YIELD, PHYSICAL CHARACTERISTICS AND CHEMICAL COMPOSITION OF LEAF PROTEIN CONCENTRATES AND BAGASSE OBTAINED FROM GMELINA (G*melina aborea,* Roxb. ex.sm) LEAVES USING TWO DIFFERENT METHODS

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BENIN CITY, NIGERIA

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA, IN PARTIAL FUFILMENT 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 **Laura Ovbiokhan USI-AGBONTAEN (Miss)** with Matriculation Number, **AGR1900146** 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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(Project Supervisor)	**(Head of Department)**
DATE:________________	**DATE:________________**

DEDICATION

This Project work is dedicated to the Almighty God, for His provisions, grace, favour, wisdom, understanding and never ending love throughout the course of this Research and Study.

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ABSTRACT

The Experiment was conducted to determine the physical properties and nutritional composition of the Leaf protein concentrates and bagasse obtained from Gmelina (*Gmelina arborea*) leaves using alum precipitation and heat coagulation methods.

The extraction process of leaf protein from Gmelina leaf was carried out in the main Laboratory of the faculty of Agriculture, University of Benin. After extraction it was observed that GLPC obtained from alum precipitation was light green with fine and powedery feeling while the GLPC obtained from heat coagulation was dark green in colour with fine and powedery feeling.

The yield of GLPC obtained using alum precipitation method (8.13%) was obviously higher (5.24%) than that obatined from heat coagulation method. The bagasse yields were (22.47 and 25.14%) respectively for both alum precipitation and heat coagulation method. The result from the expirement shows higher dry matter, crude protein, crude fibre and crude ash contents in GLPC extracted using alum precipitation method compare to those extracted using heat coagulation method. Crude fat and NFE on the contrary were higher significantly in GLPC extracted using heat coagulation method. Elemental Phosphorus, potassium, nitrogen, zinc, iron, manganese and copper were higher in GLPC extracted using alum precipitation method than that obtained in GLPC from heat coagulation method. Other parameters assayed for mineral components of the LPC were higher in those extracted using heat coagulation method. It is concluded based on the above findings that Gmelina leaf protein concentrates obtained using alum precipitation would be preferred as a result of its high crude protein content and qualitative mineral composition should therefore be considered as a viable substitute for more expensive conventional protein feed materials like soya bean meal etc. in livestock feed formulation.

CHAPTER ONE

1.0 INTRODUCTION

Approximately 815 million to one billion people worldwide, predominantly in developing countries, suffer from malnutrition or protein deficiency (FAO, 2015). In 2000 alone, hunger or the result of hunger caused the premature death of around 36 million undernourished people (Gasperini and Maguire, 2002; Ghaly and Alkoaik, 2010). With current average birth rates, it is estimated that the global population will reach 9.8 billion by the year of 2050 (United Nations, 2017). As a result, malnutrition and food security will continue to be a critical concern.

There is a shortage of supply of good quality protein for meeting the requirement of increasing animal and human population, which has necessitated the search or additional sources (Isuosuo, 2012). In order to solve protein deficiency and related malnutrition problems, novel sources of protein need to be identified. Leaf protein from many grass species are considered to be edible (Lim, 2016) and an underutilized fraction of biomass. Leaf Protein Concentrates (LPCs) are further abundant and available potentially for human diets (Tenorio *et al.,* 2017). Numerous studies on the isolation of leaf proteins have been conducted, and related technologies have been developed to varied extents (Fiorentini and Galoppini, 1983; Castellanos *et al.,* 1994; Rao *et al.,* 2007). The nutritional value of LPCs has further been found to be comparable to animal protein isolates and superior or similar to seed proteins (Badar and Kulkarni, 2011).

Trees have been suggested as a potential source of leaf protein concentrate and the production of proteins is advantageous over crops as they do not involve the recurring cost of cultivation (Suman *et al.,* 2014). Leaf protein concentrate (LPC) is a concentrated form of the proteins found in the leaves of plants, leaf protein concentrates was first introduced as a human food in the 1960's but it has not archived much success despite early promises because of certain palatability issues (Pirie, 1971; Signh, 1984). Leaf protein concentrate obtained from a green crop fractionation is free from indigestible fibre and it contains high proportion of proteins which are nutritionally superior. For this reason, its use in the nutrition of human beings and other non-ruminants has been advocated. Apart from proteins, it contains appreciable amounts of vitamin A, minerals, fat and vitamin E. to enhance yield and usability, several methods for the preparation of LPC from leaf juice have been advocated for which include, heating, acidification, natural acidification due to anaerobic fermentation of juice. It is reported that the yield of the leaf protein concentrate depends on the method used in extraction (Sayyed, 2011). Example of such leaf to be assessed for its nutritional value in this study is the leaf from the *Gmelina arborea* tree.

Gmelina arborea is a tree species of Lamiaceae family that thrives in a different forest habitat. The species grow well in the tropical regions but are adaptive to a wide range of elevation ranging from 0 to 1200 m above the mean sea level. The species is native to South Asian Countries like India, Myanmar, Thailand, Laos, Cambodia, South province of China, Vietnam, Sri Lanka, Pakistan, etc. In India, it is distributed in the foothills of

North Western Himalayas, Western Ghats and widespread through the Deccan Peninsular region. Again, it is also found with good population in the Andaman Islands (Berry *et al.,* 2021; Ashalata and Sankh 2014). In India, the species has gained great importance due to its use in the ancient therapeutic system like the Ayurveda. Apart from its therapeutic values, each part of the tree including flowers, leaves, fruits, wood, bark, and root has species uses that benefit not only human but also birds and bees. The trees over the years has been established and domesticated in Africa having most of its existence in Nigeria. It grows mainly or cultivated majorly in the Western part of Nigeria. It is fell majorly for its trunk and research has found that the leaves could be a source of protein for livestocks if properly processed.

1.1 Objectives of the study

The broad objectives of the study was to determine the physical properties and nutritional composition of the Leaf protein concentrates and bagasse obtained from Gmelina (*Gmelina arborea*) leaves using two different methods of extraction.

1.2 Specific objectives

The specific objectives were to:

1. determine the yield of leaf protein concentrates and bagasse from *Gmelina arborea* leaves obtained using two different methods of processing;

2. physical characteristics of leaves protein concentrates and bagasse obtained from *Gmelina arborea* leaf using two different methods of extractions;
3. determine the proximate and some mineral composition of the leaf protein concentrates and bagasse obtained from *Gmelina arborea* leaf using two different methods of extraction.

CHAPTER TWO

2.0 LITERATURE REVIEW

Gmelina (*Gmelina arborea*) is an evergreen perennial fast growing medicinal tree, which is used as laxative and anthelmintic, improves appetite, is useful in hallucination, piles, abdominal pains, burning sensations, fevers, and urinary discharge (Kumaresan *et al.,* 2014). The leaves are unconventional materials that can be explored for the production of feedstuff. The leaf is one of the tree leaves considered an important source of nutrient for ruminants and non-ruminants, especially in those areas with pronounced dry season. Previous records by Nkwocha *et al.* (2014) and Offor (2014) on the nutrient profile of Gmelina arborea have shown that the leaf meals contained 18.00-20.05% crude protein, 14.40-15.05% crude fibre, ash 4.55% and fat 0.79%. Metabolizable energy values were found to be appreciable (1368 Kcal kg-1) - an indication of its suitability as an energy source for livestock diets (Amata, 2014). The leaves, flower, roots and bark are used in medicine. They are useful in the treatment of blood diseases;

2.1 Origin, Distribution and Habitat

Gmelina arborea, (Beechwood, Gmelina, Goomar Teak, Kashmir tree, Malay Beechwood, White teak, yamane), locally known as gamhar, is a fast-growing deciduous tree in the Family *Lamiaceae*. *Gmelina arborea* grows naturally throughout India, Myanmar, Thailand, Laos, Cambodia, Vietnam and in southern provinces of China. It is found at altitudes from sea level to 1,500 metres. Since the 1960s, it has been introduced extensively as fast-growing timber trees in Brazil, Gambia, Honduras, Ivory Coast, Malaysia, Malawi, Nigeria, the Philippines, and Sierra Leone. It is also planted in Gardens and Avenues.

The Family *Lamiaceae* comprises around 236 genera and 7173 species distributed worldwide (Stevens, 2012). The genus *Gmelina* includes 38 species of trees occurring in tropical Asia, Australia and New Guinea (Flora of China Editorial Committee, 2016). Gmelina is related to the large genus Vitex, which also includes several Timber trees. Vitex can be distinguished by its more narrowly funnel-shaped corolla and less fleshy drupe (Flora of China Editorial Committee, 2016; PROTA, 2016).

The genus *Gmelina*, here assigned to the family *Lamiaceae*, is sometimes placed in the *Verbenaceae*. It was first described by Roxburgh in 1832 and later in a more complete form by Hooker in 1885 (Brandis, 1924). *G. arborea* var. canescens and var. glaucescens (Haines 1910, cited by Greaves, 1981) are differentiated from the type variety by their lamina features, i.e. stellately-hairy beneath (type), subcoriaceous and Grey-pubescent with simple hairs (var. canescens) and glaucous and glabrous beneath (var. glaucescens). The genus name *Gmelina* was named by Carl Linnaeus in honour of the German Botanist, Johann Georg Gmelin. The specific name means treelike, from the Latin 'arbor' (tree). *Gmelina arborea*, (in English beechwood, gmelina, goomar teak, Kashmir tree, Malay beechwood, white teak, yamane, locally known as *gambhari*...

2.2 Description of *Gmelina arborea*

G. arborea is a medium-size tree which produces a clear, but frequently bent, bole. It reaches 30 m tall (e.g. in Myanmar) on favourable sites. However, it can become shrubby in drier environments, such as the Punjab and other parts of India. *G. arborea* has low, obscure buttresses and a yellowish-grey, smooth bole. The branches are numerous and spread to form a large shading crown. The corky bark is yellow inside and greyish-white outside when young, turning brown with age. The old bark then sheds, producing patches of paler bark on the bowl (China Editorial Committee, 2016).

2.2.1 Foliage

Leaves are simple, normally cordate, broad-ovate acuminate, about 10-25 cm long and 5-18 cm broad, denticulate, with about 3-5 primary veins originating from the base and 4-6 pairs of secondary veins. The petiole is about 7.5-12.0 cm long. A pair of shining glands is located at the intersection of the blade and petiole, along the lower half of the midrib (China Editorial Committee, 2016).

2.2.2 Inflorescences, flowers and fruits

The flowers are numerous and arranged in a panicle about 30 cm long, corolla brown-yellow, upper lip shortly bifid, larger than the lower. The fruit is a drupe with sweet flesh; fruit turning yellow while ripening, 2.0-2.5 cm long and with a bony endocarp (stone it is rare for all 4 cells to be fertile and each fruit usually has only 2-3 seeds (China Editorial Committee, 2016).

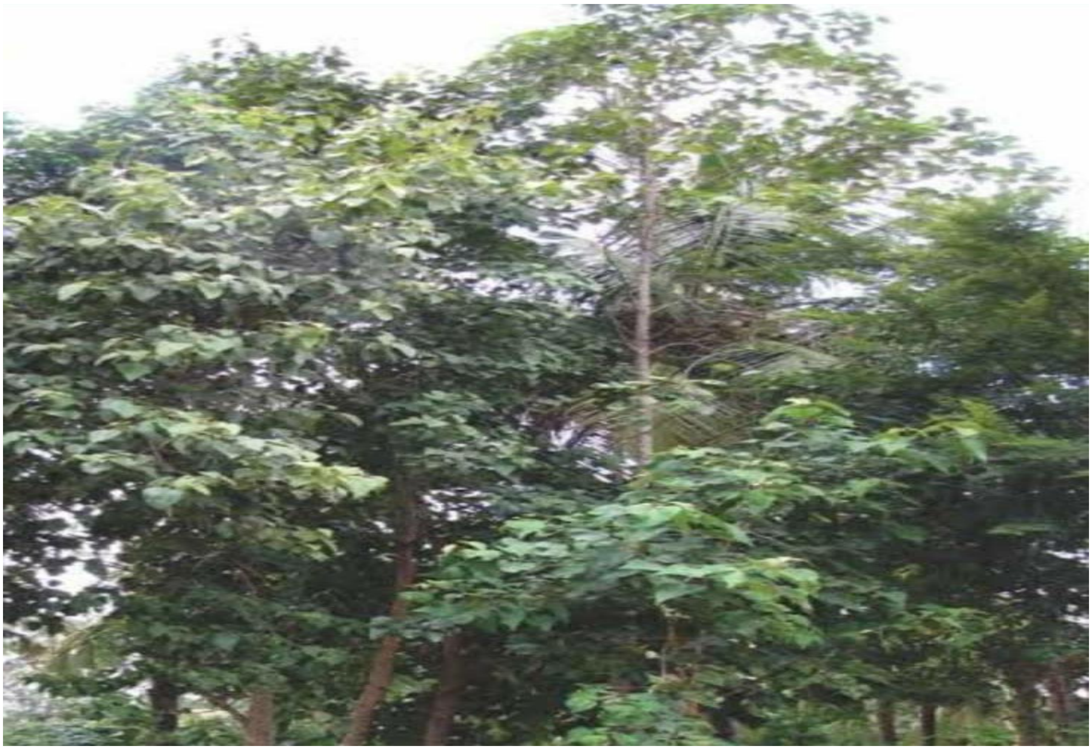

Figure 2.1: *G. arborea* Tree.
Source: Wikipedia.

Figure 2.2: *G. arborea* Leaves.
Source: Wikipedia.

Figure 2.3: *G. arborea* Fruits.
Source: Wikipedia.

2.2.3 Nutrient and anti-nutrient composition of Gmelina leave, fruits and seeds

The dry matter, protein content, and nitrogen free extract of the fruit obtained is slightly higher than the values reported by Amata (2012). For ripe Gmelina fruits, this variation can be accounted by differences in geographical location, soil and climatic factors and even analytical procedure. However, the protein content of the seed meal has qualifies it to be an alternative cheaper protein source for feeding of livestock especially monogastric animals. Banerjee (2005) further buttressed that any feed material with crude protein of 18% and above is classified as protein feed material for livestock. This therefore revealed that *Gmelina arborea* fruit has promising potentials as livestock feed resource. This was further supported by Azor and Abimbola (2003) who reported that processed Gmelina fruit can be fed to animals without adverse effects.

Table 2.1: Proximate Composition of *Gmelina arborea* Fruits and Leaves Meal

Nutrient %	Fruits	Leaves
Dry matter (DM)	91.80 +_3.21	89.80 +_7.33
Crude protein (CP)	21.50+_2.09	13.20+_4.00
Crude fibre (CF)	11.20 +_4.61	16.50+_3 .16
Ether extract (EE)	9.20+_2.19	4.50+_1.15
Ash	7.40+_2.77	6.00 +_2.88
Nitrogen -free extract (NFE)	23.57 +_5.21	19.60 +_3.90

Source: Amata (2012).

Gmelina leaves have good amino acid profile which indicates that the leaves could be a good source of protein for domestic animals (Amata, 2012).. The protein content of the leaves is however, within the range of 12 to 30% for most tropical trees as reported by Norton (1998). However, the protein content and ether extract recorded in this study is slightly lower than the values of 14.6 and 12.7% as reported by Amata and Lebari (2011). These differences could be attributed to variations in soil and climatic factors and geographical locations. Ubani and Tewe (2001) reported the crude protein content of the leaves of some multipurpose trees and shrubs: *Afezelia bella* (10.30%), *Dialum guineese* (11.73%), Newbouldia (17,86%), Baphantida (19.36%) and Albizia zygia (24.10%) of which only *Afezelia bella* and *Dialum guineense* showed lower protein values when compared to *Gmelina arborea* leaves. The fruit and the leaves were observed to have fair content of ash, ether extract and nitrogen-free extract (Table 2.2) indicating that they have promising nutritional value as livestock feed resources.

The leaves and fruits indicated good distribution of amino acid profile (Table 2.1) indicating it's potential as alternative protein feed ingredient for domestic animals. However, the seed meal had fairly high lysine threonine, leucine, Phenylalanine and valine content (Amata, 2012).

Table 2.2: Amino acid Profile of *Gmelina arborea* Leaves and Fruits

Amino acids (g/100g)	Leaves	Fruits
Lysine	3.07+_1.07	5.20+_1.52
Methionine	1.35+_0.11	2.30+_0.97
Threonine	2.30+_0.97	4.15+_1.18
Isoleusine	1.99+_0.21	3.64+_ 1.76
Leusine	3.45+_1.06	6.21+_2.43
Phenylalanine	2.08 ± 0.55	5.20 +_ 1.95
Valine	1.32 ± 0.02	4.30 +_ 1.04
Histidine	1.90 ± 0.05	2.85 +_0.17
Arginine	1.55 ± 0.12	3.20 +_1.42
Serine	1.31 ± 0.31	3.27+_1.06
Cysteine	0.80 ± 0.02	1.22 +_0.02
Tyrosine	2.09 ± 1.01	4.62+_1.85
Alanine	2.95 ± 0.71	5.02+_2.59
Glutamic	3.30 +_1.24	4.25+_1.83
Glycine	3.75 ± 1.38	8.54 +_3.67

Source: Amata (2012).

The results of the chemical analysis further indicated that the fruit and Leaves contain some toxic components (Table 2.3) such as tannins, oxalates, flavonoids, phytates and saponins. This finding is in line with the report of Amata (2012) who observed similar anti-nutritional factors in ripe Gmelina fruit. These toxic factors may be harmful to livestock when consumed beyond certain thresh hold in addition may limit nutrient utilization and consequently affect animal Performance.

Table 2.3: Levels of Anti-nutritional Factors of *Gmelina arborea* Fruits and Leaves

Anti -nutrients (g/100g)	Fruits	Leaves
Tannins	3.20+_1.25	1.20+_ 0.97
Oxalates	6.70 +_2.71	1.80+_0.27
Flavonoids	5.30 +_2.33	3.10 +_1,54
Phytates	5.80+_2.09	0.85+_0.07
Saponins	4.60+_1.78	0.72+_0.04

Source: Amata (2012).

2.3 Uses

The species is fast-growing and thus raised in large-scale plantations to produce wood for construction, crafts, paper pulp, fuel, and charcoal. It produces high-quality wood used in the manufacture of furniture and to make plywood, matches and agricultural implements. The nectar of the flowers yields high-quality honey. The Lion Throne, the most important, and last surviving, of the eight royal thrones of Myanmar, now in the National Museum in Yangon, is carved from *Gmelina arborea* wood (Wong and Khoo, 1980).

2.4 Leaf Protein Concentrates (LPCs)

Leaf proteins consist of two major categories: chloroplastic proteins (green leaf proteins) and cytoplasmic proteins (white leaf proteins). Both types of protein occur at approximately the same amounts in leaves, and cytoplasmic proteins are generally considered to have higher nutritional value than green proteins (Urribarrí *et al.,* 2005). Previous research on leaf protein extractions have mainly focused on the soluble protein fraction (green leaf proteins such as RuBisCO) of plant biomass, which could be a limiting factor for the further utilization of leaf proteins (Tamayo, 2016). Methods have been developed to increase the extractability of white leaf proteins. By treating leaf biomass with ammonia, a 4.5 fold increase in protein yield has been achieved in dwarf elephant grass (Urribarrí *et al.,* 2005).

Mechanical pressing is the most common method of separating leaf protein from cell wall components, in which fresh biomass material is pulped and pressed to produce a protein rich "juice". The leaf "juice" extract contains proteins, sugars, salts, lipids, vitamins, and water (Badar and Kulkarni, 2011). LPCs have traditionally been separated by heat coagulation or acidification to pH 4 (Badar and Kulkarni, 2011). These methods have been studied since the 1940s, when concerns over food supply led to attempts to find alternative protein sources for human use (Bals, 2012). To date, however, largescale utilization of LPCs for human consumption has not been realized. The lack of development in LPCs is most likely due to limited improvement for removal of anti-quality factors (tannins, etc.).

An alternative method for LPC isolation yielding significantly lower antiquality factors is ultrafiltration, in which the biomass is processed through an extraction column, filter compression, and ultra-filtration drying (Bals, 2011). This method does not require high energy costs and can be performed on dried material; however, reagent costs are high and column fouling from protein aggregation is common. Activated carbon treatment to protein extracts has been reported to effectively remove phenolic contents and improve flavor of the protein product (How and Morr, 1982; Seo and Morr, 1985). Utilizing activated carbon for differential separation of proteins and tannins requires selection of appropriate pH. Rubisco has an isoelectric point approximately 5.5 to 6 (Kung, 1984; Stone, 2014), therefore, conditioning of the activated carbon to a high pH can help ensure the protein does not adsorb to carbon. LPCs recovered by traditional heat coagulation methods consist of a green protein rich curd (Badar and Kulkarni, 2011), in which proteins are separated from the fibre of leafy materials, and which has the potential to be a high-value replacement for soyabean. Depending on plant species and extraction methods, the total protein content in LPCs varies from 40 to 70% (Badar *et al.,* 2011). Significant levels of minerals and vitamins, including β-carotene (pro-vitamin A) and vitamin E, are also recovered in LPCs (Badar *et al.,* 2011). LPCs can therefore be used as protein-vitamin-mineral supplements in animal or human diets, and studies have demonstrated successful LPC incorporation into poultry (Ameenuddin, 1983), fish (Ngugi, 2017), calf (Odwongo and Mugerwa, 1980) and human nutrition (Pirie, 1978). Previous reports have shown that certain napiergrass varieties developed in Nigeria have appreciable amounts of vitamin C (ascorbic acid) and pro-vitamin A (carotene) (Ukpabi, 2015), which render its leaf protein products high in nutritional value.

2.4.1 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 60^0C. To it, 50 ml fresh juice was slowly added with stirring so as to maintain the temperature of juice to 60^0C. The juice was kept for heating at this 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 ± 5℃ 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 H_2SO_4 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.

(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).

2.5 Anti-Nutritional Factors

Promising data from previous reports have indicated significant potential of LPCs for animal and human consumption (Sinclair, 2009; Ukpabi, 2015); however, current extraction methods are not able to significantly remove antinutritional factors in LPCs. These constituents have different mechanisms and metabolic interactions with crucial chemicals that affect nutrient uptake (Makkar, 1993) or cause unfavorable taste in LPCs (Huisman and Tolman, 1992). Common antinutritional substances found in plants include phytates, saponins, tannins, lectins, protease inhibitors, oligosaccharides and non-starch polysaccharides, glucosinolates, phytoestrogens, alkaloids, antigenic compounds, gossypols, cyanogens, mimosine, cyclopropenoid fatty acids, canavanine, antivitamins, and phorbol esters (Francis *et al.,* 2001).

2.5.1 Oxalates

Oxalates are also antinutritional factors widely found in plants. Although ruminants tend to have more tolerance to oxalates than non-ruminants, large and sudden consumption of oxalates can induce toxicity in grazing animals (Rahman *et al.,* 2013). Cases of oxalate poisoning have been reported for livestock upon feeding with napiergrass, in which calcium deficiency was caused by oxalate intake (Sidhu *et al.,* 1996).

2.5.2 Phytates

Phytates, most commonly found in plant seed (Francis *et al.,* 2001), cause growth reduction when fed with high phytate diets (Wallace *et al.,* 1998). The ability of phytate to form complexes with minerals (such as Cu2+, Zn2+, Co2+, Mn2+, Fe2+) makes these dietary minerals unavailable for uptake (Harland and Oberleas, 1985) and negatively affects feed digestibility (Nwokolo and Bragg, 1977).

2.5.3 Tannins, Alkaloids and Saponins

Tannins, alkaloids and saponins are all known to have antinutritional effects (Francis *et al.,* 2001). Tannins comprises a diverse group of secondary compounds that have various biological properties in animals (Brouard *et al.,* 1988), which in many cases, have been reported to cause growth depression (Davis, 1981). Alkaloids are also unsuitable for animal consumption, since they are known to interfere with nerve function (Peterson, 1982). Saponins in leaf protein products can also impart hemolytic activities on red blood cell (Jones and Elliot, 1969). Previous research has shown that while napiergrass contains high level of tannins, it has much lower amounts of alkaloids, saponins, cyanogenic glycosides, and oxalates. It is further almost devoid of phytates (Okaraonye and Ikewuchi, 2009).

2.6 Protein Purification

Protein purification utilizes methods or processes designed with the intention to isolate the desirable protein fraction from the feedstock source. Protein purification techniques have been performed for over 200 years (Healthcare, 2010). The goal of protein purification processes are not only the removal of unwanted materials, but also to concentrate and transfer the target protein (or proteins) to an environment where it remains stable and ready for the intended application. The development of protein purification is often related to the latest discovery and studies of protein. Filtration, precipitation and crystallization have been important protein separating methods since the early days of protein chemistry (Scopes, 2013), and are mostly involved with protein relative solubility (Healthcare, 2010; Scopes, 2013). Protein precipitation is considered one of the simplest approaches to obtain protein isolates from unwanted contaminants. Protein precepitation with acetone and Trichloroacetic Acid (TCA) are described and widely used in studies for quantification or determination of proteins (Stepanchenko, 2011).

2.6.1 Heat Coagulation

Temperature and pH are often the key factors in protein precipitation procedures (O'Connell and Fox, 2000). Thermal precipitation of protein is also referred to as 'heat coagulation', and has been described as a reaction taking place between protein and water with different temperature coefficients, depending on the types of protein, acidity, and salinity condition of the solution (Chick, 1910). 'Heat coagulation' of protein is generally considered an irreversible process (Anson and Mirsky, 1931; Wright and Humphrey, 2002); however, studies have reported the reversibility of coagulated proteins under certain conditions (Anson and Mirsky, 1931). Thermal precipitation or heat coagulation of protein can rarely be performed in biochemical studies due to denaturation of most proteins upon heating (Neurath *et al.,* 1944). Capture and maintenance of protein isolates with their active status is often a strict requirement for protein purification, especially for functional studies and other analyses that require native structure of the protein (Healthcare, 2010). Some proteins are more difficult to separate from the complex mixture of cellular contents depending on the type of protein. For example, insoluble proteins such as thylakoid proteins (membrane proteins), unstable protein complexes, and proteins with certain post-translational modifications will be more difficult to maintain in an active and stable form after purification (Kaufmann, 1997). In some chemical composition analyses, active protein structure may not be as crucial, and harsher purification methods such as strong denaturants, extreme pH and heat, and salt concentration can be utilized.

2.6.2 Chromatography

At the onset of the twentieth century, the term Chromatography' was introduced by Mikhail Tswett (Lederer and Lederer, 1953). Chromatographic Separation has proven to be a very efficient technique and utilized in numerous common protein purification methods (Janson, 2012). These include affinity chromatography, immobilized metal ion affinity chromatography, ion exchange chromatography, size-exclusion chromatography (gel filtration), hydrophobic interaction chromatography, reversed phase chromatography and chromato focusing (Healthcare, 2010). The advantage of chromatography is that it can be used to separate the interference of components in a complex-mixture without knowing in advance the nature and quantities of the chemicals presented in the solution (Rocklin and Johnson, 1983). Physical or biochemical properties of proteins are used in different chromatographic purification methods. Differences in size, positive and negative charges, hydrophobicity and other biospecific binding properties are used to purify desirable proteins from other proteins and non-protein contaminants (Karlström and Hober 2006). Soluble protein extracts can further be concentrated by using ultrafiltration. Removal of anti-quality factors, including phytate and oligosaccharide content, using ultrafiltration have been reported (Omosaiye *et al.,* 1978; Omosaiye and Cheryan, 1979). Centrifugal ultrafiltration has been widely used in Laboratory-scale protein purification due to its low energy requirement, high flexibility on the selection of membranes, low contamination potential, and ease of operation (Xu *et al.,* 2005).

2.7 Leaf Chlorophyll Content

The measurement of leaf chlorophyll concentration has been utilized in many aspects of crop production, as well as research activities including crop breeding, pest control, irrigation management, environmental stress evaluation, and nutrient analysis (Wood *et al.,* 1992; Rodriguez and Miller, 2000; Yuan *et al.,* 2016; Ravier, 2017). Therefore, the quantification of leaf chlorophyll concentration has potential to be studied for its association with leaf protein content and used as a phenotyping tool. Leaf chlorophyll content can be most accurately determined by *in vitro* measurements of leaf extracts using a Spectrophotometer. The Kjedahl method and Dumas method for determining total nitrogen content are also used routinely by Analytical Laboratories to estimate protein content (Jung *et al.,* 2003). However, faster, cheaper and nondestructive optical techniques would be preferred and are becoming more popular in determining relative indication of leaf chlorophyll concentration (Parry *et al.,* 2014).

2.8 Leaf Nitrogen and Proteins

Chlorophyll content meters are also widely used for monitoring the nitrogen (N) levels of crops in agricultural systems (Xiong *et al.,* 2015). According to previous studies, approximately 80% of leaf nitrogen can be found inside chloroplasts, and around half of the total leaf nitrogen is involved in photosynthetic activities by forming chlorophyll-protein complexes or other enzymatic associations (Xiong *et al*., 2015; Le Roux, 1999). The chloroplast is also known to contain at least 1000 different types of protein (with a concentration as high as 300 mg mL-1) which are associated with photosynthesis or other critical functions (Robinson *et al.,* 2001). Nitrogen involved in photosynthesis can be divided into two group: soluble proteins and thylakoid proteins (Evans, 1989). Soluble proteins are mostly Rubisco, along with a smaller fraction of other chloroplast enzymes, carbonic anhydrase, ribosomal proteins and photorespiratory enzymes in the mitochondria and peroxisomes (Evans, 1989).

2.9 Rubisco

Ribulose-1,5-bisphosphate carboxylase- oxygenase, or Rubisco, is an important green leaf protein due to its relationship with photosynthetic productivity in plants. Being essential for atmospheric carbon fixation, Rubisco is the most common protein in the world and constitutes the largest fraction of leaf proteins (Bals, 2012). Approximately one quarter of total leaf nitrogen is contributed by RuBisCO, while other associated photosynthetic enzymes account for another 25% (Chapin *et al.,* 2002). Rubisco consists of comparatively higher amounts of lysine, which is an amino acid limited in non-ruminant diet (Bals, 2012).

2.10 Thylakoid proteins

Thylakoid proteins are considered intergral membrane proteins, which carry out important light-dependent reactions of photosynthesis on the Thylakoid membrane.

These proteins include four major multisubunit membrane proteins: pigment-protein complexes Photosystem I (PSI) and Photosystem II (PSII) (Evans, 1989), Cytochrome b6f complex (cyt b6f) and ATP synthase (FOF1) (Kurisu *et al.,* 2003; Junge and Nelson, 2015).

2.11 Processing of leaf protein concentrate

Processing has proven important in improving the nutritional value of several feed ingredients. According to Agbede *et al*. (2008), the leaf protein fractionation process enhanced crude protein and gross energy by 34.8% and 22% respectively. However, many food processing techniques have been highlighted as possible means of reducing or totally eliminating the anti-nutrient levels in plants food sources to an innocuous levels that can be tolerated by animals particularly in monogastric nutrition (Fasuyi and Aletor, 2005). One of the effective ways of processing leaves to improve their utilization by monogastrics is by processing into leaf curd (leaf protein concentration). The basic technology for separating the leaf protein from the fibrous part of plants have been available for years, but the technology for large scale production is a recent development. According to abo Bakr *et al*. (1984), since the 1950s, significant development have been made in the wet green crop fractionation to produce leaf protein concentrate. The leaves are harvested and allowed to air dry to eliminate surface moisture. According to Fellow (1987), the leaves are pulped and the pulp is pressed in a twin screw press. Depending on available technology, the leaves could be chopped, crushed and pressed to extract the juice. The residue after juice extraction is basically fibre and is known as a bagasse. The leaf protein curd is coagulated from the juice by either of two methods or a combination of the methods. Fellow (1987) recommended the juice be heated to 90^0c within approximately 10 minutes to coagulate the protein. Protein is easily coagulated by quickly heating the juice to even as low as 70^0c depending on the leaf source. According to Pirie (1971), sudden heating produces a compact, coherent curd that is easy to handle and it inactivate leaf enzymes before they have time to act. The protein can be coagulated by acidification. Hydrochloric acid could be added to the juice till a pH of 4 is reached, and then the protein would coagulate. The coagulated protein are separated from the whey by filtering through cloth stockings and pressed overnight in a wooden beam press (Fellow, 1987). Pirie (1971) stated that acid coagulated leaf protein (LP) is troublesome on a filter but can be sedimented in a centrifuge. A combination of acidification as well as heating has been employed and documented. Castellanos *et al*. (1994) employed both heat and acidification in coagulating protein from cassava (*Manihot esculenta*) leaves. The leaf protein curd is then air or sun dried into leaf protein concentrate. The leaf protein yield is reported to be higher in younger leaves according to Walter *et al*. (*1978*), because less protein was available for extraction in the later stages of growth. They reported the same for Xantholylls which decreases on maturity of the leaves.

2.12 Use of leaf protein concentrates in animal feed

Using leaf protein in experimental rabbit diet led to Isuosuo *et al*. (2014) to conclude that 100% of soyabean meal (SBM) could be replaced by pumpkin leaf protein concentrates with no significant difference in weight, feed consumption, feed conversion ratio, blood parameters and some carcass characteristics. Accordingly, the best performance in mean weight and feed conversion was achieved with SBM replaced by pumkin leaf protein concentrate (PLPC) at 20% while for best result in blood building qualities (PVC, RBC and haemoglobin), growing rabbits should be fed 80-100% replacement with PLPC. Akaeze *et al*. (2014) reported that there was no significant difference in the feed intake, average daily weight gain and feed conversion ratio of the rabbits fed up to 60% partial replacement of soyabean meal substituted with rubber leaf protein concentrate. Akaeze *et al*. (2014) also recommended raising rabbit with diets containing 30% rubber leaf protein of rabbit production by mini livestock farmers. Using leaf protein in experimental pullet diet led Kokoshnikova *et al*. (1989) to conclude that 50% of fishmeal could be replaced by leaf protein concentrates with no significant problems. Leuceana leaf protein concentrate (LLPC) could replace 25% of fish meal for starting broiler with weight gain being 80.6% of the control weight gain (Agbede, 2003).

Tobacco leaf protein concentrate (TLPC), according to Miles *et al*. (1989), could be used up to 15% in the diet of broiler without having any significant effect on feed conversion, feed intake, liver and intestinal weight but decreased weight gain, while kidney weights were increased. The maximum inclusion level still needs to be further investigated. There is limited information on the use of leaf protein concentrates in other monogastric animal diets especially for pigs. Tartari *et al*. (1992) reported though that the inclusion levels of Lucerne leaf protein concentrate of up to 6% had no significant effect on weight gain and carcass quality. Rabbits tried on leaf protein concentrate from water hyacinth (*Eichhormia crassipes*) showed that replacing soyabean meal (SBM) with up to 50% of water hyacinth leaf protein concentrate increased daily fed intake, growth performance index, economic efficiency and improved feed conversion ratio (El-adawy and borhami, 1999). When Berseem (*Trifolium alexandrinum*) leaf protein concentrate (BLPC) completely replacing SBM showed that growth performance and economic efficiency tended to be lowered. Digestibility was not affected, though with 50% BLPC replacing SBM (El-adawy and Borhami, 1999).

CHAPTER THREE

3.0 MATERIALS AND METHODS

This study was conducted in two stages; first stage was to determine the yield of Gmelina *Gmelina arborea* Leaf Protein Concentrates (GLPC) and Gmelina Leaf bagasse (GLB) prepared by heat coagulation method (Pirie, 1971 and Sayyed, 2011). Followed by determination of their physical and chemical compositions such as proximate (dry matter, crude protein, crude fibre, ether extract ash and nitrogen free extract) and some of their mineral content. Secondly, was to also determine the yield of Gmelina *Gmelina arborea* Leaf Protein Concentrates (GLPC) and bagasse prepared by alum precipitation method (Sayyed, 2011), followed by determination of their physical and chemical compositions such as proximate (dry matter, crude protein, crude fibre, ether extract ash and nitrogen free extract) and some of the minerals it contains.

3.1 Experimental Site

The Research was carried out at the Faculty of Agriculture Main Laboratory, 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 degree, 30 minutes and 0 second North ($6^{0}30'00''$N) of the equator and longitude 5 degree 40 minutes and 6 seconds, East ($5^{0}40'06''$ E) of the Greenwich meridian in the humid rain forest zone with mean monthly temperature of 27.6^{0}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.

3.1.1 Calibration Procedure and Measurements:

- Harvest fresh *Gmelina arborea* 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 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 *Gmelina arborea* leaves use 8ml of water. Thus for 800g of leaves, 6400 ml of water will be used.

- Cut the *Gmelina arborea* leaves with a knife into smaller chunks before grinding.
- 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 or Alum to extract the LPC and Whey.

3.2 Processing and characterization of Gmelina Leaf Fractions

3.2.1 Production of Gmelina Leaf Protein Concentrate (GLPC) Using Heat Coagulation Method

Fresh Gmelina leaves were harvested early in the morning and were immediately taken to the laboratory for processing. The leaves were washed to remove debris and sand particles, and then chopped to smaller sizes before weighing. The leaves were then processed by grinding into slurry using a belt-driven Grinder.Each sample of slurry was placed on a sieve cloth and pressed strongly to seperate the juice from the chaff (Bagasse). 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 Whatman filter paper and the sample of LPC from the processed gmelina leaf was taken and yield recorded.

3.2.2 Production of Gmelina Leaf Protein Concentrate 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 was added. The curd of the leaf protein concentrate resulted due to the coagulation of proteins in the juice by the alum solution which was then filtered using Whatman filter paper and the LPC sample was recorded. The steps involved in Gmelina leaf protein concentrate and bagasse production is as shown in the Flow-Chart presented in Figure 3.1.

Figure 3.1: Schematics of the production of leaf protein concentrates.

Sources: Pirie (1987), modified by Olomu and Nwokoro (2015).

3.3 Fractions obtained From the Processed Gmelina (*Gmelina arborea*) Leaves

Bagasse: This was the fibre fraction obtained after the first Separation, which was sundried for 2-5 days.

Juice: This was the liquid (filtrate) obtained after the first Separation, it is usually greenish in colour.

Curd: This was the highly portentous fraction, the residue obtained after the second Separation or filtration. The curd was obtained from the processed Gmelina leaf after Separation and weighed. It was emptied into a petri dish and dried under the sun at (30-35^{0}C) for 2-4 days. The curd was considered dried when it break in scrip when touched.

Whey: This was the liquid that drain through the cloth during the second separation. It is brownish in colour and it's sometimes regarded as brown liquid.

Figure 3.2: Sieving of *Gmelina arborea* slurry.

Figure 3.3: Baggasse from *Gmelina arborea*.

Figure 3.2:

A. Whey,
B. LPC
C. Baggasse

Figure 3.3: LPC Extraction during Heat Coagulation

3.4 Data Collection

3.4.1 Physical characteristics of Gmelina Leaf Protein concentrate (GLPC)

The physical characteristics of GLPC, such as colour, texture and weight proportions of the fractions were recorded from the leaves samples obtained. During the period data were collected from the leaves samples obtained. The Gmelina leaf samples collected were processed into Gmelina leaf protein concentrate (GLPC) and Gmelina Leaf Bagasse (GLB). The samples were analysed for Proximate and mineral composition and phyto-chemical screening.

3.4.2 Proximate composition of GLPC

The proximate composition of Gmelina leaf protein concentrate was analysed to determine the dry matter, crude protein, crude fibre, crude fat, crude ash and nitrogem free extract content of the samples as described by AOAC (2016).

3.4.3 Mineral Analysis of GLPC

Minerals were analysed after first dry-ashing 1g of the curd and bagasse at 550^0C in a Muffle furnance 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, sulphur, manganese, iron and copper were determined using an atomic absorption spectrophotometer.

3.5 Statistical Analysis

Data collected from the study were subjected to analysis of variance using the GENSTAT 12th edition for windows package at 5% ($p < 0.05$). The means with significant difference were separated using Duncan multiple range test (Duncan, 1955).

CHAPTER FOUR

4.0 RESULTS

4.1 Evaluation of Physical Properties of Gmelina (*Gmelina arborea*) Leaf Protein Concentrate (GLPC) and Gmelina Leaf Bagasse (GLB) extracted using Heat Coagulation and Alum Precipitation Method

The results of Gmelina Leaf Protein Concentrate (GLPC) and Gmelina Leaf Bagasse (GLB) with some of their physical characteristics is shown in Table 4.1. The dried Gmelina leaf protein concentrate obtained through Alum precipitate had a bright green appearance (colour), the one obtained through heat coagulation had a dark green colour all at solid state while the bagasses were light green in colour whey which is the residual liquid appeared to be golden-brownish in colour for those from Alum precipitation while a dark brown colouration was observed in those obtained in heat coagulation. The texture as shown on Table 4.1 revealed that the leaf protein concentrate from the two extraction methods are fine (granules) and the bagasse are coarse (fibrous).

Table 4.1: Physical characteristics of Gmelina (*Gmelina arborea*) Leaf protein Concentrate, Bagasse and Whey of the Fresh Gmelina leaves Obtained using the two extraction methods

Fractions	State	Colour	Texture
Fresh Gmelina Leaves	Solid	Green	Mildly Coarse surface
Gmelina Leaf Protein (Alum)	Solid	Light Green	Fine and powdery
Gmelina Leaf Protein (Heat)	Solid	Dark Green	Fine and powdery
Gmelina Leaf Bagasse	Solid	Pale green	Coarse and fibrous
Gmelina leaf whey (Alum)	Liquid	Golden Brown	Liquid Portion
Gmelina leaf whey (Heat)	Liquid	Bark Brown	Liquid Portion

4.2 Percentage Yield of Gmelina (*Gmelina arborea*) Leaf Protein Concentrate (GLPC) and Gmelina Leaf Bagasse (TALB) extracted using Heat Coagulation and Alum Precipitation Method

The percentage yield of GLPC produced using the following methods; Heat coagulation and Alum precipitation method and its bagasse are shown in Table 4.2. The result shows that the yields of the two methods of extraction vary significantly from one other. Higher value (8.13%) was recorded in (GLPC) Obtained using Alum precipitation while lowest value of (5.24%) was observed in (GLPC) extracted using Heat coagulation Method. The percentage yield of baggase (22.07%) obtained from the Heat coagulation was lower than (25.14%) recorded for bagasse obtained from that extracted using Alum precipitation method.

Table 4.2: Percentage Yield of Gmelina (*Gmelina arborea*) GLPC and GLB extracted using Alum Precipitation and Heat coagulation Methods

Samples	Yield (%)
Gmelina Leaf Protein (Alum) ($GLPC_a$)	8.13
Gmelina Leaf Protein (Heat) ($GLPC_h$)	5.24
Gmelina Leaf Bagasse (Alum) (GLB_a)	25.14
Gmelina Leaf Bagasse (Heat) (GLB_h)	22.47

4.3 Proximate composition of Gmelina (*Gmelina arborea*) Leaf Protein Concentrate (GLPC) and Bagasse (GLB) Processed using two extraction methods

The proximate composition of Gmelina (*Gmelina arborea*) Leaf Protein Concentrate and Bagasse Processed using two extraction methods is shown in Table 4.3. From the result presented, it was observed that the dry matter content for GLPC was higher (94.62%) for heat coagulation extraction method when compared to that obtains from alum precipitation (37.58%). Bagasse recorded a percentage dry matter content value of 38.56%. Percentage crude protein content was higher (32.15%) from those obtained from alum precipitation method than (28.41%) obtained from those from heat coagulation method. The Bagasse recorded a percentage crude protein content value of 22.68%. Crude fibre was higher (6.87%) in those obtained from heat coagulation than (5.18%) obtained from those from alum precipitation method. The Bagasse recorded a percentage crude fibre content value of 14.86%. Crude fat was higher (5.50%) in those obtained from Alum precipitation than (5.20%) obtained from those from heat coagulation method. The Bagasse recorded a percentage crude fat content value of 2.80%. Crude ash content was higher (6.11%) in those obtained from alum precipitation than (5.13%) obtained from those from Heat coagulation method. The Bagasse recorded a percentage crude ash content value of 3.50%. Nitrogen free extract was higher (54.39%) in those obtained from Heat coagulation than (51.06%) obtained from those from alum precipitation method. The Bagasse recorded a percentage nitrogen free extract value of 56.16%.

Table 4.3: Proximate composition of Gmelina (*Gmelina arborea*) Leaf Protein Concentrate (GLPC) and Bagasse (GLB) Processed using two extraction methods.

Parameters	**$GLPC_a$**	**$GLPC_h$**	**GLB**	**SEM±**
Dry Matter Content	94.62±5.16	37.58±3.20	38.56±4.08	4.27
Crude Protein	32.15±4.25	28.41±2.12	22.68±3.11	1.35
Crude Fibre	5.18±0.07	6.87±0.04	14.86±1.53	0.02
Crude Fat	5.50±0.04	5.20±0.03	2.80±0.03	0.04
Crude Ash	6.11±2.24	5.13±1.88	3.50±0.17	0.19
Nitrogen Free Extract	51.06±2.67	54.39±3.42	56.16±2.32	3.65

4.4 Some Mineral composition of Gmelina (*Gmelina arborea*) Leaf Protein Concentrate (GLPC) and Gmelina Leaf Bagasse (GLB) Processed using two extraction methods

The mineral composition of Gmelina (*Gmelina arborea*) Leaf Protein Concentrate (GLPC) and Gmelina leaf Bagasse (GLB) Processed using two extraction methods is shown in Table 4.4. It reveals a wide range differential in the analysed values of elemental Phosphorus, calcium, magnesium, potassium, nitrogen, zinc, sodium, iron, manganese and copper. The Phosphorus recorded a numerical value of (295.31mg/100g) which was higher in GLPC extracted using alum precipitation method and lowest value (119.28mg/100g) observed in GLPC extracted using heat coagulation method. The Phosphorus value (126.88mg/100g) was obtained in Gmelina leaf baggasse. Calcium value (308.65mg/100g) was higher in GLPC extracted using heat coagulation method and lower (186.64mg/100g) in GLPC extracted using alum precipitation method. The calcium value (486.03mg/100g) was obtained in Gmelina leaf baggasse. The elemental magnesium recorded a numerical value of (155.80mg/100g) which was higher in GLPC extracted using heat coagulation method followed by a lower value of (92.50mg/100g) observed in GLPC extracted using alum precipitation method.

The Phosphorus value (164.51mg/100g) was obtained in Gmelina leaf baggasse. The potassium value (615.43mg/100g) was higher in GLPC extracted using alum precipitation method and lower (517.33mg/100g) in GLPC extracted using Heat coagulation method. The potassium value (475.85mg/100g) was obtained in Gmelina leaf baggasse. The Sodium value (144.73mg/100g) was higher in GLPC extracted using heat coagulation method and slightly lower (143.84mg/100g) in GLPC extracted using Heat coagulation method. The sodium value (119.65mg/100g) was obtained in Gmelina leaf baggasse. The Nitrogen recorded a numerical value of (5144mg/100g) which was higher in GLPC extracted using heat coagulation method and lower value (4546.03mg/100g) observed in GLPC extracted using alum precipitation method. The nitrogen value (3628.80mg/100g) was obtained in Gmelina leaf baggasse. The zinc value (2.06mg/100g) was higher in GLPC extracted using alum precipitation method and lower (1.82mg/100g) in GLPC extracted using heat coagulation method. The zinc value (1.08mg/100g) was obtained in Gmelina leaf baggasse. The iron recorded a numerical value of (136.29mg/100g) which was higher in GLPC extracted using alum precipitation method and lowest value (38.82mg/100g) observed in GLPC extracted using heat coagulation method. The iron value (41.95mg/100g) was obtained in Gmelina leaf baggasse. The Manganese value (2.11mg/100g) was higher in GLPC extracted using alum precipitation method and lower (1.93mg/100g) in GLPC extracted using Heat coagulation method. The manganese value (1.11mg/100g) was obtained in the Gmelina leaf baggasse. The copper value (0.61mg/100g) was higher in Gmelina LPC extracted using heat coagulation method and lower (0.52mg/100g) GLPC extracted using alum precipitation method. The copper value (0.38mg/100g) was obtained in Gmelina leaf baggasse.

Table 4.4: Some Mineral composition of Gmelina (*Gmelina arborea*) Leaf Protein Concentrate (GLPC) and Bagasse (GLB) Processed using two extraction methods

Parameters	$GLPC_a$	$GLPC_h$	GLB
Phosphorus (mg/100g)	295.31±6.44	119.28±4.43	126.88±7.23
Calcium (mg/100g)	186.64+11.28	308.65+12.46	486.03+10.25
Magnesium (mg/100g)	92.50+8.62	155.80+8.07	164.61+8.42
Potasium (mg/100g)	615.43+12.28	517.33+11.28	475.85+12.27
Sodium (mg/100g)	143.84+6.26	144.73+7.12	119.65+7.64
Nitrogen (mg/100g)	5144.00+26.43	4546.03+24.05	3628.80+22.05
Zinc (mg/100g)	2.06+0.03	1.82+0.03	1.08+0.02
Iron(mg/100g)	136.29+8.28	38.82+8.45	41.95+8.33
Manganese (mg/100g)	2.11+0.03	1.93+0.02	1.11+0.01
Copper (mg/100g)	1.93+0.01	0.61+0.02	0.38+0.02

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physical Properties of Gmelina (*Gmelina arborea*) Leaf Protein Concentrate (GLPC) and leaf Bagasse (GLB) extracted using Heat Coagulation and Alum Precipitation Method

The physical characteristics of Gmelina leaf protein concentrates processed using the two different extraction methods observed during the study showed significant variation in the colour of the leaf protein concentrate obtained in Gmelina leaf from the Alum precipitation being moss green and that obatined from heat coagulation method appeared to possess a darker forest shade of green. This variation in clour could be as ascribed to the heat treatment that caused the changes the colour of the LPC. This finding corroborates the report of Akaeze and Paul-Osagie (2023) who reported similar finding when Cassava leaf protein concentrate and bagasse were extracted using three different methods of extraction. The bagasse obtained was fibrous and pale green then turns dark green after sundrying. The whey obtained from the Heat coagulation was darker in colour which is a pointer of the heat treatment on the LPC. However the whey obtained from the alum precipitation tend to be lighter in colour. This compares with what was reported for cassava LPC by Akindele (2019) for cassava LPC processed using heat and alum precipitation methods. This similarity is consistent as the leaves where collected from the same geographical location as the leaves used in the present study. The finding also corobatate the findings of Mowah (2004) For *Telfairia* *occidentalis*, Agbonghae (2016) for *Carica papaya*, and Onwukaike (2017) for plaintain leaves.

5.2 Percentage Yield of Gmelina Leaf Protein Concentrate and Bagasse extracted using Alum Precipitation and Heat coagulation Methods

Percentage yield of Gmelina leaf protein concentrate using Alum precipitation method was higher on weight for weight basis than Cassava leaf concentrate as reported by Sayyed (2011), using same method. This significant difference in yield could be as a result of the nature of the plant material and the nature of the component proteins, as protein denaturation is determined by a large extend to the nature of the individual protein molecules. Gmelina leaf protein concentrate using Alum precipitation method was higher than rubber leaf protein concentrate reported by Akaeze *et al.* (2015) and also higher than that recorded in pumpkin leaf protein concentrate reported by Isuzu (2014) using same method. The yield from the Alum precipitation is higher than that from acid coagulation and heat coagulation, this could be as a result of the Sulphur content of the alum reacting with the surphur containing amino acid leading to their precipitation and hence more protein concentrates is produced. It is however noteworthy that the bagasse yield was statistically highest based on weight. The difference in yield is important in establishing what method could best be adopted in processing leaf protein concentrates for animal production, as the goal of any livestock production venture is to maximize the utilization of all materials (Akaeze, 2019).

5.3 Proximate composition of Gmelina (*Gmelina arborea*) Leaf Protein Concentrate (GLPC) and Bagasse (GLB) Processed using two extraction methods

G. arborea produces an important and valuable creamy-white timber which is sometimes called white teak. It is a fast-growing species that demands good site conditions (Durant, 1941; Fox, 1967; Nanagas and Serna, 1970; Fasehun, 1975; Greaves, 1981; Webb et al., 1984; Luna, 1996). Native to Asia, the species has great plantation potential and is widely planted, especially in Africa. Large-scale plantations of *G. arborea* are found in Nigeria, Sierra Leone and Malawi (Streets, 1962).

Compared with other high-quality timber species, *G. arborea* can be managed for relatively short rotations of 15 to 20 years (Fox, 1967). With recent developments in wood processing technology for utilizing smaller diameter logs, *G. arborea* plantations are often managed on a less than 15-year rotation; this is especially the case when fuelwood is the main end-product. *G. arborea* is also valuable as a multipurpose tree (Tiwari *et al.,* 2007) and is economically important in various agroforestry systems. Different parts of the tree are used in traditional medicine (Nayak *et al.,* 2011). This observed variation in the dry matter content with higher value recorded from Gmelina LPC extracted using Alum precipitation method might have resulted from geographic, climatic and seasonal variations. Morton, 1987 stated that the low moisture content was indicative of high dry matter content and possible shelf life. Proximate composition of using different methods revealed that the crude protein obtained from GLPC obtained from Alum precipiation method was higher than that in LPC (32.15%) reported by

Akaeze *et al*. (2015) but lower than that in LPC of *Amaranthus hybridus* leaf (34.80%) reported by Adeyeye and Omolayo (2011), and 44.92% LPC gotten from Alum precipitation method was higher than that in LPC of *Amaranthus hybridus* leaf (34.8%) reported by Adeyeye and Omolayo (2011).

The crude fibre value for Alum precipitation was higher compared to that of the GLPC extraxted using the Heat coagulation method. The crucial role crude fibre plays in the digestive process and ultimately in the proper functioning of the physiological system cannot be overemphasized. It has been reported that it helps lower the blood pressure and thus reducing the risk of cardiovascular diseases, it also helps in proper bowel functioning and movement, thereby ensuring that waste in moved through the intestine. It is reported that inadequate fibre in the diet of rabbits could lead to a condition known as GI stasis (De blas *et al*., 1999). The crude fibre was present in appreciable amount in the bagasse of GLPC. The crude fat of GLPC from alum precipitation method (5.50%) was lower than 9.6% in *Amaranthus hybridus* and 8.13% in RLPC as reported by Adeyeye and Omolayo (2011) and Odoh (2019) respectively. The bagasse was 2.80% was lower than 5.98% in RLPC reported by Odoh (2019).

The NFE of GLPC from heat coagulation method (54.39%) was higher than 23.58% from *Vernonia amygdalina* and NFE of GLPC from alum precipitation method (41.28%) was found to be higher than 23.58% from *Vernonia amygdalina* LPC as reported by Sodamade *et al.* (2014).

5.4 Some Mineral composition of Gmelina (*Gmelina arborea*) Leaf Protein Concentrate (GLPC) and Bagasse (GLB) Processed using two extraction methods

The potassium in the GLPC from Heat coagulation was higher than that in the GLPC extracted using alum coagulation method. This finding was higher that those reported in Luceme LPC by Siebrits *et al*. (1986). Potassium helps in maintaining balance in the body fluid, facilitates nervous functions and maintain healthy body sugar. Calcium on the other hand was higher in the GLPC from Alum precipitation than that in the GLPC extracted using heat coagulation method. This finding was also higher that those reported by Siebrits *et al.* (1986). Higher value of calcium recorded is a pointer to the fact that the LPC will support optimum bone formation and development if fed to livestocks as calcium plays a significant role in bone formation (Olusanya, 2008). Other macro minerals such as potassium were higher among GLPC extracted using alum precipitate while magnesium values was higher in GLPC extracted using heat coagulation. These values recorded were however higher than 116mg/kg and 457mg/kg of *Amaranthus hybridus* LPC reported by Adeyeye and Omolayo (2011).

The sodium content in GLPC from heat coagulation 144.73mg/100g was almost same as 143.84mg/100g from those extracted using alum precipitation. These values reported for sodium and nitrogen were lower than 312mg/kg and 5144mg/kg in *Telferia occidentalis* LPC reported by Adeyeye and Omolayo (2011). Higher zinc and iron values were recorded among the gmelina LPC extracted using alum precipitation. These higher values show that the LPC is rich in supporting the optimum blood quality of the animals if consumed in recommended quantity. These values were higher than those reported by Siebrits *et al.,* (1986) in Lucerne LPC. Manganese, however, in GLPC gotten from heat coagulation (2.11mg/100g) was lower than manganese (50mg/kg) and copper analyzed in Lucerne LPC as reported by Siebrits *et al.* (1986) and (Akaeze and Paul-Osagie, 2023). The GLPC from heat coagulation method revealed 0.61mg/100g, which was far lower that the value reported by (Akaeze and Paul- Osagie, 2023).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The study was carried out to determine the yield, physical and chemical composition of leaf protein concentrate and bagasse of Gmelina Leaf using two extraction method. From the study, it was observed that both the Gmelina leaf protein concentrate can be substituted in feed as study has shown that it contain high level of nutrient which the body needs.

The physical characteristics observed in this study shows that GLPC is dark green in colour when extracted using heat coagulation method. The proximate composition of GLPC as presented in Table 4.3 shows that the dry matter content is high, high level of crude protein when extracted using alum precipitation method, average ash content and low fibre content. Also proximate composition of Gmelina Leaf Bagasse in Table 4.3, shows that dry matter is high, crude protein is low, ether extract is high, low ash content and high fibre content.

From the results obtained from the proximate composition of GLPC (Table 4.3) and GLB (Table 4.3), it shows that GLPC and GLB can serve as alternative source of protein in animal diets.

6.2 Conclusion

The results from this study shows that the yields and chemical composition of Gmelina leaf protein concentrates precipitated using heat and alum methods were quite comparable with those obtained from other plants, therefore it holds prospect as a viable substitute for more expensive conventional protein feed ingredients like soya bean meal, groundnut meal and other protein sources in livestock feed formulation. The Bagasse yield was also high and could be used as feed for ruminants and also ensiled and used for dry season feeding when feed is scarce.

6.3 Recommendation

From the study, due to the promising quantitative values in the parameters assessed in Gmelina Leaf Protein Concentrate and Bagasse, there is need to:

1. develop Gmelina leaf protein concentrates in the diet of poultry and mini livestocks such as rabbits for better performance;
2. local poultry farmers should be sensitized on the phytobiotic potentials of Gmelina leaf protein concentrates as partial replacement for soya bean meal in livestock feeding;

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