PROXIMATE COMPOSITION: PHYTATE AND OXALATE OF SOME LEGUMES

BY

Promise Chukwuemeka UNINI

AGR2004328

FACULTY OF AGRICULTURE

DEPARTMENT OF ANIMAL SCIENCE

UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

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PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA, IN PARTIAL FUFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF AGRICULTURE DEGREE (B.AGRIC) IN ANIMAL SCIENCE.

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ABSTRACT

This research project is to explore the nutritional benefits of legumes and identify the presence of anti nutritional compounds such as Phytate and oxalate. Various legumes were considered such as Soybean, Jack bean, Velvet bean, Faba bean and Black gram, and a comparative study was undertaken to ascertain which was more edible and contains health beneficial compounds.

It was discovered that Soybean proved to contain highest of ash, crude protein and ether extract. While Velvet bean and Faba bean showed moderate presence of oxalate and phytate and therefore proved to be much edible for consumption as there are little or no risk of kidney stone formation and low mineral bioavailability. This work encompasses the introduction, literature review, method and materials, results, discussion, recommendation and conclusion.

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CERTIFICATION

This is to certify that the work titled **"**THE DETERMINATION OF ANTIGENS IN LEGUMES", carried out by Promise Chukwuemeka UNINI with Mat. No AGR2004328, of the Department of Animal Science, Faculty of Agriculture, University Of Benin, Edo State, Nigeria.

PROJECT SUPERVISOR:

Name: Dr. G. I. O. ODAFE-SHALOME
Signature and Date ....

HEAD OF DEPARTMENT:
Name: Dr. N. C. Akaeze
Signature and Date ....

DEDICATION

This project is dedicated to God almighty for his protection, provision and preservation towards me throughout my stay in the University of Benin and also my family who was there for me all the way and also to my lecturers for their commitment to teach.

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CHAPTER ONE

1.1 INTRODUCTION

Legumes or pulses are flowering plants belonging to the leguminosae family (Albala, 2007). Traditionally, legumes have been considered as foods with beneficial effects for human health (MA Martin, 2019).

Legumes provide energy, dietary fibre, protein, minerals and vitamins required for human health and endue well-balanced essential amino acid profiles when consumed with cereals and other foods rich in sulphur-containing amino acids and tryptophan. Because of the high protein content and beneficial nutritional value, legumes (lupin, peas, beans, and soybeans) play an important role in human diet (C. Carranca, 2013).

Some antinutritional compounds, found in legumes, can be toxic, unpalatable or indigestible, but their elimination can be achieved by selection of plant genotypes or through post-harvest processing. The production of legume protein concentrates or isolates is of growing interest to food industry because of their functional properties and ability to improve the nutritional quality of food products. For that, various techniques are used to extract protein concentrates/isolates with different features (C. Carranca, 2013).

Legume proteins have gained increasing importance because of desired functional properties, including gelling and emulsifying properties, and could be proposed as a potential supplement in a great number of food applications (C. Carranca, 2013).

Legumes are known to produce primary and secondary metabolites and other phytochemicals such as pharmaceuticals, pesticides and industrial products (Oboh, 2006). They are excellent sources of nutraceutical constituents such as fibre, phytic acid and polyphenols isoflavones, flavonoids, lignans and tannis. They contain bioactive compounds whose beneficial effects need to be explored for exploitation of the unconventional legume resource to meet the protein requirement of developing countries (Oboh, 2006).

The bean seed coat colour is attributed to the presence and quantity of polyphenols such as flavonol glycosides, condensed tannins and anthocyanins. These compounds have antioxidant, antimutagenic and anticarcinogenic activities and also free radical scavenging properties. Yes, legume seeds contain both phytates and oxalates, which are naturally occurring compounds that can impact nutrient absorption. (Oboh, 2006).

Phytate (myo- inositol hexakisphosphate) is the primary storage form of phosphorus in many seeds, grains, and legumes. It has gained attention for its role as an **anti-nutrient** due to its ability to bind essential minerals like calcium, iron, zinc, and magnesium, which decreases their bioavailability and reduces the absorption of these nutrients in the human digestive system (Ojo, Akinmoladun, & Oyetayo, 2020). Phytates are particularly abundant in legume seeds, where they can constitute up to 2.9% of the dry weight of the seeds (Ojo et al., 2020). Despite its nutritional drawbacks, phytate also serves beneficial roles in plants, such as being a significant phosphorus reserve for seedling growth.

Various processing methods, such as soaking, sprouting, and fermentation, can reduce phytate content in legumes, improving mineral bioavailability (Schlemmer et al., 2009).

Oxalates (oxalic acid and its salts) are another group of compounds found in many plants, including legumes. Oxalates can bind to calcium and form insoluble salts, such as calcium oxalate, which reduces calcium absorption and may contribute to the formation of kidney stones in susceptible individuals (Gioia et al., 2022). Legumes like soybeans, beans, and lentils contain varying levels of oxalates, with total oxalate content in legumes ranging between 13.9 and 547.9 mg per 100g (Noonan & Savage, 1999).

Despite their potential negative effects on mineral absorption, oxalates are a normal component of many plant-based diets and can be reduced through cooking and other food processing methods. Both phytates and oxalates are naturally occurring compounds that influence the nutritional quality of legumes, especially in terms of mineral absorption. Understanding their presence and potential impacts on human health is important, particularly for individuals who rely heavily on legumes as a primary source of nutrition.

1.2 JUSTIFICATION

Legume seeds are many and varied. There is an interesting need to analyze both the conventional and unconventional food legumes, which include Jack bean, Soya bean, red gram, velvet bean and black gram. The analysis of these plants will give an understanding of their composition and possible methods in utilizing them as food stuffs. But we are concerned with understanding the content level of phytates and oxalates in these legumes which in turn determine the kind of processing to adopted for their consumption.

1.3 OBJECTIVES OF THE STUDY

The objectives of this study are to

1. determine the proximate composition of Soya Bean, Jack Bean, Faba bean, velvet bean and Black Gram in their raw state.
2. analyze the phytochemical contents of the different legumes
3. determine the phytates and oxalates levels as critical phytochemicals in the various legumes
4. compare the chemical composition of the under-utilized legumes and that of the conventional legume soya bean.
5. determine from the chemical composition whether the under-utilized legumes can utilized as food legumes or for industrial purposes.

CHAPTER TWO

LITERATURE REVIEW

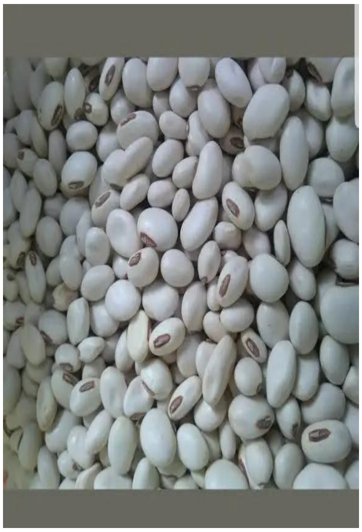

PLATE 1: JACK BEAN PLATE 2: BLACK GRAM PLATE 3: SOYABEANS

PLATE 3: VELVET BEANS PLATE 4: FABA BEAN

2.1 LEGUMES

Legumes are plants in the family **Fabaceae** (or Leguminosae) that produce seeds in pods. They are an important part of human diets and agriculture due to their high nutritional value, particularly as sources of protein, fiber, vitamins, and minerals. Legumes include beans, peas, lentils, chickpeas, and soybeans, among others, and they play a key role in soil fertility due to their ability to fix nitrogen (Wang 2017).

2.1.1 Soybean *(Glycine max)*

Soybeans are one of the most widely cultivated leguminous crops globally. They are rich in protein, essential amino acids, and oils, making them an important source of plant-based protein in human diets and animal feed. Soybeans are also utilized in a variety of products like tofu, soy milk, and soy protein isolates. They play a significant role in agriculture due to their ability to fix nitrogen in the soil (Ghosh 2020).

2.1.2 Jack Bean *(Canavalia ensiformis)*

Jack beans are part of the legume family and are commonly grown in tropical and subtropical regions. These beans are known for their high protein content but must be processed before consumption, as raw seeds contain toxic substances. They are also used as green manure to improve soil quality and fertility (Hedge 2018).

2.1.3 Faba Bean *(Vicia faba)*

Faba beans, also known as broad beans, are an important legume crop, grown primarily for their high-protein seeds. They are used in both human and animal diets, providing essential nutrients such as fiber, vitamins, and minerals. Faba beans are also known for their role in crop rotation, as they improve soil nitrogen levels (Jadhav 2021).

2.1.4 Velvet Bean *(Mucuna pruriens)*

Velvet bean is a tropical legume known for its high protein content and use as a cover crop. It is particularly famous for its high concentration of L-dopa, a precursor to dopamine, which is beneficial in treating Parkinson's disease. Velvet beans also serve as a nitrogen-fixing crop and are used in both human consumption and as a livestock feed (Gyaneshwar 2020).

2.1.5 Black Gram *(Vigna mungo)*

Black gram, commonly known as urad bean, is a significant pulse in many Asian countries, particularly in India. It is known for its rich protein content and is commonly used in various traditional dishes, including dals and curries. Black gram is also a good source of iron, calcium, and other essential nutrients (Singh 2019).

2.2 NUTRITIONAL COMPOSITION OF LEGUMES

Legumes are rich in protein and dietary fiber, and offer moderate amounts of carbohydrate while generally being low in fat (except for oil-rich legumes such as soybeans). For example, a half-cup serving of cooked legumes provides about 8 g protein, 7-9 g fiber, ~20 g carbohydrate, and ~1 g fat. On a dry-weight basis, legumes may contain protein values ranging from ~20-45%, carbohydrates as the main energy source, and dietary fibre between ~5-37% depending on species and processing. Legumes contain B-group vitamins (e.g., folate, thiamine, riboflavin), minerals such as iron, zinc, magnesium, potassium, calcium and copper (Kumar 2017).

Compared to cereals, legumes often have lower lipid content, though soybeans are a notable exception with ~19% oil, ~36% protein, ~35% carbohydrate on a dry-weight basis. They are also a good source of complex carbohydrates and resistant starch, low in saturated fat and cholesterol-free when plant-based (Frontiers 2024).

The exact nutrient composition varies by legume type, growing conditions, variety, and processing/cooking methods. For example, cooking/canning can reduce protein, fiber, magnesium and phytate contents in pulses. For example, in the study of pulses consumed in France: cooked products ranged from ~6.9-9.7 g/100 g protein and showed large variation in vitamins and minerals. In the study “Sustainable Strategies…” legumes (dry beans) contained about 23.7- 25.9 g protein per 100 g dry beans, and 8.23- 9.01 g protein per 100 g cooked beans, with fibre and other nutrients in appreciable amounts (Margier 2018).

2.3 NUTRITIONAL IMPORTANCE OF LEGUMES

2.3.1 High in Protein: Legumes are an excellent plant-based source of protein, essential for muscle repair, immune function, and overall growth. They are especially beneficial for vegetarians and vegans seeking to meet their protein needs without animal products (Papas, 2019).

2.3.2 Rich in Fiber: Legumes are an abundant source of dietary fiber, which aids in digestion, helps to regulate blood sugar levels, and supports heart health by lowering cholesterol (Slavin, 2013). The high fiber content also helps with weight management by promoting satiety.

2.3.3 Low Glycemic Index: The carbohydrate content in legumes is complex, resulting in a low glycemic index. This makes them ideal for individuals with diabetes or those looking to maintain steady blood sugar levels (Micha et al., 2017).

2.3.4 High in Micronutrients: Legumes are a good source of essential vitamins and minerals, such as folate, iron, magnesium, and potassium. Folate is crucial for cell division and proper brain function, while iron supports the production of hemoglobin and helps prevent anemia (Beto, 2015).

2.3.5 Antioxidant Properties: Many legumes, such as lentils, chickpeas, and beans, contain antioxidants like flavonoids and polyphenols that help reduce oxidative stress in the body, potentially lowering the risk of chronic diseases like cancer and heart disease (Almario et al., 2017).

2.3.6 Cholesterol-Lowering Effects: The soluble fiber in legumes binds to cholesterol and helps in its removal from the body, thereby lowering LDL cholesterol levels and contributing to cardiovascular health (Anderson et al., 2009).

2.3.7 Heart Health: Legumes are rich in unsaturated fats, particularly polyunsaturated fats, which support cardiovascular health by reducing harmful cholesterol and inflammation (He et al., 2012). The legumes of interest to us are Soya bean, Jack bean, Velvet bean, Black gram and Faba bean.

2.4 Phytochemicals In Soya Bean, Faba Bean, Jack Bean, Black Gram, And Velvet Bean

Phytochemicals are bioactive compounds found in plants that provide health benefits beyond basic nutrition. These compounds include flavonoids, phenolic acids, alkaloids, saponins, and other antioxidant compounds that may contribute to the prevention of chronic diseases. Below are the key phytochemicals found in soya bean, faba bean, jack bean, black gram, and velvet bean.

2.4.1 Soya Bean (*Glycine max*)

Isoflavones: Soya beans are rich in isoflavones (genistein, daidzein, and glycitein), which are phytoestrogens. These compounds have been studied for their potential role in reducing the risk of breast cancer, improving bone health, and alleviating menopausal symptoms (Messina, 2010).

Saponins: Soya beans contain saponins, which have antioxidant and anti-inflammatory properties and may contribute to lowering cholesterol levels (Kim et al., 2009).

Phenolic Acids: Ferulic acid and caffeic acid are the primary phenolic acids in soya beans, which are known for their antioxidant activity (Wang et al., 2008).

2.4.2 Faba Bean (Vicia faba)

Flavonoids: Faba beans contain flavonoids such as quercetin and kaempferol, which have antioxidant and anti-inflammatory effects (Hussain et al., 2016).

Saponins: Like soya beans, faba beans also contain saponins, which contribute to their antioxidant properties and potential cholesterol-lowering effects (Sharma et al., 2015).

Alkaloids: The faba bean also contains alkaloids like vicine and convicine, which have been studied for their role in providing protective effects against certain diseases, though they can be toxic at high levels (Diaz et al., 2015).

2.4.3 Jack Bean (Canavalia ensiformis)

Protease Inhibitors: Jack beans are rich in protease inhibitors that have been shown to possess anticancer properties (Jha et al., 2017).

Flavonoids: This legume contains flavonoids, particularly quercetin, which are known for their antioxidative and anti-inflammatory properties (Patil et al., 2012).

Tannins: Jack beans also contain tannins, which have demonstrated antimicrobial and antioxidant activities (Patil et al., 2012).

2.4.4 Black Gram (Vigna mungo)

Phenolic Compounds: Black gram contains phenolic compounds like flavonoids and tannins, which exhibit strong antioxidant properties (Prakash et al., 2018).

Saponins: Black gram contains saponins that contribute to its cholesterol-lowering and immune-boosting effects (Sharma et al., 2015).

Lectins: This legume also contains lectins, which have been studied for their antinutritional and immunomodulatory effects (Pusztai, 1991).

2.4.5 Velvet Bean (Mucuna pruriens)

L-DOPA (Levodopa): Velvet bean is known for its high content of L-DOPA, a precursor of dopamine. It is used for its potential in treating Parkinson's disease and improving cognitive functions (Bhat et al., 2012).

Saponins: Velvet bean also contains saponins, which contribute to its antioxidant and anti-inflammatory properties (Wang et al., 2011).

Tannins: Similar to jack beans, velvet bean contains tannins, which have been found to exhibit antimicrobial, anti-inflammatory, and antioxidant activities (Bhat et al., 2012).

2.5 PHYTOCHEMICALS: THEIR IMPORTANCE AND EFFECTS

2.5.1 Saponins

Definition: Saponins are a group of naturally occurring compounds found in plants, especially legumes, which have soap-like properties (they form foam when mixed with water). Saponins consist of a sugar molecule attached to a triterpene or steroidal aglycone (non-sugar) part.

Importance and Effects:

Health Benefits: Saponins have been linked to numerous health benefits, including immune system support, lowering cholesterol, and anti-inflammatory effects. They may also have antioxidant properties, which help reduce oxidative stress and prevent chronic diseases like heart disease and cancer (Francis et al., 2002).

Toxicity: At high concentrations, saponins can be toxic to humans, causing irritation to the gastrointestinal system, especially when consumed in raw plant form (Venkatesan et al., 2013).

2.5.2 Tannins

Definition: Tannins are polyphenolic compounds that have the ability to bind and precipitate proteins. They are found in many plants, especially in the bark, leaves, and seeds of certain species.

Importance and Effects:

Health Benefits: Tannins have been shown to exhibit antioxidant, anti-inflammatory, and antimicrobial properties. They may help protect against oxidative stress, reducing the risk of chronic conditions such as cancer and heart disease (Salah et al., 1995).

Potential Drawbacks: Excessive intake of tannins may interfere with the absorption of essential minerals, particularly iron, and may cause gastrointestinal irritation in sensitive individuals (Makkar, 2003).

2.5.3 Flavonoids

Definition: Flavonoids are a large group of plant compounds that belong to the polyphenol family. They are commonly found in fruits, vegetables, tea, and wine, and are known for their antioxidant and anti-inflammatory properties.

Importance and Effects:

Health Benefits: Flavonoids, including quercetin, catechins, and anthocyanins, are well known for their antioxidant activity, which can help protect the body from oxidative stress, a key factor in aging and the development of chronic diseases. They also have potential anti-cancer and heart-protective effects (Grosso et al., 2017).

Bioavailability: While beneficial, the bioavailability of flavonoids can be affected by factors such as food preparation, metabolism, and interactions with other dietary components (Mertens-Talcott et al., 2003).

2.5.4 Phenols

Definition: Phenolic compounds are a diverse group of chemicals containing a phenol structure. They are widely distributed in the plant kingdom and contribute to the color, flavor, and nutritional value of many fruits, vegetables, and beverages.

Importance and Effects:

Health Benefits: Phenolic compounds, including phenolic acids like ferulic acid, have significant antioxidant properties, which help neutralize free radicals and reduce inflammation, thus lowering the risk of cardiovascular disease, diabetes, and cancer (Arts & Hollman, 2005).

Antimicrobial: Many phenols also exhibit antimicrobial properties, making them useful in food preservation and as part of the body's defense mechanism against pathogens (Cushnie & Lamb, 2005).

2.5.5 Phytate

Definition: Phytates, also known as phytic acid, are the primary storage form of phosphorus in many seeds, grains, and legumes. Phytate is considered an anti-nutrient because it binds to essential minerals like iron, zinc, calcium, and magnesium, thereby reducing their bioavailability and impairing their absorption in the human digestive system.

Types

Phytates, also known as phytic acid, are the storage form of phosphorus in many seeds, including legumes. Phytates are most commonly found in the seed coats or hulls and can bind to essential minerals such as calcium, iron, zinc, and magnesium, thereby reducing their bioavailability for absorption in the human digestive system.

Properties

Phytates are water-insoluble compounds and primarily act as an anti-nutrient, meaning they can interfere with the absorption of essential minerals, which is particularly a concern in populations reliant on plant-based diets. However, they also have beneficial properties, including antioxidant effects and potential cancer-fighting properties. Additionally, phytic acid may contribute to reducing the risk of kidney stones and cardiovascular diseases due to its ability to bind certain metals.

Distribution

Phytates are widely distributed in legumes, including beans, peas, lentils, and chickpeas. The concentration of phytic acid varies depending on the legume species, with higher concentrations typically found in the seed coat or bran of the legumes.

Usefulness

Despite being considered an anti-nutrient, phytates also have antioxidant properties, contribute to reducing oxidative stress, and may reduce the risk of developing chronic diseases. Furthermore, they have been explored for their potential in cancer prevention and in lowering cholesterol levels. In the context of agriculture, phytates can play a role in soil fertility by acting as a phosphate reservoir for plants (Reddy 2018).

Effects to Humans

Health Benefits: Phytates are considered anti-nutritional factors due to their ability to decrease mineral absorption. However, they also have antioxidant properties, which help reduce oxidative stress and may contribute to cancer prevention and other health benefits (Reddy, 2003).

Positive Effects: Some studies suggest that phytates may help prevent kidney stones and reduce the risk of colorectal cancer (Reddy et al., 1996).

2.5.6 Oxalates

Definition: Oxalates (or oxalic acid) are naturally occurring compounds found in many plants, including legumes, vegetables, and fruits. They can bind to minerals like calcium to form calcium oxalate, which is poorly absorbed in the body and can contribute to the formation of kidney stones in susceptible individuals.

Types

Oxalates are organic acids found in many plants, including legumes, often in the form of calcium oxalate crystals. They can be classified into two types: soluble oxalates and insoluble oxalates. Soluble oxalates are absorbed by the body and can potentially contribute to the formation of kidney stones in susceptible individuals, while insoluble oxalates do not pose such risks but can contribute to the binding of calcium and other minerals in the gut.

Properties

Oxalates are naturally occurring compounds that can interfere with mineral absorption, particularly calcium, by forming insoluble complexes such as calcium oxalate. While oxalates are considered an anti-nutrient, their role in plants is thought to help deter herbivory due to their bitterness and ability to crystallize in tissues. In moderate amounts, oxalates do not pose significant health risks, but high concentrations in the diet can contribute to kidney stones, particularly in individuals predisposed to oxalate kidney stones.

Distribution

Oxalates are found in various amounts in legumes such as beans, lentils, and soybeans. The concentration of oxalates can vary significantly depending on the legume variety, growth conditions, and the processing methods used. For example, some beans such as soybeans contain moderate levels of oxalates, whereas other legumes like chickpeas contain lower amounts.

Usefulness

Although oxalates are considered anti-nutrients due to their interference with calcium absorption, they also have some potential health benefits. They have been shown to possess antimicrobial and antioxidant properties, contributing to the protection of plants against oxidative damage. Additionally, the role of oxalates in plants may involve regulation of mineral balance and preventing excessive accumulation of certain minerals. However, from a dietary standpoint, reducing oxalate content through soaking, boiling, or fermenting legumes can minimize the negative effects associated with high oxalate intake (Noonan 2019).

Effects

Health Effects: While oxalates are generally considered safe when consumed in moderation, high levels of oxalates can interfere with calcium absorption and increase the risk of kidney stones, especially in susceptible individuals (Li et al., 2011).

Health Benefits: Despite their negative effects, oxalates also have potential health benefits, including antimicrobial and anti-inflammatory properties (Rauf et al., 2015).

CHAPTER THREE

METHOD AND MATERIALS

3.1 SOURCES OF MATERIALS

Raw sample of jack bean (*Canavalia ensiformis*), velvet bean (*Mucuna pruriens*), Faba (Vicia faba), Black Gram (Vigna mungo), and Soyabean (Glycine max), were obtained from the Food Science and Nutrition Department Food Bank, University of Benin, Benin City, Nigeria. The seeds were cleaned to remove stones, broken seeds, and other foreign materials. Clean and undamaged seeds were selected, labeled properly, and stored in airtight containers at room temperature until required for analysis

3.2 SAMPLE PREPARATION

Seeds were cleaned, air-dried, milled into fine flour using a mortar and pistle, and sieved through a 60-mesh sieve. The flour was stored in airtight containers at 4°C until used for proximate analysis.

3.3 PROXIMATE ANALYSIS

The proximate composition of the jack bean, velvet bean, Faba bean, Black gram and Soyabean samples was determined following the procedures of the Association of Official Analytical Chemists (AOAC, 2019). The parameters analyzed included moisture, ash, crude protein, Ether extract, crude fiber, and nitrogen free extract. Proximate analysis of experimental materials was carried out in the Central Laboratory, Faculty of Agriculture, University of Benin, Benin-City, Edo state, with little modification by Isikhuemen and Efenudu *et al* (2020).

3.3.1 Reagents and Chemicals

All reagents used were of analytical grade. The major reagents included hydrochloric acid (HCl), sodium hydroxide (NaOH), petroleum ether, sulphuric acid, boric acid, Kheldahl catalyst.

3.3.2 Apparatus and Equipment

Analytical weighing balance, mortar and pistle, 60-mesh sieve, Soxhlet extractor, Kjeldahl digestion and distillation unit, muffle furnace, centrifuge, water bath (105°C), Heating mantle, and standard glassware.

3.3.3 DRY MATTER AND MOISTURE DETERMINATION

Moisture is determined by the loss in weight that occurs when a sample is dried to a constant weight in an oven. About 1g of each legume sample is weighed into separate crucibles previously dried and weighed. Each sample is then dried in an oven for 105°C for 24 hours, cooled in a desiccator and weighed. The drying and weighing is repeated until a constant weight is achieved.

Samples are usually compared for their nutrient content on moisture free or dry matter (DM) basis.

Moisture (%) = [(W1 - W2) / (W1 - W0)] × 100

Where;

(W_0) = weight of empty crucible
(W_1) = weight of crucible + sample before drying
(W_2) = weight of crucible + sample after drying

% Dry matter (DM) = 100 - %Moisture

3.3.4 ASH DETERMINATION

Ash is the inorganic residue obtained by burning off the organic matter of a sample at 550-600°C in Muffle furnace for 6 hours. 1g of each sample is weighed into a pre-heated crucible. The crucible is placed into the Muffle furnace at 550-600°C for 6 hours or until whitish-grey ash is obtained. The crucible is then placed in the desiccator and weighed.

The ash content is expressed as;

Weight of ash/weight of Sample×100/1

3.3.5 ETHER EXTRACT DETERMINATION

The ether extract represents the fat and oil in the legume sample. Soxhlet apparatus is the equipment used for the determination of ether extract. It consist of 3 major components: an extractor, comprising the thimble which holds the sample; condenser for cooling and condensing the ether vapor and a 250ml flask.

Procedure: About 150ml of an anhydrous diethyl ether (petroleum ether) of boiling point of 40-60°C is placed in the flask. 1g of the sample is weighed into a thimble and the thimble is plugged with cotton wool. The thimble with content is placed into the extractor. The ether in the flask is then heated. As the ether vapor reaches the condenser through the side arm of the extractor, it condenses to liquid form and drop back into the sample in the thimble. The ether soluble substances are dissolved and are carried into solution through the siphon tube back into the flask. The extraction continues for at least 4 hours. The thimble is removed and most of the solvent is distilled from the flask into the extractor. The flask is then disconnected and placed in an oven at 75°C for 1hour 30mins then cooled in a desiccator and weighed.

%Ether extract= weight of fat/weight of sample ×100/1

3.3.6 CRUDE FIBRE DETERMINATION

The organic residue left after sequential extraction of legume sample with ether can be used to determine the crude fibre. However, if a fresh sample is used, the fat in it could be extracted by adding petroleum ether, stir, allow it to settle and decant. Do this three times. The fat-free material is then transferred into a flask or beaker. 200ml of pre-heated 1.25% H_2SO_4 is added and the solution is gently boiled for about 30 minutes, maintaining constant volume of acid by the addition of hot water. The Buckner flask funnel fitted with Whatman filter is pre-heated by pouring hot water into the funnel. The boiled acid sample mixture is then filtered hot through the funnel under sufficient suction. The residue is then washed several times with boiling water (until the residue is neutral to litmus paper) and transferred back into the beaker. Then 200ml of pre-heated 1.25% Na_2SO_4 is added and boiled for another 30 minutes. Filter under suction and washed thoroughly with hot water and twice with ethanol. The residue is dried at 100°C until a constant weight is known. It is then transferred into a crucible and placed in Muffle furnace (500-600°C) and ashed, then cooled in a desiccator and weighed.

%Crude fiber =weight of crude fiber/weight of original sampled×100/1

3.3.7 CRUDE PROTEIN DETERMINATION

Crude protein is determined by measuring the nitrogen content of the sample and multiplying it by a factor of 6.25. This factor is based on the fact that most protein contains 16% nitrogen. Crude protein is determined by Kjeldahl method. The method involves: Digestion, Distillation and Titration.

Digestion: Weigh about 1g of the sample into a Kjeldahl flask and add 5 ml of concentrated sulphuric acid. Apply heat using a heater. Continue to digest for 45 minutes until the digesta become clear pale green. Leave until completely cool and rapidly add 50ml of distilled water. Rinse the digestion flask 2-3 times and add the rinsing to the bulk.

Distillation: Steam up the distillation apparatus and add about 5 ml of the digest into the apparatus via a funnel and allow it to boil. Add 5ml of sodium hydroxide from the measuring cylinder so that ammonia is not lost. Distil into 5ml of 2% boric acid containing screened methyl red indicator.

Titration: The alkaline ammonium borate formed is titrated directly with HCl. The titre value which is the volume of acid used is recorded. The volume of acid used is fitted into the formula which becomes:

%CP= NA × 14/1000 ×volume of acid ×100/volume of digest used ×100/weight of sample

% Crude protein = %N x 6.25

3.3.8 NITROGEN FREE EXTRACT (NFE)

NFE is determined by mathematical calculation. It is obtained by subtracting the sum of percentages of all the nutrients already determined on dry matter basis from 100.

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

NFE represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in the sample.

3.4 ANALYSIS OF PHYTOCHEMICALS

3.4.1 *PREPARATION OF SAMPLE*

1.0g of the sample was weighed and dissolved in 50ml of cool boiled-out distilled water in a 100ml beaker. This was transferred to a 100ml standard flask. The beaker was rinsed into the standard flask three times with about 10ml of the boiled out distilled water. It was then made up to mark with the same distilled. The flask was corked and inverted four times for proper mixing and then set aside for analysis. This solution has a concentration of 10000μg/ml.

3.4.2 DETERMINATION OF TOTAL PHENOLIC CONTENTS

The amount of total phenolics in the extract was determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi(1965) with slight modification using tannic acid as a standard.

Briefly, 1.0ml of extract solution (250 ug/ml) was added in a test tube. Then, 1.0 mL of Folin–Ciocalteu reagent was added, and the contents of the flask were mixed thoroughly. After 5 min, 15.0 ml Na2CO3 (20 %) was added and allowed to stand for 2 hours. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as Ug of tannic acid equivalent(TAE) using an equation obtained from the standard tannic acid calibration graph.

3.4.3 FLAVONOID CONTENT DETERMINATION

The flavonoid content was determined on triplicate aliquots of the homogenous cabbage extract (1.5 g) (Ilahy et al., 2011). Thirty-microliter aliquots of the methanolic extract were used for flavonoid determination. Samples were diluted with 90 µl methanol, 6 µl of 10% Aluminum chloride (AlCl3), 6µl of 1mol/l Sodium acetate (CH3CO2Na) were added and finally 170 µL of methanol was added. The absorbance was read at 415 nm after 30 min. Quercetin was used as a standard for calculating the flavonoid content (mg Qe/kg).

3.4.4 ESTIMATION OF TOTAL SAPONINS CONTENT

Estimation of total saponins content was determined by the method described by Makkar et al. based on vanillin-sulphuric acid colorimetric reaction with some modifications. About 50 µL of plant extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800mg of vanillin in 10Ml of 99.5% ethanol) was added. Then 2.5Ml of 72%sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°Cfor 10min. After 10min, it was cooled in ice cold water and the absorbance was read at 570nm. 0- 25 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly as test samples. The values were expressed as mg/kg.

3.4.5 ESTIMATION OF TANNINS CONTENT

Exactly 0.20 mL of sample was added to 20 mL of 50% methanol and placed in a water bath at 77˚C - 80˚C for 1hr and shaken. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and 20 mL of distilled water, 2.5 mL Folin-Denis reagent and 10 mL 17% Na2CO3 were added and mixed. The mixture was allowed to stand for 20 min. A series of standard tannic acids solutions were prepared in methanol and their absorbance as well as samples was read after colour development on a UV/ Visible spectrophotometer at a wavelength of 760 nm. Total tannin content was calculated from calibration curve.

3.4.6 *PHYTATE DETERMINATION*

4g of the samples was taken and soaked in 100 ml of 2% HCl for 3 hours; it was then filtered through Whatman filter paper. 25 ml of the filtrate was placed in a 250 ml conical flask, followed by the addition of 5 ml of 0.3% Ammonium thiocyanate solution as an indicator. 53.5 ml of the distilled water was added to give the desired acidity. This was then titrated with standard iron (III) chloride solution, which contains about 0.00195g of iron per ml, until a brownish yellow persists for 5 minutes. % Phytic Acid = 8.24t ×100/1000 × wt of sample, where: t = titre value

3.4.7 *OXALATE DETERMINATION*

About 1g of the sample was added to 75 ml of 1.5N H_2SO_4, and the solution was carefully stirred using a magnetic stirrer for 1 hour before being filtered using Whatman

No. II filter paper. 25 ml of the extract was collected and titrated when hot against 0.1N $KMnO_4$ solution to a faint pink colour endpoint. Oxalate = (titre value × 0.9004) mg/g

3.5 STATISTICAL ANALYSIS

All determinations were performed in **triplicates**, data obtained from the experiment was subjected to statistical analysis of variance (ANOVA) using GENSTAT 12th edition. Means were separated determine significant differences between jack bean and velvet bean samples using Duncan Multiple Range Test (DMRT) at significance level of $p<0.05$.

PLATE 6: ETHER EXTRACT ANALYSIS: DISTILLATION PROCESS.

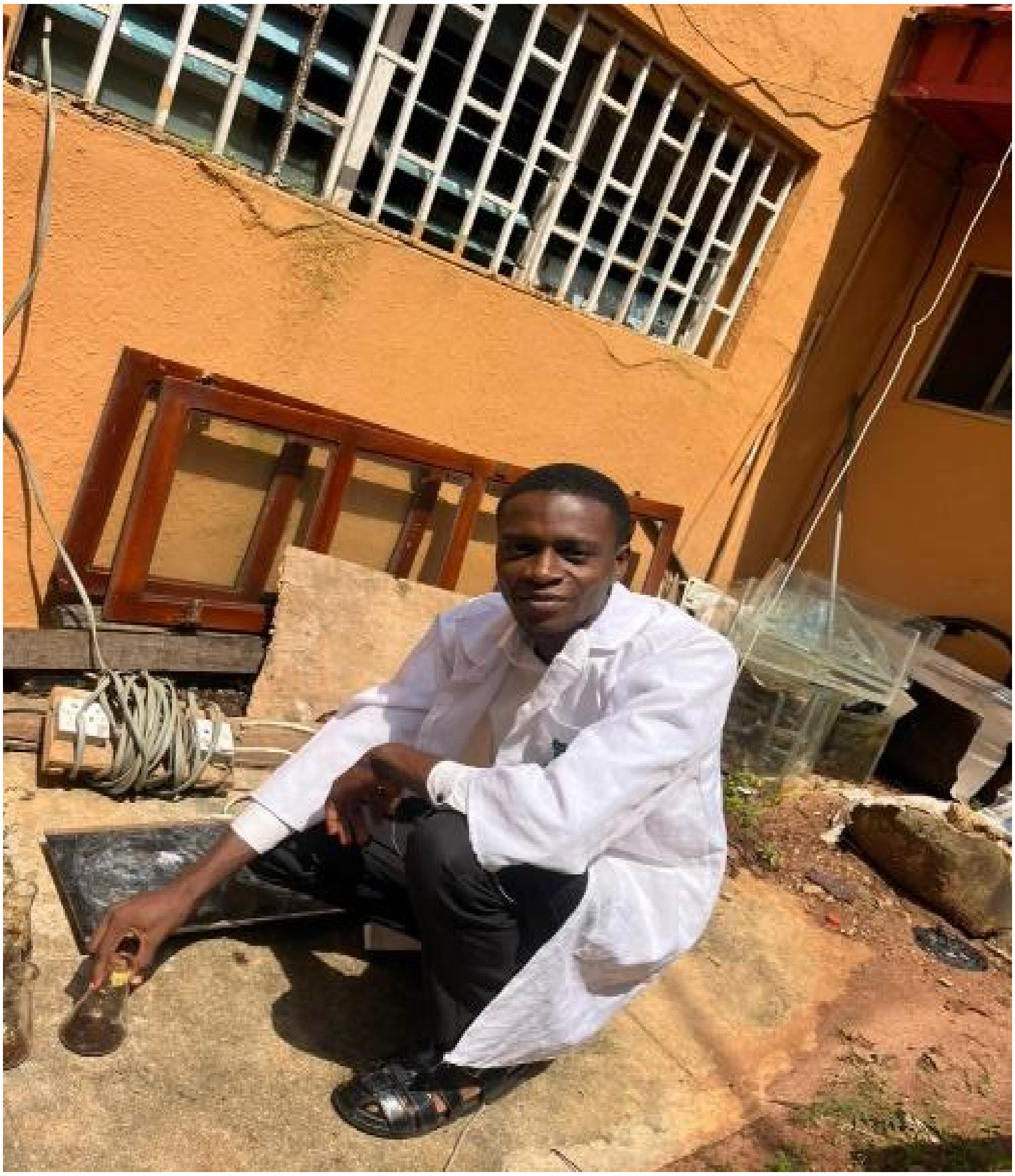

PLATE 7: CRUDE PROTEIN ANALYSIS: DIGESTION PROCESS

CHAPTER FOUR

RESULT

Table 4.1 shows the result of the proximate analysis of Soybean, Jack bean, Faba bean, Velvet bean, and Black gram. Soy bean contains 4.53% ash, 42.00% CP, 6.3% CF, 15.68% EE, 27.88% NFE. Jack bean contains 1.92% ash, 17.33% CP, 8.85% CF, 9.71% EE, 56.37% NFE. Faba bean contains 3.03% ash, 25.74% CP, 7.9% CF, 8.96% EE, 48.5% NFE. Velvet bean contains 3.79% ash, 25.74% CP, 8.4% CF, 5.65% EE, 55.12% NFE. Black gram contains 1.6% ash, 20% CP, 8.75% CF, 6.1% EE, 58.76% NFC.

Soybean has more ash 4.53%, CP 42.00%, and EE 15.68% content than the other legumes. Black gram contains least ash content 1.60%, while Jack bean has the least CP 17.33%. For EE, velvet bean contains the lowest proportion 5.65%. Jack bean contains more CF 8.85%, with soy bean having the lowest CF 6.3%. NFE has the highest value in Black gram 58.76% and lowest value in Soy bean 27.88%.

Table 4.2 shows the result of the phytochemical contents of Soybean, Jack bean, Faba bean, Velvet bean, and White bean. Total phenolic is highest in Jack bean 163.4TAE/kg and lowest in Faba bean 87.5TAE/kg. Soybean contains 50.5TAE/kg tannin as the highest with white bean having the least 21.6TAE/kg. For flavonoid, Jack bean holds the highest 111.5g/kg, and with the lowest in Velvet bean 15.04g/kg. Saponin is more in Velvet bean 5.67g/kg, and less in Faba bean 3.5g/kg. Oxalate is highest in Soybean

1791.1mg/100g and lowest in Velvet bean 3.79mg/100g. Phytate is more in Jack bean 2593.3mg/100g and less in Velvet bean 91.16mg/100g.

Table 4.1: The Result Of The Proximate Analysis Of Various Legume Seed

SAMPLE	SOYA BEAN	JACK BEAN	FABA BEAN	VELVET BEAN	BLACK GRAM
MOISTURE CONTENT	3.61	5.82	5.87	1.30	4.79
ASH	4.53	1.92	3.03	3.79	1.60
CRUDE PROTEIN	42.00	17.33	25.74	25.74	20.00
CRUDE FIBRE	6.3	8.85	7.9	8.4	8.75
ETHER EXTRACT	15.68	9.71	8.96	5.65	6.10
NFE	27.88	56.37	48.5	55.12	58.76

TABLE 4.2: THE RESULT OF THE PHYTOCHEMICAL CONTENTS OF VARIOUS LEGUME SEED EXTRACTS

SAMPLE	SOYA BEAN	JACK BEAN	FABA BEAN	VELVET BEAN	WHITE BEAN
Total phenolic content (g TAE/kg)	150.1^{c}	163.4^{bc}	87.5^{b}	55.89^{b}	97.1^{c}
Flavonoid content (g QE/kg)	84.8^{d}	111.5^{cd}	56.3^{bc}	15.04^{d}	57.0^{d}
Total Tannins content (g TEA/kg)	50.5^{e}	22.4^{de}	32.7^{cd}	27.17^{c}	21.6^{e}
Saponin (g/kg)	5.6^{f}	4.8^{e}	3.5^{d}	5.67^{e}	4.6^{f}
Oxalate (mg/100g)	1791.1^{a}	248.7^{b}	13.1^{d}	3.79^{e}	340.1^{b}
Phytate (mg/100mg)	225.1^{b}	2593.3^{a}	465.2^{a}	91.16^{a}	1808.0^{a}
SEM	2.4	32.3	10.6	1.3	3.4

CHAPTER FIVE

DISCUSSION

Proximate Composition of the Various Legumes

Among the various legumes of interest, it is seen that Soybean has higher crude protein (42.00%) than the rest. This shows that the consumption of Soybean (fully processed to reduce phytochemicals) will supply more protein required for body growth and tissue repair and development. This proportion is followed by Faba and Velvet beans of equal amount (25.74%). And these beans are considerably consumed in Europe and rarely used as staple food in Africa. Black gram follows closely in proportion (20.00%) and finally Jack bean (17:33%). These two beans are not widely consumed owing to their high phytochemical contents. For them to be safely eaten, they must undergo thorough processing.

But in Fibre content, both Jack bean and black gram are high (8.85% and 8.75% respectively). Which means they offer some physiological effect on the gastrointestinal tract of both humans and farm animals (when used as feed for these animals).

Ash content represents the mineral matter in the food material. It is used as a tool to measure the mineral content in any sample. So Soybean holds the highest in ash content (4.53%) as compared to the rest. Minerals to be gotten from processed Soybean. Cooked Soybean shows lower level of ash. This is why other processing methods are to be adopted.

Ether extract is more also in Soybean and as well decreases with heat treatment. Thus more better processing of this legume should be encouraged to conserve considerable amount of the supposed ether extract content present.

Phytochemical Composition

Exclusive to the phytochemicals of interest, which are phytate and oxalate. White which later was included out of curiosity was discovered to contain more oxalate (340.1mg/100g), followed by Jack bean (248.7mg/100g), Soybean(179.1mg/100g), Faba bean (13.1mg/100g) and Velvet bean (3.79mg/100g).

Oxalate are anti nutrients that can bind to calcium and other minerals forming insoluble complexes such as calcium oxalate. So higher proportion increases the risk of kidney stones. Therefore those will lower amount poses no threat of such. While those with more quantity should be thorough processed before consumed.

While phytate is higher in Jack bean (2593.3mg/100g) than in white bean (1808.0mg/100g), Faba bean (465.2mg/100g), Soybean (225.1mg/100g) and Velvet bean (91.16mg/100g).

Phytate are anti nutrients whose high amount can reduce the bioavailability of essential nutrients such Adirondack and Zinc, potentially leading to nutrient deficiencies.

Therefore, processing such as soaking, sprouting, or fermentation, boiling and cooking with calcium can help reduce these anti nutrients. Overall, the choice of legume depends on the health goals of individuals, preparation methods used, and specific dietary requirements. Understanding the phytochemical content, particularly oxalates and phytates, is crucial in making the most nutritionally balanced decisions when incorporating legumes into the diet.

CHAPTER SIX

6.1 CONCLUSION

In conclusion, legumes are a vital component of the human diet, providing essential nutrients like protein, fiber, vitamins, and minerals. They offer numerous health benefits, including their role in improving heart health, aiding digestion, and contributing to overall well-being. However, the presence of antinutritional compounds such as phytates and oxalates in legumes can impact mineral absorption and pose health risks, particularly for individuals prone to conditions like kidney stones or mineral deficiencies.

The study of legume composition, focusing on both conventional and under-utilized varieties such as jack bean, velvet bean, black gram, and faba bean, reveals significant variations in their nutritional and phytochemical content. While legumes like soybeans have higher levels of oxalates and phytates, which can hinder mineral absorption, other legumes like velvet beans and faba beans offer lower concentrations of these compounds, making them safer alternatives for those concerned with these antinutrients.

Processing techniques such as soaking, sprouting, and fermentation can effectively reduce the levels of phytates and oxalates, enhancing the bioavailability of essential minerals. This emphasizes the importance of incorporating such methods into legume preparation to maximize their nutritional benefits.

Ultimately, understanding the phytochemical content of legumes and their health implications is crucial for making informed dietary choices. By selecting and preparing legumes carefully, their nutritional potential can be fully realized, supporting health, food security, and sustainable diets, particularly in regions where legumes are a primary food source.

6.2 RECOMMENDATION

Soybean and white bean are highly recommended legumes as source of plant protein for this teeming population of the world today as it is helpful in reducing nutritional related problems such as protein-calorie malnutrition in Africa since animal protein is very expensive. But should undergo strict processing so as to minimize phytochemicals. Other non conventional legumes such as Velvet beans, Faba bean, Jack bean and Black gram bean, are to be evaluated more and subjected to more intense processing to allow for a wider consumption.

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