PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (8) 1733-1738 August 2023

Physicochemical Composition of Leaf Protein Concentrates and Bagasse Obtained from Cassava (*Manihot esculenta*) Leaves Using Three Different Processing Methods

***AKAEZE, NC; PAUL-OSAGIE, MA**

Department of Animal Science, Faculty of Agriculture, University of Benin, Benin City Nigeria

**Corresponding Author Email: nneamaka.akaeze@uniben.edu; Tel: +234) 7032452193*
Co-Author Email: paulosagiemiracle@gmail.com

ABSTRACT: The experiment was conducted to determine yield, physical and chemical composition of leaf protein concentrates (LPC) and bagasse obtained from Cassava leaves using three different methods. The process of extraction was done using heat coagulation, alum precipitation and acid coagulation methods, then the leaf protein concentrates and bagasse obtained were analysed for their chemical compositions. The yield of LPC obtained via alum precipitation was statistically ($p<0.05$) higher than that obtained from heat coagulation method and acid coagulation method. The bagasse yield was (19.47%). The chemical analysis reveals that ether extract from alum precipitated LPC was higher than that of heat coagulation method and acid coagulation method, the crude protein from alum precipitation method was also higher than that from heat coagulation method and acid coagulation. The CP and EE of the bagasse were low (30.92% and 6.32%), respectively. The Ash of the heat coagulation method was lower than that from alum precipitation method but higher than that from the acid coagulation. The CF of the LPC obtained from heat coagulation method was higher than that of the LPC obtained from alum precipitation method but lower than that of the LPC obtained from acid coagulation. The CF of bagasse was high; while the Ash of the bagasse was low. The minerals, potassium, phosphorus and calcium were higher in LPC heat coagulation method than that obtained in LPC from the other two methods ($p<0.05$). Cassava leaf protein concentrates obtained using alum precipitation would be preferred as a result of its high crude protein content.

DOI: https://dx.doi.org/10.4314/jasem.v27i8.17

Cite this paper as: AKAEZE, N. C; PAUL-OSAGIE, M. A. (2023). Physicochemical Composition of Leaf Protein Concentrates and Bagasse Obtained from Cassava (*Manihot esculenta*) Leaves Three Different Methods. *J. Appl. Sci. Environ. Manage.* 27 (8) 1733-1738

Dates: Received: 10 July 2023; Revised: 25 July 2023; Accepted: 14 August 2023 Published: 30 August 2023

Keywords: Chemical composition; Cassava; Leaf protein concentrates; Bagasse; Whey

The increasing reliance on feedlot based animal rearing to satisfy human appetites for meat has increased demand for cheaper vegetable protein sources. This has recently led to renewed interest in Leaf Protein Concentrate to reduce the use of human-edible vegetable protein sources in animal feed (Akaeze, 2015). There is shortage of supply of good quality protein for meeting the requirement of increasing animal and human population, which has necessitated the search for additional sources (Isuosuo, 2012). Leaf protein concentrate (LPC), a concentrated form of proteins derived from the foliage of plants is an expensive and most abundant source of available protein. Their protein value equals that of must animal products. Trees have been suggested as a potential source of leaf protein concentrate and the production of proteins is advantageous over crops as they do not involve the recurring cost of cultivation (Suman *et al.*, 2014). Leaf protein concentrate (LPC) is a concentrated form of the proteins found in the leaves of plants, leaf protein concentrates was first introduced as a human food in the 1960's but it has not archived much success despite early promises because of certain palatability issues (Pirie, 1971; Signh, 1984). Leaf protein concentrate obtained from a green crop fractionation is free from indigestible fiber and it contains high proportion of proteins which are nutritionally superior. For this reason, its use in the nutrition of human beings and other non-ruminants has been advocated. Apart from proteins, it contains appreciable amounts of vitamin A, minerals, fat and vitamin E. to enhance yield and usability, several methods for the preparation of LPC from leaf juice have been advocated for which include, heating, acidification, natural acidification due to anaerobic fermentation of juice. It is reported that the yield of the leaf protein concentrate depends on the method used in extraction (Sayyed, 2011). Proteins from leaf

sources are fast gaining prominence, because they are readily available and cheap (Fasuyi and Aletor, 2005). Although leaves contain proteins and other essential elements, the presence of anti-nutritional factor and the high fibre content of the leaves pose a restriction to their utilization by non-ruminant animals. So, an alternative way to fully utilize leaves in non-ruminants is to process them into Leaf Protein Concentrates (LPC) which is rich in protein, low in fibre and anti-nutritional factors (Olomu and Nwokoro, 2009; Aletor and Adebayo, 2012). Leaf protein concentrates on the other hand have proven to be a viable source. Akaeze (2010) reported that leaf protein concentrates (LPC) makes for a more effective utilization of the vegetables. Cassava leaves are large and palmate, having five to seven lobes or more born on a long slender petiole. They grow only towards the end of the branches. They are dark green above and light green below. Cassava leaf is a simple, formed by the lamina and petiole (USDA, 2016). The high protein content and nutritive value of cassava leaves are well documented. Cassava leaf yields amounting to as much as 4.60 tons dry matter per hectare. According to Allen (1984), cassava leaf meal has 93.0% dry matter, 21.0% (16.7- 39.9%) crude protein, 5.5% (3.8%-10.5%) crude fat, 20.0% (4.8-29.0%) crude fiber and 8.5% (5.7-12.5%) ash. This wide variability is related to difference in cultivars, stage of maturity, sampling procedure, soil fertility and climate (Eggum, 1970). Therefore, the objective of this paper is to evaluate the Physicochemical Composition of Leaf Protein Concentrates and Bagasse Obtained from Cassava (*Manihot esculenta*) Leaves using three different methods

MATERIALS AND METHODS

The Research was conducted in three phases; firstly, was to determine the yield of *M. esculenta* Leaf Protein Concentrates (LPC) and bagasse prepared by heat coagulation method (Pirie, 1971 and Sayyed, 2011). Followed by determination of their physical and chemical compositions such as proximate (dry matter, crude protein, crude fibre, ether extract ash and nitrogen free extract) and some of their mineral content. Secondly, was to determine the yield of *M. esculenta* Leaf Protein Concentrates (LPC) and bagasse prepared by alum precipitation method (Sayyed, 2011), followed by determination of their physical and chemical compositions such as proximate (dry matter, crude protein, crude fibre, ether extract ash and nitrogen free extract) and some of the minerals it contains.

Lastly, was to determine the yield of *M. esculenta* Leaf Protein Concentrates (LPC) and bagasse prepared by acid coagulation method (Sayyed, 2011), followed by determination of their physical and chemical compositions such as proximate (dry matter, crude protein, crude fibre, ether extract ash and nitrogen free extract) and some of the minerals it contains.

Production of Cassava Leaf Protein Concentrate Using Heat Coagulation Method: Fresh cassava leaves were harvested at the dawn of the day and were immediately take to the laboratory for processing. The leaves were washed to remove dirt and sand particles, and then chopped to smaller sizes before weighing. The leaves were then processed by grinding into slurry using an electric blender. Each sample of slurry was placed on a sieve cloth and pressed strongly to distinguish the juice from the chaff (Bagasse). The juice was the heated and the curd separated leaving the whey fraction. Different temperatures were taken during heating for the curd formation. The curd was separated from the whey using Whatman filter paper and the sample of LPC was taken and yield recorded.

Production of Cassava Leaf Protein Concentrate Using Alum Precipitation Method: After separating the juice from the mixture using sieve cloth, the separated juice was poured into a bowl. To every 100ml of juice, 2g of alum was added. The curd of the leaf protein concentrate resulted due to the coagulation of proteins in the juice by the alum solution which was then filter using Whatman filter paper and the LPC sample was recorded.

Production of Cassava Leaf Protein Concentrate Using Acid Coagulation Method: After processing the leaves by milling, the bagasse and juice were separated using a sieve and sieve cloth, the separated juice was poured into a bowl. To 100ml of fresh juice, a known acid 10N $H_2S0_4$was added with stirring till the pH value decreased to 3.5. The curd of the LPC resulted due to the coagulation of proteins in juice by acid was filtered using Whatman filter paper and the sample of LPC was taken for recording the yield.

Mineral Analysis: Minerals were analysed after first dry-ashing 1g of the curd and bagasse at 550^0C in a Muffle furnance and dissolved in de-ionised water to standard volume. Sodium and potassium were determined by flame photometry and phosphorus by vanadomolybdate method of AOAC (2010). Magnesium, calcium, sulphur, manganese, iron and copper were determined using an atomic absorption spectrophotometer.

Data Collection: Data were collected on the yield, proximate and mineral composition of the cassava leaf protein concentrates processed using the different methods.

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

RESULT AND DISCUSSION

Percentage Yield of CLPC and Bagasse Extracted Using Acid Coagulation, Alum Precipitation and Heat Coagulation Method: The percentage yield of LPC produced using the following methods; Acid coagulation, alum precipitation and heat coagulation method and its bagasse are shown in Table 1. The result shows that the yields of the three methods of extraction are significantly ($p<0.05$) different from each other with alum precipitation (5.65%) having the highest yield while acid coagulation (4.43%) is higher than heat coagulation (3.69%).

Table 1: Percentage Yield of CLPC and Bagasse Extracted Using Acid coagulation, Alum Precipitation and Heat Coagulation methods

Sample	Yield (%)
Bagasse	19.47
Acid	4.43[a]
Alum	5.65[b]
Heat	3.61[c]
SEM	0.31

Means with same letters are not significantly ($p>0.05$) different; SEM= standard error of the mean; CLPC= Cassava Leaf Protein Concentrate.

Percentage yield of cassava leaf protein concentrate using Acid coagulation (4.43%) was higher than 2.21% *Raphanus sativus* leaf protein concentrate as reported by Sayyed (2011), using same method. This significant difference in yield could be as a result of the nature of the plant material and the nature of the component proteins, as protein denaturation is determined by a large extend to the nature of the individual protein molecules. Thus 4.43kg will be the yield from 100kg cassava leaves processed into leaf protein concentrate using acid coagulation method. Percentage yield of cassava leaf protein concentrate using heat coagulation method (3.69%) was lower than 5.6% rubber leaf protein concentrate reported by Akaeze *et al.* (2015) and also lower than 4.19% pumpkin leaf protein concentrate reported by Isuzu (2014) using same method. Percentage yield of cassava leaf protein concentrates using alum precipitation (5.65%) was higher than 2.93% *Raphanus sativus* leaf protein concentrates reported by Sayyed (2011) using same method. The yield from the alum precipitation is higher than that from acid coagulation and heat coagulation, this could be as a result of the Sulphur content of the alum reacting with the surphur containing amino acid leading to their precipitation and hence more protein concentrates is produced. The percentage yield of bagasse 19.47% was lower than 23.62% recorded in bagasse yield gotten from pawpaw leaf as shown by Agbonghae (2016). It is however important to note that the bagasse yield was statistically significant ($p<0.05$). The difference in yield is important in establishing what method could best be adopted in processing leaf protein concentrates for animal production, as the goal of any livestock production venture is to maximize the utility of all materials (Akaeze, 2019).

Physical Characteristics of Cassava leaf protein concentrates: The physical characteristics of cassava leaf protein concentrates processed using the three different methods is presented in Table 2. The bagasse obtained was fibrous and light green then turns dark green after drying under the sun. The LPC obtained from fresh cassava leaves using heat coagulation method before and after drying under the sun was light green and dark green respectively. The same was observed for alum precipitation and acid coagulation. The whey obtained from all three methods of extraction was light yellow. This compares with what was reported for cassava LPC by Akindele (2019) for cassava LPC processed using heat and alum precipitation methods. This similarity is consistent as the leaves where collected from the same geographical location as the leaves used in the present study. Results shown in Table 2 shows that the physical characteristics of the different fractions- leaf protein concentrate, bagasse and whey were in agreement with that reported by Mowah (2004) For *Telfairia occidentalis*, Agbonghae (2016) for *Carica papaya*, and Onwukaike (2017) for plaintain leafs

Table 2: Some Physical Characteristics of Whole Leaf, LPC, Bagasse and Whey of *Manihot esculenta* After Drying under the Sun.

Alum Treatment				
Characters	Leaf	LPC	Bagasse	Whey
Colour	Green	Dark-green	Dark-brown	Brown
Texture	Smooth	Coarse	Fibrous	N/A
State	Solid	Solid	Solid	Liquid
Acid Treatment				
Characters	Leaf	LPC	Bagasse	Whey
Colour	Green	Black	Dark-brown	Dark brown
Texture	Smooth	Coarse	Fibrous	N/A
State	Solid	Solid	Solid	Liquid
Heat Treatment				
Characters	Leaf	LPC	Bagasse	Whey
Colour	Green	Very dark green	Dark-brown	Pale brown
Texture	Smooth	Coarse	Fibrous	N/A
State	Solid	Solid	Solid	Liquid

Chemical Composition of Cassava LPC and Bagasse Using Three Different Methods Proximate Composition: The results of the proximate and mineral composition of cassava leaf protein concentrates are presented on Table 3 and 4. Results from the proximate analysis of cassava LPC and bagasse using three

different methods revealed that the crude protein revealed to be 37.33% LPC obtained from heat coagulation method and was higher than that in LPC of rubber (32.64%) reported by Akaeze *et al*. (2015) and as well higher than that in LPC of *Amaranthus hybridus* leaf (34.8%) reported by Adeyeye and Omolayo (2011), and 44.92% LPC gotten from alum precipitation method was higher than that in LPC of *Amaranthus hybridus* leaf (34.8%) reported by Adeyeye and Omolayo (2011). Crude protein was 42.58% revealed for acid coagulated LPC was higher than that in Pumpkin (11.75%) and Amaranthus (10.70%) LPC reported by Ghaly (2010). Although, these values were lower than crude protein content of *Telfaira occidentalis* leaf LPC (54.80%) reported by Sodamade *et al*. (2014), while the bagasse was 30.92% which is higher than 28.64% in bagasse of *Musa sapientum* as shown by Udegbe (2007) and 13.00% reported by Odoh (2019) for *Hevea brasiliensis.*

Table 3: Proximate Composition of CLPC and Bagasse Extracted using Heat coagulation, Alum precipitation and Acid Coagulation Methods

Parameters	Heat Coagulation	Alum Coagulation	Acid Coagulation	Bagasse	SEM
MC (%)	5.95[a]	6.20[a]	5.82[a]	8.14	0.314
CP (%)	37.33[a]	6.20[a]	42.58[b]	30.92	0.825
ASH (%)	4.47[b]	4.76[b]	2.45[a]	4.07	0.123
EE (%)	10.21[a]	14.67[b]	13.61[b]	6.32	0.323
CF (%)	0.63[a]	0.50[a]	1.28[b]	26.02	0.046
NFE (%)	41.34[c]	28.95[a]	34.25[b]	25.53	1.157

Means with same letters on the same column are not significantly ($p<0.05$) different.
SEM= Standard Error Mean, MC= Moisture Content, CP= Crude Protein, CF= Crude Fiber, EE= Ether Extract, NFE= Nitrogen Free Extract, CLPC= Cassava Leaf Protein Concentrate.

Crude fibre which could be affected by the materials used for sieving, inherent nature of plant materials as well as foreign materials that could contaminate the LPC and bagasse during the process of sun drying. The crude fibre was 0.63% for LPC gotten from heat coagulation method, which was lower than that in LPC of *Amaranthus hybridus* (1.7%) and (1.8%) as reported by Akaeze *et al*. (2015), and 0.50% for alum precipitation method which is lower than that in LPC of *Amaranthus hybridus* (1.7%) as reported by Adeyeye and Omolayo (2011), while the bagasse was 26.02% as the bagasse is usually the fibrous part of the leaf. The crude fibre value for acid coagulation was 1.28% of the LPC which is higher than heat coagulation by higher than alum precipitation. The crucial role crude fibre plays in the digestive process and ultimately in the proper functioning of the physiological system cannot be overemphasized. It has been reported that it helps lower the blood pressure and thus reducing the risk of cardiovascular diseases, it also helps in proper bowel functioning and movement, thereby ensuring that waste in moved through the intestine. It is reported that inadequate fibre in the diet of rabbits could lead to a condition known as GI stasis (De blas *et al.,* 1999). Ash percentage of the CLPC using heat coagulation method was 4.47% and is lower than CLPC of alum precipitation (4.76%) but higher than that of acid coagulation method (2.45%). They are all lower than ash (17.2%) in *Amaranthus hybridus* LPC and (12.3%) *Telfairia occidentalis* LPC reported by Adeyeye and Omolayo (2011). The crude protein and ash content has been shown to have a relationship in which the higher the ash content, the lower the crude protein as shown in *Amaranthus flavus* (CP 18.4% and ash 32.1%) and *A. mantegazzianus* (CP 23.4% and ash 25.2%) reported by Cheeke *et al.* (1981) and this conforms with the results from the CLPC using acid coagulation (CP 42.58% and ash 2.45%) heat coagulation (CP 37.33% and ash 4.47%) and alum method (CP 44.92% and ash 4.76%), while the bagasse was 4.07% which was higher than 3.0% in bagasse of *Musa paradisiaca* reported by Udegbe (2007) and 5.69% RLPC reported by Odoh (2017).

The ether extract of CLPC from heat coagulation method (10.21%) was higher than 9.6% in *Amaranthus hybridus* and 8.13% in RLPC while Ether extract from alum precipitation (14.67%) was higher than 10.7% from *Telfaria occidentalis* LPC and 8.12% from RLPC as reported by Adeyeye and Omolayo (2011) and Odoh (2019) respectively. The ether extract of acid coagulation (13.61%) was higher than the values for heat coagulation and but lower than the value for alum precipitation. The bagasse was 6.32% was higher than 5.98% in RLPC reported by Odoh (2019). The NFE of CLPC from heat coagulation method (41.34%) was higher than 23.58% from *Vernonia amygdalina* and NFE of CLPC from alum precipitation method (28.95%) was found to be higher than 23.58% from *Vernonia amygdalina* LPC as reported by Sodamade *et al.* (2014). The NFE of CLPC using acid precipitation (34.25%) was higher than alum precipitation but lower than heat coagulation while the bagasse was 25.53%.

Mineral Composition: Results shown in Table 4 indicate that Potassium and calcium in the LPC from heat coagulation method produced the highest with 2436mg/kg and 852mg/kg while the potassium and calcium in the LPC from alum precipitation was 636mg/kg and 304.3mg/kg respectively. The potassium and calcium in the LPC from acid coagulation was 1293mg/kg and 577.77mg/kg and were both higher than 5.0g/kg and 0.4g/kg for calcium and magnesium in LPC of Lucerne reported by Siebrits *et al.* (1986).

Table 4: Mineral Composition of CLPC and Bagasse Extracted from Heat Coagulation, alum Precipitation and Acid coagulation (mg/kg)

Parameters	Heat	Alum	Acid	SEM
Na	4.61^{c}	1.48^{a}	3.60^{b}	0.15
K	2436^{b}	636^{a}	129.3^{a}	19.6
Mg	606^{c}	293.2^{a}	577.9^{a}	1.33
Ca	852^{c}	304.3^{a}	604.2^{a}	2.9
P	867.2^{c}	205.5^{a}	401.9^{a}	2.35
Mn	88.27^{c}	33.12^{a}	64.9^{b}	0.58
Zn	152.8^{c}	53.6^{a}	107.1^{b}	2.45
Cu	21.87^{c}	7.54^{a}	16.60^{b}	0.70
Fe	206.67^{c}	73.93^{a}	151.3^{b}	0.78
Cl	1.807^{c}	0.660^{a}	1.317^{b}	0.03

Means with same letters on the same row are not significantly (p>0.05) different; SEM= Standard Error Mean, CLPC= Cassava Leaf Protein Concentrate.

They are required for formation of bones and teeth, formation of blood clot, formation of cyclic AMP and other second messengers, for body mechanisms, etc. (Olusanya, 2008). Other macro minerals phosphorus and magnesium were 401.9mg/kg and 577.77mg/kg in CLPC from acid coagulation method, 205.5mg/kg and 293.23mg/kg in CLPC from alum precipitation method and 567.2mg/kg and 606.83mg/kg for heat coagulation method respectively and were all higher than 116mg/kg and 457mg/kg of *Amaranthus hybridus* LPC reported by Adeyeye and Omolayo (2011). The sodium content in CLPC from acid coagulation 3.60mg/kg, 1.48mg/kg from alum precipitation and 4.61mg/kg from heat coagulation respectively were lower than 312mg/kg in *Telferia occidentalis* LPC reported by Adeyeye and Omolayo (2011). The micro minerals: copper (21.87mg/kg), iron (206mg/kg), and zinc (152.8mg/kg) in CLPC from heat coagulation method were all higher than the values of copper (5mg/kg), iron (35mg/kg), and zinc analyzed in Lucerne LPC as reported by Siebrits *et al.* (1986). Manganese, however, in CLPC gotten from heat coagulation (88.27mg/kg) was higher than manganese (50mg/kg) analyzed in Lucerne LPC as reported by Siebrits *et al.* (1986). The CLPC from alum precipitation method revealed 7.54mg/kg, 73.93mg/kg, 33.12mg/kg, 53.6mg/kg and 0.660mg/kg for copper, iron, manganese, zinc and chlorine respectively. The CLPC for acid coagulation method revealed 16.60mg/kg, 151.3mg/kg, 64.9mg/kg, 107.1mg/kg and 1.317mg/kg for copper, iron, manganese, zinc and chlorine respectively.

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

REFERENCE

Adeyeye, EI; Omolayo, FO (2011). Chemical Composition and Functional Properties of Leaf Protein Concentrate of *Amaranthus hybridus* and *Telfairia occidentalis*. *Agric. Biotechnol. J. Nutri.* 2(3):499-511

Agbonghae, WO (2016*). Physical and Chemical Properties of Leaf Protein Concentrates of Pawpaw and its Utilization by Growing Rabbits*. M.SC. Thesis, University of Benin, Benin City, Nigeria.

Akaeze, N (2010). *Physical Characteristics and Chemical Composition of Heava brasiliensis leaf protein concentrate and bagasse obtained from three locations in Edo State, Nigeria* Undergraduate Project, Department of Animal Science, University of Benin Pp 40-41.

Akaeze, NC; Nwokoro, SO; Imasuen, J.A. (2014). Performance of Growing Rabbit Offerer Rubber Leaf Protein Concentrates Replacement for Soyabean Meal, *Nigerian Journal of Agriculture, Food and Environment.* 10(4): 55-59.

Akaeze, NC; Nwokoro, SO; Imasuen, JA (2015). Replacement of Soyabean Meal with Rubber Leaf Protein Concentrate in the Diets of Growing Rabbits, Effect on Physiological Performance. *Nig. J. Agric. Food Envi.* 11(1): 1-6.

Akindele, OK (2019). *Physical Characteristics and Chemical Composition of Cassava leaf protein concentrate and bagasse obtained using two methods in Edo State, Nigeria* Undergraduate Project, Department of Animal Science, University of Benin Pp 47-58.

Aletor, VA; Adebayo, AO (2012). Nutritive and physiochemical characteristics of the leaves and leaf protein concentrates from tow edible vegetables: a comparative evaluation. *World J. Dairy Food Sci.* 7(1): 54-58.

AOAC (2010). *Official Method of Analysis*.20th Edition, Association of Analytical Chemists, Washington, D.C.

Cheeke, PR; Carsson, R; Kohler, GO (1981). Nutritive value of leaf protein concentrates prepared from *Amaranthus* species. *Can. J. Ani. Sci.* 61: 199-204.

De Blas, C; Garcia, J; Carabano, R (1999). Role of fibre in rabbit diets. A review. *Ann Zootech*, 48: 3-9.

Duncan, DB (1955). Multiple ranges and multiple F-test. *Biometrics, 11: 1-42*.

Eggum, L. (1970). The Protein Quality of Cassava Leaves. *British Journal of Nutrition* 24: 761-769.

Fasuyi, AO; Aletor, VA (2005). Varietals Compostion and Financial Properties of Cassava (*Manihot esculenta, Crantz*) Leaf Meal and Leaf protein Concentrates. *Food Chemistry Toxicology* 48 (8-9): 2227-2238.

Ghaly, A (2010). Extraction of Protein from Common Plant Leaves for Use as Human Food. *American Journal of Applied Sciences.* 7. 10.3844/ajassp.2010.323.330.

Isuosuo, IO (2012). T*he Physical and Chemical Properties of Pumpkin (Telfairia occidentalis) Leaf Protein Concentrate and Bagasse and their utilization in rabbit feeding*. M.Sc. Thesis, University of Benin, Benin City, Nigeria.

Isuzu, PC; Njoku, E; Nzediegwu, AA; Ayuk, C; Nzediegwu, IU; Efenudu,I; Erhayimwen MA (2014). Anti-Nutrient Composition of Pumpkin Leaf (*Telfiaria occidentalis*) at Three Temperature Regimes. *Pakistan Journal of Nutrition*, 13: 678-682.

Mowah, IM (2004). *Physical and Chemical Composition of Protein Concentrate of Leaves of Telferia occidentalis obtained from Three Locations in Edo State*. Undergraduate project of the Department of Animal Science, Faculty of Agriculture, University of Benin, Benin-city, Nigeria. Pp. 223-231.

NAA (2014). Meteorological Department, Nigeria Airport Authority, Benin City, Edo State, Nigeria.

Nwokoro, SO (2015). From the known to the unknown: Some Glimpse and Dances of a Scientist. 160th inaugural Lecture Series of the University of Benin, Pp 22-26.

Odoh, C (2017). *Yield, physical and Chemical Properties of Leaf Protein Concentrates and Bagasse of rubber (Hevea brasilliensis) tree*. B.SC. Project University of Benin, Benin city, Nigeria.

Olomu, CB; Nwokoro, SO (2009). Extraction rates and some chemical composition of *Manihot esculenta* leaf protein concentrate obtained from top, middle and buttom leaves using two different extraction methods. Proceedings of *14th Annual Conference of Animal Science Association of Nigeria (ASAN) September 14-17, 2009, LAUTECH, Ogbomoso, Nigeria*, pp 167-169.

Olomu, JM (2011). *Monogastric Animal Nutrition: Principle and Practice.* Second edition.ST. Jackson Publishing, Benin City, Nigeria. pp 87.

Olusanya, JO (2008). Essential of Food and Nutrition. *1st edition Apex book limited*, Pp. 62-78, 125-130.

Onwukaike, O.K. (2017). *Performance, blood and carcass characteristics of growing Rabbits fed Banana (Musa sapientum) Leaf Protein Concentrates as partial replacement for Soyabean meal*. M.Sc. Thesis University of Benin, Benin City, Nigeria. Pp 57-63.

Pirie, NW (1971). Ed. "Leaf protein: its Agronomy, Preparation, Quality and Use" IBP Handbook No. 20, *Blackwell Scientific Publications*, Oxford and Edinburgh.

Sodamode, A; Bolaji, OS; Adeboye, OO (2014). Proximate analysis, mineral contents and functional properties of *Moringa oleifera* leaf protein concentrate. *Journal of Applied Chemistry*4(6): 47–51.

Sayyed, IU (2011). Study of LPC and PCR prepared From Radish (*Rpahanus sativus Linn*) *Plant Science Feed* **1** (6): 88-92.

Siebrits, FK; Christina, D; Esterhuyse, I; Kemm, E (1986). Animal and Dairy Science Research Institute, Irene. *The Relative Nutritive value of Lucerne Leaf Protein Concentrate (LPC) Coagulated by means of steam or hot water.*

Signh, N (1984). *Progress in Leaf Protein Research, Today and Tomorrow.* Printer and Publishers, New Dehli.

Suman, S; Varshney.VK; Wahi,N ; Khan,LH (2014). Isolation and Biochemical Analysis of Leaf Protein concentrates from the Leaves of *Shorea robusta. Pakistan Journal of Nutrition* 13 (9): 546-553.

Udegbe, NC (2007). *Some physical and Chemical Properties of Leaf Protein Concentrates and Bagasse of plantain and banana leaves in Benin City*. B.SC. Project University of Benin, Benin city, Nigeria.

USDA, (2016). Cassava raw. *Nutrition Nutrient Database for Standard References Release 28.* Agricultural Research Service, US Department of Agriculture.

World Health organization, WHO (1985). *Energy and Protein Requirement.* WHO Technical Report Series 724:121.