

**TOTAL ATPASE ACTIVITY AND HISTOPATHOLOGY OF THE HEART IN WISTAR
RATS FED WITH PALM OIL OF VARYING FREE FATTY ACIDS LEVELS**



BY

AKINFULIE SAMUEL AYOKUNLE

MATRIC NO: LSC2009759

**A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY,
FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY;**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF
BACHELOR OF SCIENCES (B.SC) IN BIOCHEMISTRY.**

JANUARY, 2025.

CERTIFICATION

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

PROF. G. ERIMYAMREMU

Project Supervisor

DATE

DR, S. I. OJEABURU

Project Coordinator

DATE

PROF. CHUKWU E. ONYENEKE

Head of Department

DATE

EXTERNAL EXAMINER

DATE

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.

ACKNOWLEDGEMENT

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.

My heartfelt gratitude and appreciation goes to my family, especially my parents MR AND MRS AKINFULIE for their financial, emotional, spiritual and mental support. I am also grateful to my loved ones and friends for their love and care especially the ones made in school.

Special thanks goes to my project supervisor PROF GEORGE ERINYAMREMU for his guidance and fatherly love throughout the period of his tutelage. My gratitude also goes to MR NATHANIEL for his great supervision and support throughout this project work. Thank you so much sir.

TABLE OF CONTENTS

TITLE PAGE	i
CERTIFICATION	ii
DEDICATION	iii
AKNOWLEDGEMENT	iv
TABLE OF CONTENTS	v-vii
LIST OF FIGURES AND TABLES	viii
ABSTRACT	ix
CHAPTER ONE	
1.0 INTRODUCTION AND LITERATURE REVIEW	1
1.1 INTRODUCTION	5
1.1.1 JUSTIFICATION OF STUDY	14
1.1.2 AIM OF THE STUDY	6
1.1.3 OBJECTIVES OF STUDY	7
1.2 LITERATURE REVIEW	16
1.2.1 THE OIL PALM	9
1.2.1.1 PROCESSING OF FRESH FRUIT BUNCHES	14
1.2.1.2 PRIMARY PRODUCTS OF THE OIL PALM	15

1.2.2 PALM OIL	17
1.2.3 CHEMICAL COMPONENTS OF PALM OIL	19
1.2.4 PHYSIO-CHEMICAL PROPERTIES OF PALM OIL	19
1.2.5 HEALTH BENEFITS OF PALM OIL	20
1.2.6 OXIDATION OF PALM OIL	22
1.3 TOTAL ATPASE ACTIVITY	23
1.3.1 ROLE OF $\text{Na}^+\text{-K}^+$ ATPASE, Ca^{2+} ATPASE AND Mg^{2+} ATPASE IN THE HEART	25
1.4 HISTOPATHOLOGICAL CHANGES IN CARDIAC TISSUE	27
CHAPTER TWO	
2.0 MATERIALS AND METHODS	36
2.1 MATERIALS	37
2.1.1 REAGENTS AND CHEMICALS	37
2.1.2 PALM OIL SAMPLES	38
2.2 ANIMALS	38
2.2.1 ANIMAL STUDIES	38
2.2.2 EXPERIMENTAL DESIGN	39
2.2.3 ANIMAL SACRIFICE	39
2.2.3 PREPARATION OF TISSUE HOMOGENATES	40
2.3 ESTIMATION OF TOTAL ATPASE ACTIVITY	40

2.3.1 PROCEDURE	41
2.4 HISTOPATHOLOGICAL ANALYSIS	42
2.5 STATISTICAL ANALYSIS	42
CHAPTER THREE	
3.0 RESULTS	43
3.1 MEAN VALUES OF THE BODY WEIGHT OF WISTAR RATS	44
3.2 MEAN VALUES OF THE ORGAN WEIGHT AND ORGAN TO BODY WEIGHT RATIO OF THE HEART	45
3.3 MEAN VALUES OF TOTAL ATPASE ACTIVITY IN THE HEART OF WISTAR RATS	46
3.4 HISTOPATHOLOGY AND HISTOPATHOLOGY OF THE HEART	47
CHAPTER FOUR	
4.0 DISCUSSION AND CONCLUSION	52
4.1 DISCUSSION	52
CONCLUSION	56
REFERENCES	57
APPENDIX	67

LIST OF FIGURES

Figure 1.1	7
------------	---

LIST OF TABLES

Table 1	34
Table 2	35
Table 3	36

ABSTRACT

Palm oil is widely consumed worldwide, particularly in Africa and Asia, where improper storage often leads to increased levels of free fatty acids (FFA). Elevated FFA levels can induce oxidative stress and impair organ function, including the heart. The study aimed to determine the influence of varying FFA levels in palm oil on body weight, total ATPase activity and the histopathological integrity of cardiac tissue in rats. Palm oil samples with FFA levels ranging from 0.4% to 42.7% were fed to six groups of Wistar rats for four weeks. The study observed significant differences in weight gain across groups consuming palm oil with different FFA levels, the control group show moderate weight gain as baseline for comparison, the low level FFA group (0.4% FFA) show a significant weight gain suggesting optimal intake of nutrient while the higher level FFA group (28.4% FFA) showed reduce weight gain particular in the 8.4% FFA group. Total ATPase activity was assessed using standard spectrophotometric methods, while histopathological analysis of cardiac tissue was conducted to evaluate structural changes. The study revealed an initial increase in ATPase activity in groups fed moderate FFA levels (4.8%), reflecting potential adaptive metabolic responses. However, higher FFA levels ($\geq 8.4\%$) led to suppressed ATPase activity, likely due to oxidative damage. Despite the increase observed in ATPase activity and tissue structure ,no evidence of acute myocardial damage was found in the control and experimental groups. Histopathological analysis showed normal cardiac architecture in the control and palm oil fed groups. The finding underscore the importance of proper palm oil storage to limit FFA accumulation and prevent potential adverse effects on cardiac health.

CHAPTER ONE

1.0. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

The only crop that produced edible oil until the late nineteenth century was the olive, which was primarily used in the Mediterranean region. The rest of the continent relied on animal fats for cooking oil. More recently, vegetable oils—which are oils primarily composed of glycerides of fatty acids obtained solely from plant sources—have been introduced in Europe. The consumption of olive oil and other vegetable oils has increased as a result of technological advancements, increased food production, and more affordable transportation (Cordain et al., 2005). Dietary fatty acids can be found in significant amounts in vegetable oils. Triacylglycerol (TAG), a storage lipid that serves as an important commodity for industrial and culinary applications, makes up the majority of it. Oilseeds like sunflower, rapeseed, and soybeans can be mechanically or solvently extracted to produce vegetable oils, as can fruits high in oil like palm and olive (Vidrih et al., 2010). Triglycerides, which are triesters produced by the interaction of glycerol and fatty acids, make up the majority of it (about 98 g/100 g) (Qian et al., 2020), with minor components (Gnanaprakasam et al., 2013). Soybeans, palm, sunflower, and rapeseed are the main sources of oils in the human diet worldwide, though there is a lot of variation because of local traditions. These oils are typically used as salad dressing, frying oil, or baking oil. According to Brahmī et al. (2020), vegetable oils and fats are vital parts of food and an important part of our daily diet. Monounsaturated fatty acids (MUFAs) (n-9) and polyunsaturated fatty acids (PUFAs) (n-6 and n-3)

are both abundant in vegetable oils. One special substance linked to the use of olive oil is hydroxytyrosol, which is thought to have numerous health benefits. At least one carboxyl ($-\text{C}(=\text{O})\text{OH}$, $-\text{COOH}$, or $-\text{CO}_2\text{H}$) group and an extended carbon chain—which may have single bonds in saturated fatty acids or double bonds in unsaturated fatty acids—define fatty acids (FAs), which are organic acids. Depending on whether a double bond is present or absent, they are categorised as saturated or unsaturated (Baum et al., 2012). Triglycerides and phospholipids are the main sources of fatty acids, which make up the majority of dietary fats. Most naturally occurring fatty acids feature a linear chain including an even number of carbon atoms, ranging from 4 to 28. The fatty acid catalogue divides fatty acids into three categories based on the quantity of double bonds they contain: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs).

Plasmids use acyl carrier protein (ACP) and acetyl-CoA as the main substrate for the synthesis of fatty acids. The enzymatic action of thioesterase then eliminates the fatty acids from ACP. After translocating to the cytosol, free fatty acids are subsequently added to the phosphatidylcholine (PC) and/or acyl-CoA pools. Before these pools amalgamate into triacylglycerol (TAG), they undergo additional modifications such as desaturation, hydroxylation, and epoxylation. Plant cells' endoplasmic reticulum (ER) is where the subsequent processes take place (Bates et al., 2013). The main storage type found in seeds is TAGs. Through the Kennedy pathway, it is produced in the endoplasmic reticulum using acyl-CoA and glycerol-3-phosphate as substrates. Glycerol-3-phosphate acyltransferase (GPAT) is the main enzyme that acylates the glycerol backbone at position sn-1, producing lysophosphatidic acid (LPA). Lysophosphatidic acid acyltransferase (LPAAT), the second enzyme in the cycle, acylates at the sn-2 position to produce phosphatidic acid (PA), which

phosphatidic acid phosphatase (PAP) subsequently transforms into diacylglycerol (DAG). Using acyl-CoA as a substrate, another acyltransferase called diacylglycerol acyltransferase (DGAT) converts DAG into TAG (Bates et al., 2013).

Cells receive fatty acids, which are used as energy sources for general metabolism and muscle contraction (Philip, 2015). Essential biological elements for human metabolism, health, and illness are fatty acids. They supply edible fatty acids (saturated, monounsaturated, or polyunsaturated), which are necessary for cellular metabolism and serve as a source of energy when needed as well as an energy store. It is well known that fatty acids are essential for cellular growth and division. They are essential parts of hormones, neurotransmitters, cell membranes, and a number of other biological materials.

Depending on the length of the saturated fatty acid chain, different foods are the main sources of each individual saturated fatty acid in the diet. For instance, red meat, dairy fats, and plant oils are the main sources of medium and long chain SFAs, whereas dairy fats are the main source of short chain SFAs (Ratnayake and Galli, 2009). The hydrogen atoms in saturated fatty acids (SFAs) are fully saturated. Linear hydrocarbon chains with an even number of carbon atoms make up the majority of saturated fatty acids.

One carbon-carbon double bond, which can be positioned in various ways, is present in monounsaturated fatty acids (MUFAs). The primary monoenes have a cis double bond and a chain length of 16–22. This indicates that the direction of orientation of the hydrogen atoms next to the double bond is the same. Trans isomers can be produced in ruminant digestive tracts and during the

industrial hydrogenation of unsaturated oils. The acyl chain's mobility at that location is restricted by a double bond. 90% of all MUFAs in the diet are oleic acid, making it the most abundant MUFA (Schwingshackl and Hoffman, 2012). The chemical structure is distorted by the cis arrangement, and cis fatty acids are less thermodynamically stable than their trans counterparts. Compared to trans fatty acids and their saturated counterparts, cis fatty acids have lower melting points. The first double bond in polyunsaturated fatty acids (PUFAs), also known as n-3 or omega-3 fatty acids, is located between the third and fourth carbon atoms from the n carbon. These are known as n-6 fatty acids when the first double bond is formed between the sixth and seventh carbon atoms. A methylene group separates double bonds in polyunsaturated fatty acids (PUFAs). Only plants and phytoplankton produce polyunsaturated fatty acids (PUFAs), which are vital for all higher organisms, including fish and mammals. According to Abdelhamid et al. (2018), PUFAs are commonly found in fish and plant oils, with fish having a high omega-3 content and plant oils having a high omega-6 content. Both n-3 and n-6 fatty acids are necessary nutrients that cannot be converted. The body further breaks down PUFAs through desaturation (the removal of hydrogen atoms) and elongation (the addition of carbon atoms). Desaturases found in mammals have the ability to remove hydrogens only from carbon atoms that are situated between a carboxyl group and an existing double bond. Both mitochondria and peroxisomes can undergo β -oxidation of fatty acids, a catabolic process that involves the breakdown of fatty acids.

Monoacylglycerols and free fatty acids (FFAs) are released and absorbed in the small intestine during digestion. Free fatty acids undergo re-esterification in intestinal mucosal cells to form triacylglycerols, which are then carried to the bloodstream as chylomicrons via lymphatic channels. Either integrated into lipoproteins or bound to albumin, fatty acids are carried through the

bloodstream (Glatz et al., 2010). Fatty acid-binding proteins (FABP) carry free fatty acids (FFAs) intracellularly after they are primarily taken up by cells through protein transporters in the plasma membrane. The acyl-CoA-binding protein (ACBP) transports fatty acids to mitochondria or peroxisomes for β -oxidation after they have been activated to acyl-CoA.

1.1.1. Study Justification

One of the most widely used edible oils in the world, especially in Asia and Africa, where it is an essential part of many diets, is palm oil. Triglycerides degrade into free fatty acids (FFAs) under improper storage conditions, and these FFAs build up over time. When consumed, high levels of free fatty acids may cause histopathological changes and organ failure in a variety of organs, such as the kidney, liver, intestinal mucosa, and heart (Alaam et al., 2012; Boniface et al., 2014). Particularly in metabolically active organs like the heart, they are known to cause oxidative stress, inflammation, and impaired organ function. Reactive oxygen species (ROS) may be produced as a result of lipid peroxidation brought on by elevated levels of free fatty acids in palm oil. DNA, lipids, and biological proteins can all be harmed by reactive oxygen species. Reactive oxygen species can cause arrhythmias and myocardial failure in cardiac tissue by interfering with mitochondrial function, decreasing ATP synthesis, and impairing ion transport. Free fatty acid buildup in dietary oils has been shown to suppress Na^+/K^+ ATPase activity, resulting in ionic imbalance and erratic cardiac electrical activity.

An essential biomarker for evaluating ion transport across cellular membranes and energy metabolism is total ATPase activity. The accumulation of free fatty acids and variations in total ATPase activity can impair cardiac contractility, disturb cellular homeostasis, and ultimately lead to

cardiac disorders like hypertrophy or failure. An understanding of the molecular mechanisms underlying FFA-induced cardiac failure can be gained by measuring the ATPase activity in Wistar rats given palm oil with varying concentrations of free fatty acids.

Histopathology sheds light on cellular and structural heart damage, such as inflammation, necrosis, and fibrosis. A comprehensive assessment of the effect of FFAs on cardiac tissue is made possible by the combination of histological evaluation and ATPase activity analysis (Alaam et al., 2012). Despite palm oil's widespread use, most research focusses on how it affects the liver and kidneys (Boniface et al., 2014), ignoring its impact on the heart. Few studies look at the relationship between cardiac ATPase function or histology and dietary free fatty acids. Not enough research has been done on the effects of diets high in FFA on heart health, particularly in developing countries where palm oil consumption is high. This study provides a thorough assessment of cardiac health in response to dietary oils of different levels of free fatty acids by combining biochemical (ATPase activity) and structural (histopathological) evaluations.

1.1.2. Aim of Study

This study is directed at evaluating the impact of varying free fatty acid (FFA) levels in palm oil on total ATPase activity and the histopathology of the heart in Wistar rats fed with palm oil.

1.1.3. Objectives of Study

- i. To measure the total ATPase activity in the heart of Wistar rats fed palm oil containing varying levels of free fatty acids palm oil.

- ii. To assess the histopathological changes in the cardiac tissue of Wistar rats subjected to diets containing varying levels of free fatty acids palm oil.
- iii. To evaluate the role of free fatty acids in disrupting ATPase-mediated ion homeostasis, particularly Na⁺/K⁺ ATPase and Ca²⁺ ATPase functions.
- iv. To determine the relationship between free fatty acid concentration in palm oil and cardiac oxidative stress.

1.2. Literature Review

1.2.1. The Oil Palm

The African oil palm (*Elaeis guineensis*) is a widely recognized tropical species mostly farmed for palm oil production. It is the highest-yielding and most economical vegetable oil in the world, rendering it the favored cooking oil for millions worldwide and a source of biodiesel (Vijay *et al.*, 2016). It is the preeminent tropical vegetable oil worldwide, both in terms of production and trade significance, constituting one-third of vegetable oil production in 2020. It belongs to the Arecaceae family, which encompasses coconut and date palms (Gnanesh *et al.*, 2014). It is a vital component of the global palm family, holds considerable economic importance, is thoroughly studied, and is economically exploited (España *et al.*, 2018). Researchers generally agree that the oil palm *Elaeis guineensis* is native to the western and southwestern regions of Africa, particularly the area between Angola and Gambia (Reddy *et al.*, 2019). Domestication is believed to have originated in its native habitat, likely in Nigeria, and subsequently spread over tropical Africa approximately 5000 years ago.

The oil palm fruit (OPF), originating from the palm tree, is a drupe that forms in compact, spiny

clusters (Mba *et al.*, 2015). The oil palm produces fruits in clusters known as fresh fruit bunches (FFB) (Figure 2). These clusters have densely packed spikelets bearing fruits and can weigh up to 50 kg, containing several hundred to several thousand fruits (Nair, 2010). Each fruit comprises multiple distinct layers, including an outer 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, contains palm oil, but the kernel encases oil within its central nut. A typical palm fruit measures approximately 3.5 cm in length and has a mass ranging from 3.5 to 4.0 g. OPF is characterized by its reddish coloration and clustered growth pattern. Each fruit consists of two main components: the oily, fleshy layer known as the mesocarp, and a single seed, termed the palm kernel or endosperm. The oil extracted from the mesocarp is referred to as crude palm oil (CPO), whereas the oil recovered from the kernel is called palm kernel oil (Mba *et al.*, 2015).



Fig 1.1 (a) Oil palm tree and its palm fruits

1.2.1.1. Processing of Fresh Fruit Bunches into Crude Palm oil

The first clusters of fruits start to ripen after the third year of cultivation. Oil synthesis starts about 180 days after inflorescence development starts, and it picks up speed significantly two weeks into maturation (Rao et al., 2010). Sterilisation of fresh fruit, fruit detachment, digestion, oil extraction, and clarification are some of the intricate steps involved in the production of palm oil. In order to inactivate lipolytic enzymes, such as lipases, that are present in the fruit mesocarp, sterilising fresh fruit is a crucial procedure that requires moisture absorption and heat treatment (Wondi et al., 2019; Wondi et al., 2020; Tan et al., 2023). According to Rao et al. (2010), these enzymes may raise the levels of free fatty acids (FFAs), which could lead to problems with quality during processing, refining, and storage (Mahmod et al., 2023).

There are two types of milling techniques used to produce palmoil: artisanal and industrial. The yield and quality of oil are determined by the extraction process, which uses a variety of techniques. From small mechanical units and artisanal methods to medium-sized and large industrial mills, the extraction techniques can be divided into groups based on their processing capacity and level of complexity.

The oldest technique for separating oils is artisanal palm oil extraction, which typically uses standard machinery. Harvested fruit bunches are left for a few days before the oil extraction process to aid in fruit detachment. This increases lipase activity and causes the hydrolysis of palm oil triglycerides (Godswill et al., 2017). A manual or automated press is then used to extract the fruits after they have been cooked in a drum.

Two main techniques are used to extract industrial palm oil: physical or dry processes, like mechanical pressing, and chemical or wet processes, which include solvent extraction. 75% to 90% oil extraction efficiencies can be attained with these methods (Alam et al., 2020; Gibon et al., 2009). The crude oil's acidity and quality, as well as regional constraints, will determine which of these two processes is best.

Using water or steam, oil is extracted from the oil palm's ruptured cells as part of the solvent extraction process. This procedure hydrolyses any possible starch, glue, or gum and coagulates proteins (Chai et al., 2023). When frying, these substances may result in oil foaming. Most phosphatides and free fatty acids are eliminated during the alkaline neutralisation step of chemical refining. Materials that have hydrolysed and coagulated are eliminated during the ensuing oil clarification procedure. Moisture evaporation produces crude palm oil (CPO) (Awere et al., 2023).

Dry extraction breaks up the oil cells using a centrifuge, screw press, or hydraulic press. While the hydraulic press is frequently used in batch or semi-batch extraction systems, the screw press is usually better suited for continuous extraction systems. After pressing, crude palm oil is extracted from the fibrous mesocarp, while the remaining fibre components contain 5–6% (w/w) oil. Initial oil and moisture contents, operating temperature, heating time, and applied pressure all affect the extracted oil's yield and quality (Zamanhuri et al., 2022). In order to keep fruit kernels from rupturing, the pressure is frequently lowered, which increases oil retention in the mesocarp biomass to about 10–12%.

Because of their nutritional value, oils must contain triacylglycerol (neutral lipid), carotenoids, phytosterols, and vitamin E (tocopherol and tocotrienols). Both industrial and artisanal extraction methods produce a variety of compounds besides oil, including hydrocarbons, pigments, vitamins, sterol glycosides, protein fragments, dioxins, pesticide residues, free fatty acids, partial acylglycerols, phosphatides, sterols, tocopherols, tocotrienols, and heavy metals. According to Abdeltawab and Khattab (2018), the refining process aims to lower the FFA level to 0.3% and reach a moisture content of less than 10%. On the other hand, gums, phospholipids, and free fatty acids are regarded as contaminants and are not desirable chemically. Processing is required to give palm oil the purity qualities required for human consumption (Silva et al., 2014). Refined, bleached, and deodorised (RBD) palm oil is the end product of the efficient removal of impurities through the chemical or physical refining process. Metrics like free fatty acid content, iodine value, peroxide value, moisture content, saponification value (SV), and impurity level are used mainly to evaluate the quality of refined palm oil. In chemical refining, soap is separated by centrifugation (sludge) and free fatty acids are eliminated by alkali treatment. A sodium hydroxide or sodium carbonate solution is used to wash chemically refined crude palm oil in order to eliminate phospholipids and other polar lipids and reduce free fatty acids. However, not all potentially hazardous chemical components may be removed by alkali refining alone (Alhaji et al., 2016).

Using a stripping technique, physical refining eliminates free fatty acids and other substances. The properties of each oil determine which refining method is best. Benefits of physical refining include reduced effluent discharge and chemical consumption. Because physical refining lowers neutral oil loss and operating costs, it is appropriate for crude palm and palm kernel oils with low initial

phosphatide concentrations and high levels of carotene and free fatty acids (FFA) (Alhaji et al., 2016; Sampaio et al., 2013). While reducing the production of undesirable trans fatty acids, processing conditions can be changed to enhance the retention of beneficial secondary components like tocopherols and tocotrienols. Physical refinement of crude palm oil requires bleaching and deodorisation procedures that require high temperatures (Tan et al., 2021). The adsorption process known as bleaching is carried out at temperatures ranging from 95 to 135 degrees Celsius. To get rid of colours, metals, oxidation products, and soaps, this method uses neutral or acid-activated bleaching earth (Almeida et al., 2019). Certain carotenes are eliminated during the bleaching process, and the remaining carotenes degrade at temperatures of 240 °C or higher during the deodorisation process (Ribeiro et al., 2018). Through volatilisation, the thermal bleaching method eliminates free fatty acids, aldehydes, and ketones. According to Vispute and Dabhade (2018), oils with low DOBI and high levels of FFA and peroxides are more susceptible to bleaching. The DOBI value, which shows the proportions of carotenes and oxidised carotenes, is calculated by dividing the oil absorption at 446 nm by that at 268 nm. A higher DOBI score indicates less oxidised carotenes, which improves the bleaching action of the oil. The bleaching process is made more difficult by DOBI readings below 2.0, which indicate poor quality, while readings between 2.5 and 4.0 indicate moderate to good crude oil quality. Because of the increased heat during these stages, the high temperatures used in refining operations may cause harmful chemical changes. According to Almeida et al. (2024), the use of bleaching earth may result in the production of oxidation products, which raise the levels of foams, colourants, free fatty acids, and viscous materials. Unwanted byproducts of refining include glycidyl esters (gEs) and 3-monochloropropane-1,2-diol (3-MCPD), which are pollutants produced by the process (Rahn and

Yaylayan, 2011). These substances are poisonous, and eating them has been connected to the growth of tumours.

Temperature has a major impact on the formation of 3-MCPD and gEs, particularly during the oil deodorisation process (Jędrkiewicz et al., 2016). In virgin, unrefined oils, 3-MCPDs are very undetectable (Arris et al., 2020). Using neutral bleaching clays, reducing exposure to and duration of high temperatures during processing, and treating acid-activated clays with hydrochloric acid (Oey et al., 2019).

During storage, transportation, and use, refined oil may progressively turn darker; this is known as colour reversal (Chen and Sun, 2023). In general, poor oil quality or insufficient degumming and bleaching procedures are linked to colour reversal. The final colour of the oil is significantly influenced by the presence of vivid pigments and oxidising agents, which also causes colour reversal. In addition to causing carotenes to brown, temperature also promotes oxidation, which results in the formation of molecules with distinct colours. Tocopherol oxidation products, such as γ -tocopherol and γ -tocopherol-5,6-quinone, can stabilise other pigments and prevent their removal by adsorption, giving the oil a more intense yellowish or red tint (Igile et al., 2013).

1.2.1.2. principal oil palm products.

Palm fruit yields two types of oil: palm kernel oil, which is obtained from the seeds, and palm oil, which is obtained from the mesocarp. A variety of methods, generally categorised as wet or dry processes, can be used to extract the edible oil found in the mesocarp of palm fruit (Mba et al., 2015). The oil obtained through these methods contains both beneficial compounds, such as triacylglycerols (TAGs), vitamin E, carotenoids, and phytosterols, as well as impurities, such as phospholipids, free fatty acids (FFAs), gums, and lipid oxidation products, the latter of which can be removed through

refining procedures. Following centrifugation and drying, the palm oil is purified; the desiccated oil is then cooled and placed in appropriate storage containers. Depending on their intended use, palm oil and palm kernel oil have different physical and chemical characteristics (Mba et al., 2015). While palm oil (PO) contains 50% SFAs, mainly palmitic acid (44%) and smaller amounts of stearic acid (5%), 40% monounsaturated fatty acids (MUFAs), mainly oleic acid, and 10% polyunsaturated fatty acids (PUFAs), mostly linoleic acid, palm kernel oil contains 85% SFAs, primarily lauric and myristic acids (Gee, 2007). The main saturated fatty acid found in vegetable and animal fats, palmitic acid (PA) is also the main ingredient in human milk fat. Palm oil's stability and nutritional value are increased by the most abundant natural source of carotenoids (500–700 ppm), tocopherols, and tocotrienols (600–1200 ppm) (Souganidis et al., 2013). According to Sen et al. (2007), their antioxidant properties, which specifically target reactive oxygen species (ROS), aid in the prevention of cancer, cardiovascular disease (CVD), and ageing. According to Sen et al. (2007), tocotrienols are naturally occurring inhibitors of cholesterol synthesis. While low-quality oils are used in non-edible industries like biofuels, candles, cosmetics, and soap production, high-quality palm oil is suitable for the edible oil sector due to its low levels of free fatty acids (FFAs), minimal impurities, and effective bleaching.

1.2.2. Palm oil

The mesocarp of oil palm fruits yields palm oil (PO), which has a balanced makeup of saturated (mostly palmitic) and unsaturated (mostly oleic) fatty acids. It is the most widely used vegetable oil in the world and is an essential and versatile oil that is used as a raw material for both food and non-food products. For thousands of years, it has been used in a wide variety of culinary applications and

preparations. According to Basiron (2007), about 80% of palm oil products are used in cooking. In 2013, the percentage of palm oil used in food rose to 32.5%, and it is expected to soon surpass 34%. Palm oil is one of the most widely used oils by food manufacturers because of its competitive pricing and unique characteristics, including its high nutritional value, unique solid content profile, exceptional oxidative stability, lack of trans fatty acids and cholesterol, antioxidant properties, and non-genetically modified status.

The remarkable stability of palm oil over time is one of its main benefits. Because palm oil has a low concentration of polyunsaturated fatty acids and a high concentration of monounsaturated fatty acids (oleic acid) and vitamin E, it has exceptional oxidative stability. As a result, foods produced or processed using palm oil have a longer shelf life and are less likely to go bad. Because palm oil has better oxidation stability than other vegetable oils, it keeps the flavour of the product throughout its shelf life. Deodorised palm oil's neutral flavour and aroma allow it to be used in a variety of culinary applications without altering flavour; this neutrality makes sure that the oil doesn't overpower the flavours of other ingredients like milk, chocolate, and hazelnuts. Since palm oil is semi-solid, it does not require hydrogenation, which raises the saturated fatty acid content of liquid oils and produces trans fatty acids, prior to use in food formulations. The US Food and Drug Administration's 2003 requirement for trans fatty acid labelling makes this particularly important for the food industry (Tarrago-Trani et al., 2006). Because trans fatty acids have a negative impact on cardiovascular health, there is increasing concern about their presence in hydrogenated products. For vegetable oils, such as canola, soybean, cottonseed, and maize oils, to achieve oxidative stability and solid fat content (SFC) equivalent to palm oil, hydrogenation is necessary. The demand for food

products made with natural oils and fats or those free of trans fatty acids has increased as a result of consumer awareness of the harmful health effects linked to trans fatty acids from partially hydrogenated oils (O'Brien and Wakelyn, 2005). Therefore, the best substitutes for partially hydrogenated fats in food formulation and processing are palm oil and its trans-fatty acid-free fractions. Technological developments in fractionation have made it possible to produce a wide range of hard palm fractions (palm stearin, POs) with an iodine value (IV) below 10. These hard fats would be useful as a texturizer to fully replace trans fats in margarine and shortening formulations. Furthermore, the physical characteristics of palm oil are similar to those of animal fats like tallow and lard. A semi-solid fat rich in palmitic and oleic acids (consisting of 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 are among the many bioactive components of palm oil fruit, whose phytochemistry has been thoroughly studied. Because of its nutritional, antioxidant, and various therapeutic benefits, palm oil is recommended by numerous scientific studies (Absalome et al., 2020; Mancini et al., 2015; Oyewole et al., 2010).

1.2.3. Chemical Components of Palm Oil

A notable feature of palm oil is its high concentration of oleic acid and the high levels of antioxidant compounds, such as carotenes and tocopherols, which improve its oxidative stability and suitability for frying, thus meeting the requirements of the food industry. Palm oil is frequently criticised for having high concentrations of saturated fatty acids (SFA), especially palmitic acid, which are linked to health problems like obesity, cardiovascular diseases, diabetes, and cancers (Montoya et al., 2013).

Along with polyunsaturated fatty acids (PUFA), oleic acid, a monounsaturated fatty acid (MUFA) found in palm oil, may lower dangerous cholesterol levels and offer protection against cardiovascular disease (Ribeiro et al., 2018). Omega (ω) unsaturated fatty acids, which include ω -3 (linolenic acid), ω -6 (linoleic acid), and ω -9 (oleic acid) fatty acids, are classified based on the carbon's location in the unsaturated chain. About 95% of the composition of palm oil is made up of a mixture of triacylglycerols. In addition to antioxidants and important substances like carotenoids, vitamin A precursors, tocopherols (tocopherols and tocotrienols), and phenolic compounds, the oil also contains a number of minor components, such as free fatty acids (FFA), monoacylglycerols (MAG), diacylglycerols (DAG), metals, phospholipids, peroxides, and chlorophylls (Norhaizan et al., 2013; Machado et al., 2023). The long chains of carotenoids, which are vital minor constituents of palm oil, have conjugated double bonds and greatly affect the oil's colour, which ranges from yellow to orange-red. The characteristic orange hue of the oil that is extracted from the mesocarp is caused by liposoluble pigments. α - and β -carotenes make up about 90% of the carotenes in the oil (Zurawik et al., 2021). Because carotenoids have health-promoting antioxidant qualities, palm oil is crucial for preventing cancer, heart disease, and vision impairments (Yeung et al., 2022). Carotenoids are precursors of vitamin A, and the most effective provitamin A (retinol) is β -carotene. In addition to their health benefits, carotenes have a significant impact on oil oxidation by inhibiting $1O_2$ (singlet molecular oxygen), and their effectiveness increases with the number of double bonds in the chain. These substances are extracted from the oil during the refining process to produce a lighter colour, which enhances consumer appeal in numerous industrial applications in addition to their nutritional benefits. On the other hand, because they prevent oxidation, residual carotenoids in post-bleaching palm oil

are crucial (Tan et al., 2021).

Tocopherols and tocotrienols, or tocots, are an essential minor component of palm oil. These substances may work as antioxidants in concert with carotenes to improve the oil's oxidative stability (Almeida et al., 2019). Together with carotenes and tocots, palm oil is abundant in biologically active substances such as phospholipids, phenolic compounds, and notable amounts of squalene and phytosterols that enhance nutritional absorption and promote cognitive function (Loganathan et al., 2010).

1.2.4. Palm Oil's Physio-chemical Characteristics

The fatty acid composition of palm oil and how these acids are arranged within the triglyceride structure are what give it its distinct qualities and health advantages over other vegetable oils (Mancini et al., 2015). The physical and chemical characteristics of palm oil at the time of purchase are the main factors determining its suitability in the global market. Free fatty acids (FFA), iodine value (IV), peroxide value (PV), moisture content, impurity content, colour, taste, aroma, melting point, and the amount of tocopherols and tocotrienols are usually among the characteristics taken into account (Zarezadeh et al. 2021b). The iodine value and saponification value are two elements that have an indirect connection to the makeup of edible oils.

1.2.5. Advantages of Palm Oil for Health

About 800 parts per million of vitamin E compounds, such as tocopherols and tocotrienols, are present in palm oil, which is also high in pro-vitamin A carotenoids (Carneiro et al., 2023). The antioxidant qualities of carotenes, tocopherols, and tocotrienols give palm oil its nutritional advantages. They offer tissues and cells defence against oxidative stress brought on by free radicals. These substances function as agents that counteract free radicals that are created during biological

metabolism or as a result of exposure to hazardous chemicals and pollutants in the environment. Triacylglycerols, which make up the majority of dietary fats and oils, have twice the energy per gramme compared to carbohydrates. Free radicals are associated with ageing processes, the onset of chronic degenerative diseases like atherosclerosis and arthritis, and the mechanisms underlying carcinogenesis (Ribeiro et al., 2018). The provision of fat-soluble vitamins, namely vitamins A, D, E, and K, depends on fats and oils. The vitamin E in palm oil provides protection against Alzheimer's disease, atherosclerosis, cancer, cellular ageing, and arthritis.

1.2.6. Palm Oil Oxidation

Like other edible oils, palm oil is greatly impacted by lipid oxidation, which causes significant degradation in its chemical, sensory, and nutritional qualities (Velasco & Dobarganes, 2002). Due to the production of toxic or physiologically active compounds, the oxidative breakdown of oils and fats results in the formation of undesirable off-flavors as well as the breakdown of vitamins (A, D, E, K, and C), essential fatty acids, chlorophylls, carotenes, amino acids, proteins, and enzymes. Compared to animal fats, which are mostly saturated and less likely to undergo chemical reactions, unsaturated vegetable oils are more reactive. In the early stages, oxidation frequently proceeds slowly, but it intensifies when exposed to heat, light, and trace metals. The oxidation process will proceed more quickly if the dissolved oxygen concentration (DOC) rises. Since the presence of double bonds in unsaturated fatty acids can cause more oxidation, more double bonds are associated with less stable oil. Because palm oil has a lower concentration of unsaturated fatty acids than polyunsaturated oils like soybean and maize oils, it is therefore more stable. Carotenoids and vitamin E are found in crude palm oil in concentrations of 500–700 ppm and 600–1000 ppm, respectively. These essential dietary components primarily serve as antioxidants, which

are compounds that prevent oxidation.

The oxidation of palm oils is also influenced by additional chemical processes that occur during storage, such as autoxidation and photosensitised oxidation. Unsaturated fatty acids, whether free or esterified as triglycerides, react with oxygen to form autoxidation. These reactions result in hydroperoxides, which quickly break down into alcohols, hydrocarbons, furans, lactones, ketones, aldehydes, and esters (Choe and Min, 2006; Adetola et al., 2016), reducing the oil's value and flavour. Apart from oxidative rancidity, oil breakdown is accelerated by hydrolytic processes that are triggered by lipases derived from food or microbes. Proper packaging, sanitation, refrigeration, and effective transportation can all help to reduce hydrolytic reactions. On the other hand, auto-oxidation can be minimised by freezing or refrigeration, using inert gases to replace oxygen, and vacuum packaging.

According to Almeida et al. (2018), changes in oil quality brought on by inappropriate storage conditions continue to pose a serious health risk. Furthermore, most oils that are incorrectly identified as fresh palm oil are actually photooxidized (Beshel and Beshel, 2019). Long-term consumption of these oils has been shown to cause electrolyte imbalance (Beshel et al., 2018; Beshel and Beshel, 2019); decrease glomerular filtration rate and raise blood pressure (Beshel et al., 2014); induce renal glycosuria and change renal function parameters (Beshel et al., 2019).

1.3 Total Activity of ATPase

Enzymes known as adenosine triphosphatases (ATPases) help break down ATP into ADP and inorganic phosphate (Pi). These are enzymes that cleave ATP's gamma-phosphate to produce inorganic phosphate (Pi). Enzymes use the energy produced by the hydrolysis and release of Pi to power energy-dependent chemical reactions. Maintaining cell membrane potential, promoting ion

transport, and controlling cellular volume all depend on the enzyme ATPase. Every aspect of life depends on this process. Significantly exergonic, ATP hydrolysis releases energy used in a number of cellular functions. There are many different kinds of ATPases, such as Ca^{2+} ATPase, $\text{H}^{+}/\text{K}^{+}$ ATPase, and $\text{Na}^{+}\text{-K}^{+}$ ATPase. The lack of mitochondrial ATPase is important; for example, a lack of Ca^{2+} -ATPase causes exertional muscle pain syndrome, while a lack of $\text{Na}^{+}\text{-K}^{+}$ ATPase aggravates anxiety-related behaviour. For the diagnosis and mechanistic research of some diseases, accurate measurement of ATPase activity is crucial. Cation-transport ATPases, including the sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA) (Moller et al., 2005) and $\text{Na}^{+}\text{-K}^{+}$ ATPase (Jørgensen et al., 2003), are the main representative members of this large family. These proteins associate the movement of ionic species against their electrochemical gradient with the hydrolysis of ATP. Ion translocation and ATP hydrolysis are commonly associated through a cyclic sequence of chemical reactions known as the "enzymatic cycle." The enzymatic cycle begins with cation-induced enzyme activation, followed by ATP consumption to produce a phosphorylated intermediate. The phosphoenzyme then uses the free energy from ATP to undergo a conformational change that facilitates the displacement and release of the bound cation. When the enzyme is dephosphorylated due to the binding of counter-transported ions, the counterions are released through a conformational shift back to the initial state. The hydrolysis of ATP is facilitated by ATPase enzymes, which release energy for a variety of cellular processes, including protein synthesis, ion transport, and muscle contraction. A sample's overall ability to use ATP for energy-dependent cellular processes is reflected in its total ATPase activity, which is the aggregate rate at which all of the ATPase enzymes in that sample (such as a cell or tissue extract) hydrolyse ATP into adenosine diphosphate (ADP) and inorganic phosphate.

Pi detection is therefore crucial for evaluating the rate of Pi generation by ATPases and the related enzyme activity, a crucial functional parameter. The measurement is established by quantifying the amount of inorganic phosphate released per unit of time. The in vitro ATPase activity of isolated proteins has been measured using a variety of techniques. The measurement of free inorganic phosphate (Pi) is the foundation of most quantification techniques. Malachite green is used as the calorimeter substrate in the most common method. Furthermore, there are a number of techniques that use radioactive and fluorescent substrates to detect free phosphate in the ATPase reaction (Shiue et al., 2006).

1.3.1 The Function of Ca²⁺ and Na⁺-K⁺ ATPases in the Heart

In order to create an electrochemical gradient across the cell membrane and maintain cation homeostasis in cardiomyocytes, the Na⁺-K⁺ ATPase, which is embedded within the sarcolemmal membrane, primarily transports three sodium ions and two potassium ions through an energy-dependent process. When this enzyme is blocked, other cardiotonic steroids, such as endogenous ouabain and marinobufagenin, are known to affect heart contractility. This enzyme acts as a receptor for exogenous cardiac glycosides, such as digoxin and ouabain. As a signal transducer, Na⁺-K⁺ ATPase modulates numerous metabolic processes in cardiomyocytes, including cellular development, differentiation, survival, and apoptosis. Its regulatory function with regard to its structure–function relationship, pathological implications, and therapeutic aspects has been extensively studied (Galougahi et al., 2012; Silva and Soares-da-Silva, 2012; Obradovic et al., 2017; Bartlett et al., 2018; Figtree et al., 2012; Liu et al., 2013; Gheorghide et al., 2011). Although Na⁺-K⁺ ATPase activation is thought to be a mechanism for cell survival, significant inhibition of this enzyme has been linked to cardiac dysfunction, cellular damage, arrhythmias, and

cell death. Furthermore, while high doses of cardiac glycosides, which significantly inhibit Na⁺-K⁺ ATPase, are linked to cardiotoxicity in heart failure, low doses of these agents, which moderately inhibit Na⁺-K⁺ ATPase, are known for their beneficial inotropic effects and advantages. The SERCA2 gene encodes the predominant isoform of sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase 2a, or SERCA2a, which is expressed in both developing and adult mammalian hearts. Several research groups have developed transgenic (TG) animal models with increased SERCA pump expression in the heart to determine the effect of elevated Sarcoplasmic levels on cardiac function. According to studies conducted on transgenic animal models with the myocardium overexpressing SERCA2a or SERCA1a, the overexpression of the SERCA pump resulted in increased contractility and Ca²⁺-ATPase activity (Mørk et al., 2007). The SERCA pump is 2.5 times higher in the murine heart when the skeletal muscle isoform, SERCA1a, is overexpressed than in non-transgenic (NTG) control hearts. However, these mice's endogenous SERCA2a levels dropped to 50%, suggesting a limit on the SR's storage capacity. Mice with high SERCA pump levels do not exhibit cardiac disease and their in vivo cardiac function is normal. Increased SR Ca²⁺ transport brought about by SERCA pump overexpression increased the rates of cardiac contraction and relaxation (Mørk et al., 2007). When taken as a whole, these studies showed that increased SERCA expression is well tolerated and can be used to treat conditions such as heart failure, which is characterised by decreased SERCA levels.

1.4 Histological Alterations in Heart Tissue

According to histology, cardiomyocytes and connective tissue make up the majority of the heart. The cardiac/fibrous skeleton has two dense connective tissues with elastic fibres. These components are easier to see with certain stains, such as Masson's elastic trichrome stains. An inner serous layer and

an outer fibrous layer make up the pericardium. Fibrous connective tissue makes up the fibrous layer. Two layers make up the serous layer: an inner layer that covers the myocardium and an outer layer that is fused with the fibrous pericardium. A continuous layer of mesothelial cells with microvilli pointing towards the pericardial cavity makes up the layers, which are histologically identical. The parietal pericardium is made up of the surrounding serous pericardium and the fibrous pericardium. The epicardium includes the inner serous pericardium, also referred to as the visceral pericardium. The pericardial fluid produced and reabsorbed by the microvilli on the mesothelial cells is found in the pericardial cavity, which is located between the outer and inner serous layers. The myocardium makes up a sizable section of the heart wall. This layer is created by the accumulation of cardiomyocytes. The striations of these cardiomyocytes resemble those of skeletal muscle myocytes. They are branching, have intercalated discs, and are frequently mononucleated, in contrast to skeletal muscle cells. Moreover, they cannot regenerate. After an injury, like a myocardial infarction, scar tissue replaces the necrotic area. Under light microscopy, the histological change is visible because Masson's elastic trichrome stain gives the fibrous portion of the scar tissue a blue hue. The heart chambers are lined with a single layer of endothelial cells, which makes up the endocardium. On occasion, the endocardium may also contain trace amounts of smooth muscle. Because of the increased pressure from the pulmonary veins, the left atrium has a thicker endocardium than the right. Between the myocardium and endocardium is the subendothelial layer, which is made up of loose elastic tissue, collagen bundles, nerves, and occasionally blood vessels. Specialised cardiac cells and fibres make up the conduction system, which aids in the start and spread of impulses. Nodal (P) and transitional (T) cells make up the SA node. Though they have fewer myofibrils, these cells resemble cardiac cells. This area is insulated and separated from the rest

of the atria by dense connective tissue. Certain muscle fibres in the atrioventricular (AV) node, which is next to the heart's fibrous structure, receive impulses from the SA node. The epicardium contains Purkinje fibres, which are extensions of the atrioventricular nodes. According to Murphy and Lazzara (2016), these fibres have a lower number of myofibrils and are higher in glycogen. The ventricularis, fibrosa, and spongiosa are the three layers that make up a valve. It can be easier to orient the valves during microscopy if these layers are recognised. The artery side of the semilunar valves or the atrial side of the atrioventricular valves are where the spongiosa is found. While the fibrosa, an extension of the cardiac skeleton, has dense irregular connective tissue, the spongiosa layer is distinguished by significant amounts of proteoglycans, including glycosaminoglycans, and loose connective tissue. The ventricularis, which is located on the valve's ventricular aspect, is lined with endothelium and has elastic fibres. The chordae tendineae are extensions of the ventricular myocardium found in the atrioventricular valves. Collagen elastic fibres and dense regular connective tissue make up the majority of the chordae tendineae, which shield these valves from excessive pressure (Misfeld and Sievers, 2007).

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

Tris-HCl buffer (pH 7.4), ethylene di-amino tetra-acetic acid (EDTA), sodium chloride (NaCl), potassium chloride (KCl), magnesium sulphate ($MgSO_4$), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), trichloroacetic acid (TCA), adenosine triphosphate (ATP), 1-amino, 1-naphthol-4-sulphonic acid (ANSA), ammonium molybdate, ouabain. 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 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 rats (Wistar strain) (130-150 g), obtained from the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, were used in this study. The animals were kept in clean cages in a 12 h 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. Handling of animals was in accordance with the care and use of laboratory animals' manual. Weights of the rats were monitored throughout the duration of the experiment.

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

The experiment was designed in a way that rats were grouped into control and treatment groups. Thirty-six rats were used for this experiment which were grouped into six groups of six rats each as shown below.

Group I: normal control

Group II: (Experimental control); given freshly milled crude palm oil of 0.4 % FFA 480mg/kg body weight.

Group III: Crude palm oil of 4.8 % FFA 480mg/kg body weight.

Group IV: Crude palm oil of 8.4 % FFA 480mg/kg body weight.

Group V: Crude palm oil of 21.9% FFA 480mg/kg body weight.

Group VI: Crude palm oil of 42.7 % FFA 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. Estimation of Total ATPase activity using the method of Evan, (1969)

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.

Reagent preparation : 0.1M Tris-HCl buffer (pH 7.4). This was prepared by dissolving 16.34g of trichloroacetic acid in 100 mL of distilled water and adjusting the pH to 7.4 with HCl. 1mM EDTA. This was prepared by dissolving 0.040g of EDTA in 100 mL of distilled water. 120mM NaCl. This was prepared by dissolving 0.701g of NaCl in 100mL of distilled water. 2mM KCl. This was prepared by dissolving 0.015g of KCl in 100 mL of distilled water. 3mM MgSO₄. This was prepared by dissolving 0.074g of MgSO₄ in 100mL of distilled water. 25mM MgCl₂. This was prepared by dissolving 0.15g of MgCl₂ in 100mL of distilled water. 2mM CaCl₂ in 100ml of distilled water. 3mM ATP. This was prepared by dissolving 0.152g of ATP in 100ml of distilled water. 10% TCA. This was prepared by dissolving 10g of TCA in distilled water and making up the volume to 100mL mark in the volumetric flask. 0.5% ammonium molybdate. This was prepared by dissolving 0.5g of ammonium molybdate in 100mL of distilled water. 0.25% ANSA (1-amino-2-naphthol-4-sulphonic acid). This was prepared by dissolving 0.25g of ANSA in 100ml of distilled water. 1mg/L of ouabain. This was prepared by dissolving 0.0003g of ouabain in 1L of distilled water.

2.3.1. Procedure

To 1.5 mL of 0.1M Tris-HCl buffer, was added 0.1 mL of each of tissue, NaCl, KCl, MgCl₂, CaCl₂, ATP solution. The tubes were incubated at 37°C for 20 minutes. The reaction was arrested by addition of 1 mL of 10% TCA. A standard tube containing all reagent, tissue and ouabain was made.

The tubes were centrifuged at 1500×g for 10 minutes and the phosphorous content in the supernatant was determined by the method of Fiske and Subbarow (1925). The supernatant and aliquots of standards were made up to 5 ml with 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: Total ATPase activity

$$S_a \times R_v$$

$$S_v \times T$$

Where:

S_a = amount of phosphate

R_v = reaction volume

S_v = sample volume

T = reaction time

2.4. Histopathological Examination of Tissues

The organs for histopathological examination were excised, blot-dried on tissue paper before preserving in 10% buffered formalin solution. The organs were processed 48 hours later, through

graded ethanol, followed by xylene, and embedded in paraffin wax. Tissue sections 6 microliters thick were prepared and stained with hematoxylin-eosin (H and E). The stained sections were analyzed and photographed under a light microscope to ascertain histopathological properties (Drury et al., 1976).

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 \pm 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. RESULT

This chapter show the findings on the effect of free fatty acid (FFA) level and store palm oil on the body weight, serum alkaline phosphate (ALP), and serum gamma -glutamyl transferase (GGT) in the Wistar rat. The result are reported as \pm standard error of the mean (SEM) and include statistical significance where applicable ($p < 0.05$) These findings show the liver-biliary function in response to varying dietary FFA level. After the administration, three (3) mice from each group were sacrificed, and their serum were obtained through centrifugation of the blood samples.

3.1 The Effect of Palm Oil on Body Weight of Wistar rats.

The findings shows that the control group exhibited an 8.54g increase in weight, rats fed with 0.4% FFA experienced the most significant weight gain (11.10g) while groups exposed to intermediate FFA levels (4.8%, 8.4%) exhibited reduced weight changes, the 8.4% FFA group showed the least weight gain (3.13g). While, the highest FFA group (42.7%) showed a moderate weight gain (7.69g).

Table3. 1: Mean Values of the Body Weights of Wistar Rats

Group	Body Weight Before Treatment (g)	Body Weight After Treatment (g)	Difference in Weight (g)
I- Control	136.50 ± 0.11 ^a	148.07 ± 0.14 ^b	8.54 ± 0.05 ^c
II- 0.4 % FFA	141.30 ± 0.11 ^c	157.00 ± 0.11 ^e	11.10 ± 0.08 ^f
III- 4.8 % FFA	149.50 ± 0.11 ^f	161.60 ± 0.11 ^f	8.06 ± 0.06 ^d
IV-8.4 % FFA	140.30 ± 0.11 ^b	144.70 ± 0.11 ^a	3.13 ± 0.08 ^a
V-21.9 % FFA	142.30 ± 0.11 ^d	150.70 ± 0.11 ^c	5.90 ± 0.08 ^b
VI- 42.7 % FFA	143.00 ± 0.11 ^c	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 the Organ Weight and Organ To Body Weight Ratio of the Heart

The weight of the heart was measured and a ratio was gotten from the wight of the organ to the body weight of the Wistar rats. Table 2 summarizes the mean values and standard error of mean of the organ weight and organ to body weight ratio of the heart of Wistar rats.

Table 2 : Mean Values of the Organ Weight and Organ To Body Weight Ratio of the Heart

Group	Organ Weight (g)	Organ to Body Weight Ratio (g)
I- Control	0.5200 ± 0.00 ^{bc}	0.0035 ± 0.00 ^b
II- 0.4 % FFA	0.5400 ± 0.00 ^c	0.0034 ± 0.00 ^b
III-4.8 % FFA	0.5333 ± 0.00 ^c	0.0032 ± 0.00 ^a
IV-8.4 % FFA	0.4800 ± 0.00 ^a	0.0034± 0.00 ^b
V-21.9 % FFA	0.4800 ± 0.00 ^a	0.0031 ± 0.00 ^a
VI-42.7 % FFA	0.5067± 0.00 ^b	0.0032 ± 0.00 ^a

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 Total ATPase Activity In The Heart Of Wistar Rats

The total ATPase activity in the heart of Wistar rats were assessed for the control group and experimental groups fed with palm oil of varying free fatty acid (FFA) levels. Table 3 shows the mean values of total ATPase activity in the heart of Wistar rats.

Table 3 : Mean Values of Total ATPase Activity In The Heart Of Wistar Rats

Group	Total ATPase Activity x10⁻⁷ (μmole/Pi/min/mg protein)
I- Control	1.10 \pm 0.00 ^b
II- 0.4 % FFA	1.10 \pm 0.17 ^b
III-4.8 % FFA	1.93 \pm 0.83 ^b
IV-8.4 % FFA	0.40 \pm 0.00 ^a
V-21.9 % FFA	1.13 \pm 0.03 ^b
VI-42.7 % FFA	0.83 \pm 0.03 ^b

Data with the same superscript are not significantly different ($p \geq 0.05$) while data with different superscript are significantly different ($p \leq 0.05$).

3.4 HISTOPATHOLOGY AND HISTOPATHOLOGY OF THE HEART

Histopathology is the microscopic examination of tissue samples to study disease manifestations. It involves the preparation of tissue sections, staining, and analysis under a microscope to detect structural and cellular abnormalities. Histopathology is crucial for diagnosing diseases, understanding pathological mechanisms, and assessing the impact of external factors on tissues.

Histopathology of the Heart focuses on structural and cellular changes in cardiac tissue due to disease, toxins, or dietary influences. In this project, histopathological analysis was conducted to assess the effect of varying free fatty acid (FFA) levels in palm oil on the heart of Wistar rats. The findings revealed that:

- Control and low FFA-fed groups exhibited normal cardiac architecture with intact myocytes.
- Moderate FFA intake (4.8%) led to increased ATPase activity, suggesting adaptive metabolic responses.
- Higher FFA levels ($\geq 8.4\%$) caused oxidative stress, reduced ATPase activity, and structural damage such as myofibrillar disorganization, necrosis, and inflammation.
- Despite these alterations, no acute myocardial damage was observed.

3.4. Histopathological Analysis

Histopathological analysis was conducted to evaluate the structural and cellular effects of feeding

Wistar rats with palm oil of varying free fatty acid levels, Representative images from the analysis are presented below.

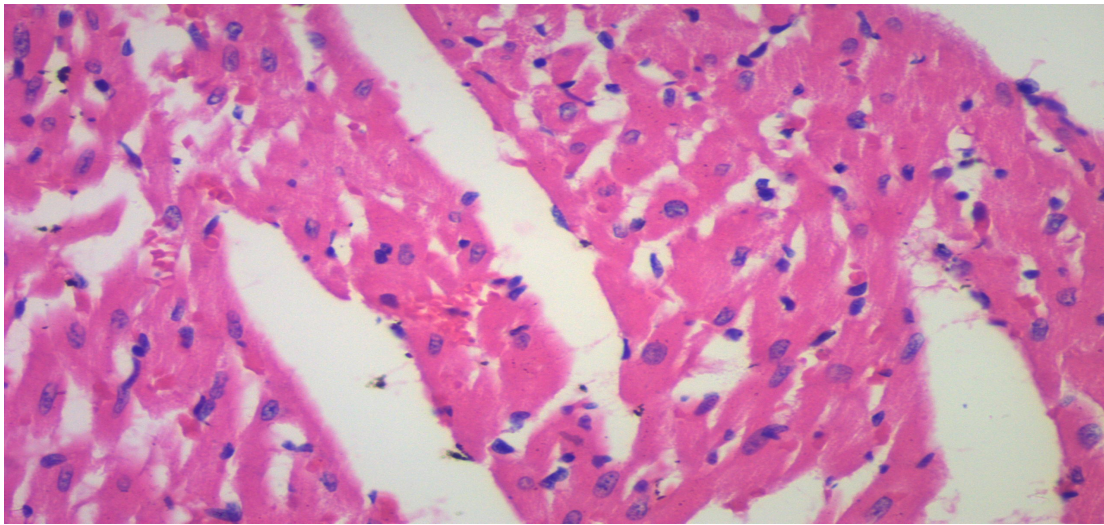


Plate 1(control) : Section of cardiac muscle shows myocytes (arrow) with peripheral placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with NORMAL MYOCYTES.

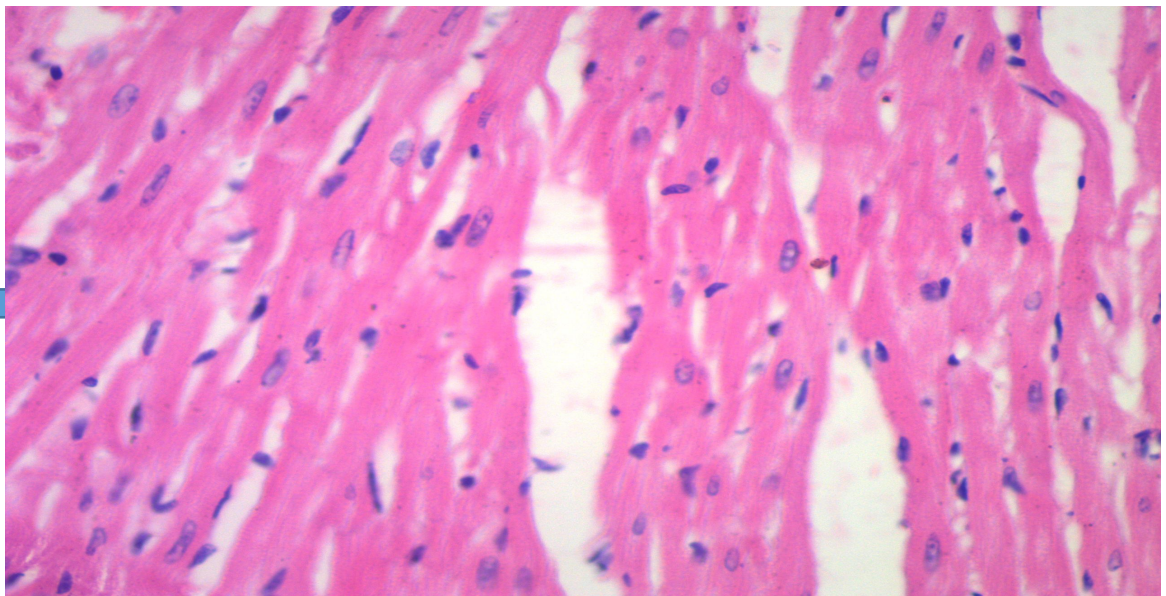
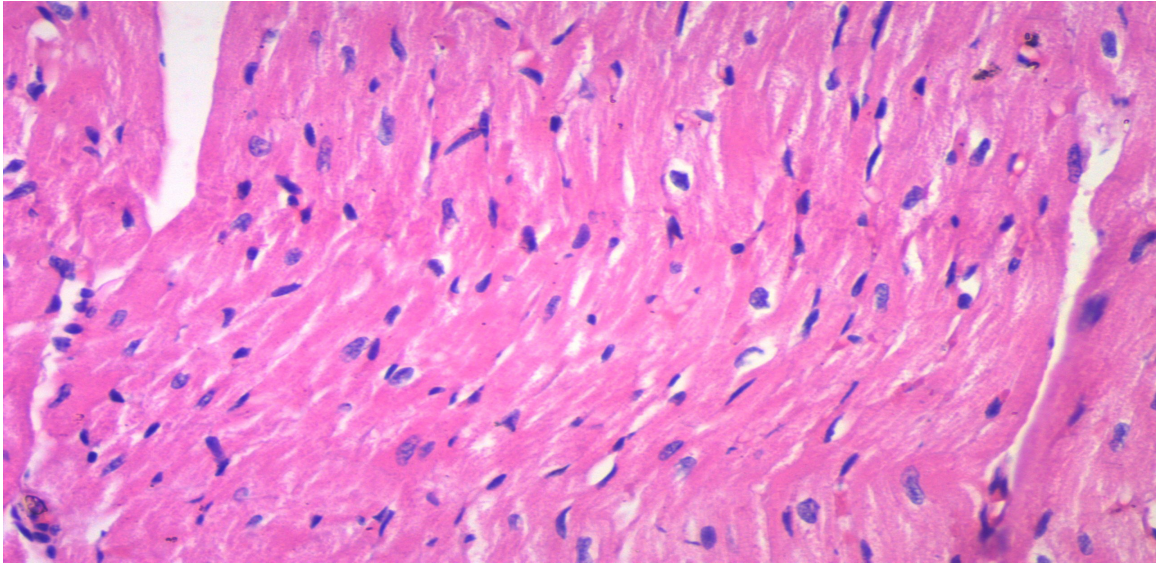


Plate 2 (0.4% FFA) : Section of cardiac muscle shows myocytes (arrow) with peripheral places nuclei

surroun
ded by
eosinop
hilic
cytopla
sm.

Feature
s are in
keeping
with



NORMAL MYOCYTES.



Plate 3(4.8% FFA) : Section of cardiac muscle shows myocytes (arrow) with peripheral places nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with NORMAL MYOCYTES.

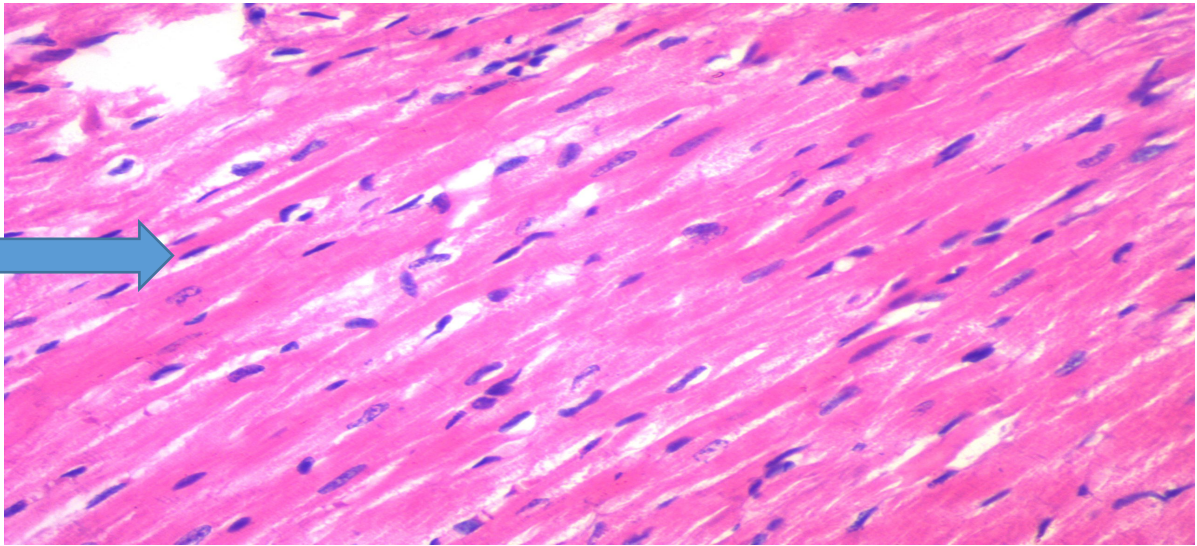


Plate 4(8.4% FFA) : Plate 3 : Section of cardiac muscle shows myocytes (arrow) with peripheral places nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with NORMAL MY

OC
YT
ES.

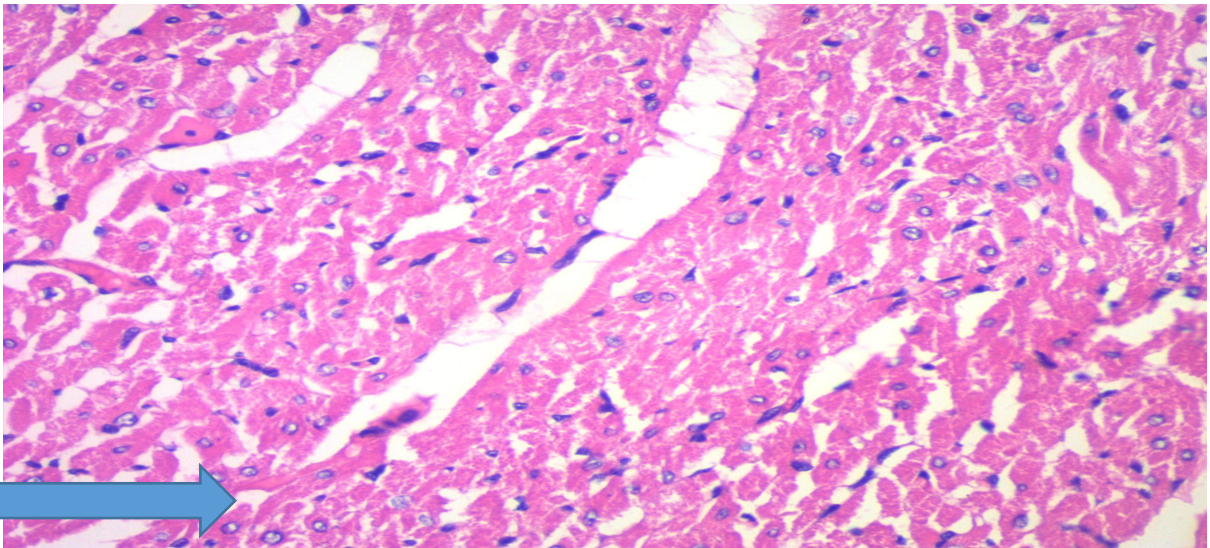


Plate 5(21.9% FFA) : Plate 3 : Section of cardiac muscle shows myocytes (arrow) with peripheral places nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with NORMAL MYOCYTES.

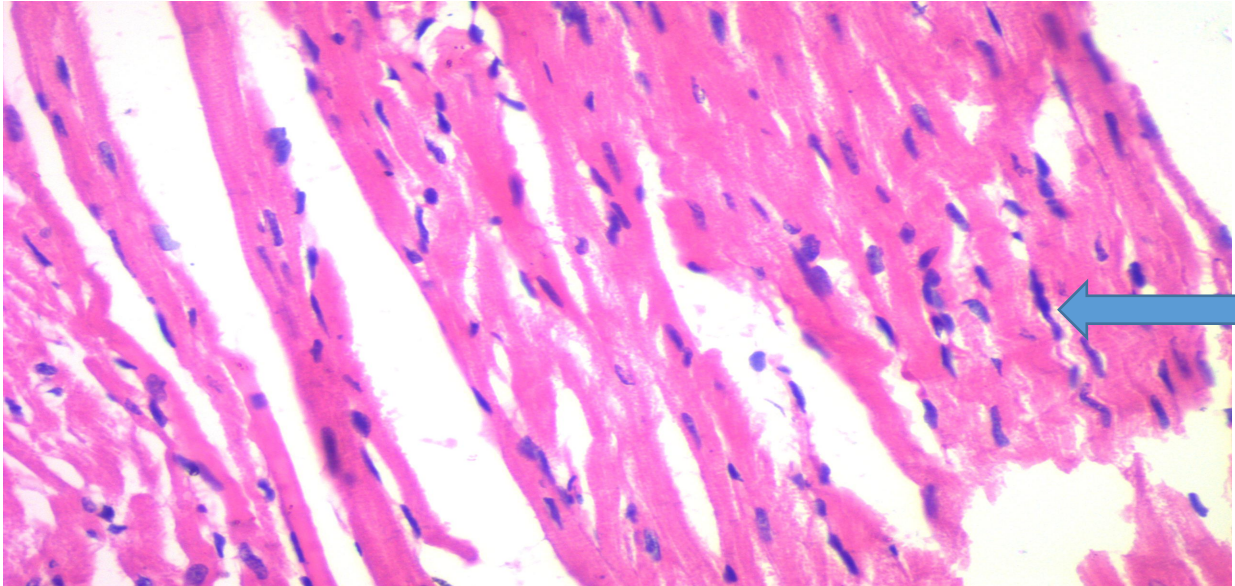


Plate 6)(42.7% FFA) : Plate 3 : Section of cardiac muscle shows myocytes (arrow) with peripheral places nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with NORMAL MYO

CHAPTER FOUR

4.0 DISCUSSION.

4.1 Discussion

Inadequate processing or storage conditions may result in an increase in the quantity of free fatty acids (FFA) in palm oil. When ingested in its oxidised state, this dietary palm oil can be toxic to the body. When consumed, high levels of free fatty acids may cause histopathological changes and organ failure in a variety of organs, such as the kidney, liver, intestinal mucosa, and heart (Alaam et al., 2012; Boniface et al., 2014). Additionally, it affects the body's ATPases, which results in an ionic imbalance.

An essential biomarker for evaluating ion transport across cellular membranes and energy metabolism is total ATPase activity. Particularly in metabolically active organs like the heart, they are known to cause oxidative stress, inflammation, and impaired organ function. Reactive oxygen species (ROS) may be produced as a result of lipid peroxidation brought on by elevated levels of free fatty acids in palm oil. DNA, lipids, and biological proteins can all be harmed by reactive oxygen species. Reactive oxygen species can cause arrhythmias and myocardial failure in cardiac tissue by interfering with mitochondrial function, decreasing ATP synthesis, and impairing ion transport. The accumulation of free fatty acids and variations in total ATPase activity can impair cardiac contractility, disturb cellular homeostasis, and ultimately lead to cardiac disorders like hypertrophy or failure. A comprehensive assessment of the effect of FFAs on cardiac tissue is made possible by the combination of histological evaluation and ATPase activity analysis (Alaam et al., 2012). According to Table 3's findings, the control group had the lowest total ATPase activity (0.04 ± 0.01), while group 3 fed palm oil with 4.8% FFA had the highest (1.13 ± 0.09). This suggests that ATPase activity may be upregulated by moderate levels of free fatty acids, which may also improve membrane fluidity and act as an energy substrate. Because free fatty acids can be used as a metabolic fuel in physiological settings, the observed peak in ATPase activity in group 3 may represent an

adaptive response to moderate lipid exposure. When compared to the control group, Group 2's total ATPase activity significantly increased after being fed fresh palm oil that contained 0.4% FFA. This demonstrates how fresh palm oil's high antioxidant content helps to maintain membrane potential and energy metabolism (Almeida et al., 2019). Group 4 fed stored palm oil with FFA levels of 8.4% (0.60 ± 0.11) and above showed a decrease in ATPase activity, while groups 5 and 6 fed stored palm oil with 21.9% and 42.7% FFA, respectively, showed comparatively constant activity. This demonstrates how free fatty acids' inhibitory effects on ATPase activity become noticeable at higher concentrations. Longer storage of oxidised free fatty acids produces reactive oxygen species (ROS), which can harm membrane proteins and interfere with ATPase activity (Boateng et al., 2016). Ion balance is upset by this damage, which impairs cardiac function. Group 1 (control) and group 6 (42.7 % FFA), group 1 (control) and group 5 (21.9 % FFA), group 1 (control) and group 4 (8.4 % FFA), group 1 (control) and group 2 (0.4% FFA), and group 1 (control) and group 3 (4.8% FFA) all showed significant differences ($p \leq 0.05$). Group 6 (42.7 percent FFA), group 21.9 percent FFA, and group 4 (8.4 percent FFA) did not differ significantly ($p \geq 0.05$). According to the histopathological analysis results shown in Figures 1 through 6, there was a progressive rise in histopathological damage as free fatty acid levels rose, which was correlated with changes in ATPase activity. Histopathological analysis of the control group showed normal cardiac architecture, including intact nuclei, well-aligned myofibrils, and no signs of necrosis or inflammation. Using this as a starting point to evaluate the effects of fed and stored palm oil, it was found that group 2 likewise showed no signs of inflammation or necrosis. Nevertheless, myofibrillar disarray, necrosis, inflammatory infiltration, and cellular degeneration are among the notable histopathological alterations seen in the cardiac tissues of rats in groups 3 through 6 that were fed

stored palm oil with rising levels of free fatty acids. This suggests that elevated levels of free fatty acids in palm oil may have a harmful impact on the heart through processes like oxidative stress and lipid peroxidation. The decrease in ATPase activity in the heart tissues of rats given a lot of free fatty acid palm oil is consistent with the histopathological alterations that were seen. ATPases are essential for energy production, cardiac function, and ion homeostasis maintenance (Jørgensen et al., 2003). Necrosis and inflammation, which indicate structural damage to cardiac cells, most likely interfere with the activity of these enzymes. In earlier studies, Narang et al. (2004) showed how palm olein in the diet affected ischaemia reperfusion injury (IRI) using a rat isolated heart model. Three Wistar rat groups were employed. Different amounts of palm olein (5% and 10%, respectively) were given to the two groups. The third group was the control group, which was given a typical diet. After 30 days, each group was split in half, and each half was subjected to global ischaemia for 20 minutes and reperfusion for 40 minutes. After that, the researchers showed that the amount of antioxidants in the myocardium increased in the rats that were fed the 5% olein-supplemented diet. When contrasted with the rats given the control diet, which showed severe oxidative damage without a corresponding rise in antioxidant activity, this was noteworthy (Narang et al., 2004). However, they were unable to detect a dose-dependent effect. Their research, which is consistent with this new study, offered more proof of the advantages of a diet high in palm oil in defending the heart against oxidative stress and tissue damage.

CONCLUSION

The study demonstrated that the consumption of palm oil with varying levels of free fatty acids (FFA) significantly influences total ATPase activity and cardiac histology in Wistar rats. The study also observed significant differences in weight gain across groups consuming palm oil with different FFA levels, the control group show moderate weight gain as baseline for comparison, the low level FFA group (0.4% FFA) show a significant weigh gain suggesting optimal intake of nutrient while the higher level FFA group (28.4% FFA) showed reduce weight gain particular in the 8.4% FFA group. Moderate FFA levels enhanced ATPase activity, potentially reflecting adaptive physiological responses. However, higher FFA levels caused oxidative stress and lipid peroxidation, leading to suppressed ATPase activity and histopathological changes in the cardiac tissue. Despite the observed changes in ATPase activity and tissue structure, no evidence of acute myocardial damage was found in the control and experimental groups. The findings underscore the importance of proper palm oil storage to limit FFA accumulation and prevent potential adverse effects on cardiac health

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 Science* , 11(4) :157-164.
- Absalome, M.A., Massara, C.C., Alexandre, A.A., Gervais, K., Chantal, G.G.A., Ferdinand, D., Rhedoor, A.J., Coulibaly, I., George, T.G., Brigitte, T. and Marion, M. (2020). Biochemical properties, nutritional values, health benefits and sustainability of palm oil. *Biochimie*, 178 : 81-95.
- 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.
- Alaam, M. H., Yasin, N. M., Hafez, S. A. and Mohammed, H. H. (2012). Biological and histological evaluations of palm oil and its fractions. *World Journal of Dairy and Food Sciences*, 7(2):120-130.
- 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.
- 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.
- 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.
- Ashaari, A., Ahmad, T., Awang, S.R. and Shukor, N.A. (2021). A graph-based dynamic modeling for palm oil refining process. *Processes* , **9** : 523.
- 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.
- Bartlett, D.E., Miller, R.B., Thiesfeldt, S., Lakhani, H.V., Shapiro, J.I. and Sodhi, K. (2018). The role of Na/K-ATPase signaling in oxidative stress related to aging: implications in obesity and cardiovascular disease. *International Journal of Molecular Sciences*, **19**(7) : 2139.
- Basiron, Y. (2007). Palm oil production through sustainable plantations. *European Journal of Lipid Science and Technology*, **109**: 289-295.
- 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.
- Beshel, F. N. and Beshel, I.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. 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., 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.
- Boniface, M. N., Ejimofor, O. C. E. A. and Ezissi, A. I. (2014). The effects of thermally oxidized palm oil on the kidney of adult Wistar rats. *Journal of Medical Science and Clinical Research*, **2**(4):759-767.
- 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** :112456.
- 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.
- 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.

- Chen, X. and Sun, S., 2023. Color reversion of refined vegetable oils: a review. *Molecules*, **28**(13) : 5177.
- 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.
- 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.
- Figtree, G.A., Karimi, G.K., Liu, C.C. and Rasmussen, H.H. (2012). Oxidative regulation of the Na⁺-K⁺ pump in the cardiovascular system. *Free Radical Biology and Medicine*, **53**(12) : 2263-2268.
- Galougahi, K.K., Liu, C.C., Bundgaard, H. and Rasmussen, H.H. (2012). β -Adrenergic regulation of the cardiac Na⁺-K⁺ ATPase mediated by oxidative signaling. *Trends in Cardiovascular Medicine*, **22**(4) : 83-87.
- 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.
- Gheorghiade, M., Ambrosy, A.P., Ferrandi, M. and Ferrari, P. (2011). Combining SERCA2a activation and Na-K ATPase inhibition: a promising new approach to managing acute heart failure syndromes with low cardiac output. *Discovery Medicine*, **12**(63) : 141-151.
- 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 Reviews*, **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.
- 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.
- 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.
- Jorgensen, P.L., Håkansson, K.O. and Karlsh, S.J. (2003). Structure and mechanism of Na, K-ATPase: functional sites and their interactions. *Annual Review of Physiology*, **65**(1) : 817-849.
- Liu, C.C., Fry, N.A., Hamilton, E.J., Chia, K.K., Garcia, A., Galougahi, K.K., Figtree, G.A., Clarke, R.J., Bundgaard, H. and Rasmussen, H.H. (2013). Redox-dependent regulation of the Na⁺-K⁺ pump: new twists to an old target for treatment of heart failure. *Journal of Molecular and Cellular Cardiology*, **61** : 94-101.
- 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): 678-685.
- 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., Orrú, S. and Buono, P. (2015). Biological and nutritional properties of palm oil and palmitic acid: effects on health. *Molecules*, **20**: 17339-17361.
- 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.
- Misfeld, M. and Sievers, H. H. (2007). Heart valve macro- and microstructure. *Philosophy and Transmission Reviews on Society of London Books of Biological Science*, **362**(1484):1421-1436.
- Møller, J. V., Nissen, P., Sørensen, T. L. M and Le Maire, M. (2005). Transport mechanism of the sarcoplasmic reticulum Ca²⁺ -ATPase pump. *Current Opinions on Structural Biology*, **15**: 387–393
- 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.
- Mørk, H.K., Sjaastad, I., Sande, J.B., Periasamy, M., Sejersted, O.M. and Louch, W.E. (2007). Increased cardiomyocyte function and Ca²⁺ transients in mice during early congestive heart failure. *Journal of Molecular and Cellular Cardiology*, **43**(2): 177-186.
- Murphy, C. and Lazzara, R. (2016). Current concepts of anatomy and electrophysiology of the sinus node. *Journal of Intervenous Cardio Electrophysiology*, **46**(1):9-18.
- Narang , D., Sood, S., Thomas, M. K., Dinda, A. K. and Maulik, S. K (2004). Effect of dietary palm oil olein oil on oxidative stress associated with ischemic-reperfusion injury in isolated rat heart. *BMC Pharmacology*, **4** : 29.
- 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.
- O'brein, R. D. and Wakelyn, P. J. (2005). Cottonseed oil: an oil for trans-free option. *Food Technology*, **16**: 677-679.
- Obradovic, M., Stanimirovic, J., Panic, A., Bogdanovic, N., Sudar-Milovanovic, E., Cenic-Milosevic, D. and R Isenovic, E. (2017). Regulation of Na⁺/K⁺-ATPase by estradiol and IGF-1 in cardio-metabolic diseases. *Current Pharmaceutical Design*, **23**(10) : 1551-1561.
- 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.
- Oyewole, O.E. and Amosu, A.M. (2010). Public health nutrition concerns on consumption of red palm-oil (RPO): the scientific facts from literature. *African Journal of Medical Science* , **39** : 255-262.
- 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.
- 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.
- 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.
- Schwingshackl, 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.
- Sen, C.K., Khanna, S. and Roy, S. (2007). Tocotrienols in health and disease: the other half of the natural vitamin E family. *Molecular Aspects of Medicine*, **28**(5-6) : 692-728.
- Shiue, S.J., Kao, K.M., Leu, W.M., Chen, L.Y., Chan, N.L. and Hu, N.T. (2006). XpsE oligomerization triggered by ATP binding, not hydrolysis, leads to its association with XpsL. *The EMBO Journal*, **25**(7):1426-1435.
- Silva, E. and Soares-da-Silva, P. (2012). New insights into the regulation of Na⁺, K⁺-ATPase by ouabain. *International Review of Cell and Molecular Biology*, **294** :.99-132.
- 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.
- Souganidis, E., Laillou, A., Leyvraz, M. and Moench-Pfanner, R. (2013). A comparison of retinyl palmitate and red palm oil β -carotene as strategies to address vitamin A deficiency. *Nutrients*, **5**(8) : 3257-3271.

- 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.
- Tarrago-Trani, M. T., Phillips, K. M, Lemar, L. E. and Holden, J. M. (2006). New and existing oils and fats used in products with reduced trans-fatty acid content. *Journal of American Dietetic Association*, **106**: 867-880.
- Velasco, J. and Dobarganes, C. (2002). Oxidative stability of virgin olive oil. *European Journal of Lipid Science and Technology*, **104**(9-10): 661-676.
- 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.
- Vijay, V., Pimm, S. L., Jenkins, C. N. and Smith, S. J. (2016). The impacts of oil palm on recent deforestation and biodiversity loss. *PLoS One*, **11**(7) : e0159668.
- 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.
- Wondi, M.H., Shamsudin, R. and Yunus, R. (2019). A review of palm mesocarp maceration and separator machine. *Konvensyen Kebangsaan Kejuruteraan Pertanian Dan Makanan* , **9** :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.

- 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 Analytical Methods*, **14**:2199–2210.
- Žurawik, A., Jadcak, D., Panayotov, N. and Žurawik, P. (2021). Antioxidant properties of pepper (*Capsicum annuum L.*) depending on its cultivar and fruit colouration. *Plant, Soil & Environment*, **67**(11) : 567-571.