PROXIMATE COMPOSITION AND AMINO ACID PROFILE OF SCENT LEAF (*Ocimum gratissimum L.*) PROTEIN CONCENTRATE

BY

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BENIN CITY, NIGERIA

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY; NIGERIA
IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR BACHELOR OF AGRICULTURE, DEGREE IN ANIMAL SCIENCE (BAGANS) OF THE UNIVERSITY OF BENIN, BENIN, BENIN CITY, NIGERIA

OCTOBER, 2023

CERTIFICATION

This is to certify that this project work was carried out by Jeffrey Efe GIDIAGBA with matriculation number AGR1700105 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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Prof. S.O. Nwokoro Date
Project supervisor

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Dr. O. W. Agbonghae Date
Co-supervisor

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Prof. J. A. Imaseun Date
Head of Department

DEDICATION

I want to dedicate this project work to everybody out there that is trying or struggling to make a positive impact in this world.

ACKNOWLEDGEMENT

I want to express my profound gratitude to God The Father Almighty for His overwhelming love, guidance and protection throughout my stay in the University of Benin. I am also really grateful to my project supervisor, Prof. S.O. Nwokoro, my co project supervisor, Dr Wisdom Agbonghae, the Dean of the Faculty of Agriculture, Prof. E.R. Orhue, my amiable HOD, Prof J.A. Imasuen, and all the lecturers and staff that made it possible for me to complete this lap of my academic pursuit. And certainly I wish to express my deepest gratitude to my family, especially my parents, Mr. and Mrs. Gidiagba and my siblings. I also really want to appreciate and acknowledge every single person who has ever assisted me in this life, and anyone out there who has ever assisted someone else.

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ABSTRACT

This research work was conducted to evaluate the proximate composition and amino acid profile of scent leaf protein concentrate and scent leaf meal. Wet milling method was used for the extraction of the scent leaf protein concentrate. A grinder was used in the processing of the scent leaf meal. The amino acid profile of the scent leaf was determined using high performance liquid chromatography (HPLC). The Proximate analysis was carried out in triplicate with different procedures for moisture content determination, crude protein determination, crude fibre determination, ash determination, ether extract determination, and nitrogen free extract determination. From the result of the proximate analysis, the scent leaf meal contained a significant portion of crude protein, but the crude protein content of the scent leaf protein concentrate was significantly higher ($p<0.05$). It was also observed that the crude fibre content of scent leaf meal was significantly higher ($p<0.05$) than that of scent leaf protein concentrate. It was also observed that there is more crude fat in scent leaf protein concentrate when compared to scent leaf meal ($p<0.05$). The scent leaf protein concentrate also has more ash content when compared to the scent leaf meal. From the result of the amino acid profile, it was observed that both the scent leaf protein concentrate and the scent leaf meal have 18 amino acids, including essential amino acids, with scent leaf protein concentrate being higher in lysine and methionine than that of scent leaf meal. This study shows that scent leaf meal and scent leaf protein concentrate can be incorporated into animal and human diet.

CHAPTER ONE

1.0 INTRODUCTION

Amino acids are any of a group of organic molecules that consist of a basic amino group (—NH2), an acidic carboxyl group (—COOH), and an organic R group (or side chain) that is unique to each amino acid (Michael, 2023). The term amino acid is short for α-amino [alpha-amino] carboxylic acid. Each molecule contains a central carbon (C) atom, called the α-carbon, to which both an amino and a carboxyl group are attached. The remaining two bonds of the α-carbon atom are generally satisfied by a hydrogen (H) atom and the R group. There are three classes of amino acids; essential amino acids, non essential amino acids and conditionally essential amino acids (www.medlineplus.gov (2023)). Essential amino acids are amino acids that cannot be synthesized by the body of a human or an animal, as a result, they must come from food / feed. The 9 essential amino acids are: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Nonessential amino acids are amino acids that can be synthesized by the body system of a human or an animal. Nonessential amino acids include: alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine. Conditionally essential amino acids are amino acids that are usually not essential, except in times of illness and stress. Conditionally essential amino acids include: arginine, cysteine, glutamine, tyrosine, glycine, proline, and serine. Amino acids are the building blocks of protein (Jillian, 2023), and protein is an Integral fraction of the ingredients used in feed formulation for livestock. It is important to note

that feed Ingredients account for 75% of the total cost of production of livestock (Nwokoro *et al.*, 2022).

To maximize food production and meet protein requirements in Nigeria, alternative options need to be explored and evaluated (Jiya *et al.*, 2013). Alternative protein sources for animal nutrition refer to non-traditional sources of protein that can be used to replace or supplement conventional protein sources like soybean meal, fishmeal, and meat and bone meal (Dawn, 2023). Alternative protein sources can offer various benefits for animal feed, such as reducing the environmental impact, improving the nutritional quality, diversifying the feed ingredients, and lowering the feed costs (Dawn (2023), Ana (2020). Examples of alternative protein sources include insects, yeast derivatives, single-celled bacteria protein, pea protein, algae, microalgae, seaweed, duckweed, distiller's grain, rice bran, wheat bran, hydrolysed feather meal, spirulina, mushrooms, leaf meal, leaf protein concentrates (LPC), e.t.c. (feedandadditive.com (2023), feedstradegy.com (2020), Animal Feed Science and Technology (volume 268, 2020).

1.1 JUSTIFICATION

Leaf protein concentrate (LPC) is an extremely nutritious food product made by mechanically separating (through a process of juicing, boiling, and drying leaves) indigestible fiber and soluble anti-nutrients from certain fresh green plant leaves, and as evidenced by its name, LPC contains extraordinary levels of high-quality protein (Catherine Webb, 2020). On a dry weight basis, which describes the percentage of a

nutrient in a substance after removing the moisture, LPC is roughly 50-65% protein with significant amounts of calcium, iron, and vitamin E, as well as other vitamins and minerals (Catherine , 2020). It is a good source of folic acid (vitamin B9, Ascorbic acid) (Lliyas and Badar, 2010). It is rich in iron, beta carotene and high quality protein (Kennedy, 1993). Surprisingly, LPC has a higher dry weight of protein than beef (Catherine , 2020). Leaf protein has been assessed for human or animal food sources because it is potentially the cheapest source of available protein (Tripathi, 2011). Leaf protein is the most abundant source of protein by volume, not only is it a sustainable source of plant protein, it can also aid in the regeneration of degraded farmland and help reverse climate change (The Leaf Protein Co). Among the unconventional sources of protein, leaf protein concentrate appears to have better potential in light of excessive photosynthesis and its abundant availability as green vegetation (Nisha *et al*., 2014). It is very useful to monogastric animals because some of the anti -nutrients like nitrates, hydrocyanic and free oxalic acid that limit the usefulness of many leafy crops in the human diet are almost completely removed when the leaves are converted to leaf protein concentrate (Kennedy, 1993). It was reported by Akaeze *et al.* (2015) that rubber leaf protein concentrate can be used to replace soybean meal in the diets of rabbits as an unconventional protein source. It was reported by Agbonghae and Nwokoro (2023) that pawpaw leaf protein concentrate is an alternative source of protein in rabbit feeding. Other examples of leaves that can be used as sources of protein concentrates include; radish leaf, cauliflower, broccoli, cabbage, beetroot leaf, nettle leaf, clover, legumes,

scent leaf, e.t. c. However the quality of the protein of many of these leaves that can be used as sources of protein concentrate has not been known.

1.2 OBJECTIVE OF STUDY

The broad objective of this study was to determine the proximate composition and amino acid profile of scent leaf meal and scent leaf protein concentrate.

The specific objectives were to:

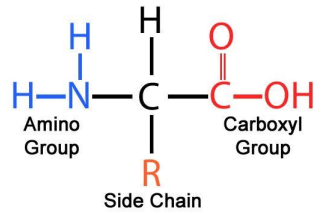
1. Determine the proximate composition of scent leaf meal and scent leaf protein concentrate.
2. Determine the amino acid profile of scent leaf meal and scent leaf protein concentrate.

CHAPTER TWO

2.0 LITERATURE REVIEW

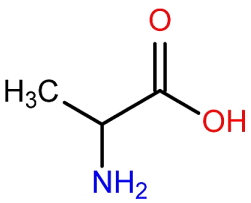
2.1 CHARACTERISTICS OF AMINO ACIDS

The amino acid has a structure:



SOURCE: www.astrochem.org

The amino acid has a chemical formula:



SOURCE: www.thoughtco.com

Amino acids are organic molecules that have both an amino group (-NH2) and a carboxylic acid group (-COOH) attached to a central carbon atom (called the alpha-carbon) (Michael, 2023). They are the building blocks of protein and peptides. There are

20 common amino acids found in proteins, and each one has a unique side chain (called the R group) that determines its chemical and physical properties (chem.libretexts.org). Most amino acids are chiral, meaning they exist in two mirror-image forms (L and D enantiomers), but in biological proteins, only L-amino acids are commonly found (Garrett, and Grisham. (2016)) .Amino acids can be classified into four groups based on the characteristics of their side chains: nonpolar, polar, acidic, or basic (chem.libretexts.org). Some examples of amino acids are glycine, which has the simplest side chain (a hydrogen atom), and threonine, which has a polar hydroxyl group on its side chain (chem.libretexts.org).

Some other characteristics of amino acids are:

- They are colorless, crystalline solids that melt and decompose at high temperatures (above 200°C).

- They are soluble in water and insoluble in organic solvents.

- They exist as zwitterions in neutral solutions, which means they have both a positive and a negative charge on different atoms.

- They can form peptide bonds with other amino acids by linking their carboxyl group with the amino group of another amino acid, releasing a molecule of water.

- They have different optical activities, which means they can rotate the plane of polarized light in different directions. Most amino acids found in proteins are L-amino acids, which have the same configuration as L-glyceraldehyde34.

- Amino acids play vital roles in metabolism, including serving as precursors for neurotransmitters, hormones, and other important molecules.

SOURCES: Gaura (2018), Devlin (2010), www.sigmaaldrich.com

2.1.2 IMPORTANCE OF AMINO ACIDS

Amino acids play a crucial role in various biological processes and are of significant importance in human and animal physiology. Some of the Importance of amino acids include :

1. Protein Synthesis: Amino acids are the building blocks of proteins. The sequence of amino acids in a protein is determined by the genetic code (Nelson and Cox, 2008)

2. Growth and Tissue Repair: Amino acids are essential for growth, development, and tissue repair in organisms (Berge *et al*. 2019).

3. Enzyme Function: Amino acids can serve as cofactors or coenzymes for enzymes, facilitating biochemical reactions (Voet, and Pratt, 2016)

4. Neurotransmitters: Amino acids like glutamate and gamma-aminobutyric acid (GABA) act as neurotransmitters in the nervous system, enabling communication between nerve cells (Siegel, Albers, and Brady (2011))

5. Hormone Production: Some amino acids are precursors for the synthesis of hormones, such as thyroxine and insulin.
Reference: Devlin (2010)

6. Immune Function: Amino acids are necessary for the production of antibodies and immune system components, aiding in the body's defense against pathogens. (Calder, Yaqoob (2007))

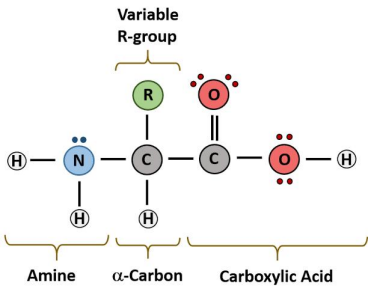
7. Energy Production: Amino acids can be metabolized to produce energy when the body's energy needs are not met by carbohydrates or fats (Murray, Bender, Botham (2012))

8. Nutritional Balance: Balanced intake of essential amino acids is crucial for maintaining good health, and amino acid deficiencies can lead to various health issues (World Health Organization (WHO). (2007))

9. Muscle Maintenance: Amino acids, particularly branched-chain amino acids (BCAAs), are important for muscle maintenance, repair, and growth (Blomstrand, Eliasson, Karlsson, and Köhnke (2006))

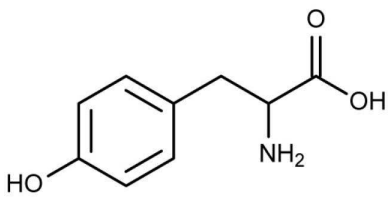
2.2 CHARACTERISTICS OF PROTEIN

The structure of protein is:



SOURCE: https://wou.edu/chemistry/courses/online-chemistry-textbooks/ch450-and-ch451-biochemistry-defining-life-at-the-molecular-level/chapter-2-protein-structure/

The chemical formula of protein is:



SOURCE: www.socratic.org

Proteins have a diverse range of structures, from simple linear chains to complex three-dimensional shapes (Alberts, Johnson, Lewis *et al.,* 2002. Proteins are composed of amino acids linked together by peptide bonds. The sequence of amino acids determines the protein's structure and function (Berg, Tymoczko, and Stryer (2002)). Proteins serve diverse functions, including enzymes that catalyze biochemical reactions, structural components, transporters, receptors, and signaling molecules (Voet, Voet and Pratt (2016)). Proteins exhibit specificity in their interactions with other molecules, recognizing specific substrates or ligands (Nelson,and Cox (2008)). Proteins can undergo denaturation, where their native structure is disrupted, leading to loss of function. This can be caused by factors such as heat, pH, or chemicals (Lehninger, Nelson, & Cox, (2007)). Proteins play a central role in the regulation of various biological processes, including gene expression, cell signaling, and metabolism (Lodish, Berk, Zipursky. *et al.* 2000)). Many proteins are enzymes that catalyze specific chemical reactions, facilitating the conversion of substrates into products (Stryer 1988)). Proteins fold into specific three-dimensional structures through a process driven by interactions between amino acid side chains (Branden and Tooze 1999)). Protein solubility varies depending on the

amino acid composition and environmental conditions such as pH and salt concentration (Creighton 2010)). Proteins, such as hemoglobin and ferritin, are involved in the transport and storage of essential molecules like oxygen and iron (Devlin (2010)).

Some other characteristics of proteins are:

- The solubility of proteins depends on their amino acid composition, pH, and ionic strength of the solution (Creighton 2010)

- Proteins vary in molecular weight, ranging from small peptides to large macromolecule (Alberts, Johnson and Lewis *et al.* 2002) .

- The isoelectric point is the pH at which a protein has no net charge and is least soluble. It depends on the distribution of acidic and basic amino acids (Voet, Voet, and Pratt. (2016)).

- The temperature at which a protein denatures (loses its native structure) depends on its stability and can vary widely among proteins (Lehninger, Nelson, and Cox 2007) .

- Proteins are optically active due to the chiral nature of amino acids, which can rotate plane-polarized light (Berg, Tymoczko, and Stryer 2002)).

- The presence of proteins in a solution can affect its viscosity, particularly at high protein concentrations (Voet, Voet, and Pratt 2016) .

- Proteins can exhibit characteristic absorption spectra in the ultraviolet (UV) and visible regions due to the presence of aromatic amino acids like tryptophan and tyrosine (Branden, and Tooze 1999) .

2.2.1 IMPORTANCE OF PROTEINS

Proteins are essential for life in humans and animals. They have many important functions such as:

1. Growth and maintenance of tissues: The body system of humans and animals needs protein to repair and build new cells. Protein also helps in wound healing, muscle strength, bone health, and hair and skin quality.

2. Biochemical reactions: Proteins act as enzymes that speed up or regulate chemical processes in your body. Enzymes are involved in digestion, energy production, blood clotting, muscle contraction, and many other functions.

3. Messenger molecules: Proteins act as hormones that communicate signals between cells, tissues, and organs. Hormones control your growth, development, metabolism, and reproduction.

4. Transport and storage of molecules: Proteins carry and store substances in blood and cells. For example, heamoglobin is a protein that transports oxygen in blood. Ferritin is a protein that stores iron in the liver.

5. Immune system defense: Proteins act as antibodies that fight off infections and diseases. Antibodies recognize and bind to foreign invaders, such as bacteria and viruses, and mark them for destruction by other immune cells.

6. Energy source: Proteins can provide energy for your body when carbohydrates and fats are not available. However, this is not the main role of proteins and should be avoided as much as possible, as it can lead to muscle loss and other problems.

SOURCES: Gavin (2023), Harvard T.H Chan (School of Public Health).

2.3 CHARACTERISTICS OF SCENT LEAF PLANT

The aromatic perennial shrub Ocimum gratissimum has been widely distributed throughout the world's tropical and subtropical climates. It has escaped cultivation and can be found growing as a weed in distributed sites, waste areas, pasture and along roadsides. In this species seeds are small, numerous and are easily dispersed by gravity, animals, human activities and as a contaminant in soil and garden debris. Scent leaves go by many names: O gratissimum. in Latin, scent leaf, clove basil or tea bush in Nigeria English, and a host of other names in various dialects. The Yorubas call it effirin, the Igbos call it Ncho-anwu, the Hausas call it daidoya. It is also known as tree basil. Probably as a result of its woody stem (Abdullahi *et al.*, 2003).

2.3.1 DESCRIPTION OF THE PLANT

It is a herbaceous perennial shrub (or subshrub), 1 - 3 m tall, with an erect stem, and it is woody at the base. It has opposing leaves 2 - 4.5 cm petiole long (PROSEA, 2018). The leaves are broad and narrowly ovate, usually 5 - 13 cm long and 3 - 9 cm wide. It is a fragrant shrub with lime green leaves (USDA, 2008). A typical scent leaf has a curvy peak with serrated / wavy edges. The leaf itself is not smooth, both back and front. The older larger leaves are hardy and woody in flavor compared with the fresh, young and smaller ones. The flowers are fragrant with white to greenish-yellow spikes (Obidike, 2021).

2.3.2 TAXONOMY OF Ocimum gratissimum

Domain: *Eukaryota*

Kingdom: *Plantae*

Phylum: *Spermatophyta*

Sub-phylum: *Angiospermae*

Class: *Dicotyledonae*

Order: *Lamiales*

Family: *Lamiaceae*

Genus: *Ocimum*

Specie: *gratissimum*

Source : CABI (2021)

2.3.3 LEAF PROTEIN CONCENTRATE (LPC)

Leaf Protein Concentrate is an extremely nutritious foodstuff made by mechanically separating indigestible fibres and soluble anti nutrients from much of the protein, vitamins, and minerals in certain fresh green plant leaves. Because it is rich in beta carotene, iron and high quality protein, leaf protein concentrate is very effective in combating malnutrition, especially anaemia and vitamin A deficiency which are prevalent among children and pregnant women in most developing countries. 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).

2.3.4 ADVANTAGES OF LEAF CONCENTRATE

1. Leaf concentrate is an extremely nutritious food. It is richer in iron and vitamin A than any other commonly available food.

2. It is an effective way of using land to produce food, yielding roughly three times as much protein per hectare as grain crops, and five to ten times as much per hectare as animal raising.

3. Leaf concentrate is relatively easy to make. People with little training or education can make it in rural villages.

4. 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).

2.3.5. DISADVANTAGES OF LEAF CONCENTRATE

1. Good leaf yields require a steady supply of water. In many locations there are long dry seasons and irrigated land is at a premium.

2. Most people are not accustomed to eating many dark green foods.

3. Fresh leaves are very perishable. They must be processed soon after they are harvested or the quality and yield of the leaf concentrate goes down.

4. 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.

5. The Vitamin C in fresh leaves is lost during processing (Kennedy, 1993).

2.3.6 NUTRITIONAL VALUE OF LEAF CONCENTRATE

Leaf concentrate has been considered to be a very good source of protein. It has a high protein content (24%) when compared to other food sources; beef (17.4%), Chicken (22.5 %), Milk (3.5%), Eggs (12. 9%) and beans (8.1%). Leaf concentrate also has other nutrients such as lipids (20 - 25%), starch (5 - 10%), minerals (including iron, magnesium, and calcium), and vitamins (including vitamin A, Vitamin E, Niacin and folic acid)

SOURCE: FAO / WHO (1985)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 LOCATION AND DURATION OF THE STUDY

The experiment was carried out in the main laboratory of the faculty of Agriculture, University of Benin, Ugbowo campus, Benin city, Nigeria. University of Benin is located between latitude 6°30°N of the equator and longitude 5°40° and 6°E of the Greenwich Meridian in the forest zone with an average temperature of 27.6°C (NAA 2014), Google Earth (2023) .

3.2 EXPERIMENTAL MATERIALS

The materials used for the experiments were freshly harvested scent leaves, thermometer, weighing balance, plastic bowls, spoons, stainless steel, pot, gas measuring cylinder, grinding machine, paper foil, sieve cloth, e.t.c.

3.3 PRODUCTION OF SCENT LEAF PROTEIN CONCENTRATE USING WET MILLING METHOD

Freshly harvested scent leaf was washed to remove dirt and insect attached to the leaves. Thereafter the leaves were weighed and milled using a known volume of water to obtain scent leaf slurry. The scent leaf slurry was then sieved to separate the bagasse from the leaf juice. The scent leaf protein concentrate was extracted from the juice using heat coagulation method following the procedure of Sayyed (2011). The

leaf protein concentrate and bagasse obtained were sun-dried to remove moisture. The dried sample was observed for physical properties, and kept in an airtight container and then used for chemical analysis.

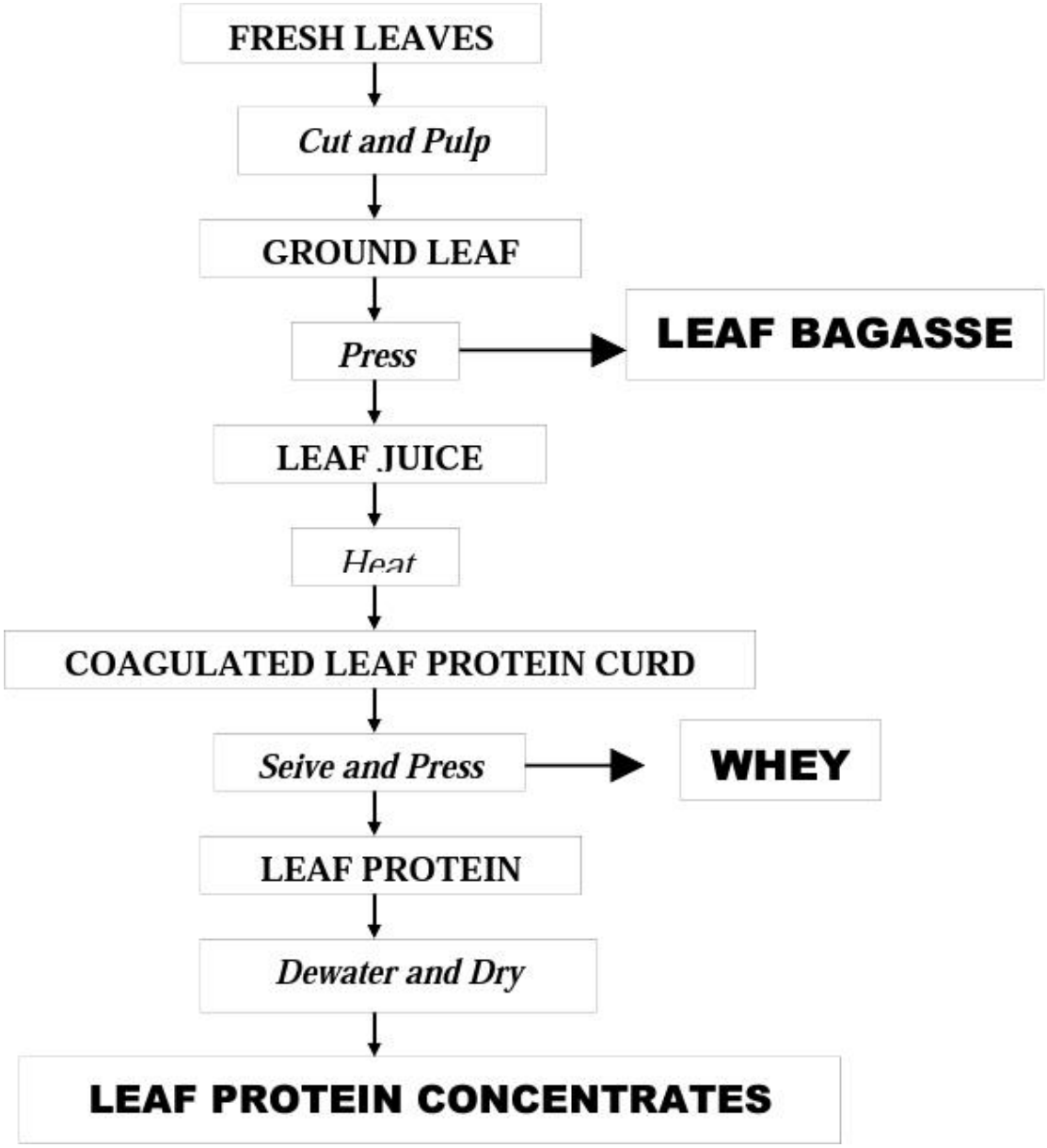


Figure 3.1: Flow chart of the production of leaf protein concentrates using wet milling method.

SOURCE: Pirie (1987), as modified by Nwokoro (2015)

3.4 PRECAUTION TAKEN DURING THE PRODUCTION OF LEAF PROTEIN CONCENTRATE (LPC) the

1. The sieve cloth used had very small pores in order not to allow the fibres to pass through.

2. The LPC should be air dried rather than oven dried.

3. The material used for the LPC experiment was washed before and after use.

4. Matured and fresh leaves were used for the experiment.

5. The leaves were processed immediately after harvesting to prevent wilting.

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

3.5 CHEMICAL ANALYSIS

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

3.5.1 Proximate Analysis

3.5.1.1 Moisture content determination

Materials used: Weighing balance / Sensitive scale, oven and dessicator.

Procedure:

2g of the sample was measured into a silica dish that had been previously weighed. Then it was placed in an oven at 100°c for 24 hours, and then to constant weight. The sample

was cooled in a desiccator before it was weighed again, the weighing was continued until a constant weight was obtained. Then calculations were made.

Calculations

1. Weight of moisture= weight of crucible sample - weight of Crucible and sample after drying.

2. %Moisture = weight of Moisture / weight of sample x 100/1.

3. Dry matter= 100 - %moisture.

3.5.1.2 Crude Protein (CP) Determination

2g of prepared LPC and bagasse were weighed and transferred into clean digestion flasks, the digestion mixture; copper, selenium or mercury catalyst plus potassium or sodium sulphate was then added to raise the boiling point 30ml of concentrated sulphuric acid was added to the digestion flask containing the other

mixture and the sample digested for 2 hours. The flask was cooled then diluted with water and was made to 100ml in a volumetric flask. 20 ml of 2% boric acid plus indicator was pipetted into a 100 ml Erlenmeyer flask. The 100 ml 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 .10 ml aliquots of the samples were then pipetted into the distillation unit and 100 ml of 40% NaOH was added. The samples were distilled with standard HCI

(0.01N) until the blue colour disappeared. A blank determination was first carried out and the crude protein value was determined using the following formulae.

Calculations

%N of sample = Net volume of acid x concentration of acid x 14 x 100 x 10 / weight of sample.

% crude protein = Net vol. of acid x 14 x 100 x 10 x 6.25 / weight of sample.

3.5.1.3: Crude Fibre (CF) Determination

2g of the prepared LPC and bagasse were 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 free of acid. Also NaOH (sodium hydroxide) solution which had been previously boiled was added, filtered while hot using a Whatman filter paper and the residue was allowed to drain and transferred to a pyrex heater and dried, weighed and ashed. The residue was cooled in the desiccator and weighed after 1 hour. The loss in weight due to ashing was calculated as the crude fibre content.

Calculations

Sample size = A (2g)

Weight before ashing = P

Weight after ashing = Z

% Crude fibre = P - Z / A x 100 / 1

3.5.1.4 Ash Determination

2g of the prepared LPC and bagasse were weighed and put in a weighed crucible and was ignited at 550°C for 6 hours in the furnace for ashing. Then the samples were removed and allowed to cool in a desiccator for about 30 minutes then re-weighed and the value was calculated.

Calculations

Sample weight = A

Sample weight before ashing = P

Sample weight after ashing = Z

% Ash = P-Z / A x I00 / 1

3.5.1.5. Ether Extract (EE) Determination

2g of the prepared LPC and bagasse were weighed into a fat free extraction tumble. It was then corked tightly with cotton and placed in the extractor, petroleum

ether was then 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 samples 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 tumble removed and dried. The flask was detached, while the samples were removed and dried in an oven to constant weight.

Calculation

%EE = Initial weight - final weight / weight of sample x 100/1.

3.5.1.6 Nitrogen Free Extract (NFE) Determination

This is determined from the subtraction of the addition of % ash, ether extract, crude protein and crude fibre from 100. The difference is the Nitrogen Free Extract.

Calculation

NFE = 100 (%CP + %EE + %CF + %Ash)

3.6 AMINO ACID PROFILE ANALYSIS

The amino acid profiles of the scent leaf was determined using the method of Benitez LV. The scent leaves were dried to constant weight and defatted using chloroform and methanol of ratio 2:1.They were evaporated in a rotary evaporator and loaded into the Applied Bio-Systems, PTH Amino Acid Analyzer. About 300mg of the leaves were put in an extraction thimble and extracted for 15 hours in a Soxhlet extraction apparatus. The tryptophan in the samples was hydrolyze

using 4.2 N sodium hydroxide.

3.7 STATISTICAL ANALYSIS

Data obtained from the proximate and amino acid analysis were subjected to analysis of variance using the GENSTAT 12th edition for Windows Package at 5% ($p<0.05$). The means with significant difference were separated using

the same Statistical Package.

CHAPTER FOUR

4.0 RESULT

4.1 PROXIMATE COMPOSITION OF SCENT LEAF PROTEIN CONCENTRATE AND SCENT LEAF MEAL.

The Proximate composition of Scent Leaf Protein Concentrate and Scent Leaf Meal is presented in Table 4.1.

The result showed that the Dry Matter (DM) content in Scent Leaf Protein Concentrate (86.80%) is significantly lower than that of Scent Leaf Meal (88.41%), which is due to the fact that the bagasses which contain much dry matter have been extracted from the Scent Leaf Protein Concentrate, while that of the Scent Leaf Meal remain intact. The Crude Protein (CP) content of Scent Leaf Protein Concentrate (40.96%) is significantly higher than that of the Scent Leaf Meal (16.94%), which is due to the fact that the Scent Leaf Protein Concentrate is a product of extraction, a substance which has been concentrated into a proteinous mass, while the Scent Leaf Meal contain other parts of the leaf that are less proteinous. The Crude Fibre (CF) content of Scent Leaf Protein Concentrate (1.54%) is significantly lower than that of Scent Leaf Meal (7.07%), which is due to the fact that the bagasses which contain most of the fibre in the scent leaf has been extracted from the Scent Leaf Protein Concentrate, while that of the Scent Leaf Meal remain intact. The Ether Extract content of the Scent Leaf Protein Concentrate (8.008%) is significantly higher than that of the Scent Leaf Meal (5.735%), which is due to the fact that while the primary objective is to concentrate proteins, some lipids

may also be concentrated in the Leaf Protein Concentrate as a result of the removal of water and other non-lipid components. The Ash content of Scent Leaf Protein Concentrate (10.72%) is significantly higher than that of Scent Leaf Meal (6.988%), which is due to the fact that while the primary goal of producing Leaf Protein Concentrate is to concentrate protein, some minerals and ash-forming elements may also become more concentrated in the Leaf Protein Concentrate as a result of the removal of water and non-protein components. The Nitrogen Free Extract (NFE) content of Scent Leaf Protein Concentrate (25.57%) is significantly lower than that of Scent Leaf Meal, which is due to the fact that NFE is the portion of the sample that remains after subtracting the percentages of protein (of which is a major component of Leaf Protein Concentrate), fat,

ash, and crude fiber from 100%.

Table 4.1: Proximate composition of Scent Leaf Meal and Scent Leaf Protein Concentrate obtained using wet milling.

PROXIMATE COMPOSITION PARAMETERS	LEAF MEAL (g/100g)	LEAF PROTEIN CONCENTRATE (g/100g)
DRY MATTER	88.41[a]	86.80[b]
CRUDE PROTEIN	16.94[b]	40.96[a]
CRUDE FIBER	7.07[a]	1.54[b]
EITHER EXTRACT	5.735[b]	8.008[a]
ASH	6.988[b]	10.720[a]
NFE	51.67[a]	35.57[b]

[ab] – Means with different superscript letters indicate significant differences at $p<0.05$

4.2 AMINO ACID PROFILE OF SCENT LEAF PROTEIN CONCENTRATE AND SCENT LEAF MEAL

The amino acid profile of scent leaf meal and scent leaf protein concentrate is presented in Table 4.2. The amino acid compositions in scent leaf protein concentrate were significantly higher ($p<0.05$) that those in scent leaf meal, except for tryptophan and serine, in which the amino acids were lower in scent leaf protein concentrate than in scent leaf meal. However, there was no significant difference ($p>0.05$) between the scent leaf protein concentrate and scent leaf meal in valine. Scent leaf protein concentrate contained

a significantly higher ($p<0.05$) amount of total and essential amino acids than scent leaf meal.

Table 4.2: Amino acid profile of Scent Leaf Meal and Scent Leaf Protein Concentrate.

AMINO ACID (g/100g)	**SCENT LEAF MEAL**	**SCENT LEAF PROTEIN CONCENTRATE**	**LSD**
Lysine	3.37^{b}	4.76^{a}	0.51
Threoline	1.60^{b}	2.62^{a}	0.20
Valine	4.10	4.14	0.26
Tryptophan	5.09^{a}	1.09^{b}	0.11
Methionine	1.86^{b}	2.18^{a}	0.12
Isoleucine	2.52^{b}	4.07^{a}	0.13
Leucine	4.88^{b}	8.56^{a}	0.25
Phenyla lanine	2.81^{b}	4.07^{a}	0.20
Histidine	1.74^{b}	2.54^{a}	0.08
Arginine	3.44^{b}	4.93^{a}	0.17
Aspartic acid	6.12^{a}	8.86^{a}	0.14
Serine	5.38^{a}	4.54^{b}	0.29
Glutamic acid	8.27^{b}	12.71^{a}	0.66
Proline	1.93^{b}	3.96^{a}	0.05
Glycine	2.14^{b}	2.92^{a}	0.28
Alanine	2.26^{b}	3.52^{a}	0.24
Cysteine	1.37^{b}	1.65^{a}	0.07
Tyrosine	2.52^{b}	3.42^{a}	0.05
Total Amino acid	27.96^{b}	34.03^{a}	0.89
Total Essential amino acid	61.39^{b}	80.54^{a}	0.99

^{ab}- Mean with different superscript in each row are significantly different ($P<0.05$)

CHAPTER FIVE

5.0 DISCUSSION

The total Crude Protein (CP) contents of scent leaf meal and scent leaf protein concentrate indicate that they are good food and feed resources for humans and animals, respectively. The scent leaf meal and scent leaf protein concentrate also contain fats, vitamins, minerals and fibre.

The Crude Protein (CP) content of scent leaf protein concentrate in this study is higher than those obtained for pumpkin leaf (*Telfairia occidentalis*) and green amaranth (*Amaranthus hybridus*) leaf meals (Aja *et al*., 2021), although that of the scent leaf meal is comparable with them.

Amino acids are the basic building materials of proteins and the nitrogenous backbones of substances such as neurotransmitters and hormones. The total amino acid contents in scent leaf meal and scent leaf protein concentrate indicate that they are good food and feed resources for humans and animals, respectively. The higher amino acid content in scent leaf protein concentrates than in leaf meal corroborates the protein contents of the food/feed materials.

The total amino and total essential amino acids obtained in scent leaf protein concentrate in this study are comparable with that obtained for pumpkin leaf (*Telfairia occidentalis*) and green amaranth (*Amaranthus hybridus*) leaf meals (Aja *et al*., 2021). Lysine and methionine are limiting amino acids in monogastric animals, particularly poultry and pigs. The lysine and methionine contents of the species of scent leaf (*Ocimum gratissimum*) in

this study were higher than those reported for holy basil (*Ocimum sanctum*) (Alikwe *et al.*, 2013).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

This research work was conducted to know the proximate composition and amino acid profile of scent leaf protein concentrate and scent leaf meal. For the proximate composition, the leaf meal has a dry matter content of 88.41%, crude protein of 16.94%, crude fibre of 7.07%, ether extract of 5.735%, ash of 6.988%, and NFE of 51.67%, while the leaf protein concentrate has a dry matter content of 86.80%, crude protein of 40.96%, crude fibre of 1.54%, ether extract of 8.008%, ash of 10.720% and NFE of 35.57%. For the amino acid profile, the scent leaf meal and the scent leaf protein concentrate has a total amino acid (lysine, threoline, valine, tryptophan, methionine, isoleucine, leucine, phenyl lanine, histidine, arginine, aspartic acid, serine, glutamic acid, proline, glycine, alanine, cysteine, tyrosine) of 27.96% and 34.03% respectively, with the essential amino acid in the total amino acid being 61.39% and 8.54% respectively.

6.2 CONCLUSION

Based on the result of the proximate analysis and the amino acid profile, scent leaf protein concentrate and scent leaf meal can be added as sources of protein to the diet of both livestock and humans respectively.

Lysine and methionine (which are found in scent leaf protein concentrate) are limiting amino acids in monogastric animals, particularly poultry and pigs, hence scent leaf protein concentrate can be used in compounding feed for these animals.

Scent leaf protein concentrate may serve as substitutes for more expensive protein feedstuffs like groundnut meal and soybean meal.

6.3 RECOMMENDATION

I recommend that scent leaf protein concentrate should be prepared using dry milling method in order to know if it would present higher yield and other nutrients than that from wet milling method. I also recommend the experimentation of feed formulation with scent leaf protein concentrate and the prolonged trial of these feeds on livestock in order to ascertain its full effects and potentials.

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