THE PHYSICAL PROPERTIES AND NUTRITIONAL COMPOSITIONS OF LEAF PROTEIN CONCENTRATES AND BY PRODUCTS FROM BUSH MANGO (*Irvingia gabonensis baill*) PLANT OBTAINED FROM OVIA NORTH EAST LGA, EDO STATE, NIGERIA.

BY

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DEPARTMENT OF ANIMAL SCIENCE
FACULTY OF AGRICULTURE
UNIVERSITY OF BENIN
BENIN CITY, NIGERIA

NOVEMBER, 2025.

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A RESEARCH 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)

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CERTIFICATION

This is to certify that this Project work was carried out by **Regina Iziegbe OHENHEN (Miss)** with Matriculation Number, **AGR2005897** under the guidance of the Project Supervisors approved by the Department of Animal Science, Faculty of Agriculture, University of Benin, Benin City, Nigeria.

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(Head of Department)

DEDICATION

This Project work is dedicated to God Almighty, for His Provisions, Grace, Favour, Wisdom, Understanding and never ending Love throughout the course of this Research, work and my Study.

And to my incredible Parents (Mr. and Mrs. Ohenhen), Rev. Fr. Idahosa Amadasu, my amazing Siblings, other Family and Special Friends who through their Financial, Spiritual, Emotional, Physical, Psychological, Physiological support and Continuous Encouragements saw me through the duration of my Study.

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ABSTRACT

This study examined the Extraction of Leaf Protein Concentrates (LPC) from *Irvingia gabonensis* (Bush Mango) leaves using three different methods: Heat Coagulation, Alum Precipitation, and Acid Coagulation to evaluate their effects on the Physical Properties, Nutritional Composition, and Phytochemical Constituents of the products. Fresh Leaves collected from a part of Edo State were processed into a Slurry, and the resulting LPC and Bagasse were analyzed for Proximate, Mineral, and Phytochemical Contents using Standard Laboratory Procedures (AOAC, 2019). Results showed that the Extraction Method significantly influenced the quality and Nutrient Content of the LPC. The Alum Precipitation method produced the highest Crude Protein (32.95%) and ash (8.50%) values, while the Acid Coagulation method gave the highest Moisture (20.20%) and Carbohydrate (45.54%) levels.

The Heat Coagulation method recorded the highest Fat Content (23.33%). Mineral Analysis indicated that LPC from the Alum method contained the most Magnesium (9.67 mg/kg), Potassium (399.33 mg/kg), and Phosphorus (62.75 mg/kg). Phytochemical Screening revealed the presence of Flavonoids, Tannins, Phenols, Steroids, Cardiac Glycosides, Coumarins, and Alkaloids across all methods, with the Alum-treated LPC showing the highest Concentration of these compounds. The high Protein, Fat, and Mineral Contents, together with the presence of beneficial Phytochemicals, make the LPC nutritionally rich and suitable for use as a plant-based protein source in Food and animal Feed Formulation. The Alum Precipitation method proved to be the most effective for producing high-quality *Irvingia gabonensis* LPC with superior Nutritional and Functional Properties.

CHAPTER ONE

1.0 INTRODUCTION

Irvingia Species, commonly known as Bush Mango, as well as in their Local names namely, *dikka*, *dika nut*, *dika bread tree*, *dika*, *ogbono*, or *iba-tree*, are Multipurpose Fruit Trees growing in Africa and Asia. They belong to the family *Irvingiaceae* (Tsobeng *et al*., 2021).

The Genus is composed of Seven Species, native to Different Regions. *Irvingia wombolu* and *Irvingia grandifolia* are native to Central Africa. *Irvingia gabonensis*, *Irvingia robur*, *Irvingia smithii* and *Irvingia tenuinucleata* are native to West and Central Africa, and *Irvingia malayana* is native to South-East Asia (Nguena-Dongue *et al*., 2023; Kew Science, 2024).

The *Irvingia* Trees commonly grow in Rainforests and Humid Zones in Dry Ground, except for *Irvingia smithii*, which always grows beside Water on Riverbanks in Savannah Regions, Seasonally flooded and Riverine Forest, and of *Irvingia malayana*, which occurs also in Deciduous dipterocarp Forest and Dry Evergreen Forest (Tsobeng *et al*., 2021).

Irvingia Trees have multiple uses. They bear edible Mango-like Fruits and are valued for their Fat and Protein-rich Nuts, in addition to Vitamin C, β-carotene and Fibre Contents.

The Bark and Kernel have Medicinal Uses. Bush Mango Oil is used in the Cosmetics Industry.

Irvingia Trees have multiple uses. They bear edible Mango-like Fruits and are valued for their Fat and Protein-rich Nuts, in addition to Vitamin C, β-carotene, and Fibre Contents (Mgbemena *et al*., 2019). The Bark and Kernel have Medicinal Uses and Bush Mango Oil is used in the Cosmetics Industry (Otitolaiye *et al*., 2023).

Scientists (Okafor *et al*., 2014) have proven that Bush Mango Seeds contain Calories, Dietary Fibres, Fat, Carbohydrates, Protein, Amino Acids (like Tryptophan), Water, Minerals, such as Calcium, Iron, Potassium, Zinc, Sodium, Magnesium and Phosphorus, Fatty acids (Myristic acid, Lauric acid, Oleic acid, Palmitic acid and Stearic acid), Antioxidants, and Vitamins B and C (Mgbemena *et al*., 2019; Ogunsina *et al*., 2018). Preliminary Phytochemical Screening of the Aqueous Leaf Extract of *Ogbono* (*Irvingia gabonensis*) shows that it contains Phylobatanins, Saponins, Phenols and Tannins (Abdulrahman *et al*., 2004). Studies have shown that *Ogbono* aids Digestion and prevents Digestive Disorders, including Constipation, by adding bulk to the stool and improving Bowel Functioning (Ugochukwu and Babady, 2005). African Mango Extracts inhibits Ulcer Formation in rats by increasing mucus production in the stomach (Abdulrahman *et al*., 2004). The Dietary Fibre in *Ogbono* helps in reducing “bad” Low-Density Lipoprotein–LDL Cholesterols, simply by binding to Bile acids located in the Intestine (Ngondi *et al*., 2009). The presence of Catechins make *Ogbono* Seed very effective in regulating insulin levels and lowering Blood Sugar Levels, which in turn lowers the risk of Diabetes and assures safety in Obesity (Ngondi *et al*., 2009; Onakpoya *et al*., 2013).

Several studies have, respectively, supported the Assertion that *Dika nut* is good for Weight Loss because it blocks the action of Amylase, causing the body to absorb less sugar; Inhibits Glycerol-3-phosphate Dehydrogenase, preventing the conversion of Blood Sugar into Fat; and helps in increasing the levels of the hormone Adiponectin and Leptin, both of which reduce appetite and promote Weight Loss (Ngondi *et al*., 2009; Ngondi *et al*., 2005). The Fibre content of *Dika nut* also promotes Weight Loss by suppressing Appetite (Onakpoya *et al*., 2013).

Eating this Fruit can also help control Anxiety, Stress, Depression, and other factors that may affect the overall Mood because of the presence of Tryptophan, which enhances the Production of the Neurotransmitter (Serotonin) associated with the Promotion of good Mood, Libido, Concentration, and Sleep quality (McLendon *et al*., 2013).

Studies also reveal that the Water Extract of the Bark can help reduce pain caused by both heat and pressure. Thus, the leaves of this tree are used, mainly by Igbos, to prepare a concoction that is used in treating Fevers. The Methanolic Extract of *Irvingia gabonensis* can be used for treating Bacterial and Fungal Infections. The Powdered Kernels can be used as Astringent applied to the skin to soothe burns and also to reduce bleeding from Minor Abrasions. The Stems of the Tree can be used as Chewing Sticks for cleaning Teeth (Ngozika, 2022).

1.1 Objectives of study

The main aim of this Study is to determine the Physical Properties and Nutritional Compositions of Leaf Protein Concentrated and By products from *Irvingia gabonensis* obtained from three different Methods.

The specific objectives were to:

i. Determine the Physical Properties and Nutritional Compositions of Leaf Protein Concentrated and By products from *Ogbono* plant using Heat Coagulation Method.
ii. Determine the Physical Properties and Nutritional Compositions of Leaf Protein Concentrated and By products from *Ogbono* plant using Alum Precipitation Method.
iii. Determine the Physical Properties and Nutritional Compositions of Leaf Protein Concentrated and By products from *Ogbono* plant using Acid Coagulation Method.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Ogbono* plant and parts

2.1.1 CLASSIFICATION

Bush Mango, *Irvingia* Species is scientifically classified as follows:

Kingdom: *Plantae*

Phylum: *Tracheophyta*

Class: *Magnoliopsida*

Order: *Malpighiales*

Family: *Irvingiaceae*

Genus: *Irvingia*

Species (Selected):

Irvingia gabonensis (sweet African mango)

Irvingia wombolu (bitter African mango)

Irvingia excelsa

Irvingia malayana (Southeast Asia)

Irvingia smithii

Source: Mialoundama *et al*., 2023

2.1.2 *IRVINGIA* TREE PLANT

Irvingia Species, commonly referred to as African Mango or Bush Mango, are a group of Tropical Trees prized for both their Ecological and Economic Significance. They bear edible Mango-like Fruits and their occurrences in Sub-Saharan African are mostly in South-Western Nigeria and Southern Cameroon and also in Cote d'Ivoire, Ghana, Togo and Benin (Atoyebi *et al*., 2020). Some Species are also found in parts of Southeast Asia. Typically growing up to 40 meters in height, *Irvingia* Trees are Large, Evergreen, and feature a straight Bole and spreading Crown. The Leaves are Simple, Glossy, and Elliptic to oblong, with alternate Phyllotaxy. The Trees produce Small, Greenish-White Flowers that are arranged in axillary Racemes, followed by Large, Fibrous drupes that turn Yellowish or Greenish when ripe (Tsobeng *et al*., 2021).

The Fruit pulp is either sweet or bitter depending on the species—*Irvingia gabonensis* is known for its Sweet, Edible pulp, while *Irvingia wombolu* produces a more Fibrous and Bitter Fruit. Despite differences in taste, both Species yield Seeds (commonly called "dika nuts" or "ogbono") that are widely used in Culinary traditions across Africa.

2.1.3 *IRVINGIA* SEEDS

The Seeds of *Irvingia* Species are Economically valuable due to their high Oil and Protein content. Once the hard Shells are cracked, the Kernels are used as thickening agents in Soups and Stews, valued for their Mucilaginous Properties when ground and they form a thick Viscous Sauce Base when heated (Orugun *et al*., 2023). In addition to their Nutritional value, *Irvingia* Seeds contain Fats (up to 70%) and Proteins, making them important in both Local Diets and Industrial Applications, such as in the production of Vegetable Oil and Cosmetic products and also, the Vitamin C rich Fruits is consumed fresh or dried, and used to produce Juice and Wine, or as a Flavourant (Mateus-Reguengo *et al*., 2019). Pulp extract from the species also has Beneficial Therapeutic Potential, including but not limited to Weight Loss (Anaduaka *et al*., 2022). *Irvingia* Seed extracts have also been found to improve Blood Sugar Levels, increase High Density Lipoprotein (HDL), Cholesterol and reduce Total Cholesterol, Low Density Lipoprotein (LDL), and therefore they can be used as Anti-diabetic and Anti-obesity Agents (Yang *et al*., 2020). Agriculturally, *Irvingia* Species are promoted in Agroforestry Systems due to their Adaptability and Contribution to Food Security, Income Generation, and Forest Conservation (Leakey *et al*., 2003). Efforts to Protect, Domesticate and Cultivate these Trees are ongoing, with initiatives aimed at selecting superior Fruit and Seed traits for propagation. This underscores the Ecological Importance and Socio-economic Potential of *Irvingia Species* across their Native Range.

2.2 Leaf Protein Concentrate (LPC)

Concentrate has been defined by Olomu (2011) as a Food or Feed used together with another Feed in order to improve its Nutritional Status. Also, it is intended to be diluted or mixed further to produce a complete Food or Feed. High Quality Protein can be extracted from the Leaves of Plants and used to improve the Nutritional Status of a Food or Feed Material and to also produce a Food or Feed.

Leaf Protein Concentrate (LPC) is obtained from processing Pulped Leaves after undergoing some processes. The products obtained are Green in Colour and palatable when included in Feed (Taylor, 2003). The Green Colour varies sometimes, from light Green to dark Green to blackish-Green, depending on the method of Extraction. Fresh, moist leaf protein concentrate has the consistency of crumbly cheese and can be used immediately (fresh) and can be preserved, usually by Sun-drying and Granulating, after which it is ground to a Fine Powdery Form, for Future use.

Leaf Protein Concentrate can be easily combined with other variety of inexpensive foods to make culturally acceptable dished needed by the people in developing countries; it can be used in Soups, Fruit Drinks, Baked Foods, Stew, etc. (Kennedy, 1993). LPC was first suggested as Human Food in the 1960s, but it did not achieve much success despite early promises because of Specific palatability issues. However, Norman Pirie was highly recognized by the UK Government in 1975 for his Work on the benefits of Leaf Protein Concentrates, which later brought the subject to the limelight (Singh, 1984). Belitz *et al*. (2009) reported that the value of Leaf Protein Concentrate lies between that of Soyabean and Milk. Exhaustive study on plant Leaf Protein Concentrate has been undergone at the University of Winconsin, Madison, where several Biochemists, Agronomists, Nutritionist were involved and significant results were published (Dale, 1974; Russel *et al.*, 1974; Koegel and Bruhn, 1972; Stahmann, 1968). Several methods for the preparation of LPC from leaf juice have been advocated which include heating (Pirie, 1971, 1978), Acidification (Miller *et. al.*, 1975) and natural Acidification due to an Anaerobic Fermentation of Juice (Stahmann, 1968; Ajibola, 1981; Ajibola *et al.*, 1984). The yield and quality of the Leaf Protein Concentrate (LPC) depends on the method by which it is prepared (Sayyed, 2011). It is considered that *Ogbono* Leaves have a great potential of Protein Concentrate Extraction. Research has shown that high amount of Protein can be extracted from the Leaves of various edible Plants and fed to Humans and Animals also. This Extract called, Leaf Protein Concentrate, LPC, is primarily composed of Protein, ranging from 40% to 65% of the Dry Weight. The Protein profile varies depending on the Plant source and Extraction Process, but often consists of Essential Amino Acids, although Lysine may be limiting. Besides Protein, LPC also contains Carbohydrates, Fibre, Minerals, and other Bioactive Compounds. Akaeze (2010) reports that Leaf Protein Concentrate makes for effective utilisation of Vegetables in his Work on Hevea brasiliensis.

2.2.**1 Methods of extraction of Leaf Protein Concentrate from plants**

The Fresh Juice Samples extracted from Plants are used for the preparation of LPC by following eight (8) Methods according to Sayyed (2011):

(1)**Differential heat coagulation**: 20ml distilled water was heated in a beaker to 60℃. To it, 50 ml fresh Juice was slowly added with stirring so as to maintain the Temperature of Juice to 60℃. The Juice was kept for heating at this Temperature for 5 min. It was then filtered through Whatman Filter Paper to separate Green Chloroplastic Leaf Protein Concentrate (LPC), which received 2-3 washings with water. The Filtrate was collected and heated to boiling. This resulted into the precipitation of Cytoplasmic Proteins resulting into White Cytoplasmic LPC. The Cytoplasmic fraction was recovered by Filtration through Whatman Filter Paper and the Deproteinised Juice (DPJ) released as a filtrate was collected. The samples of green chloroplastic LPC, white cytoplasmic LPC (resulting due to the heating of filtrate to over 95℃) and the Deproteinised Juice (DPJ) left after recovering Chloroplastic and Cytoplasmic Fractions were collected for further analysis. The samples of LPC were sun dried and further dried in oven at 65 ± 5℃ to record the yield.

(2) **Heat coagulation**: To about 20ml of boiling water, 50 ml of fresh juice was slowly added with constant stirring. It was heated till boiling to a temperature exceeding 95℃. The Proteins in juice got coagulated due to the heat treatment and resulted into a curd referred to as Leaf Protein Concentrate (LPC). The LPC was separated from remaining

portion of the juice - the Deproteinised Juice (DPJ) by filtration through Whatman Filter Paper with 2 to 3 Hot Water washings. The sample of LPC was collected, Sun dried and further dried in Oven at $65 \pm 5^{\circ}$ C and the yield of LPC dry matter (LPC – DM) was recorded.

(3) **Heat coagulation followed by acid wash**: The LPC from plant juice was prepared by heat coagulation as described above and suspended in acidic water with a pH value of 3.5 for 10 minutes and filtered. The samples of LPC and DPJ were collected for recording the yield and further analysis.

(4) **Acid coagulation**: To 50 ml fresh juice, 5 N H2SO4 was added with stirring till the pH value decreased to 3.5. The curd of LPC resulted due to the coagulation of proteins in juice by acid was filtered through Whatman filter paper and the sample of LPC was taken for recording the yield.

(5) **Fermentation**: Fresh juice (about 120 ml) was placed in 125 ml Conical flask. The mouth of the Flask was capped with Rubber Cork. A 25 to 35 cm long Capillary Rod (1 mm i.d.) was inserted through the Cork and mouth of the Conical Flask was sealed with Wax. The Flask was left at Room Temperature for Fermentation of Juice till six days. The Capillary Rod, on the top of the Flask, allowed Carbon dioxide formed during Fermentation to escape out and also to exclude Air. After 6 days, the Cork was removed and loss in the volume of Juice due to Fermentation was made up with Distilled Water.

The Fermented Juice showed Precipitation of Proteins in it due to Acidification during Fermentative Process. It was filtered through Whatman Filter Paper and the sample of LPC was kept in oven to record the yield of LPC.

(6) **Alum precipitation**: 2g of Alum was dissolved in 100 ml Distilled Water to prepare Alum Solution. To 50 ml fresh Juice, Alum Solution was added with stirring. The Curd of LPC resulted due to the coagulation of Proteins in Juice by Alum Solution was filtered through Whatman Filter Paper and the Sample of LPC was taken for recording the yield.

(7) ***Flocculation***: 100mg of Praestol (a Flocculent) was dissolved in 100 ml distilled water by constant stirring on Magnetic stirrer for 30 minutes. To 50 ml fresh Juice, the Flocculent Solution was added with Stirring. The curd of LPC resulted due to the Coagulation of Proteins in Juice by Flocculent was filtered through Whatman Filter Paper and the Sample of LPC was taken for recording the yield of LPC.

(8) **Flocculation at pH 3.5**: To 50 ml fresh Juice the Flocculent Solution prepared as above was added with stirring. To it 5N HCL was slowly added with stirring till the pH value dropped to 3.5. The Curd of LPC resulting due to the Coagulation of Proteins in the Juice, was filtered through Whatman Filter Paper and Sample of LPC was taken to record the Yield (Sayyed, 2011).

2.2.2 Chemical composition of leaf protein concentrate obtained from some plants

Further Studies on Leaf Protein Concentrate have been carried out on Leaves from Plants. The Proximate Analysis reveals great Nutritional Compositions. Aletor (2002) conducted a Research on Leaves from four leafy Vegetables Species. *Vernonia amygdalina* (Bitter leaf), *Solanum africana*, *Amaranthus hybridus* (Green tete) and *Telfairia occidentalis* (Fluted pumpkins) were subjected to proximate analysis and determination of energy values and Nutritionally valuable minerals. Thereafter, Leaf Protein Concentrates (LPCs) were produced from the different Species using Low-cost Fractionation Techniques. The LPCs were subsequently characterised with respect to their Proximate Composition, Mineral Constituents and Functional Properties. On average, the Leafy Vegetables contained 33.3 g/100 g DM Crude Protein (range 31.7–34.6 g/100 g) and 8.4 g/100 g DM (range 7.4–9.8 g/100 g DM crude fibre. Gross Energy averaged 378 k cal/100 g. The Protein Extracts contained, on average, 47.2 g/100g DM Crude Protein (range 35.1–54.9 g/100 g) 1.4 g/100 g DM Crude Fibre, 7.9 g/100 g DM Ether Extract; 15.7 g/100 g DM Ash and a Gross Energy of 439 kcal/100 g. Ca, Mg, Na and K were the most abundant Minerals in the Leaf Meals and Leaf Protein Concentrates while P and Cu were the least abundant (Aletor, 2002).

Table 1: Provides Information on the result of the Composition of *Irvingia* Species Leaf Meals, Leaf Protein Concentrates and Leaf Protein Concentrates Residues (whey).

Table 1: Analyzed compositions of the Leaf Meals, Leaf Protein Concentrates and Leaf Protein Concentrate Residues of *Irvingia* Species Leaf Products (Dry Matter Basis)

Proximate Composition (%)	Leaf Meal	Leaf Protein Concentrate (gkg-^{1} DM)	Leaf Protein Concentrate-Residue
Crude protein	18.50–20.40	45.60 – 50.20	10.80 – 12.60
Ash	9.20 – 10.40	10.90 – 12.10	12.80 – 14.00
Ether extract	4.50–5.80	4.20 – 5.00	2.40 – 3.00
Crude fibre	13.20–15.80	5.90 – 7.10	19.50 – 22.00
Nitrogen free extract	49.00–52.0	28.00 – 30.50	48.00 – 51.00
Moisture content			
Metabolizable energy (kcal/kg)	7.50 – 8.80	6.00 – 6.80	7.00 – 7.80
	+-2,450-2,520	~2,600 – 2,750	~2,050 – 2,200
Mineral content (mgkg-^{1} DM):			
Sodium	20 – 38	25 – 40	22 – 30
Calcium	320 – 410	450 – 560	270 – 350
Magnesium	190 – 240	250 – 300	180 – 220
Phosphorus	95 – 125	130 – 150	90 – 110
Potassium	950 – 1120	1200 – 1350	880 – 980
Iron	15 – 20	22 – 28	13 – 18
Zinc	3.8 – 5.1	6.2 – 7.5	3.2 – 4.0

Source: Fasuyi *et al*., (2006)

2.2.3 Advantages of Leaf Protein Concentrate (LPC)

1. Leaf Protein Concentrate is a very nutritious Food with low Prices, compared to other highly Nutritious and Protein rich Foods like Eggs, Meat, Beans, Milk, etc.

2. There is little or no waste in the production process of Leaf Protein Concentrate. The residual Fibre can be an excellent feed for Cows, Goats, and other Ruminants able to utilize Fibre. The leftover liquid can be used to enrich the soil. It can also be used in the production of biogas for kitchen use, can also be formulated to distill alcohol.

3. It is a very efficient way of using Land to produce Food, yielding roughly three times as much Protein per Hectare as grain Crops and five to ten times as much per Hectare as Animal raising.

4. Leaf Protein Concentrate is an extremely Nutritious Food, rich in Vitamin A and Iron compared to commonly available Foods. It is also an excellent source of high quality Protein, Calcium and several other essential Nutrients (Aletor and Adebayo, 2012).

5. It has potential for Commercialization in the Food and Feed Production Industry for the production of Novel Food and Feed Products.

2.2.4 Disadvantages of Leaf Protein Concentrate

1. Carcasses of Animals fed with Diet formulated with Leaf Protein Concentrate are usually Yellow due to the presence of beta carotene. This Yellow colouration causes Market resistance in some Countries.

2. Good leaf yield require a steady supply of Water which may not be feasible all year round in some parts of the World that experience Water Scarcity, Drought or long-Dry Seasons, etc. This may affect the Leaf Yield of Plants and the Production Level of LPC.

3. Most people are not accustomed to eating Dark Green Food. This reduces the acceptability level of Leaf Protein Concentrate because it is mostly dark Green in Colour.

4. The processing of Leaf Protein Concentrate can be done on Small-Scale or Domestic Level with inexpensive Grinders and Blenders. However, Large Scale Production is costlier. Home of the Equipment must be custom-built.

5. Fresh Leaves are highly perishable. Therefore, they must be Processed soon after they are harvested; otherwise, the quality and yield of the Leaf Concentrate will drop.

2.3 Bagasse

This is the Chaffy material that is left after the pressing out of Juice from ground or blended Plant parts such as Leaves. It is the fibrous Residue after the milling of Plant stalks, Leaves or Roots, and their Extract or Juice is removed. It contains 9-10% CP on its Dry Matter depending on the Leaf used for Fractionation (Badar and Kulkarni, 2011).

Bagasse, as shown in Table 2, is mainly composed of cellulose (40–50%), hemicellulose (20–30%), and lignin (18–25%), with smaller amounts of ash (1–4%) and extractives (1–3%). This composition indicates that Bagasse is a high-fibre, low-nutrient material. The large proportion of cellulose and hemicellulose explains its fibrous and bulky nature, which makes it useful as a roughage source in animal feed and suitable for bedding or litter material because it can absorb moisture effectively.

However, the relatively high lignin content reduces its digestibility and palatability, especially for non-ruminant animals, since lignin forms complex bonds with cellulose that limit microbial breakdown. The low levels of ash and extractives also show that Bagasse contains little mineral and soluble nutrient content, which aligns with its low protein (9–10%) and energy value as mentioned earlier.

Therefore, the chemical composition presented in Table 2 supports the understanding that while Bagasse serves as a valuable fibrous by-product useful for gut health, bedding, and waste absorption, it has limited nutritional quality and often requires supplementation or treatment before being used as feed.

2.3.1 Advantages of Bagasse

According to Omologe (2021), Bagasse is a non-Forage Roughage and a by-product of Crop Processing. Olabimitan (2021) suggests that it could serve as a Fibrous Feed, which enhances Digestion and Excretion of Feed Materials. It is important for Gut Health and Nobility, Caecotrophy and Appetite Stimulation (Halls, 2010). It can also be used as Breeding Materials in Nest Box for Does approaching Parturition. It could be a Bedding Material for Deep Litter Poultry System and Ruminant Pens, because it is Animal friendly, i.e., if eaten, it will not hurt the Animal due to the fact that it's from Plant Material, it isn't going to raise Dust and it can absorb Waste and provides a Soft Cushion to make Animals Comfortable. It maintains Gastro-Intestinal Health (Ograin, 2011)

2.3.2 Disadvantages of Bagasse

It loses its palatability if not fed fresh or properly-dried. Improper handling can result in Off-Flavours or Fermentation, which reduces Voluntary Intake by Animals (Mekasha *et al*., 2021).

It is not equally liked by all animals. For instance, Cattle are more likely to accept it than Ruminant animals like Rabbits and Goats. The Coarse Texture and high fibre content makes it less desirable for Non-Ruminant or Monogastric Animals (Elghandour *et al*., 2020).

Due to its poor Protein and Energy Content, Bagasse often requires Supplementation with Protein Sources or Chemical/Biological Treatment to improve its Digestibility and Nutritional Profile before feeding (Adegbeye *et al*., 2020).

Table 2: Chemical composition of Bagasse Fibre

Components	**Percentage (% dry basis)**
Cellulose	40-50
Hemi cellulose	20-30
Lignin	18-25
Ash	1-4
Extractives	1-3

Source: Singh *et al*., (2020)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location and Duration of the Study

The Experiment on the Yield, Physical Characteristics and Chemical Composition of Leaf Protein Concentrate and Bagasse obtained from Bush Mango Leaves was carried out in the Faculty of Agriculture Main Laboratory, University of Benin, Ugbowo Campus, Benin City, Edo State, Nigeria. University of Benin is located between Latitude 6 30'N of the Equator and Longitude 5 40' and 6 E of the Greenwich Meridian in the Forest Zone with an average Temperature of 27.6 C (National Airport Authority, 2014). The Scope of this Study lasted for about 8 months, which started in February 20 and ended in October 20, 2025.

3.2 RESEARCH DESIGN

The Research was conducted in Different Phases indicated below:

3.2.1 PHASE ONE:

The first Phase involved the Preliminary Trials of the Methods of Extraction of LPC from leaves.

3.2.2 PHASE TWO:

The second Phase involved the determination of the Yield and Physical Characteristics of *Irvingia gabonensis* and Leaf Protein Concentrates (LPC) prepared by Heat Coagulation Method.

3.2.3 PHASE THREE

The third Phase involved the determination of the Yield and Physical Characteristics of *Irvingia gabonensis* and Leaf Protein Concentrates (LPC) prepared by Alum Precipitation Method.

3.2.4 FOURTH PHASE:

The fourth Phase involved the determination of the Yield and Physical Characteristics of *Irvingia gabonensis* and Leaf Protein Concentrates (LPC) prepared by Acid Coagulation Method.

3.2.5 FIFTH PHASE:

The fifth Phase involved the determination of the Proximate Analysis (to determine Dry Matter, Crude Protein, Crude Fibre, Ether Extract, Ash and Nitrogen Free Extract) and as well as the Mineral analysis (5 Macro-minerals, e.g., Magnesium, Potassium, Phosphorus, Sodium, Calcium and 3 Microminerals, e.g., Manganese, Iron, Zinc).

3.3 COLLECTION AND SELECTION OF EXPERIMENTAL MATERIALS

The Bush Mango Leaves for this Research were harvested from two Variety of Bush Mango which are *Irvingia gabonensis* and *Irvingia wombolu* at the Forestry Arboretum, Faculty of Agriculture, University of Benin, Ugbowo Campus, Benin City, Nigeria.

The Stalks were removed from the freshly harvested Leaves, and the Leaves were rinsed with distilled water and blended to prepare the Leaf Protein Concentrates (LPC).

3.3.1 Equipment Used:

Weighing Balance

pH meter

Belt-driven Grinder

Heat Source (Heating Mantle, Electric heater etc.).

3.3.2 Materials:

Freshly harvested Bush Mango leaves.

Plastic bowls

Calibrated plastic buckets

Hand gloves

Steel spoon

Measuring Cylinder

Mortar and Pestle

Aluminum foil

Sieve cloth

Stainless Steel Pot

Alum

5N H_2SO_4 Acid

Masking Tape for labelling samples

3.3.3 Calibration Procedure and Measurements:

Harvest fresh bush mango leaves as early as 8 am in the morning.

Immediately take to the Laboratory for processing.

Rinse leaves carefully to remove dirt, sand and other particles.

Spread rinsed leaves out on a clean flat surface under fan to allow to dry off quickly. This may take up to 5 minutes.

Weigh the leaves afterwards.

Measure water to be used to grind the leaves to a pulp.

For optimum yield, for every 1g of bush mango leaves use 8ml of water. Thus, for 800g of leaves, 6400 ml of water will be used.

Grind into a slurry using a Blender or Grinder.

For more bagasse yield, use a Blender or adjust the Grinder to coarse grinding.

For more LPC yield, use a Grinder set to fine grinding.

For large scale extraction, a Grinder is more effective and recommended.

Sieve and press the slurry using an appropriate sieve cloth to obtain two extracts: Bagasse and Juice.

The juice is then subjected to Heat, Alum or Acid to extract the LPC and Whey fraction

Figure 1 shows the flowchart of the production of Leaf Protein Concentrates by Osagie and Nwokoro, 2023

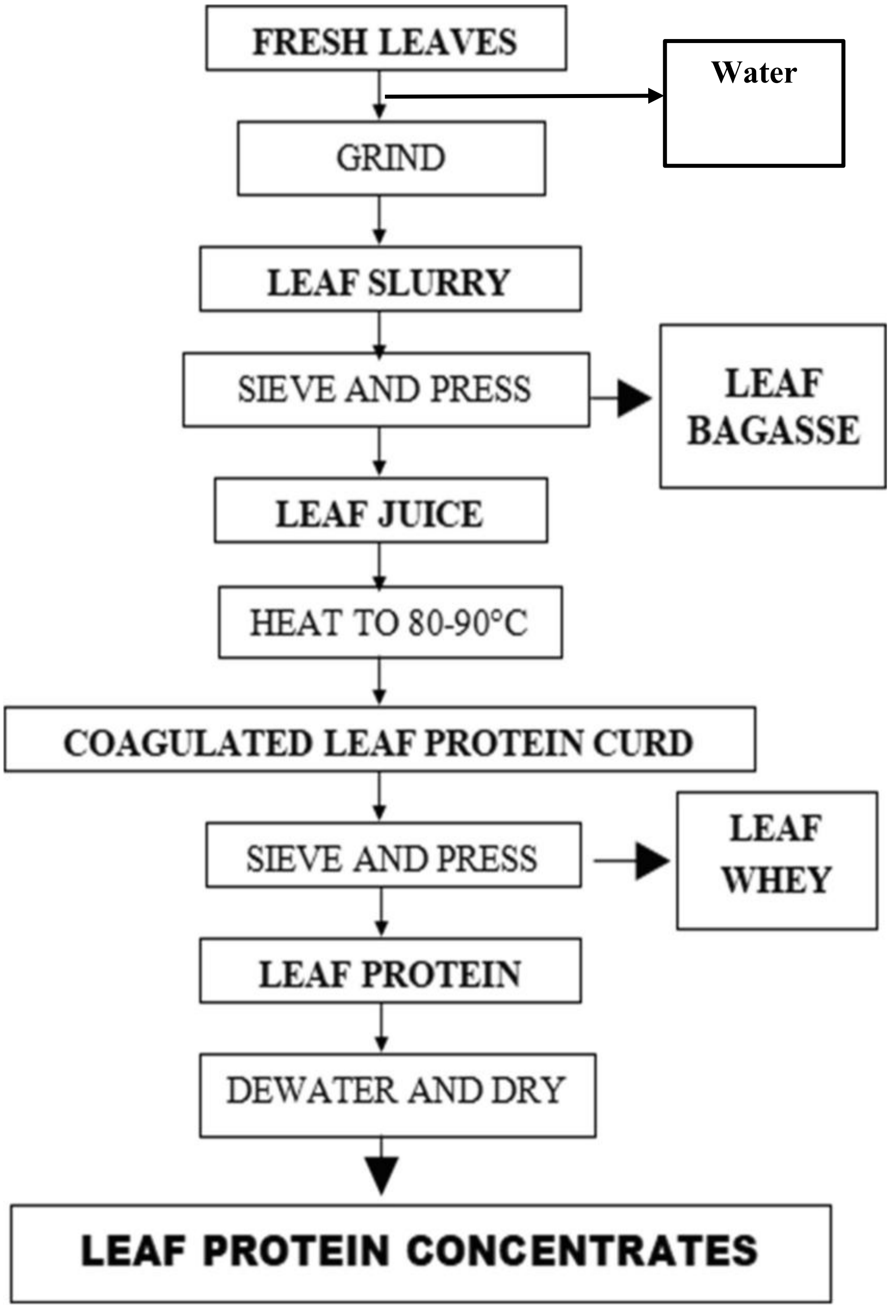

Figure 1: Flowchart of the production of leaf protein concentrates

Source: ResearchGate (Osagie and Nwokoro, 2023)

3.4 PRODUCTION OF BUSH MANGO LEAF PROTEIN CONCENTRATE (BM- LPC) USING THE HEAT COAGULATION METHOD

Fresh Bush Mango Leaves were harvested early in the Morning and promptly transported to the Laboratory for Processing. The Leaves were thoroughly washed to remove dirt, sand, and other impurities, after which they were weighed and chopped into smaller pieces. The chopped Leaves were ground into a Slurry using a Belt-driven Grinder. The resulting Slurry was transferred onto a Sieve Cloth and firmly pressed to extract the Green Juice. The Residue retained on the sieve was identified as Bagasse.

A 100 mL portion of the Extracted Juice was heated, and after approximately five Minutes, the formation of a Protein Curd was observed on the Liquid Surface. Temperature readings were taken during the heating process to monitor the Curd Formation. The Curd, also known as the Leaf Protein Concentrate (LPC), was gently scooped out, while the remaining liquid fraction, referred to as Whey, was allowed to cool for about 10 minutes. The Whey was then filtered through a Cheesecloth to separate any remaining LPC.

The wet LPC Samples were collected, and their yield was recorded. The Concentrate was subsequently Sun-dried on a clean Plastic or Aluminium Foil surface until a constant weight was achieved, after which the final weight and physical characteristics were documented.

3.5 PRODUCTION OF BUSH MANGO LEAF PROTEIN CONCENTRATE (BM- LPC) USING THE WET MILLING METHOD

Freshly harvested Bush Mango Leaves were washed thoroughly to eliminate Dust, Insects, and other Contaminants. The Leaves were weighed and milled with a measured volume of water to obtain a Leaf Slurry. The Slurry was Sieved to separate the Bagasse from the Juice. The Leaf Protein Concentrate was then extracted from the Juice using the Heat Coagulation Method, following the procedure described by Osagie and Nwokoro (2023)

Both the Leaf Protein Concentrates and the Bagasse obtained were Sun-dried to remove Residual Moisture. The Dried Samples were observed for Physical Properties, stored in Airtight Containers, and later subjected to Chemical Analysis.

3.6 PRODUCTION OF BUSH MANGO LEAF PROTEIN CONCENTRATE (BM- LPC) USING THE ALUM PRECIPITATION METHOD

Following wet milling, the leaf juice was separated from the bagasse using a Sieve Cloth. To precipitate the Leaf Protein Concentrate (LPC) from the Juice, an Alum solution was prepared by dissolving 1g of Alum in 50mL of Distilled Water to yield a homogeneous Solution. The Alum Block was first crushed into smaller Granules with a Mortar and Pestle to enhance dissolution.

For each 100mL of Leaf Juice, 150mL of Alum solution (equivalent to 3g of Alum dissolved in 150mL of Distilled Water) was added while stirring continuously to ensure uniform mixing. The addition of Alum caused the Coagulation of Proteins, forming a Curd of Leaf Protein Concentrate. The coagulated LPC was filtered using Cheesecloth or Whatman Filter Paper to separate it from the Liquid Phase.

The recovered LPC sample was sun-dried to a constant weight, after which all weights and physical observations were recorded.

3.7 PRODUCTION OF BUSH MANGO LEAF PROTEIN CONCENTRATE (BM- LPC) USING ACID COAGULATION METHOD

Freshly harvested Bush Mango Leaves were collected early in the morning and promptly transported to the Laboratory for Processing. The Leaves were thoroughly washed to remove Dust, Sand, and other Extraneous Materials, after which they were weighed and cut into smaller pieces. The chopped Leaves were ground into a Slurry using a belt-driven Grinder. The resulting Slurry was then transferred onto a Sieve Cloth and pressed firmly to extract the Juice, while the Fibrous residue retained on the Sieve was referred to as Bagasse.

A 5N solution of concentrated Sulfuric Acid (H_2SO_4) was prepared from a Stock Solution with a Normality of 35.644N, Density of 1.84 g/mL, Formula weight of 98.08 g/mol, and Concentration of 95% w/w. To prepare 1000mL of 5N H_2SO_4, 140.275mL of the Stock Acid was carefully added to 250mL of Deionized Water with constant Stirring, followed by dilution to a final Volume of 1000mL using Deionized Water.

The prepared 5N Acid was introduced dropwise into 100mL of the Extracted Leaf Juice with continuous stirring until the pH decreased to between 3.0 and 3.5, as verified using a Calibrated pH Meter. The formation of Curd indicated Protein Coagulation. The Coagulated Protein (Curd) was separated from the liquid fraction using a Cheese Cloth or Whatman Filter Paper. The resulting Leaf Protein Concentrate (LPC) was then Sun-dried to a constant weight, and the final Weight and Physical characteristics were recorded.

3.8 PRECAUTIONS TAKEN DURING THE PRODUCTION OF LPC

b) All materials used were thoroughly washed after use to prevent error by contamination.

c) Fresh Leaves were used during the period of Research.

d) Hand Gloves were worn on both hands during the use of H_2SO_4 Acid for Acid Coagulation method to prevent irritation to Skin.

e) Ensure to take the correct Readings from the Weighing Balance.

f) Ensure that all Laboratory Rules were followed, including the use of a Coat while working in the Laboratory.

g) The Leaves were chopped into smaller bits before Grinding.

h) All LPC and Bagasse obtained from the procedure was properly Sun-dried to prevent growth of Mould. The Sieve Cloth used had small Pores to allow for the passage of only the Juice

i) The Time at which the Curd formed during Heat Coagulation Method was noted.

j) All Products obtained were properly labelled to avoid Mix Up.

k) The Grinder used was rinsed with sufficient water before the start of any Grinding and after grinding in order to prevent contamination.

l) The wet and dry weight of the Bagasse and LPC were properly recorded.

3.9 CHEMICAL ANALYSIS

The Chemical composition of the produced Bush Mango Leaf Protein Concentrates (BM-LPC) was determined through Laboratory Analyses. All Analyses were carried out in Triplicates to ensure Precision and Reproducibility. The Analytical Procedures adopted were as described below.

3.9.1 PROXIMATE ANALYSIS

In the field of Nutrition, understanding the composition of Food is crucial for both Researchers, Scientists, Practitioners and Nutritionists. One of the most widely used Methods to achieve this is Proximate Analysis.

Proximate Analysis is a Method used to determine the basic Nutritional Components of Food or Feeds. It is categorized into:

a) Moisture Content

b) Crude Ash

c) Crude Fats

d) Crude Fibre

e) Crude Protein

f) NFE – Nitrogen Free Extracts (digestible Carbohydrates)

3.9.1.1 DETERMINATION OF MOISTURE CONTENT

The moisture content of the Bush Mango Leaf Protein Concentrate (BM-LPC) was determined following the Oven Drying method as described by the Association of Official Analytical Chemists (AOAC, 2019). The procedure was conducted as follows:

1. A clean, Dry Crucible was placed in a hot air Oven and preheated at 100°C for 30 Minutes to remove any Residual Moisture. The Crucible was then transferred into a Desiccator using a pair of Tongs and allowed to cool to Room Temperature.

2. The Empty Crucible was weighed using an Analytical Balance, and the weight recorded as W_1.

3. Approximately 2g of the finely ground Sample was accurately weighed into the Crucible, and the Total Weight (Crucible + Sample) was recorded as W_2.

4. The Crucible containing the Sample was placed in a Hot Air Oven maintained at 105°C for three (3) hours to ensure complete removal of Moisture.

5. After Drying, the Crucible was removed and immediately transferred into a Desiccator to cool to Room Temperature, after which it was reweighed and the final weight recorded as W_3.

Residual Moisture calculation;

$W_3 - W_2 / W_1$ X 100

W_3 = Weight of crucible + sample

W_2 = weight of oven dried sample

W_1 = weight of sample

Calculation;

% Dry matter = weight of Dried Sample / weight of Original Sample X 100

To get % Dry Matter= 100 - % Moisture

3.9.1.2 DETERMINATION OF CRUDE ASH

1. Place Crucible in the Oven to dry for 1hr then cool in the Desiccator and weigh the empty crucible
2. Add 2g of sample in the crucible
3. Ash it in the muffle furnace for 3hrs at 600^{o}C. Leave in the muffle furnace till the next day. With a pair of tongs, transfer into a desiccator to cool.
4. Take the weight.

Calculation;

$$\frac{\text{Weight of ashed Sample} - \text{Weight of Crucible}}{\text{Weight of Sample}} \times 100$$

Weight of ashed Sample is the weight after the Sample has been in the Furnace for 3hrs at 600^{o}C.

3.9.1.3 ETHER EXTRACTS/CRUDE FATS

Determination of Ether Extract (Crude Fat)

Ether-soluble Materials in Feed consist of various Organic compounds that are soluble in Organic solvents such as Diethyl Ether. In Animal Feed Analysis, the Ether Extract Fraction typically comprises Fats, Fatty Acid Esters, Waxes, and Fat-Soluble Vitamins, and is collectively referred to as Crude fat. The primary objective of Ether Extraction is to quantify the fraction of Feedstuff with high caloric value, as Fats are the most Energy-dense Nutrients.

In this procedure, a portion of the Dried Feed Sample is subjected to continuous extraction with anhydrous ether for approximately four hours using a Soxhlet Apparatus. The Ether dissolves the Lipid components of the Sample, leaving behind the Fat-Free Residue. The Solvent is then Evaporated, and the remaining Lipid Fraction is dried and weighed.

The weight of the residue represents the Ether Extract, which is taken as the measure of Crude Fat content in the Sample. Since Fats and Fatty acid esters are soluble in Ether, the Ether Extract value effectively reflects the Total Lipid content of the Feed.

Calculations;

$W_2 - W_1 / W_0 \times 100$

W_2 = weight of Filter Paper + Sample

W_1 = weight of Sample after Oven drying

W_0 = weight of Sample

3.9.1.4 DETERMINATION OF CRUDE FIBRE

Crude Fibre represents the portion of Feed that is resistant to both mild Acid and Alkaline digestion, primarily consisting of Cellulose, Hemicellulose, and Lignin. The determination of Crude Fibre involves a sequential Acid-Alkali Digestion Process designed to mimic the chemical conditions within the Animal Digestive System.

In this Method, a Dried Feed Sample is first boiled in 1.25% Sulfuric acid (H_2SO_4) for a specified period to remove Acid-Soluble Components. The mixture is then filtered, and the Residue is subsequently boiled in 1.25% Sodium Hydroxide (NaOH) to dissolve the Alkali-soluble Materials. After filtration, the remaining Residue is washed, dried, and ashed in a Muffle Furnace to remove the Organic Matter.

The Crude Fibre content is calculated as the difference in weight between the dried Residue before Ashing and the Residue after ashing. This process provides an estimate of the Indigestible portion of the Feed, although it does not account for Enzymatic Digestion that occurs in the Gastrointestinal Tract.

3.9.1.5 DETERMINATION OF CRUDE PROTEIN

The procedure to estimate Crude Protein was developed by a Danish Chemist, Johan Kjeldahl and is commonly known as "Kjeldahl" Procedure. The Kjeldahl Analysis depend on the measurement of Nitrogen (N) in the Test Material. To convert the measured to Crude Protein, a calculation factor of 6.25 (N X 6.25) is applied. This is based on the fact that all Proteins contain about 16%N (100/16 = 6.25) or 16g of N comes from 100g protein, or 1g of N is associated with 100/16 = 6.25g of Protein.

Note; Nitrogen (N) X 6.25 = Crude Protein (C.P)

Crude Protein Analysis is in 3 stages:

1. Digestion Stage
2. Distillation Stage
3. Titration/calculation Stage.

Crude Protein Procedure

a. **FIRST STAGE**: Digestion Stage

i. take 1g of Food Sample into a 250ml Conical Flask

ii. add one Catalyst Tablet or 2 Spatulas full of Catalyst Mixture

iii. carefully add 20ml of Concentrated Sulfuric Acid (H_2SO_4)

iv. place bent Glass Funnel on the mouth of the Conical Flask.

v. Then take it to the heating mantle and heat for 1hr to 1:30 mins. Then place on your working table to allow it to cool down after which distilled water is added. The mixture is transferred to a graduated Sample Bottle and make it to 100ml using Distilled Water.

b. SECOND STAGE: Distillation

From your digest which is also called Aliquot

i. Take 10ml of your digest or aliquot into the Kjeldahl Flask

ii. Add 15ml of 40% Sodium Hydroxide (NaOH).

iii. Add 50ml of Distilled Water

iv. Measure 5ml of Boric Acid indicator into 50ml Conical Flask

Nitrogen is collected via the Kjeldahl Distiller.

c. THIRD STAGE: Titration

i. After collecting Nitrogen via the Kjeldahl Distiller, Titrate with 0.1N HCl

ii. Take your Burette Readings, then do the calculation. The Titre Value (volume of Acid used is obtained by Titration).

Calculation;

% CP = Nacid X 14/1000 X vol of Acid X V_1/V_2 X 100/W X 6.25

Where;

Nacid = Normality of Acid

14/100 = Atomic Weight of Nitrogen

Vacid = Titre Volume of acid used

V_1 = volume of volumetric flask used

V_2 = volume of digest used

W = weight of sample

6.25 = conversion factor

3.9.1.6 NITROGEN-FREE EXTRACT (NFE)

The term Nitrogen-Free Extract (NFE) is somewhat inaccurate since no nitrogen determination or extraction process is involved. NFE is not directly measured in the Laboratory; instead, it is calculated by difference. It represents the Soluble Carbohydrate Fraction of Feed Materials, including Starches and Sugars. The value is obtained by subtracting the sum of the percentages of Moisture, Ether Extract, Crude Protein, Crude Fibre, and Ash from the Total Weight of the Sample. Consequently, NFE tends to accumulate errors from these other determinations and usually provides an overestimated value of the Actual Soluble Carbohydrate Content.

3.9.1.7 PROCEDURE FOR MINERAL DETERMINATION

To determine the Mineral content, 10 mL of Nitric acid was added to the Ash residue obtained from the Crude Ash determination. The Mixture was then heated gently in a Water Bath to aid dissolution. Afterward, the Solution was filtered into a 100 mL Volumetric Flask and made up to the mark with Distilled Water.

3.10 PHYTOCHEMICAL SCREENING (QUALITATIVE ANALYSIS)

Phytochemicals are bioactive compounds present in Medicinal Plants which, though not classified as Nutrients, play significant roles in plant defense and Therapeutic Applications. The Methanolic Extracts of the Samples were qualitatively analyzed to identify various Bioactive constituents such as Flavonoids, Tannins, Cardiac Glycosides, Saponins, Steroids, Terpenoids, Phenols, Phlobatannins, Coumarins, Anthraquinones, and

Alkaloids. Phytochemical Screening was performed following Standard Analytical Procedures as described by Harborne (1973), Trease and Evans (1989), and Sofowora (1993) to detect the presence of these Secondary Metabolites.

3.10.1 METHANOL EXTRACT PREPARATION:

A Pulverized Sample (150 g) was accurately weighed and transferred into Clean Containers. The Samples were soaked in absolute Methanol for 72 hours to allow for efficient extraction of the Bioactive Compounds. After Maceration, the Supernatant was filtered through a Muslin cloth, and the Solvent was removed using a Rotary Evaporator. The resulting Dried Extracts were then weighed and stored in Sterile Universal Bottles under Refrigeration at −4°C until required for Analysis. Aliquot Portions of the Crude Extract Residue were subsequently weighed and utilized for Phytochemical Screening.

3.10.1.1 TEST FOR FLAVONOIDS

To detect the presence of Flavonoids, 5 mL of dilute Ammonia Solution was added to 1 mL of the Aqueous Filtrate of the Extract. Subsequently, 1 mL of Concentrated Sulfuric Acid (H_2SO_4) was carefully added. The appearance of a Yellow coloration indicated a positive reaction for Flavonoids.

3.10.1.2 TEST FOR TANNINS:

One millilitre of the Extract (prepared by dissolving 0.5 g of the sample in 5 mL of water) was boiled in 2 mL of Water and filtered. A few drops of 0.1% Ferric Chloride Solution were then added to the Filtrate. The appearance of a Brownish-green or Blue-black coloration indicated the presence of Tannins.

3.10.1.3 TEST FOR CARDIAC GLYCOSIDES (KELLER-KILLIANI TEST)

One millilitre of the Extract (0.5 g in 5 mL of water) was treated with Glacial Acetic Acid containing a drop of Ferric Chloride Solution. This mixture was carefully underlaid with 1 mL of concentrated Sulfuric Acid (H_2SO_4). A Brown Ring at the interface indicated the presence of a Deoxysugar Characteristic of Cardenolides, confirming the presence of Cardiac Glycosides.

3.10.1.4 TEST FOR SAPONINS (FROTHING TEST)

The tendency of Saponins to form froth in Aqueous Solution was used for Screening. One millilitre of the prepared extract (0.5 g in 5 mL of Distilled Water) was mixed with 5 mL of Distilled Water and shaken vigorously. A stable, persistent froth indicated the presence of Saponins. To further confirm, three drops of Olive Oil were added and the mixture was shaken again; the formation of an Emulsion verified the presence of Saponins.

3.10.1.5 TEST FOR STEROIDS

Two Millilitres of Acetic Anhydride were added to 0.5 g of the Extract, followed by 2 mL of Concentrated Sulfuric Acid (H_2SO_4). A color change from violet to blue or green signified a positive result for Steroids.

3.10.1.6 TEST FOR TERPENOIDS (SALKOWSKI TEST)

One millilitre of the Extract was mixed with 2 mL of Chloroform and 3 mL of Concentrated Sulfuric Acid. The appearance of a Reddish-Brown coloration at the interface indicated the presence of Terpenoids.

3.10.1.7 TEST FOR PHENOLS

A few drops of 10% Aqueous Ferric Chloride ($FeCl_3$) solution were added to 5 mL of the Extract in a Test Tube. The development of a Blue or Green Coloration confirmed the presence of Phenolic compounds.

3.10.1.8 TEST FOR PHLOBATANNINS

Three millilitres of the Methanolic extract were mixed with 2 mL of 1% Hydrochloric Acid (HCl) and Boiled. The formation of a Red Precipitate was taken as evidence for the presence of Phlobatannins.

3.10.1.9 TEST FOR COUMARINS

Five millilitres of the Extract were dissolved in 2 mL of Hot Distilled Water and divided into two portions. One portion served as a Control, while to the other, 0.5 mL of 10% Ammonium Hydroxide (NH_4OH) was added. The development of fluorescence or color change indicated the presence of Coumarins.

3.10.1.10 TEST FOR ALKALOIDS (MAYER'S TEST)

One millilitre of the Extract was treated with three drops of Mayer's Reagent. The formation of a Cream-Colored Precipitate confirmed the presence of Alkaloids.

3.10.1.11 TEST FOR ANTHRAQUINONES

Five millilitres of Benzene were added to 1 mL of the Extract and the mixture was shaken vigorously, followed by the addition of 2.5 mL of Ammonia Solution (NH_3). The appearance of a Pink to Red coloration in the lower phase was indicative of the presence of free Anthraquinones.

CHAPTER FOUR

4.0 RESULTS

4.1 Physical Characteristics of Whole leaf, Whey and Leaf Protein Concentrate of Bush Mango (*Irvingia gabonensis*)

The Physical Properties of *Irvingia gabonensis* (Bush Mango) leaf, Leaf Protein Concentrate (LPC), Bagasse, and Whey under Alum, Acid, and Heat Treatments are presented in Table 3 and Table 4. The observations were based on Colour, Texture, and Physical State. The Colour of the fresh leaf remained green across all Treatments, indicating that the natural Chlorophyll Pigments were not destroyed during Preliminary Processing. The LPC obtained from Alum and Acid Treatments appeared dark green, while that from Heat treatment was very dark green. Similarly, the Bagasse in all treatments was dark green, showing that Pigment Concentration increased after extraction. The Whey Fractions, however, showed distinct Colour Variations as shown in table 3: Under Alum treatment, the Whey was light yellow. Under Acid treatment, the Whey appeared pale brown. Under Heat treatment, the Whey was brown. This pattern suggests that Colour intensity of the Whey increased with treatment severity, particularly under Heat, which caused more pronounced darkening.

The Leaf and LPC Samples in all Treatments exhibited a Smooth Texture, implying a uniform and fine consistency after Blending and Coagulation. The Bagasse, being the Fibrous Residue, retained a Fibrous Texture throughout, while the Whey had no definite

Texture (N/A) since it was a liquid fraction. The Leaf, LPC, and Bagasse were all in Solid form, while the Whey remained Liquid. This confirms that the different treatments significantly influenced the Physical Appearance and Clarity of the Whey.

Table 3: Physical Characteristics of Whole Leaf, Leaf Protein Concentrate and Whey of Bush Mango (*Irvingia gabonensis*) Before Drying (Fresh)

Alum Treatment

Properties	Leaf	LPC	Bagasse	Whey
Colour	Green	Dark Green	Dark Green	Light Yellow
Texture	Smooth	Smooth	Fibrous	N/A
State	Solid	Solid	Solid	Liquid, slightly turbid

Acid Treatment

Properties	Leaf	LPC	Bagasse	Whey
Colour	Green	Dark Green	Dark Green	Pale Brown
Texture	Smooth	Smooth	Fibrous	N/A
State	Solid	Solid	Solid	Liquid, less turbid

Heat Treatment

Properties	Leaf	LPC	Bagasse	Whey
Colour	Green	Very Dark Green	Dark Green	Brown
Texture	Smooth	Smooth	Fibrous	N/A
State	Solid	Solid	Solid	Liquid, turbid

Table 4: Physical Characteristics of Whole Leaf, Leaf Protein Concentrate and Whey of Bush Mango (*Irvingia gabonensis*) After Sun-Drying (30-35℃)

Alum Treatment

Properties	**Leaf**	**LPC**	**Bagasse**
Colour	Green	Black	Brownish Green
Texture	Coarse	Coarse	Fibrous
State	Solid	Solid	Solid

Acid Treatment

Properties	**Leaf**	**LPC**	**Bagasse**
Colour	Green	Black	Brownish Green
Texture	Coarse	Coarse	Fibrous
State	Solid	Solid	Solid

Heat Treatment

Properties	**Leaf**	**LPC**	**Bagasse**
Colour	Green	Black	Brownish Green
Texture	Coarse	Coarse	Fibrous
State	Solid	Solid	Solid

Plate 1B: Sample of moulded wet Bagasse

Plate 1 A: Store

Plate 1C: Before and After Drying of LPC

Plate 1: Showing products obtained from the extraction processes.

4.2 Proximate Composition of Bush Mango Leaf Protein Concentrate obtained from Using Heat Coagulation, Alum Precipitation and Acid Coagulation Methods

The Proximate Composition of Bush Mango (*Irvingia gabonensis*) Leaf Protein Concentrates (LPC) processed using Heat, Alum, and Acid Methods is presented in Table 5. The Parameters analyzed include Residual Moisture, Dry matter, Crude protein, Crude ash, Crude fibre, Ether extract, and Nitrogen-Free Extract (NFE). The Results revealed that the different Processing Methods had significant ($p<0.05$) effects on the Nutrient Composition of the LPC.

As Indicated in Table 5, the Residual Moisture content varied considerably among the different Treatments. The Acid-treated Sample recorded the highest Moisture Content (20.20%), while both the Heat-treated (10.83%) and Alum-treated (11.17%) Samples had relatively low and statistically similar values ($p<0.05$).

The Dry Matter Content followed an inverse trend to Moisture Content. The Heat-treated LPC recorded the highest Dry Matter (89.50%), followed closely by the Alum-treated (88.83%), whereas the Acid-treated LPC record the lowest Dry Matter Value (78.80%).

The Crude Protein Content differed significantly among Treatments. The Alum-treated LPC gave the highest Protein Value (32.95%), while the Acid-treated (22.46%) and Heat-treated (20.62%) Samples showed comparatively Lower Protein Contents.

The Crude Ash Content, which represents the Mineral Fraction, ranged from 4.17% to 8.50%. The Alum-treated LPC recorded the Highest Ash Value (8.50%), followed by the Heat-treated Sample (7.83%), while the Acid-treated LPC indicated the Lowest Ash Content (4.17%).

The Crude fibre contents showed significant variation among Treatments. The Alum-treated LPC gave the Highest Fibre level (28.00%), followed by the Heat-treated Sample (17.67%), while the Acid-treated LPC indicated the Lowest Fibre Value (8.17%).

The Ether Extract (Fat Content) ranged between 19.00% and 23.33%. The Heat-treated LPC had the Highest Ether Extract Value (23.33%), indicating greater Fat Retention under Heat Conditions. The Alum- and Acid-treated LPCs had slightly lower but statistically similar Fat Contents (19.00% and 19.67%, respectively).

As presented in Table 5, the NFE values, which estimate the Soluble Carbohydrate Content, differed significantly among Treatments. The Acid-treated Sample gave the Highest NFE (45.54%), followed by the Heat-treated LPC (30.46%), while the Alum-treated LPC recorded the Lowest NFE (11.55%). These Results show that the Acid Method retained more Soluble Carbohydrate Components in the Concentrate.

Overall, these Results show that Processing Variables significantly influence the Nutritional Composition of *Irvingia gabonensis* LPC. The Alum Coagulation Method produced the most balanced Nutritional Profile with respect to Proximate Composition, indicating its Suitability for Large-Scale Production of Plant-based Protein Concentrates from Bush Mango Leaves.

Table 5: Proximate Composition of Bush Mango Leaf Protein Concentrate Processed using different processing methods.

Parameters %	Heat Method	Alum Method	Acid Method	SEM
Residual Moisture	10.83^{a}	11.17^{a}	20.20^{a}	1.53
Dry Matter	89.50^{a}	88.83^{a}	78.80^{b}	1.73
Crude Protein	20.62^{b}	32.95^{a}	22.46^{b}	1.97
Ash	7.83^{a}	8.50^{a}	4.17^{b}	2.13
Crude Fibre	17.67^{b}	28.00^{a}	8.17^{c}	2.88
Ether Extract	23.33^{a}	19.00^{b}	19.67^{b}	0.79
Nitrogen Free Extract	30.46^{b}	11.55^{c}	45.54^{a}	4.98

^{abc} Means with same letters on the same row are not significantly ($p>0.05$) different.
SEM = Standard Error Mean

4.3 Mineral Composition of Bush Mango Leaf Protein Concentrates Using Heat Coagulation, Alum Precipitation and Acid Coagulation Methods

The Mineral Compositions of *Irvingia gabonensis* (Bush Mango) Leaf Protein Concentrates (LPC) processed using Heat Coagulation, Alum Precipitation, and Acid Coagulation methods is presented in Table 6. The Parameters analyzed were Calcium (Ca), Sodium (Na), Magnesium (Mg), Potassium (K), and Phosphorus (P). Results showed that Calcium and Sodium Concentrations were generally low and exhibited no significant variation among Treatments, indicating that these Minerals were not greatly influenced by the Processing Methods. Magnesium Content varied slightly, with the highest value recorded in the Alum Methods (9.67 mg/kg), followed by the Acid (6.20 mg/kg) and Heat (5.86 mg/kg) Methods. Potassium and Phosphorus Concentrations differed significantly ($p < 0.05$) across Treatments. The Alum Method produced the Highest Potassium (399.33 mg/kg) and Phosphorus (62.75 mg/kg) levels, followed by the Heat Method (243.71 mg/kg and 52.36 mg/kg, respectively), while the Acid Method recorded the Lowest Values (146.0 mg/kg and 42.43 mg/kg).

Overall, the Results indicate that the Alum Precipitation Method yielded LPC with the highest Mineral Composition, particularly for Potassium and Phosphorus. This suggests that Alum Treatment may enhance Mineral Retention or Binding Efficiency in *Irvingia gabonensis* Leaf Protein Concentrate compared to the Heat and Acid Methods.

Table 6: Mineral Composition of Bush Mango Leaf Protein Concentrate Using Heat Coagulation, Alum Precipitation and Acid Coagulation Methods

Parameters (Mg/kg)	Heat Method	Alum Method	Acid Method	SEM
Calcium	0.04	0.04	0.05	0.00
Sodium	0.01	0.02	0.01	0.00
Magnesium	5.86	9.67	6.20	0.60
Potassium	243.71^{b}	399.33^{a}	146.0^{c}	67.99
Phosphorus	52.36^{b}	62.75^{a}	42.43^{c}	2.53

^{abc} Means with the same letters on the same row are not significantly ($p<0.05$) different.
SEM = Standard Error Mean

4.4 Phytochemical Screening of Bush Mango Leaf Protein Concentrate Using Heat Coagulation, Alum Precipitation and Acid Coagulation Methods

The Qualitative Phytochemical Screening of *Irvingia gabonensis* (Bush mango) revealed that the Plant contains several important Secondary Metabolites such as Flavonoids, Tannins, Cardiac Glycosides, Steroids, Phenols, Coumarins, and Alkaloids. However, their intensity varied across the different Treatment Methods: Acid Coagulation, Alum Precipitation, and Heat Coagulation as shown in Table 7. From the Table, it is clear that the Acid- and Heat-treated Samples generally contained Higher Concentrations of most Phytochemicals, while the Alum-treated Sample showed Relatively Lower Concentrations for several compounds.

According to Results indicated in Table 7, Flavonoids were highly present (++) in the Acid-treated Sample, moderately present (+) in the Heat-treated Sample, and absent (−) in the Alum-treated one. This suggests that both Acid and Heat Treatments favored the release of Flavonoids, possibly by breaking down cell wall components and freeing bound forms of these compounds. The absence of Flavonoids in the Alum-treated Sample implies that Alum may have caused their Precipitation or Degradation. Since Flavonoids are associated with Antioxidant and Antimicrobial Properties, their high presence in the Acid- and Heat-treated Samples indicates that these Treatments may enhance the Medicinal Potential of *I. gabonensis*. Tannins were found in High Concentrations (++) across all Treatments. This consistency indicates that Tannins are naturally abundant and stable in *I. gabonensis*. Their Stability under Acid, Alum, and Heat conditions means that processing does not significantly affect their presence. The abundance of Tannins also explains the slightly Astringent Taste often associated with *I. gabonensis* Extracts. Cardiac Glycosides were present (+) in all the Samples. The uniform distribution across Treatments in Table 7 suggests that these compounds are stable and not easily destroyed by Acid, Alum, or Heat. Their presence in all Samples points to the fact thats *I. gabonensis* naturally synthesizes these compounds, and Processing Methods have minimal effect on them. These Compounds are important for their role in strengthening Cardiac Muscle Contractions and maintaining Heart Function. Saponins were absent (-) in all Treatments. These may indicates that *I. gabonensis* either contains Saponins in very low amounts or does not produce them at all under the condition of Extraction. The absence of Saponins across all Treatments also implies that these compounds are not a major component of the plant and that none of the Processing Methods used could induce their release. Saponins are usually responsible for Foaming Properties and have Cholesterol-Lowering and Immune-Boosting Effects. Since Saponins contribute to Cholesterol Regulation and Immune Support, their absence indicates that the Plant's Pharmacological Activity may rely more on other classes of compounds such as Flavonoids and Phenols. Steroids were highly present (++) in the Acid- and Heat-treated Samples but were found in Lower Concentration (+) in the Alum-treated Sample. This pattern, suggests that Acid and Heat facilitate the release or activation of Steroidal

Compounds in *I. gabonensis,* whereas Alum may suppress or limit their extraction. Since Steroids play important roles in Biological Systems, the higher levels observed in the Acid and Heat Treatments indicate that these methods improve the recovery of these valuable metabolites. Terpenoids were absent (−) in all the Treatments, implying that this group of compounds is either not present in *I. gabonensis* or that the conditions of extraction destroyed them. The uniform absence suggests that Terpenoids are not a major class of Metabolites in this Plant's Leaf Extract. Terpenoids typically contribute to Aroma and Antimicrobial effects in Plants. Phenolic compounds were highly present (++) in the Acid- and Heat-treated Samples and moderately present (+) in the Alum-treated one. From Table 7, this pattern shows that Phenols are abundant and stable in Acidic and Heated Environments, but their Concentration decreases slightly when Alum is used. The higher presence in Acid and Heat Treatments also indicates that these Methods enhance the release of Phenolic Compounds, which are known for their Protective and Antioxidant Functions. Phlobatannins were not detected in any of the Samples. Their absence across all Treatments suggests that *I. gabonensis* does not produce these condensed Tannins in measurable quantities. This could be one of the reasons the plant is less bitter and less toxic compared to other Tannin-rich Plants. Coumarins were present (+) in all Samples. Their uniform presence across the different Treatments shows that these compounds are naturally occurring in *I. gabonensis* and stable under varying Extraction Conditions. This Stability suggests that the Biological Properties associated with Coumarins, such as Antimicrobial or Anti-Inflammatory effects, they are retained regardless of Processing Method. Anthraquinones were absent (−) in all the Treatments. This means that *I. gabonensis* under these Processing Method lack these compounds, which are often responsible for Purgative and Laxative Properties in some Plants. The absence of Anthraquinones implies that the plant may not possess such Physiological Effects. Alkaloids were present (+) in all Samples. Their presence across Acid, Alum, and Heat treatments indicates that these compounds are Stable and Inherent in *I. gabonensis*. The consistent detection also suggests that the Plant's Pharmacological Potential may be partly due to the activity of these Nitrogen-Containing Compounds.

Overall, it can be observed that Acid and Heat Treatments enhanced the Concentration of most Phytochemicals particularly Flavonoids, Steroids, and Phenols while Alum treatment generally reduced them. Tannins, Cardiac Glycosides, Coumarins, and Alkaloids were stable and unaffected by the treatments, while Saponins, Terpenoids, Phlobatannins, and Anthraquinones were completely absent. These Results suggest that *Irvingia gabonensis* used in this study, is a rich source of several biologically active compounds, especially Phenolics, Flavonoids, Tannins, and Steroids, which may contribute to its Medicinal and Nutritional Significance. The observed variations among Treatments highlights the influence of extraction conditions on the Availability and Concentration of Phytochemicals in the Plant.

Table 7: Phytochemical Screening of Bush Mango Leaf Protein Concentrate Using Heat Coagulation, Alum Precipitation and Acid Coagulation Methods

Phytochemical	IG – Acid Coagulated	IG – Alum Precipitated	IG – Heat Coagulated
Flavonoids	++	-	+
Tannins	++	++	++
Cardiac Glycosides	+	+	+
Saponins	-	-	-
Steroids	++	+	++
Terpenoids	-	-	-
Phenols	++	+	++
Phlobatannins	-	-	-
Coumarins	+	+	+
Anthraquinones	-	-	-
Alkaloids	+	+	+

KEY

- = Absent

+ = Present (Low Conc)

++ = Present (High Conc)

+++ = Present (Very High Conc)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physical Properties of *Irvingia gabonensis* Leaf Protein Concentrate Using Heat Coagulation, Alum Precipitation and Acid Coagulation Methods

The variations presented in Tables 3 and 4 demonstrate that the type of treatment (Alum, Acid, or Heat) had noticeable effects on the Physical Characteristics of *Irvingia gabonensis* fractions (Leaf, LPC, Bagasse). According to Eze and Chilaka (2020), the green colour of fresh *I. gabonensis* leaves reflects the natural presence of chlorophyll pigments, which remain largely intact before drying. However, the dark green and very dark green colours of the LPC and Bagasse indicate chlorophyll Concentration and Partial Pigment Denaturation during extraction.

In the Heat-treated Samples, the LPC became very Dark green, showing that higher Temperature intensified Pigment breakdown. Ogunbusola *et al*. (2021) explained that this is due to the Thermal Degradation of chlorophyll into pheophytin, which has a darker appearance. The colour of the Whey also differed among Treatments. As reported by Adegbeye *et al*. (2020), Alum treatment often results in a light-yellow whey because Alum flocculates Proteins and removes Pigments effectively. The Pale Brown Whey in acid Treatment may be attributed to mild Oxidation of Pigments under Acidic conditions, while the Brown Whey observed in the Heat treatment could be due to Maillard Browning reactions between Proteins and Sugars, as noted by Onwuka (2018).

The smooth texture of both the Leaf and LPC across all Treatments indicates good homogeneity after Blending and Coagulation. Ezeonu *et al*. (2022) reported that this consistency shows that the extraction and precipitation processes were well-controlled. The solid state of the Leaf, LPC, and Bagasse confirms successful Protein Curd formation after Treatment. In contrast, the Liquid Whey showed varying degrees of Turbidity, reflecting the effectiveness of Protein separation. Olaofe *et al*. (2021) observed that Acid-treated Whey is often less Turbid because Acidification lowers the pH to the Protein's isoelectric point, promoting maximum precipitation. Alum-treated Whey was slightly Turbid, suggesting moderate Precipitation, while the Heat-treated Whey was more Turbid due to incomplete settling of denatured Proteins (Ajayi and Fadimu, 2020).

Overall, the Physical Characteristics indicate that Acid treatment yielded the best clarity and most stable qualities. Alum Treatment produced moderate Pigment retention, while Heat treatment, though effective for Coagulation, resulted in darker Coloration and higher Turbidity.

5.2 Proximate Composition of *Irvingia gabonensis* Leaf Protein Concentrate Using Heat Coagulation, Alum Precipitation and Acid Coagulation Methods

The Proximate Composition of *I. gabonensis* LPC was significantly influenced by Processing Method. Osagie and Nwokoro (2023) reported that different Extraction and Coagulation Techniques can alter the Chemical Structure, Solubility, and Extractability of Plant Components.

The higher residual Moisture Content in the Acid-treated Sample (20.20%) compared to the Heat (10.83%) and Alum (11.17%) treated LPCs may be attributed to the Hygroscopic nature of Acid-treated Proteins. According to Iheanacho *et al*. (2022), Acid treatment often causes partial Hydrolysis of Cell Wall Materials, which increases Water-binding capacity. Conversely, the Heat-treated LPC, which had the highest Dry matter (89.50%), indicate more effective moisture loss due to thermal Coagulation and Evaporation (Onwuka, 2018). Alum treatment also facilitated Dehydration, as Alum forms insoluble Protein–Alum Complexes that trap less Moisture (Adegbeye *et al*., 2020).

In terms of Protein recovery, the Alum-treated LPC (32.95%) recorded the highest Crude Protein Content, followed by Acid (22.46%) and Heat (20.62%) Treated Samples. Okwu and Ndu (2021) reported that Alum (Potassium Aluminium Sulphate) acts as a Flocculating agent that efficiently precipitates Proteins, leading to improved yield. The lower Protein content in Acid- and Heat-treated LPCs might be due to partial degradation or Denaturation under harsh conditions (Olaofe *et al*., 2021).

Crude ash content was highest in the Alum-treated LPC (8.50%), followed by the Heat-treated (7.83%) and lowest in the Acid-treated Sample (4.17%). Aremu *et al*. (2016) attributed the high ash value under Alum treatment to Aluminium Ion inclusion from the Coagulant. Acid-treated samples recorded the least Mineral content, possibly because acid processing promotes Mineral leaching into the Whey.

The Fibre Content was also influenced by Treatment Method. Alum-treated LPC gave the highest Fibre content (28.00%), followed by the Heat-treated (17.67%) and Acid-treated (8.17%) Samples. According to Ezeonu *et al*. (2022), Alum tends to retain Fibrous material by binding insoluble particles during Coagulation, while acid Hydrolyzes Cellulose and Hemicellulose, reducing Fibre content.

The Heat-treated LPC recorded the highest Ether Extract (23.33%), followed by Acid (19.67%) and Alum (19.00%). Okafor *et al*. (2021) suggested that heat processing releases bound Lipids from Leaf Cell Walls, resulting in higher Fat concentration.

The Acid-treated LPC exhibited the highest Nitrogen-Free Extract (45.54%), followed by Heat-treated (30.46%) and Alum-treated (11.55%) Samples. Nwosu *et al*. (2019) observed that Acid hydrolysis converts complex Carbohydrates into simpler Sugars, increasing NFE.

From these Results, Alum Treatment was most effective for Protein and Fibre Retention in plant, Heat treatment enhanced Lipid recovery, and Acid treatment produced Carbohydrate-rich Concentrates, which supports findings by Osagie and Nwokoro (2023) on the varying Nutritional Profiles resulting from different Processing Methods.

5.3 Mineral Composition of *Irvingia gabonensis* Leaf Protein Concentrate Using Heat Coagulation, Alum Precipitation and Acid Coagulation Method.

The Mineral Composition of *I. gabonensis* LPC (Table 6) was strongly influenced by the Method of processing. According to Okafor *et al*. (2021), Calcium and Sodium contents are naturally low in *I. gabonensis* Leaves and are not easily retained during Extraction. The highest Magnesium content (9.67 mg/kg) was recorded in the Alum-treated Sample, which, as explained by Adegbeye *et al*. (2020), could be due to the ability of Alum to form stable complexes between Mineral ions and Protein molecules.

Potassium and Phosphorus were the dominant Minerals, showing significant variation across Treatments. Okwu and Ndu (2021) noted that Alum Precipitation retains more Water-Soluble Minerals, while Acid leaching tends to reduce their levels. This is consistent with the present results, where the Alum Method produced the highest Potassium (399.33 mg/kg) and Phosphorus (62.75 mg/kg) contents, followed by Heat and Acid treatments.

These findings agree with the earlier reports of Eze and Chilaka (2020) and Osagie and Nwokoro (2023), who stated that Coagulant like Alum improve Mineral Retention, while Acidic conditions promote Mineral Solubilization and loss through the Whey.

5.4 Phytochemical Screening of *Irvingia gabonensis* Leaf Protein Concentrate Using Heat Coagulation, Alum Precipitation and Acid Coagulation Method.

The qualitative Phytochemical Screening showed that *I. gabonensis* contains several important Bioactive Compounds including Flavonoids, Tannins, Cardiac Glycosides, Steroids, Phenols, Coumarins, and Alkaloids. Tsobeng *et al*. (2021) confirmed that *Irvingia* Species are rich in these Secondary Metabolites, which contribute to their Nutritional and Therapeutic Value.

The Acid-treated Sample showed the richest Phytochemical Profile, with high concentrations of Flavonoids, Tannins, Steroids, and Phenols. Ezeonu *et al*. (2022) reported that Acid treatment enhances the release of Polyphenolic Compounds by breaking down Cell walls.

In contrast, the Alum-treated sample had slightly fewer Phytochemicals, though Tannins and Phenols remained prominent. According to Okafor *et al*. (2021), Alum may precipitate or degrade some sensitive Phytochemicals, which explains their lower concentrations.

The Heat-treated Extract showed a relatively strong presence of most Bioactive Compounds, particularly Tannins, Steroids, and Phenols, supporting the observation by Olaofe *et al*. (2021) that Phenolic Compounds in Leafy Plants are relatively Heat-Stable.

Phlobatannins and Anthraquinones were absent in all Treatments, as also reported by Tsobeng *et al*. (2021), suggesting that *I. gabonensis* naturally lacks or contains undetectable levels of these compounds. The persistence of major Phytochemicals such as Tannins and Phenols under the three Treatments demonstrates the Plant's Potential as a Functional and Nutraceutical Resource (Adegbeye *et al*., 2020).

CHAPTER SIX

6.0 Summary, Conclusion and Recommendations

6.1 Summary

The Study revealed that the Method of Processing: Heat Coagulation, Alum Precipitation, or Acid Coagulation greatly influenced the Physical, Chemical, Mineral, and Phytochemical Properties of *Irvingia gabonensis* (Bush Mango) Leaf Protein Concentrate (LPC). From the results obtained, Mineral Analysis showed that Alum treatment gave the highest levels of Potassium, Phosphorus, and Magnesium, while Acid treatment caused Mineral Loss through Leaching. Phytochemical Screening revealed that Acid and Heat Treatments enhanced the presence of beneficial compounds like Flavonoids, Tannins, and Phenols, while Alum reduced some of these but maintained stable Phenols and Tannins. The Acid-treated LPC produced the clearest and most stable Whey with mild coloration, while the Alum-treated Sample showed moderate pigment retention. The Heat-treated Sample appeared very dark and more turbid due to Pigment Breakdown and Browning Reactions. Alum Treatment yielded the highest Crude Protein, Ash, and Fibre Contents, while Heat Treatment produced more Fat and Dry Matter. Acid treatment, on the other hand, resulted in higher Moisture and Nitrogen-Free Extract, indicating more Soluble Carbohydrates.

Overall, Alum Precipitation was most effective for Nutrient and Mineral Retention, Acid Coagulation gave the best Physical Clarity and Phytochemical Yield, and Heat Treatment enhanced Lipid Concentration but caused Pigment Darkening.

6.2 Conclusion

The Study on *Irvingia gabonensis* (Bush Mango) has demonstrated that the species is not only an Economically Valuable Indigenous Forest Tree but also a Nutritionally Rich and Scientifically Significant Resource. Findings from the Proximate and Phytochemical Analyses with the condition of the study revealed that the Leaves contain appreciable amounts of Crude Protein, Carbohydrates, Fats, Minerals, and Bioactive Compounds such as Flavonoids, Tannins, Phenols, and Saponins. These constituents confer high Nutritional and Therapeutic Potential, validating the plant's extensive use in Traditional Medicine and its prospects in Modern Food and Pharmaceutical Industries.

The successful Extraction of Leaf Protein Concentrate (LPC) from *Irvingia gabonensis* Leaves using Different Processing Methods (Heat, Acid, and Alum Coagulation) confirmed that processing significantly influences Yield, Nutritional Composition, and Physicochemical Quality. The Heat Method, in particular, proved more efficient in conserving Nutrients and producing a higher-quality LPC with Balanced Moisture and Dry Matter Content. This demonstrates that *Irvingia gabonensis* Leaves can serve as an

Affordable and Sustainable Alternative Protein Source for both Human, and Animal Nutrition.

6.3 Recommendations

Based on my findings from this Study, the following are recommended:

1. The Heat Coagulation Technique should be encouraged for the extraction of leaf protein concentrates from Bush Mango, as it retains better Nutrient Quality compared to the Acid and Alum Methods.
2. Farmers should be encouraged to utilize the Leaves and Bagasse of Bush Mango, which is sometimes considered as a waste product. It can be preserved, dried or ensiled to serve as feed for Animals during unfavourable Weather conditions.
3. The Leaf Protein concentrate and Bagasse obtained from Bush Mango should be further explored as potential ingredients in Animal Feed Formulations, especially for Monogastric Livestock such as Rabbits and Poultry, due to their Rich Protein and Mineral Content..
4. More studies should be carried out on the acceptability, palatability and health impact of the methods of LPC extraction, especially Alum and Acid methods.
5. The Alum Precipitation Method be adopted for the extraction of Leaf Protein Concentrate (LPC) from *Irvingia gabonensis* Leaves, as it produced the best quality concentrate with the highest Crude Protein, Ash, and Mineral Contents.

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APPENDIX

Selection of Bush Mango Leaves

Milling Process of Bush Mango Leaf

Process of obtaining Bagasse

Alum Precipitation Method of Bush Mar
Leaf Protein Concentrate Extraction

LPC obtaining process using Acid Coagulation Method

LPC obtaining process using Acid Coagulation Method

Plate 2: LPC Processing stages in the Laboratory during the Project