enriched margarines and dietary supplements formulated to support heart health.

2.5 REFINING OF CRUDE PALM OIL

Researchers reported that the refining of crude palm oil (CPO) was a systematic, multistep process designed to eliminate undesirable substances such as free fatty acids (FFAs), phospholipids, gums, colour pigments, odorous compounds, moisture, and trace metals. These refining steps were said to be essential for improving the edibility, shelf life, flavour, and nutritional quality of palm oil, particularly for its application in the food, pharmaceutical, and cosmetic industries.

According to Gibon *et al.* (2007) and Choo *et al.* (2009), each stage of the refining process involved carefully controlled physical, chemical, or thermal treatments optimized to preserve

beneficial constituents while effectively removing unwanted or unstable components. The refining process was reported to be broadly classified into two main methods:

I. Chemical Refining – which involved neutralization using an alkali solution (typically sodium hydroxide, NaOH), followed by bleaching and deodorization.

II. Physical Refining – which avoided the use of alkali and instead relied on steam distillation after degumming and bleaching stages.

The choice of refining method was said to depend largely on the quality of the crude palm oil and the desired characteristics of the final product.

2.5.1 Degumming

Degumming was reported to be the first stage of the refining process, primarily aimed at the removal of phospholipids, commonly referred to as gums, which were known to cause cloudiness, foaming, and oxidative instability in palm oil. These compounds were also said to interfere with subsequent refining stages if not properly removed.

According to Nielsen (2010), hydratable phospholipids such as lecithin were typically removed by the addition of water or dilute acids (such as citric or phosphoric acid), which caused them to hydrate, precipitate, and separate from the oil. Non-hydratable phospholipids, however, were reported to require enzymatic or acid pre-treatment to achieve complete removal.

Efficient degumming was said to enhance oil clarity, improve oxidative stability, and reduce operational issues during later stages such as bleaching and deodorization. Researchers further indicated that the effectiveness of degumming could be evaluated through phosphorus content analysis, centrifugation testing, and emulsion stability measurements.

2.5.2 Bleaching

Bleaching was reported to serve as a critical refining stage aimed at the removal of pigments, residual phospholipids, trace metals, soaps, and oxidation products through adsorption mechanisms. The process was said to be conducted under vacuum conditions, generally at temperatures between 80°C and 110°C.

Researchers stated that activated bleaching earth (ABE) or acid-activated clays were the most commonly used adsorbents due to their large surface area and high adsorption capacity. The efficiency of bleaching was reported to depend on several factors, including the adsorbent's surface area and pore size, its chemical properties, the contact time, temperature, and the intensity of agitation during the process.

Sampaio *et al.* (2018) observed that bleaching effectively removed carotenoids and chlorophyll, thereby improving oil colour and reducing impurities such as residual FFAs and peroxides, which in turn enhanced oxidative stability. However, it was also noted that excessive bleaching could lead to the loss of essential micronutrients such as tocopherols and tocotrienols.

To assess bleaching performance, analytical techniques such as spectrophotometry were reported to be used for pigment quantification, while peroxide value (PV) and free fatty acid (FFA) levels were measured to monitor oxidative changes and refining efficiency.

2.5.3 Deodorization

Deodorization was described as a high-temperature steam distillation process performed under vacuum conditions, typically between 2–6 mmHg, and at temperatures ranging from 220°C to 260°C. The main objective of this process was reported to be the removal of volatile odour and

flavour compounds such as aldehydes, ketones, and hydrocarbons as well as the reduction of residual FFAs and colour-reversion substances that may compromise oil quality.

According to Zhang *et al.* (2019), the deodorization process involved direct injection of steam into the heated oil under vacuum conditions. The volatile compounds were stripped from the oil and condensed separately, after which the deodorized oil was cooled and filtered before final packaging.

While deodorization was recognized as an essential step for achieving taste neutrality, oxidative stability, and consumer safety, researchers also cautioned that the high temperatures used could lead to the degradation of heat-sensitive micronutrients like tocopherols and tocotrienols. Additionally, the process was said to potentially result in the formation of 3-monochloropropane-1,2-diol (3-MCPD) esters, a compound of toxicological concern.

To ensure process control and product safety, analytical monitoring using Gas Chromatography–Mass Spectrometry (GC–MS) and High-Performance Liquid Chromatography (HPLC) was reported to be standard practice in deodorization studies.

2.5.4 Neutralization (Alkali Refining)

Neutralization, often referred to as alkali refining, was reported to involve the removal of free fatty acids by converting them into soap stock through reaction with a caustic solution, typically sodium hydroxide (NaOH). The reaction was summarized as:

FFAs + NaOH → Soap (removed by centrifugation or washing)

This process was said to also aid in the removal of other impurities such as phospholipids, trace metals, and minor oxidation products.

According to Gibon *et al.* (2007), laboratory evaluation of the neutralization process involved the determination of acid value (AV) before and after treatment to assess the efficiency of FFA removal. Additional quality parameters such as soap content, moisture content, and residual phosphorus concentration were also measured to ensure compliance with industrial refining standards.

Although physical refining was reported to be increasingly preferred for high-quality crude palm oils due to its lower chemical waste and better nutrient retention, alkali refining remained an important technique, particularly for crude oils with high FFA content or when minimal bleaching loss was desired.

2.6 HEALTH BENEFITS OF PALM OIL

Researchers have reported that the health effects of palm oil have long been a subject of scientific discussion, primarily due to its relatively high content of saturated fatty acids. However, recent nutritional and biochemical studies have indicated that moderately refined or cold-pressed palm oil especially red palm oil can provide significant health benefits when consumed as part of a balanced diet. These benefits were attributed not only to its fatty acid composition but also to its rich concentration of bioactive micronutrients such as tocotrienols, carotenes, phytosterols, and coenzyme Q10.

Sundram *et al.* (2003) and Choo *et al.* (2009) reported that these compounds possess strong antioxidant, anti-inflammatory, cardioprotective, and neuroprotective properties, making palm oil not merely a cooking medium but also a potential functional food and nutraceutical agent. However, the overall health effects were said to depend greatly on factors such as the processing method, consumption level, and general dietary pattern. It was observed that while highly refined

or repeatedly heated palm oil may present health risks, minimally processed palm oil could support health and wellness when consumed moderately.

2.6.1 Cardiovascular Health

Early nutritional studies once suggested that the relatively high saturated fat content of palm oil could negatively influence heart health. However, more recent research has indicated that palm oil, when consumed in moderate amounts, does not significantly elevate low-density lipoprotein (LDL) cholesterol levels the so-called “bad” cholesterol.

Researchers noted that the presence of oleic acid (~39%), a monounsaturated fatty acid, helps to maintain healthy lipid profiles, while phytosterols in palm oil reduce plasma cholesterol by inhibiting intestinal cholesterol absorption. Although palmitic acid (~44%) is a saturated fat, it was reported to be balanced by the unsaturated fatty acids, oleic and linoleic acids, thereby reducing the risk of hyperlipidaemia when the oil is consumed appropriately (Kritchevsky, 2000).

It was further emphasized that replacing trans fats or animal-derived fats with palm oil in food formulations could contribute positively to cardiovascular function and reduce the risk of atherosclerosis and other heart-related conditions.

2.6.2 Antioxidant and Anti-Cancer Properties

Palm oil was reported to exhibit remarkable antioxidant potential due to its rich composition of tocotrienols and carotenes. These bioactive molecules were said to act as scavengers of free radicals and reactive oxygen species (ROS), which are known to damage cellular components, promote aging, and initiate cancerous changes.

Qureshi *et al.* (2000) observed that tocotrienols in palm oil inhibited cancer cell proliferation and induced apoptosis in experimental cancer models. Similarly, Beta-carotene one of the major carotenoids in paj8 njjjjjjlm oil was described as a potent provitamin A compound that supports DNA integrity, immune function, and normal cell growth.

Nesaretnam *et al.* (2007) further reported that various forms of tocotrienols, particularly α-, γ-, and δ-tocotrienols, exhibited significant anti-cancer activities in studies involving breast, pancreatic, and prostate cancers. These findings highlighted palm oil's potential role as a dietary component that could help mitigate oxidative stress-related diseases, including cancer, diabetes, and cardiovascular disorders.

2.6.3 Neuroprotection

Tocotrienols derived from palm oil have been widely recognized for their neuroprotective properties. Researchers reported that these compounds inhibit glutamate-induced neurotoxicity and enhance blood circulation in the brain, thereby protecting neural tissues from oxidative stress and ischemic damage.

Sen *et al.* (2006) found that tocotrienols may slow the progression of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Coenzyme Q10 and squalene, also present in palm oil, were said to contribute to mitochondrial energy metabolism and neuronal stability, providing additional neuroprotective benefits.

2.6.4 Immune and Skin Health

Palm oil was reported to support immune function and skin health through its high levels of bioactive micronutrients, particularly carotenes and tocotrienols. Carotenes were said to play a vital role in preventing vitamin A deficiency, especially among children and pregnant women in developing regions.

Tocopherols and tocotrienols, forms of vitamin E present in palm oil, were reported to protect skin lipids from UV-induced oxidation, acting as natural photoprotective agents. Ng *et al.* (2004) stated that palm oil-based cosmetic formulations, particularly those derived from red palm oil, exhibited hydrating, anti-aging, and restorative properties due to the presence of these natural antioxidants.

2.6.5 Metabolic and Liver Health

Experimental findings have suggested that moderate consumption of palm oil may exert beneficial effects on metabolic and liver health. Researchers reported that palm oil improved glucose tolerance, enhanced insulin sensitivity, and helped regulate lipid metabolism by suppressing HMG-CoA reductase, the enzyme responsible for cholesterol biosynthesis (Qureshi *et al.*, 2000).

It was also observed that palm oil could reduce hepatic fat accumulation and lower the risk of non-alcoholic fatty liver disease (NAFLD). These findings indicated that palm oil, when consumed as part of a balanced diet, might contribute to the prevention or management of metabolic disorders such as obesity, type 2 diabetes, and liver dysfunction.

2.6.6 Risks and Moderation

Despite the numerous reported benefits, researchers emphasized that excessive or improper use of palm oil could pose certain health risks. Overconsumption was said to contribute to high caloric intake, weight gain, and elevated LDL cholesterol levels especially when combined with diets low in fibre and antioxidants.

Additionally, repeated heating of palm oil, particularly in deep frying, was reported to produce toxic oxidation products, deplete antioxidants, and increase the risk of inflammation and cardiovascular diseases.

Experts recommended that consumers should:

I. Prefer cold-pressed or minimally refined palm oil varieties when possible;

II. Avoid reusing palm oil multiple times during cooking; and

III. Incorporate palm oil as part of a diet rich in vegetables, fruits, and whole grains while limiting processed fats and animal-derived saturated fats.

These recommendations were said to help ensure that the health-promoting attributes of palm oil are maximized while minimizing potential health risks associated with poor handling or excessive intake.

CHAPTER THREE

MATERIALS AND METHODS

3.1 APPARATUS AND MATERIALS

Beaker (250 ml)

Black nylon and Twine

Conical flasks (250 ml)

Container (2 litres)

Cotton wool

Distilled water

Filter paper (Whatman filter paper)

Funnel

Masking tape

Measuring cylinder (100 ml, 1000 ml)

Plastic bucket (5 litres)

pH paper

Sample container (30 ml)

Stirring wooden ladle

Test tubes

Volumetric flask (1000 mL).

3.2 REAGENTS

The reagents used in this experiment were obtained from Pyrex LG Scientific Supply Company, Benin City.

Benzene (98% pure) (British Drug House Chemicals Ltd, England)

Copper (II) sulfate (Loba Chemie Pvt. Ltd, India)

Ethanol (99.7% pure) (JHD Chemicals, China)

Hexane (Sigma-Aldrich, USA)

Hydrochloric acid (Merck KGaA, Germany)

Methyl red Indicator (Kermel Chemical Reagent Co. Ltd, China)

Phenolphthalein Indicator (Kermel Chemical Reagent Co. Ltd, China)

Potassium hydroxide (KOH) (British Drug House Chemicals Ltd, England)

Sodium hydroxide (NaOH) (96% pure) (CDH Laboratory Chemicals, New Delhi, India)

Sulfuric acid (Sigma-Aldrich (St. Louis, Missouri, USA)

3.3 EQUIPMENT

Analytical Weighing Balance (Mettler Toledo, Switzerland)

Digital Rotary Viscometer (Brookfield Engineering, USA)

Hot Plate (Stuart Equipment, England)

Laboratory Drying Oven (Memmert GmbH, Germany)

UV–Vis Spectrophotometer (Shimadzu Corporation, Japan)

Water Bath (Grant Instruments Ltd, England)

3.4 METHODOLOGY

3.4.1 Preparation of Reagent (NaOH)

The reagent used in this experiment was obtained from Pyrex LG Scientific Supply Company, Benin City.

1.02 g, 2.04 g, and 3.06 g of the alkali reagent were weighed using an analytical weighing balance and transferred into separate 250 mL beaker, distilled water was added. Each sample was dissolved in distilled water, after which the solutions was then transferred into a 1000 mL volumetric flask and made up to 1000ml distilled water. This procedure was repeated for 2.04g and 3.0g and labelled as 0.025 M, 0.050 M, and 0.075 M solutions of NaOH. These solutions served as the alkali concentrations used during the bleaching of crude palm oil.

3.4.2 Method of Bleaching Process of Palm Oil

The crude palm oil (CPO) used for this study was sourced from Ring Road, Benin City, Nigeria. Exactly 1 L of CPO was measured and poured into three (3) separate 5 L plastic buckets, each designated for one of the three prepared alkali concentrations. The buckets were properly labelled to correspond to their respective molar concentrations (0.025 M, 0.050 M, and 0.075 M).

Subsequently, 50 mL of each alkali solution was measured using a 100 mL graduated measuring cylinder and added to the corresponding 5 L bucket containing the crude palm oil. The mixtures were thoroughly stirred for 10–15 minutes using a wooden ladle to ensure proper homogenization.

After mixing, each bucket was securely sealed using polythene nylon, twine, and a tight-fitting cover to prevent contamination. The samples were allowed to stand for 12–24 hours before being reopened and stirred again for 10 minutes to maintain even distribution. The sealed samples were then left to ferment naturally for one (1) week under ambient room conditions.

At the end of the fermentation period, 1000 mL of oil was measured from each treated sample using a 1000 mL measuring cylinder. The measured oil samples were then subjected to thermal treatment in an oven at 150 °C to facilitate the bleaching process.

After 1–2 weeks of controlled heating, the bleached oils were filtered using a funnel lined with Whatman filter paper to remove residual impurities. The resulting filtrates were collected into clean 1 L containers, properly sealed, and stored for subsequent physicochemical and biochemical analyses.

3.4.3 Determination of Acid Value or Acid number

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 ≡ Molecular weight of potassium hydroxide (56.1g).

N≡ Normality of potassium hydroxide solution (0.1 N).

V≡ Volume of potassium hydroxide solution used in titration.

W ≡ Weight of oil sample.

3.4.4 Determination of Viscosity

The Brookfield NDJ-5S Rotary viscometer was used in the determination of viscosity. The appropriate spindle number was identified and selected for the test sample and gently mounted on the machine. A 250ml beaker was cleaned, and the sample was poured up to the 200ml mark. The beaker was then placed on a water bath with the temperature preset at a constant 30 °C and allowed to equilibrate for 10 minutes. The spindle and the temperature sensor of the machine were then lowered into the sample, and the power button was turned on. The appropriate spindle number and speed were selected on the display screen, followed by the run button. The machine was then allowed to read the viscosity until a stable value was obtained and recorded.

3.4.5 Determination of Sugar Content

1 g of the reference sample was weighed using a weighing balance in a 250ml conical flask.

0.5M NaOH was prepared and 100ml was measured using a 100ml measuring cylinder and was added to the conical flask containing the oil. The conical flask was placed in the heating mantle for 10minutes.The sample then underwent neutralization by adding 20% HCl and using PH filter paper to check if the sample has been neutralized.

Dried test tubes were added 0 to 3ml standard sugar, and the volumes of test tubes were made up to 3ml with distilled water, thereby making concentrations ranging from 0 to 750 mg. 1mL DNS reagent was added to all the test tubes and mixed gently. The test tube was plugged with cotton or marble and placed in a boiling water bath for 5 minutes. The tubes were then removed, allowed to cool to ambient temperature, and immediately placed in a cuvette, and the concentration of sugar was determined in a UV- Vis Spectrophotometer at 540 nm using distilled water as the blank. Total sugar concentrations were estimated from a standard curve by comparing the absorbance of the standard glucose solution to that of the unknown sugar solution.

3.4.6 Determination of Beta carotene

The Beta-carotene value of a food item usually refers to the concentration of beta-carotene in that food, expressed in a unit such as micrograms (µg) or milligrams (mg) per serving or 100 grams of the food.

Method:

UV spectrophotometric method according to Alonge and Odokuma-Alonge, 2019. The evaluation of beta-carotene in each of the samples was carried out using a UV-visible spectrophotometer set at an absorbance of 474 nm with n-hexane as the solvent.

Procedure:

Beta-carotene in the bleached and unbleached oil samples was measured by dissolving 1 ml of the oil in 10 ml n-hexane. The spectrophotometer was set at an absorption wavelength of 474 nm. A quantity of n-hexane was poured into the cuvette and placed in the sample holder for reading. The procedure was repeated for all the samples. Beta-carotene present in the samples is calculated as:

Beta-carotene (mg/kg) = 343 × A /l × C

Where:

A = absorbance of a given mass of oil

C = mass of the oil in grams dissolved in 100 cm^3 of the solvent

l = path length of the cells used

3.4.7 Crude Protein Determination

Crude protein (CP) was determined by the Kjeldahl method (Gregory, 2005). About 5 g of sample in a conical flask was digested with 20ml H_2SO_4 and $CuSO_4$ as a catalyst using a hot plate for several minutes until a clear solution was obtained. It was cooled and diluted with 100 mL of distilled water. The tube containing the diluted sample was connected to the distillation unit, and a conical flask containing 25ml of 4% boric acid solution was attached to the condenser outlet. 50ml of 40% NaOH was dispensed into the conical flask, and distillation was carried out for 10 minutes. The ammonium borate solution formed was titrated with 0.1M HCl to a purplish–grey endpoint using methyl red as an indicator.

$$\%CP = \frac{(S - B)N(1.4007)}{w}$$

N is the normality of acid

S is titre value of sample

B is titre value of blank

CHAPTER FOUR

RESULTS

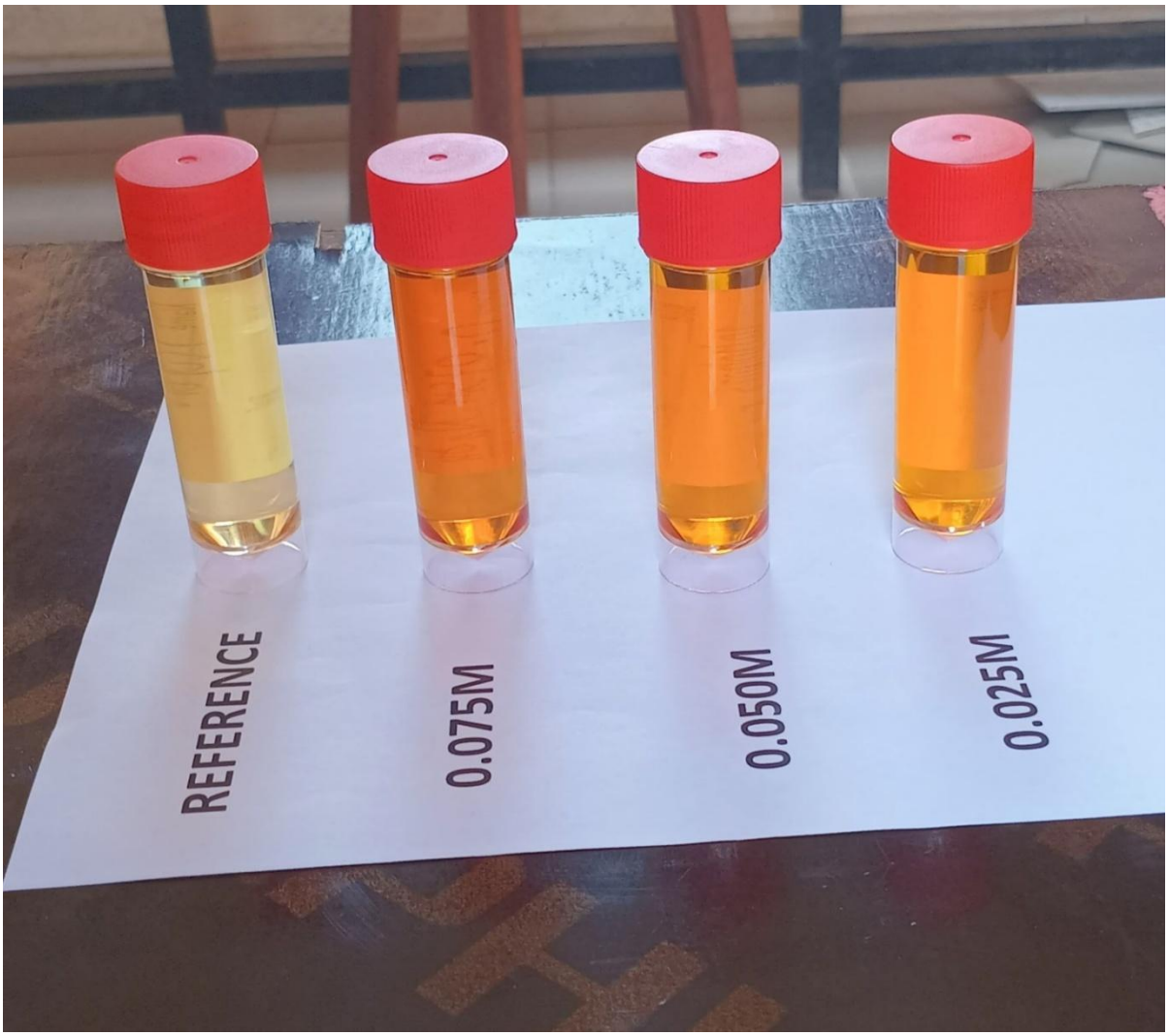

Plate 4.1. Comparative Visual Appearance of Bleached Crude Palm Oil Samples fermemted with Varying Concentrations of NaOH (0.025 M-0.075 M) against a Reference Commercial Vegetable Oil.

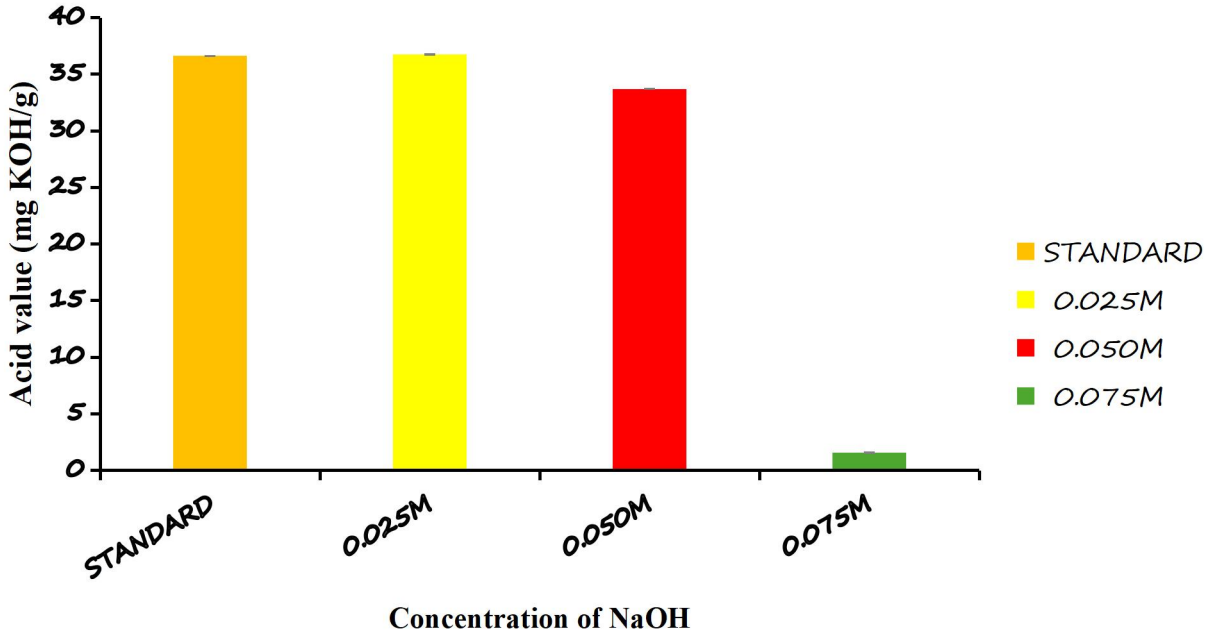

Figure 4.1: Acid Value (mg KOH/g) of Standard, bleached CPO with different concentrations of 0.025M, 0.050M, and 0.075M at 150°C.

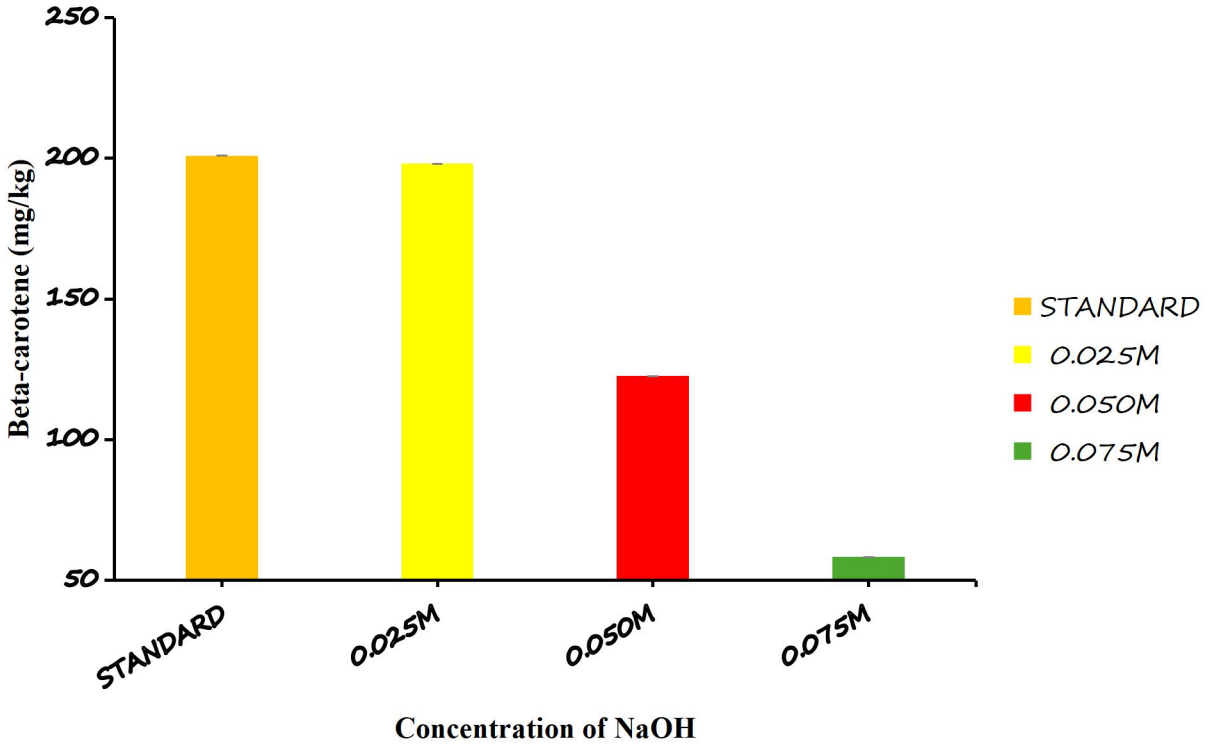

Figure 4.2: Beta-Carotene (mg/kg) of Standard, bleached CPO with different concentrations of 0.025M, 0.050M, and 0.075M at 150°C.

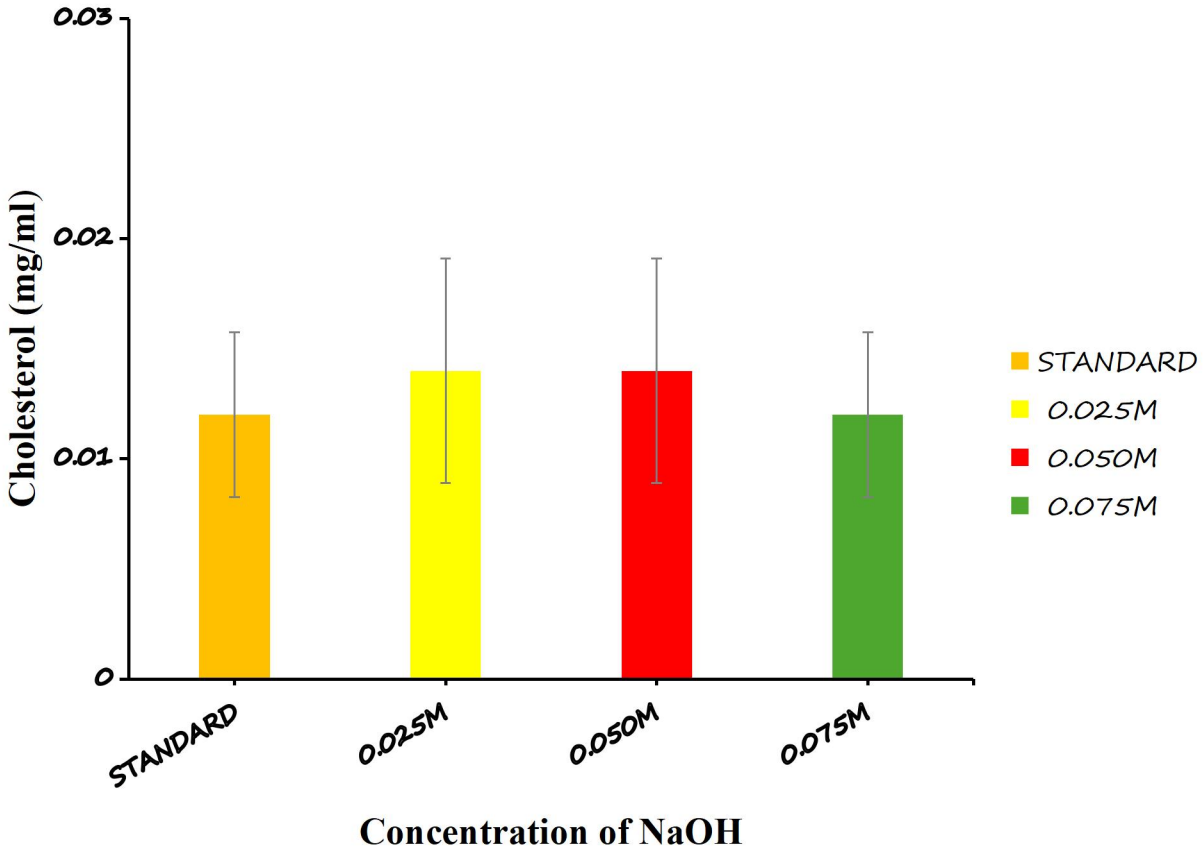

Figure 4.3: Cholesterol (mg/ml) of Standard, bleached CPO with different concentrations of 0.025M, 0.050M, and 0.075M at 150°C.

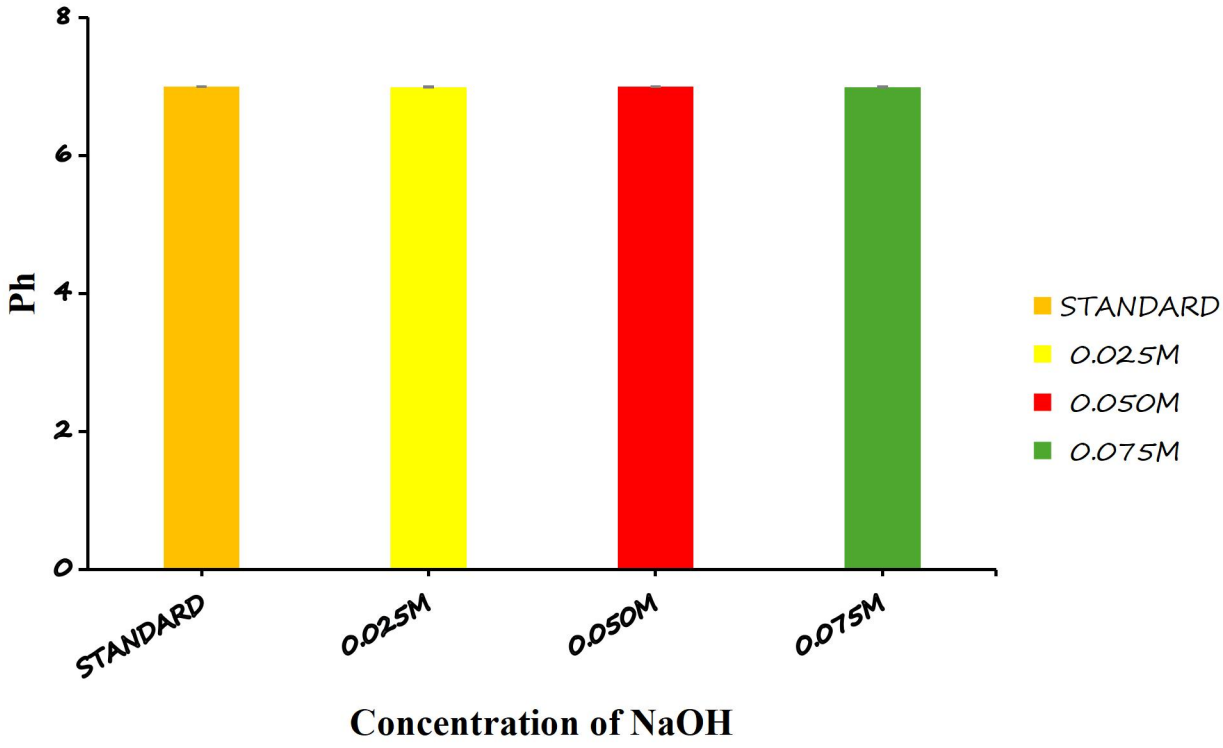

Figure 4.4: Ph of Standard, bleached CPO with different concentrations of 0.025M, 0.050M, and 0.075M at 150°C.

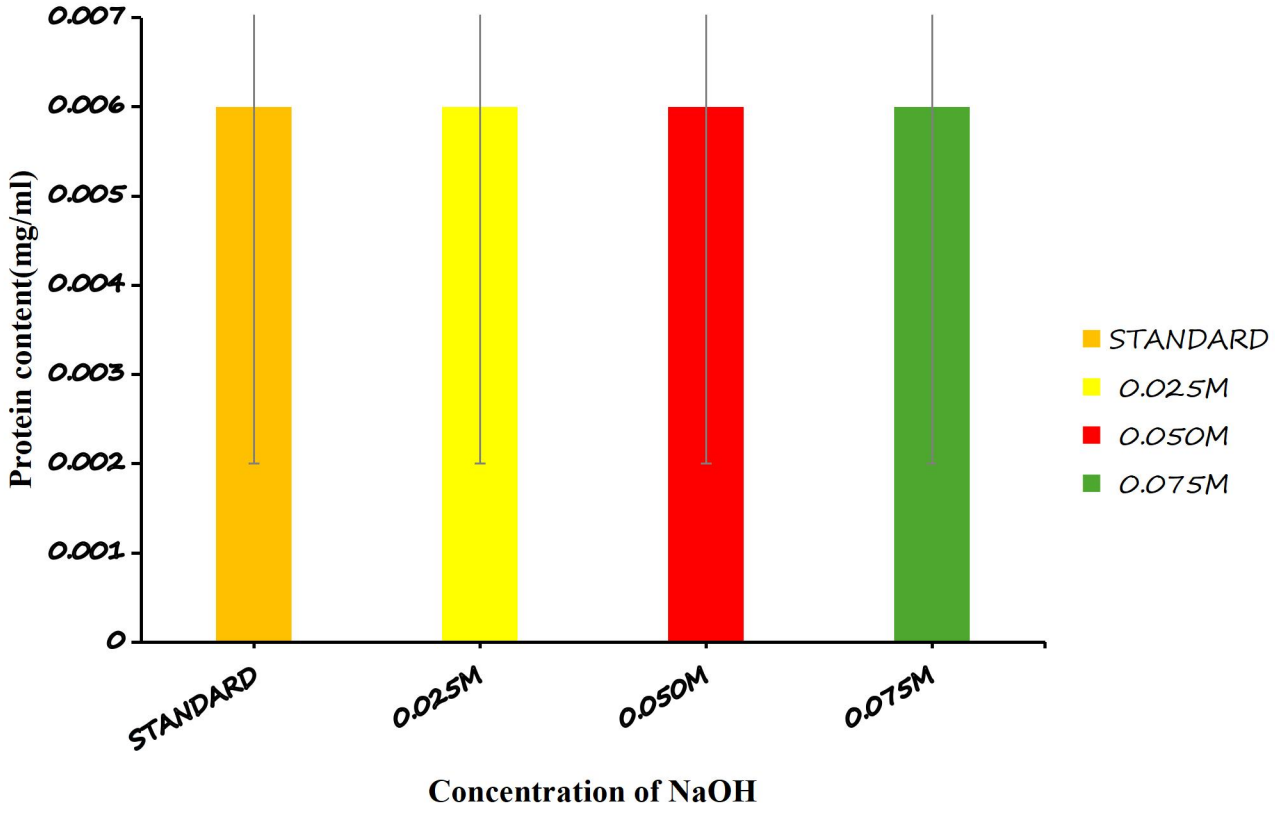

Figure 4.5: Protein Content (mg/ml) of Standard, bleached CPO with different concentrations of 0.025M, 0.050M, and 0.075M at 150°C.

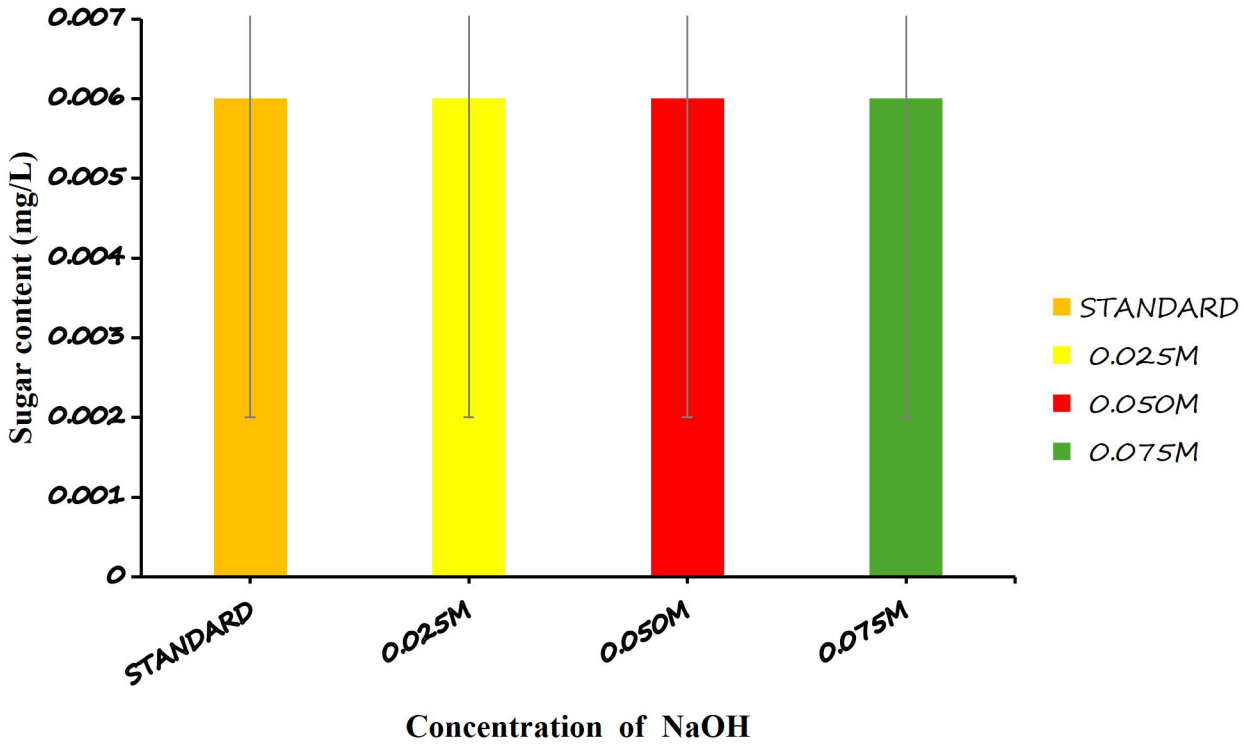

Figure 4.6: Sugar Content (mg/L) of Standard, bleached CPO with different concentrations of 0.025M, 0.050M, and 0.075M at 150°C.

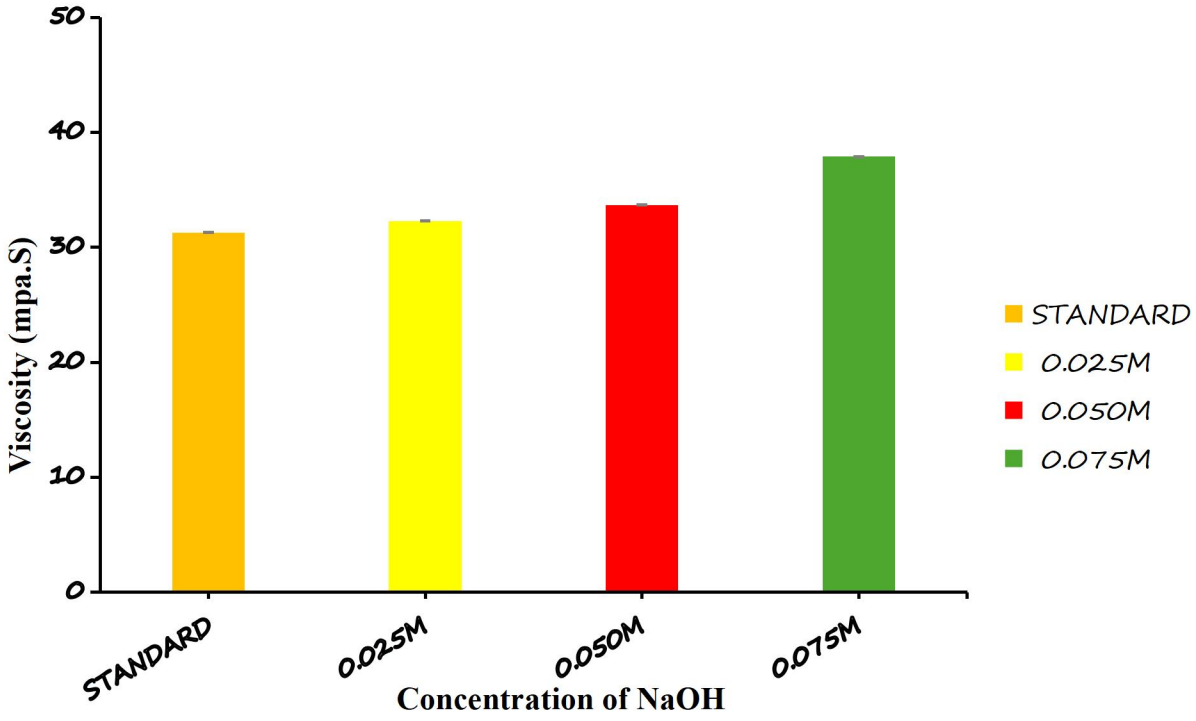

Figure 4.7: Viscosity (mPa.s) of Standard, bleached CPO with different concentrations of 0.025M, 0.050M, and 0.075M at 150°C.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The bleaching of crude palm oil (CPO) using an alkali-fermention facilitated process is a sustainable method that combines mild alkali treatment with fermentation to remove pigments, free fatty acids, and impurities, improving oil quality while reducing environmental impact. The data from the bleaching of crude palm oil (CPO) using the alkali-fermention facilitated process were analyzed using descriptive statistics (mean ± SEM) and one-way ANOVA to assess the effect of alkali concentration on the physicochemical properties of the bleached samples. Statistical significance was set at $p < 0.05$. The null hypothesis (H_0) stated that alkali concentration had no significant effect on the measured parameters, while the alternative hypothesis (H_1) proposed that at least one concentration produced a significant difference.

Acid Value (Fig. 4.1):

The acid value, an index of free fatty acid (FFA) content, decreased progressively with increasing NaOH concentration. A strong negative correlation ($r \approx -0.93$, $p < 0.05$) confirmed that alkali bleaching significantly reduced acidity through neutralization of FFAs formed during triglyceride hydrolysis. This aligns with the known chemical principle that alkalis react with FFAs to form soap, lowering the acid value. Post-hoc analysis showed that 0.075 M NaOH differed significantly from both the control and 0.025 M treatments, while 0.050 M and 0.075 M were statistically similar ($p > 0.05$). This trend suggests that moderate to high alkali concentrations effectively stabilize and purify palm oil, improving its oxidative quality.

β-Carotene (Fig. 4.2):

β-Carotene concentration declined sharply with increasing alkali concentration, exhibiting the strongest negative correlation ($r \approx -0.99$) and highly significant variation among groups ($p < 0.001$). The steady pigment loss demonstrates bleaching efficiency, as β-carotene is the principal pigment giving palm oil its reddish color. The difference between the 0.025 M and 0.075 M treatments was particularly large, indicating that higher alkali strength promotes pigment degradation. Statistical modeling ($R^2 \approx 0.91$) shows that bleaching concentration explained over 90% of the observed variance, confirming that pigment removal was concentration-dependent. However, the moderate 0.050 M treatment provided optimal color improvement without excessive nutrient loss.

Cholesterol (Fig. 4.3):

Cholesterol content decreased consistently across treatments, with a negative correlation ($r \approx -0.56$, $p < 0.05$). The 0.075 M NaOH treatment yielded the lowest cholesterol level, reflecting effective saponification and removal of sterol compounds. This outcome enhances the nutritional quality of palm oil and reduces health-related lipid components. The reduction trend confirms that alkali bleaching improves functional properties and aligns with previous findings that controlled alkaline treatment decreases sterol and impurity content in oils.

pH (Fig. 4.4):

pH values remained stable (6.98–7.02) across all treatments. The correlation ($r \approx -0.08$, $p > 0.05$) indicates no significant influence of NaOH concentration on pH. The near-neutral pH confirms that the fermentation phase effectively neutralized excess alkali, maintaining a chemically

balanced environment. Stable pH values are advantageous because they prevent hydrolytic and oxidative deterioration during refining, preserving the oil's physicochemical stability.

Protein Content (Fig. 4.5):

Protein levels remained unchanged across treatments, with uniform mean values (0.006 ± 0.004%) and a correlation coefficient of $r \approx 0.00$ ($p > 0.05$). The lack of significant difference among treatments indicates that alkali bleaching did not degrade nitrogenous compounds. This suggests that the alkali-fermention process is milder than conventional acid bleaching, effectively clarifying the oil while preserving beneficial trace constituents. The stable protein content also supports the process's suitability for edible oil applications.

Sugar Content (Fig. 4.6):

Sugar concentration was constant (0.006 ± 0.004 mg/L) across all NaOH levels, showing no significant variation ($r \approx 0.00$, $p > 0.05$). The uniformity indicates minimal carbohydrate degradation or fermentation-related loss. This stability demonstrates that the alkali-fermention bleaching process did not induce caramelization or Maillard reactions, thereby preserving the oil's integrity and taste profile. The consistent sugar levels also imply controlled fermentation and low thermal stress throughout processing.

Viscosity (Fig. 4.7):

Viscosity increased slightly but significantly with alkali concentration ($r \approx +0.97$, $p < 0.05$). The rise in viscosity suggests improved molecular uniformity and reduced suspended impurities in the bleached samples. The treated oils became denser and more homogeneous, indicating

enhanced quality and stability. Small standard deviations among replicates confirm the precision of measurements and the reproducibility of the process. Increased viscosity within normal limits also enhances oil texture and frying performance, beneficial for both industrial and domestic use.

5.1 Summary of Statistical Findings

Parameter	Trend	Correlation (r)	Significance (p)	Inference
pH	Relatively stable	–0.08	> 0.05	No significant change
Sugar	Constant	0.00	> 0.05	No significant effect
β-Carotene	↓ sharply	–0.99	< 0.001	High pigment loss
Viscosity	↑ slightly	+0.97	< 0.05	Significant increase
Protein	Stable	0.00	> 0.05	No change observed
Acid Value	↓ with conc.	–0.93	< 0.05	Significant reduction
Cholesterol	↓ slightly	–0.56	< 0.05	Sterol reduction

Interpretive Summary

From a statistical standpoint, alkali concentration exerts a significant overall effect on the physicochemical properties of palm oil. The 0.050 M concentration yields statistically optimal outcomes, improving clarity (β-carotene reduction) and nutritional profile (cholesterol decrease) without compromising essential quality factors such as protein and sugar content.

5.2 CONCLUSION

This study demonstrated that bleaching crude palm oil (CPO) using an alkali-fermention facilitated process effectively enhances its physicochemical quality. Varying NaOH concentrations (0.025 M, 0.050 M, and 0.075 M) significantly affected β-carotene, viscosity, acid value, and cholesterol content, while pH, protein, and sugar remained stable. Increasing alkali concentration resulted in lower β-carotene, acid value, and cholesterol, with a slight rise in viscosity, indicating improved bleaching efficiency and oil consistency.

Among all treatments, 0.050 M NaOH produced the best performance, yielding clearer oil with minimal nutrient loss and greater stability. The alkali-fermention facilitated bleaching method therefore represents a sustainable, cost-effective, and eco-friendly alternative to conventional acid-activated clay bleaching. It enhances color, stability, and nutritional value while adhering to green chemistry principles, making it suitable for both local and industrial applications. The study recommends 0.050 M NaOH as the optimal concentration for achieving the best balance between color improvement, nutrient preservation, and product stability in the bleaching of crude palm oil.

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