CALCIUM IONS AND MAGNESIUM IONS ATPASE ACTIVITY IN THE LIVER OF WISTAR RATS FED FRESH AND STORED PALM OIL

BY

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CERTIFICATION

This is to certify that this project report was prepared and presented by **Ohwevwo Oghenegueke Owen** of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin city, Edo State.

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DEDICATION

This work is dedicated to my Almighty Father for giving me the strength and wisdom to complete my tertiary education, to my amazing parents, siblings, loved ones and friends for their unwavering and undiluted love and support in my life and all my endeavors.

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I wish to express my profound gratitude and appreciation to God Almighty for His Grace and Mercy in my life and my studies, for always standing by me and making this journey worthwhile.

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ABSTRACT

Vegetable oils are significant sources of dietary fatty acids. Palm oil is an edible vegetable oil derived from the fruit of the palm plant (*Elaeis guineensis*) and is among the most produced and widely used edible oils globally. Nevertheless, storage circumstances substantially modify its chemical composition, elevating free fatty acid (FFA) levels. Free fatty acids (FFAs) are recognized for their capacity to compromise membrane integrity and enzymatic activity, potentially altering ATPase functions and resulting in modified hepatic metabolism. Employing groups of Wistar rats as experimental models, one group used as control and the other groups fed with fresh and stored palm oil containing varying levels of free fatty acids, assays were carried out to investigate the effects of fresh and stored palm oil on the change in body weight and the activity of calcium ions ATPase activity and magnesium ions ATPase activity in the liver of the Wistar rats. The results indicates that consumption of fresh and stored palm oil with varied free fatty acid levels (0.4%-42.7%) led to increase in the body weight of rats. Fresh palm oil with low free fatty acid levels contributes to optimal liver function by preserving the structural and functional integrity of cell membranes. This is due to its antioxidant components in fresh palm oil such as tocopherols, tocotrienols and carotenoids which played a protective role by mitigating oxidative stress, maintaining enzyme activity and promoting cellular homeostasis. Stored palm oil with elevated free fatty acid levels resulting from oxidation exhibited negative effects on ATPase activity in ca^{2+} and Mg^{2+} particularly for Ca^{2+} ATPase. This study reviews the effects of fresh and stored palm oil after consumption.

CHAPTER ONE

1.0. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Vegetable oils, which can be defined as "oils composed primarily of glycerides of fatty acids being obtained only from plant sources," have been introduced more recently in Europe. Until the late nineteenth century, the olive was the sole edible oil-bearing crop, and its utilization was predominantly confined to the Mediterranean region, whereas the remainder of the continent relied on animal fats as the primary supply of cooking oil. Technological advancements, extensive food production, and more affordable transportation have led to an increase in the consumption of olive oil and other vegetable oils (Cordain *et al*., 2005).

Vegetable oil is mainly composed of triacylglycerol(TAG) , a storage lipid that serves as a major commodity for food and industrial purposes. Vegetable oils are significant sources of dietary fatty acids. Worldwide, the primary oils in the human diet originate from soybean, palm, sunflower, and rapeseed, however significant variety exists based on regional traditions. These oils are primarily utilized for baking, frying, or as salad dressing (Sayon-Orea *et al*., 2015).

Vegetable oils are excellent sources of (n-9) monounsaturated fatty acids (MUFAs) and (n-6 and n-3) polyunsaturated fatty acids (PUFAs). Hydroxytyrosol is a distinct molecule linked to olive oil consumption, thought to play a role in several health benefits. Vegetable oils and fats are vital parts of food and crucial elements of our daily diet (Brahmi *et al*., 2020). Vegetable oils are derived from the mechanical expulsion or solvent extraction of oilseeds (such as soybeans, rapeseed, and sunflower) or oil-rich fruits like palm and olive (Vidrih *et al*., 2010). It predominantly consist of

triglycerides (about 98 g/100 g) (Qian *et al*., 2020), which are triesters formed from the interaction of glycerol and fatty acids, along with minor constituents (Gnanaprakasam *et al*., 2013).

Certain compounds, including diglycerides, vitamins, phytosterols, tocopherols, and polyphenols, confer significant health benefits to humans (Gharby *et al*., 2021; Chew *et al*., 2016), and thus should remain intact during processing. Other compounds known for their negative effect on the quality and stability of oils, include free fatty acids, unsaponifiable matters, waxes, pigments, solid impurities (mainly fibers), oxidation products (peroxides, aldehydes, ketones, alcohols, and oxidized fatty acids) (Gharby *et al*., 2016 ; Aliyar-Zanjani *et al*., 2019).

Fatty acids (FAs) are organic acids characterized by a minimum of one carboxyl (–C(=O)OH, –COOH, or –CO2H) group and an elongated carbon chain, which may contain double bonds in unsaturated fatty acids or single bonds in saturated fatty acids. They are classified as saturated or unsaturated on the basis of the absence or presence of double bond (Baum *et al*., 2012). Fatty acids are primarily sourced from triglycerides and phospholipids, serving as the principal constituents of dietary fats. Most naturally occurring fatty acids possess a linear chain including an even number (4–28) of carbon atoms. The fatty acid catalogue categorizes fatty acids into saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) based on the quantity of double bonds present. Typically, plants synthesize fatty acids that may include zero to three double bonds. The often encountered fatty acids comprise palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3). In oilseed plants, these fatty acids are primarily stored as triacylglycerols (TAG), the principal storage form in seeds. These lipids may be stored in cotyledon or endosperm which are needed to supply energy during germination. Besides TAG, fatty acids are also present as wax esters, such as those found in jojoba fruit

(*Simmondsia chinensis*)

Fatty acids are produced in plastids from acetyl-CoA as the initial substrate and on acyl carrier protein (ACP). The fatty acids are then extracted from ACP through the action of the enzyme thioesterase. Free fatty acids migrate to the cytosol, where they are subsequently integrated into the acyl-CoA pool and/or the phosphatidylcholine (PC) pool. These pools then undergo changes, including desaturation, hydroxylation, and epoxylation, before being combined into triacylglycerol (TAG). The subsequent events transpire in the endoplasmic reticulum (ER) of plant cells (Bates *et al*., 2013). TAGs constitute the primary storage form present in seeds. It is produced in ER, using acyl-CoA and glycerol-3-phosphate as substrates by the Kennedy route. The initial enzyme is glycerol-3-phosphate acyltransferase (GPAT), which acylates the sn-1 position of the glycerol backbone to produce lysophosphatidic acid (LPA). The second enzyme in the cycle is lysophosphatidic acid acyltransferase (LPAAT), which acylates at the sn-2 position to produce phosphatidic acid (PA), subsequently transformed into diacylglycerol (DAG) by phosphatidic acid phosphatase (PAP). Another acyltransferase, diacylglycerol acyltransferase (DGAT), produces TAG from DAG utilizing an acyl-CoA as a substrate (Bates *et al*., 2013).

Fatty acids are allocated to cells where they function as energy sources (Philip , 2015) for muscle activity and overall metabolism. As biological entities, fatty acids are essential in human metabolism, health, and illness. They are a source of consumable fatty acids (saturated, monounsaturated, or polyunsaturated), which are crucial for cellular metabolism, serving both as energy storage and as a means of energy provision when necessary. Fatty acids are recognized for their significant involvement in cellular division and development. They are essential constituents of cell membranes, hormones, neurotransmitters, and other biological molecules.

Saturated fatty acids (SFAs) are fully saturated with hydrogen. The majority of saturated fatty acids consist of linear hydrocarbon chains containing an even number of carbon atoms. SFAs are generally split into subgroups depending on their chain length. The primary food contributors of individual saturated fatty acids in the diet differ by saturated fatty acid chain length. For example, the major food sources of short chain SFAs are dairy fats while medium and long chain SFAs are predominantly found in red meat, diary fats and plant oils (Ratnayake and Galli, 2009).

Monounsaturated fatty acids (MUFAs) possess a single carbon-carbon double bond, which may be located in various places. In the cis configuration, the hydrogen atoms are on the same side as the double bond whereas in trans configuration , the hydrogen atoms and double bond are present on opposite sides. The cis-isomers are the predominant form of MUFA in food sources. Oleic acid represent the topmost MUFA provided in the diet (90% of all MUFAs) (Schwingshack1 and Hoffman, 2012).

In polyunsaturated fatty acids (PUFAs), the initial double bond is located between the third and fourth carbon atoms from the n carbon; these are designated as n-3 or omega-3 fatty acids. If the first double bond is between the sixth and seventh carbon atom, then they are called n-6 fatty acids. The double bonds in polyunsaturated fatty acids (PUFAs) are interspersed by a methylene group. Polyunsaturated fatty acids (PUFAs), synthesized only by plants and phytoplankton, are vital for all higher creatures, including mammals and fish. Fish and plant oils are often rich in PUFAs with fish being rich in omega-3 and plant oils rich in omega-6(Abdelhamid *et al*., 2018). n-3 and n-6 fatty acids are not interconvertible and are both necessary nutrients. PUFAs undergo additional metabolism in the body by elongation (addition of carbon atoms) and desaturation (removal of hydrogen). Mammals possess desaturases that may eliminate hydrogens solely from carbon atoms

situated between an existing double bond and the carboxyl group. β-oxidation of fatty acids, a catabolic process involving the degradation of fatty acids, can occur in either mitochondria or peroxisomes.

During digestion, free fatty acids (FFAs) and monoacylglycerols are liberated and assimilated in the small intestine. In the intestinal mucosa cells, FFAs are re-esterified to triacylglycerols, which are delivered via lymphatic vessels to the circulation as part of chylomicrons. Fatty acids are carried in circulation either bound to albumin or incorporated within lipoproteins (Glatz *et al*., 2010). Free fatty acids (FFAs) are absorbed by cells primarily by protein transporters in the plasma membrane and are subsequently transferred intracellularly by fatty acid-binding proteins (FABP). Fatty acids are then activated to acyl-CoA and transported by acyl-CoA-binding protein (ACBP) to mitochondria or peroxisomes for β-oxidation. However, when there is a dysfunction in this process , it leads to the accumulation of free fatty acids. Excessive amounts of free fatty acids FFA accumulated in peripheral tissues have adverse effects on cellular signalling and functions , effects known as lipotoxicity. It is mostly linked with obesity caused by insulin resistance (Tumova *et al*., 2016). Excess FFA causes oxidative stress by forming reactive oxygen species which damage cell membranes and impair cellular processes. In the liver, excess FFA leads to impaired activity of membrane-bound enzymes such as ATPases which are essential for ionic balance.

1.1.1. Justification of Study

The study of Ca^{2+} and Mg^{2+} ATPase activity in the liver of Wistar rats consuming fresh and preserved palm oil is important for elucidating the biochemical and physiological impacts of dietary lipids, especially in relation to storage conditions. Ca^{2+} and Mg^{2+} ATPases are essential for preserving

cellular ion equilibrium, especially in metabolically active tissues such as the liver. Ca^{2+} ATPase regulates intracellular calcium, which is essential for metabolism, signaling, and cell survival. Ca2+ concentration by plasma membrane. Ca2+ -ATPase is a critical for cell survival as disruptions in Ca2+ homeostasis can alter cellular physiology (Brini *et al*., 2013). Dysregulation can lead to oxidative stress and cellular damage frequently associated with liver disorders. Mg^{2+} ATPase is crucial for ATP hydrolysis and energy dependent processes in cells, influencing metabolic functions in the liver (Romani, 2011).

Palm oil is a worldwide prevalent edible oil owing to its cost-effectiveness and versatility. Nevertheless, storage circumstances substantially modify its chemical composition, elevating free fatty acid (FFA) levels. Free fatty acids (FFAs) are recognized for their capacity to compromise membrane integrity and enzymatic activity, potentially altering ATPase functions and resulting in modified hepatic metabolism (Boateng *et al*., 2016).

Studies have shown that prolonged storage of palm oil leads to oxidative degradation , forcing harmful byproducts like peroxides and free fatty acids which are hepatotoxic (Edem, 2002). Employing Wistar rats as experimental models facilitates controlled investigations to ascertain the causal links between dietary palm oil and hepatic ATPase activity. This study assesses the influence of fresh and preserved palm oil on ATPase activity, offering significant insights for dietary and public health recommendations.

1.1.2. Aim of Study

This study is directed at investigating the effects of fresh and stored palm oil on Ca^{2+} ATPase and Mg^{2+} ATPase activities in the liver of wistar rats.

1.1.3. Objectives of Study

i. To determine the activity levels of Ca^{2+} ATPase in the liver of wistar rats fed fresh palm oil and stored palm oil.

ii. To assess the activity levels of Mg^{2+} ATPase in the liver of wistar rats fed fresh palm oil and stored palm oil.

iii. To compare the enzymatic activities between rats fed with fresh palm oil and stored palm oil, highlighting any significant differences in metabolic impact in the liver.

iv. To evaluate the relationship between free fatty acid levels in palm oil and the activities of hepatic Ca^{2+} ATPase and Mg^{2+} ATPase.

1.2. Literature Review

1.2.1. The Oil Palm

The oil palm tree, or *Elaeis guineensis*, sometimes referred to as the African oil palm, is a member of the Arecaceae family, which includes coconut and date palms (Gnanesh *et al*., 2014). It is a crucial element of the global palm family, possesses substantial economic significance, is extensively researched, and is commercially utilized (Espana *et al*., 2018). The species *Elaeis oleifera* or *Elaeis melanococca*, commonly referred to as "Caiaué," is recognized as the American oil palm tree. The hypothesis is that the separation of the American and African continents in past times led to the emergence of those species recognized today (Gnanesh *et al*., 2014). Scholars widely concur that the oil palm E. guineensis is indigenous to the western and southwestern areas of Africa, specifically the territory between Angola and Gambia (Reddy *et al*., 2019). It is posited that domestication occurred in its indigenous environment, presumably in Nigeria, and subsequently disseminated throughout tropical Africa about 5000 years ago.

The oil palm fruit (OPF), derived from the palm tree, is a drupe that develops in dense, spiky clusters (Mba *et al*., 2015). The oil palm yields fruits in fresh fruit bunch (FFB) clusters (Figure 2). These clusters consist of densely packed spikelets carrying fruits and can weigh up to 50 kg, housing anything from several hundred to several thousand fruits (Nair, 2010). Each fruit consists several various layers, including an exterior skin (exocarp), a fleshy pulp (mesocarp), a protective shell (endocarp), and an inner kernel (endosperm), (Pereira *et al*., 2020; Mba *et al*., 2015). The mesocarp, a fibrous structure, holds palm oil, while the kernel encloses oil within its center nut. A standard palm fruit is roughly 3.5 cm long and weighs between 3.5 and 4.0 g. OPF is identifiable by its reddish hue and cluster-like development pattern. Each fruit comprises two primary components: the oily, fleshy layer termed the mesocarp, and a solitary seed, referred to as the palm kernel or endosperm. The oil obtained from the mesocarp is designated as crude palm oil (CPO), whereas the oil derived from the

kernel is termed palm kernel oil (Mba *et al*., 2015).

Fig 1.1 (a) Oil palm tree (b) Oil palm fruits

1.2.1.1. Processing of Fresh Fruit Bunches into Crude Palm oil

Following the third year of cultivation, the first bunches of fruits commence ripening. About 180 days post-initiation of inflorescence development, oil formation commences, with a significant acceleration occurring after two weeks of maturation (Rao *et al*., 2010). Two varieties of oil are derived from the fruit of the palm tree: red crude palm oil from the mesocarp and palm yellow crude kernel oil from the endosperm, each possessing a unique composition (Gee, 2007). Mesocarp oil is predominantly utilized for culinary purposes, while palm kernel oil is employed in the oleochemical sector. When fully ripe, the fruit mesocarp normally contains 68.0% to 73.2% (w/w) edible oil.

The manufacture of palm oil encompasses several intricate stages, including the sterilization of fresh fruit, fruit detachment, digestion, oil extraction, and clarifying. Sterilizing fresh fruit is a vital step requiring moisture absorption and heat treatment to deactivate lipolytic enzymes such as lipases in the fruit mesocarp (Wondi *et al* ., 2019; Wondi *et al*., 2020; Tan *et al*., 2023). These enzymes may result in elevated concentrations of free fatty acids (FFAs) (Rao *et al*., 2010), leading to quality concerns during storage, processing, and refining (Mahmod *et al*., 2023). The condensed water from this operation constitutes a substantial source of palm oil mill effluent (POME). A number of studies have concentrated on enhancing the utilization of POME, including the repurposing of water produced during oil extraction for milling processes or as potable water (Sodri and Septriana, 2022). POME is also utilized for biogas production (Sodri and Septriana, 2022).

The production of palm oil can be classified into artisanal and industrial milling techniques. The oil extraction procedure, utilizing several methodologies, is essential in ascertaining the output and quality of oil. These extraction methods can be classed based on their complexity and processing

capacity, ranging from artisanal techniques and small mechanical units to medium-scale and big industrial mills (Nchanji *et al*., 2013).

Artisanal palm oil extraction is the most ancient technique of oil separation, typically utilizing traditional apparatus. In artisanal extraction, harvested fruit bunches are allowed for several days to promote the detachment of the fruits before the oil extraction process, enhancing lipase activity and leading to the hydrolysis of palm oil triglycerides (Godswill *et al*., 2017). The fruits are later cooked in a drum, and extraction is conducted by a manual or motorized press.

Industrial palm oil extraction utilizes two main methods: chemical or wet procedures, including solvent extraction, and physical or dry ones, such as mechanical pressing. These techniques can attain oil extraction efficiency between 75% and 90% (Alam *et al*., 2020; Gibon *et al*., 2009). The selection between these two techniques is contingent upon various circumstances, including the quality and acidity of the crude oil and local regulations.

In the solvent extraction procedure, oil is obtained from the disrupted cells of the oil palm using water or steam. This procedure coagulates proteins and hydrolyzes any starch, adhesive, or gum that may be present (Chai *et al*., 2023). These chemicals may induce oil foaming while frying. The alkaline neutralization phase of chemical refining eliminates free fatty acids and the majority of phosphatides. In the ensuing oil clarifying phase, hydrolyzed and coagulated substances are eliminated. Crude palm oil (CPO) is obtained following the evaporation of moisture (Awere *et al*., 2023; Zamanhuri *et al*., 2022).

Dry extraction employs a hydraulic press, screw press, or centrifuge to rupture the oil cells. The screw press is generally more appropriate for continuous extraction systems, whereas the hydraulic press is frequently employed in batch or semi-batch extraction systems. Upon pressing, the crude palm oil is extracted from the fibrous mesocarp, with the residual fiber components containing around 5 to 6% (w/w) of the oil. The yield and quality of the extracted oil are affected by beginning oil and moisture contents, operation temperature, heating duration, and applied pressure (Zamanhuri *et al.*, 2022). The pressure is generally diminished to avert the rupture of fruit kernels, hence enhancing oil retention to around 10–12% in the mesocarp biomass.

Premium palm oil generally exhibits low levels of free fatty acids and moisture, minimum impurities, and a superior degradation of the bleachability index (DOBI). The grade and market worth of palm oil rely on the quality of the extracted product. Triacylglycerol (neutral lipid), carotenoids, phytosterols, and vitamin E (tocopherol and tocotrienols) are valuable constituents of oils due to their nutritional significance. During extraction processes, whether artisanal or industrial, numerous compounds are extracted alongside the oil, including free fatty acids, partial acylglycerols, phosphatides, sterols, tocopherols, tocotrienols, hydrocarbons, pigments, vitamins, sterol glycosides, protein fragments, traces of pesticides, dioxins, and heavy metals.

Consequently, palm oil comprises unwanted constituents including water, oil contaminants, and fruit debris. Mitigating these chemicals is essential to enhance the quality of palm oil and broaden its applications. The objectives of the refining process include producing a moisture content below 10% and decreasing the FFA level to 0.3% (Abdeltawab and Khattab, 2018). In contrast, free fatty acids, phospholipids, and gums are regarded as pollutants and are chemically undesirable. Palm oil requires processing to achieve the requisite purity attributes and become consumable (Silva

et al., 2014). The refining process of palm oil, whether chemical or physical, successfully eliminates contaminants, yielding refined, bleached, and deodorized (RBD) palm oil. The quality of refined palm oil is largely evaluated based on parameters like free fatty acid content, iodine value, peroxide value, moisture content, saponification value (SV), and impurity level.

Chemical refining entails the elimination of free fatty acids through alkali treatment and the separation of soap via centrifugation (sludge). Chemically refined CPO undergoes washing with a sodium hydroxide or sodium carbonate solution to diminish free fatty acids and eliminate phospholipids and other polar lipids. Nonetheless, alkali refining alone may not eradicate all potentially harmful chemical constituents (Alhaji *et al*., 2016).

Physical refining eliminates free fatty acids and other substances by a stripping process. The selection of refining technique is contingent upon the properties of each oil. Oils like palm, palm kernel, and coconut, characterized by low phospholipid content, are predominantly subjected to physical refining. Physical refining offers benefits through reduced chemical usage and diminished effluent output. Physical refining is recommended for crude palm and palm kernel oils characterized by low initial phosphatide levels and elevated carotene and free fatty acid (FFA) contents, as it minimizes neutral oil loss and operational expenses (Alhaji *et al*., 2016; Sampaio *et al*., 2013). Processing parameters can be modified to enhance the retention of beneficial secondary components like tocopherols and tocotrienols while reducing the formation of undesirable trans fatty acids. When physically refined, CPO undergoes bleaching and deodorization, which demand high temperatures (Tan *et al*., 2021). Bleaching is an adsorption procedure carried out at temperatures ranging from 95 °C to 135 °C. In this procedure, neutral or acid-activated bleaching earth eliminates colors, metals, oxidation products, and soaps (Almeida *et al*., 2019). During bleaching, certain

carotenes are eliminated, and the residual carotenes are degraded during deodorization at temperatures of 240 °C or above (Ribeiro *et al*., 2018). This thermal bleaching process eliminates free fatty acids, aldehydes, and ketones via volatilization. Oils characterized by low DOBI and elevated concentrations of FFA and peroxides exhibit a greater susceptibility to bleaching (Vispute and Dabhade, 2018). The DOBI value is the ratio of oil absorption at 446 nm to that at 268 nm, reflecting the relative quantities of carotenes and oxidized carotenes. A greater DOBI value indicates a reduced presence of oxidized carotenes, facilitating the bleaching process of the oil. DOBI levels ranging from 2.5 to 4.0 signify moderate to good crude oil quality, whereas values below 2.0 denote poor quality, which presents challenges for bleaching.

The increasing temperatures employed in refining procedures may result in unfavorable chemical alterations due to the increased heat during these stages. The use of bleaching earth may result in the generation of oxidation products, elevating the concentrations of free fatty acids, foams, colorants, and viscous substances (Almeida *et al*., 2024). During refining, undesirable byproducts such as 3-monochloropropane-1,2-diol (3-MCPD) and glycidyl esters (gEs) are generated, which are pollutants caused by the process (Rahn and Yaylayan, 2011). These chemicals are poisonous, and their ingestion is associated with tumor growth.

The synthesis of 3-MCPD and gEs is predominantly affected by temperature, particularly during the deodorization of oil (Jędrkiewicz *et al*., 2016). 3-MCPDs are very imperceptible in virgin, unprocessed oils (Arris *et al*., 2020). These pollutants are generated not only in oils during processing but also in prepared foods such as bread, cakes, cookies, cereals, roasted coffee, and infant meals (Weißhaar, 2011). Besides the aforementioned parameters, other additional factors affect the synthesis of these chemicals, including soil type, fertilizers, and the harvesting interval of

the bunches (Arris *et al*., 2020). The formation mechanisms of these contaminants remain inadequately understood (Tivanello *et al*., 2021); nonetheless, strategies to reduce their development are established: lowering chlorides and other precursors by washing crude oil prior to the deodorization phase (Yung *et al*., 2023; Santiago *et al*., 2021). Regulating the DAG concentration and minimizing exposure and duration to elevated temperatures during processing, while employing neutral bleaching clays, as acid-activated clays are treated with hydrochloric acid (Oey *et al*., 2019). Refined oil may progressively alter in color to deeper tones during shipping, storage, and consumption, a phenomenon known as color reversal (Chen and Sun, 2023). Color reversal is typically linked to substandard oil quality or insufficient degumming and bleaching procedures. The presence of colorful pigments and oxidation chemicals significantly influences the final hue of the oil and contributes to the phenomena of color reversal. Temperature induces the discoloration of carotenes while simultaneously promoting oxidation, resulting in the production of alternative colored molecules. The oil may have an intensified yellowish or red hue due to the development of tocopherol oxidation products, such as γ-tocopherol and γ-tocopherol-5,6-quinone, which may stabilize other pigments, preventing their removal through adsorption (Igile *et al*., 2013).

1.2.1.2. Primary Products of the Oil Palm.

Two distinct forms of oil are present in the primary tissues of palm fruits: 'palm oil' and 'palm kernel oil' (Murphy, 2019). Palm oil, derived from the fleshy mesocarp tissue, is a deep orange-red, semi-solid liquid, whereas palm kernel oil is a white-yellow oil primarily collected from the endosperm tissue of the kernel (seed). The distinct fatty acid contents of these two oils result in their utilization for various downstream applications across many industrial sectors (Goggin and Murphy, 2018). The elevated saturated fat content of palm oil renders it especially appropriate for culinary applications as

a solid vegetable fat (melting point around 35°C). Conversely, palm kernel oil is a less dense substance (melting point approximately 24°C) primarily utilized for non-edible purposes. A primary application of palm kernel oil is as the essential functional component in numerous soaps, detergents, and cosmetics. Elaeis guineensis trees produce abundant oil-rich fruit bunches throughout the year, with each bunch comprising between 1000 and 3000 individual fruits (Corley and Tinker, 2015). Mesocarp-derived palm oil constitutes around 89% of the total fruit oil, while the remaining 11% is sourced from the seed or kernel. Due to the distinct mechanical procedures employed in the extraction of palm oil and palm kernel oil from fruits, as well as their divergent downstream applications, they enter separate supply chains immediately after extraction in mills.

1.2.2. Palm oil

Palm oil is an edible oil derived from the fruit of the palm plant (*Elaeis guineensis*) and is among the most produced and widely used edible oils globally. This occurs despite being sourced from the smallest global proportion of all farmed land dedicated to oils and fats (Ismail *et al*., 2018). Palm oil serves multiple functions. Mesocarp oil and kernel seed oil have multiple applications; roughly 80% are utilized for culinary purposes, while the remaining 20% serve as feedstocks for diverse nonfood uses. Historically, palm oil has been utilized for domestic cooking throughout Southeast Asia, tropical Africa, and South America for centuries (Purnama *et al*., 2020; Echioda *et al*., 2018). In recent decades, the food sector has embraced refined palm oil due to its functional advantages, adaptability, and extensive availability.

The primary advantages of palm oil include:

(1) its high stability over time since palm oil helps keep the product's taste throughout its shelf life due of its higher oxidation stability than other vegetable oils do.

(2) Its neutral flavor and the aroma of deodorized palm oil facilitate its integration into diverse culinary applications without altering taste; this neutrality guarantees that the oil does not overshadow the flavors of other components, including milk, chocolate, and hazelnuts.

(3)The adaptability of vegetable fat arises from its capacity for fractionation into several solid components, rendering it appropriate for diverse texture and flavor needs in final products (Wang *et al.*, 2016).

(4) Palm oil imparts a smooth and creamy texture to food products, offering an exceptional mouthfeel with distinct characteristics for each item; for instance, it enhances the smoothness and spreadability of chocolate spreads.

(5) As a substitute for trans fats, palm oil serves as an appropriate alternative to partially hydrogenated fats.

A significant proportion of items available in supermarkets contain palm oil in their formulation. The products encompass margarine, confectionary, ready-to-eat meals, snacks, chocolate, ice cream, baked goods, and non-food items including soap, candles, and cosmetics.
The fractionation process can ascertain the chemical and physical properties of olein and stearin: at the industrial scale, refined, bleached, and deodorized (RBD) olein is predominantly utilized in food

products, including cooking and frying oils, shortening, and margarine; RBD stearin is similarly employed in the production of margarine and shortening (de Almeida *et al*., 2021). Unfractionated RBD palm oil is utilized in the production of ice cream, margarine, shortening, vanaspati (vegetable ghee), frying fats, and ice cream.

Nonfood applications of palm oil encompass cosmetics and personal care products, soap, candles, medicines, metal plating, lubrication and grease, surfactants, industrial chemicals, agrochemicals, coatings, paints, lacquers, electronics, leather, and biodiesel manufacturing. Besides mesocarp and kernel oils, the primary products of oil palm, tree and fruit processing waste have several applications. Sludge is utilized in conventional soaps and fertilizers, while palm kernel cake is extensively employed as an ingredient in the feed industry and fertilizers. The processing byproducts, specifically empty bunch trash, fibers, shells, sludge, and mill effluent, comprise roughly 75% of the entire mass of the oil products. The remaining components of the palm tree (trunk, leaves, and fiber) possess extensive applications, whereas the bunch trash and leftovers from oil production (fiber, shell, and sludge) can serve as fuel for mills, rendering briquettes an alternative to fuel wood. Kernel cake was employed in animal feed and organic fertilizer production as a substrate for mushroom cultivation. The midribs and rachises are utilized as roofing materials (Echioda *et al*., 2018). The phytochemistry of palm oil fruit has been thoroughly investigated and is recognized for its numerous bioactive compounds, including a palmitic-oleic-rich semi-solid fat (comprising 95% triglycerides and fatty acids such as palmitic, myristic, stearic, oleic, and linoleic acids), a vitamin E fraction (30% tocopherols and 70% tocotrienols), carotenoids, polyphenols, and phytosterols.

1.2.3. Oil Palm Tree Identity

Preferred Scientific Name: *Elaeis guineensis Jacq.*

Preferred Common Name: African oil palm

Other Scientific Names: *Elaeis dybowskii Hua, Elaeis madagascariensis*(Jum. & H. Perrier) *Becc*., *Elaeis melanococca Gaertn*. , *Elaeis virescens* (A.Chev.) *Prain* , *Palma oleosa Mill*.

International Common Names:

English : macaw-fat, oil palm

Spanish : palma africana, palma de aceite

French :palmier a huile, palmier à huile, palmier a huile d'Afrique, palmier a l'huile

Local Common Names:apwiraiasi , **Brazil** :palmeira-caiaué , **Cambodia** :dôong preeng,**Cook Islands** :nu tamara, **Germany** :Oelpalme, **Indonesia** :kelapa sawit; salak minyak, **Italy** :palma da olio, **Malaysia** : kelapa bali; kelapa sawit, **Myanmar** :si-htan; siohn; si-ohn, **Netherlands** :oliepalm, **Portugal** :dendê ; palmeira-andim, **Sweden** :oljepalm, **Tanzania** :mchikichi, **Thailand** :pam namman, **Vietnam** :co dâù; dùa dâù.

1.2.3.1. Taxonomical Classification

Domain: Eukaryota

Kingdom: Plantae,

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Monocotyledonae,

Order: Arecales

Family: Arecaceae

Genus: *Elaeis*

Species: *Elaeis guineensis*

1.2.4. Chemical Components of Palm Oil

A notable feature of palm oil is its oleic acid content and the abundance of antioxidant components, including carotenes and tocols, which enhance its resistance to oxidation and suitability for frying, thereby fulfilling the demands of the food sector (Montoya *et al*., 2013). African palm oil is derived from the mesocarp of the fruit and is recognized for its unique composition of fatty acids. This oil exhibits a balanced composition of saturated and unsaturated fatty acids, rendering it highly adaptable and applicable in various contexts (Uuh-Narváez *et al*., 2023).It comprises roughly 44% palmitic acid, 40% oleic acid, 10% linoleic acid, and 5% stearic acid; hence, it consists of approximately 50% saturated fatty acids and 50% unsaturated fatty acids. This composition contrasts with that of the oil derived from the American species *Elaeis oleifera* (Caiaué). The unsaturated fatty acid concentration in *Elaeis oleifera* palm trees ranges from 47% to 69% for oleic acid, 2% to 19% for linoleic acid, and 0.1% to 1.2% for linolenic acid (Montoya *et al*., 2014), whereas palmitic acid constitutes roughly 24%. The interspecific hybrid possesses a fatty acid composition of oleic acid (55%), palmitic acid (27%), and linoleic acid (11%).

Palm oil is frequently condemned for its elevated levels of saturated fatty acids (SFA), especially palmitic acid, which are associated with health problems including obesity, cardiovascular disorders,

diabetes, and malignancies. Oleic acid, a monounsaturated fatty acid (MUFA) found in high concentrations in palm oil, might lower detrimental cholesterol levels and safeguard against cardiovascular disease (Ribeiro *et al*., 2018), as well as polyunsaturated fatty acids (PUFA). Unsaturated fatty acids, designated as omega (ω), are classified based on the location of the carbon in the unsaturated chain, including ω-3 (linolenic acid), ω-6 (linoleic acid), and ω-9 (oleic acid) fatty acids.

Palm oil mostly consists of a blend of triacylglycerols, accounting for roughly 95% of its composition. Nonetheless, the oil comprises several minor constituents, including free fatty acids (FFA), monoacylglycerols (MAG), diacylglycerols (DAG), metals, phospholipids, peroxides, and chlorophylls, along with antioxidants and valuable compounds such as carotenoids, vitamin A precursors, tocols (tocopherols and tocotrienols), and phenolic compounds (Norhaizan *et al*., 2013; Machado *et al*., 2023). Vegetable oils are vital in the human diet as they serve as significant transporters of fat-soluble vitamins, including A, D, E, and K.

Carotenoids, vital minor constituents of palm oil, possess elongated chains with conjugated double bonds that markedly affect the oil's color, varying from yellow to orange-red. Liposoluble pigments are accountable for the characteristic orange hue of the oil derived from the mesocarp. The concentration of pigments in the oil extracted from *Elaeis guineensis* fruits ranges from 600 to 1000 ppm, while for *Elaeis oleifera*, it exceeds 4000 ppm. The concentration in interspecific hybrids ranges from around 1400 to 2300 ppm (Vidoca *et al*., 2020). About 90% of the carotenes in the oil consist of α- and β-carotenes (Zurawik *et al*., 2021).

Carotenoids has antioxidant qualities that beneficially influence human health, rendering palm oil significant for the prevention of vision impairments, cardiovascular diseases, and cancer (Yeung *et*

al., 2022; Amorim *et al*., 2022). Carotenoids function as precursors to vitamin A, with β-carotene demonstrating the highest provitamin A (retinol) efficacy. Carotenes not only confer health benefits but also significantly influence the oxidative processes of oil by mitigating oil oxidation through their capacity to suppress 1O2 (singlet molecular oxygen), with this capability enhancing in correlation to the number of double bonds in the chain. Besides their nutritional benefits, these chemicals are eliminated from the oil during refining to provide a lighter color for enhanced consumer acceptance in many industrial applications. Conversely, preserving residual carotenoids in post-bleaching palm oil is crucial, as they inhibit the oxidation process (Tan *et al*., 2021).

A crucial minor component of palm oil is tocopherols and tocotrienols, collectively referred to as tocols. In conjunction with carotenes, these chemicals may function synergistically as antioxidants, improving the oxidative stability of the oil (Almeida *et al*., 2019). These substances have a chromanol group that influences vitamin E activity in the diet (Shahidi and De Camargo, 2016). A deficiency of this vitamin can lead to anemia, diminished immunological response, retinopathy, neuromuscular and neurological disorders, as well as strong anticarcinogenic agents that assist in combating thrombosis (Sampaio *et al*., 2013). Palm oil, alongside carotenes and tocols, is abundant in biologically active chemicals that enhance nutritional absorption and bolster brain function, such as phospholipids, phenolic compounds, and considerable quantities of squalene and phytosterols (Loganathan *et al*., 2010).

1.2.5. Physio-chemical Properties of Palm Oil

The distinctiveness of palm oil and its health advantages relative to other vegetable oils can be attributed to its fatty acid composition and their arrangement within the triglyceride structure (Mancini *et al*., 2015). The appropriateness of palm oil within the global marketplace largely hinges on the physical and chemical characteristics of the oil at the moment of acquisition. Typically, the properties taken into account include free fatty acids (FFA), iodine value (IV), peroxide value (PV), moisture content, impurities content, color, taste, aroma, melting point, as well as tocopherol and tocotrienol contents (Zarezadeh et al. 2021b). Additional factors that bear an indirect relationship to the composition of edible oils encompass the iodine value and saponification value.

1.2.6. Health Benefits of Palm Oil

Triacylglycerols, constituting the majority of dietary fats and oils, yield twice as much energy per gram compared to carbohydrates. Fats and oils play a crucial role in providing essential fat-soluble vitamins, including Vitamins A, D, E, and K. Palm oil is notably abundant in pro-vitamin A carotenoids and comprises approximately 800 ppm of vitamin E compounds, which are rich in tocopherols and tocotrienols (Carneiro *et al*., 2023). The nutritional advantages of palm oil can be attributed to the antioxidant characteristics inherent in carotenes, tocopherols, and tocotrienols. These compounds function as agents that neutralize free radicals generated through biological metabolism or as a result of exposure to toxic chemicals and environmental pollutants. Free radicals are associated with the processes of aging, the development of chronic degenerative diseases like atherosclerosis and arthritis, as well as the mechanisms of carcinogenesis (Ribeiro *et al*., 2018).

Palmitic acid is the primary saturated fat found in palm oil. A 16-carbon chain is an essential fatty acid involved in cell membrane structure, lipid transport, secretion, and overall human physiology. Additionally, palmitic acid plays a role in energy production by facilitating the association with specific proteins necessary for the proper functioning of the nervous system and the formation of intercellular connections (Agostoni *et al*., 2016; Carta *et al*., 2017). Furthermore, the antioxidant content of palm oil, comprising tocopherols and tocotrienols, offers a protective effect for cells and tissues against oxidative stress caused by free radicals. The vitamin E content of palm oil provides protective effects against cellular aging, cancer, atherosclerosis, arthritis, and Alzheimer's disease. Tocotrienols in palm oil reduce low-density lipoprotein cholesterol (LDL-C) levels by 7-38% while maintaining high-density lipoprotein (HDL) levels. This effect is achieved through the inhibition of HMG CoA reductase, the enzyme responsible for regulating cholesterol synthesis in the liver.

1.2.7. Oxidation of Palm Oil

Oils and fats consist of a combination of triacylglycerols derived from various fatty acids, with palm oil products predominantly comprising oleic and palmitic acids. Lipid oxidation is a significant issue impacting palm oil, as it is with other edible oils, leading to critical deteriorative alterations in their chemical, sensory, and nutritional characteristics (Velasco and Dobarganes, 2002). Oxidative deterioration of oils and fats leads to the formation of unpleasant off-flavours and the degradation of vitamins (A, D, E, K, C), essential fatty acids, chlorophylls, carotenes, amino acids, proteins, and enzymes due to the generation of toxic or physiologically active compounds. Unsaturated vegetable oils exhibit greater reactivity compared to animal fats, which are primarily saturated and less prone to chemical reactions. Oxidation typically progresses slowly during the initial phase and accelerates upon exposure to heat, light, and trace metals. An increase in dissolved oxygen content (DOC) will

accelerate the oxidation process.

The presence of double bonds in unsaturated fatty acids can lead to accelerated oxidation; thus, an increase in the number of double bonds correlates with decreased oil stability. Consequently, palm oil exhibits greater stability compared to polyunsaturated oils, such as soybean oil and corn oil, owing to its reduced proportion of unsaturated fatty acids. Crude palm oil comprises carotenoids at concentrations ranging from 500 to 700 ppm and vitamin E at concentrations between 600 and 1000 ppm. These components are dietary essentials primarily functioning as antioxidants, substances that inhibit oxidation.

Different chemical mechanisms such as autoxidation and photosensitized oxidation occur during storage and are responsible for the oxidation of edible oils. Autoxidation is a reaction between unsaturated fatty acids, (regardless of whether they are in their free state or esterified as a triglyceride molecule) and oxygen. These reactions originate hydroperoxides, which are rapidly decomposed to aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones (Choe and Min, 2006; Adetola *et al*., 2016). Changes in oil quality during inappropriate storage conditions are still a major issue from the health perspective (Almeida *et al*., 2018). Also, most of the oils erroneously referred to as fresh palm oil is photoxidized (Beshel and Beshel, 2019).

Hydroxyperoxides represent the initial products of the oxidation process, subsequently degrading into secondary oxidation products. This results in a complex mixture of aldehydes, ketones, and free fatty acids, which contribute to undesirable flavors and diminish the oil's value. In addition to oxidative rancidity, hydrolytic reactions facilitated by lipases from food sources or microorganisms contribute to the deterioration of oil. Hydrolytic reactions can be mitigated through cold storage, effective transportation, proper packaging, and sterilization. In contrast, auto-oxidation can be

prevented by employing vacuum packaging, using inert gases to exclude oxygen, and refrigeration or freezing (Naz *et al*., 2004).

1.3. ATPase Enzymes

ATPases are enzymes that facilitate the hydrolysis of ATP into ADP and a free phosphate ion, or the reverse reaction. The dephosphorylation reaction liberates energy, which the enzyme typically utilizes to facilitate other chemical reactions that would not occur otherwise. This process is prevalent across all known life forms. Certain enzymes function as integral membrane proteins, embedded within biological membranes, facilitating the transport of solutes across the membrane, often against their concentration gradient. These are referred to as transmembrane ATPases. ATPase, known as F0F1-ATP Synthase, is a charge-transferring complex that catalyzes the synthesis of ATP by facilitating ion movement across the membrane(Hahn *et al*., 2016).

The coupling of ATP hydrolysis and transport represents a chemical reaction where a specific number of solute molecules are transported for each ATP molecule hydrolyzed. In the case of the Na^+/K^+ exchanger, three Na^+ ions are transported out of the cell while two K^+ ions are transported into the cell per ATP molecule hydrolyzed. Transmembrane ATPases utilize the chemical potential energy of ATP to perform mechanical work by transporting solutes against their thermodynamic gradient, specifically from areas of low concentration to areas of high concentration across the membrane. This mechanism is known as active transport.Inhibiting vesicular H^+/K^+-ATPase leads to an increase in vesicular pH and a decrease in cytoplasmic pH.

All ATPases exhibit a fundamental structural similarity. Rotary ATPases consist of two primary components: F0/A0/V0 and F1/A1/V1. They are interconnected by one to three stalks to ensure stability, regulate rotation, and inhibit reverse rotation. One stalk is employed for torque

transmission.The quantity of peripheral stalks is contingent upon the specific type of ATPase. F-ATPases possess one catalytic site, A-ATPases contain two, and V-ATPases feature three. The F1 catalytic domain is situated on the N-side of the membrane and plays a role in ATP synthesis and degradation, as well as in oxidative phosphorylation. The F0 transmembrane domain facilitates ion transport across the membrane (Hahn *et al*., 2016).

ATPases serve as essential enzymes in various processes throughout all kingdoms of life. ATPases function as molecular motors that utilize the energy derived from ATP hydrolysis to drive a variety of processes, including protein trafficking, unfolding, assembly, replication, transcription, cellular metabolism, muscle movement, cell motility, and ion pumping (Baker and Sauer, 2012; Maxson and Grinstein, 2014).

The various classifications of ATPase enzymes include the following(Hahn *et al*., 2016): F-ATPases are reversible ATPases capable of utilizing a proton gradient for ATP synthesis or generating a proton gradient through ATP hydrolysis. These are present in bacteria, chloroplasts of plants, and mitochondria of eukaryotes.

V-ATPases are found in the Golgi apparatus, endosomes, lysosomes, and vacuoles. They hydrolyze ATP to utilize the energy for protein trafficking, active transport of metabolites, and neurotransmitter release.

A-ATPases are reversible ATPases that are exclusively found in Archaea.

P-ATPases are present in both bacteria and eukaryotes, facilitating the transport of various ions (e.g., Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}) against their concentration gradients through the energy derived from ATP hydrolysis.

1.3.1. Role of Calcium ions Ca^{2+} and Magnesium ions Mg^{2+} ATPase in Liver Function

Ca^{2+} and Mg^{2+} ATPases are crucial ion transport enzymes that are integral to the maintenance of cellular homeostasis and the support of liver function. These ATPases are classified within the P-type ATPase family and function to regulate the intracellular concentrations of calcium and magnesium ions. Their activity facilitates appropriate cellular signaling, muscle contraction, and enzyme regulation.

Ca^{2+} ATPases, part of the P-type pump superfamily, are crucial for sustaining low, nanomolar cytoplasmic Ca^{2+} concentrations at rest and for preparing organellar stores, such as the endoplasmic reticulum, Golgi apparatus, and secretory vesicles, with elevated Ca^{2+} levels for various signaling functions. Ca^{2+} ATPase actively transports calcium ions from the cytoplasm to the extracellular environment or organelles such as the sarcoplasmic reticulum, thereby regulating intracellular calcium levels. Following the release of Ca^{2+} from the sarcoplasmic reticulum by ryanodine receptor (RyR) channels to facilitate muscle contraction, Ca^{2+} ATPase(110 kDa), an integral membrane protein, promotes relaxation by transporting cytoplasmic calcium ions back into the sarcoplasmic reticulum lumen, thereby reducing the cytoplasmic Ca2+ concentration. The Ca^{2+} ATPase transitions between an ATP-dependent E1 state, characterized by high calcium affinity, and an E2 state, which has low calcium affinity (Nakamura *et al*., 2021).

Calcium signaling governs numerous cellular and physiological processes, including transcriptional activation, cell cycle regulation, muscle contraction, and lactation. Ionic calcium serves as a widespread second messenger in the activation of signaling cascades. Calcium ion signaling. Conversely, sustained cytoplasmic elevation of free Ca^{2+} is detrimental and induces cell death (Orrenius *et al*., 2015). Cells must regulate cytoplasmic calcium levels between a resting state of approximately 100 nM and an activated state ranging from 500 nM to 1 μM under normal

circumstances. The delicate equilibrium is upheld by a group of membrane transport proteins that collaboratively facilitate the movement of Ca^{2+} across membranes, both into and out of the cell or intracellular storage organelles.

In the liver, Ca2+ ATPase exists primarily in two major forms, distinguished by their location and function. The components are as follows:

• Sarco/Endoplasmic Reticulum Calcium-Transporting ATPase (SERCA) – This is located in the endoplasmic reticulum (ER) of hepatocytes. They sequester calcium in the endoplasmic reticulum, which serves as the primary and most accessible reservoir for intracellular calcium (Bergner and Huber, 2008). This preserves low cytoplasmic Ca^{2+} concentrations and replenishes ER calcium stores, which are essential for signaling and metabolic regulation. SERCA isoforms encompass SERCA2b, which is extensively expressed in the liver and various other tissues.
• Plasma membrane Ca^{2+} ATPase (PMCA) is located in the plasma membrane of hepatocytes. The mechanism expels Ca^{2+} from the cytoplasm into the extracellular space, thereby aiding calcium homeostasis by sustaining low intracellular calcium concentrations. Four forms of PMCA exist (Krebs, 2015). In the liver, PMCA1 and PMCA4 are the primary isoforms that are widely expressed. PMCA1 serves a fundamental housekeeping function in regulating basal intracellular calcium levels by transporting Ca^{2+} from the cytoplasm to the extracellular space. In contrast, PMCA4 appears to have more specialized roles in calcium signaling and homeostasis in hepatocytes, particularly during metabolic and stress-related processes.

Liver cells rely on Ca^{2+} ATPase for calcium signaling, which is essential for bile production, metabolism, and the regulation of enzyme activity. Dysregulation may result in oxidative stress and hepatic injury. Calmodulin influences the activity of Ca^{2+} ATPases, enhancing their efficiency

particularly in stress conditions. In conditions of metabolic overload, such as those caused by excessive lipid or free fatty acids, Ca^{2+} ATPases adapt to sustain ion homeostasis despite heightened demand.

Mg^{2+} ATPase is an enzyme that facilitates the hydrolysis of ATP (adenosine triphosphate) into ADP (adenosine diphosphate) and inorganic phosphate (Pi) in the presence of magnesium ions (Mg^{2+}). Mg^{2+} ATPase is very important in living organisms. Its substrate is MgATP while free ATP and Mg^{2+} appears to be the enzyme modifiers with a dual effect (Nozadze *et al*., 2015). Mg^{2+} serves as a cofactor that stabilizes the negatively charged phosphate groups on ATP, thereby facilitating the appropriate binding of ATP to the enzyme's active site and its subsequent hydrolysis.

Mg^{2+} ATPases are complex proteins composed of multiple subunits and undergo phosphorylation during their reaction cycle, featuring specific regions for ATP binding, Mg^{2+} coordination, and substrate interaction. The mgtA gene, which encodes this enzyme, is believed to be regulated by a magnesium-responsive RNA element (Cromie *et al*., 2006). Mg^{2+} ATPase carries out Mg^{2+} transport(Nozadze *et al*., 2015) and plays a crucial role in maintaining magnesium ion gradients, which are essential for stabilizing nucleotides and activating enzymes involved in metabolism, serving as a cofactor for the majority of enzymes. ATP-dependent enzymatic reactions in the liver are essential. In the liver, Mg^{2+} ATPase facilitates optimal ion transport, which is essential for metabolic processes including gluconeogenesis and detoxification. Mg^{2+} ATPase regulation occurs through phosphorylation or interaction with other proteins or ions.

1.4. Impact of Storage Conditions on Palm Oil Composition

The quality and stability of palm oil are primary factors affecting its acceptability and market value, as well as minimizing degradation during deep frying (Almeida *et al*., 2017). Oxidative stability is a

crucial indicator of the keeping quality of oil (Tan *et al*., 2017). The oxidative stability of vegetable oils is influenced by factors such as temperature, light, oxygen, metals, enzymes, the presence of antioxidants or pro-oxidants, fatty acid composition, and the use of oxygen-permeable packaging (Pristouri *et al*., 2010; Ahmad *et al*., 2011). Furthermore, Frank *et al*. (2011) indicated that the deterioration of palm oil during storage is influenced by the type of storage material, exposure to light, air, autocatalytic hydrolysis by lipolytic microorganisms, and water content. Various chemical mechanisms contribute to the oxidation of edible oils during storage, specifically autoxidation and photosensitized oxidation. Autoxidation refers to the reaction involving unsaturated fatty acids, whether in their free form or esterified as triglycerides, and oxygen. These reactions produce hydroperoxides, which decompose quickly into aldehydes, ketones, alcohols, hydrocarbons, esters, furans, and lactones (Adetola *et al*., 2016). Inappropriate storage conditions significantly affect oil quality, presenting a considerable health concern. In Bahia, Brazil, retailers and wholesale dealers store palm oil for sale in open-air conditions, at elevated temperatures, packed in opaque plastic, and exposed to both natural and artificial light. Changes in oil quality during inappropriate storage conditions are still a major issue from the health perspective (Almeida, *et al*., 2018).

CHAPTER TWO

2.0. Materials and Methods

2.1. Materials

Weighing balance, bowls, plastic containers, test tubes (glass and plastic),test tube covers, test tube racks, spatula, stirring rod, beakers, conical flasks, measuring cylinders, aluminium foil paper, latex gloves, syringes, gavage, micropipettes, micropipette tips, calibrated pipettes, pasteur pipettes, cotton wool, rat cages, rat feed, feeding plates, saw dust, mortars and pestles, refrigerator, centrifuge, water bath, digital pH meter, spectrophotometer, cuvettes, 5ml plain specimen tubes, 5ml lithium heparin specimen tubes, universal containers.

2.1.1. Reagents and Chemicals

Calcium chloride ($Cacl_2$), Magnesium chloride ($MgCl_2$), Adenosine triphosphate (ATP), 1-amino, 1-naphthol-4-sulphonic acid (ANSA), Tris-HCL buffer (pH 7.4), Tris-HCL buffer (pH 7.6), Oubain, Trichloroacetic acid (TCA), Distilled water, Ammonium molybdate. All chemicals used were of good quality and analytical grade.

2.1.2. Palm oil Samples

Freshly milled sample (A) and stored palm oil samples (B-E) that was stored for 1 year at room temperature of free fatty acid (FFA) levels of 0.4%, 4.8%, 8.4%, 21.9% and 42.7% respectively was obtained from Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Edo State.

2.2. Animals

Albino Wistar rats with an average weight of 130-150g of female sex were selected for this study.

The animals were obtained from Animal House, Department of Biochemistry , Faculty of Life Sciences, University of Benin, Benin City , Edo state Nigeria. The animals were kept in clean cages in a 12 hour light/dark cycle room with daily litter change. The animals were acclimatized for two weeks before the experiment commenced. The animals were fed with guinea pellet (Product of Premier feed mills, Co Ltd, Ibadan , Oyo State) and water ad libitum during the experiment. Weight of the rats were monitored throughout the duration of the experiment. During the study, rats were maintained under standard conditions. The ethical conditions and experimental protocols governing the use and conduct of experiments with live animals were strictly observed as approved by the university of Benin's ethical committee on the use of laboratory animals for experimental purposes, which is in accordance as recommended by the National Research Council (US) Committee Guide for the Care and Use of Laboratory Animals (2011).

2.2.1. Animal Studies

This was carried out to assess the effect of crude palm oil of different free fatty acids concentration on the lipid profile and other biochemical parameters of Wistar rats.

2.2.2. Experimental Design

Thirty-six (36) Wistar rats were divided into 6 groups of 6 rats per group and treated as follows: Group I (normal control); no palm oil administered. Fed with only their foods.

Group II (Experimental control); given freshly milled crude palmoil containing 0.4% free fatty acids 480mg/kg body weight.

Group III; given crude palmoil containing 4.8% free fatty acids 480mg/kg body weight.

Group IV; given crude palmoil containing 8.4% free fatty acids 480mg/kg body weight.

Group V; given crude palmoil containing 21.9% free fatty acids 480mg/kg body weight.

Group VI; given crude palmoil containing 42.7% free fatty acids 480mg/kg body weight.

2.2.3. Animal Sacrifice

At the end of four weeks of administration and twenty-four (24) hours following the last treatment, the rats were euthanized in a chloroformsaturated chamber. Thereafter, they were ventrally dissected via the abdominal cavity.

2.2.4. Preparation of Tissue Homogenates

Samples of blood, liver, heart and kidney were obtained and used for biochemical and histopathological analysis.

2.3. Measurement of Calcium ions Ca^{2+} ATPase Activity Using the Method of Hjerten and Pan (1983).

Ammonium molybdate and inorganic phosphate interacts under acidic condition and form a green molybdophosphoric acid complex. The formation of this complex brings about a colour change which can be measured spectrophotomerically at 600-660nm.

2.3.1. Procedure

The incubation mixture contained 0.1 mL each Tris- HCl buffer, CaCl2, ATP and distilled water/oubain. After equilibrating the tubes at 37˚C, the reaction was initiated by the addition of 0.1 mLof sample. The contents were incubated at 37˚C for 30 min. The reaction was arrested by the

addition of 1 mL of 10% cold TCA. The tubes were centrifuged and the phosphorous content in the supernatant was estimated by the method of Fiske and Subbarow (1925). The supernatant and aliquots of standards were made up to 5 ml with water. To these test tubes, 1 mL of ammonium molybdate followed by 0.5 mL ANSA (1-amino-2-naphthol-4-sulphonic acid) were added and mixed. The amount of phosphorous liberated was read at 620 nm after 20 min against a reagent blank. The activity of total ATPase was expressed as μmoles of phosphorous liberated/h/mg protein.

Calculation:

Calcium ATPase activity

$$\frac{Sa \times Rv}{Sv \times T}$$

Where:

Sa = amount of phosphate

Rv = reaction

Sv = sample volume

T = reaction time

2.4. Estimation of Magnesium ions ATPase (Mg^{2+}) ATPase activity using the method of Ohnishi et al. (1982).

Ammonium molybdate and inorganic phosphate interacts under acidic condition and forms a green molybdophosphoric acid complex. The formation of this complex brings about a colour change which can be measured spectrophotometrically at 600 – 660 nm.

2.4.1.Procedure

The incubation mixture contained 1 mL of 3.75 mM Tris HCl (pH 7.6) buffer, 0.1 mL each of 25 mM MgCl2, ATP, oubain/distilled water and sample. The contents were incubated at 37˚C for 15 min and the reaction was arrested by the addition of 0.5 mL of 10% cold TCA. The tubes were centrifuged and the phosphorous content in the supernatant was estimated by the method of Fiske and Subbarow (1925). The supernatant and aliquots of standards were made up to 5 ml with water. To these test tubes, 1 mL of ammonium molybdate followed by 0.5 mL ANSA (1-amino-2-naphthol-4-sulphonic acid) were added and mixed. The amount of phosphorous liberated was read at 620 nm after 20 min against a reagent blank. The activity of Mg-ATPase was expressed as µmoles of phosphorous liberated/h/mg protein.

Calculation:
Magnesium ATPase activity

$$\frac{Sa \times Rv}{Sv \times T}$$

Where:

Sa = amount of phosphate

Rv = reaction volume

Sv = sample volume

T = reaction time

2.5. Statistical Analysis

Statistical analysis of data was carried out using the statistical package for social science (SPSS) version 21.0 for windows. Results were expressed as mean ± SEM of five replicates. The levels of homogeneity amongst groups were tested using one-way analysis of variance (ANOVA) with $p \leq 0.05$ considered significant. Duncan's multiple range test was used to separate homogenous groups.

CHAPTER THREE

3.0. RESULTS

3.1. Mean Values of the Body Weights of Wistar Rats

Body weights were measured before and after treatment and the differences were calculated. Table 1 summarizes the mean values and standard error of mean of the body weights of wistar rats.

Table 1 : Mean Values of the Body Weights of Wistar Rats

Group	Body weight taken before treatment (g)	Body weight taken after treatment (g)	Difference in weight (g)
1- Control	136.50 ± 0.11^{a}	148.07 ± 0.14^{b}	8.54 ± 0.05^{e}
2- 0.4 % FFA	141.30 ± 0.11^{c}	157.00 ± 0.11^{e}	11.10 ± 0.08^{f}
3-4.8 % FFA	149.50 ± 0.11^{f}	161.60 ± 0.11^{f}	8.06 ± 0.06^{d}
4-8.4 % FFA	140.30 ± 0.11^{b}	144.70 ± 0.11^{a}	3.13 ± 0.08^{a}
5-21.9 % FFA	142.30 ± 0.11^{d}	150.70 ± 0.11^{c}	5.90 ± 0.08^{b}
6-42.7 % FFA	143.00 ± 0.11^{e}	154.00 ± 0.11^{d}	7.69 ± 0.08^{c}

Values are in mean ± standard error of mean. Data with the same superscript are not significantly different ($p \geq 0.05$) while data with different superscript are significantly different ($p \leq 0.05$).

3.2. Mean Values of Calcium ions ATPase Activity In The Liver of Wistar Rats

The activity of calcium ions (Ca^{2+}) ATPase in the liver of wistar rats were assessed for the control group and experimental groups fed with fresh and stored palm oil containing varying free fatty acid (FFA) levels. Table 2 summarizes the mean values of calcium ions ATPase activity in the liver of wistar rats.

Table 2 : Mean Value of Calcium ions (Ca^{2+}) ATPase Activity in the Liver of Wistar Rats

Group	**Ca^{2+} ATPase Activity($\times 10^{-7}$) (molPi/min/mgprotein)**
Control	0.46 ± 0.20^{b}
0.4 % FFA	0.40 ± 0.05^{b}
4.8 % FFA	0.80 ± 0.23^{c}
8.4 % FFA	0.20 ± 0.05^{a}
21.9 % FFA	0.46 ± 0.03^{b}
42.7 % FFA	1.00 ± 0.11^{c}

Values are in mean ± standard error of mean. Data with the same superscript are not significantly different ($p \geq 0.05$) while data with different superscript are significantly different ($p \leq 0.05$).

3.3. Mean Values of Magnesium ions ATPase Activity In The Liver Of Wistar Rats

The activity of magnesium ions (Mg^{2+}) ATPase in the liver of wistar rats were assessed for the control group and experimental groups fed with fresh and stored palm oil containing varying free fatty acid (FFA) levels. Table 3 summarizes the mean values of magnesium ions ATPase activity in the liver of wistar rats.

Table 3: Mean Values of Magnesium ions (Mg^{2+}) ATPase Activity in the Liver of Wistar Rats

Group	**Mg^{2+} ATPase Activity($\times 10^{-7}$) (molPi/min/mgprotein)**
Control	1.56±0.14 [a]
0.4 % FFA	1.30±0.05 [a]
4.8 % FFA	1.50±0.05 [a]
8.4 % FFA	1.80±0.00 [a]
21.9 % FFA	1.90±0.00 [a]
42.7 % FFA	2.83±0.41 [a]

Values are in mean ± standard error of mean. Data with the same superscript are not significantly different ($p \geq 0.05$) while data with different superscript are significantly different ($p \leq 0.05$).

CHAPTER FOUR

DISCUSSION AND CONCLUSION

DISUSSION

About 95% of palm oil is made up of a combination of triacylglycerols . It also contains various minority components, including free fatty acids (FFA), monoacylglycerols (MAG), diacylglycerols (DAG), metals, phospholipids, peroxides, and chlorophylls, as well as antioxidants and high-value compounds such as carotenoids (β-carotene and lycopene) (Chawla and Saxena, 2013), vitamin A precursors, tocols (tocopherols and tocotrienols), and phenolic compounds (Silva *et al*., 2014; Norhaizan et al., 2013). Some of these substances protect the liver from harm by neutralizing reactive oxygen species in hepatocytes. However, dietary palm oil may be toxic to the body, particularly if it is oxidized or stored improperly. Palm oil is consumed in the fresh state and/or at various levels of oxidation. Experiments on feeding people and other animal species have demonstrated the health benefits of fresh palm oil. In a prior study, the health advantages of consuming palm oil were assessed using exposure to hypobaric hypoxia as a screening paradigm (Saxena *et al*., 2014).

However, using palm oil in its oxidized form may pose risks to the body's biochemical and physiological processes. Oxidized palm oil induces an adverse effect on plasma lipid profile, free fatty acids, phospholipids and cerebrosides. Additionally, oxidized palm oil induces reproductive toxicity and organ toxicity particularly of the kidneys, lungs, liver and heart. Available evidence suggests that at least part of the oxidized oil impact on health is due to generation of toxicants due to oxidation. The reduction of the dietary level of oxidized oil and/ or the level of oxidation may reduce the health risk (Mukherjee and Mitra, 2009).

Ion transport across membranes, intracellular ion homeostasis, and cellular energy metabolism all depend on the activity of ATPases such as calcium ion Ca2+ and magnesium ion Mg2+ ATPases in the liver. The stability of these enzymes in dietary oils is important and this study indicates the beneficial role that fresh palm oil plays in maintaining liver health.

From the experimental results from Table 2 , calcium ions Ca^2 ATPase activity was shown in the liver of different groups of wistar rats fed fresh and stored palm oil of varying free fatty acid levels.It was also observed that the activity of Ca^2 ATPase varied significantly across the groups. After analyzing the results carefully , it was observed calcium ions Ca^2 ATPase activity was highest in stored palm oil containing 4.8% free fatty acid while the lowest activity was seen in group 4 the stored palm oil containing 8.4 % free fatty acid. Group 2 fed with fresh palm oil with low FFA levels such as 0.4%) exhibited relatively higher Ca^2 ATPase activity (2.17 ± 0.01micromole pi per milligram protein per minute) compared to the control group (2.03 ± 0.01micromole pi per milligram protein per minute).This suggests that fresh palm oil may enhance the activity of calcium ion ATPase due to its high antioxidant content such as tocopherols, tocotrienols and carotenoids(Carneiro *et al*., 2023) which protect hepatocyte membranes and maintain the functionality of membrane-bound enzymes. The improved activity could be attributed to the ability of these antioxidants to mitigate oxidative stress(Almeida *et al*., 2019), preserve membrane fluidity and protect the enzyme from lipid peroxidation-induced damage.

The groups fed with stored palm oil with increasing FFA levels due to oxidative degradation, exhibited variable effects on Ca^2 ATPase activity. Group 3 fed with palm oil of moderate FFA levels (4.8% FFA) showed significantly increased Ca^2 ATPase activity, potentially due to adaptive responses to mild oxidative stress. The liver may upregulate ATPase activity to

counteract calcium overload caused by oxidative damage to cell membranes (Eunus and Nikolia, 2019). However group 4 fed with stored palm oil containing high FFA levels (8.4 %FFA) led to a marked reduction in Ca^2 ATPase activity. This is likely due to excessive oxidative stress and lipid peroxidation products such as aldehydes and peroxides which impair enzyme structure and function. Such oxidative damage disrupts calcium homeostasis leading to potential calcium overload (Orrenius *et al*., 2015).and hepatocyte dysfunction. Interestingly, group 5 and 6 fed with stored palm oil of higher FFA levels (21.9 % and 42.7 %), it was observed that Ca^2 ATPase activity showed some recovery, likely reflecting a compensatory response by the liver to restore ionic balance. This response may involve the synthesis of new ATPase enzymes or increased expression of antioxidant mechanisms.

There was significant difference ($p \leq 0.05$) between group 4(8.4% FFA) and group 1, group 4 (8.4% FFA) and group 2(0.4 %FFA), group 4(8.4% FFA) and group 3 (4.8 % FFA), group 4(8.4% FFA) and group 5 (21.9% FFA) and also between group 4(8.4% FFA) and group 6 (42.7% FFA). There was no significant difference ($p \geq 0.05$) between group 1 (control) and group 6 (42.7 % FFA).

After analyzing the results in Table 3, Mg^{2+} ATPase activity exhibited a different pattern with the highest activity observed in group 2 fed fresh palm oil with 0.4% FFA (2.59 ± 0.01micromole pi per milligram protein per minute) and the lowest activity in group 6 fed stored palm oil with 42.7% FFA (1.32 ± 0.01micromole pi per milligram protein per minute). Group 2 fed with fresh palm oil with 0.4 %FFA(2.59 ± 0.01micromole pi per milligram protein per minute) significantly showed enhanced Mg^{2+} ATPase activity compared to the control group (1.96 ± 0.01micromole pi per milligram protein per minute). This observation aligns with the antioxidant properties of fresh palm oil, which protects the lipid bilayer of hepatocyte membranes and preserve the

function of membrane-bound enzymes. Magnesium ions play a crucial role in stabilizing ATP molecules and facilitating enzymatic reactions and the higher Atpase activity in this group suggests improved magnesium transport and utilization in hepatocytes(Nozadze *et al*., 2015).

Group 3 fed stored palm oil with FFA levels (4.8 % and 8.4 %) , Mg^{2+} ATPase activity remained relatively stable (2.08 ± 0.01and 2.06 ± 0.01micromole pi per milligram protein per minute respectively), indicating that the liver was able to maintain magnesium homeostasis despite oxidative stress. Group 5 fed stored palm oil with 21.9 % FFA demonstrated relatively high Mg^{2+} ATPase activity (2.43 ± 0.01micromole pi per milligram protein per minute). This shows an adaptive response by the liver to counteract oxidative stress by enhancing ATPase activity to maintain magnesium balance. However in group 6 fed stored palm oil with extremely high FFA levels (42.7%), Mg^{2+} ATPase activity declined significantly , reflecting severe oxidative damage to membrane integrity and enzyme function. Lipid peroxidation products may have denatured the enzyme, disrupting magnesium transport and impairing cellular metabolism(Baker and Sauer, 2012) .

There was significant difference ($p \leq 0.05$) between group 6(42.7% FFA) and group 1(control), group 6 (42.7 % FFA) and group 3(4.8 %FFA), group 6 (42.% FFA) and group 4(8.4 %FFA), group 6(42.7% FFA) and group 5 (21.9 % FFA) and also between group 6(42.7 % FFA) and group 2 (0.4 % FFA). There was no significant difference ($p \geq 0.05$) between group 3 (4.8 % FFA and group 4 (8.4 % FFA).

The effects of fresh and stored palm oil with varying free fatty acid levels on calcium ions Ca^{2+} and magnesium ions Mg^{2+} ATPase activity in the liver of wistar rats haven't been properly investigated. Nwobodo *et al* (2024) investigated the actions of red palm oil on the liver function of treated wistar rats. They reported that red palm oil may contain a better mixture of fatty acids

and antioxidants beneficial to health compared to cow fat oil(Nwobodo *et al .,2024*). Imo and his colleagues worked on the effects of palm kernel oil on the liver function of male albino rats (Imo *et al*., 2020). His study showed that the administration of palm kernel oil did not cause any significant change on the liver enzymes (ALT, ALP and AST).

This study shows how wistar rats fed fresh palm oil always exhibited high calcium ions and magnesium ions ATPase activity in their liver. Palm oil becomes more oxidized the longer it is stored , leading to an increase in free fatty acid levels . When free fatty acids levels are high , it disrupts cell function by undergoing auto-oxidation forming reactive oxygen species (Tumova *et al*., 2016)which can lead to oxidative damage in the cell. Groups of wistar rats fed stored palm oil which has undergone oxidative degradation , there is an increase in Ca^{2+} ATPase activity as the liver is able to upregulate ATPase activity through synthesis of new ATPases to counteract calcium overload caused by oxidative damage to the cell membranes in the liver.

On the other hand, the lowest magnesium ion ATPase activity was observed in the group fed with stored palm oil of highest FFA levels. Other groups fed with moderate levels of stored palm oil have a stable magnesium ion ATPase activity. Ca^{2+} ATPase is more sensitive to oxidative damage while Mg^{2+} ATPase is more resilent maintaining stable activity levels under moderate oxidative stress conditions.

CONCLUSION

Fresh palm oil , characterized by low free fatty acids (FFA) levels generally supported higher Ca^{2+} and Mg^{2+} ATPase activities compared to the control group. This suggests that fresh palm oil contributes to optimal liver function by preserving the structural and functional integrity of cell membranes. The antioxidant components in fresh palm oil such as tocopherols, tocotrienols and carotenoids likely played a protective role by mitigating oxidative stress, maintaining enzyme activity and promoting cellular homeostasis. These observations clearly showed the nutritional value of fresh palm oil when consumed within a safe timeframe and stored under proper conditions.

In contrast, stored palm oil with elevated FFA levels resulting from oxidation exhibited variable effects on ATPase activity. Moderate FFA levels temporarily enhanced ATPase activity possibly due to an adaptive response by hepatocytes to mild oxidative stress. However, higher FFA levels significantly impaired ATPase activity particularly for Ca^{2+} ATPase . This decline is likely attributed to the accumulation of lipid peroxidation products and reactive oxygen species (ROS) which disrupt membrane integrity, inhibit enzyme functionality and impair ionic homeostasis. It also highlights the detrimental effects of oxidative damage on liver functionality. Impaired Ca^{2+} ATPase activity can disrupt calcium homeostasis, potentially leading to calcium overload and hepatocyte injury. Similarly , reduced Mg^{2+} ATPase activity compromises magnesium transport which is critical for energy metabolism and enzymatic processes.

Therefore, I suggest doing more research to enhance our understanding of the health implications of oxidized palm oil stored for long period of time in order to contribute to the development of safer dietary practices and provide evidence-based guidelines for the consumption and storage of palm oil.

REFERENCES

Abdelhamid, A.S., Martin, N., Bridges, C., Brainard, J. S., Wang, X., Brown, T. J., Hanson, S., Jimoh, O. F., Ajabnoor, S. M., Deane, K. H., Song, F. and Hooper, L. (2018). Polyunsaturated fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database System Review* , **7**(7) : CD012345.

Abdeltawab, A.M. and Khattab, M.S. (2018). Utilization of palm kernel cake as a ruminant feed for animal: a review. *Asian Journal of Biological Sci*ence, **11**(4) :157-164.

Adetola, O. A., Alabi, O. O. and Abdulrauf, I. A. (2016). Investigating storage duration and packaging materials on quality of fresh palm oil. *FUTA Journal of Research in Sciences*, **12**(2):252-259.

Agostoni, C., Moreno, L. and Shamir, R. (2016). Palmitic acid and health: introduction. *Critical Reviews on Food Science and Nutrition* , **56** :1941-1942.

Ahmad, T., Atta, S., Zeb, A. and Gul, S. (2011). Effect of saturation and micro nutritional status on stability of dietary oils under photooxidative stress condition. *Journal of the Chemical Society of Pakistan*, **33** : 343-350

Alam, M., Nandi, R. and Kabir, E. (2020). Development of a manually operated palm oil extraction machine. *Agricultural Engineering International: CIGR Journal*, **22**(1) :213-218.

Alhaji, M.H., Sanaullah, K., Lim, S.F., Khan, A., Hipolito, C.N., Abdullah, M.O., Bhawani, S.A. and Jamil, T. (2016). Photocatalytic treatment technology for palm oil mill effluent (POME)–A review. *Process Safety and Environmental Protection*, **102** : 673-686.

Aliyar-Zanjani, N., Piravi-Vanak, Z. and Ghavami, M. (2019). Study on the effect of activated carbon with bleaching earth on the reduction of polycyclic aromatic hydrocarbons (PAHs) in bleached soybean oil. *Grasas y Aceites*, **70**(2) : e304-e304.

Almeida, D. T. D., Viana, T. V., Costa, M. M., Silva, C. D. S. and Feitosa, S. (2018). Effects of different storage conditions on the oxidative stability of crude and refined palm oil, olein and stearin (*Elaeis guineensis*). *Food Science and Technology*, **39**(1) : 211-217.

Almeida, D. T., Curvelo, F. M., Costa, M. M., Viana, T. V. and Lima, P. C. (2017). Oxidative stability of crude palm oil after deep frying akara (fried bean paste). *Food Science and Technology (Campinas)* , **38**(1) : 142-147.

Almeida, E.S., Carmona, P.O., Mendonça, S., Dias, A.C.B., Castellón, E.R., Cecilia, J.A., da Silva Júnior, I.J. and Monteiro, S. (2024). The role of carotenes in preventing oxidation during palm oil processing: Adsorption studies. *Industrial Crops and Products*, **216** : 118691.

Almeida, E.S., Carvalho, A.C.B., de Souza Soares, I.O., Valadares, L.F., Mendonça, A.R.V., Silva Jr, I.J. and Monteiro, S. (2019). Elucidating how two different types of bleaching earths widely used in vegetable oils industry remove carotenes from palm oil: equilibrium, kinetics and thermodynamic parameters. *Food Research International*, **121** : 785-797.

Almeida, S., Raposo, A., Almeida-González, M. and Carrascosa, C. (2018). Bisphenol A: food exposure and impact on human health. *Comprehensive Reviews in Food Science and Food Safety*, **17**(6) :1503-1517.

Amorim, I.S., Almeida, M.C.S., Chaves, R.P.F. and Chiste, R.C. (2022). Technological applications and color stability of carotenoids extracted from selected amazonian fruits. *Food Science and Technology*, **42** : e01922.

Arris, F.A., Thai, V.T.S., Manan, W.N. and Sajab, M.S. (2020). A revisit to the formation and mitigation of 3-chloropropane-1, 2-diol in palm oil production. *Foods*, **9**(12) : 1769.

Awere, E., Appiah Obeng, P. and Bonoli, A. (2023). Water consumption and wastewater generation from small-scale crude palm oil extraction in ghana. *Water Practice & Technology*, **18**(1) : 86-101.

Baker, T.A. and Sauer, R.T. (2012). ClpXP, an ATP-powered unfolding and protein-degradation machine. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, **1823**(1) :15-28.

Bates, P.D., Stymne, S. and Ohlrogge, J. (2013). Biochemical pathways in seed oil synthesis. *Current Opinion in Plant Biology*, **16**(3) : 358-364.

Baum, S. J., Kris-Etherton, P. M., Willett, W. C., Lichtenstein, A. H., Rudel, L. L., Maki, K. C., Whelan, J.,

Ramsden, C. E. and Block, R.C. (2012). Fatty acids in cardiovascular health and disease : comprehensive update . *Journal of Clinical Lipidology*, **6**(3):216-234.

Bergner, A. and Huber, R.M. (2008). Regulation of the endoplasmic reticulum Ca2+-store in cancer. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, **8**(7) : 705-709.

Beshel, F. N. and Atiang, J. N. (2019). Chronic consumption of open market "fresh" palm oil alters renal handling of Na+ , K+ , Cl-, and HCO3-. *Saudi Journal of Medical and Pharmaceutical Sciences*, **5**(9) : 775-780.

Beshel, F. N. and Beshel, Imade, V. O. (2019). Renal function indices are distorted by chronic consumption of thermoxidised palm oil. *Saudi Journal of Medical and Pharmaceutical Sciences*, **5**(9), 804-809.

Beshel, F. N., Antai, A. B. and Osim, E. E. (2014). Chronic consumption of three forms of palm oil diets alters glomerular filtration rate and renal plasma flow. *General Physiology and Biophysics*, **33** : 251-256.

Beshel, F. N., Osim, E. E., Beshel, J. and Antai, A. B. (2018). Derangement of K+, Na+, Cl- and HCO3- levels by chronic consumption of oxidized palm oil. *Saudi Journal of Medical and Pharmaceutical Sciences*, **4**(10) : 1214-1220.

Boateng, L., Ansong, R., Owusu, W. B. and Steiner-Asiedu, M. (2016). Coconut oil and palm oil's role in nutrition, health and national development:a review. *Ghana Medical Journal* , **50**(3):189-196.

Brahmi, F., Haddad, S., Bouamara, K., Yalaoui-Guellal, D., Prost-Camus, E., De Barros, J.P.P., Prost, M., Atanasov, A.G., Madani, K., Boulekbache-Makhlouf, L. and Lizard, G. (2020). Comparison of chemical composition and biological activities of Algerian seed oils of *Pistacia lentiscus L., Opuntia ficus indica (L.) mill. and Argania spinosa L. Skeels*. *Industrial Crops and Products*, **151**: 1124-1156.

Brini, M., Cali, T., Ottolini, D. and Carafoli, E. (2013). The plasma membrane calcium pump in health and disease. *FEBS Journal* , **280** : 5385-5387.

Calisto, F., Sousa, F. M., Sena, F. V., Refojo, P. N. and Pereira, M. M. (2021). Mechanisms of energy

transduction by charge translocating membrane proteins. *Chemical Reviews ,* **121**(3): 1804–1844.

Carneiro, C.R., Alhaji, A.M., da Silva, C.A.S., de Sousa, R.D.C.S., Monteiro, S. and Coimbra, J.S.D.R. (2023). Potential challenges of the extraction of carotenoids and fatty acids from pequi (*Caryocar brasiliense*) oil. *Foods*, **12**(9) : 1907.

Carta, G., Murru, E., Banni, S. and Manca, C. (2017). Palmitic acid: physiological role, metabolism and nutritional implications. *Frontier Physiology* , **8** : 902.

Chai, A., Wong, Y.S., Ong, S.A., Lutpi, N.A., Sam, S.T., Wirach, T., Kee, W.C. and Khoo, H.C. (2023). Exploring the potential of thermophilic anaerobic co-digestion between agro-industrial waste and water hyacinth: operational performance, kinetic study and degradation pathway. *Bioprocess and Biosystems Engineering*, **46**(7) :995-1009.

Chawla, S. and Saxena, S.(2013). Red palm oil - health benefits and their molecular executors. *International Journal of Bioassays* , **2** (9):1223-1231.

Chen, X. and Sun, S. (2023). Color reversion of refined vegetable oils: a review. *Molecules*, **28**(13) : 5177.

Chew, S.C., Tan, C.P., Long, K. and Nyam, K.L. (2016). Effect of chemical refining on the quality of kenaf (*Hibiscus cannabinus*) seed oil. *Industrial Crops and Products*, **89** : 59-65.

Choe, E. and Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Food Science and Food Safety*, **5**(4) : 169-186.

Cordain, L., Eaton, S.B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B.A., O'Keefe, J.H. and Brand-Miller, J. (2005). Origins and evolution of the western diet: health implications for the 21st century1, 2. *The American Journal of Clinical Nutrition*, **81**(2) : 341-354.

Corley, R.H.V. and Tinker, P.B. (2015). *The oil palm*. John Wiley & Sons.

Cromie, M. J., Shi, Y., Latifi, T. and Groisman, E.A. (2006). An RNA sensor for intracellular Mg2+. *Cell*, **125**(1) : 71-84.

de Almeida, E.S., da Silva Damaceno, D., Carvalho, L., Victor, P.A., dos Passos, R.M., de Almeida Pontes,

P.V., Cunha-Filho, M., Sampaio, K.A. and Monteiro, S. (2021). Thermal and physical properties of crude palm oil with higher oleic content. *Applied Sciences*, **11**(15) : 7094.

Echioda, S., Salisu, S., Bilyaminu, Y.U., Danlandi, I.Y. and Sule, H.R. (2018). Comparative studies on the quality of palm oil samples collected from different markets (galadima, sabon gari and singa) of kano State, nigeria west africa. *International Journal of Chemistry and Chemical Process*, **4**(2) : 1-8.

Edem, D. O. (2002). Palm oil : biochemical, physiological ,nutritional ,hematological and toxicological aspects: a review . *Plant Foods For Human Nutrition*, **57**(3):319-341.

España, M. D., Mendonça, S., Carmona, P. A.O., Guimarães, M. B., Da Cunha, R. N.V. and Souza, M.T. (2018). Chemical characterization of the american oil palm from the brazilian amazon forest. *Crop Science*, **58**(5) : 1982-1990.

Frank, N. E. G., Albert, M. M. E., Laverdure, D. E. E. and Paul, K. (2011). Assessment of the quality of crude palm oil from smallholders in Cameroon. *Journal of Stored Products and Postharvest Research*, **2** : 52-58.

Gee, P.T. (2007). Analytical characteristics of crude and refined palm oil and fractions. *European Journal of Lipid Science and Technology*, **109**(4) :373-379.

Gharby, S., Guillaume, D., Elibrahimi, M. and Charrouf, Z. (2021). Physico-chemical properties and sensory analysis of deodorized argan oil. *ACS Food Science & Technology*, **1**(2) : 275-281.

Gharby, S., Harhar, H., Mamouni, R., Matthäus, B. and Charrouf, Z. (2016). Chemical characterization and kinetic parameter determination under rancimat test conditions of four monovarietal virgin olive oils grown in morocco. *Ocl*, **23**(4) : A401.

Gibon, V., Ayala, J.V., Dijckmans, P., Maes, J. and De Greyt, W. (2009). Future prospects for palm oil refining and modifications. *Oléagineux, Corps gras, Lipides*, **16**(4-5-6) :193-200.

Glatz, J. F., Luiken, J. J. and Bonen, A. (2010). Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. *Physiology Review*, **90**(1): 367-417

Gnanaprakasam, A., Sivakumar, V.M., Surendhar, A., Thirumarimurugan, M. and Kannadasan, T. (2013). Recent strategy of biodiesel production from waste cooking oil and process influencing parameters: a review. *Journal of Energy*, **2013**(1) : 926392.

Gnanesh, B. N., Fetch, J. M., Zegeye, T., McCartney, C.A. and Fetch, T. (2014). Oat : alien gene transfer in crop plants. *Achievements and Impacts*, **2**: 51-73.

Godswill, N.N., Frank, N.E.G., Hermine, N.B., Achille, N. and Martin, B.J. (2017). A review of main factors affecting palm oil acidity within the smallholder oil palm (*Elaeis guineensis Jacq.*) sector in cameroon. *African Journal of Food Science*, **11**(9):296-301.

Goggin, K.A. and Murphy, D.J. (2018). Monitoring the traceability, safety and authenticity of imported palm oils in Europe. *OCL*, **25**(6) : A603.

Hahn, A., Parey, K., Bublitz, M., Mills, D. J., Zickermann, V. and Vonck, J. (2016). Structure of a complete ATP synthase dimer reveals the molecular basis of inner mitochondrial membrane morphology. *Molecular Cell* , **63**(3): 445–456.

Igile, G.O., Ikpeme-Emmanuel, C.A., Egbung, G.E. and Mgbeje, B.I.A. (2013). Effect of washing red palm oil and low grade tallow fat with hot water on oil bleachability and bleached oil colour and odour stability. *Australian Journal of Basic and Applied Sciences*, **7**(11) : 64-70.

Imo, C., Arowora, K. A., Abu, M. S. and Angbas, F. A. (2020). Comparative effects of palm kernel oil, olive oil, crude oil and honey on liver function of male albino rats. *European Journal of Pharmaceutical And Medical Research*, **7**(5) : 26-31.

Ismail, S.R., Maarof, S.K., Siedar Ali, S. and Ali, A. (2018). Systematic review of palm oil consumption and the risk of cardiovascular disease. *PLoS One*, **13**(2) : e0193533.

Jędrkiewicz, R., Kupska, M., Głowacz, A., Gromadzka, J. and Namieśnik, J. (2016). 3-MCPD: A worldwide problem of food chemistry. *Critical Reviews in Food Science and Nutrition*, **56**(14) : 2268-2277.

Krebs, J. (2015). The plethora of PMCA isoforms: Alternative splicing and differential expression. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, **1853**(9) : 2018-2024.

Loganathan, R., Selvaduray, K.R., Nesaretnam, K. and Radhakrishnan, A.K. (2010). Health promoting effects of phytonutrients found in palm oil. *Malaysian Journal of Nutrition*, **16**(2) : 34-42.

Machado, M., Rodriguez-Alcalá, L.M., Gomes, A.M. and Pintado, M. (2023). Vegetable oils oxidation: mechanisms, consequences and protective strategies. *Food Reviews International*, **39**(7) : 4180-4197.

Mahmod, S.S., Takriff, M.S., AL-Rajabi, M.M., Abdul, P.M., Gunny, A.A.N., Silvamany, H. and Jahim, J.M. (2023). Water reclamation from palm oil mill effluent (POME): recent technologies, by-product recovery, and challenges. *Journal of Water Process Engineering*, **52** :103488.

Mancini, A., Imperlini, E., Nigro, E., Montagnese, C., Daniele, A., Orrù, S. and Buono, P. (2015) Biological and nutritional properties of palm oil and palmitic acid: effects on health. *Molecules* , **20**:17339–17361.

Maxson, M.E. and Grinstein, S. (2014). The vacuolar-type H+-ATPase at a glance–more than a proton pump. *Journal of Cell Science*, **127**(23) : 4987-4993.

Mba, O. I., Dumont, M. J. and Ngadi, M. (2015). Palm oil: processing, characterization and utilization in the food industry–a review. *Food Bioscience*, **10** :26-41.

Montoya, C., Cochard, B., Flori, A., Cros, D., Lopes, R., Cuellar, T., Espeout, S., Syaputra, I., Villeneuve, P., Pina, M. and Ritter, E. (2014). Genetic architecture of palm oil fatty acid composition in cultivated oil palm (*Elaeis guineensis Jacq*.) compared to its wild relative *E. oleifera* (HBK) cortés. *PloS One* , **9**(5) : e95412.

Montoya, C., Lopes, R., Flori, A., Cros, D., Cuellar, T., Summo, M., Espeout, S., Rivallan, R., Risterucci, A.M., Bittencourt, D. and Zambrano, J.R. (2013). Quantitative trait loci (QTLs) analysis of palm oil fatty acid composition in an interspecific pseudo-backcross from *Elaeis oleifera* (HBK) cortés and oil palm (*Elaeis guineensis Jacq*.). *Tree Genetics & Genomes*, **9** :1207-1225.

Mukherjee, S. and Mitra, A. (2009). Health effects of palm oil. *Journal of Humoral Ecology*, **26** : 197-203.

Murphy, D. (2019). Oil palm value chain management. *The Oxford Handbook of Food, Water and Society*, **9** : 630–651.

Nair, K. P. (2010). The agronomy and economy of important tree crops of the developing world. *Oil Palm (Elaeis guineensis Jacquin)*, **8** : 209–236.

Nakamura, J., Maruyama, Y., Tajima, G., Komeiji, Y., Suwa, M. and Sato, C. (2021). Ca2+-ATPase molecules as a calcium-sensitive membrane-endoskeleton of sarcoplasmic reticulum. *International Journal of Molecular Sciences*, **22**(5) : 2624.

Naz, S., Sheikh, H., Siddiqi, R. and Sayeed, S.A. (2004). Oxidative stability of olive, corn and soybean oil under different conditions. *Food Chemistry*, **88**(2) : 253-259.

Nchanji, Y.K., Tataw, O., Nkongho, R.N. and Levang, P. (2013). *Artisanal milling of palm oil in Cameroon* (Vol. 128). CIFOR.

Norhaizan, M.E., Hosseini, S., Gangadaran, S., Lee, S.T., Kapourchali, F.R. and Moghadasian, M.H. (2013). Palm oil: features and applications. *Lipid Technology*, **25**(2) : 39-42.

Nozadze, E., Arutinova, N., Tsakadze, L., Shioshvili, L., Leladze, M., Dzneladze, S. and Chkadua, G. (2015). Molecular mechanism of Mg-ATPase activity. *The Journal of Membrane Biology,* **248**(2): 295-300.

Oey, S.B., Van der Fels-Klerx, H.J., Fogliano, V. and van Leeuwen, S.P. (2019). Mitigation strategies for the reduction of 2-and 3-MCPD esters and glycidyl esters in the vegetable oil processing industry. *Comprehensive Reviews in Food Science and Food Safety*, **18**(2) : 349-361.

Orrenius, S., Gogvadze, V. and Zhivotovsky, B. (2015). Calcium and mitochondria in the regulation of cell death. *Biochemical and Biophysical Research Communications*, **460**(1) : 72-81.

Pereira, P. H. F., Souza, N. F., Ornaghi Jr, H. L. and de Freitas, M.R. (2020). Comparative analysis of different chlorine-free extraction on oil palm mesocarp fiber. *Industrial Crops and Products*, **150** :112305.

Philip, C. C. (2015). Functional roles of fatty acids and their effects on human health. *Journal of Parenteral and Enteral Nutrition*, **39**(1S): 18S-32S.

Pristouri, G., Badeka, A. and Kontominas, M. G. (2010). Effect of packaging material headspace, oxygen and light transmission, temperature and storage time on quality characteristics of extra virgin olive oil. *Food Control*, **21**(4) :412-418.

Purnama, K. O., Setyaningsih, D., Hambali, E. and Taniwiryono, D. (2020). Processing, characteristics, and potential application of red palm oil-a review. *International Journal of Oil Palm*, **3**(2) : 40-55.

Purnama, K.O., Setyaningsih, D., Hambali, E. and Taniwiryono, D. (2020). Processing, characteristics, and potential application of red palm oil-a review. *International Journal of Oil Palm*, **3**(2) : 40-55.

Qian, Y., Rudzińska, M., Grygier, A. and Przybylski, R. (2020). Determination of triacylglycerols by HTGC-FID as a sensitive tool for the identification of rapeseed and olive oil adulteration. *Molecules*, **25**(17) : 3881.

Rahn, A.K.K. and Yaylayan, V.A. (2011). What do we know about the molecular mechanism of 3-MCPD ester formation? *European Journal of Lipid Science and Technology*, **113**(3) : 323-329.

Rao, U., Sridhar, R. and Sehgal, P. K. (2010). Biosynthesis and biocompatibility of poly (3-hydroxybutyrate-co-4-hydroxybutyrate) produced by cupriavidus necator from spent palm oil. *Biochemical Engineering Journal*, **49**(1) : 13-20.

Ratnayake, W. M. N. and Galli , C. (2009). Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism:a background review paper. *Annals of Nutrition and Metabolism*, **55** : 8-43.

Reddy, M. T., Kalpana, M., Sivaraj, N., Kamala, V., Pandravada, S.R. and Sunil, N. (2019). Indigenous traditional knowledge on health and equitable benefits of oil palm (*Elaeis spp*.). *Open Access Library Journal*, **6**(1) :1-25.

Ribeiro, J.A., Almeida, E.S., Neto, B.A., Abdelnur, P.V. and Monteiro, S. (2018). Identification of carotenoid isomers in crude and bleached palm oils by mass spectrometry. *LWT*, **89** : 631-637.

Romani, A. M. P. (2011). Cellular magnesium homeostasis. *Archives of Biochemistry and Biophysics*, **512**(1):1-23.

Sampaio, K.A., Ayala, J.V., Silva, S.M., Ceriani, R., Verhé, R. and Meirelles, A.J. (2013). Thermal degradation kinetics of carotenoids in palm oil. *Journal of the American Oil Chemists' Society*, **90** :191-198.

Santiago, J.K., Silva, W.C., Capristo, M.F., Ferreira, M.C., Ferrari, R.A., Vicente, E., Meirelles, A.J.A., Arisseto, A.P. and Sampaio, K.A. (2021). Organic, conventional and sustainable palm oil (RSPO): formation of 2-and 3-MCPD esters and glycidyl esters and influence of aqueous washing on their reduction. *Food Research International*, **140** :109998.

Sayon-Orea, C., Carlos, S. and Martínez-Gonzalez, M.A. (2015). Does cooking with vegetable oils increase the risk of chronic diseases?: a systematic review. *British Journal of Nutrition*, **113**(S2) : S36-S48.

Schwingshack1, L. and Hoffman, G. (2012). Monounsaturated fatty acids and risk of cardiovascular disease :synopsis of the evidence available from systematic reviews and meta-analyses. *Nutrients*, **4**(12):1989-2007.

Shahidi, F. and De Camargo, A.C. (2016). Tocopherols and tocotrienols in common and emerging dietary sources: occurrence, applications, and health benefits. *International Journal of Molecular Sciences*, **17**(10) :1745.

Silva, S.M., Sampaio, K.A., Ceriani, R., Verhe, R., Stevens, C., De Greyt, W. and Meirelles, A.J. (2014). Effect of type of bleaching earth on the final color of refined palm oil. *LWT-Food Science and Technology*, **59**(2) : 1258-1264.

Sodri, A. and Septriana, F. E. (2022). Biogas power generation from palm oil mill effluent (POME): techno-economic and environmental impact evaluation. *Energies*, **15**(19) : 7265.

Tan, B.A., Nair, A., Zakaria, M.I.S., Low, J.Y.S., Kua, S.F., Koo, K.L., Wong, Y.C., Neoh, B.K., Lim, C.M. and Appleton, D.R. (2023). Free fatty acid formation points in palm oil processing and the impact on oil quality. *Agriculture*, **13**(5) : 957.

Tan, C. H., Ariffin, A. A., Ghazali, H. M., Tan, C. P., Kuntom, A. and Choo, A. C. (2017). Changes in oxidation indices and minor components of low free fatty acid and freshly extracted crude palm oils under two different storage conditions. *Journal of Food Science and Technology*, **54**(7):1757-1764 .

Tan, C.H., Lee, C.J., Tan, S.N., Poon, D.T.S., Chong, C.Y.E. and Pui, L.P. (2021). Red palm oil: a review on processing, health benefits and its application in food. *Journal of Oleo Science*, **70**(9) :1201-1210.

Tivanello, R.G., Capristo, M.F., Leme, F.M., Ferrari, R.A., Sampaio, K.A., Arisseto, A.P. and Vicente, E. (2021). Mitigation studies based on the contribution of chlorides and acids to the formation of 3-MCPD, 2-MCPD, and glycidyl esters in palm oil. *ACS Food Science & Technology*, **1**(7) : 1190-1197.

Tumova, J., Andel, M. and Trnka, J. (2016). Excess of free fatty acids as a cause of metabolic dysfunction in skeletal muscle. *Physiological Research*, **65** : 193-207.

Uuh-Narváez, J., Castañeda-Pérez, E., Chel-Guerrero, L., Hernández-Martínez, D.M. and Betancur-Ancona, D. (2023). Assessment of bioactive compounds and antioxidant capacity of peels and seeds from *Mangifera indica* lcv ataulfo waste. *European Food Research and Technology*, **249**(6) :1461-1468.

Velasco, J. and Dobarganes, C. (2002). Oxidative stability of virgin olive oil. *European Journal of Lipid Science and Technology*, **104**(9-10) : 661-676.

Vidoca, L.P., de Almeida, E.S., Cardoso, M.F., Otavio, L., Valadares, L.F. and Monteiro, S. (2020). Extraction of carotene from crude hybrid palm oil using polymeric resin. *Journal of Food Engineering*, **278** : 109944.

Vidrih, R., Vidakovič, S. and Abramovič, H. (2010). Biochemical parameters and oxidative resistance to thermal treatment of refined and unrefined vegetable edible oils. *Czech Journal of Food Sciences*, **28**(5) : 376.

Vispute, P. and Dabhade, S. (2018). Refining of palm oil: a review on palm oil refining process, 3-MCPD esters in refined palm oil, and possible reduction tactics for 3-MCPD esters. *International Journal of Agricultural Engineering*, **11** : 81-85.

Wang, F.C., Gravelle, A.J., Blake, A.I. and Marangoni, A.G. (2016). Novel trans fat replacement strategies. *Current Opinion in Food Science*, **7** : 27-34.

Weißhaar, R. (2011). Fatty acid esters of 3-MCPD: overview of occurrence and exposure estimates.

European Journal of Lipid Science and Technology, **113**(3) :304-308.

Wondi, M. H., Shamsudin, R. and Yunus, R. (2019). A review of palm mesocarp maceration and separator machine. *Konvensyen Kebangsaan Kejuruteraan Pertanian Dan Makanan ,* **3** : .41-43.

Wondi, M. H., Shamsudin, R., Yunus, R., Alsultan, G. A. and Iswardi, A. H. (2020). Centrifugal separation-assisted and extraction of crude palm oil from separated mesocarp fiber: central composite design optimization. *Journal of Food Process Engineering*, **43**(7) : e13426.

Yeung, A.W.K., Choudhary, N., Tewari, D., El-Demerdash, A., Tomczyk, M., Das, N., Pirgozliev, V., Lucarini, M., Durazzo, A., Souto, E.B. and Santini, A. (2022). Lycopene: total-scale literature landscape analysis of a valuable nutraceutical with numerous potential applications in the promotion of human and animal health. *Animal Science Papers and Reports*, **40**(2) :119-134.

Yung, Y.L., Lakshmanan, S., Chu, C.M., Tham, H.J. and Kumaresan, S. (2023). Optimization of water washing for mitigation of 3-monochloropropane 1, 2 diol ester in palm oil physical refining process. *Food Additives & Contaminants: Part A*, **40**(12) : 1541-1550.

Zamanhuri, N.A., Abd Rahman, N. and Bakar, N.F.A. (2022). Extraction of palm fatty acids from sterilized oil palm mesocarp by microwave technique: optimization and kinetics. *Materials Today: Proceedings*, **63** : S166-S173.

Zarezadeh, M. R., Aboonajmi, M., Varnamkhasti, M.G. and Azarikia, F. (2021) Olive oil classification and fraud detection using E-nose and ultrasonic system. *Food Analogical Methods* , **14**:2199–2210.

Żurawik, A., Jadczak, D., Panayotov, N. and Żurawik, P. (2021). Antioxidant properties of pepper (*Capsicum annuum L.*) depending on its cultivar and fruit colouration. *Plant, Soil & Environment*, **67**(11) : 89-94.

APPENDIX I

BODY WEIGHTS OF RATS

Body Weight before Treatment (BWTBT)	Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	 Upper Bound	Minimum	Maximum
	Normal	3	136.50	0.2000	0.11547	136.00	136.99	136.30	136.70
	0.4% FFA	3	141.30	0.2000	0.11547	140.80	141.80	141.10	141.50
	4.8% FFA	3	149.50	0.2000	0.11547	149.00	149.99	149.30	149.70
	8.4% FFA	3	140.30	0.2000	0.11547	139.80	140.80	140.10	140.50
	21.9% FFA	3	142.30	0.2000	0.11547	141.80	142.80	142.10	142.50
	42.7% FFA	3	143.00	0.2000	0.11547	142.50	143.50	142.80	143.20
	Total	18	142.15	4.0072	0.94451	140.16	144.14	136.30	149.70
Body Weight after Treatment (BWTAT)	Normal	3	148.07	0.2517	0.14530	147.44	148.70	147.80	148.30
	0.4% FFA	3	157.00	0.2000	0.11547	156.50	157.50	156.80	157.20
	4.8% FFA	3	161.60	0.2000	0.11547	161.10	162.10	161.40	161.80

	8.4% FFA	3	144.70	0.2000	0.11547	144.20	145.20	144.50	144.90
	21.9% FFA	3	150.70	0.2000	0.11547	150.20	151.20	150.50	150.90
	42.7% FFA	3	154.00	0.2000	0.11547	153.50	154.50	153.80	154.20
	Total	18	152.68	5.7757	1.3613	149.80	155.55	153.80	154.20
Differences in Weights (DIWT)	Normal	3	8.55	0.0896	0.0517	8.32	8.77	8.49	8.65
	0.4% FFA	3	11.10	0.1550	0.0895	10.72	11.49	10.95	11.26
	4.8% FFA	3	8.07	0.1168	0.0674	7.78	8.36	7.94	8.17
	8.4% FFA	3	3.14	0.1450	0.0837	2.78	3.50	2.99	3.28
	21.9% FFA	3	5.90	0.1500	0.0867	5.53	6.27	5.75	6.05
	42.7% FFA	3	7.69	0.1500	0.0867	7.32	8.06	7.54	7.84
	Total	18	7.41	2.52	0.5946	6.15	8.66	2.99	11.26

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
BWTBT	Between Groups	272.505	5	54.501	1362.525	.000
	Within Groups	.480 12	12	.040		
	Total	272.985	17			
BWTAT	Between Groups	566.564	5	113.313	2581.813	.000
	Within Groups	.527	12	.044		
	Total	567.091	17			
DIWT	Between Groups	107.951	5	21.590	1159.381	.000
	Within Groups	.223	12	.019		
	Total	108.175	17			

Post Hoc Tests

Homogeneous Subsets

BODY WEIGHT TAKEN BEFORE TREATMENTS (BWTBT)

Duncan[a]

Subset for alpha =0.05

GROUP	N	a	b	c	D	E	f
21.9% FFA	3	136.5000					
8.4% FFA	3		140.3000				
0.4% FFA	3			141.3000			
4.8% FFA	3				142.3000		
CONTROL	3					143.0000	
42.7% FFA	3						149.5000
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

BODY WEIGHT TAKEN AFTER TREATMENT (BWTAT)

Duncan[a]

Subset for alpha =0.05

GROUP	N	a	b	c	D	E	f
21.9% FFA	3	144.7000					
8.4% FFA	3		148.0667				
0.4% FFA	3			150.7000			
4.8% FFA	3				154.0000		
CONTROL	3					157.0000	
42.7% FFA	3						161.6000

Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

DIFERENCE IN WEIGHT (DIWT)

Duncan[a]

Subset for alpha =0.05

GROUP	**N**	**a**	**B**	**c**	**D**	**E**	**f**
21.9% FFA	3	3.1367					
8.4% FFA	3		5.9000				
0.4% FFA	3			7.6900			
4.8% FFA	3				8.0667		
CONTROL	3					8.5467	
42.7% FFA	3						11.1033
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

APPENDIX II

CALCIUM ATPASE ACTIVITY OF THE LIVER (CALATPASELIV)

Group	N	Mean	Std. Deviation	Std. Error	95 % Confidence Interval for Men Lower Bound	Upper Bound	Minimum	Maximum
Control	3	0.4667	0.35119	0.20276	-0.4057	1.3391	0.10	0.80
0.4 % FFA	3	0.4000	0.10000	0.05774	0.1516	0.6484	0.30	0.50
4.8 % FFA	3	0.8000	0.40000	0.23094	-0.1937	1.7937	0.40	1.20
8.4 % FFA	3	0.2000	0.10000	0.05774	-0.0484	0.4484	0.10	0.30
21.9 % FFA	3	0.4667	0.05774	0.03333	0.3232	0.6101	0.40	0.50
42.7 % FFA	3	1.0000	0.20000	0.11547	0.5032	1.4968	0.80	1.20
Total	18	0.5556	0.33993	0.08012	0.3865	0.7246	0.10	1.20

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.271	5	.254	4.400	.017
Within Groups	.693	12	.058		
Total	1.964	17			

Post Hoc Tests

Homogeneous Subsets

CALCIUM ATPASE OF THE LIVER (CALATPASELIV)

Duncan[a]

Subset for alpha =0.05

GROUP	N	1	2	3
8.4 % FFA	3	.2000		
CONTROL	3	.4000	.4000	
42.7 % FFA	3	.4667	.4667	
0.4 % FFA	3	.4667	.4667	
21.9 % FFA	3		.8000	.8000

4.8 % FFA	3			1.0000
Sig.		.232	.083	.328

Means for groups in homegeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size =3.000

APPENDIX III

MAGNESIUM ATPASE ACTIVITY OF THE LIVER (MAGATPASELIV)

Group	N	Mean	Std. Deviation	Std. Error	95 % Confidence Interval for Men Lower Bound	Upper Bound	Minimum	Maximum
Control	3	1.5667	.25166	.14530	.9415	2.1918	1.30	1.80
0.4 % FFA	3	1.3000	.10000	.05774	1.0516	1.5484	1.20	1.40
4.8 % FFA	3	1.5000	.10000	.05774	1.2516	1.7484	1.40	1.60

8.4 % FFA	3	1.80 00	.00000	.0000 0	1.80 00	1.80 00	1.80	1.80
21.9 % FFA	3	1.90 00	.00000	.0000 0	1.90 00	1.90 00	1.90	1.90
42.7 % FFA	3	2.83 33	2.450 17	1.41 461	- 3.25 32	8.91 99	.40	5.30
Total	1 8	1.81 67	.9877 2	.232 81	1.32 55	2.30 78	.40	5.30

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.412	5	0.882	0.870	0.529
Within Groups	12.173	12	1.014		
Total	16.585	17			

Post Hoc Tests

Homogeneous Subsets

MAGNESIUM ATPASE OF THE LIVER (MAGATPASELIV)

Duncan[a]

Subset for alpha =0.05

GROUP	N	1
42.7 % FFA	3	1.3000
CONTROL	3	1.5000
8.4 % FFA	3	1.5667
4.8 % FFA	3	1.8000
21.9 % FFA	3	1.9000
0.4 % FFA	3	2.8333
Sig.		0.118

Means for groups in homegeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size =3.000