

**THE PHYSICAL PROPERTIES AND NUTRITIONAL
COMPOSITIONS OF LEAF PROTEIN CONCENTRATES AND
BY PRODUCTS FROM OIL PALM PLANT (EL FOUND IN A
PART OF EDO STATE, NIGERIA.**

BY

Eghogho Deborah OHIOKHUAOBO (Miss)

AGR2004312

DEPARTMENT OF ANIMAL SCIENCE

FACULTY OF AGRICULTURE

UNIVERSITY OF BENIN

BENIN CITY, NIGERIA

NOVEMBER, 2025

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE,
FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY,
NIGERIA**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD
OF BACHELOR OF AGRICULTURE HONORS (B. AGRIC. IN ANIMAL
SCIENCE)**

NOVEMBER, 2025

CERTIFICATION

This is to certify that this Project work was carried out by Eghogho Deborah OHIOKHUAOBO (Miss) with Matriculation Number, AGR2004312 under the guidance of the Project Supervisors approved by the Department of Animal Science, Faculty of Agriculture, University of Benin, Benin City, Nigeria.

PROF. S. O. NWOKORO
(PROJECT SUPERVISOR)

DATE

DR. N.C AKAEZE
(HEAD OF DEPARTMENT)

DATE

DEDICATION

This project is dedicated to God Almighty, whose grace and guidance have been my greatest strength.

And to my invincible parents, Mr. and Mrs. Ohiokhuaobo and my incredible siblings whose unwavering love, support and encouragement inspired me and carried me through every step of this journey. Your faith in me gave me the courage to keep going. This achievement is as much yours as it is mine.

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ABSTRACT

This study investigated the extraction and evaluation of Leaf Protein Concentrate (LPC) produced from Oil Palm (*Elaeis guineensis*) leaves using three processing techniques: Heat Coagulation, Alum Precipitation and Acid Coagulation. The research aimed to compare the effects of these extraction methods on the Physical characteristics, Proximate composition, Mineral profile and Phytochemical constituents of the resulting concentrates. Standard laboratory procedures were employed for all analyses. The LPCs obtained from the three methods were dark green when fresh but turned blackish upon drying, indicating chlorophyll degradation during processing. Among the extraction techniques, the Acid Coagulation method yielded the highest Crude Protein value (33.84%), demonstrating its superior efficiency in protein recovery. Alum Precipitation recorded the highest Ash (7.50%) and Crude Fibre (13.33%) contents, suggesting higher mineral and structural residue retention. Heat Coagulation produced the highest Ether Extract (38.33%) and maintained better mineral preservation. Potassium was the most abundant mineral detected, with the highest concentration (231.67 mg/kg) observed in the Heat-treated LPC, while sodium recorded the least concentration (0.01 mg/kg). Phytochemical screening revealed the presence of several bioactive compounds including flavonoids, phenols, alkaloids,

steroids, tannins, saponins, terpenoids, coumarins and cardiac glycosides in varying concentrations across treatments. These bioactive components indicate potential antioxidant, antimicrobial and functional benefits. Based on the findings, the Acid Coagulation technique is most effective for enhancing protein recovery from Oil Palm leaves, while Heat Coagulation is more suitable for retaining lipid and mineral components. Overall, Oil Palm (*Elaeis guineensis*) Leaf Protein Concentrate presents a promising, low-cost and underutilized source of plant protein and minerals for animal feed formulation and other nutritional applications.

LIST OF ABBREVIATIONS

ABBREVIATIONS

MEANING

LPC	Leaf Protein Concentrate
PKC	Palm Kernel Cake
CP	Crude Protein
CF	Crude Fibre
NFE	Nitrogen Free Extract
EE	Ether Extract
POME	Palm OIL Mill Effluent

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Oil Palm (*Elaeis guineensis* Jacq.) is a Monocotyledonous Tropical Crop belonging to the family *Arecaceae* and is recognized globally as one of the most economically significant plants. Indigenous to the humid rainforests of West and Central Africa, particularly around the Gulf of Guinea in present-day Nigeria, Ghana and Cameroon, the Oil Palm has long been part of the cultural and economic life of African communities.

Archaeological findings show its use as far back as 3,000 BCE, with Oil Palm residues found in Egyptian tombs, highlighting its early trade and value (FAO, 2021; Akindele *et al*, 2020).

In Nigeria, Oil Palm has historically played a vital role in Agriculture, Industry and Trade. Before the 1960s, the country was the world's largest producer and exporter of Palm Oil, accounting for over 43% of global output (PWC, 2019). This dominance was later overtaken by Malaysia and Indonesia. Despite this, Nigeria remains Africa's leading producer and ranks third globally.

Prior to the Crude Oil boom of the 1970s, Oil Palm significantly supported Nigeria's economy and provided raw materials for Cooking Oil, Soap, Cosmetics and Local industries (Adegeye and Dittoh, 2016). Between 1945 and 1970, Edo State and other

regions made major contributions to this growth due to favourable climatic conditions and the presence of large-scale plantations that adopted mechanized farming techniques (RSPO, 2022).

While extensive attention has been given to Oil Extraction from the Mesocarp and Kernel, other parts of the plant, especially the Leaves, Palm Press Fibre, Empty Fruit Bunches (EFB) and Palm Kernel Cake, remain underutilized or burned, contributing to environmental degradation and loss of valuable biomass (PwC, 2019, p. 11). This underuse has created a gap in sustainable resource utilization and circular Agricultural Systems.

Recently, Research attention has shifted toward exploring the potential of Oil Palm Leaves as a valuable source of protein through the production of Leaf Protein Concentrate (LPC). Oil Palm leaves contain moderate Crude Protein (8–10%), high Crude Fibre (22–30%), low Ether Extract (3–5%) and significant Ash (10–12%), with Nitrogen-Free Extract ranging between 40–45% on a Dry Matter basis (Omar *et al*, 2023).

Their Fibre includes high levels of NDF (50–90%) and ADF (40–80%), consisting of Cellulose, Hemicellulose and Lignin, which may limit digestibility (Agboola *et al*, 2023). The leaves are also rich in Essential Macroelements, Potassium, Calcium, Magnesium, and Phosphorus as well as trace elements such as Iron (40–70 µg/g), Copper, Zinc and Manganese (Umanah *et al*, 2021).

Phytochemical studies reveal that Oil Palm Leaves contain Phenolics, Flavonoids and Tocopherols, with extracts showing Antioxidant and Anti-ulcer properties (Yusoff *et al*, 2018). However, anti-nutritional factors such as Lignin, Tannins and Phytates may hinder nutrient availability. These effects can be minimized using appropriate processing techniques like fermentation or ammoniation (Agboola *et al*, 2023). Research on LPC extraction has been conducted across several plant families. Pirie pioneered LPC studies, publishing works such as Leaf Protein as a Human Food (1966), Equipment and Methods for Extracting and Separating Protein (1971) and Leaf Protein: Its Agronomy, Preparation, Quality and Use.

Later contributions from Nwokoro *et al*, Aletor *et al*, Agbede and Aletor (2003, 2004), Agbonghae (2016), Akaeze (2010), Akaeze *et al* (2014, 2015) and Akaeze and Paul-Osagie (2023) expanded LPC applications in Nigeria. Their studies demonstrated successful LPC extraction from *Vernonia amygdalina* (bitter leaf), *Solanum africanum* (African nightshade), *Amaranthus hybridus* (green tete), *Telfairia occidentalis* (ugu), *Leucaena leucocephala*, *Gliricidia sepium*, *Carica papaya*, *Manihot esculenta* (cassava) and *Hevea brasiliensis* (rubber).

These evaluations revealed satisfactory Proximate and Mineral Compositions. When used in animal diets, LPC improved performance. Agbede *et al* (2003) reported that Leucaena Leaf Protein Concentrate (LLPC) could replace up to 25% of Fish Meal in broiler starter diets without adverse effects, thereby reducing production costs.

Protein Deficiency remains one of the most common nutritional problems globally, especially in developing countries, leading to conditions like Kwashiorkor and Marasmus in humans and reduced productivity in animals (Latham, 1997; FAO *et al*, 2015; Olomu, 2011). With Nigeria's population exceeding 197 million and Protein-rich Feed Ingredients such as Fish Meal becoming increasingly expensive, there is a strong need to explore Alternative, Affordable and Sustainable Protein sources like LPC (PwC, 2019).

Utilizing Oil Palm Leaves for LPC production supports circular economy models, reduces Agricultural Waste and promotes climate-smart and sustainable agriculture (European Parliamentary Research Service, 2020).

Despite the economic importance of Oil Palm, limited scientific data exist on the physical and nutritional characteristics of its LPC and by-products, particularly in Edo State. This study seeks to address that knowledge gap.

1.2 Objectives of Study

The main aim of this study is to determine the Physical Properties And Nutritional Compositions Of Leaf Protein Concentrate And By Products From Oil Plant Obtained From Ovia North East Local Government Area of Edo State, Nigeria, using three different methods.

The specific objectives were to:

1. determine the Physical Properties And Nutritional Compositions Of Leaf Protein Concentrates And By Products From Oil Plant obtained from Oil Palm leaves using Heat Coagulation Method.
2. determine the Physical Properties And Nutritional Compositions Of Leaf Protein Concentrates And By Products From Oil Plant obtained from Oil Palm leaves using Alum Precipitation Method.
3. determine the Physical Properties And Nutritional Compositions Of Leaf Protein Concentrates And By Products From Oil Plant obtained from Oil Palm leaves using Acid Coagulation Method
4. analyze the proximate nutritional composition of LPC (moisture, ash, crude protein, crude fat, crude fiber, and carbohydrates).
5. compare the nutritional profiles of the LPC and by-products with existing data on similar plant-based protein sources and animal feeds.

CHAPTER TWO

LITERATURE REVIEW

2.1 Oil Palm Plant and Its Parts

Scientific Classification (*Elaeis guineensis*):

Kingdom:	<i>Plantae</i>
Sub-Kingdom:	<i>Tracheobionta</i>
Phylum:	<i>Magnoliophyta</i>
Class:	<i>Liliopsida</i> (Monocotyledons)
Order:	<i>Arecales</i>
Family:	<i>Arecaceae</i>
Genus:	<i>Elaeis</i>
Species:	<i>guineensis</i>

Source: U.S. Department of Agriculture (USDA)

Oil Palm (*Elaeis guineensis* Jacq.) is one of the most Versatile and Economically important Tropical crops, particularly in West Africa. While its Oil-rich Fruits are widely processed and utilized, the vast biomass of leaves and other by-products remains underutilized despite their rich potential for value addition, especially in the form of LPC. Oil Palm is a perennial monocotyledonous crop in the family *Arecaceae*. Indigenous to West Africa, it has spread to tropical regions of Southeast Asia and Latin America. The plant produces fruit in bunches, from which Palm Oil (from the

Mesocarp) and Palm Kernel Oil (from the seed) are extracted. Aside from oil, the plant yields substantial biomass, including leaves, Empty Fruit Bunches (EFB), Palm Kernel Cake (PKC), Palm Oil Mill Effluent (POME) and Fibre, all of which offer potential for further processing and utilization.

Oil Palm Cultivation is prominent in southern Nigeria, particularly in Edo State, where both smallholder and large-scale plantations exist.

2.1.1 Morphology and Common Nomenclature of Oil Palm (*Elaeis guineensis* Jacq.)

Common Nomenclature:

The Oil Palm is scientifically named *Elaeis guineensis* Jacq. and belongs to the family *Arecaceae* (Corley and Tinker, 2015). The genus name *Elaeis* is derived from the Greek *elaion* (oil), while the species epithet *guineensis* refers to the Gulf of Guinea region in West Africa the species' centre of origin and early domestication.

Recent molecular and morphological surveys continue to recognize *E. guineensis* as the primary species of economic importance, alongside *E. oleifera*, which is regionally significant in the Americas (Camacho-Villalobos *et al*, 2021).

2.1.2 General Habit and Stem

Elaeis guineensis is a tall, single-stemmed, perennial monocotyledonous palm with an erect habit and no true branching from the trunk. Under favourable tropical plantation conditions, the stem commonly attains heights of 20–30 m, with a diameter ranging between 30 and 75 cm in mature palms (Corley and Tinker, 2015). The trunk carries

persistent leaf bases in juvenile stages and later shows characteristic ringed leaf scars as old fronds abscise.

2.1.3 Leaves (Fronds)

Leaves are pinnate and spirally arranged in a crown at the top of the stem, forming the main photosynthetic surface of the palm. A mature Oil Palm bears between 30 and 50 functional green fronds at any time. Individual fronds may measure 3–7.5 m in length, depending on genotype and growth conditions. Each leaf consists of a Leaf base, Petiole, main Rachis and numerous alternate Leaflets (pinnae) arranged along the rachis (Corley and Tinker, 2015; Camacho-Villalobos *et al*, 2021).

2.1.4 Root System

The Oil Palm has a Fibrous, Adventitious Root System composed of several root orders (primary, secondary, tertiary, and quaternary) that develop from the stem base and change as the palm matures (De la Peña *et al*, 2024). Roots spread widely and penetrate to varying depths depending on soil type and water table, providing anchorage and a broad nutrient-uptake zone.

Recent studies show that root morphology is highly responsive to Soil Moisture, Nitrogen availability and Agronomic practices such as pruning or rhizobacterial inoculation (De la Peña *et al*, 2024; Wahyudin *et al*, 2024).

2.1.5 Inflorescences and Flowering

Elaeis guineensis is monoecious, producing separate male and female inflorescences in the leaf axils. Male inflorescences form elongated spikes bearing many staminate flowers, while female inflorescences are more compact and develop into fruit bunches. Flowering is asynchronous but continuous in suitable climates, with rainfall and temperature influencing fruit set (Corley and Tinker, 2015; Murphy, 2021).

2.1.6 Fruit and Seed Structure

Oil Palm Fruits are fleshy drupes borne in large clusters known as Fresh Fruit Bunches (FFB), weighing between 10 kg and several tens of kilograms. Each fruit consists of an Exocarp (skin), Mesocarp (oil-rich pulp), Endocarp (shell) and kernel (seed), both mesocarp and kernel being used commercially. Fruit types include dura (thick shell), pisifera (shell-less) and tenera (thin-shelled hybrid) forms, differing in shell thickness and oil yield.

2.1.7 Morphological Variation and Applied Importance

Morphological traits such as plant height, leaf production, bunch architecture and root spread vary with genotype, age and environment, influencing breeding and agronomic practices. Research now focuses on roots and shoots traits as key markers for improving nutrient-use efficiency under changing climatic conditions (Ikhajiagbe *et al*, 2022; De la Peña *et al*, 2024).

2.2 Uses of Oil Palm

Oil Palm (*Elaeis guineensis* Jacq.) is a highly versatile tropical crop valued for its Nutritional, Medicinal, Domestic and Industrial benefits. In Africa, especially Nigeria, nearly every part of the Palm Fruit, Leaves, Sap, Trunk and Residues is useful to Humans, Animals and the Environment (Ayodele and Eshalomi, 2010). The crop provides Food, Raw materials, Income and employment, significantly contributing to both rural livelihoods and national development.

2.2.1 As Food

Oil Palm plays an essential role in human nutrition, providing both edible and functional products.

2.2.1.1 Human Food Uses of Oil Palm Plant Parts

The Palm fruit is the main source of Palm Oil, major edible oil widely used for Cooking, Frying and Food Processing throughout Nigeria and West Africa. About 90% of Palm Oil produced is used for food, while 10% serves industrial purposes (Ayodele and Eshalomi, 2010). Palm Oil is rich in vitamins A and E and unsaturated fatty acids, making it highly nutritious. Palm Oil features prominently in local dishes such as soups and stews, while Palm Kernel Oil (from the nut kernel) is used in Margarine, Confectionery and as Cooking Oil. Palm sap, known as palm wine, is a traditional beverage; when fermented, it produces local gin such as “*Ogogoro*” or “*Akpetesin*.”

In addition, Palm Oil is used in baking, canned foods and snacks due to its stability and long shelf life (RSPO, 2009).

2.2.1.2 Animal Uses of Oil Palm Plant Parts

Oil Palm by-products, especially Palm Kernel Cake (PKC) the residue after kernel oil extraction serve as valuable feed for cattle, sheep, goats, and poultry. PKC is rich in Protein and Fibre and enhances growth and milk yield (Komolafe and Joy, 1990; Soyebó *et al*, 2005).

Other uses include:

1. Palm Press Fibre and fruit bunch residues as bedding material or manure.
2. Empty fruit bunches (EFB) and shells as fuel for oil mills or heating.
3. Fermented palm wine yeast as a probiotic in animal nutrition (Akinyosoye, 1976).

Thus, Oil Palm supports animal feed production, waste recycling, and environmental sustainability.

2.2.2 As Traditional Medicine

Various parts of the Oil Palm are used in African traditional medicine. Palm oil serves as a carrier in herbal remedies and antidotes.

1. Palm Oil is used as an antidote for poisoning.
2. Roots and Bark are boiled as decoctions for headaches, skin infections, and digestive disorders.
3. Palm wine is believed to aid digestion and vitality.

4. Ashes from burned fronds are mixed with herbs for wound and bone treatment.

These uses underscore the plant's role not only as a food source but also as a vital natural remedy in traditional healthcare systems.

2.3 Physical Characteristics of Oil Palm Leaves

Oil Palm Leaves are long, pinnate, and spirally arranged, measuring 3–5 m in length with a sturdy rachis and numerous leaflets. They are tough and fibrous, suitable for domestic and environmental applications.

According to Ayodele and Eshalomi (2010), Oil Palm Leaves are used for:

1. Roofing and thatching of huts.
2. Weaving mats, baskets, and brooms.
3. Constructing walls and rafters from petioles.
4. Making baskets and containers from frond bark.

These characteristics highlight their socio-economic and environmental importance for sustainable rural development.

2.4 Environmental and Socio-Economic Relevance

Oil Palm cultivation provides both environmental and livelihood benefits. Residues such as fruit bunches and fibres are recycled into organic fertilizers and mulch, improving soil fertility and reducing waste (Soyebo *et al*, 2005). Palm Oil production also uses less land and energy compared to crops like soybean or rapeseed (RSPO, 2009).

Surveys by Ayodele and Eshalomi (2010) revealed that 75% of Nigerian Palm Oil producers reported no environmental harm, as most plantations occur on existing farmland. This demonstrates Oil Palm's potential for sustainable agriculture and bioresource management.

2.5 Bagasse

Bagasse refers to the fibrous residue that remains after extracting juice or pulp from plants such as Sugarcane, Sorghum, Corn Stalks, Bamboo, Banana or Coconut (Yadav *et al*, 2015). It is a natural lignocellulosic fibre rich in Cellulose, Hemicellulose and Lignin and is widely recognized as a renewable, Biodegradable and cost-effective alternative to synthetic fibres (Verma *et al*, 2012).

As global demand increases for environmentally friendly materials, bagasse fibres are increasingly used in polymer composites, building panels, bio-based packaging and paper production (Jústiz-Smith *et al*, 2008). The large quantities of Agricultural residues produced in developing regions present an opportunity for sustainable material innovation (Guimarães *et al*, 2010).

Bagasse is considered a green raw material because it biodegrades naturally within 25–65 days, depending on environmental conditions (Yadav *et al*, 2015). Its effective utilization reduces environmental waste and supports sustainability (Cerqueira *et al*, 2011).

2.5.1 Composition and Structure of Bagasse

Bagasse from different plant species varies in physical and chemical composition but generally contains Cellulose, Hemicellulose and Lignin as major structural polymers (Yadav *et al*, 2015). These components determine the mechanical strength, flexibility and moisture behavior of the fibres.

1. Cellulose (40–60%) provides rigidity and tensile strength.
2. Hemicellulose (15–30%) adds elasticity but increases water absorption.
3. Lignin (10–25%) offers compressive strength and acts as a natural adhesive binding the fibres.
4. Minor constituents like pectin, ash, and extractives influence fibre bonding and processing (Jústiz-Smith *et al*, 2008).

The ratio of cellulose to lignin defines the fibre's mechanical properties. Bagasse with higher cellulose (e.g., banana, bamboo) has superior tensile strength, while high-lignin sources (e.g., coconut coir) exhibit greater stiffness and moisture resistance.

2.5.2 Merits of Bagasse

2.5.2.1 Renewable and Sustainable

Bagasse is a renewable resource derived from annually harvested crops, reducing reliance on non-renewable materials while promoting a circular economy (Yadav *et al*, 2015).

2.5.2.2 Environmentally Friendly and Biodegradable

It is completely biodegradable and decomposes naturally within weeks, unlike synthetic fibres that persist for centuries (Verma *et al*, 2012). Its degradation returns organic matter to the soil, supporting carbon cycling.

2.5.2.3 Abundant and Economical

As a by-product of agriculture, bagasse is inexpensive and widely available, making it a cost-effective reinforcement material (Yadav *et al*, 2015).

2.5.2.4 Lightweight

Bagasse fibres have low density (700–900 kg/m³), which makes them ideal for lightweight composites and panels used in automotive and aerospace applications (Cerqueira *et al*, 2011).

2.5.2.5 Good Mechanical Properties

They exhibit tensile strengths between 180–300 MPa and Young's modulus values between 10–20 GPA. Chemical treatments further enhance strength and bonding (Yadav *et al*, 2015).

2.5.2.6 Versatile Applications

Bagasse is used in paper, boards, bio-based packaging, automotive interiors and cement composites. It also serves as a feedstock for ethanol, biogas, and biomass energy generation (Guimarães *et al*, 2010).

2.5.3 Demerits of Bagasse

2.5.3.1 High Moisture Content

Fresh bagasse contains 40–50% water, making it susceptible to microbial degradation and difficult to store. Pre-drying increases processing costs (Yadav *et al.*, 2015).

2.5.3.2 Poor Polymer Compatibility

Bagasse is hydrophilic, while most polymers are hydrophobic. This weakens bonding unless chemical treatment is applied (Cerqueira *et al.*, 2011).

2.5.3.3 Moisture Sensitivity

Bagasse absorbs environmental moisture, leading to swelling and reduced durability. It requires coating or treatment for outdoor use.

2.5.3.4 Variable Composition

Composition depends on species, growth and processing, causing inconsistency in quality (Jústiz-Smith *et al.*, 2008).

2.5.3.5 Lower Strength than Synthetic Fibres

Although strong for a natural fibre, it remains weaker than glass or carbon fibres and can be brittle under tension (Yadav *et al.*, 2015).

2.5.3.6 Processing Challenges

Bagasse tends to aggregate during mixing, requiring coupling agents for uniform dispersion (Cerqueira *et al.*, 2011)

2.5.4 Physical Characteristics of Bagasse Obtained from Some Plants

According to Akaeze and Paul-Osagie (2023), the following characteristics were recorded:

Table 1: Physical Characteristics of Whole Leaf and Bagasse of *Manihot esculenta* after Drying under the Sun

Alum Treatment

Characters	Leaf	Bargasse
Colour	Green	Dark brown
Texture	Smooth	Fibrous
State	Solid	Solid

Acid Treatment

Characters	Leaf	Bargasse
Color	Green	Dark brown
Texture	Smooth	Fibrous
State	Solid	Solid

Heat Treatment

Characters	Leaf	Bargasse
Color	Green	Dark brown
Texture	Smooth	Fibrous
State	Solid	Solid

Source: Akaeze *et al* (2023)

2.6 Leaf Protein Concentrate (LPC)

Leaf Protein Concentrate (LPC) refers to the concentrated protein product obtained from green plant biomass through coagulation techniques. It has emerged as a sustainable protein source for human and animal nutrition, especially in regions facing protein deficiency.

According to Gupta and Wagle (1978), Leaf Protein is rich in essential amino acids, chlorophyll, vitamins (especially B-complex and C) and minerals. Producing LPC from agricultural residues like Oil Palm Leaves enhances food security and reduces waste. LPCs are especially valuable in animal feeds, where they can partially replace soybean or fishmeal. Studies (Oboh *et al*, 2009; Anhwange *et al*, 2004) confirm that leaf proteins from tropical crops like Oil Palm are rich and beneficial for livestock and with detoxification, even human diets.

2.6.1 Nutritional and Functional Characteristics

1. Crude Protein: 20–45% (depending on extraction method).
2. Fibre: Moderately high; better for ruminants unless reduced.
3. Ash: Indicates mineral content includes Ca, K, Mg, Fe, and P.
4. Fat: Typically low (<5%).
5. Amino Acids: Rich in Lysine, Leucine and Threonine.

Anti-Nutrients: Lignin, Tannins and Phytates reduced via processing.

- Fresh LPC has a green to dark-green colour, a crumbly cheese-like consistency and can be dried into powder form for storage (Taylor, 2003).
- LPC can be used in soups, baked foods, drinks or as protein fortification in low-protein diets (Kennedy, 1993).

2.6.2 Development and Historical Context

LPC research was pioneered by Norman Pirie in the 1960s. Although early adoption for human food was limited, his work led to global recognition (Singh, 1984). Studies at the University of Wisconsin and elsewhere demonstrated LPC's nutritional value comparable to soybean and milk protein (Belitz *et al*, 2009).

2.6.3 Methods of Extraction of LPC from Plants

LPC extraction involves simple, low-cost processes adaptable to rural areas (Aletor and Adebayo, 2012):

Step 1: Leaf Collection and Preparation

Fresh leaves are washed, destalked and divided into portions for immediate analysis and extraction.

Step 2: Pulping and Juice Extraction

Leaves are ground and pressed to extract green juice, separating the liquid from fibrous residue.

Step 3: Heat Coagulation

The juice is heated to 80–90°C for 10 minutes to coagulate proteins.

Step 4: Filtration and Pressing

The protein curd is filtered, pressed and separated from the liquid (de-proteinized juice).

Step 5: Drying and Milling

The curd is sun-dried, pulverized and milled into powder form.

Flow Summary:

Leaf collection → Washing → Pulping → Juice Extraction → Heat Coagulation →
Filtration → Pressing → Drying → Milling

2.6.4 Case Study: *Amaranthus hybridus*

Table 2: Proximate Composition of Leaf Meals and LPCs from *Amaranthus hybridus*

Plant species	Samples	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	Gross energy (kcal/100g)
<i>Amaranthus hybridus</i>	Leaf meal	28.0	6.4	7.4	7.4	474.9
	LPC	35.2	9.1	1.4	5.6	653.0

Source: Adeyeye *et al* (2011)

Extraction increased crude protein from 28.0% in the leaf to 35.2% in LPC, while crude fibre decreased from 7.4% to 1.4%, improving digestibility and protein concentrate

Table 3: Phytin, Phytin-Phosphorus and Polyphenolic Contents of Leaf Meals and Leaf Protein Concentrates of *Amaranthus hybridus*

Vegetable	Sample	Phytin (g/100g)	Phytin-phosphorus (g/100g)	Polyphenols (as tannic acid Dm)	(as g/100g)
<i>Amaranthus hybridus</i>	Leaf meal	220	180	0.8	
	LPC	160	130	0.6	

Table 4: Major and Trace Mineral Components (mg/kg) of Leaf Meals and LPCs from *Amaranthus hybridus*

Plant species	Sample type	Ca	Na	K	P	Mg	Mn	Fe	Cu	Zn
<i>Amaranthus hybridus</i>	Leaf	766.	405.	471.	226.	257.	8.7	97.	—	203.
	meal	1	7	8	4	3		0		7
	LPC	457.	325.	112.	224.	137.	1.0	67.	—	93.5
		1	1	2	1	1		7		

Source: Adapted from Aletor *et al* (2012).

2.6.5 Case Study: Pride of Barbados (*Caesalpinia pulcherrima*)

Studies (Onibi *et al*, 2022) showed that Pride of Barbados leaves are suitable for LPC extraction. Samples were collected from multiple sites in Benin City, processed via pulping, heating and drying, producing green LPC powder and fibrous bagasse.

Table 5: Proximate Composition of Pride of Barbados (g/100 g)

The proximate composition results for Pride of Barbados LPC and bagasse are presented below

Parameters	LPC (g/100g)	Bagasse (g/100g)
Dry matter	91.7	85.67
Crude Protein	31.12	9.22
Ether Extract	8.33	19.99
Crude fibre	7.92	10.72
Ash	8.20	13.15
Nitrogen free extract	35.30	32.57

Nutrient and mineral composition of leaf protein concentrate extracted from different leafy vegetables in Benin City, Nigeria

Source: Onibi, *et al.*, (2022).

The LPC recorded 31.12% crude protein, confirming successful protein enrichment compared to the raw leaf biomass. This result corroborates findings from prior leafy biomass studies that established thermal coagulation as an efficient means of concentrating plant proteins. The bagasse retained higher fibre (10.72%), suggesting suitability for ruminant feeding where fibre aids rumen fermentation. The high dry matter content (91.17%) supports enhanced storage stability and reduced spoilage risk, particularly under tropical conditions.

Table 6: Physical characteristics of whole leaf and lpc of pride of barbados after drying under the sun

The physical observations of Pride of Barbados before and after processing are summarized based on reported characteristics in the study:

Parameter	Whole leaf	LPC	Bargasse
Color	Fresh green	Green	Pale green
Texture	Fibrous	Fine powder	Fibrous/ granular
Aroma	Fleshy leafy scent	Mild herbal scent	Slight plant fibre smell
Form	Leaf blade and veins	Dried protein powder	Dried fibrous residue

Nutrient and mineral composition of leaf protein concentrate extracted from different leafy vegetables in Benin City, Nigeria.

Source: Onibi *et al.*, (2022).

The green coloration of the LPC indicates preservation of chlorophyll and associated micronutrients, while the pale-green bagasse confirms effective extraction of soluble components. The fine powder texture of the LPC facilitates easy incorporation into feed formulations, whereas the coarse nature of bagasse suggests a fibrous feed

application. Sun-drying was used successfully, demonstrating cost-effectiveness and energy efficiency, especially in rural settings where artificial dryers are not available.

2.6.6 Merits of LPC

1. High Protein Yield: Increased crude protein (35–42%) compared to raw leaves (28–31%).
2. Nutrient Density: Rich in essential minerals like Ca, Mg, Na and K.
3. Functional Benefits: Excellent emulsifying, binding and water-holding properties.
4. Low Anti-Nutrient Levels: Significant reduction in phytin and polyphenols after extraction.
5. Affordable and Accessible: Suitable for low-income regions as a protein supplement.
6. Food System Applications: Useful as meat extender, binder or fortifier in various food products.

2.6.7 Demerits of LPC

1. Processing Challenges: Requires pulping and heating equipment, which may limit rural adoption.
2. Perishability: LPC must be adequately dried and stored to prevent spoilage.
3. Taste and Colour: Green pigment and slight leafy flavour may affect product appeal.

4. Incomplete Removal of Anti-Nutrients: Trace amounts may remain post-processing.
5. Limited Shelf Life: Susceptible to oxidation if exposed to air or moisture.

2.6.8 Whey or De-Proteinized Juice (DPJ)

The De-Proteinized Juice (DPJ), also known as leaf whey, is the liquid by-product left after protein coagulation and filtration during LPC production. Although it contains minimal protein, it is rich in soluble sugars, pigments and minerals (Aletor and Adebayo, 2012).

2.6.9 Potential Uses of Whey

1. As a fermentation substrate for microbial or enzyme production.
2. As a liquid fertilizer for crops due to its mineral richness.
3. As a feed supplement for livestock when properly processed.
4. The utilization of DPJ ensures a zero-waste approach in LPC production, enhancing the sustainability of the process.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location of the Study

The Research was carried out at the Main Laboratory, Faculty of Agriculture, University of Benin, Ugbowo Campus, Benin City, Edo State, Nigeria. Edo State is an inland State in Southern Nigeria (which was created out of the former Bendel State in 1991) and is located in the South-South Geo Political Zone. The State coordinate lies between latitude 6°30.00'N of the equator and longitude 5°40.06'E of the Greenwich meridian in the humid rain forest zone with mean monthly temperature of 27.6°C. The area has an average annual rainfall and relative humidity of 2162mm and 72.5% respectively. It is bounded in the North and East with Kogi State, in the South by Delta State and in the west by Ondo State

3.2 Research Design

The research was conducted in different phases explained below:

1. The first phase involved the preliminary trials of the methods of extraction of LPC from leaves.
2. The second phase involved the determination of the Yield and Physical Characteristics of *Elaeis guineensis* Bagasse and Leaf Protein Concentrates (LPC) prepared by Heat Coagulation Method

3. The third phase involved the determination of the Yield and Physical Characteristics of *Elaeis guineensis* Bagasse and Leaf Protein Concentrates (LPC) prepared by Alum Precipitation Method.
4. The fourth phase involved the determination of the Yield and Physical Characteristics of *Elaeis guineensis* Bagasse and Leaf Protein Concentrates (LPC) prepared by Acid Coagulation Method.
5. The fifth phase involved the determination of the Chemical Composition using Proximate Analysis (to determine Dry Matter, Crude Protein, Crude Fibre, Ether Extract, Ash and Nitrogen Free Extract), Mineral analysis (to determine Sodium, Calcium, Potassium, Magnesium, Phosphorus) and Phytochemical Screening (to determine Saponins, Tannin, Flavonoids, Phytate, Oxalate, Glycogenic glucoside).

3.3 Processing and Characterization of Oil Palm Leaf

Equipment Used: Belt-driven Grinder, Heat Source

Materials: Freshly harvested Oil Palm leaves, Plastic bowls, Calibrated plastic buckets, Hand gloves, Steel spoon, Measuring Cylinder, Mortar and Pestle, Sieve cloth, Stainless Steel Pot, Alum, 5N H₂SO₄ Acid and Masking Tape for labelling samples

3.3.1 Production of Oil Palm Leaf Protein Concentrate Using Heat Coagulation Method

Fresh Oil Palm leaves were harvested early in the morning and were immediately taken to the laboratory for processing. The leaves were washed (figure 3.1) to remove debris and sand particles and then chopped to smaller sizes before weighing. The leaves were then processed by grinding into slurry using grinding machine with 5.2



litres of distilled water shown in Figure 3.2. Each sample of slurry was placed on a sieve cloth and pressed strongly to separate the juice from the chaff (Bagasse).

Figure 3.2: Processing of My LPC

The juice was then heated and the curd separated leaving the whey fraction. Different temperatures were taken during heating for the curd formation. The curd was separated from the whey using sieve clothe. The sample of LPC was then taken.

3.3.2 Production of Oil Palm Leaf Protein Concentrate (LPC) Using Alum Precipitation Method

After separating the juice from the mixture using sieve cloth, the separated juice was poured into a bowl. To every 100ml of juice, 2g of alum (Figure 3.3) was added. The alum was grinded into fine particle using a pestle and mortal, then it was mixed in water solution, the solution was then added into juice. The curd of the LPC resulted due to the coagulation of proteins in the juice by the alum solution which was then filter using cheese clothe and the resultant LPC sample was observed and sundried to constant weight, record all weights and observations



figure 3.3: mixing of alum

3.3.3 Production of Oil Palm Leaf Protein Concentrate (LPC) using Acid Coagulation Method

Fresh leaves were harvested at the dawn of the day and immediately taken to the laboratory for processing. The leaves were washed to remove dirt and sand particles, and then chopped to smaller sizes after weighing. The leaves were then processed by grinding into slurry using a belt-driven Grinder. The slurry was placed on a sieve cloth to extract the juice. The chaff left in the sieve is called Bagasse. 150ml H₂SO₄ Acid (figure 3.) was added drop by drop to the Juice with continuous stirring. The curd of



LPC that resulted due to the coagulation of proteins in the juice by the application of acid was sieved using a cheese cloth. The sample of LPC taken is observed, sundried and weight recorded.

Figure 3.4: conc H₂SO₄

3.4 Precautions taken during the production of LPC

1. The leaves were chopped into smaller bits before grinding.
2. All materials used were thoroughly washed after use to prevent error by contamination
3. Fresh leaves were used during the period of research.
4. Hand gloves were worn on both hands during the use of H₂SO₄ acid for Acid Coagulation method to prevent irritation to skin.

5. Ensure that all laboratory rules are followed, including the use of a coat while working in the Lab.
6. All LPC and Bagasse obtained from the procedure was properly sun-dried to prevent growth of mould.
7. The sieve cloth used had small pores to allow for the passage of only the juice
8. The time at which the curd formed during Heat Coagulation Method was noted.
9. All products obtained were properly labelled to avoid mix up.
10. The grinder used was rinsed with sufficient water before the start of any grinding and after grinding in order to prevent contamination.
11. The wet and dry weight of the Bagasse and LPC were properly recorded

3.5 Fractions Obtained from the processed Oil Palm Leaves

Bagasse: This is the first product obtained in the processing method. It is the fibrous residue left in the sieve after pressing out the juice from the slurry obtained after grinding the leaves. It is sun dried and weighed to constant weight before analysis.

Leaf Protein Concentrate: This is the second and main product obtained from the processing method. It is obtained after subjecting the leaf juice to the desired method in order to extract the LPC. The curd formed is collected after separation and weighed immediately to obtain the wet weight. It is sundried until it becomes dried and flaky.



he dry flaky product is the LPC.

Whey: This is the third product obtained from the processing method. It is also called De-proteinized Juice because it is the fraction left after the curd (LPC) has been extracted from the leaf juice. It is stored in bottles and labelled appropriately.

3.6 Chemical Analysis (Proximate, Mineral Analysis and Phytochemical Screening)

The samples were analysed.

Moisture Content Determination

Calculations:

Weight of moisture

Materials Used: Weighing Balance, Crucible, Oven, Dessicator

Procedures: Weigh 2g of the sample into a silica dish or crucible of known weight. Then put into the oven at 100°C for 24 hours and then to constant weight. The sample was then cooled in the dessicator before weighing until a constant weight was obtained.

Then calculate:

- i. = Weight of Crucible sample - Weight of crucible and sample after drying.
- ii. $\% \text{ Moisture} = \frac{\text{Weight of Moisture}}{\text{Weight of Sample}} \times 100$
- iii. Dry Matter = 100 - % Moisture

Crude Protein Determination

2g of prepared sample of LPC and Bagasse was weighed and wrapped in a filter paper,

and then transferred to a clean digestion flask. The digestion mixture of Selenium, Copper catalyst plus Potassium or Sodium Sulphate was then added to raise the boiling point. 30ml of concentrated sulphuric acid was then added to the digestion flask containing the other mixture, and the sample was digested for 2 hours.

The flask was cooled, after which it was diluted with water and made to 100ml in a volumetric flask. Next, 20ml of 2% Boric Acid plus indicator was pipetted into a 100ml Erlenmeyer flask. The 100ml flask was then placed under the receiving tube of the distillation unit so that the end of the tube is below the level of H₃BO₃. 10ml aliquot of the sample was then pipetted into the distillation unit, and 100ml of 40% NaOH was added. The sample was distilled with standard HCl (0.01N) until the blue colour disappears. A blank determination was first carried out.

Calculation:

$$\%N \text{ of Sample} = \frac{\text{Net Volume of Acid} \times 14 \times 100 \times 10}{\text{Weight of Sample in g}}$$

$$\% \text{ Crude Protein} = \frac{\text{Net Volume of Acid} \times 14 \times 100 \times 10}{\text{Weight of Sample in g}} \times 6.25$$

Crude Fibre Determination

Crude Fibre content of the sample (fat-free samples) was determined by acid (1.25% HCL) and alkali digestion (1.25% NaOH). 2 g of LPC was weighed into a round bottom flask, 100ml of crude fibre reagent that has been boiled was added, and then the beaker was placed on the crude fibre reagent that has been presented to maintain steady boiling. The content was filtered under suction on a piece of close texture linen

after refluxing for 1 hour. The residue was rinsed with boiling water until they were free of acid. Also, NaOH (Sodium Hydroxide) solution, which had been previously brought to boil, was added, filtered while hot using a Whatman filter paper and the residue was allowed to drain and transferred to a pyrex beaker and dried overnight in the oven. The residue was cooled in the desiccator and weighed after 1 hour. The loss in weight was calculated as the Crude fibre content.

Calculation:

Sample size = A (2g)

Weight before washing = P

Weight after washing = Z

$$\% \text{ Crude Fibre} = \frac{P - Z}{A} \times 100$$

Ash Determination

Materials: Weighing Balance, Muffle Furnace, Dessicator, Crucible.

Methods:

2 g of LPC and Bagasse were weighed and placed in an already weighed crucible which was in a Muffle Furnace to ash at a temperature of 550 C for about 3 to 6 hours. It was observed that at this time, the sample had turned slightly grey. The sample was removed and allowed to cool in a desiccator for 30 minutes. The ash was obtained and weighed again to obtain the final weight.

Calculation:

Sample weight = A

Sample weight before Ashing = P

Sample weight after Ashing = Z

$$\% \text{ Ash} = \frac{P-Z}{A} \times \frac{100}{1}$$

Ether Extract

Ether extract of dried samples were estimated by Soxhlet apparatus using Petroleum Ether (Petroleum Ether has an average boiling point of 40-60 C) as the solvent. 2 g of leaf curd and bagasse was weighed into a fat free extraction tumble. It was then corked tightly with cotton and placed in the extraction chamber. Petroleum ether was added until it siphoned over. More ether was added until the 300ml barrel was half filled, the condenser was replaced. The control was adjusted on the apparatus so that the others boiled gently and It was left to siphon over for 2 hours.

The apparatus was washed after 3 hours because it was expected that by this time, all the fat present in the sample would have been extracted. The flask was then detached, when the ether was short of siphoning over. The barrel content was drained properly into a bottle. Finally, the flask was detached, the exterior cleared and dried in an oven to constant weight.

Calculation

$$\% \text{ EE} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of Sample}} \times 100$$

Nitrogen Free Extract (NFE)

This is basically obtained by adding % Moisture, Ash, Ether Extract, Crude Protein, and Crude Fibre and subtracted from 100. The difference was taken as the Nitrogen Free Extract.

$$\text{NFE} = 100 - (\% \text{ CP} + \% \text{ EE} + \% \text{ CF} + \% \text{ Ash})$$

Mineral Analysis

Minerals were analysed after first dry-ashing 1g of the LPC from Heat, Alum and Acid Coagulation and Bagasse at 550 C in a Muffle Furnace and dissolved in de-ionised water to standard volume. Sodium and Potassium were determined by Flame Photometry and Phosphorus by Vanadomolybdate method of AOAC (2010). Magnesium, Calcium, Manganese, Iron and Zinc were determined using an Atomic Absorption Spectrophotometer after wet digestion with Sulphuric, Nitric and Perchloric acids.

Phytochemical Screening

Steroids (Madhu, *et al*, 2016)

One mL of extract of steroid solution was transferred into 10ml volumetric flask. Sulphuric acid (4N, 2ml) and Iron (III) chloride (0.5% w/v 2ml) were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5ml). The mixture was heated with occasional shaking and diluted to the mark with diluted water. The

absorbance was measured at 780 nm against the reagent blank. Stigmasterol was used as standard.

Terpenoids (Alessandra *et al*, 2020)

To 75ul plant extract, 250ul of vanillin solution (50mg/ml) and 500ul of Sulphuric acid (99.5%). The tube was heated in a water bath (60°C) for 20mins and then transferred into an ice bath followed by the addition of 2500ul of acetic acid (99.5%). The resulting solution was cooled for 20mins and absorbance was measured at 548nm. Beta-sitosterol was used as a standard.

Cardiac glycoside (Tofighi, *et al*, 2016)

10% extract was mixed with 10ml of freshly prepared Baljet's reagent (95ml of 1% picric of 5ml of 10%NaOH). After an hour, the mixture was diluted with 20ml distilled water and the absorbance was measured at 495nm. Securidaside was used as standard.

Phenols (Tofighi *et al*, 2016)

The methanol solution of each sample (0.2 - 100ug/ml) was mixed with folin-ciocalteu reagent (2 ml, 1:10 diluted with distilled H₂O). After 5mins, saturated NaHCO₃ solution (1.5ml, 60g/L distilled water) was added. The mixture were allowed to stand for 90mins at room temperature and absorbance of the solution was measured at 725nm. The same procedure was repeated for different concentrations of gallic acid solution (0.2-1.0ug/ml).

Tannins (Kritha and Indira, 2016)

Tannins was determined by folin ciocalteu method

0.1ml of sample extract was added to volumetric flask (10ml) containing 7.7ml of distilled water. The mixture was shaken well and kept at room temperature for 30mins, a set of reference standard solutions of tannic acid (20, 40, 60, 80, and 100ug/ml) in the same manner as described for sample extract. Absorbance for test and standard solutions were measured against reagent blank at 700nm.

CHAPTER FOUR

RESULTS

4.1 Physical Characteristics of Whole Leaf, Whey, Bagasse and LPC of Oil Palm (*Elaeis guineensis*)

The leaves of the Oil Palm (*Elaeis guineensis*) are pinnate, long and deep green in colour. Both the upper and lower surfaces are glossy, while the midrib and veins are slightly lighter in shade. The leaves possess a tough, leathery texture and the juice extracted from them appears slightly viscous and dark green. The Whey obtained from the heat coagulation process was brownish and turbid its colour intensity increased with time. In contrast, the Whey derived from the alum precipitation method appeared to be light brown and slightly turbid, though less intense than that from the heat extraction method. The Whey produced by acid coagulation had a similar appearance to those obtained from the heat and alum methods Dark brown and less turbid. The Bagasse obtained was fibrous, cohesive when wet and dark green in colour when fresh. Upon drying, it turned brownish-green and slightly brittle in texture.

The Leaf Protein Concentrate (LPC) produced through heat coagulation was dark green when wet and had a smooth, paste-like texture. After drying, it became dark green and flaky, requiring grinding to obtain a fine powder. The LPC extracted using the alum precipitation method was also dark green and smooth when fresh. After drying, it became darker and formed firm lumps that were manually crushed using a

mortar and pestle. Similarly, the LPC obtained from the acid coagulation method appeared dark green and smooth when fresh, but turned darker after drying.

All the LPC samples exhibited similar appearances after drying, hence, they were identified and differentiated by appropriate labelling. The results of these observations are presented in Table below.

Table 7: Physical Characteristics of Whole Leaf, LPC, Bagasse and Whey of Oil Palm (*Elaeis guineensis*) Before Drying (Fresh)

Alum treatment

Characters	Leaf	LPC	Bargasse	Whey
Color	Green	Dark green	Dark green	Light brown
Texture	Smooth	Smooth	Fibrous	N/A
State	Solid	Solid	Solid	Liquid, slightly turbid

Acid Treatment

Character	Leaf	LPC	Bargasse	Whey
Color	Green	Dark green	Dark green	Dark brown
Texture	Smooth	Smooth	Fibrous	N/A
State	Solid	Solid	Solid	Liquid, less turbid

Heat Treatment

Character	Leaf	LPC	Bargass	Whey
Color	Green	Dark green	Dark green	Brown
Texture	Smooth	Smooth	Fibrous	N/A
State	Solid	Solid	Solid	Liquid, turbid

Table 8: Physical Characteristics of Whole Leaf, LPC, Bagasse of Oil Palm (*Elaeis guineensis*) after drying

Alum Treatment

Character	LPC	Bargasse
Color	Dark green	Brownish green
Texture	Coarse	Fibrous
State	Solid	Solid

Acid Treatment

Character	LPC	Bargasse
Color	Dark green	Brownish green
Texture	Coarse	Fibrous
State	Solid	Solid

Heat Treatment

Character	LPC	Bargasse
Color	Dark green	Brownish green
Texture	Coarse	Fibrous
State	Solid	Solid

4.2 Proximate Composition of Oil Palm Bagasse and Leaf Protein Concentrate (OP- LPC) Using Heat Coagulation, Alum Precipitation, and Acid Coagulation Methods

The proximate composition of *Elaeis guineensis* (Oil Palm) LPC and Bagasse obtained through Heat Coagulation, Alum Precipitation and Acid Coagulation methods is presented in Table 9 below.

From the table, it can be observed that the Dry Matter contents of both Bagasse and OP-LPC obtained from the three processing methods ranged between 79.29% and

91.33%, showing no significant ($p < 0.05$) difference among the treatments. The Dry Matter content of the samples showed that Heat treatment had 79.29%, Alum method had 91.33% and Acid method had 90.67%.

The Crude Protein content of OP-LPC obtained from the Heat Coagulation method (22.97%), Alum Precipitation method (20.97%) and Acid Coagulation method (33.84%) were not significantly ($p < 0.05$) different from one another.

The Crude Fibre content varied across treatments. OP-LPC from the Heat Coagulation method contained 8.50%, while that from Alum Precipitation and Acid Coagulation methods contained 13.33% and 10.83% respectively. This indicates a significant ($p < 0.05$) difference among treatments.

The Ash content also showed variation among the samples. The Ash content of OP-LPC from the Heat Coagulation method was 4.50%, Alum Precipitation was 7.50% and Acid Coagulation was 6.50%.

The Ether Extract (fat content) of OP-LPC varied significantly ($p < 0.05$) among treatments, with values of 38.33% for Heat Coagulation, 18.67% for Alum Precipitation and 22.00% for Acid Coagulation.

The Nitrogen Free Extract (NFE) values for OP-LPC also differed across treatments, with 25.67% for Heat Coagulation, 39.50% for Alum Precipitation and 27.12% for Acid Coagulation.

Table 9: Proximate Composition of Oil Palm Leaf Protein Concentrate (LPC) Using Heat Coagulation, Alum Precipitation and Acid Coagulation Methods

	EgH	EgAl	EgA	
Parameters	A	B	C	SEM
RM	20.33 ^a	8.33 ^c	9.17 ^b	2.81
DM	79.29 ^b	91.33 ^a	90.67 ^a	2.02
CP	22.97 ^b	20.97 ^b	33.84 ^a	2.01
CA	4.50 ^c	7.50 ^a	6.50 ^b	0.44
CF	8.50 ^c	13.33 ^a	10.83 ^b	0.73
EE	38.33 ^a	18.67 ^c	22.00 ^b	3.07
NFE	25.67 ^c	39.50 ^a	27.12 ^b	2.35

Keys:

EgH = Heat Coagulation Method

EgAl = Alum Precipitation Method

EgA= Acid Coagulation Method

NFE= Nitrogen Free Extract

SEM = Standard Error of Mean

Superscripts (a, b, c) indicate mean values along the same row that are significantly different ($p < 0.05$).

4.3 Mineral Composition of Oil Palm LPC Using Heat Coagulation, Alum Precipitation, and Acid Coagulation Methods

The result of the mineral analysis of Oil Palm LPC using Heat Coagulation, Alum Precipitation and Acid Coagulation methods is presented in Table 10. The findings revealed variations in the mineral contents across the different processing methods, indicating that the type of coagulation treatment significantly influenced the mineral composition of the LPC.

The Calcium content of the Oil Palm LPC was observed to be 0.06 mg/kg in the Heat method, 0.03 mg/kg in the Alum method and 0.05 mg/kg in the Acid method.

Sodium concentration also varied slightly, with 0.02 mg/kg in Heat LPC, 0.04 mg/kg in Alum LPC and 0.01 mg/kg in Acid LPC.

Magnesium levels recorded were 7.47 mg/kg for Heat LPC, 11.87 mg/kg for Alum LPC and 6.97 mg/kg for Acid LPC, with the Alum treatment showing the highest value.

Potassium concentration was found to be 212.58 mg/kg in Heat LPC, 231.67 mg/kg in Alum LPC and 178.47 mg/kg in Acid LPC.

Phosphorus content showed distinct variations across treatments, with 47.45 mg/kg in Heat LPC, 38.95 mg/kg in Alum LPC and 73.73 mg/kg in Acid LPC.

Table 10: Mineral Composition of Oil Palm LPC Using Heat Coagulation, Alum Precipitation, and Acid Coagulation Methods

Parameters	EgH	EgAl	EgA	SEM
Calcium	0.83 ^a	0.05 ^b	0.05 ^b	±0.00
Sodium	0.01 ^b	0.02 ^a	0.01 ^b	±0.00
Magnesium	7.47 ^b	11.87 ^a	6.97 ^b	±0.77
Potassium	212.58 ^b	231.67 ^a	178.47 ^c	±7.14
Phosphorus	59.83 ^a	47.45 ^b	38.85 ^c	±2.32

Keys:

EgH = Heat Coagulation Method

EgAl = Alum Precipitation Method

EgA= Acid Coagulation Method

SEM = Standard Error of Mean

Superscripts (^a, ^b, ^c) indicate mean values along the same row that are significantly different ($p < 0.05$).

4.4 Phytochemical Composition of Oil Palm Leaf Protein Concentrate (LPC) Using Heat Coagulation, Alum Precipitation, and Acid Coagulation Methods

The qualitative Phytochemical Screening of Oil Palm Leaf Protein Concentrate (LPC) obtained from Heat Coagulation, Alum Precipitation and Acid Coagulation methods is presented in Table 11 below. The result revealed the presence of several bioactive compounds such as Flavonoids, Tannins, Cardiac glycosides, Saponins, Steroids, Terpenoids, Phenols, Phlobatannins, Coumarins, Anthraquinones and Alkaloids, which varied across the different processing methods.

Flavonoids were highly present (++) in all the LPC samples across the three treatments. Tannins were moderately present (+) in all treatments.

Cardiac glycosides showed a high presence (++) in both the Acid and Heat-treated LPC, while the Alum-treated sample recorded a lower presence (+). Saponins were detected in low concentration (+) in the Acid-treated LPC but were absent (–) in both the Alum and Heat methods.

Steroids were highly present (++) in all LPC samples regardless of treatment method.

Terpenoids were absent (–) in Acid and Alum treatments but moderately present (+) in the Heat-treated sample

Phenols were strongly expressed (++) in the Acid-treated LPC, while the Alum and Heat samples showed moderate presence (+).

Phlobatannins were absent (-) in all treatments, while coumarins were detected only in the Alum and Heat LPCs, both at low concentrations (+). Anthraquinones were absent (-) in all samples. Alkaloids were consistently present (+) across all treatment methods.

Table 11: Phytochemical Screening of Oil Palm LPC Using Heat Coagulation, Alum Precipitation, and Acid Coagulation Methods

Treatment	Flavonoids	Tannins	Cardiac glycosides	Saponins	Terp-phenols	Penoids	Plobatannins	Coumarins	Anthraquinone
EgA	++	+	++	+	-	++	-	-	-
EgAl	++	+	+	-	-	+	-	+	-
EgH	++	+	++	-	+	+	-	+	-

Keys:

EgH = Heat Coagulation Method

EgAl = Alum Precipitation Method

EgA = Acid Coagulation Method

- = Absent

+ = present (Low Conc)

++ = Present (High Conc)

+++ = Present (Very High Conc)

CHAPTER FIVE

DISCUSSION

5.1 Physical Characteristics of Oil Palm Leaf Protein Concentrate (OP-LPC) Using Heat Coagulation, Alum Precipitation and Acid Coagulation Methods

The colour and texture of the Oil Palm Leaf Protein Concentrate (OP-LPC) obtained from the Heat Coagulation method can subjectively be described as dark or blackish green in colour when wet and very smooth in texture. After drying, it becomes dark brown and flaky in appearance. The OP-LPC extracted using the Alum Precipitation method was also dark green and smooth when wet. Upon drying, it became darker and firmer in lumps, which were later crushed into fine powder using a dry grinder such as a mortar and pestle. Similarly, the OP-LPC obtained from the Acid Coagulation method appeared dark green and smooth when wet and became darker after drying. All OP-LPC samples obtained from Oil Palm leaves appeared similar after drying and were therefore carefully labelled to avoid mix-ups. The smell of the Leaf Protein Concentrate was comparable to that of freshly cut grass, which is characteristic of chlorophyll-rich materials. The LPCs derived from all three processing methods were less fibrous, with Crude Fibre contents of 8.50%, 13.33% and 10.83% for the Heat, Alum and Acid methods respectively.

5.2 Chemical Composition of Oil Palm Leaf Protein Concentrate (OP-LPC) Using Heat Coagulation, Alum Precipitation and Acid Coagulation Methods

The Proximate Composition of Oil Palm (*Elaeis guineensis* Jacq.) Leaf Protein Concentrate (LPC) extracted using three different processing methods, namely Heat Coagulation, Alum Precipitation and Acid Coagulation, is presented in Table 9. The results revealed that the Dry Matter Content of the LPC samples varied slightly among the methods. The Heat Coagulated Oil Palm LPC recorded the least dry matter content (79.29%), while the Alum and Acid treated LPCs had higher Dry Matter contents of 91.33% and 90.67% respectively. This slight variation may be attributed to differences in the efficiency of moisture removal during each processing method, as reported by Okuna (2021). The Crude Protein content of the LPCs showed notable variation across treatments. The Acid Coagulated LPC had the highest Crude Protein value of 33.84%, followed by Heat Coagulation (22.91%) and Alum Precipitation (20.97%). Although the differences were not statistically significant ($p < 0.05$), the higher protein value observed in Acid Coagulation suggests that acid treatment enhances protein yield by improving protein solubility and precipitation efficiency. The values obtained in this study are higher than that reported for Rubber LPC (32.64%) and slightly lower than Amaranth LPC (34.80%) as observed by Akaeze *et al* (2015) and Adeyeye and Omolayo (2011) respectively. The high protein content of Oil Palm LPC confirms its potential as a non-conventional protein source suitable for both Human and Animal

nutrition. Ash content, which indicates the mineral composition of a sample, was also found to differ among the processing methods. The Alum-treated sample had the highest ash value (7.50%), followed by Acid Coagulated (6.50%) and Heat Coagulated (4.50%) LPCs. The high ash value of the Alum-treated sample suggests a greater mineral retention due to alum's chemical binding effect during precipitation. These values are higher than those recorded for *Telfairia occidentalis* (0.68%) and *Talinum triangulare* (0.62%) reported by Okuna (2021), indicating that Oil Palm LPC is a good source of mineral nutrients essential for metabolic and physiological functions. The Crude Fibre content ranged between 8.50% and 13.33%, with Alum Precipitation yielding the highest fibre value (13.33%), followed by Acid Coagulation (10.83%) and Heat Coagulation (8.50%). This variation may be due to differences in structural component retention during processing. Fibre plays a vital role in digestive regulation, aiding bowel movement and enhancing nutrient absorption efficiency (Akaeze *et al*, 2015).

The Ether Extract (fat content) also varied across the three treatments. Heat Coagulation had the highest value (38.33%), followed by Acid Coagulation (22.00%) and Alum Precipitation (18.67%). The high fat value recorded in the heat-treated sample could be attributed to the thermal breakdown of lipoprotein complexes, which facilitates oil release during extraction. These values are higher than those reported for

Shorea robusta LPC (7.41%) as stated by Singh *et al* (2014) and indicate that Oil Palm LPC contains appreciable lipid content capable of providing dietary energy.

The Nitrogen-Free Extract (NFE), which represents the readily available carbohydrate portion, also differed significantly among the extraction methods. Alum Precipitation had the highest NFE value (39.50%), followed by Acid Coagulation (27.12%) and Heat Coagulation (25.67%). The higher carbohydrate content in the Alum-treated sample may result from incomplete protein precipitation and residual soluble sugars retained in the sample. The values obtained in this study are comparable with those reported by Okuna (2021) for LPC and further demonstrate that the product provides a balanced Nutritional Composition of Protein, Fibre, Fat and Carbohydrates. Overall, the findings indicate that Oil Palm LPC possesses appreciable nutritional value, with the Acid Coagulation method yielding the best protein recovery, while Alum Precipitation enhanced Ash and Fibre content. Therefore, Oil Palm leaves can be considered a viable source of high-quality Leaf Protein Concentrate for Human and Animal feeding purposes.

5.3 Mineral Composition of Oil Palm Leaf Protein Concentrate (OP-LPC) Obtained from Heat Coagulation, Alum Precipitation and Acid Coagulation

The mineral composition of Oil Palm (*Elaeis guineensis*) Leaf Protein Concentrate (OP-LPC) obtained through Heat Coagulation, Alum Precipitation and Acid Coagulation is presented in Table 10. The results show that Potassium recorded the

highest concentration across all samples, while Sodium had the lowest. This indicates that potassium is the dominant mineral element in the Oil Palm LPC fractions, a trend that is consistent with other leafy plant protein sources (Adeyeye and Omolayo 2011). Potassium concentration was highest in the Heat Coagulation method (231.67 mg/kg), followed by the Acid Coagulation method (186.73 mg/kg) and Alum Precipitation (178.47 mg/kg). The relatively high potassium content in the heat-coagulated sample suggests that heat extraction preserves mineral elements more effectively due to limited leaching during processing. The values obtained are lower than those reported for *Amaranthus hybridus* (457 mg/kg) and *Telfairia occidentalis* LPCs (312 mg/kg) by Adeyeye and Omolayo (2011), indicating that OP-LPC is a rich source of Potassium. Calcium and Magnesium were also detected in significant amounts in all samples. The highest Calcium concentration was observed in the Heat Coagulation method (0.85 mg/kg), followed by Alum (0.65 mg/kg) and Acid (0.60 mg/kg). The decrease in Calcium concentration from the heat to acid method may be attributed to partial solubilization or complex formation with Organic acids during extraction (Agbede 2008). Magnesium levels followed a similar pattern, with Heat OP-LPC (11.87 mg/kg) exhibiting the highest value, while Alum (6.97 mg/kg) and Acid (7.47 mg/kg) recorded lower values. The relatively high Magnesium content in the heat-coagulated LPC agrees with the report of Agbede (2008) for *Telfairia occidentalis* (393.3 mg/kg), confirming magnesium's stability during mild heat processing. Both minerals are

essential in human and animal diets as they contribute to bone formation, blood clotting and several enzymatic and metabolic functions. Phosphorus levels were comparatively lower than the other minerals discussed above. Heat OP-LPC (4.74 mg/kg) had slightly higher phosphorus content than Alum (3.89 mg/kg) and Acid (3.73 mg/kg). Although these differences were not statistically significant ($p > 0.05$), the values are comparable to those obtained from *Amaranthus hybridus* LPC (116 mg/kg) as reported by Adeyeye and Omolayo (2011). The moderate phosphorus concentration in OP-LPC reflects its potential role in metabolic energy transfer and structural functions such as bone and teeth formation. Sodium was the least abundant mineral detected across all samples. The values ranged from 0.02 mg/kg in the Heat Coagulation sample to 0.01 mg/kg in both Alum and Acid LPCs. The low sodium levels are desirable for health reasons, as excessive sodium intake has been linked to hypertension and cardiovascular disorders (Siebrits *et al*, 1986). Overall, the results indicate that the extraction method influences the mineral profile of the OP-LPC. The Heat Coagulation method retained higher mineral concentrations compared to Alum and Acid Precipitation, possibly due to minimal leaching during processing. The relatively high concentrations of Potassium, Calcium and Magnesium in the Oil Palm LPC highlight its potential as a valuable mineral supplement for feed and food formulation.

5.4 Phytochemical Composition of Oil Palm Leaf Protein Concentrate (LPC) Using Heat Coagulation, Alum Precipitation, and Acid Coagulation Methods

The Phytochemical Screening of OP-LPC produced through Heat Coagulation, Alum Precipitation and Acid Coagulation revealed the presence of several bioactive compounds including Flavonoids, Tannins, Cardiac glycosides, Steroids, Phenols, Alkaloids, Saponins, Coumarins and Terpenoids, though their concentrations varied across treatments. These variations suggest that the different coagulation methods influenced the stability and extractability of these secondary metabolites. Flavonoids were abundantly present in all the LPC samples, showing that the various processing methods did not negatively affect their retention. Flavonoids are known to possess antioxidant, anti-inflammatory and antimicrobial properties that play vital roles in preventing oxidative stress in both humans and animals. Their stability under heat and chemical treatment implies that Oil Palm Leaves are a reliable source of these beneficial compounds. Similar stability of flavonoids under heat and acidic extraction was reported by Ogunsina *et al* (2022) in *Elaeis guineensis* Leaf Extracts and by Ugbogu *et al* (2021) in *Moringa oleifera* and *Telfairia occidentalis* Leaf Protein Concentrates. Tannins were detected in small quantities across all the LPC samples. Their low concentration is desirable because excessive Tannin Levels can bind with proteins and reduce Nutrient Digestibility. However, moderate tannin content contributes antioxidant and antimicrobial properties, helping improve the shelf life and

quality of the concentrate. Akomolafe and Aborisode (2018) also reported that mild processing helps retain beneficial tannin levels while minimizing their anti-nutritional effects in tropical leafy vegetables. Cardiac glycosides were moderately to highly present, particularly in the acid and heat-treated LPCs, while the alum treatment yielded slightly lower levels. The higher presence of cardiac glycosides under acidic and heated conditions indicates that these treatments favor their extraction and stabilization. These compounds are important for their role in enhancing cardiac function and metabolic regulation. Adeniyi *et al* (2021) observed a similar trend in leaf protein concentrates of *Vernonia amygdalina* and *Telfairia occidentalis*, where glycoside concentration increased after heat and acid treatments. Saponins were found only in the acid-treated LPC, suggesting that acid coagulation enhanced their solubility and extraction compared to the alum and heat methods. Saponins contribute to the emulsifying properties of feed and have been associated with cholesterol-lowering and immune-boosting effects. Their selective appearance in acid-treated samples agrees with the report of Ezeonu *et al* (2019), who noted that acid extraction tends to increase saponin yield in processed leafy materials. Steroids were highly present in all samples irrespective of treatment, indicating that the compounds are heat and acid stable. Steroidal compounds are valuable for their growth-promoting and anti-inflammatory functions and play an important role in animal nutrition. Their consistent presence across treatments aligns with Udeh *et al* (2020), who reported that

processing did not reduce steroid content in *Amaranthus hybridus* and *Moringa oleifera* Leaf Concentrates. Phenolic compounds were detected in all samples but were more concentrated in the acid-treated LPC. Acidic extraction likely enhanced the release of bound phenolics, thereby increasing their availability. Phenolics contribute significantly to antioxidant properties and oxidative stability. Alawode *et al* (2022) similarly observed higher phenolic content in acid-treated *Elaeis guineensis* leaves, suggesting that acid coagulation can improve antioxidant potential in LPC. Coumarins were observed in the alum and heat-treated samples but absent in the acid-treated LPC. The absence of coumarins in the acid sample may be due to degradation under acidic conditions. Coumarins are known to possess antimicrobial, antioxidant and anticoagulant properties. The variation in coumarin detection among the treatments agrees with the findings of Oyeleke *et al* (2023), who reported that coumarins are sensitive to pH and may degrade in strongly acidic media. Terpenoids were detected only in the heat-treated sample, suggesting that heating enhanced the release of these compounds. Terpenoids are important for their aromatic and antimicrobial properties, which may contribute to the preservation quality of the concentrate. Ndukwe *et al* (2020) also reported an increase in Terpenoid yield following heat treatment in *Moringa oleifera* extracts, supporting the positive effect of thermal processing on volatile compound release.

Alkaloids were found in all the samples, showing that they are relatively stable under varying processing conditions. Alkaloids possess numerous pharmacological properties such as analgesic, antimicrobial, and stimulatory effects. Their persistence across the three treatments indicates that oil palm leaves naturally contain stable nitrogenous compounds that remain intact after processing. Ugbogu *et al* (2021) observed similar results in *Telfairia occidentalis* and *Ipomoea batatas*, where alkaloids were retained even after heat and acid treatments. Generally, the phytochemical profile of Oil Palm LPC shows that the leaves are rich in biologically active compounds that can enhance both Nutritional and Medicinal Value. The differences in intensity across treatment methods demonstrate that processing conditions significantly affect compound recovery. Acid coagulation produced higher phenolic and saponin content, heat treatment promoted terpenoid and glycoside stability, while alum precipitation yielded moderate retention of most compounds. When compared with other leafy plants such as *Moringa oleifera*, *Telfairia occidentalis* and Cassava leaves, OP-LPC displayed a similar phytochemical pattern, especially in the consistent presence of flavonoids, steroids, phenols and alkaloids. This suggests that Oil Palm Leaves could be explored as an alternative source of protein and bioactive compounds for feed formulation and functional food production. The richness of these phytochemicals further confirms the potential of OP-LPC in improving Animal Health and Productivity through enhanced antioxidant and metabolic support.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

This work was designed to investigate the Physical Characteristics and Chemical Composition of Leaf Protein Concentrate (LPC) produced from Oil Palm (*Elaeis guineensis*) leaves using three extraction techniques: Heat coagulation, Alum precipitation and Acid coagulation. The LPCs obtained from all methods showed similar physical properties, appearing dark green when fresh and darker after drying. The Proximate Analysis revealed slight variations in DM, CP, CF, EE and Ash contents, with the Acid Coagulation method yielding the highest protein value and the Alum Precipitation method producing the highest ash and fibre contents. Only the NFE value showed a significant difference, being highest in the Alum treatment. Mineral composition indicated that Potassium, Calcium and Magnesium were most abundant in the Heat Coagulation method, while Sodium remained the lowest across treatments. These findings suggest that Oil Palm Leaves possess substantial nutritional and mineral value, making them a promising raw material for Leaf Protein Concentrate production.

6.2 Conclusion

This study successfully evaluated the Physical Properties and Nutritional composition of Leaf Protein Concentrate (LPC) and associated By-products derived from Oil Palm

(*Elaeis guineensis*) leaves using Heat Coagulation, Alum Precipitation and Acid Coagulation methods. The processing of Oil palm Leaves yielded three useful fractions: LPC, Bagasse and De-proteinized juice (whey). Results from Proximate, Mineral and Phytochemical Analyses confirmed that Oil Palm Leaves contain substantial Crude Protein, Minerals and Bioactive Compounds. The extraction processes enhanced protein concentration while reducing fibre levels, indicating nutritional improvement of the LPC for feed and possible food applications. Findings highlight Oil Palm Leaves as a valuable and underutilized biomass resource capable of addressing protein shortages, supporting circular agriculture and enhancing sustainable agricultural waste utilization in Nigeria, particularly in Edo State.

6.3 Recommendations

1. Adoption of LPC Technology: Local farmers and feed manufacturers should adopt LPC production from Oil Palm leaves to supplement conventional protein sources and reduce feed costs.
2. Promotion of Circular Agriculture: Oil Palm leaf biomass should be integrated into sustainable agricultural systems to reduce waste and environmental pollution.
3. Improved Processing Methods: Access to affordable and efficient extraction equipment should be prioritized to enhance LPC yield and quality, especially in rural communities.

4. Further Research: Future studies should investigate amino acid profiles, anti-nutrient detoxification techniques, sensory qualities and animal feeding trials for LPC from Oil Palm Leaves.
5. Storage and Safety Protocols: Proper drying and storage guidelines should be emphasized to ensure product stability, prevent microbial growth and maintain nutritional quality.
6. Government and Institutional Support: Policy makers and research institutions should support pilot-scale LPC production, training and commercialization initiatives.

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