YIELD, PHYSICAL AND CHEMICAL COMPOSITION OF SWEET ORANGE (*Citrus sinensis* L.Osbeck) LEAF PROTEIN CONCENTRATES AND BAGASSE EXTRACTED USING THREE DIFFERENT METHODS

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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

ABSTRACT

This study was carried out to determine the yield, physical properties and chemical composition of sweet orange leaf protein concentrates and bagasse extracted using three different methods.

Heat coagulation, alum precipitation and acid coagulation methods were used for the extraction of sweet orange leaf protein concentrates. Thereafter, the yield, physical properties and chemical composition were determined.

The results obtained showed that the yield obtained via the three methods are 2.675% for acid coagulation, 6.60% for alum precipitation and 7.838% for heat coagulation.

The chemical analysis performed on the leaf protein concentrates and bagasse, CP, EE and Ash content from LPC obtained from acid and alum precipitation were statistically the same ($p<0.05$). The CP and EE of Bagasse (17.48% and 4.50% respectively) were significantly lower ($p<0.05$) than the CP and EE obtained from all three methods.

For the minerals, potassium content from alum precipitation (1351 mg/kg) and heat coagulation (2148 mg/kg) were not significantly different ($p<0.05$).

CERTIFICATION

This is to certify that this Project work was carried out by **Uche Anthony CHIME** with matriculation number, **AGR1500078** 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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ACKNOWLEDGEMENT

I give glory and thanks to God of the heavens and to Jesus Christ who gave me the grace, and strength and good health in peace to oversee the conclusion of the final phase of my university undergraduate programme.

My immense gratitude goes to my diligent and indefatigable supervisor, (Prof. S.O. NWOKORO) whom I highly regard as a pinnacle of innovative research, working and contributing massively and relentlessly to improve human life. I want to specially appreciate my co-Supervisor (Mr. O.W. AGBONGHAE) of whom I hold in high esteem for his unwavering care, attention and dedication throughout the period of my undergraduate project and my whole study, for always offering his time services unconditionally, always checking on our progress, inspecting, auditing, verting and encouraging me during the period of my undergraduate project. I am eternally grateful to my co-Supervisor (Dr. N.C. AKAEZE) for his wisdom and dedication towards the planning, overseeing and finalizing of my undergraduate project, for putting me through the difficult processes and always ensuring that I was always able to reach him in spite of how tedious his schedule always was. I also want to appreciate all the members of staff of the Department of Animal Science and Faculty of Agriculture, and especially to my HOD (Prof J.A. IMASUEN). My special thanks go to Prof J.M. OMOYAKHI for his wisdom and fatherliness and his gracious show of love and discipline that has helped to mould me and carry me along throughout my undergraduate years and especially in my final year.

My love and sincerest gratitude go to my sweet mother (Mrs. Patricia CHIME) who always single-handedly devoted her time and efforts in every circumstance to ensure that I had the best experience a child could ever have. For every single privilege I enjoy today will not be possible without God's divine care and dedication through my sweet hardworking mother.

To my dear uncle (Mr. Emeka ENEDU), words will fail me to express how grateful I am, for your love, care, assistance and dedication towards my undergraduate journey. You are indeed a gem sir,

To my friends, Victor, Merit, Precious, Miracle, Bigard, to mention a few, you have added so much joy, value and essence to my undergraduate journey. Thank you so much. I love you guys dearly.

To, my dear, beloved friends resting in the Lord, Omigie Favour ITOHAN and Michael Oluwaseun AKINADE, death wouldn't let you witness this fulfilment of all the hard work we all passed through, but I am rest assured that you are both resting in the bosom of Christ. Thank you so much for all the wonderful times we shared. I miss you dearly.

Finally, to everyone that showed me love and prayed for me and supported me in all my endeavors, may the Good Lord bless you all.

DEDICATION

This work is dedicated to my Mum, (Mrs. Patricia CHIME) and to all those who through their love, dedication, moral and financial support kept me all through the course of this Study.

TABLE OF CONTENT

CONTENT

PAGE

Title page ii

Abstract i

Certification ii

Acknowledgement iii

Dedication v

Table of Content vi

List of Tables x

List of Figures xii

CHAPTER ONE

1.0: Introduction 1

1.1: Justification 3

1.2: Objectives of the Study. 7

CHAPTER TWO

2.0: Literature Review 8

2.1: Sweet Orange 8

2.1.1: Description 8

2.1.2: Distribution 9

2..1.3: Classification of *Citrus sinensis* (L. Osbeck) 10

2.2.1: Leaf Protein Concentrate 11

TABLE OF CONTENT CONT'D

CONTENT	PAGE
2.2.2: Leaf Protein Concentrate Limitations	12
2.2.3: Methods of Preparation of Leaf Protein Concentrate. . .	16
2.3: Bagasse	22
CHAPTER THREE	
3.0: Materials and Methods	25
3.1: Location and Duration of the Study	25
3.2: Experimental Materials.	25
3.3: Preliminary Trials	25
3.4: Calibration Procedures.	26
3.5: Production of Sweet Orange LPC Using Heat Coagulation Method .	26
3.6: Production of Sweet Orange LPC Using Alum Precipitation Method	28
3.7: Production of Sweet Orange LPC Using Acid Coagulation Method	28

TABLE OF CONTENT CONT'D

CONTENT **PAGE**

3.8: Precautions Taken During the Production of LPC. . . . 28

3.9: Chemical Analysis. 29

3.10: Mineral analysis 33

3.11: Statistical Analysis. 34

3.12: Yield Determination 34

CHAPTER FOUR

4.0: Results 35

4.1: Percentage Yield of Sweet Orange LPC and Bagasse using Heat Coagulation, Acid Coagulation and Alum Precipitation Methods. 35

4.2: Physical Properties of the Whole Leaf, Bagasse, LPC and Whey of Sweet Orange. 37

4.3: Proximate Composition of Sweet Orange LPC and Bagasse Using Acid Coagulation, Alum Precipitation and Heat Coagulation Methods .. 40

4.4: Mineral composition of Sweet Orange LPC and Bagasse Using Alum Precipitation and Heat Coagulation Methods . . . 42

TABLE OF CONTENT CONT'D

CONTENT **PAGE**

CHAPTER FIVE

5.0: Discussion 44

5.1: Yield of Sweet Orange LPC and Bagasse Using Three Different Methods 44

5.2: Chemical Composition of Sweet Orange LPC and bagasse using three different methods 45

5.2.1: Proximate Composition. 45

5.2.2: Mineral Composition. 47

CHAPTER SIX

6.0: Summary, Conclusion and Recommendations . . . 49

6.1: Summary. 49

6.2: Conclusion 50

6.3: Recommendations 51

References 52

Appendix 55

LIST OF TABLES

TABLE	TITLE	PAGE
1:	Nutrient composition of ProXan - Alflafa (Medicago sativa) LPC.	5
2:	Amino Acid Contribution of ProXan - Alflafa (Medicago sativa) LPC.	6
3:	Protein and energy content of a 100g portion of some food . .	13
4:	The chemical composition of Alfalfa LPC.	15
5:	Preparation Of Lpc From Leaf Juice Of *Raphanus sativus*. . .	19
6:	Essential Amino Acid Composition of Various Foods (as percentage of total protein)	20
7:	Vitamins and Mineral Contents of leaf protein concentrate. . .	21
8:	Chemical composition of bagasse fibre.	23
9:	Percentage yield of Sweet orange LPC and bagasse using Acid Coagulation Alum Precipitation and Heat Coagulation	36
10:	Some Physical Characteristics of Whole Leaf, LPC, Bagasse and Whey of *Citrus sinensis* (L. Osbeck) Before Drying (Fresh). .	38
11:	: Some Physical Characteristics of Whole Leaf, Bagasse and LPC of *Citrus sinensis* (L. Osbeck) After Sun Drying (30 – 35oC) .	39

LIST OF TABLES CONT'D

TABLE	TITLE	PAGE
12:	Proximate Composition of Sweet Orange LPC and Bagasse using Acid Coagulation, Alum Precipitation and Heat Coagulation	41
13:	Mineral Composition of Sweet Orange LPC and Bagasse Using Acid Coagulation, Alum Precipitation and Heat Coagulation Extraction Methods(mg/Kg).	43

LIST OF FIGURE

FIGURE	TITLE	PAGE
1:	Flow Chart of the Production of Leaf Protein Concentrates . .	27

CHAPTER ONE

1.0 INTRODUCTION

Leaf protein concentrate as an alternative protein source was first suggested as a human food in the 1960s (Tripathi *et al.,* 2011) and research to develop a potential product was conducted in the 1960s and 1970s (Gilani and Lee, 2003).

Statistics have shown that there is an increasing rate of protein deficiency globally which has continued to intensify with the rapidly increasing global population growth rate, currently at 1.04% (David, 2021). This increase in population has indirectly made the availability of traditional sources of protein scarce, especially in the tropics where population growth rate and malnutrition are more intensified.

In livestock production, Provision of the adequate feed composition required by farm animals remains a challenge, owing to the inadequacy or unavailability of feed materials to maintain livestock. The major protein feed sources such as soybean meal, fish meal and groundnut cake for monogastric farm animals are all conventional protein sources for farm animals, and the increase in demand for these commodities has led to their increased cost. These protein feed commodities as well serve as potential human food, and due to their limited availability, there is competition for these commodities to be prioritized for human or livestock nutrition (Akaeze *et al.,* 2015).

It is very difficult for monogastric animals to utilize leaf meal as protein sources for nutrition This is due to the presence of non-digestible fiber and anti-nutritional factors

such as tannins, phytates, oxalates, cyanide and more which induce varying degrees of unfavourable neurological and metabolic consequences to these animals (Shipra *et al.,* 2011)

There have been several suggestions concerning very accessible, and economical sources of protein; of these, Leaf protein concentrate (LPC) stands to be one of the most promising avenues (Shipra *et al.,* 2011)

Leaf protein concentrate is a novel alternative to utilize leaves in non-ruminants due to its rich protein content and infinitesimal amount of fiber and anti-nutritional factors (Nwokoro and Olomu, 2009). Leaf protein concentrate (LPC) is a product resulting from green crop fractionation whereby freshy harvested young leaves are macerated to a pulp containing some amount of fiber, mineral and proteinaceous material of leaf origin, chloroplasts or fragments of it and about 90% water (Baraniak and Waleriańczyk, 2003). It is a highly advantageous utility in the curbing of protein deficiency and malnutrition in both human and livestock populations

(Shipra *et al.,* 2019).

Sweet Orange, scientifically known as *Citrus sinensis* (L. Osbeck) *(*family: *Rutaceae),* is an important fruit globally. It is supposed to have originated in the Southeast Asia, but is today also found growing in other parts of the world. (Sowmya *et al.,* 2015). The pulp and juice prepared from it are important dietary sources of vitamin C, limonoids, synephrine, hesperidin, polyphenols, pectin, calcium, potassium, thiamine, niacin, and magnesium. (Sowmya *et al.,* 2015) These biologically active compounds aid in

prevention against arteriosclerosis, cancer, kidney stones and stomach ulcers in humans. They have also been known to reduce cholesterol levels. Potassium contained in the fruit helps to prevent high blood pressure and stroke. The fruit juice and leaves also contain large amounts of a chemical called citrate which might help to prevent kidney stones. Citrate tends to bind with calcium before it can form a stone. (Sowmya *et al.,* 2015).

1.1 Justification

To meet up with the demands of the ever-increasing population, and for affordability in every economic condition, there is need to have diverse alternative sources of protein.

Leaf protein concentrate extraction was initiated to address the need for more economic means of obtaining protein, to increase the protein resource availability in food and animal feed industry (Arellano and Gonzalez-Perez, 2009).

This research on *Citrus sinensis* (L. Osbeck) will form a base for comparison between its counterparts such as *Moringa olifera* (Moringa Plant)*, Medicago sativa* (Alfalfa) and other species that have been extensively studied and are well documented. LPC prepared from alfalfa has given growth performance similar to that obtained with soybean meal with swine (Myer *et al.,* 1975; Cheeke *et al.,* 1977b) and with poultry (Kuzmicky and Kohler, 1977). This will help decipher if the extracts from Sweet Orange leaves are capable of yielding a relatively sufficient amount of Leaf protein concentrate per hectare;

because aside the relatively cheap extraction process, the yield and quality must be capable of meeting up with global demands on a large-scale production.

Bagasse, the chaffy material that is left after the removal of the extract or juice can serve as a source of indigestible fiber for rabbits. Instead of disposing bagasse, it can be useful for a number of important functions: It enhances easy digestion and excretion of other feed materials. Bagasse from *Citrus sinensis* (L. Osbeck) can serve as bedding material for ruminant pens; it can absorb waste and provide a soft cushion to make animals more comfortable (Cheeke, 1987).

Table 1: LPC Nutrient composition of ProXan - Alflafa (Medicago sativa) LPC.

% dry matter basis

Nutrient	ProXan #1	ProXan #1 W	ProXan #2	ProXan
Dry matter				
Protein	96.34	99.67	97.25	93.02
Fat (ether extract)	62.81	66.94	51.37	67.75
Crude fiber	9.41	10.31	6.96	9.62
Ash	2.03	1.95	1.21	1.64
Si02	11.26	7.75	13.23	7.28
Calcium	3.57	4.06	2.65	3.62
Phosphorus	1.55	.18	2.07	.22
Sodium	.60	.31	.56	.32
Potassium	.06	.01	.19	.01
Magnesium	1.07	.06	2.84	.08
Chloride	.43	.12	.77	.13
Total sugars	.17	.08	.93	.02
Reducing sugars	.10	.05	1.89	.04
Starch	.10	.03	1.18	.04
Water soluble	.60	.08	27.09	.37
solids	8.05	.44	87.8	.53
Protein digestibility[2]	90.5	95.5		97.2

Prepared as follows: Pro-Xan coagulum pressed and hot air dried; Pro-Xan #1 W, dilute acid washed and freeze-dried Pro-Xan ; Pro-Xan #2, coagulum drained and freeze-dried; Pro-Xan #2W, dilute acid washed and freeze-dried Pro-Xan #2.

In vitro protein digestibility as determined by the pepsin-trypsin method of Saunders *et al.* (1973).

Source: Bickoff (1975)

Table 2. Amino Acid Contribution of ProXan - Alflafa (Medicago sativa) LPC

Amino acid	ProXan #1	ProXan	ProXan #2	ProXan
Arginine	6.45	6.86	6.18	6.60
Cystine	1.18	1.21	1.31	1.31
Glycine	5.52	5.66	5.30	5.81
Histidine	2.32	2.59	2.40	2.83
Isoleucine	5.60	5.75	5.28	5.86
Leucine	9.29	10.04	9.18	10.12
Lysine	5.92	6.50	6.30	6.83
Methionine	2.30	2.62	2.16	2.29
Phenylalanine	5.94	6.47	5.86	6.56
Threonine	5.10	5.47	5.07	5.54
Tyrosine	4.75	4.90	4.43	5.06
Valine	6.33	7.19	6.54	7.08
Alanine	6.28	6.43	6.11	6.52
Aspartic acid	10.03	10.42	10.26	10.60
Glutamic acid	11.35	12.00	11.39	12.04
Proline	4.86	4.77	4.68	5.10
Serine	4.33	4.56	4.40	4.68

Source: Bickoff (1975)

1.2 Objectives

The objective of this study was to determine the yield, physical and chemical composition of leaf protein concentrate and bagasse extracted from sweet orange (*Citrus sinensis* (L. Osbeck) using three extraction methods.

The specific objectives were:

1. Determine the yield of leaf protein concentrates and bagasse from Sweet Orange leaves using three different methods.
2. Determine the physical and chemical compositions of the leaf protein concentrates and bagasse obtained from Sweet Orange leaves extracted using two different methods of extraction.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Citrus sinensis* (L. Osbeck)

2.1.1 Description

Description of *Citrus sinensis* (L. Osbeck) *Plant*

Orange, *Citrus sinensis* (L. Osbeck), is an evergreen tree in the family Rutaceae grown for its edible fruit. The orange tree is branched with a rounded crown and possesses elliptical or oval leaves which are alternately arranged on the branches. The leaves have narrowly winged petioles, a feature that distinguishes it from bitter orange, which has broadly winged petioles. The fruit is a spherical berry with a green-yellow to orange skin covered in indented glands and a segmented pulpy flesh and several seeds. Orange trees can grow to a height of 6–15 m (16–49 ft) and can live for periods in excess of 100 years. Most plantations have an economic lifespan of around 30 years. (Plantvillage, 2019)

The orange blossom is the fragrant flower of the *Citrus sinensis* (L. Osbeck) (orange tree). Orange flowers are white and are distinguished by their very fragrant aroma. They have 5 petals and numerous stamens. The outstanding feature of orange flowers and flowers of related citrus species is their pleasant fragrance. (Britannica, 2020).

2.1.2 Distribution

Citrus sinensis (L. Osbeck) or sweet orange originated from south East Asia (Etebu and Nwauzoma, 2014). The orange has become the most commonly grown tree fruit in the world. It is an important crop in the Far East, the Union of South Africa, Australia, throughout the Mediterranean area, and subtropical areas of South America and the Caribbean. The United States is the biggest producer, having an annual yield of more than 200 million boxes, except when freezes occur which may reduce the crop by 20 or even 40% (Figure 1.1). California, Texas and Arizona follow in that order, with much lower production in Louisiana, Mississippi, Alabama and Georgia. Other major producers are Brazil, Spain, Japan, Mexico, Italy, India, Argentina and Egypt. (Perea, 2016).

It is the most widely planted fruit tree in the world. According to FAO statistics in 2009, the global acreage for citrus was nine million hectares with production put at 122.3 million tonnes. Oranges are an all-season fruit that grows abundantly in late April and late May. Nigeria is one of the largest producers of citrus in Africa with about 14 states being major producers of this vitamin C-rich fruit (Plantvillage, 2019)

2.1.3 Classification of *Citrus sinensis* (L. Osbeck)

- Domain: *Eukaryota*
- Kingdom: *Plantae*
- Phylum: *Spermatophyta*
- Subphylum: *Angiospermae*
- Class: *Dicotyledonae*
- Order: *Rutales*
- Family: *Rutaceae*
- Genus: *Citrus*
- Species: *Citrus sinensis* (L. Osbeck)

Source: CABI (2021)

2.2.1 Leaf Protein Concentrate

Leaf protein concentrate (LPC), a concentrated form of the proteins obtained from plant leaves has been assessed for human or animal food source, because it is potentially the cheapest, most abundant source of available protein (Tripathi *et al.,* 2011). Leaf proteins consist of two major categories: chloroplastic proteins (green leaf proteins) and cytoplasmic proteins (white leaf proteins). Both types of protein occur at approximately the same amounts in leaves, and cytoplasmic proteins are generally considered to have higher nutritional value than green proteins (Urribarrí *et al.,* 2005).

Leaf protein concentrate (LPC) is an extremely nutritious food made by mechanically separating indigestible fibre and soluble anti-nutrients from much of the protein, vitamins, and minerals in certain fresh green plant leaves. Because it is so rich in beta-carotene, iron, and high-quality protein, leaf concentrate is very effective in combating malnutrition, especially the anemia and vitamin A deficiency which are prevalent among children and pregnant women in most developing countries. It is easily combined with a variety of inexpensive foods to make culturally acceptable dishes. Because it takes more direct advantage of solar energy, a leaf crop can produce more nutrients per hectare than any other agricultural system (Graham and Telek, 1983). Leaf concentrate is relatively easy to make. People with little training or education can make it in rural villages. It offers a very nutritious food at prices below what foods like meat, cheese, eggs, or powdered milk cost. It is usually the cheapest dietary source of vitamin A and iron wherever it is made (Kennedy, 1993).

2.2.2 Leaf Protein Concentrate Limitations

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

Table 3: Protein and Energy Content of a 100g Portion of Some Food

Food	Protein (%)	Energy (kcal)
Beef	17.4	301
Chicken	22.5	124
Beans	23	118
LPC	24.0	138
Milk	3.5	66
Eggs	163	12.9

Source: FAO/WHO (1985)

The table above reveals that LPC had a higher protein content compared to beef, chicken, milk, beans and eggs while its energy level is only than that of beef.

LPC is a good source of cyanocobalamine (Vitamin B12), ascorbic acid (Vitamin C) and folic acid or Vitamin B9 (Iliyas and Badar, 2010a) and also estimation of thiamine, riboflavin and pyridoxine were found in some plants (Iliyas and Badar, 2010b). The chemical composition of Alfalfa LPC is given below.

Table 4: The Chemical Composition of Alfalfa LPC

Mineral	Quantity
Calcium	5.0g
Sodium	0.5g
Potassium	1.8g
Phosphorus	4.0g
Magnesium	0.4g
Selenium	0.04mg
Iron	35mg
Zinc	12mg
Iodine	0.15mg
Manganese	50mg
Niacin	15mg

Source: Siebrits *et al.* (1986)

2.2.3 Methods of preparation of leaf protein concentrate

Quite a number methods have been used in the preparation of Leaf protein concentrate. According to Sayyed (2011) Radish (Raphanus sativus) was prepared using eight different methods and the yield results are as shown in Table 2.4. These methods include:

1. Differential heat coagulation:

Twenty ml distilled water was heated in a beaker to 60°C. To it, 50 ml fresh juice was slowly added with stirring so as to maintain the temperature of juice to 60°C. The juice was kept for heating at this temperature for 5 min. It was then filtered through Whatman filter paper to separate green chloroplastic leaf protein concentrate (LPC), which received 2-3 washings with water. The filtrate was collected and heated to boiling. This resulted into the precipitation of cytoplasmic proteins resulting into white cytoplasmic LPC. The cytoplasmic fraction was recovered by filtration through Whatman filter paper and the deproteinised juice (DPJ) released as a filtrate was collected. The samples of green chloroplastic LPC, white cytoplasmic LPC (resulting due to the heating of filtrate to over 95°C) and the deproteinised juice (DPJ) left after recovering chloroplastic and cytoplasmic fractions were collected for further analysis. The samples of LPC were dried in oven at 65 ± 5°C to record the yield (Pirie, 1971; Sayyed, 2011).

2. Heat coagulation:

To about twenty ml boiling water 50 ml of fresh juice was slowly added with constant stirring. It was heated till boiling to a temperature exceeding 95°C. The proteins in juice got coagulated due to the heat treatment and resulted into a curd referred to as leaf protein

concentrate (LPC). The LPC was separated from remaining portion of the juice; the deproteinised juice (DPJ) by filtration through Whatman filter paper with 2 to 3 hot water washings. The sample of LPC was collected, dried in oven at 65 ± 5°C and the yield of LPC dry matter (LPC – DM) was recorded (Pirie, 1987).

3. Heat coagulation followed by acid wash:

The LPC from Lucerne juice was prepared by heat coagulation as described above and suspended in acidic water with a pH value of 3.5 for 10 minutes and filtered. The samples of LPC and DPJ were collected for recording the yield and further analysis (Sayyed, 2011).

4. Acid coagulation:

To 50 ml fresh juice, 5N H_2SO_4 was added with stirring till the pH value decreased to 3.5. The curd of LPC resulted due to the coagulation of proteins in juice by acid was filtered through Whatman filter paper and the sample of LPC was taken for recording the yield (Singh and Singh, 1985; Sayyed, 2011).

5. Fermentation:

Fresh juice (about 120 ml was placed in 125ml conical flask. The mouth of the flask was capped with rubber cork. A 25 to 35 cm long capillary rod (1 mm internal diameter) was inserted through the cork and mouth of the conical flask was sealed with wax. The flask was left at room temperature for fermentation of juice till six days. The capillary rod, on the top of the flask, allowed carbon dioxide formed during fermentation to escape out and also to exclude air. After 6 days, the cork was removed and loss in the volume of juice

due to fermentation was made up with distilled water. The fermented juice showed precipitation of proteins in it due to acidification during fermentative process. It was filtered through Whatman filter paper and the sample of LPC was kept in oven to record the yield of LPC (Szakacs and Madas, 1979; Sayyed, 2011).

6. Alum precipitation:

Two gm of alum was dissolved in 100 ml distilled water to prepare alum solution. To 50 ml fresh juice, alum solution was added with stirring. The curd of LPC resulted due to the coagulation of proteins in juice by alum solution was filtered through Whatman filter paper and the sample of LPC was taken for recording the yield (Sayyed, 2011).

7. Flocculation:

One hundred mg of prestol (a flocculant) was dissolved in 100 ml distilled water by constant stirring with magnetic stirrer for 30 minutes. To 50 ml fresh juice flocculant solution was added with stirring. The curd of LPC resulted due to the coagulation of proteins in juice by flocculant was filtered through Whatman filter paper and the sample of LPC was taken for recording the yield of LPC (Bray and Humphries, 1979; Sayyed, 2011).

8. Flocculation at pH 3.5:

To 50 ml fresh juice the flocculant solution prepared as above was added with stirring. To it 5N HCL was slowly added with stirring till the pH value dropped to 3.5. The curd of LPC resulting due to the coagulation of proteins in the juice, was filtered through Whatman filter paper and sample of LPC was taken to record the yield (Sayyed, 2011).

TABLE 5: Preparation of LPC from Leaf Juice of *Raphanus Sativus*

Method of LPC Preparation	Yield of LPC (g/100ml)	Total N (mg/100ml)	N IN DPJ (mg/100ml)	N IN LPC (mg/100ml)
Differential heat Coagulation				
A) Chloroplastic	1.731	-	-	-
B) Cytoplasmic	0.569	-	-	-
Total	2.300	550.0 (100)	37.5 (6.8)	512.0 (93.2)
Heat coagulation	2.213	550.0 (100)	87.5 (16.0)	462.5 (84.0)
Heat coagulation followed by acid wash	2.306	550.0 (100)	150.0 (27.3)	400.0 (72.7)
Acid coagulation	2.213	550.0 (100)	137.5 (25.0)	412.5 (75.0)
Fermentation	2.613	550.0 (100)	112.5(20.5)	437.5 (79.5)
Alum precipitation	2.931	550.0 (100)	100.0 (18.2)	450.0 (87.8)
Flocculation	2.536	550.0 (100)	137.5 (25.0)	412.5 (75.0)
Flocculation at pH 3.5	2.731	550.0 (100)	112.5 (20.5)	437.5 (79.5)
Mean	2.355	550	109.3	440.5
S.D	0.24	-	33.51	31.37
C.V	10.25	-	30.65	7.60

- = not applicable, SD= Standard Deviation, CV= Coefficient of Variation, LPC= Leaf Protein Concentrate, N= Nitrogen, N in DPJ= Nitrogen in De-Proteinised Juice

Source: Sayyed. (2011)

Table 6: Essential Amino Acid Composition of Various Foods (as percentage of total protein)

	Milk	Egg	Beef	Chicken	Cod	Trout	Bean	Lpc	Fao/std
Isoleucine	5.45	5.61	5.12	4.63	5.29	5.27	4.16	5.22	2.88
Leucine	10.00	8.29	8.01	7.51	8.45	8.48	7.67	9,34	2.88
Lysine	8.18	6.26	9.10	8.98	9.73	9.73	7.17	6.51	6.80
Tyrosine	10.00	9.10	8.32	8.80	7.65	7.66	7.87	10.00	6.30
Threonine	4.85	5.12	4.64	4.63	4.77	4.78	4.03	5.02	3.40
Trptophan	1.42	1.79	1.27	1.12	1.15	1.14	0.95	2.14	1.1
Valine	7.27	7.56	5.30	4.78	5.75	5.76	4.66	6.33	3.50
Total essential amino acid	47.17	43.64	41.72	39.65	42.78	42.82	36.53	45.55	29.5

Source: WHO/FAO, (1985)

Table 7: Vitamins and Mineral Contents of Leaf Protein Concentrate

	Amount per 100g of moist curd	**Amount per 100g of dry curd**
Vitamin A	34.70mg	86.70mg
Vitamin D	ND	ND
Vitamin E	10.70IOU	26.70mg
Vitamin K	0.40mg	1.00mg
Thiamin	0.18mg	0.44mg
Riboflavin	0.22mg	0.56mg
Vitamin B6	0.40mg	1.00mg
Pantothenic acid	1.80mg	4.40mg
Biotin	20mg	40mg
Folic acid	132mg	40mg
Ascorbic acid	0.90mg	2.20mg
Calcium	7.46mg	1865mg
Phosphorus	242mg	604mg
Magnesium	154mg	384mg
Iron	40mg	99mg
Potassium	285mg	713mg
Zinc xx	3.16mg	9.10mg
Copper	0.90mg	2.20mg
Manganese xx	2.70mg	6.70mg
Iodine xx	8.80mg	22mg

Source: FAO/WHO. (1985)

ND - not determined

xx - amount will depend on availability on local soil

2.3 Bagasse

This is the fibrous matter or chaffy material that remains after the extraction of juice from plant parts such as the leaves or stalk. It is used industrially as a raw material in pulp production; for making papers and paper products as well as building materials. It is a non-forage roughage, hence, this residue is nowhere near ideal for ruminant nutrition, although it can aid in digestion; as it serves a roughage due to its fibre content (Cheeke, 1981).

Table 8: Chemical Composition of Bagasse Fibre

Parameter	Percentage composition
Cellulose	45-55
Hemi cellulose	20-25
Lignin	18-24
Pectin	0.6-0.8
Ash	1-4
Extractives	1.5-9

Source: Sachin *et al.* (2015)

Bagasse can serve as a bedding material or the deep-litter poultry system and also in ruminant pens due to its characteristics that improve the comfort of the animal making it friendlier and easier to attend to. It can also be dried and used later as animal feed, or employed as green manure.

If Bagasse is not fed fresh or well dried, it loses its palatability and will not encourage the animal to consume the feed, also Bagasse does not have equal acceptability for all animals; Cows tend to like it better than goats and rabbits (Kennedy, 1993). It is therefore a utility by-product that has a decent number of useful qualities.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location and Duration of the Study

The experiment was undertaken in the main Laboratory of Faculty of Agriculture in Ugbowo Campus, University of Benin (Uniben), Edo State, Nigeria. University of Benin, Ugbowo 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 an average temperature of 27.6 ºC (Google Earth, 2022; NAA, 2014).

3.2 Experimental Materials

The materials used for this experiment were freshly harvested *Citrus sinensis* (L. Osbeck) leaves, weighing balance, laboratory thermometer, Whatman filter paper, knife, stainless steel pot, plastic bowls, heating stove, measuring cylinder, grinder, masking tape and paper foil.

3.3 Preliminary Trials

Seven 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 *Citrus sinensis* (L. Osbeck) leaves were harvested in the morning and were taken immediately to the Laboratory for processing to prevent them from wilting. The leaves were washed thoroughly to remove dirts, sand particles and other contaminants. Then 250g was weighed for each trial respectively for the different samples. For each respective trial, a known volume of water was used.

3.5 Production of Sweet Orange Leaf Protein Concentrate Using Heat Coagulation Method

Fresh sweet orange leaves were harvested in the morning and were taken immediately to the Laboratory for processing as described and illustrated by Pirie (1987). The leaves were thoroughly washed to remove dirt and sand particles. Then the leaves were then processed by grinding to slurry using a generator powered grinder. The slurry of each sample was poured on a sieve cloth and the cloth was wringed thoroughly to separate the juice from the fibrous chaff (Bagasse). The juice was heated and after a few minutes, 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 LPC was taken and the yield, recorded.

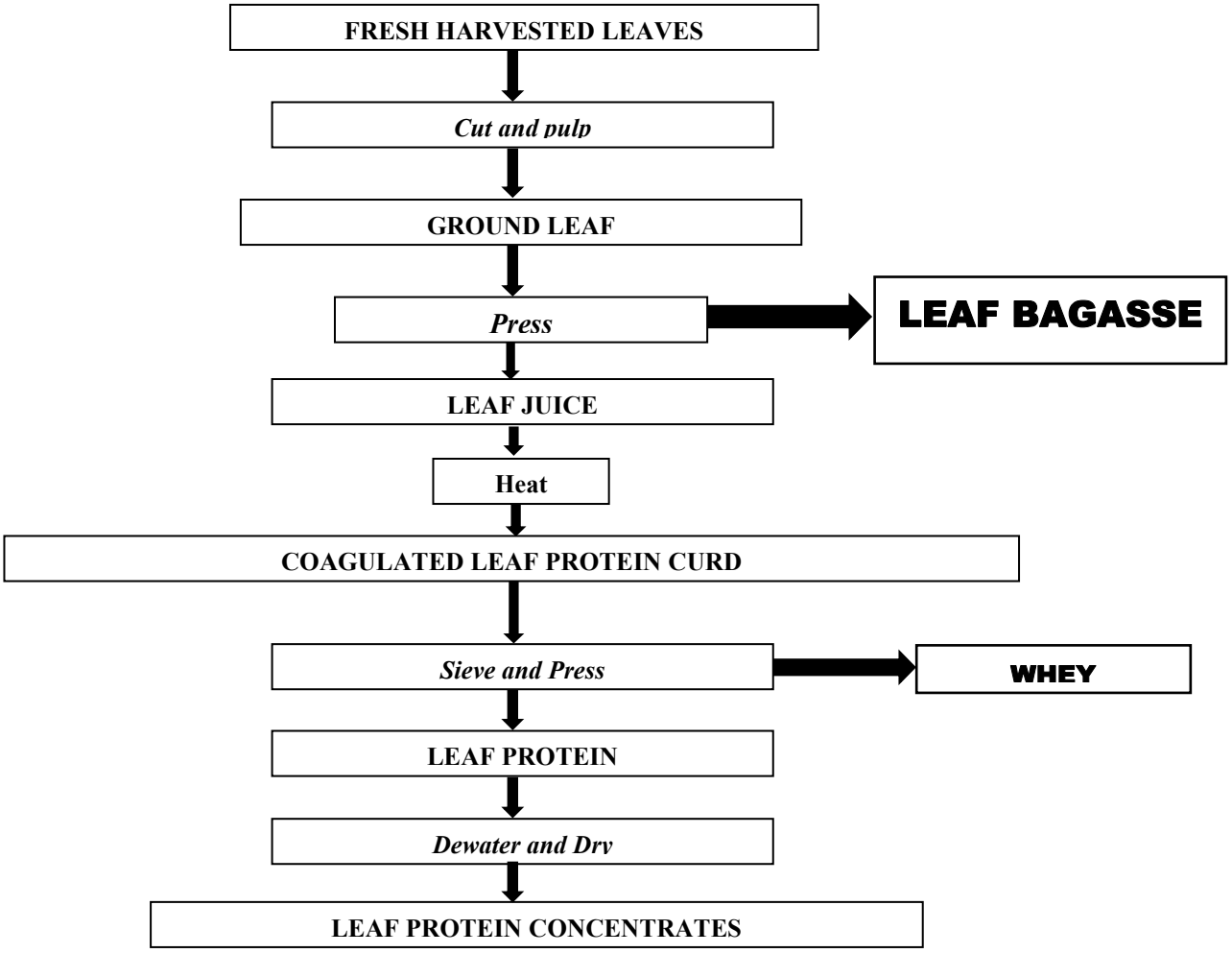

Figure 1: Flow chart of the production of leaf protein concentrates

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

3.6 Production of *Citrus sinensis* (L. Osbeck) 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 200ml of the juice, 4g of alum was added. The curd of LPC precipitated as a result of the coagulation of proteins in juice by alum solution which was then filtered through Whatman filter paper and the sample of LPC taken and the yield was recorded.

3.7 Production of *Citrus sinensis* (L. Osbeck) protein concentrate using acid coagulation method

After the leaves were milled and the bagasse extracted, leaving the juice, 5N H_2SO_4 was added in drops to 50ml of the juice with continuous stirring till the PH value decreased to 3.0. Then the curd of the LPC was precipitated from the juice as a result of the coagulation of proteins by the Acid. The curd was filtered through Whatman filter paper and the LPCs sample weight was recorded and taken for further analysis

3.8 Precautions taken during the production of LPC

1 The leaves were processed immediately after harvest to prevent wilting

2 The LPC in the Whatman filter paper should be scooped out before drying to prevent it from sticking to the filter paper

3 The leaves were cut into smaller bit for easy blending

4 Matured leaves free of physical injury were used

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

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

3.9 Chemical Analysis

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

3.9.1 Moisture Content Determination

Materials used: crucible, weighing balance, oven and desiccators

Procedures

Weigh 2g of the sample into a silica dish that has been previously weighed. Then place 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

3.9.2 Crude Protein Determination

Two grams of the prepared curd and bagasse was weighed and then 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, and then 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

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

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

3.9.3 Crude Fibre Determination

Two grams 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 pyrexheater 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

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

3.9.4 Ash Determination

Two grams 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

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

3.9.5 Ether Extract

Two grams 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:

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

3.9.6 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)

3.10 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.

3.11 Statistical Analysis

Data collected from the study were subjected to analysis of variance using GENSTAT computer statistical package at 5% ($p<0.05$). The means with significant difference were separated using Duncan's multiple range tests (Duncan's, 1955).

3.1.2 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 4

4.0 RESULTS

4.1 Percentage Yields of Sweet Orange LPC and Bagasse Using Heat Coagulation, Acid Coagulation and Alum Precipitation Methods

LPC and bagasse percentage yields are presented in Table 9 below showing their respective yields for heat coagulation, alum precipitation and acid coagulation methods. At $p<0.05$ there were significant differences in the yields of LPC from the respective extraction methods. From the results observed, heat coagulation had the highest yield of LPC ($p<0.05$) at 7.838% followed by alum precipitation at 6.660% and acid coagulation at 2.765%. Inference deducted during the extraction indicated that the heat coagulation method was the most expensive and elaborate process, requiring more materials and equipment for extraction, including heat sources and also demanding constant checks to avoid wastage from fuming.

Table 9: Percentage Yield of Sweet Orange LPC Using Acid Coagulation, Alum Precipitation and Heat Coagulation Methods

Sample	Yield (%)
Acid Coagulation	2.765[a]
Alum Precipitation	6.660[b]
Heat Coagulation	7.838[c]
SEM	0.293

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

4.2 Physical Properties of the Whole Leaf, Bagasse, LPC and Whey of Sweet Orange

Sweet orange leaves are lime green in colour usually having small winglike extensions on their petioles. They were harvested during the dry season in the year and the leaves exhibited dark green coloration prior to washing, with the younger leaves having a little lighter colouration. Their lime green colour was then exposed after washing. The bagasse obtained was lime green in colour, fibrous and retained its colour even after drying. The leaf protein curd obtained after extraction also had a light green colour, was significantly smaller and smoother and less fibrous compared to bagasse. There was no significant variation in colour of LPC extracted from the three methods. The whey was brown to greenish brown in colour and had a very unique and intense fragrance.

Table 10: Some Physical Characteristics of Whole Leaf, LPC, Bagasse and Whey of *Citrus sinensis* (L. Osbeck) Before Drying (Fresh)

Character	Leaf	Bagasse	LPC	Whey
Colour	Lime Green	Lime Green	Lime Green	Greenish brown to brownish
Texture	Smooth	Fibrous	Smooth	Consistent
State	Solid	Solid	Semi solid	Liquid

Table 11: **Some Physical Characteristics of Whole Leaf, Bagasse and LPC of *Citrus sinensis* (L.Osbeck) After Sun Drying (30 – 35^{o}C)**

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

4.3. Proximate Composition of Sweet Orange LPC and Bagasse Using Acid Coagulation, Alum Precipitation and Heat Coagulation Methods

Table 12 shows the proximate composition of sweet orange LPC and bagasse using acid coagulation, alum precipitation and heat coagulation method. There was no significant ($p>0.05$) difference in the dry matter content of sweet orange LPC using acid coagulation (91.70) and alum precipitation (91.79%), but there was significant ($p>0.05$) difference with heat coagulation. For crude protein, acid coagulation (43.41%) and alum precipitation (43.58%) exhibited no significant difference but were both significantly lower than heat coagulation (89.98%). For crude fiber, there was no significant difference between acid coagulation (1.30%), alum precipitation (1.318%) and heat coagulation (1.303%). The ether extract of sweet orange LPC had both acid coagulation (14.47%) and alum precipitation (14.39%) not significantly different, but both were significantly lower than the results from heat coagulation (16.20%).The ash content from both acid coagulation (5.815%) and alum precipitation (6.137%) showed no significant difference, but both were significantly lower than the results form heat coagulation (7.267%). NFE content of acid coagulated (26.70%) and alum precipitated (26.37%) LPC were not significantly different but their values were significantly higher than values from heat coagulation method (16.88%).

Table 12: Proximate Composition of Sweet Orange LPC and Bagasse using Acid Coagulation, Alum Precipitation and Heat Coagulation

Parameters	%DM	%CP	%CF	%EE	%Ash	%NFE
Acid	91.70[b]	43.41[b]	1.30	14.47[b]	5.815[a]	26.70[b]
Alum	91.79[b]	43.58[b]	1.318	14.39[b]	6.137[a]	26.37[b]
Heat	89.98[a]	48.33[c]	1.303	16.20[c]	7.267[b]	16.88[a]
SEM	0.392	0.655	0.0919	0.263	0.365	1.311
Bagasse	90.47[a]	17.48[a]	1.423	4.50[a]	7.762[b]	59.31[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 Sweet Orange LPC and Bagasse Using Alum Precipitation and Heat Coagulation Methods

Table 13 contains compositions of various minerals of sweet orange LPC. Calcium content derived from all three methods, heat coagulation (942.2mg/kg), alum precipitation (614.41mg/kg) and acid coagulation (1242.4 mg/kg) are all significantly different. For magnesium, the results from heat coagulation (178.9 mg/kg), alum precipitation (117.3 mg/kg) and acid coagulation (233.6 mg/kg) are all significantly different from each other. The Sodium content of sweet orange LPC from all three methods shows no significant difference between the other; heat coagulation (24.86 mg/kg), alum precipitation (14.80 mg/kg) and acid coagulation (31.76). Mineral analysis of potassium showed that the potassium content from alum precipitation (1351 mg/kg) and heat coagulation (2148 mg/kg) were not significantly different but these two values were lower than the potassium content from acid coagulation (3526 mg/kg). For phosphorus, there was no significant difference in results from extraction of acid coagulation (307.6 mg/kg), alum precipitation (221.4 mg/kg) and heat coagulation (597.0 mg/kg). For the micro minerals, Iron showed no significant difference between the values from acid coagulation (134.31 mg/kg) and heat coagulation (131.48 mg/kg), but the values were significantly higher than that of alum precipitation (17.41 mg/kg)

Table 13: Mineral Composition of Sweet Orange LPC and Bagasse Using Acid Coagulation, Alum Precipitation and Heat Coagulation Extraction

Methods(mg/Kg)							
Minerals	**Ca**	**Mg**	**Na**	**K**	**P**	**Fe**	**Mn**
Acid	1242.4^{c}	233.6^{c}	31.76^{c}	3526^{b}	307.6	134.31^{b}	48.16^{c}
Alum	614.41^{a}	117.3^{a}	14.80^{a}	1351^{a}	221.4	17.41^{a}	5.18^{b}
Heat	942.2^{b}	178.9^{b}	24.86^{b}	2148^{a}	597.0	131.48^{b}	42.66^{c}
SEM	8.51	1.648	1.072	229.7	202.5	3.57	1.072

Means with same alphabet on the same column are not significantly ($p < 0.05$) different.

SEM= Standard Error Mean

CHAPTER FIVE

5.0 DISCUSSION

5.1 Yield of Sweet Orange LPC and Bagasse Using Three Different Methods

The percentage yield of sweet orange leaf protein concentrate (2.765%) obtained from acid precipitation was significantly lower than the LPC yield of *Pongamia pinnata* (6.63%) as reported by Khan and Varshney (2015). This could have resulted from the relatively fibrous nature of the plant material. Therefore, it is expected that 2.77kg of dry matter of sweet orange LPC will be yielded from 100kg of dry matter of sweet orange leaves via acid coagulation extraction method.

Percentage yield of sweet orange leaf protein concentrates (6.660%) was higher than yield of leaf protein concentrates in *Raphinus sativus* (2.391%) based on the work of Sayyed (2011) and also higher than the yield of leaf protein concentrates of *Gmelina arborea* leaves (3.30%) as reported by Oyesanmi (2019). This high yield indicates a relatively greater protein supply for animal feed from the use of leaf protein concentrates derived from acid precipitation of sweet orange

Percentage yield of sweet orange leaf protein concentrates derived from heat coagulation (7.838%) was higher than 5.6% leaf protein concentrate from rubber leaf as reported by Akaeze *et al.,* (2015) and Isuosuo (2014) and lower than LPC yields of Pawpaw (7.12%) as reported by Agbonghae and Nwaokoro (2019).

The percentage yield of bagasse obtained (28.30%) was higher than the recorded bagasse yield of pawpaw leaf as reported by Agbonghae (2016).

5.2 Chemical Composition of Sweet Orange LPC and bagasse Using Three Different Methods

5.2.1 Proximate Composition

From the results of the proximate analysis of sweet orange LPC and bagasse using three different methods, the crude fibre mass could be affected by certain external factors like the apparatuses; sieves and Whatman filter paper which would contribute their content and contaminate the LPC and bagasse during the process of sun drying. Crude fibre was found to have compositions of 1.3% from acid coagulation, 1.318% from alum precipitation and 1.303% from heat coagulation and were all lower 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 43.41% from acid coagulation, 43.58% from alum precipitation and 48.33% from heat coagulation, all values which 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 in the LPC of *Amaranthus hybridus* (34.8%) from the works of Adeyeye and Omolayo (2011). The

crude protein content obtained from the bagasse (48.33%) was found to be lower than 28.64% bagasse of *Musa sapientum* reported by Udegbe (2007).

The ash content of heat coagulated sweet orange LPC (7.267%) and those from alum precipitation (6.137%) and acid coagulation (5.815%) were found to be lower than ash content of LPC of *Amaranthus hybridus* (17.2%) as reported by Adeyeye and Omolayo (2011). Ash content analysed from sweet orange leaf bagasse (7.762%) was higher than *Musa paradisica* bagasse (3.0%) from works of Udegbe (2007). The ash content of both the LPC and the bagasse 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.

Ether extract composition of sweet orange LPC obtained from acid coagulation (14.47%), alum precipitation (14.39%) and heat coagulation (16.20%) were found to be higher than 9.6% in *Amaranthus hybridus,* from reports from Adeyeye and Omolayo (2011). From bagasse, the ether extract was found to have (4.50%) composition

The NFE composition of LPC from acid coagulation (26.70%), alum precipitation (26.37%) and heat coagulation (16.88%) were found to be higher than the composition from alum precipitation of *Vernonia amygdalina* LPC (23.58%) and lower than the composition from heat coagulation of *Vernonia amygdalina* LPC (24.95%) as analysed by Sodamade *et al.* (2013)

5.2.2 Mineral Composition

With results from Table above, calcium (1242.4 mg/kg) and potassium (3526 mg/kg) from acid coagulation had the highest composition. Calcium (942.2 mg/kg) composition from LPC extracted via heat coagulation was higher than calcium derived from heat coagulated LPC of *Caesalpinia pulcherrima* (817.23 mg/kg) while the magnesium content of sweet orange LPC (178.9 mg/kg) was lower compared to *Caesalpinia pulcherrima* (554.54 mg/kg) as reported by Nwokoro *et al.* (2022). Calcium and magnesium are the chief macro elements that are required for bone building and formation as well as in clotting of blood and other essential physiological processes.

The potassium content 2148 mg/kg from sweet orange LPC via heat coagulation was higher than the potassium content (269.8 mg/kg) from LPC of *Caesalpinia pulcherrima* and also the phosphorus content of heat coagulated sweet orange LPC (597.0.0 mg/kg) is lower than the phosphorus content from heat coagulated LPC and potassium (1387.0 mg/kg) from the work of Nwokoro *et al.,* (2022). From alum precipitation, the LPC potassium content, 1351 mg/kg was higher than potassium (116mg/kg), while the phosphorus content (221.4 mg/kg) was lower than the LPC phosphorus content (457mg/kg) both of *Amaranthus hybridus* reported by Adeyeye and Omolayo (2011).

The sodium composition in sweet orange LPC from heat coagulation and alum precipitation method, 14.80 mg/Kg and 24.86 mg/Kg respectively were both lower than

312mg/kg of sodium in *Telfilaria occidentalis* LPC as reported by Adeyeye and Omolayo (2011).

Microminerals, iron and manganese were analysed and the iron (131.48 mg/kg) and manganese (42.66 mg/kg) composition of the sweet orange LPC form heat coagulation methods were higher than the LPC iron content (88.5 mg/kg) and manganese content (18.0 mg/kg) of *Caesalpinia pulcherrima* LPC reported by Nwokoro *et al.* (2022).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

This research work was undertaken in three phases: In the first phase, the yield of sweet orange leaf protein concentrates and bagasse was determined with heat coagulation method, then followed by the determination of their proximate and mineral composition. The second phase involved the application of alum precipitation method for extraction and analysis leaf protein concentrate and bagasse of sweet orange followed by the determination of their proximate and mineral composition. In the third phase the acid coagulation method was applied to determine the yield of sweet leaf protein concentrate and bagasse, then followed by determination of their proximate and mineral composition.

The resultant yields of sweet orange leaf protein concentrate obtained from the three methods are 7.838% from heat coagulation, 6.660% form alum precipitation and 2.765% from acid coagulation method. This means that for every 100kg of dry matter (100%), sweet orange leaf protein concentrate will produce 7.9kg, 6.7kg and 2.8kg form heat coagulation, alum precipitation and acid coagulation respectively. The CP and EE content of the LPC obtained from heat coagulation method yielded the highest values, while the CP and EE obtained from bagasse was lower than their contents in LPC obtained from all three extraction methods. Ash and NFE content from the bagasse was found to be

relatively higher than the Ash and NFE content from the LPC obtained from all the extraction methods used.

Calcium, magnesium, potassium and sodium of the macro minerals analysed yielded the highest in the LPC obtained from acid coagulation method. Phosphorus yielded the highest from the LPC obtained via heat coagulation.

Of the micro minerals, Iron and manganese were deducted to have yielded the highest the LPC obtained via acid coagulation method.

From the results, sweet orange leaf protein concentrates obtained using acid coagulation and alum precipitation possess quite similar proximate compositions, but the heat coagulation method yields the highest CP and EE content amongst all four comparisons and from the mineral composition, acid coagulation method has the highest mineral yield amongst the four variables of LPC and bagasse compared.

6.2 Conclusion

Results from the study show that the yields of sweet orange LPC from all three extraction methods are quite comparable, ranging between 43.41% and 48.33% CP and their percentage CP composition is relatively similar to more convenient high crude protein sources like Full fat soya bean (38% CP) and Groundnut cake (48% CP). In this case, heat coagulation method will be preferred for extraction as it yields the highest %CP

content (48.33% CP), but if more emphasis is on the mineral content, then the acid coagulation method will produce the best yield of minerals in the LPC.

Bagasse yield was also very high, and this can be used effectively as feed for ruminants. The bagasse can also be ensiled so as to make feed available in the dry season, when there is scarce vegetation to feed on.

6.3 Recommendation

There should be investments into more research methodologies that will aid to improve the yield and nutritional values of leaf protein concentrates with very little alterations on its chemical composition. I will also encourage the use of sweet orange leaf protein concentrates in diet formulation, due to its high crude protein yield.

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APPENDIX

APPENDIX I

Analysis of variance

Variate: DM

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	3	14.6858	4.8953	5.31	0.007
Residual	20	18.4447	0.9222		
Total	23	33.1305			

APPENDIX II

Analysis of variance

Variate: ASH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	3	15.2443	5.0814	6.36	0.003
Residual	20	15.9741	0.7987		
Total	23	31.2184			

APPENDIX III

Analysis of variance

Variate: FAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	3	510.3316	170.1105	410.36	<.001
Residual	20	8.2907	0.4145		
Total	23	518.6223			

APPENDIX IV

Analysis of variance

Variate: FIBER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	3	0.06181	0.02060	0.41	0.750
Residual	20	1.01435	0.05072		
Total	23	1.07616			

APPENDIX V

Analysis of variance

Variate: PROTEIN

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	3	3528.987	1176.329	456.37	<.001
Residual	20	51.552	2.578		
Total	23	3580.539			

APPENDIX VI

Analysis of variance

Variate: NFE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	3	6202.82	2067.61	200.54	<.001
Residual	20	206.21	10.31		
Total	23	6409.03			

APPENDIX VII

Analysis of variance

Variate: Ca

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	2	1184993.2	592496.6	1363.64	<.001
Residual	15	6517.4	434.5		
Total	17	1191510.7			

APPENDIX VIII

Analysis of variance

Variate: Mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	2	40624.95	20312.48	1245.81	<.001
Residual	15	244.57	16.30		
Total	17	40869.52			

APPENDIX IX

Analysis of variance

Variate: Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	2	872.847	436.424	63.26	<.001
Residual	15	103.482	6.899		
Total	17	976.329			

APPENDIX X

Analysis of variance

Variate: K

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	2	14531506.	7265753.	22.96	<.001
Residual	15	4747278.	316485.		
Total	17	19278784.			

APPENDIX XI

Analysis of variance

Variate: P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	2	464333.	232167.	0.94	0.411
Residual	15	3690897.	246060.		
Total	17	4155231.			

APPENDIX XII

Analysis of variance

Variate: Fe

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	2	53373.45	26686.72	349.46	<.001
Residual	15	1145.49	76.37		
Total	17	54518.94			

APPENDIX XIII

Analysis of variance

Variate: Mn

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	2	6564.775	3282.387	476.18	<.001
Residual	15	103.397	6.893		
Total	17	6668.172			

APPENDIX XIV

Analysis of variance

Variate: YIELD%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
LPC	2	84.5964	42.2982	82.30	<.001
Residual	15	7.7094	0.5140		
Total	17	92.3058			