THE YIELD, PHYSICAL AND CHEMICAL COMPOSITION OF OPEPE (*Nauclea diderrichii,* De Wild. and T. Durand) LEAF PROTEIN CONCENTRATE AND BAGASSE, EXTRACTED USING THREE DIFFERENT METHODS

BY

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JANUARY, 2023

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF AGRICULTURE (B. Agric) DEGREE IN ANIMAL SCIENCE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

JANUARY, 2023

CERTIFICATION

This is to certify that this Project work was carried out by **Chinedu Joseph OZAH** with Matriculation number, **AGR1608547,** under the guidance of the Project Supervisors and approved by the Department of Animal Science, Faculty of Agriculture, University of Benin, Benin-City, Nigeria.

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DEDICATION

This work is dedicated to God Almighty, who gave me the grace to get to this point in my life, my Father (Late Mr. Ozah Chinedu), my Mother (Mrs. Ozah Chika) and to all those who through their love, trust, finance and moral support, kept me through the course of this Study.

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I want to give thanks to God almighty who has giving me the grace to get to this level today. God made it possible for me to come into this university and guided me even to this last phase of my undergraduate Program, his name be praised and glorified. I give all glory to him.

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ABSTRACT

The study was carried out to determine the yield, physical and chemical composition of Opepe leaves (*Nauclea diiderrichi*) De Wild. and T. Durand. The leaves used were harvested and blended, before the leaf Protein Concentrate was extracted using 3 different methods (Heat coagulation , acid coagulation and alum precipitation methods) . The result from the analysis showed that acid coagulation , alum precipitation and heat coagulation methods yielded 2.398% , 3.913% and 6.383% respectively. The yield of bagasse was 23.508%. The result of the proximate analysis showed that the dry matter and NFE were higher in alum precipitation method than in acid coagulation and heat coagulation methods . It also showed that CP , CF and ASH were higher in the acid coagulation method than in the alum precipitation and heat coagulation methods, while EE was higher in the heat coagulation method than in the other two methods . The mineral analysis showed that Mg , Na and P were higher in the heat coagulation method than in the acid coagulation and alum precipitation methods . It also showed that K , Fe and Mn were higher in the acid coagulation method than in the alum precipitation and heat coagulation methods . LPC result of Ca , showed that acid and heat coagulation methods yielded the same amount , which were higher than that of alum precipitation method .

CHAPTER ONE

1.0 INTRODUCTION

Proteins are the basic constituents of all living things and it is a nutritional essential for animals, which occurs in varying amounts in almost all of our food. Due to the relative abundance of protein in leaf material and basic need for protein in diet , scientists have tried to prepare edible proteins for animals from green plant tissues (Jadhao and Bhuktar , 2018). Leaves are a potential source of low cost proteins. Protein deficiency may lead to high mortality rate and lowered resistance to disease , especially in young age of animals . In view of the economic situation in the rural areas, it is essential to search other economically inexpensive sources of good quality protein that can be used as alternatives source to expensive animal protein. It was felt that a local substitute such as leaf protein concentrate might decrease the cost of animal protein (Emmanuel and Folasade , 2011) .

Leaves are the largest source of proteins in the world (Ellis, 1979; Fiorentini and Galoppini, 1983). Plant leaves represent a vast source of proteins that in many cases are utilized by grazing livestock but potentially could be used for animal feeding purposes. Leaf protein concentrate (LPC) is a concentrated form of proteins obtained from plant leaves and has been assessed as human or animal food source, because it is potentially a cheap and abundant source of available protein. This has recently led to renewed interest in LPC to reduce the use of human-edible vegetable protein sources in animal feed. However, the challenges that have to be overcome before establishing LPC a viable protein source for humans include the high fiber content and other antinutritional factors such as phylate, cyanide and tannins. As such the area requires adequate scientific and technological intervention so as to develop feasible and cost-effective

methods for enhancing LPC production while minimizing the possibility of denaturation and eliminating antinutritional factors (by Y.C. Tripathi , academia.edu).

The development of new protein sources has been a high priority research goal from past few decades (Hall *et al.,* 1975) . The significant procedures for utilizing protein in green plant tissue for animals is the production of leaf protein concentrate (LPC), which is prepared by expressing plant juice with a press and coagulating soluble protein in juice.

1.1 Experiments on leaf protein concentrates (LPC) is very important because:

The livestock sector in the world is growing rapidly in order to meet the high demand for meat and dairy products, which has increased 1.5-fold over a 50 years period (i.e. 1960–2010) (Godfray *et al.*, 2010 ; McMichael *et al.*, 2007) . Leaf protein is the most abundant source of protein by volume and sustainable source of protein (The leafprotein.com , 2020). Leaf proteins can also aid the reduction in cost of feeding our livestock, thereby increasing their efficiency in meat and milk production.

Presently, the world is coming to recognize the grim truth that ultimately the population growth will outstrip food suppliers with apocalyptic results and near about 36 million people die per year due to hunger or as a result of hunger. Approximately, 60% of the 10.9 million deaths each year among children are bellow the age of five in the developing countries are attributed to malnutrition and protein deficiency is one of the major nutritional problems in the developing world (Jadhao and Anil, 2018). The development of novel protein sources such as Fish Protein Concentrate (FPC), Single Cell Protein (SCP) and Soybean Protein (SBP) as well as Leaf Protein Concentrate (LPC) has made significant contributions toward the alleviation of the world protein deficiency (Ghaly and Alkoaik, 2010). The protein shortage

cannot be alleviated by conventional agriculture alone and there is need of an additional source of protein, the leaf protein concentrates (LPC) should be given serious attention because leaves are abundant and many have high protein content. The yield per hectare per year of leaf proteins can be at least four times higher than that of seed proteins (Lehel Telek). The green leafy vegetables have long been recognized as the cheapest and rich source of protein which has prime importance to health(Emmanuel and Folashade , 2011).

1.2 OBJECTIVES

The broad objective of this work is to determine the yield physical and chemical composition of leaf protein concentrate and bagasse obtained from OPEPE (*Nauclea diderrichii*) using 3 different extraction methods .

The specific objectives are:

1. To determine the yield of leaf protein concentrate (LPC) and bagasse obtained from OPEPE(*Nauclea diderrichii*) leaves using three methods (Heat coagulation, alom and Acid).
2. To determine the physical characteristics of leaf protein concentrate (LPC) and bagasse obtained from OPEPE (*Nauclea diderrichii*) leaves
3. To determine the chemical composition of leaf protein concentrate (LPC) and bagasse obtained from OPEPE (*Nauclea diderrichii*) leaves.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 OPEPE PLANT

2.1.1 Description

OPEPE *(Nauclea diderrichii,* De Wild. and T. Durand), is an evergreen tree that reaches a height of 30-40 m and a diameter of 0.9-1.5 m; bole cylindrical, slender, straight and branchless, rising to 20-30 m and a broad spherical crown with thick foliage. The shining leaves are 15 cm long and bigger when young, elliptic, acute at the ends, keeled towards the base, and stipulate, with a pair of distinct leafy stipules at the base. It is mostly deciduous except at the ends of shoots, and the nodes are often occupied by ants. Flowers small, green-white-yellow and tubular, in solitary terminal heads (unbranched), 3 cm across; stalks only about 1 cm. The fruit is yellow, fleshy, in a globose head deeply pitted between the deeply fused calyx lobes. There are about 250 fruit/kg . It is a tall perennial woody plant having a main trunk and branches forming a distinct elevated crown; includes both gymnosperms and angiosperms. It grows well in the humid tropical rainforest where annual rainfall ranges from 1600 to 3000mm (Dupuy and Mille, 1993; FORMECU, 1999).

2.1.2 Distribution

Nauclea diderrichii (OPEPE) is a specie of tree of the genus Nauclea in the family Rubiaceae. It is known by the common names bilinga, aloma, badi, kusia and OPEPE. Its natural habitat is subtropical or tropical moist lowland forests. It grows to around 35m to 48m tall, and 1m to 2m in diameter at breast height. The timber is known as bilinga, or Aloma in Germany and opepe in the UK and is used in joinery, flooring and marine construction (Orwa *et al.,* 2009)

Nauclea diderrichii (OPEPE) is an evergreen species native to moist evergreen and transitional-to-moist semi-deciduous forest (Orwa *et al.*, 2009). The species is found throughout the tropical rainforest of West Africa and extends south to Angola. A sun-loving species, it regenerates abundantly in gaps and openings and is often almost gregarious in the transition zone between freshwater swamp and lowland forest. The tree is harvested from the wild for local use as a medicine and source of wood. The wood is of high quality and is also traded (De Wild.)Merr. (1915).

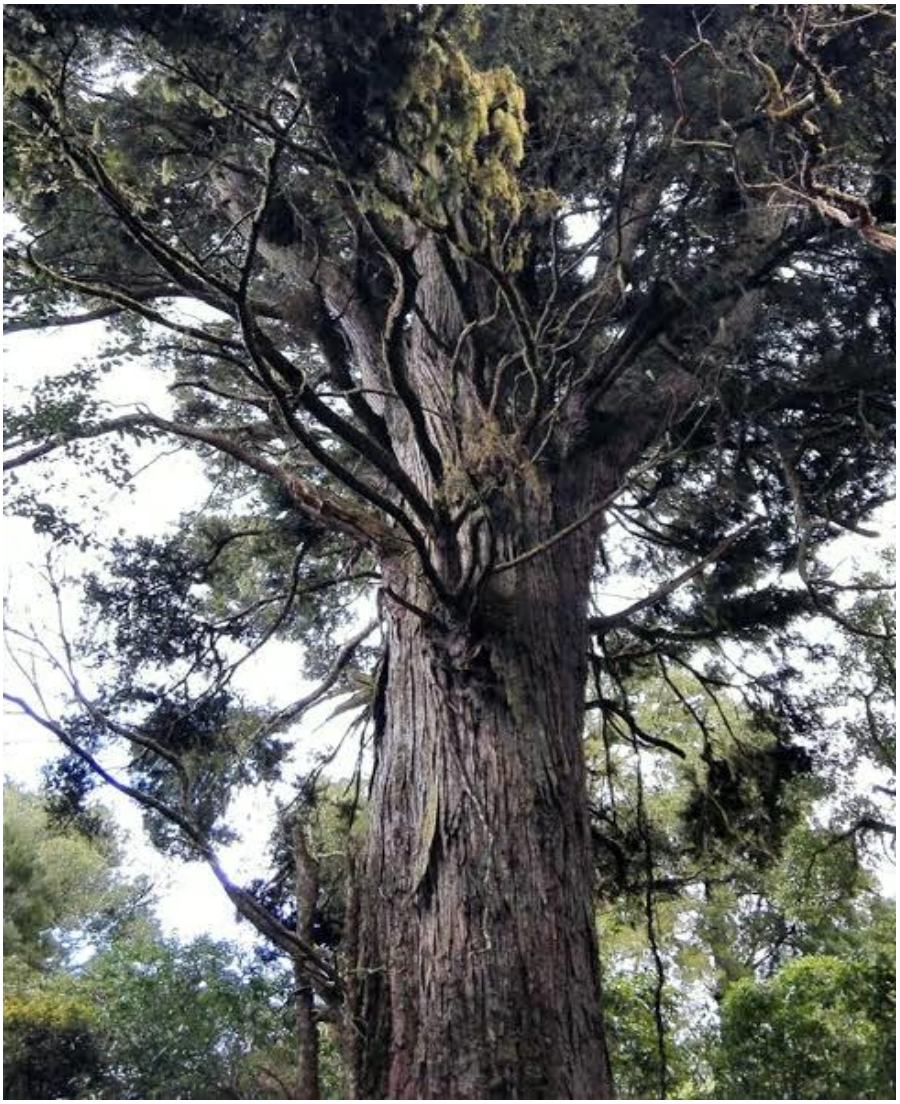


Image : OPEPE TREE

Source : Rusticstone (2016)

2.2 TAXONOMY OF OPEPE

Kingdom: *Plantae*

Subkingdom: *Viridiplantae*

Infrakingdom: *Streptophyta*

Superdivision: *Embryophyta*

Division: *Tracheophyta*

Subdivision: *Spermatophytina*

Order: *Gentianales*

Family: *Rubiaceae*

Subfamily: *Cinchonoideae*

Tribe: *Naucleeae*

Genus: *Nauclea*

Species: *Nauclea diderrichii*

Source: The Wood Database (2019)

2.3 LEAF PROTEIN CONCENTRATE

Leaf protein concentrate (LPC) is an extremely nutritious food product made by mechanically separating (through a process of juicing, boiling, and drying leaves) indigestible fiber and soluble anti-nutrients from certain fresh green plant leaves. As evidenced by its name, LPC contains extraordinary levels of high-quality protein. On a dry weight basis, which describes the percentage of a nutrient in a substance after removing the moisture, LPC is roughly 50-65% protein with significant amounts of calcium, iron, and vitamin E, as well as other vitamins and minerals. (Catherine Webb, 2020).

Aside from its potential as a high-quality protein source, the by-products of LPC have other sustainable uses. In agriculture and land management, the residual fiber and liquid whey remaining after protein extraction can serve the following purposes:

1.**Biomass energy**:	They can be used as fuel for energy generation .
2.**Animal Feed**:	They have the same nutritional value as traditionally unprocessed feed for cows, goats, sheep .
3.**Organic Fertilizer** :	They improve soil fertility, composition, water retention, and crop yields

Source : Catherine Webb (2020)

2.3.1 THE NEED FOR ALTERNATIVE PROTEIN SOURCES

The world's livestock sector is growing rapidly in order to meet the high demand for meat and dairy products, which has increased 1.5-fold over a 50 years period (i.e. 1960–2010) (Godfray *et al.,* 2010; McMichael *et al.,* 2007). In 2018, the European livestock population was approximately 333 million head (excluding poultry), and included 148 million pigs (EUROSTAT, 2020). Denmark is the fourth largest pig producer in the EU with 8.5 % of the total pigs, just after Spain, Germany and France (EUROSTAT, 2020). Definitely, the EU has a strong self-sufficiency in terms of protein production for human consumption since most of the available meat in EU is produced within the EU , however , the EU is greatly dependent on imported protein-rich feed materials, mainly soybeans and soybean meal (de Visser *et al.,* 2014).

Potein-rich sources are required for livestock feeding in animals. Protein extraction from plant leaves is a suitable solution for providing protein-rich feeds for animals, especially if the leaf protein extracts have amino acid profiles similar to soybeans (Santamaría-Fernández *et al.*, 2017). The integration of protein extraction within green biorefineries could positively influence the overall economics making protein extraction more profitable (Dale *et al.*, 2009) and could be attractive for providing more sustainable protein sources than soybeans for livestock production (Parajuli *et al.*, 2015). Green biorefineries represent integrated systems for exploitation of green crops i.e. grasses, legumes and the green part of crops, which can be utilized fresh or ensiled for the production of feed, food, chemicals, materials and biofuels (Kamm *et al.*, 2016).

LPC - Leaf protein concentrate

RUBISCO - ribulose-15-bisphosphate carboxylase or oxygenase

TCA - trichloroacetic acid

2.4 THE LEAF PROTEINS

Leaves are the largest source of proteins in the world (Ellis , 1979 ; Fiorentini and Galoppini , 1983). Most proteins in plant leaves (about 80%) are located in the chloroplasts, where about half of the proteins are soluble in the stroma and the other half are part of the thylakoid membranes (Fiorentini and Galoppini , 1983 ; Tamayo Tenorio *et al.*, 2018). The thylakoid membranes are networks of membranes containing proteins, lipids and pigments (chlorophyll and carotenoids) specialized in the photosynthesis and embedded in the chloroplasts stroma (Ellis , 1977). More than 70 different proteins are involved in the photosynthetic reactions taking place is the thylakoid membranes (Friso *et al.*, 2004). The remaining proteins in plant leaves are mostly located in the cytoplasm (about 20 %) with minor amounts found in the cell nucleus (1−2 %) or in the mitochondria (less than 5 %) (Fiorentini and Galoppini , 1983). Around 250–300 different proteins and polypeptides were detected by electrophoresis in green plant extracts (Kromus *et al.*, 2006).

Leaf proteins can be differentiated between insoluble and soluble based on their solubility in water. The insoluble protein fraction is mainly composed of proteins forming the photosynthetic complexes together with lipids and pigments in the thylakoid membranes of the chloroplast (Fiorentini and Galoppini , 1983 ; Tamayo Tenorio *et al.*, 2018). A small fraction of insoluble proteins can be also found in the cell wall attached to polysaccharides (Tamayo Tenorio *et al*., 2018). The soluble protein fraction is predominantly Rubisco (Ribulose 1,5-bisphosphate carboxylase/oxygenase), the key enzyme for the fixation of CO2 during photosynthesis that can represent up to 50% of the total soluble proteins in the leaves (Fiorentini and Galoppini , 1983 ; Tamayo Tenorio *et al*., 2018). Rubisco is a relatively large enzyme with an approximate molecular weight of 550 kDa, composed of eight large subunits (55 kDa) and eight small

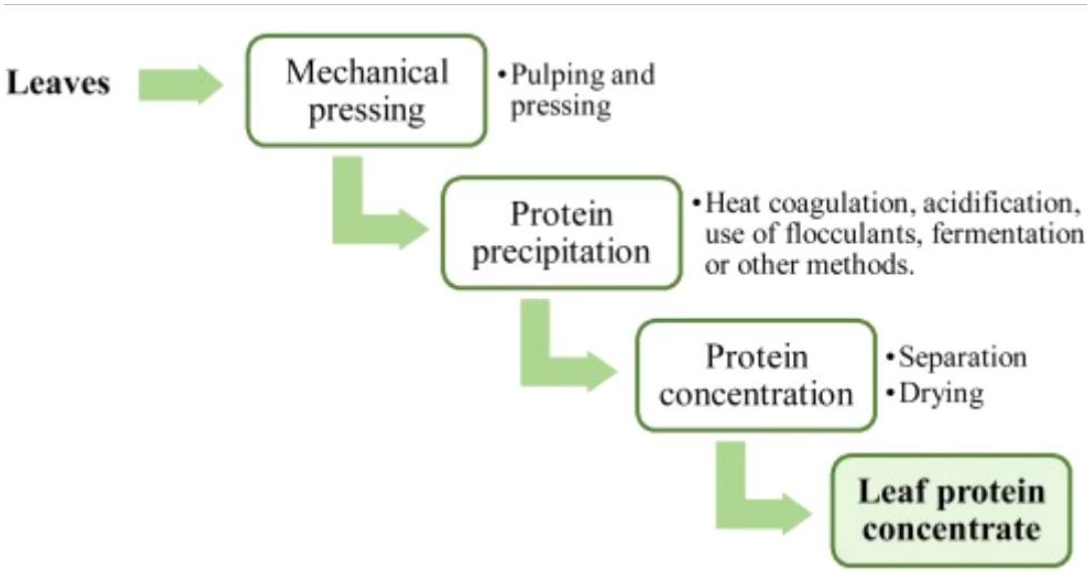
subunits (15 kDa) and located in the stroma, where it catalyzes the first step of the photosynthetic process. However, Rubisco has a very low catalytic efficiency that plants overcome by synthesizing large Rubisco amounts in the leaves (Nishimura *et al.*, 2008). The remaining soluble proteins in the leaves are enzymes involved in the synthesis of carbohydrates, lipids, proteins and other compounds as well as free amino acids and oligopeptides (Ellis , 1977 ; Fiorentini and Galoppini , 1983). Therefore, plant leaves represent a vast source of proteins that in many cases are utilized by grazing livestock but potentially could be used for animal feeding purposes .

2.5 EXTRACTION OF PROTEINS FROM LEAVES

The extraction of proteins from leaves is not a new concept since a lot of research was carried out in this field during the 20th century, especially during the Second World War. At that time, the possibility of utilizing leaf proteins as human food appeared as an alternative for providing populations with sufficient protein in the event of food shortages (Kromus *et al.*, 2006 ; Pirie , 1971). Pirie and colleagues at the Rothamsted Experimental Station (England) carried out highly valuable research in order to develop a large scale process for the extraction of proteins from fresh leaves to be used as food protein in human nutrition (Morrison and Pirie , 1961 ; Pirie , 1969, 1971, 1987). Nevertheless, leaf protein concentrates were not well-accepted by consumers because of their bitter, grassy flavor and dark green color (Chiesa and Gnansounou, 2011; Edwards *et al.*, 1975). In the last couple of decades, the interest on the protein extraction from plant leaves has been rekindled probably driven by factors such as the need for alternative protein sources with food and feed applications, the industrialization and development of biotechnologies, and the concept of biorefineries for producing wide range of commodities as alternative to oil refineries .

The extraction of proteins from leaves involves an initial mechanical pressing of the fresh material so leaf proteins are released into the pressed plant juice; followed by protein precipitation and protein concentration into the LPC .

Figure 1: Basic unit operations for the extraction of proteins from leaves.



2.5.1 MECHANICAL PRESSING

Mechanical pressing is carried out to separate the leaf proteins from the cell wall so that a protein rich juice is squeezed out from the fibers (Bals *et al.*, 2012). Traditionally, fresh leaves were firstly pulped with different mills or rollers to break up the cells and release the cellular components including soluble proteins and chloroplasts, then the juice was expressed from the fibrous pulp (or press cake) by pressing (Bals *et al.*, 2012). Been that pulping and pressing could not be performed in a single unit at large scale due to technological limitations, Pirie (1971, 1987) carried out an extensive research to develop an economic process for the plant juice extraction. For instance, several pulpers alternative to hammer mills were developed to continuously pulp the crops (Pirie, 1971). Further, Pirie (1987) focused on developing presses to press out most of the extractable juice by applying pressure very efficiently.

The degree of cell disruption is critical for the juice expression and protein recovery. However, about at least half of the proteins are retained in the press cake after the mechanical pressing. In this regard, several authors recommended the addition of water to the press cake and a secondary re-pressing in order to extract part of the retained proteins (Byers and Sturrock , 1965 ; Knuckles *et al.*, 1972; Morrison and Pirie , 1961). Morrison and Pirie (1961) reported that half of the proteins left in the press cake could be extracted by means of a secondary pulping and pressing with addition of water. The addition of water to the press cake and secondary re-pressing resulted in an increased protein recovery in the green juice from 40 % to 53 % (Knuckles *et al.,* 1972).

The addition of water likely favors the recovery of proteins in the green juice but at the same time, the concentration of proteins in the green juice is diluted. In addition, chopping the fresh plant material before the mechanical pressing has been recommended since it helps releasing

plant soluble components. However, in contrast, Morrison and Pirie (1961) recommended to carry out the pulping and pressing at alkali pH of around 8.0 for an improved extraction of proteins. Indeed, leaf protein extractability was highly influenced by pH according to Betschart and Kinsella (1973), who concluded that leaf protein is more soluble and chloroplasts are disrupted more effectively at high pH values; however, they suggested to use pH values between 7.0–8.0 to avoid the risk of protein denaturation. The temperature of the plant material before the mechanical pressing is also an important factor for the recovery of proteins during mechanical pressing, as studied by Hanna and Ogden (1980). Results showed that heating to 35°C, 50°C or 60°C before mechanical pressing was detrimental for the juice expression and protein recovery while cooling to 3°C, 7°C or 14°C had no effect compared with the ambient temperature (25°C). Also , the equipment design and operation conditions during the mechanical pressing of fresh plant material are crucial for ensuring an efficient juice expression and protein extraction into the green juice. The various extraction methods may also facilitate the co-extraction of anti-nutrients, which can influence animal performance.

2.5.2 PROTEIN PRECIPITATION

The aim of the protein precipitation step is to concentrate the proteins into a solid fraction, which can be further separated and dried into a storable product (Bals *et al.*, 2012). The precipitation of the proteins is usually performed by thermal coagulation or acidification but other methods including addition of flocculants or bacterial fermentation have been investigated and are detailed below.

The green juice contains chloroplast and cytoplasmic proteins from the plant cells (Fiorentini and Galoppini , 1983). The chloroplast proteins, also known as green proteins are the insoluble

lipoproteins mostly present in the thylakoid membranes. These proteins are easy to destabilize and coagulate more rapidly at lower temperatures resulting in a dark green concentrate with a strong grassy flavor (Edwards *et al.*, 1975; Fiorentini and Galoppini , 1983 ; Hernandez *et al.,* 1988). The cytoplasmic white proteins are soluble in the cell cytoplasm or in the stroma and are relatively stable. Precipitation of the white protein fraction results in a tasteless, odorless white/creamy precipitate (Fiorentini and Galoppini , 1983). Two different strategies can be used for the precipitation of proteins: (a) an unfractionated LPC containing both green and white proteins can be obtained , (b) alternatively, a fractional process can be performed in order to firstly precipitate the green protein fraction into a LPC suitable for animal feeding and then, the white protein fraction into a LPC suited for human food (Chiesa and Gnansounou , 2011).

2.5.3 METHODS USED IN THE EXTRACTION OF LPC .

2.5.3.1 HEAT COAGULATION

Heat coagulation has been widely applied for the precipitation of proteins from plant green juices at temperatures ranging from 60°C to 95°C (Baraniak , 1990 ; Byers and Sturrock , 1965 ; Collins , 1986 ; Edwards *et al.*, 1975 ; Koschuh *et al.,* 2004 ; Lazar *et al.*, 1971 ; Morrison and Pirie , 1961). Heating provokes the coagulation of proteins as result of opening up hydrophobic sites and protein denaturation (Bals *et al.*, 2012). According to Morrison and Pirie (1961), heat coagulation at 75−80°C by direct steam injection in the green juice was the most convenient method for producing leaf protein concentrates on a large scale .

Heat coagulation is an efficient method for the precipitation of proteins. Nevertheless, the leaf protein concentrates produced by heat coagulation might have low protein solubility due to the irreversible changes in the protein structure caused by denaturation, which could also affect other

functional properties (Betschart and Kinsella, 1973 ; Bray and Humphries , 1978 ; Lamsal *et al.*, 2007). The denaturation of Rubisco takes place at 76.2°C (Lamsal *et al.*, 2007).

2.5.3.2 ACID PRECIPITATION

The addition of acid changes the solubility of the proteins in the green juice and can lead to their precipitation. Acid precipitation has been performed in order to obtain an unfractionated LPC (Baraniak , 1990 ; Coldebella *et al.*, 2013 ; Damborg *et al.*, 2020 ; Morrison and Pirie , 1961) .

Damborg *et al.,* 2020, studied the precipitation of proteins under a pH range from 3.0–5.0 by addition of HCl to different green juices. In most cases, the precipitation efficiency (i.e. protein yield in the protein concentrate) was not pH-dependent. Furthermore, acidification showed a tendency towards a better precipitation efficiency when compared with heat coagulation (Damborg *et al.*, 2020).

2.5.3.3 ADDITION OF FLOCCULANTS

The action of flocculants derives from their ability to aggregate particles, proteins in this case, forming large complexes that easily settle and can be partitioned from the mixture. The addition of flocculants to the green juice has been proposed to precipitate the proteins and produce LPC at room temperature avoiding heat coagulation (Anelli *et al*., 1977 ; Baraniak , 1990 ; la Cour *et al.*, 2019).

2.5.3.4 OTHER PROTEIN PRECIPITATION METHODS

Direct spray-drying of green juice was proposed as an alternative method for producing LPC while preserving valuable soluble components and avoiding drying at high temperatures (Hartman *et al.*, 1967). Relatively high N recoveries from the total plant N (43–44 %) were

achieved in the freeze-dried product, which contained between 18–35 % protein and high concentration of vitamins. Furthermore, chlorophyll was removed from the spray-dried product with 95 % ethanol resulting in an increased protein content in the freeze-dried product (26–43 %). Nevertheless, direct freeze-drying of the green juice was refused by Pirie (1971) , due to the risk for the formation of indigestible complexes and the presence of harmful soluble compounds.

2.5.4 PROTEIN CONCENTRATION : SEPARATION OF PROTEINS AND DRYING

In most cases, the separation of proteins from the juice is achieved by centrifugation but filtration processes or membrane technology can be utilized as well. Afterwards, the LPC is usually dried in order to produce a stable product that can be stored and easily transported. In case that drying or freezing are not performed, growth of soil fungi like Mucus racemosus, which are not inhibited during preparation of the LPC, may cause microbial spoilage of the wet protein concentrate (Arkcoll , 1973).

Drying significantly influences the texture and the nutritional quality of the LPC(Morrison and Pirie , 1961). Indeed, a decreased nutritional value in the LPC has been observed upon drying at high temperatures (Miller *et al.,* 1972 ; Morrison and Pirie , 1961). Heat treatments of LPC can lead to Maillard reactions between reducing sugars and lysine, rendering lysine biologically unavailable and reducing the overall nutritional value (Gilani *et al.*, 2012). Moreover, heat (or alkali) treatments of food proteins can provoke racemization of amino acids to D-enantiomers and concurrent formation of lysinoalanine (LAL, an unnatural amino acid derivative) (Gilani *et al*., 2012 ; Schwass and Finley, 1984).

The protein extraction yield from the fresh plant material into the LPC is highly influenced by the extraction procedure. Mostly, an efficient mechanical pressing ensures a great release of

proteins into the juice; however, the subsequent protein precipitation and protein concentration are also important to ensure an overall high protein extraction yield. Apart from the protein extraction procedure, the yield of extractable proteins is also highly influenced by agronomic factors such as the plant species, variety, type of soil and its fertility, growth stage and age of the plant at harvesting, climate or plant density (Arkcoll and Festenstein, 1971).

2.5.5 NUTRITIONAL VALUE OF LEAF PROTEIN CONCENTRATE FOR ANIMALS

The protein concentration of LPC can vary significantly depending on the extraction procedure and the plant biomass. LPC are good sources of lipids, beta-carotene and xanthophyll. In addition, "unfractionated" LPC also contains cell debris such as fibre, broken chloroplasts, and particulates (Tamayo Tenorio *et al.*, 2018).

The feeding value of the leaf protein concentrate depends on the protein concentration and quality but also on the energy content and on the presence of anti-nutritional compounds and their concentration (Dale *et al.*, 2009). Beta-carotene and xanthophyll can be beneficial for e.g. yolk color. Depending on the age and type of animal, the presence of fibers can be unwanted i.e. young animals such as new hatched chickens, pigs and calves have very high nutritional requirements regarding digestibility during weaning and are very sensitive to anti-nutritional compounds, including fibre.

2.5.6 AMINO ACID COMPOSITION

Proteins are sources of amino acids, which can be divided into essential, semi-essential, and non-essential amino acids. Essential amino acids are those that cannot be synthetized by the organism itself, but need to be supplied with the diet in adequate amounts and ratio to each other for building up the body's own proteins (Sundrum *et al.*, 2005). The deficit of any essential amino

acid in the animal diets results in a general protein deficiency (Blair, 2008) and therefore, the adequate supply of essential amino acids is crucial in the nutrition of animals. It is likely that Rubisco is the main protein in protein concentrates extracted from plant biomass but the minor differences observed in the profile of essential amino acids reveal that other proteins are extracted as well. The relatively high proportion of essential amino acids in the protein concentrates indicates a suitable supply of essential amino acids for animals. (Steenfeldt and Hammershøj, 2015). Overall, the LPC from different plants present a similar profile of essential amino acids despite the plant biomass or the protein precipitation method, which is advantageous for commercializing the product for animal feeding purposes.

Therefore, LPC is a good source for dietary protein, with relatively high content of methionine and cysteine, for feeding animals. Possible deficiencies in specific amino acids might be compensated from the diet by preparing mixed formulations with other protein sources such as lupin, which shows a relatively good amino acid profile compared with other grain legumes. Nevertheless, the amino acid content itself does not provide enough information about the nutritional value since the bioavailability of amino acids is required in relation with digestibility as well (Sundrum *et al.*, 2005).

2.5.7 INTEGRATION OF LEAF PROTEIN EXTRACTION WITH GREEN BIOREFINERIES

The integration of leaf protein extraction within green biorefineries represents a great opportunity for the industrial development and establishment of protein extraction processes for feeding purposes of animals.

CHAPTER THREE

MATERIALS AND METHODS

3.1 LOCATION AND DURATION OF THE STUDY

The experiment was carried out in the main Laboratory of Faculty of Agriculture in Ugbowo Campus, University of Benin (Uniben), Edo State, Nigeria. The Campus is located between Latitude 6º and 30ºN of the Equator and Longitude 5º40 and 6ºE of the Greenwich Meridian in the forest zone with a temperature of 27.6 ºC (Google Earth, 2022).

3.2 EXPERIMENTAL MATERIALS

The materials used for this experiment were freshly harvested opepe leaves, laboratory thermometer, whatmann filter paper, weighing balance, knife, plastic bowls, stainless steel pot, heating stove, measuring cylinder, grinder, paper foil, masking tape.

3.3 PRELIMINARY TRIALS

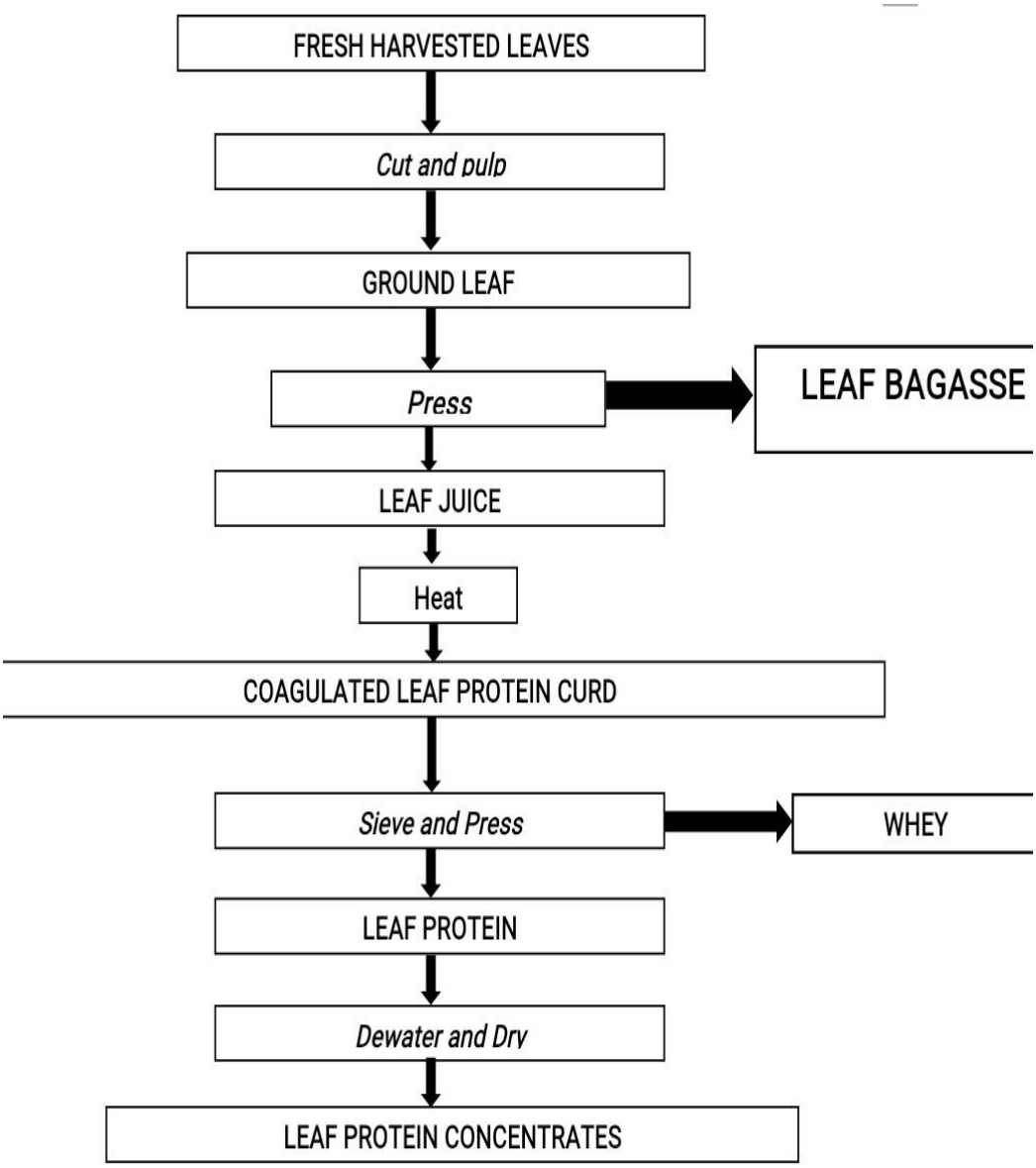
Nine preliminary trials were carried out before the final production which were done to serve as a guide and to effectively standardize the process and all the errors encountered noted.

3.4 CALIBRATION PROCEDURES

Fresh opepe leaves were harvested in the morning and were taken immediately to the Laboratory for processing to prevent wilting. The leaves were thoroughly washed to remove dirts and sand particles. Then 300g was weighed for each trial respectively for the different samples. A known volume of water was used for each trial respectively.

3.5 PRODUCTION OF OPEPE LEAF PROTEIN CONCENTRATE USING HEAT COAGULATION METHOD

Fresh opepe leaves were harvested in the morning and were taken immediately to the Laboratory for processing as illustrated by Pirie (1987). The leaves were thoroughly washed to remove dirt and sand particles. The leaves were then processed by grinding to slurry using an electric blender. The slurry of each sample was put on a sieve cloth and pressed thoroughly to separate the juice from the fibrous chaff (Bagasse). The juice was heated and the curd began to separate leaving the whey fraction. The different temperatures and time taken for the curd formation were noted. After cooling, the curd was separated from the whey using a whatmann filter paper and the sample of leaf protein concentrate was taken and the yield was recorded.



3.1: Flow chart of the production of leaf protein concentrates

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

3.6 PRODUCTION OF OPEPE LEAF PROTEIN CONCENTRATE USING ALUM PRECIPITATION METHOD

After processing the leaves by grinding, it was then extracted using a sieve to get the bagasse and curd. The juice was put in a bowl. To 100ml of the juice, 2g of alum was added. The curd of LPC resulted due to the coagulation of proteins in juice by alum solution which was then filtered through Whatman filter paper and the sample of LPC was taken and the yield was recorded.

PRECAUTIONS TAKEN DURING THE PRODUCTION OF LPC

1. The leaves were processed immediately after harvest to prevent wilting.

2. Matured leaves free of physical injury were used.

3. The leaves were cut into smaller bit for easy blending.

4. The sieve cloth used had a very small pore in order not to allow the fibre to pass through.

5. The LPC in the whatmann filter paper should be scooped out before drying to prevent it from sticking to the filter paper.

6. The LPC should be air-dried rather than oven-dried.

7. The materials used for the experiment should be washed before and after use.

3.8 CHEMICAL ANALYSIS

The samples were analyzed in triplicate, using the procedure described as follows:

3.8.1 Moisture Content Determination

Materials used: weighing balance, crucible, oven and desiccators

Procedures

Weigh 2g of the sample into a silica dish that has been previously weighed. Then put in the oven at 100°C for 24 hours and then to constant weight. The sample was then cooled in a desiccators before weighing again until a constant weight was obtained then calculate:

Calculations:

(i) Weight of moisture = wt of crucible sample – wt of crucible and sample after drying

(ii) $\%\text{Moisture} = \frac{\text{wt of moisture}}{\text{wt of sample}} \times \frac{100}{1}$

(iii) Dry matter = 100 - % moisture

3.8.2 Crude Protein Determination

2g of prepared curd and bagasse was weighed and transferred into a clean digestion flask, the digestion mixture; copper, selenium catalyst plus potassium or sodium sulphate was then added to raise the boiling point, 30ml of concentrated sulphuric acid was added to the digestion flask containing the other mixture and the sample digested for 2 hours. The flask was cooled then diluted with water and was made to 100ml in a volumetric flask. 20 ml of 2% boric acid plus indicator was pipetted into a 100ml Erlenmeyer flask. The 100ml flask was then placed under the receiving tube of the distillation unit in a way that the end of the tube is below the level of the

H_3BO_3. 10ml aliquot of the sample was then pipetted into the distillation unit and 100ml of 40% NaOH was added. The sample was distilled with standard HCl (0.01N) until the blue colour disappeared. A blank determination was first carried out and the crude protein values were determined using the following formula:

Calculation

$$\%N \text{ of sample} = \frac{\text{net vol. of acid x conc, of acid x 14 x 100 x 10}}{\text{Weight of sample in g}}$$

$$\% \text{ crude protein} = \frac{\text{net vol. of acid x 14 x 100x 10 x 6.25}}{\text{Weight of sample in g}}$$

3.8.3 Crude Fibre Determination

2 g of the curd and bagasse was weighed into a round bottom flask, 100ml of crude fibre reagent that has been boiled was added, and then the beaker placed on the crude fibre apparatus which has been presented to maintain steady boiling. The content was filtered under suction on a piece of close texture linen after refluxing for 1 hour.

The residue was rinsed with boiling water until they were finished. Also NaOH (sodium hydroxide) solution which had been previously brought to boil was added, filtered while hot using a Whatman filter paper and the residue was allowed to drain and transferred to a preheater and dried over-night in the oven. The residue was cooled in the desiccator and weighed after 1 hour cooled and weighed. The loss in weight was calculated as the crude fibre content.

Calculation

Sample size = A (2g)

Wt before washing = P

Wt after washing = Z

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

3.8.4 Ash Determination

2g of the curd and bagasse were weighed separately and put in a weighed crucible and was Ignited at 550^{o}C for 6 hours in the furnace for ashing. Then the sample was removed and allowed to cool in a dessicator for about 30minutes then re-weighed and the value was calculated.

Calculation

Sample wt = A

Sample wt before Ashing = P

Sample wt after Ashing = Z

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

3.8.5 Ether Extract

2g of leaf curd and bagasse were weighed separately into a fat free extraction tumble. It was then corked tightly with cotton and placed in the extraction petroleum ether was added until it siphoned over. More ether was added until the barrel 300ml was half filled, the condenser was replaced. The control was adjusted on the apparatus so that the others boiled gently and it was

left to siphon over for 2 hrs. The apparatus was washed after 3 hours because it was expected that by this time all the fat present in the sample would have been extracted. The flask was then detached when the ether was short of siphoning over.

The barrel content was drained properly into the bottle and the thimble removed and dried. The flask was detached the exterior cleared and dried in an oven to constant weight.

Calculation:

$$\%EE = \frac{\text{initial weight} - \text{final weight}}{\text{Weight of sample}} \times \frac{100}{1}$$

3.8.6 Nitrogen Free Extract (NFE)

This is determined from the subtraction of the addition of % ash, ether extract (EE), crude protein (CP) and crude fibre (CF) from 100. The difference is the NFE.

NFE = 100 – (%CP + %EE + %Cf + %Ash)

3.9 MINERAL ANALYSIS

Minerals were analysed after the first dry-ashing 1g of the LPC and bagasse at 550c in a muffle furnace and dissolved in de-ionised water to standard volume. Sodium and potassium were determined by flame photometry and phosphorus by vanadomolybdate method of AOAC (2010). Magnesium, calcium, manganese, iron, zinc and copper were determined using an atomic absorption Spectrophotometer.

3.10 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's multiple range tests (Duncan's, 1955).

3.11 YIELD DETERMINATION

The percentage yield of leaf protein concentrate and bagasse is derived by evaluating(dividing) the quantity of dry matter LPC or dry matter of bagasse with the dry matter leaves used multiplied by 100%

Yield determination formulas for LPC and bagasse are given below:

$$\text{LPC \%Yield} = \frac{\text{DM LPC}}{\text{DM Leaves}} \times 100$$

$$\text{Bagasse \%Yield} = \frac{\text{DM Bagasse}}{\text{DM Leaves}} \times 100$$

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield of LPC and Bagasse, using Alum Precipitation, Acid coagulation and heat coagulation method

The percentage yields of LPC and bagasse from alum precipitation, acid coagulation and heat coagulation method are presented in Table 4.1. The yields of alum precipitation LPC (3.913%), acid coagulation LPC (2.398%) and heat coagulation LPC (6.383%) were significantly ($p<0.05$) different from each other. The yield of bagasse was 23.508%. It was observed from the results obtained that LPC from the heat coagulation method (6.383%) was significantly ($p<0.05$) higher than the LPC yield (3.913%) from alum precipitation and the LPC yield (2.398%) from acid coagulation. It is also important to note that the opepe LPC from heat coagulation method was easier to extract than that of alum precipitation method, as LPC from alum precipitation sticks sometimes to the whatman filter paper.

Table 4.1: Percentage Yield of Pepped LPC Using Acid Coagulation, Alum precipitation and Heat Coagulation Methods

Sample	Yield (%)
Acid Coagulation	2.398[a]
Alum Precipitation	3.913[ab]
Heat Coagulation	6.383[b]
SEM	0.907
Bagasse	23.508[c]

SEM= Standard Error of Mean, Means with same alphabet are not significantly ($p > 0.05$) different

4.3 Proximate Composition of Opepe LPC and Bagasse Using Alum Precipitation, Acid coagulation and Heat Coagulation methods

The proximate composition of opepe LPC and bagasse using alum precipitation, acid coagulation and heat coagulation method is presented in Table 4.2. The dry matter using Heat coagulation method (88.40%), dry matter using acid coagulation method (88.66%) and dry matter using alum precipitation method (89.42%) were not significantly($p<0.05$) different from each other; and bagasse dry matter was (89.00%). Crude fibre of LPC (1.863%) using Heat coagulation method was not significantly ($p<0.05$) different from Crude fibre of LPC (1.870%) using Acid coagulation method but they were both significantly higher than crude fibre of LPC (1.172%) using alum precipitation. Crude fibre of Bagasse was (7.322%). Crude protein of the LPC using Heat coagulation method was (45.08%) and was not significantly ($p<0.05$) different from Crude protein of LPC (45.37%) using acid coagulation but they were both significantly higher from crude protein of LPC (42.13%) using alum precipitation method and crude protein of Bagasse was (24.66%). Ash of LPC (5.207%) using heat precipitation method was not significantly ($p<0.05$) different from Ash of LPC (5.005%) from the alum coagulation method but they were both significantly lower (6.022%) than that of the acid coagulation. Ash of bagasse was (7.662%). Ether Extract of LPC (17.78%) using heat coagulation method was not significantly ($p<0.05$) different from ether extract of LPC (17.52%) using acid precipitation method and ether extract of LPC (16.75%) using alum precipitation method. Ether extract of Bagasse was (4.13%). Nitrogen Free Extract of LPC (18.47%) using heat coagulation method was not significantly ($p<0.05$) different from nitrogen free extract of LPC (17.88%) using acid coagulation method but they were both significantly lower than nitrogen free extract of LPC (24.37%) using alum precipitation method; Nitrogen free extract of Bagasse was (45.43%).

Table 4 .2: Proximate Composition of Opepe LPC and Bagasse using Acid Coagulation, Alum Precipitation and Heat Coagulation

Parameters	%DM	%CP	%CF	%EE	%Ash	%NFE
Acid	88.66	45.37^{c}	1.870^{b}	17.52^{b}	6.022^{b}	17.88^{a}
Alum	89.42	42.13^{b}	1.172^{a}	16.75^{b}	5.005^{a}	24.37^{b}
Heat	88.40	45.08^{c}	1.863^{b}	17.78^{b}	5.207^{a}	18.47^{a}
SEM	0.354	0.747	0.2786	0.391	0.276	1.495
Bagasse	89.00	24.66^{a}	7.322^{c}	4.13^{a}	7.662^{c}	45.43^{c}

Means with same alphabet on the same column are not significantly ($p< 0.05$) different.
SEM= Standard Error Mean, DM= Dry Matter, CP= Crude Protein, CF= Crude Fibre, EE=Ether Extract, NFE= Nitrogen Free Extract.

4.4 Mineral composition of OPEPE LPC using Alum Precipitation and Heat Coagulation Methods

Some mineral compositions of opepe LPC using alum precipitation, acid coagulation and heat coagulation method are shown in Table 4.3. Among the macro minerals, potassium (4140 mg/kg) was the highest in heat coagulated LPC and was found to be significantly ($p<0.05$) different from that in acid coagulated LPC (4522mg/kg) and that in alum precipitated LPC (1639mg/kg). Phosphorus content (178.3mg/kg) of alum precipitated LPC was significantly different($p<0.05$) from that obtained from heat coagulated LPC (360.3mg/kg) and that obtained from acid coagulated LPC (286.5mg/kg). Sodium content (34.73mg/kg) obtained from heat was significantly ($p<0.05$) different from Sodium content (32.89mg/kg) obtained from acid coagulated LPC and sodium content (16.35mg/kg) from alum precipitated LPC. Magnesium content (243.4mg/kg) from acid coagulated LPC and magnesium content (244.9mg/kg) from heat coagulated LPC were not significantly different but they were significantly different from Magnesium content (144.0mg/kg) from alum coagulated LPC. Calcium content (1304mg/kg) from acid coagulated LPC and calcium content (1304mg/kg) from heat coagulated LPC were not significantly different but they were both significantly different from calcium content (647mg/kg) from alum precipitated LPC.

For micro minerals, iron content (123.87mg/kg) from heat coagulated LPC and iron content (124.69mg/kg) from acid coagulated LPC was not significantly ($p<0.05$) different but they were both significantly different iron content (1.78mg/kg) from alum precipitated LPC. Manganese content (36.35mg/kg) from acid coagulated LPC and manganese content (35.02mg/kg) from heat coagulated LPC was not significantly ($p<0.05$) different but they were both significantly different from manganese content (0.50mg/kg) from alum precipitated LPC.

Table 4.3: Mineral Composition of Opepe LPC and Bagasse Using Acid Coagulation, Alum Precipitation and Heat Coagulation Extraction Methods(mg/Kg)

Minerals	Ca	Mg	Na	K	P	Fe	Mn
Acid	1304[b]	243.4[b]	32.89[b]	4522[c]	286.5[b]	124.69[b]	36.35[b]
Alum	647[a]	144.0[a]	16.35[a]	1639[a]	178.3[a]	1.78[a]	0.50[a]
Heat	1304[b]	244.9[b]	34.73[c]	4140[b]	360.3[c]	123.87[b]	35.02[b]
SEM	16.21	5.14	0.603	84.5	202.5	2.32	0.478

Means with same alphabet on the same column are not significantly ($p < 0.05$) different.
SEM= Standard Error Mean

4.5: Some Physical Characteristics of Whole Leaf, LPC, Bagasse and Whey of Opepe

The leaves of opepe are evergreen and glabrous. The whey obtained from heat coagulation is dark and brown and gets darker with time while the whey from alum precipitation and acid coagulation is quite transparent and lighter than the whey from heat coagulation. The bagasse obtained was fibrous and dark green in color both before and after drying. The LPC obtained from heat coagulation, acid coagulation and alum precipitation were all dark green in color both before and after drying.

Table 4.4: Some Physical Characteristics of Whole Leaf, LPC, Bagasse and Whey of Opepe

Before Drying				
Character	**Leaf**	**Bagasse**	**LPC**	**Whey**
Colour	Light Green	Dark Green	Dark Green	Greenish brown
Texture	Leathery	Fibrous	Smooth	Consistent
State	Solid	Solid	Semi solid	Liquid

After drying			
Character	**Leaf**	**Bagasse**	**LPC**
Colour	Dark green	Dark green	Dark green
Texture	Coarse	Fibrous	Smooth
State	Solid	Solid	Solid

CHAPTER FIVE

5.0 DISCUSSION

5.1 Yield of Opepe LPC using heat coagulation, acid coagulation and alum precipitation methods

Percentage yield of opepe leaf protein concentrates (6.383%) using heat coagulation method was higher than the yield of rubber leaf protein concentrates (5.6%) using the same method, as reported by Akaeze *et a*l. (2015). This could be as a result of the inherent nature of the plant material. Thus, 100kg dry matter of opepe leaves is expected to yield an average of 6.383kg dry matter of opepe leaf protein concentrates using heat coagulation method. The yield of bagasse was 23.508%.

Percentage yield of opepe leaf protein concentrates (3.913%) using alum precipitation method was higher than 2.93% *Raphanus sativus* leaf protein concentrates reported by Sayyed (2011) using same method. To that effect, this could serve as an important source of protein supplementation that is needed in livestock feeding.

Percentage yield of opepe leaf protein concentrates derived from heat coagulation (2.398%) was lower than LPC yields of Pawpaw (7.12%) as reported by Agbonghae and Nwaokoro (2019).

The yield of LPC derived from heat coagulation method is significantly higher than that of alum precipitation and acid coagulation method. This could be as a result of some advantages that heat coagulation has over alum precipitation and acid coagulation method and hence, more protein concentrates is produced. It is however important to note that this difference is statistically significant ($p<0.05$).

5.2 Chemical Composition of Opepe LPC and using three different methods

5.2.1 Proximate Composition

Results obtained from the proximate analysis of opepe LPC using three different methods are shown in Table 4.5. The results show that the percentage contribution for crude fibre will be higher than its actual content due to the inherent nature of materials and other foreign materials from the filter paper and sieve that could contaminate the LPC during the process of sieving and sun drying.

The crude fibre (1.863 %) for LPC gotten from heat coagulation method was higher than the result of LPC of *Amaranthus hybridus* (1.7%) from heat coagulation method and heat coagulated LPC of rubber (1.8%) as reported by Akaeze *et al*., 2015. The LPC composition, 3.913% for alum precipitation method is higher than that in LPC of *Amaranthus hybridus* (1.7%) *as reported by* Adeyeye *and* Omolayo (2011). . Crude fibre was found to have compositions of 1.870% from acid coagulation, was higher than the LPC of rubber (1.8%) and *Amaranthus hybridus*(1.7%) from works of Akaeze *et al.* (2015) and Adeyeye and Omolayo (2011) respectively.

The LPC crude protein composition was 45.37% from acid coagulation, 42.13% from alum precipitation and 45.08% from heat coagulation, all values were higher than the crude protein content of rubber LPC (32.64%) as reported by Akaeze *et al.* (2015). The crude protein composition was also discovered to be higher than that in the LPC of *Amaranthus hybridus* (34.8%) from the works of Adeyeye and Omolayo (2011).

The ash content of heat coagulated opepe LPC (5.207%) and those from alum precipitation (5.005%) and acid coagulation (6.022%) were found to be lower than ash content of LPC of *Amaranthus hybridus* (17.2%) as reported by Adeyeye and Omolayo (2011). The ash content of

both the LPC could have been contaminated during alum precipitation. This is due to the release of aluminum and sulphide into the juice from the alum during extraction, both of which will significantly affect the ash composition of the sample.

The ether extract of opepe LPC (17.78%) from heat coagulation method was higher than 9.6% in *Amaranthus hybridus* while opepe LPC from alum precipitation (3.913%) was lower than 10.7% from *Telfaria occidentalis* LPC as reported by Adeyeye and Omolayo (2011)

The NFE of opepe LPC (18.47%) from heat coagulation method was lower than 23.58% from *Vernonia amygdalina* and NFE of opepe LPC (24.37%) from alum precipitation method was found to be higher than 23.58% from *Vernonia amygdalina* LPC as reported by Sodamade *et al.* (2013).

5.2.2 Mineral Composition

From the results shown in table 4.3, the potassium from acid coagulated LPC and the calcium from both acid and heat coagulated LPC, produced the highest with 4522mg/kg, 1304 mg/kg and 1304 mg/kg respectively. Calcium is a mineral, often associated with healthy bones and teeth and it also plays an important role in blood clotting and helping muscles to contract, while potassium helps maintain normal levels of fluid inside our cells.

Calcium(1304mg/kg) and Magnesium (244.9 mg/kg) in the LPC from heat method and were both lower than 5000mg/kg and 400mg/kg for calcium and magnesium in LPC of Lucerne respectively, as reported by Siebrits *et al.* (1986). The calcium and magnesium in the LPC from alum precipitation was 647mg/kg and 144.0mg/kg respectively, while the calcium and magnesium in the LPC from acid coagulation was 1304mg/kg and 243.4mg/kg respectively.

Magnesium is important for many processes in the body, including regulating muscle and nerve function, blood sugar levels and blood pressure, and also important in bone formation.

Sodium content in opepe leaf protein concentrate from heat coagulation (34.73 mg/kg), alum precipitation (16.35 mg/kg) and acid coagulation (32.89 mg/kg) were lower than 312mg/kg from *Telferia occidentalis* LPC as reported by Adeyeye and Omolayo (2011). Sodium is a counterpart of magnesium, it maintains food levels outside of cells and balances the amount and distribution of water in our bodies, playing a key role in the control of our blood pressure.

Phosphorus content of heat coagulated opepe LPC (360.3mg/kg), alum precipitated opepe LPC (178.3mg/kg) and acid coagulated LPC (286.5mg/kg) were lower than the LPC phosphorus content (457mg/kg) of *Amaranthus hybridus* reported by Adeyeye and Omolayo (2011).

For the micro minerals, the analysis of iron and manganese, showed that composition of iron (123.87mg/kg) and manganese (35.02mg/kg) in heat coagulated opepe LPC were higher than the composition of iron(88.5mg/kg) and manganese(18.0mg/kg) in *Caesalpinia pulcherrima* LPC respectively, as reported by Nwokoro *et al.* (2022).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

This research work was carried out in three phases: First, was to determine the yield of opepe leaf protein concentrate and bagasse using heat coagulation method followed by the determination of their proximate and some mineral composition. Second, was to determine the yield of leaf protein concentrate and bagasse using alum precipitation method followed by the determination of their proximate and some mineral composition. In the third phase, the acid coagulation method was applied to determine the yield of opepe leaf protein concentrate and bagasse, then followed by determination of their proximate and mineral composition.

The results obtained from the extraction indicated that, the yields from the three methods, are 6.383%, 3.913%, and 2.398% respectively from heat coagulation, alum precipitation and acid coagulation. What this means is that, every 100kg dry matter of opepe leaf will produce an average of 6.383kg, 3.913kg and 2.398kg of opepe leaf protein concentrate for heat coagulation, alum precipitation and acid coagulation respectively.

From the chemical analysis, CP of LPC from heat coagulation method was the best of the three methods and EE of the LPC from heat coagulation method yielded the highest of the three methods. The CF of LPC from acid coagulation method yielded better than the other two methods. The ash composition from acid coagulated LPC was found to be higher than that of

alum precipitated and heat coagulated LPC. The NFE of the LPC from alum precipitation method yielded the highest amongst the three methods.

In the mineral analysis, Potassium, Iron and Manganese in the LPC from acid coagulation yielded higher than in alum precipitated and heat coagulated LPC. Calcium in the LPC from alum precipitation was lower than calcium in both LPC extracted from acid and heat coagulation. Magnesium, Sodium and Phosphorus gotten from the heat coagulated LPC was found to be the highest of the three methods.

6.2 CONCLUSION

The results from the experiments show that the yields of opepe LPC which were extracted using the alum precipitation, heat coagulation and acid coagulation methods were not significant when compared. LPC extracted from either of the three methods may serve as substitute for the more expensive protein food like soya bean meal, palm kernel meal, groundnut meal etc., although, opepe LPC from acid coagulation would be more preferred because of its quite high CP (45.37%) and minerals like Ca, K, Fe and Mn content.

Bagasse yield was quite high and could be used as feed for ruminants and also ensiled and used for feeding when feed is scarce, especially in the dry season.

6.3 RECOMMENDATION

More research on extraction methods to reduce contamination and wastage of LPC should be carried out. Also, from the inference of this study, opepe leaf protein concentrates should be encouraged in diet formulation.

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APPENDIX

APPENDIX I

Analysis of variance for yield.

Variate: %YIELD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	3	1720.707	573.569	116.13	<.001
Residual	20	98.778	4.939		
Total	23	1819.485			

APPENDIX II

Analysis of variance for Ash.

Variate: ASH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	3	26.2695	8.7565	19.21	<.001
Residual	20	9.1166	0.4558		
Total	23	35.3862			

APPENDIX III

Analysis of variance for Dry Matter.

Variate: DM

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	3	3.5610	1.1870	1.58	0.226
Residual	20	15.0340	0.7517		
Total	23	18.5950			

APPENDIX IV

Analysis of variance for Fat.

Variate: FAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	3	790.4108	263.4703	287.26	<.001
Residual	20	18.3434	0.9172		
Total	23	808.7541			

APPENDIX V

Analysis of variance for Fibre.

Variate: FIBRE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	3	147.4540	49.1513	211.02	<.001
Residual	20	4.6585	0.2329		
Total	23	152.1125			

APPENDIX VI

Analysis of variance for Nitrogen Free Extract.

Variate: NFE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	3	3010.72	1003.57	74.86	<.001
Residual	20	268.10	13.41		
Total	23	3278.83			

APPENDIX VII

Analysis of variance for Protein.

Variate: PROTEIN

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	3	1790.663	596.888	178.11	<.001
Residual	20	67.026	3.351		
Total	23	1857.689			

APPENDIX VIII

Analysis of variance for Calcium.

Variate: Ca

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	2	1790611.	895306.	568.03	<.001
Residual	15	23642.	1576.		
Total	17	1814254.			

APPENDIX IX

Analysis of variance for Potassium.

Variate: K

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	2	29417710.	14708855.	343.33	<.001
Residual	15	642631.	42842.		
Total	17	30060342.			

APPENDIX X

Analysis of variance for Iron.

Variate: Fe

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	2	60027.02	30013.51	930.63	<.001
Residual	15	483.76	32.25		
Total	17	60510.78			

APPENDIX XI

Analysis of variance for Magnesium.

Variate: Mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	2	40137.7	20068.9	126.61	<.001
Residual	15	2377.6	158.5		
Total	17	42515.3			

APPENDIX XII

Analysis of variance for Manganese.

Variate: Mn

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	2	4955.836	2477.918	1805.77	<.001
Residual	15	20.583	1.372		
Total	17	4976.420			

APPENDIX XIII

Analysis of variance for Sodium.

Variate: Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	2	1229.821	614.910	281.80	<.001
Residual	15	32.731	2.182		
Total	17	1262.552			

APPENDIX XIV

Analysis of variance for Phosphorus.

Variate: P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	2	100590.	50295.	26.77	<.001
Residual	15	28179.	1879.		
Total	17	128770.			