YIELD, PHYSICAL CHARACTERISTICS AND CHEMICAL COMPOSITION OF LEAF PROTEIN CONCENTRATES AND BAGASSE OBTAINED FROM OIL PALM (*Elaeis guinensis* jacq.)LEAVES USING THREE DIFFERENT PROCESSING METHODS

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A PROJECT WORK SUBMITED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE BACHELOR OF AGRICULTURE (B. Agric) DEGREE IN ANIMAL SCIENCE

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ABSTRACT

The experiment was conducted to determine the yield, physical and chemical composition of leaf protein concentrates and bagasse obtained from oil palm leaf using three different processing methods. The process of extraction were heat coagulation, acid coagulation, and alum precipitation methods. The leaf protein concentrates and bagasse obtained were further analysed for their chemical and mineral compositions.

The yield of LPC and bagasse obtained from acid coagulation, alum precipitation, and heat precipitation were comparable. But, the yield of OLPC was not significantly ($p>0.05$) different in the acid and alum processing methods, but was significantly ($p<0.05$) different for the heat processing method. Result, from the chemical analysis shows that the CP and EE of acid coagulation method were significantly ($p<0.05$) different from alum and heat processing methods. At the same time, the MC and NFE of OLPC for heat were significantly ($p<0.05$) different from alum precipitation method and acid coagulation method.

From the results obtained from this study, Oil palm leaf protein concentrates obtained using heat coagulation, acid coagulation and alum precipitation methods were comparable but heat coagulation would be preferable as a result of its high Ca (633.0), Cu (15.67), Mn (66.83), Zn (108.77), Na (3.46), Mg (501.63), P (417.67), Fe (154.30) and Cl (1.307) values.

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CERTIFICATION

This is to certify that this project work was carried out by Rapheal Voke OKUNA (AGR1600228) 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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DEDICATION

This work is dedicated to God Almighty, the maker and sustainer of men. Also, to my family and loved ones who, through their love, trust, finance and moral support, kept me all through the course of this study.

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CHAPTER ONE

1.0 INTRODUCTION

Protein is the basic building block of life, and it constitutes several enzymes and hormones in the body. According to FAO, the recommended minimum per capita daily protein intake is 53.8g for proper health and development and maintenance of the tissue and other systems (Iyangbe and Orewa, 2009). Generally, dietary protein is obtained from animal origin (meat, poultry, eggs and cheese, milk, fish, etc.) and plant origin (beans, leafy greens, grasses, etc.). However, despite the diversity of animals and plants species, Nigeria cannot meet the minimum annual protein having a 45.4g per capita daily protein intake (Iyangbe and Orewa, 2009).

The primary reason for not meeting the daily protein requirement is attributed to the high cost of production and the demands for these products by man for food industrial raw material. Thus, an alternative source of cheaper protein is required (Akaeze *et al.,* 2015).

Leaf protein concentrate (LPC) is a cheaper alternative source of protein. It is the concentrated form of protein found in the leaves of plant parts. The nutritional value of LPC extracted from green foliage is comparable to protein isolates of animal origin and superior to seed protein (Olomu, 2011).

Leaf protein concentrate makes for the effective utilization of foliage (Akaeze, 2010). As unprocessed leaves fed to livestock provide protein, but certain factors such as high fiber of the plant materials and toxic components in the leaves often hindered protein utilization. Leaf protein concentrate has been successfully extracted from a wide variety of plants, some of which include; Rubber (*Hevea brasilensis*) by Akaeze (2010), Avocado (*Persea americana*) by Okafor (2017), Pawpaw (*Carica papaya)* by Ogunje (2017), Bambo (*Bambusa vulgaris*) by Uti (2019), amongst others.

Several studies have been carried out on LPC usage and its growth effects on different farm animals. For example, Agbede *et al.* (2003) recommended that Leucaena Leaf Protein Concentrate (LLPC) can successfully replace 25% of fish meal in the diet of broiler starter without any adverse effects. Thus, reduce the cost of production and increase profit optimization of poultry birds.

Ogunsipe *et al.* (2011) experimented with growing rabbits to determine the optimum dietary inclusion level of Glyricidia leaf protein concentrate (GLPC). It was reported that a 20% inclusion level of GLPC has no significant difference obtained for the feed intake, average weight gain, and feed conversion ratio. Akaeze *et al.* (2014) recommended that

mini livestock farmers in Nigeria who want to maximize rabbit economic potential production can incorporate the rubber leaf protein concentration of about 30% to substitute for soya beans.

Okafor and Ezebuo (2014) recommended that Moringa leaf protein concentrates up to 20% inclusion level produced no adverse effect on the performance of broiler chicks. Thus showing that Moringa LPC is an excellent alternative source of protein with no negative impact on the chick mortality rate. Furthermore, Agbonghae *et al.* (2016) showed that 60% of soya bean meals could be replaced with pawpaw leaf protein concentrate in the diet of a growing rabbit without any adverse effect on the performance parameter measured.

LPC is prepared by grinding young leaves to a pulp, pressing the paste then isolating a liquid fraction containing protein by filter or centrifuge. Herbaceous plants and legumes such as clover and Lucerne have been reported to produce higher yields of protein concentrate than most perennial grasses. Also, the protein quality of some leaf protein concentrates has been found to approach that of the soybean, the most protein-rich of the oilseeds (Agbede *et al.,* 2003).

It is important to note that the yield and quality of the leaf protein concentrate depend on the method by which it is prepared (Sayyed, 2011). Therefore, all leaf protein concentrates require supplements because they are deficient in two of the nutritionally essential amino acids (lysine and methionine). Hence, this study seeks to determine the

yield, physical and chemical composition of leaf protein concentrate and bagasse extracted from oil palm using three different methods.

1.1 Objectives of the Study

This study aims to determine the yield, physical characteristics, and chemical composition of oil palm (*Elaeis guineensis* Jacq.*)* leaf protein concentrates and bagasse extracted using three different methods (Heat coagulation, acid coagulation, and Alum precipitation).

The specific objectives were to determine;

1. Percentage yield of oil palm (*Elaeis guineensis)* leaf protein concentrate and bagasse obtained by using the three processing methods.
2. Physical composition of oil palm (*Elaeis guineensis)* leaf protein concentrate and bagasse obtained from the three processing methods.
3. Chemical composition of oil palm (*Elaeis guineensis)* leaf protein concentrate and bagasse obtained from the three processing methods.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Distribution of oil Palm Plant.

It is generally agreed that the oil palm originated from Africa's equatorial tropical rain forest region, precisely along the gulf of guinea. The oil palm has been domesticated long ago by many West African dwellers before the arrival of white men to Africa (Naher *et al*., 2013).

Its distribution runs from the main belt through Cameroon's southern latitude, Cote d'Ivoire, Ghana, Liberia, Nigeria, Sierra leone, Togo, the equatorial region, Angola, and Congo (Ofosu-Budu, and Sarpong, 2013). The spread of the oil palm seed is majorly done by human farming activity. Another method of the spread may have been nomadic animal species (cow) either deliberately or accidentally. However, humans are clearly the most factor in the distribution of oil palm (Zeven, 1965; Okolo, 2019).

The oil palm grove in West Africa consists of a collection of palm trees of varying ages randomly scattered over an area where shifting cultivation was the dominant agricultural system. Zeven classified groves based on palm and tree diversity.

Table 2.1: Classification of Oil Palm groves and Tree Diversity by Zeven.

Type	Characteristics
Secondary forest	Low yield (1.50 of fresh fruit bunch (FFB) tonnes/ha/year); low palm density; slow palm growth
Palm bush	75-150 palms/ha; yields over 2.0 tonnes FFB/ha/year; farming relatively frequent
Dense grove	Almost pure stand of plants; high density with arable land
Thinned grove	Palms deliberately thinned to allow in more light to achieve higher yields of food crops.
Sparse grove	40 palms/ha; frequent arable land cropping with attendant soil degradation, leading to derived Savanna with few isolated palms

Source: Okolo (2019)

2.2 Description

The Oil Palm tree (*Elaeis guinensis*) belongs to the palm family Arecaceae. Oil palm is a large, pinnate-leaved palm (3 – 5m long) having a solitary columnar stem with short internodes. There are short spines on the leaf petiole and within the fruit bunch (Ataga and Vossen, 2007; Ecocrop, 2011).

The palm is normally monoecious with male and female, but sometimes mixed; inflorescences develop in the leaves' axil. The fruit is borne on a large compact bunch. The fruit pulp which produces the palm oil surrounds a nut, the shell of which encloses the palm kernel (Ataga and Vossen, 2007; Ecocrop, 2011). Mature palms are single-stemmed and can grow up to 20m tall. Although the tree can live up to 200 years, its productive life is about 25 – 35 years, and as the tree grows old, it becomes too tall and impractical to harvest (Ecocrop, 2011).

The tree has a short trunk of up to 75cm diameter tall; it has a crown made of 40 – 100 fronds (leaves), spirally arranged at the top, and an adventitious root system (Ataga and Vossen, 2007). The compound leaves are up to 8m long with 250 – 350 leaflets irregularly inserted on the rachis (Ataga and Vossen, 2007).

Large and heavy fruit is developed after pollinating the female inflorescence (Ataga and Vossen, 2007; Ecocrop, 2011). Two to six bunches of fruit are produced yearly. The fruit is a fibrous, ovoid drupe 2 -5cm long and about 2cm broad and usually orange in color when ripe. The fruit has a thin epicarp, a fleshy and oily mesocarp, and a hard endocarp containing an oil-rich endosperm.

The fruit yield different types of oils: Palm oil which is the first type of oil extracted from the fleshy mesocarp, and it is rich in palmitic acid (42 – 47%) and Oleic acid (37 – 41%), and another type of oil extracted is palm kernel oil which is extracted from the kernel and is rich in lauric acid which is about 44 – 51% (Basiron, 2005)

2.3 Scientific Classification of African Oil Palm

Kingdom:	*Plantae*
Sub – Kingdom:	*Tracheobionta*
Super Division:	*Spermatophyta*
Division:	*Magnoliophyta*
Class:	*Liliopsida*
Sub – Class:	*Arecidae*
Order:	*Arecales*
Family:	*Aracaceae*
Genus:	Elaeis Jacq
Species:	guineensis
Binomial Name:	*Elaeis guineensis. Jacq.*

Source: United States Department of Agriculture (USDA)
www.plants.usda.gov/java/Classification

2.4 Leaf Protein Concentrate (LPC)

Olomu (2011) defined concentrate as a food or feed used together with another feed in other to improve its nutritional status. Also, it is intended to be diluted further and mixed to produce a complete food or feed. LPC is obtained from processing a pulped leaves after undergoing extraction. The products obtained are green in color and palatable when included in feed (Taylor, 2003).

Therefore, Leaf protein concentrate is the concentrated form of protein obtained from plant leaves (Olomu, 2011). Leaf protein concentrate is an extremely nutritious food that can be made mechanically by separating the indigestible fibre and soluble anti-nutrients from much of the protein, vitamins, and minerals in certain green plant leaves.

Its richness in beta carotene, iron, and high-quality protein effectively mitigates malnutrition, especially anemia and vitamin A deficiency prevalent among children and pregnant women in Nigeria. Also, due to the advantage of direct solar energy, leaf crops can produce more nutrients per hectare than any other agricultural system (Graham and Telek, 1983).

Leaf protein concentrate was first suggested as human food in the 1960s, but it did not archive much success despite early promises because of specific palatability issues. However, Norman Pirie was highly recognized by the UK government in 1975 for his work on the benefits of Leaf Protein Concentrates, which later brought the subject to the limelight (Singh, 1984).

2.5 Leaf Protein Concentrate as a Food for Man, Feed for Animal and Nutrition Complement

The green leaf concentrate can feed humans or serve as a milk replacement for calves, thereby saving milk for human consumption. The bagasse can be provided to rabbits, cattle, lambs, and goats. Also, green concentrate and green juice could be fed to pigs and chickens (Pirie, 1987).

In Countries, like Nigeria, the protein needs of the populace are far more than what the conventional protein sources can sustain and the relatively high cost of animal protein, which strengthens the argument (FAO/WHO, 1985). The use of LPC which is relatively cheap and as well adequate to meet these growing need of protein.

Also, a test carried out shows that regular consumption of LPC promotes good health and weight gain and increases hemoglobin and vitamin A status. It also improves general health and reduces the frequency or severity of illness (Pirie, 1987).

The extraction of leaf protein concentrate (LPC) has been widely studied in Europe, the United States, and Asia. In Africa, Byers (1983) found out that many Ghanaian plants are suitable for LPC Extraction. Likewise, Olatunbosun (1969) demonstrated the suitability of LPC For the treatment of kwashiorkor.

2.6 Advantages of Leaf Protein Concentrate

1. Leaf protein concentrate is an extremely nutritious food rich in vitamin A and iron compared to commonly available foods. Leaf concentrate is also an excellent source of high-quality protein, calcium, and several other essential nutrients (Aletor and Adebayo, 2012).
2. Leaf protein concentrate offers very nutritious food at low prices compared to food materials like meat, cheese, eggs, or powdered milk. Therefore, it is usually the cheapest dietary source of vitamin A and iron (Fasuyi, 2005).

3. Little or no waste in the production of Leaf protein concentrate. The residual fiber can be an excellent feed for cows, goats, sheep, horses, rabbits, or guinea pigs. It can also enrich the soil, as the leftover liquid is rich in nitrogen and potassium and makes a good fertilizer. In the kitchen, it can be used in the production of biogas for cooking.
4. Leaf concentrate is easy to preserve compared to other vegetable leaves. It can also be dried and converted to pasta, made into drink mixes or syrups, salted or pickled.
5. Most of the anti-nutrients present in leafy foods are extracted out through the leaf concentrate process. In addition, harmful chemicals such as hydrocyanic acid and free oxalic acid that generally limit the usefulness of many leaf crops in the human diet are almost entirely removed when the leaves are converted to leaf concentrate.
6. Leaf concentrate uses far less fuel when prepared than Cowpea, the leading high protein food of the world's poor population (Fasuyi, 2005).
7. Leaf concentrate is relatively easy to make. People with little skills, training, or education can make it in rural villages.
8. There have not been any known cases of allergic reaction to leaf concentrate since 1975 when the standard processing heat was raised to a minimum of 90 ° C.

2.7 Disadvantages of Leaf Protein Concentrate

1. Good leaf yields require a steady supply of water. However, there are long dry seasons in many locations, and irrigated land is at a premium. In arid lands, the water requirements of lush leaf crops are usually excessive and focusing on improving water-thrifty crops like sorghum, millet, buffalo gourd and acacia is a more realistic strategy (Fasuyi, 2005).
2. Most people are not accustomed to eating many dark green foods.
3. Fresh leaves are very perishable. Therefore, they must be processed soon after they are harvested, or the quality and yield of leaf concentrate go down.
4. Fresh leaves are heavy, as are the residual fibre and 'whey.' This means transportation costs will be high unless processing can be done very close to the leaf crop field.
5. While domestic-scale production can be done with inexpensive commercial grinders and blenders, larger-scale equipment is not currently available commercially and must be custom-built.
6. The vitamin C in fresh leaves is lost during processing.

Table 2.2: Proximate Composition (g/100DM) of four leafy vegetables

Vegetable Species	Family	DM	CP	EE	CF	ASH	NFE	GE
S. Africana	Solanceae	96.4±0.3	46.1±1.2	8.7±0.1	1.3±0.1	19.4±0.2	20.3	1798
A. Hybridus	Amaranthaceae	95.5±0.2	35.1±1.3	5.6±0.3	1.1±1.4	22.3±0.1	31.4	1584
T. occidentalis	Curcurbitaceae	97.3±0.3	54.9±1.3	11.9±0.2	1.8±0.3	11.4±0.4	17.3	2077
V. amygdalina	Compositaceae	94.5±0.4	52.2±2.4	5.6±0.37	1.5±0.6	95±0.1	25.7	1896
	MEAN	96.0	47.2	7.1	1.4	15.7	23.7	439
	S>D	1.2	8.8	3.0	0.3	6.2	6.6	0.3
	CV(%)	1.3	18.6	37.9	20.7	39.4	28.2	68.0

Source: Aletor *et al.* (2002)

Table 2.3: Chemical composition (%) of some Leaf Protein Concentrate

	Cassava	Oil Palm	Pumpkin	Siam	Leucaena	Paw Paw
Dry matter	94.00	91.10	90.75	94.00	95.00	90.00
Crude protein	35.35	18.10	43.40	30.80	37.40	38.20
Crude fat	23.00	0.45	12.46	29.00	4.70	0.65
Ash	6.80	7.60	2.80	3.20	14	7.05
Crude fibre	2.15	16.20	0.04	0	1.75	2.75
NFE	26.70	48.75	31.85	31.00	37.15	41.35
Ca	-	0.01	-	-	-	-
P	-	0.50	-	-	-	-

Source: Olomu (2011)

2.8 Nutritional Value of Leaf Protein Concentrate

Leaf protein concentrate is an extremely rich source of essential nutrients vital for many life's processes (WHO, 1985). The nutritional composition of leaf protein concentrate could be summarized as follow;

2.8.1 Energy

Leaf protein concentrate contains a medium or average range of energy content, and it accounts for about 138kcal from a 100g portion. Therefore, it is approximately the same as cereal grains and pulse when considered on a dry matter basis.

Table 2.4: Energy content of a 100g portion of some food

Food	Energy (Kcal)
Leaf Protein Concentrate	138
Beef	301
Chicken	124
Milk	66
Egg	163
Beans	118
Trout	168

Source: FAO/WHO (1985)

2.8.2 Protein

Protein is the basic building block of life. They are needed daily to build and repair muscles, maintain healthy body cells and a wide range of enzymes and hormones. LPC is considered to have a high protein level, and it is particularly rich in amino acids such as Tryptophan, Threonine, Leucine, Phenylalanine, and Tyrosine, but it is limiting in Methionine (Bayers, 1983)

Pirie (1971) assembled the results of an experiment on laboratory animals (pigs and poultry), and it indicated from the amino acid analysis that LPC is a satisfactory substitute for fish meal. It also shows a satisfactory substitute for groundnut and soybean.

The digestibility of milk and egg ranges from 95% to 98%, and they are the standard from which other foods are compared. On the other hand, studies have shown that the digestibility of LPC ranges from 83% to 88% and with an average of 86% (FAO/WHO, 1985). Utilization may, however, be hindered by the presence of anti-nutritional factors (Fasuyi and Aletor, 2005).

Table 2.5: Protein content of a 100g portion of some food

Food	Energy (Kcal)
Leaf Protein Concentrate	24.0
Beef	17.4
Chicken	22.5
Milk	3.5
Egg	12.9
Beans	23.0
Trout	18.3

Source: WHO/FAO (1985)

Table 2.6: Amino acids of various foods (g/100g)

Amino acid	Milk	Egg	Beef	Chicken	LPC	Beans	Trout
Isoleucine	5.45	5.61	5.12	4.63	5.22	4.61	5.27
Leucine	10.00	8.29	8.01	7.51	9.34	7.67	8.48
Lysine	8.18	6.26	9.10	8.98	6.51	7.17	9.73
Tyrosine	10.00	9.10	8.32	8.80	10.00	7.87	7.66
Threonine	4.85	5.12	4.64	4.63	5.02	4.03	4.78
Tryptophan	1.42	1.79	1.27	1.12	2.14	0.95	1.14
Valine	7.27	7.56	5.30	4.78	6.33	4.66	5.76
Total	47.17	43.64	41.72	39.65	45.56	36.53	42.78

Source: WHO/FAO (1985)

2.8.3 Vitamins

Leaf protein concentrate is a good source of vitamin A, E, K, niacin, folic acid, and pantothenic acid. It contains a considerable amount of Vitamin B6, biotin, and good but not prominent thiamine. It is deficient in vitamin D, $B_{12,}$ and Ascorbic acid lost during the leaf concentrate preparation (FAO/WHO, 1985).

It would be expected that the water-soluble vitamins would be lost in the processing of leaf concentrate (i.e., water-soluble vitamins would be present in the whey and not in the curd). Still, in this case, water-soluble vitamins are present in both the whey and curd. The fat-soluble vitamins are concentrated nine times in the green crop, while thiamine and riboflavin are not concentrated (Byers, 1983).

Table 2.7: Vitamin content of 100g portion of some food

Food	Vit. A	Vit. E	Thiamine	Riboflavin	Niacin	Folic acid	Vit B_6	Ascorbic acid
LPC	57.8	10.7	0.18	0.22	9.7	182	0.4	0
Beef	15	Tr	0.07	0.15	10.5	11	0.35	0
Chicken	32	Tr	0.17	0.12	6.6	18	0.23	0
Milk	45	Tr	0.03	0.17	0.8	5	0.05	1
Egg	330	Tr	0.11	0.3	3.8	65	0.2	0
Bean	4	0.3	0.16	0.07	2.2	130	0.1	0

Tr = Trace

Source: FAO/WHO (1985)

2.8.4 Minerals

Leaf concentrate is a good source of calcium, magnesium but low in iodine (FAO/WHO, 1985). Also, Byers (1983) analyzed leaf protein and found out that it is a rich source of phosphorus, iron, potassium, zinc, copper, manganese.

2.8.5 Fats and Fatty Acids

About 22.5% of leaf protein concentrate is made up of lipid, and fat represents only about a third of the total (Lima *et al.,* 1965). The most prominent fatty acid found in LPC includes Linolenic acid and Linoleic fatty acids. These fatty acids are needed in proper human nutrition. Linoleic acid present in LPC is an omega –3-fatty acid that is also present in fish oil (FAO/WHO, 1985)

2.8.6 Fibre Content

According to Byers (1983) and WHO (1985), LPC contains low fibre content, usually less than half a percent. It is, therefore, suitable for non - ruminant animals such as chickens. Under fibre, a fraction can be used to feed ruminant or maybe ensiled or dried and use as animal feed. Dietary fibre alter the colonic environment in such a way as to protect it against colorectal disease

2.8.7 Anti – Nutritional Factor

The major anti-nutritional factor found in leafy green vegetables is phytic acid and oxalic acid (Graham and Telek, 1983). The high level of these acids has been known to inhibit

the absorption and utilization of mineral nutrients by an animal (Graham and Telek, 1983). Also, the biological active coumestrol present in Alfafa was found in leaf protein concentrate (Kohler *et al*., 1982).

Table 2.8: Analyzed Composition of the Leaf Meal, Leaf Protein, and Residues of *Telifairia Occidentalis*

Proximate composition (gkg^{-1} DM)	**Leaf Meal**	**Leaf protein concentrate**	**Leaf protein concentrate residue**
Crude protein	362.0±0.3	554±1.1	41.0±1.2
Ash	143±1.0	120.7±0.5	107.0±1.7
Ether extract	8.8±2.6	12.4±1.1	5.1±1.2
Gross energy ($MJKg^{-1}$)	15.6	20.0	11.6
Mineral Content ($Mgkg^{-1}$ DM)			
Sodium	270.3	289.0	288.4
Calcium	732.6	762.2	959.7
Magnesium	352.3	393.3	493.1
Phosphorus	288.8	442.4	388.4
Potassium	353.0	336.7	459.4
Iron	27.5	25.2	65.4
Copper	ND	ND	ND
Zinc	79.6	21.5	25.7
Manganese	18.1	2.33	0.8
Anti- nutrient (mg $100mg^{-1}$ DM)			
Phytate	8.2	3.2	7.5
Phytin – Phosphorus	2.3	0.9	2.1
Tanin	4.0	0.8	1.1

ND = Not detected

Source: Agbede *et al.* (2008)

2.9 Methods of Preparing Leaf Protein Concentrate

Several methods have been used in the preparation of leaf protein concentrate (Sayyed, 2011). Five methods will be discussed, and they are;

1. **Heat coagulation:** A 500ml of fresh juice was slowly added to about 20ml of boiling water and constantly stirred. It was heated continuously to a temperature above 95ºC. The protein present in the juice coagulated due to the heat treatment, and it formed a curd, also known as leaf protein concentrate (LPC). The LPC is separated from the remaining portion of the juice, and the remaining portion is known as deproteinized juice (DPJ). This was separated with a Whatman filter paper with 2 to 3 hot water washings. The sample of LPC was collected, dried in an oven, and its yield was recorded (Pirie, 1987).
2. **Differential Heat Coagulation:** A distilled water of about 20ml was heated in a beaker to 60ºC, and it was slowly added 50ml of fresh juice and was stirred continuously. The juice was kept for heating at this temperature for 5mins. It was then filtered through a Whatman filter paper to separate the green chloroplast leaf protein concentrate, and it was washed about 2 – 3 times with water. The filtrate was collected and resulting in white cytoplasmic LPC. The cytoplasmic fraction was collected by filtration through a Whatman filter paper, and the deproteinized juice (DPJ) was released as a filtrate. The sample of green chloroplastic LPC, white cytoplasmic LPC (resulting due to the heating of filtrate to over 95ºC) and

deproteinized juice (DPJ) left over after recovering chloroplastic and cytoplasmic fractions were collected for further analysis. The sample of LPC was dried in an oven, and the yield was recorded (Pirie, 1971; Sayyed, 2011).

3. **Heat coagulation followed by acid washing:** The LPC was prepared by heat coagulation as described above. It is then suspended in acidic water with a pH value of 3.5 for about 10mins, and it is filtered. The sample of LPC and DPJ were collected to record the yield and also for further analysis (Sayyed, 2011).
4. **Acid coagulation:** A 50ml of fresh juice was added to $5NH_2SO_4$, and it was stirred till the pH value decreased to 3.5. The curd of the LPC resulted due to the coagulation of the protein in the juice by acid. Afterward, it was filtered with a Whatman filter paper, and the sample of LPC was taken for recording and further analysis. (Singh, 1984; Sayyed, 2011).
5. **Alum Precipitation:** 2gm of alum was dissolved in 100ml distilled water to prepare an alum solution. The alum solution was added to 50ml of fresh juice, and the curd of LPC resulted from the coagulation of protein in juice by alum solution. It was filtered through a Whatman filter paper, and the sample of LPC was taken for the recording of the yield (Sayyed, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Location of the Study

The experiment of the yield, physical and chemical composition of leaf protein concentrates and bagasse from oil palm is carried out in the Faculty of Agriculture main laboratory, University of Benin, Benin City, Edo State, Nigeria. The scope of this study lasted for two about a year and three months, which started in February 2020. The research was conducted in different phases. The first phase was the trial methods, and six (6) calibrations were carried out for the different. The essence of the six (6) calibrations is to acquaint appropriately with the different preparation methods of Leaf Protein Concentrate. After the various trials, the second phase features the yield of the oil palm leaf protein concentrate, determination of the chemical proximate of oil palm leaf (dry matter, crude protein, crude fibre, ether extract, and Nitrogen free extract), and some mineral composition of the oil palm leaf.

3.2 Materials for the experiment

Below are the material used during the experiment;

1. Freshly harvested oil palm leaves

2. Laboratory thermometer

3. Weighing balance

4. Plastic bowls

5. Stainless steel pot and spoon

6. Measuring cylinder

7. Grinder

8. Foil paper

9. Whatman filter paper

10. Sieve cloth

11. Alum

12. Bunsen burner

3.3 Preliminary Trials

During the first trial, six (6) trials were carried out before the final phase. The six (6) trials were carried out to ensure that all the different processing methods were well acquainted with and serve as a guide to the final processing methods.

3.4 Calibration Procedures

In the early hours of the morning, fresh oil palm leaf was harvested within the University of Benin environment. The harvest was taken to the laboratory for processing, as this is to avoid wilting. In the laboratory, a bowl and water were used to wash the freshly

harvested oil palm leaf. This is to remove any available and irrelevant materials from the leaf.

After washing and drying, a 350g per sample was weighed using the weighing scale for each trial made; the result was recorded. The weighed sample was grounded into a slurry with varying amounts of water. The juice obtained from the produce of the leaf was used to produce the leaf protein curds.

3.5 Production of Oil Palm Leaf Protein Concentrate (OLPC) using Heat Coagulation Method

Fresh oil palm leaves were harvested in the early hours before sunset. The harvested leaves were washed, chopped, weighed, and grounded with clean water. The slurry obtained after grinding was put in a sieve to separate the juice from the bagasse (chaff).

The separated juice was poured into a clean pot and place on an electric stove. The juice was heated, and the curd began separating, leaving the whey fraction. After cooling, the curd (LPC) was separated from the whey using a Whatman filter paper. The LPC sample was taken in an aluminum foil paper and was sun-dried, and the yield was weighed.

3.6 Production of Oil Palm Leaf Protein Concentrate (OLPC) using Alum Precipitation Method

After the oil palm leaves have been processed, grounded, and sieved, the juice was poured into a bowl. A 1g of crushed Alum was added to every 50ml of juice. The curd of LPC was formed as a result of the Alum present in the slurry. The curd was filtered through a Whatman filter paper and was sun-dried and weighed.

3.7 Production of Oil Palm Leaf Protein Concentrate (OLPC) using Acid Coagulation Method

To every 100ml of fresh juice, a known amount of acid was added with continuous stirring till the pH value of the juice decreased to 3.5. The curd of the LPC resulted due to the coagulation of proteins in the juice. After coagulation, the LPC formed was filtered using a Whatman filter paper, sun-dried, and weighed.

3.8 Precautions Taken During the Processing

- The materials used were washed thoroughly after each calibration
- Fresh leaves were used throughout the whole calibration
- The leaves used in the experiment were mature and injury-free leaves
- The leaf juice was removed from the heat source after it reached a boiling point to avoid burning the curd, after which it was adequately separated from the whey fraction.
- The leaves were cut into smaller bits for easy grinding
- The sieve cloth used had a tiny pore to prevent the passage of the baggase
- During the grinding process, efforts were made to ensure that the leaves are appropriately ground.
- The LPC was sun-dried rather than oven-dried.
- The LPC in the Whatman filter paper was properly scooped out before sun drying.
- After each use, all equipment was adequately washed and sun-dried before re-using. This is carried out to ensure the accurate result from each experiment.

3.9 Product Handling

CURD: The curd was collected after separation and weighed; it was then placed in a petri dish and sundried until it became flaky.

WHEY: This was collected after the curd has been removed, and they were stored in bottles and labeled accordingly

BAGASSE: This is the fraction obtained in the processing stage after blending and sieving the slurry. It was also dried and weighed to a constant weight.

3.10 Chemical Analysis

All samples were analyzed in triplicates

3.10.1 Moisture Content Determination

Materials: weighing balance, crucible, oven, and desiccator

Methods: 2g of sample was weighed into a pre-weighed silica dish. The sample and container were placed in the oven for 24 hours. The sample was removed and placed in a desiccator for 30 minutes before weighing again. The samples were dried until a constant weight was obtained.

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

3.10.2 Crude Protein Determination

2g of prepared curd and bagasse was weighed and wrapped in a filter paper, and transferred into a clean digestion flask. The following was then added copper, selenium, or mercury catalyst plus potassium or sodium sulphate to raise the boiling point. 30ml of concentrated sulphuric acid was added to the digestion flask containing the other mixture, and the sample was digested for 2 hours.

The flask was cooled, after which it was diluted with water and made to 100ml in a volumetric flask. Next, 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 so 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 disappears. A blank determination was first carried out.

Calculation

$$\%\text{N of sample} = \frac{\text{Net vol. of Acid} \times \text{Conc, of Acid} \times 14 \times 100 \times 10}{\text{Weight of Sample in g}}$$

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

3.10.3 Crude Fibre Determination

2 g of curd was weighed into a round bottom flask, 100ml of crude fibre reagent that has been boiled was added, and then the beaker was placed on the crude fibre 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 of acid. Also, NaOH (sodium hydroxide) solution, which had been previously brought to boil, was added, filtered while hot rising a Whatman filter paper, and the residue was allowed to drain and transferred to a pyrex beater and dried over-right 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

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

3.10.4 Ash Determination

Materials: weighing balance, muffle furnace, desiccator crucible

Methods:

2g of curd and bagasse were weighed and placed in an already weighed crucible which was in a muffle furnace to ash at a temperature of 550^{o}c for about 3 hours. It was observed that at this time, the sample had turned slightly grey. The sample was removed and allows to cool in a desiccator for 30 minutes. The ash was obtained as the weighed again to obtain the final weight.

Calculation:

Sample wt = A

Sample wt before Ashing = P

Sample wt after Ashing = Z

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

3.10.5 Ether Extract

2 g of leaf curd and bagasse was weighed into a fat-free extraction tumble. It was then corked tightly with cotton and placed in the extraction petroleum ether was added until it siphoned over. More ether was added until the barrel 300ml was half-filled, the condenser was placed. 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. Next, the barrel content was appropriately drained into the bottle, and the thimble was removed and dried. Finally, the flask was detached, the exterior cleared and dried in an oven to constant weight.

Calculation:

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

3.10.6 Nitrogen Free Extract

This is basically obtained by adding % moisture, ash, ether extract, crude protein, and crude fibre and subtracted from 100. The difference was taken as the Nitrogen free extract.

NFE = 100 – (%CP + %EE + %CF + %Ash)

3.10.7 Mineral Analysis

After first dry-ashing 1g of the curd and bagasse at 550^{o}c in a Muffle furnace and dissolved in de-ionized water to standard volume, Minerals were analyzed. Sodium and potassium were determined by flame photometry and phosphorus by the vanadomolybdate method of AOAC (2010). Magnesium, calcium, sulphur, manganese, iron, and copper were determined using an atomic absorption spectrophotometer.

3.11 Statistical Analysis

Data collected from the study were subjected to analysis of variance using the GENSTAT 12th edition for Windows package at 5% level of probability. In addition, the means with a significant difference were separated using the same Statistical Package.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield of OLPC and Bagasse Extracted Using Acid Coagulation Method, Alum Precipitation and Heat Coagulation Method

The percentage yield of LPC using the following methods; acid coagulation, alum precipitation and heat coagulation method, and bagasse are shown in Table 4.1. The result shows that acid coagulation (3.41%) and alum precipitation (3.95%) were not significantly ($p<0.05$) different from each other. However, the yield of heat coagulation (2.49%) was significantly ($p<0.05$) different and lower than acid and alum extraction methods. It was also observed that the oil palm LPC from heat coagulation takes less time to extract than that of acid coagulation and alum precipitation. This is because the LPC from the acid and alum extraction processes takes more time to sediment before the filtration is carried out.

Table 4.1: Percentage Yield of OLPC and Bagasse Extracted Using Acid coagulation, Alum Precipitation and Heat Coagulation methods.

Sample	Yield (%)
A)	
Acid	3.41[a]
Alum	3.95[a]
Heat	2.49[b]
SEM	0.228
B)	
Bagasse	
Acid	17.43
Alum	17.81
Heat	14.48
Mean	16.57

SEM= Standard Error of the Mean, Means with same letters are not significantly ($p>0.05$) different, OLPC= Oil Leaf Protein Concentrate.

4.2 Physical Properties of Fresh Oil Palm Leaf, Juice, LPC, Bagasse and Whey of *Elaeis guinensis*

In Table 4.2 and 4.3 showing the Fresh oil palm leaves before processing and after processing. The oil palm leaves were dark green and smooth when harvested, and the juice obtained was green. The bagasse obtained was fibrous and light green, then turns dark green after drying under the sun. The LPC obtained from fresh oil palm leaves using the heat coagulation method before and after drying under the sun was light green and dark green. The same was observed for alum precipitation and acid coagulation. The whey obtained from all three methods of extraction was light yellow.

Table 4.2: Physical Characteristics of Whole Leaf, Juice, LPC, Bagasse and Whey of *Elaeis guinensis* Before Drying (Fresh)

Character	Leaf			Juice			LPC			Whey			Bagasse		
	Acid	**Alum**	**Heat**	**Acid**	**Acid**	**Heat**	**Acid**	**Alum**	**Heat**	**Acid**	**Alum**	**Heat**	**Acid**	**Alum**	**Heat**
Colour	Green	Green	Green	Green	Green	Green	Light green	Light green	Light green	Light yellow	Light yellow	Light yellow	Light green	Light green	Light green
Texture	Smooth	Smooth	Smooth	NA	NA	NA	Smooth	Smooth	Smooth	NA	NA	NA	Fibrous	Fibrous	Fibrous
State	Solid	Solid	Solid	Liquid	Liquid	Liquid	Semi Liquid	Semi Liquid	Semi Liquid	Liquid	Liquid	Liquid	Solid	Solid	Solid

NA= Not Applicable

Table 4.3: Physical Characteristics of Leaf, LPC, Juice, Bagasse and Whey of *Elaeis guinensis* After Drying under the Sun

Character	Leaf			Juice			LPC			Whey			Bagasse		
	Acid	**Alum**	**Heat**	**Acid**	**Acid**	**Heat**	**Acid**	**Alum**	**Heat**	**Acid**	**Alum**	**Heat**	**Acid**	**Alum**	**Heat**
Colour	Dark Green	Dark Green	Dark Green	NA	NA	NA	Dark green	Dark green	Dark green	Light yellow	Light yellow	Light yellow	Dark green	Dark green	Dark green
Texture	Coarse	Coarse	Coarse	NA	NA	NA	Coarse	Coarse	Coarse	NA	NA	NA	Fibrous	Fibrous	Fibrous
State	Solid	Solid	Solid	Liquid	Liquid	Liquid	Semi Liquid	Semi Liquid	Semi Liquid	Liquid	Liquid	Liquid	Solid	Solid	Solid

NA= Not Applicable

4.3 Proximate Composition of OLPC and Bagasse Extracted Using Alum Precipitation, Heat Coagulation and Acid Coagulation Methods

The proximate composition of oil palm LPC and bagasse using heat coagulation, alum precipitation and acid coagulation method is shown in Table 4.4. The result for moisture content shows there was no significant ($p>0.05$) difference between the OLPC extracted using acid coagulation (5.157%), alum precipitation (5.270%) and heat coagulation (5.353%). Although, the heat coagulation method has a higher moisture content in its LPC, and the bagasse had a (7.53%) value.

The CP of the OLPC using the acid coagulation method (46.67%) was significantly ($p<0.05$) different from the CP of alum precipitation (28.00%) and heat coagulation (26.84%). The acid precipitation method had the highest CP value, while the CP of bagasse was (15.75%).

The Ash result for OLPC had no significant ($p>0.05$) difference between the (4.25%) acid coagulation method and the (4.34%) heat coagulation method. However, the result was significantly ($p<0.05$) different from the (8.62%) alum precipitation method, which was higher and (4.51%) for the bagasse value.

The fat of OLPC was significantly ($p<0.05$) different in the alum precipitation (6.347%), acid coagulation (8.233%), and heat coagulation (4.173%) method. Also, the bagasse was (2.36%). The CF of OLPC had no significant ($p>0.05$) difference between the heat coagulation method (3.14%) and the acid coagulation method (3.10%) but were

significantly ($p<0.05$) different from the alum precipitation method (2.22%). Also, the CF for bagasse was (8.80%).

There was a significant ($p<0.05$) difference in the NFE extracted with heat coagulation method (56.16%), alum precipitation method (49.50%) and acid coagulation method (32.60%). And the NFE for bagasse was (61.05%).

Table 4.4: Proximate Composition of OLPC and Bagasse Obtained Using Heat Coagulation, Alum Precipitation and Acid Coagulation Processing Methods.

Parameters	Acid Coagulation	Alum Precipitation	Heat Coagulation	Bagasse	SEM
MC (%)	5.157[a]	5.270[a]	5.353[a]	7.53	0.022
CP (%)	46.670[a]	28.000[b]	26.840[c]	15.75	0.476
ASH (%)	4.250[a]	8.620[b]	4.340[a]	4.51	0.014
EE (%)	8.233[a]	6.347[b]	4.173[c]	2.36	0.044
CF (%)	3.100[a]	2.220[b]	3.140[a]	8.80	0.194
NFE (%)	32.600[a]	49.500[b]	56.160[c]	61.05	0.562

Means with same letters on the same row are not significantly ($p>0.05$) different,

SEM= Standard Error Mean, MC= Moisture Content, CP= Crude Protein, CF= Crude Fibre, EE= Ether Extract, NFE= Nitrogen Free Extract, OLPC= Oil pallm Leaf Protein Concentrate.

4.4 Mineral Composition of OLPC and Bagasse using Heat coagulation, Alum Precipitation and Acid Coagulation Methods

Some mineral compositions of oil palm LPC extracted and bagasse using heat coagulation, alum precipitation and acid coagulation are shown in Table 4.5. Among the macro minerals, the result for potassium (K) concentration was highest in alum precipitation (444.7mg/kg), and was found to be significantly ($p<0.05$) different in heat coagulation (131.7mg/kg) and acid coagulation (117.9mg/kg). The concentration of sodium (Na) in heat coagulation (3.46mg/kg), was significantly ($p<0.05$) different from alum precipitation (1.27mg/kg) and acid coagulation (2.73mg/kg). Magnesium (Mg) concentration was significantly ($p<0.05$) different in heat coagulation (501.63mg/kg), alum precipitation (187.67mg/kg) and acid coagulation (463.00mg/kg). A significant ($p<0.05$) difference was observed in the concentration of calcium (Ca) in heat coagulation (633.0mg/kg), alum precipitation (213.3mg/kg) and acid coagulation (584.3mg/kg). The concentration of phosphorous (P) in heat coagulation (417.67mg/kg), was significantly ($p<0.05$) different in alum precipitation (144.93mg/kg) and acid coagulation (390.67mg/kg). For the micro minerals, iron (Fe) in heat coagulation (154.30mg/kg), was significantly ($p<0.05$) different from alum precipitation (51.86mg/kg) and acid coagulation (136.97mg/kg). The concentration of zinc (Zn) in heat coagulation (108.77mg/kg), alum precipitation (36.83mg/kg) and acid coagulation (97.17mg/kg) were significantly ($p<0.05$) different. The concentration of manganese (Mn) in heat coagulation (66.83mg/kg), was significantly ($p<0.05$) different from alum precipitation

(22.77mg/kg) and acid coagulation (59.63mg/kg). The concentration of Copper (Cu) in heat coagulation (15.67mg/kg) was significantly ($p<0.05$) different from alum precipitation (5.29mg/kg) and acid coagulation (14.00mg/kg). The concentration of chloride (Cl) in heat coagulation (1.307mg/kg), was not significantly ($p>0.05$) different from acid coagulation (1.170mg/kg), but significantly ($p<0.05$) different from (0.433mg/kg) alum precipitation.

Table 4.5: Mineral Composition of OLPC and Bagasse Extracted from Heat Coagulation, Alum Precipitation and Acid coagulation (mg/kg).

Parameters	Acid Coagulation	Alum Precipitation	Heat Coagulation	SEM
Na	2.73[a]	1.27[b]	3.46[c]	0.218
K	117.9[a]	444.7[b]	131.7[c]	2.13
Mg	463.0[a]	187.67[b]	501.63[c]	1.540
Ca	584.3[a]	213.3[b]	633.0[c]	3.97
P	390.67[a]	144.93[b]	417.67[c]	0.766
Mn	59.63[a]	22.77[b]	66.83[c]	0.545
Zn	97.17[a]	36.83[b]	108.77[c]	0.296
Cu	14.00[a]	5.29[b]	15.67[c]	0.371
Fe	136.97[a]	51.86[b]	154.30[c]	0.850
Cl	1.170[a]	0.433[b]	1.307[a]	0.022

Means with the same letters on the same row are not significantly ($p>0.05$) different,

SEM= Standard Error Mean, OLPC= Oil Palm Leaf Protein Concentrate.

CHAPTER FIVE

5.0 DISCUSSION

5.1 The Physical Properties of Oil Palm Leaf Protein Concentrate (OLPC) and the Resulting Bagasse using three Extraction Methods

The leaf derived from all three processing methods is smooth and less fibrous with a CF of 3.100, 2.220 and 3.140 for the acid, alum and heat extraction method. While the bagasse is coarse and highly fibrous with a CF of 8.80 higher than that from the leaf curd; and this is in conformation to the statement made by Fellows (1987).

5.2 Chemical Composition of Oil Palm Leaf Protein Concentrate (OLPC) and Bagasse using three Extraction methods

Results from proximate analysis of oil palm LPC and bagasse using three different methods revealed that the crude fibre was 2.220 for the LPC alum precipitation method, 3.100 for LPC acid coagulation and 3.140 for the heat coagulation method. The three extraction methods were higher than that in LPC of *Amaranthus hybridus* (1.7%) and rubber (1.80%) as reported by Adeye and Omolayo (2011) and Akaeze *et al.* (2015). At the same time, the bagasse was with a higher value of 8.80. This can be attributed to the fact that the bagasse is the fibrous component of the leaf. The difference in the margin of the CF value in the curd may be due to factors like the material used during sieving, the inherent nature of the plant, and foreign material that could be contaminated to the LPC.

Crude protein was revealed to be 28.00, 46.67, and 26.84 for LPC alum precipitation, acid coagulation, and heat coagulation, respectively. The CP of the heat coagulation

method was lower than the CP for rubber leaf (32.64%) and *Amaranthus hybridus* (34.80). Alum precipitation extraction was also lower for both rubber leaf (32.64) and *Amaranthus hybridus* (34.80%). The CP of acid coagulation was also lower than Pumpkin (68.00%) and *Amaranthus hybridus* (65.00). This is reported by Akaeze *et. Al.,* (2015), and Adeyeye and Omolayo (2011), and Sayyed (2011), respectively.

Ash was revealed to be 4.250, 8.620, and 4.340 for LPC acid coagulation, alum precipitation, and heat coagulation, respectively. The ash content of the three extraction methods is lower than ash (17.2%) in *Amaranthus hybridus* LPC and (12.3%) *T. occidentalis* LPC reported by Adeyeye and Omolayo (2011). On the other hand, ash in oil palm bagasse (4.51%) was found to be higher than (3.0%) in the bagasse of *Musa paradisiaca* reported by Udegbe (2007).

The ether extract of OLPC (8.233%) from acid coagulation, (6.345%) from alum precipitation, (4.173%) from heat coagulation. Ether extract of *A. hybridus* (9.6%) and *T. occidentalis* (10.7%) was observed to be higher than all three extraction methods used on the OLPC as reported by Adeyeye and Omolayo (2011), while the bagasse was (10.78%).

The NFE of OLPC (56.160) from the heat coagulation method was higher than (23.58%) from *Vernonia amygdalina* LPC as reported by Sodamade (2013), and NFE of OLPC from the alum precipitation method (49.50) was higher than (47.2%) from *Amaranthus cruentus* HH3 LPC as reported by Cheeke *et al.* (1987), while the bagasse was (61.05%)

5.3 Mineral Composition of Oil Palm Leaf Protein Concentrate (OLPC) and Bagasse using three Extraction methods

The result from the analysis indicates that OLPC of calcium and magnesium in the LPC from heat and acid method produced the highest; with the heat method having a (633.0mg/kg) Ca, and (501.63) Mg, and acid method having (584.3) Ca, and (463.0) Mg. This is shown to be higher than 5.0g/kg and 0.4g/kg for calcium and magnesium in LPC of Lucerne reported by Siebritis *et al.* (1986) and LPC from *T. occidentalis* (762.2mg/kg and 393.3mg/kg for Ca and Mg respectively) as reported by Agede *et al.* (2008) while calcium and magnesium in the LPC from alum precipitation was 4329 and 4508mg/kg. This is because they are required to form bones and teeth, form blood clots, form cyclic AMP and other second messengers, body mechanisms, etc. (Olusanya, 2008).

Other macro minerals phosphorus and potassium were 390.67mg/kg and 117.9mg/kg from the acid coagulation method and 144.93mg/kg and 444.7mg/kg from alum precipitation method, and 417.67mg/kg and 131.7mg/kg from heat coagulation, respectively and were higher than the phosphorus (116mg/kg) *Amaranthus hybridus*. However, the potassium of the three extraction methods was lower than (457mg/kg) of *Amaranthus hybridus* LPC, as reported by Adeyeye and Omolayo (2011). Furthermore, the sodium content in OLPC from heat coagulation 3.46mg/kg and 1.27mg/kg from alum precipitation and 2.73mg/kg from acid coagulation were lower than 312mg/kg *Telferia occidentalis* LPC reported by Adeyeye and Omolayo (2011).

The micro minerals copper (15.67mg/kg), iron (154.30mg/kg), manganese (66.83mg/kg) and zinc (108.77mg/kg) in OLPC from heat coagulation method were all higher than the values of copper (5mg/kg), iron (35mg/kg), manganese (50mg/kg) and zinc analyzed in Lucerne LPC as reported by Siebrits *et al.* (1986). The OLPC from alum precipitation method revealed 5.29mg/k, 51.86mg/kg, 22.77mg/kg, 36.83mg/kg for copper, iron, manganese and zinc respectively. The OLPC from acid precipitation method revealed 14.00mg/k, 136.97mg/kg, 59.63mg/kg, 97.17mg/kg for copper, iron, manganese and zinc respectively.

Table 5.1: Summary of the Observed Differences found in the Extraction of Oil Palm LPC

Parameters	LPC Heat Coagulation ($p<0.05$)	Alum Precipitation ($p<0.05$)	Acid Coagulation ($p<0.05$)
Yield	Low	The same	The same
MC	The same	The same	The same
CP	Highest	High	Low
ASH	The same	Highest	The same
EE	Low	High	Highest
CF	The same	Low	The same
NFE	Highest	High	Low
Na	Highest	Low	High
K	High	Highest	Low
Mg	Highest	High	Low
Ca	Highest	Low	High
P	Highest	Low	High
Mn	Highest	High	Low
Zn	Highest	Low	High
Cu	Highest	Low	High
Fe	Highest	Low	High
Cl	Highest	Low	High

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The research was conducted in two different phases. The first phase was the trial methods, and six (6) calibrations were carried out for the different extraction methods (acid, alum, and heat). The Second was the production and determination of the yield of oil palm leaf protein concentrate and bagasse using acid, alum, and heat methods, followed by determining their proximate and some mineral composition.

From the result obtained, the yield from the three different methods is 3.41, 3.95, and 2.49 for acid coagulation, alum precipitation, and heat coagulation method, respectively. Therefore, in terms of yield, alum precipitation method produces more LPC than acid, and heat coagulation method.

Result, from the chemical analysis shows that the CP and EE of acid coagulation method were significantly ($p<0.05$) different from alum and heat processing methods. At the same time, the MC and NFE of OLPC for heat were significantly ($p<0.05$) different from alum precipitation method and acid coagulation method.

Result from the mineral analysis, shows that Potassium (K) was highest for the Alum precipitation method compared to other methods. However, the heat coagulation method produces higher values for Ca, Cu, Fe, Mn, Zn, Na, Mg, P, Fe, and Cl. Thus, analysis of

oil palm leaf protein concentrates using acid coagulation, alum precipitation, and heat coagulation method were comparable, but heat coagulation would be preferable.

6.2 Conclusion

The result from the study shows that the yields of OLPC using heat coagulation, alum precipitation, and acid coagulation method were slightly affected due to the different methods of extraction. Irrespective of the differences, the OLPC can be serve as an alternative source of augmenting the high cost of protein in the diet of man and animal. Also, Bagasse yield was observed to be high and, therefore, can be used as feed for ruminant animals directly or ensiled.

6.3 Recommendation

Research on more effective and efficient methods to improve yioed of leaf protein concentrates with minimal effect on its chemical composition should be promoted. Also, the use of leaf protein concentrate should be encouraged through awareness creation and sensitization of stakeholders in the feed industry.

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APPENDIX I

Plate 1: showing OLPC before drying

Plate 2: Showing Separetion of OLPC

APPENDIX II

Proximate Composition of Samples

Samples	Treatments	Replication	%MC	%Ash	%Fat	%Protein	%Fiber	%NFE
Oil palm	Acid	R1	5.16	4.25	8.11	45.5	2.65	34.33
		R2	5.17	4.23	8.25	47.25	3.54	31.56
		R3	5.14	4.27	8.34	47.25	3.1	31.9
	Alum	R1	5.23	8.63	6.32	28	2.11	49.71
		R2	5.35	8.61	6.35	28	2.23	49.46
		R3	5.34	8.62	6.37	28	2.33	49.34
	Heat	R1	5.34	4.37	4.14	26.25	3.46	56.44
		R2	5.34	4.35	4.15	26.25	3.21	56.7
		R3	5.38	4.3	4.24	28	2.75	55.33
	Baggers	R1	7.11	4.54	2.37	15.75	8.67	61.56
		R2	7.25	4.5	2.35	15.75	8.47	61.68
		R3	8.23	4.5	2.36	15.75	9.25	59.91

Minerals in sample in mg/kg

Sample	TRT	Rep.	Na	K	Ca	Mg	P	Fe	Mn	Cu	Zn	Cl
			mg/kg									
Oil palm	Acid	R1	2.8	1174	591	461	390	136.5	59.1	13.6	96.9	1.18
	Acid	R2	2.8	1174	591	461	390	136.5	59.1	13.6	96.9	1.18
	Acid	R3	2.6	1187	571	467	392	137.9	60.7	14.8	97.7	1.15
	Alum	R1	1	441	212	187	144	51.3	22.2	5.1	36.4	0.42
	Alum	R2	1	441	212	187	144	51.3	22.2	5.1	36.4	0.42

	Alum	R3	1.8	452	216	189	146.8	52.88	23.9	5.67	37.7	0.46
	Heat	R1	3.2	1316	632	500	417	153	66.3	15.2	108.6	1.34
	Heat	R2	3.2	1316	632	500	417	153	66.3	15.2	108.6	1.34
	Heat	R3	3.98	1318	635	504.9	419	156.9	67.9	16.6	108.9	1.25

APPENDIX III

Analysis of Variance

Variate: MC

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	0.058467	0.029233	19.63	0.002
Residual	6	0.008933	0.001489		
Total	8	0.067400			

Variate: ASH

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	37.4234000	18.7117000	31186.17	<.001
Residual	6	0.0036000	0.0006000		
Total	8	37.4270000			

Variate: FAT

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	24.766489	12.383244	2135.04	<.001
Residual	6	0.034800	0.005800		
Total	8	24.801289			

Variate: FIBRE

Source of variation	d.f.	s.s	m.s.	v.r	F pr.

Treatment	2	1.6049	0.8024	7.08	0.026
Residual	6	0.6797	0.1133		
Total	8	2.2846			

Variate: PROTEIN

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	743.0267	371.5133	547.45	<.001
Residual	6	4.0717	0.6786		
Total	8	747.0984			

Variate: NFE

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	885.1758	442.5879	466.32	<.001
Residual	6	5.6946	0.9491		
Total	8	890.8704			

Variate: Na

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	7.4899	3.7449	26.16	0.001
Residual	6	0.8589	0.1432		
Total	8	8.3488			

Variate: K

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	204957.68	102478.84	7498.45	<.001
Residual	6	82.00	13.67		
Total	8	205039.68			

Variate: Ca

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	316129.56	158064.78	3347.25	<.001
Residual	6	283.33	47.22		
Total	8	316412.89			

Variate: Mg

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	175876.047	87938.023	12364.35	<.001
Residual	6	42.673	7.112		
Total	8	175918.720			

Variate: P

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	135497.342	67748.671	38493.56	<.001
Residual	6	10.560	1.760		
Total	8	135507.902			

Variate: Fe

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	18037.543	9018.771	4158.59	<.001
Residual	6	13.012	2.169		
Total	8	18050.555			

Variate: Mn

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	3352.8622	1676.4311	1883.63	<.001
Residual	6	5.3400	0.8900		
Total	8	3358.2022			

Variate: Cu

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	186.3171	93.1585	225.09	<.001
Residual	6	2.4833	0.4139		
Total	8	188.8004			

Variate: Zn

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	8949.0756	4474.5378	16991.92	<.001
Residual	6	1.5800	0.2633		
Total	8	8950.6556			

Variate: Cl

Source of variation	d.f.	s.s	m.s.	v.r	F pr.
Treatment	2	1.324067	0.662033	476.66	<.001
Residual	6	0.008333	0.001389		
Total	8	1.332400			