PROXIMATE COMPOSITION, TOTAL PHENOLS AND TANNIN CONTENT OF SOME LEGUMES

BY

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CERTIFICATION

This is to certify that the work titled **"**PROXIMATE COMPOSITION, TOTAL PHENOLS AND TANNIN CONTENT OF SOME LEGUMES", carried out by Daniela Erere OJEMUDIA with Mat. No AGR2000099, of the Department of Animal Science, Faculty of Agriculture, University Of Benin, Edo State, Nigeria.

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DEDICATION

This project is dedicated to God almighty for his protection, provision and preservation towards me throughout my stay in The University of Benin.

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ACRONYMS

JB- Jack beans

VB- velvet beans

SB- Soya beans

WB- White bean

BG- Black gram

FB- Faba beans

SEM- Standard error mean

ABSTRACT

This study investigated the proximate composition and phytochemical content of five economically important legume seeds: Soya Bean (SB), Faba Bean (FB), Black Gram (BG), Jack Bean (JB), White Bean (WB), and Velvet Bean (VB), to assess their nutritional quality and suitability for food and feed applications. Standard procedures were used to determine moisture, crude protein (CP), ether extract (EE), crude fibre (CF), ash, and nitrogen-free extract (NFE). Phytochemical screening included the quantification of total phenolics, flavonoids, tannins, saponins, oxalate, and phytate. Results of the proximate analysis showed that Soya Bean was the most nutritionally dense sample, recording the highest Crude Protein (42.0%) and Ether Extract (15.68%). Conversely, Black Gram was identified as the highest energy source, dominated by Nitrogen-Free Extract (62.04%). Phytochemical analysis revealed a critical trade-off: Jack Bean had the highest levels of beneficial antioxidants (Phenolics and Flavonoids) but also the highest concentration of the anti-nutritional factor (ANF) Phytate (2593.3 mg/100 mg). Soya Bean, despite its protein superiority, exhibited alarmingly high Oxalate content (1791.1 mg/100 g). In contrast, Velvet Bean presented the lowest overall concentration of key ANFs (Phytate and Oxalate), suggesting better mineral bioavailability. The study concludes that while SB and JB offer superior macronutrient and antioxidant profiles, their utilization requires mandatory processing techniques (such as soaking and fermentation) to mitigate the adverse effects of high anti-

nutritional factors and ensure optimal nutrient absorption and safety in human and animal diets.

CHAPTER ONE

1.0: INTRODUCTION

Legumes, classified under the family *Leguminosae* (or *Fabaceae*), represent one of the most valuable groups of plant foods consumed around the world. This group includes widely eaten seeds such as cowpea (*Vigna unguiculata*), soybean (*Glycine max*), groundnut (*Arachis hypogaea*), pigeon pea (*Cajanus cajan*), lentil (*Lens culinaris*), and chickpea (*Cicer arietinum*). They are especially important in developing nations, where they supply affordable, nutrient-rich foods and play a major role in enhancing food and nutrition security (Youssef et al., 1989). Legumes are well recognized for their impressive nutrient composition, which includes substantial amounts of protein, dietary fibre, minerals, vitamins, and complex carbohydrates that support healthy diets and help address protein-energy malnutrition.). Although this study focuses mainly on four types of beans namely; jack beans (*Canvaia ensiformis),velvet beans (mucuna sloanei),*soybeans (*glycine max),* black grams beans (*vinga mungo).*

In addition to their nutritive components, legumes contain a variety of phytochemicals, particularly phenolic compounds and tannins. These compounds act as secondary plant metabolites and contribute to the protective and functional properties associated with legume consumption (Tsao, 2010). Phenolics such as flavonoids, tannins, and phenolic acids have attracted great scientific interest due to their antioxidant, anti-inflammatory, antimicrobial, and disease-preventive effects (Ganesan et al, 2017). Tannins, a subgroup

of polyphenols, are especially notable for their ability to interact with proteins, influence digestibility, and enhance antioxidant potential (Duenas et al., 2015). Analysing the proximate compositn as well as the total phenolic and tannin contents of legumes provides valuable insight into their nutritional and functional value. Proximate analysis, which measures moisture, ash, crude protein, crude fat, crude fibre, and ether extract determination, is widely used to evaluate the basic nutritional quality of foods. Such information is important for dietary assessments, food formulation, and nutritional planning.

The quantification of phenolic compounds and tannins typically involves extraction using aqueous organic solvents such as methanol, ethanol, or acetone and tannic acid, followed by spectrophotometric or chromatographic techniques. The Folin–Ciocalteu reagent is commonly used to determine Total Phenolic Content (TPC), though it lacks specificity and can be influenced by other reducing agents (Singleton et al., 1999). Tannins are usually evaluated using colorimetric assays such as the Folin–Denis or vanillin method, which provide rapid estimates of tannin levels in plant foods.

With the rising interest in plant-based diets and functional foods, legumes have gained considerable attention not only for their nutrient density but also for their bioactive compounds that support overall health. Evaluating their proximate composition alongside phenolic and tannin content is essential for understanding their nutritional potential, therapeutic relevance, and suitability for use in food and health applications. This study

focuses on selected legumes with the aim of generating detailed nutritional and phytochemical information that can benefit researchers, nutritionists, food scientists, and public health professionals.

1.1 JUSTIFICATION OF THE STUDY

Legumes are widely consumed across the world due to their affordability, nutritional diversity, and long storage life. However, their nutritional and phytochemical composition varies widely among species, varieties, and processing conditions (Marathe et al., 2011). Studying their proximate composition is therefore crucial for evaluating their contributions to daily nutrient intake, particularly in communities where legumes serve as the primary protein source.

Apart from their macronutrient content, legumes are rich in phenolic compounds and tannins, which are known for their biological and pharmacological benefits. Phenolic compounds exhibit strong antioxidant capabilities that help reduce oxidative stress, neutralize harmful free radicals, and lower the risk of chronic diseases including diabetes, cardiovascular disorders, cancer, and neurodegenerative illnesses (Ganesan & Xu, 2017). Tannins, while sometimes regarded as antinutrients due to their ability to bind proteins, also play beneficial roles by enhancing antioxidant activity and contributing to the functional quality of foods (Duenas et al., 2015).

Evaluating the proximate composition as well as total phenolic and tannin contents of legumes is justified for several reasons:

1. **Nutritional relevance:** Understanding key nutrients such as protein, fibre, and fat helps guide dietary choices and nutrition planning.
2. **Food industry application:** Detailed information on bioactive constituents supports the development of functional foods and nutritionally enhanced products.
3. **Public health significance:** Phytochemicals such as phenols and tannins can help prevent diseases driven by oxidative stress, supporting population-level health strategies.
4. **Scientific and technological value:** Nutritional and phytochemical data contribute to research on food quality, safety, and product innovation.

Thus, this study is justified as it produces essential data that aid in nutritional evaluation, food product development, dietary recommendations, and public health improvement. It also supports growing scientific interest in legumes as valuable sources of both nutrients and health-promoting compounds.

1.2 OBJECTIVES OF THE STUDY

The main aim of this research is to determine the **proximate composition**, **total phenolic content**, and **tannin concentration** of selected legume samples.

The specific objectives are to:

1. Assess the proximate composition (moisture, ash, crude protein, crude fat and crude fibre) of various legumes.
2. Quantify the total phenolic compounds present in the legumes.
3. Determine the tannin content of the selected legume species.
4. Evaluate the nutritional implications of the proximate and phytochemical findings.
5. Compare the nutrient and bioactive compound profiles across different legumes.
6. Establish the importance of legumes as functional foods rich in both essential nutrients and health-promoting phytochemicals.
7. Provide relevant scientific information useful for food processing, diet formulation, and health-related recommendations.

CHAPTER TWO

2.0: LITERATURE REVIEW

2.1 DEFINITION AND CLASSIFICATION OF LEGUMES

Legumes belong to the botanical family *Fabaceae* (also known as *Leguminosae*), a large and diverse group of flowering plants whose seeds or pods are widely exploited as food, feed, green manure and cover crops. This family is traditionally divided into three primary sub- families: *Papilionoideae*, *Caesalpinioideae* and *Mimosoideae*. Classification within these groups is typically based on floral morphology (such as the shape of the petals and arrangement of stamens) and seed- pod (legume) structure (Morris, 365). The terminology "legume" itself derives from the Latin verb *legere*, which means "to gather," reflecting the traditional agricultural practice of harvesting pods from these plants.

A distinguishing agronomic feature of legumes is their capability to establish symbiotic associations with nitrogen- fixing bacteria (such as *Rhizobium* or *Bradyrhizobium*) in their root nodules. This symbiosis enables atmospheric nitrogen to be fixed into bioavailable forms, thereby enhancing soil fertility, improving cropping system sustainability and reducing the need for synthetic nitrogen fertilizers. From a nutritional and agronomic viewpoint, legumes can be categorised into different utilisation groups: food legumes (e.g., soybeans, common beans, Chickpeas), forage or green- manure legumes (used as cover crops or animal fodder), and oil- seed legumes (e.g., soybean, groundnut). Many of the widely consumed edible legumes, including beans (common

bean, kidney bean), lentils, chickpeas, peas, soybeans and peanuts falls within the *Papilionoideae* sub-family, and they are valued for their capacity to supply complex carbohydrates, dietary fibre, high-quality plant proteins and key micronutrients such as iron, folate and magnesium (Food and Agriculture Organization). For the specific context of this study, the four legumes selected: Jack beans (*Canavalia ensiformis*), Soybeans (*Glycine max*), Velvet beans (*Mucuna pruriens*) and Black gram (*Vigna mungo*) are all classified as food-legumes in the sense that their seeds are or can be consumed as human foods. The nutritional and phytochemical evaluation of these legumes is highly relevant in the context of functional foods, dietary diversification, food security, and the development of nutrient-dense plant-based protein sources.

Plate1**: Jack Beans (*Canavalia ensiformis)***

Plate 2: Velvet Beans (*Mucuna pruriens)*

Plate 3: Soya Beans (*Glycine max)*

Plate 4: Black gram beans (Vigna mungo)

2.2 Proximate Composition of Selected Legumes

The proximate composition of food legumes refers to the major nutrient fractions: moisture, ash (which approximates mineral residue after combustion), crude protein (nitrogen content × conversion factor), crude fat (ether extractable), crude fibre (insoluble plant fibre fraction) and total carbohydrate (often calculated by difference, or more specifically through nitrogen-free extract). Assessing the proximate composition provides foundational nutritional profiling of legumes and is critical to evaluate their value as dietary staples.

2.2.1 Soybeans (*Glycine max)*

Soybeans are among the most extensively studied legumes and are widely recognised for their high protein and oil content. Proximate composition values reported for raw soybeans typically indicate crude protein in the range of 35–40 %, crude fat about 18–22 %, crude fibre approximately 4–10 % and carbohydrate (by difference) around 30–40 %, although values vary depending on variety, agronomic conditions, seed moisture, and processing (e.g., dehulling). The high protein plus fat make soybeans relatively energy-dense and desirable for food and feed applications.

2.2.2Velvet beans (*Mucuna pruriens*)

Velvet bean, an underutilised legume species, has been gaining attention for its nutritional potential and role in addressing food insecurity. One study reported that seed-flour of M. pruriens contained crude protein values ranging from approximately

24.0–31.44 %, carbohydrate content 42.79–64.88 %, crude fibre between 5.3–11.5 % and crude fat varying from 4.1–14.39 % (Ezegbe et al.2023). Moisture content for white-seed accessions was reported at around 7.9 g/100 g (Kalidass et al. 2014). The range in values illustrates a significant variation depending on genotype, growing conditions and post-harvest processing. This demonstrates that velvet bean has the potential to compare favourably with more conventional legumes in terms of protein contribution, although further evaluation is required for consistency and functional quality.

2.2.3 Jack beans (Canavalia ensiformis)

Compared to soybeans and velvet beans, research on jack beans is more limited in both quantity and depth. In one study, jack bean flour was reported to have a proximate composition that included around 19.8 % crude protein, with starch and carbohydrate dominating (Praseptiangga et al.2022). Total phenolic content in jack bean flour was measured at 0.13 % in the same study, but detailed proximate values across many studies are lacking. The limited data suggest jack bean may be less studied but still holds promise as a nutrient-rich legume.

2.2.4 Black gram (*Vigna mungo*)

Black gram is widely consumed as a pulse in many parts of Asia and increasingly in Africa. While proximate composition varies by cultivar and region, reported values generally place crude protein at around 22–26 %, crude fibre at about 5–8 %, and carbohydrate (by difference) at approximately 50–60 %. Seed coat pigmentation and

other seed-morphological features have been found to correlate with differences in phytochemical composition (i.e., phenolic content) but also may correlate with fibre and micronutrient profiles.

2.2.5 Comparative Observations

The variation observed in proximate composition among different legume species, and even within the same species, can be attributed to multiple factors: species and genotype (different varieties), agronomic and environmental conditions (soil fertility, rainfall, light and temperature), maturity at harvest, and post-harvest handling (drying, storage). Additionally, seed-coat color (pigmentation) has been correlated with higher phenolic and fibre content in many legumes (dark-seeded types tend to have higher phenolics and often higher fibre). Given the uneven depth of published data especially for jack beans and black gram, there is a clear justification for conducting systematic investigations in these species to fill the gap and provide consistent comparative data.

2.3 Total Phenols and Tannins in Legumes

Bioactive compounds especially phenolics (also referred to as polyphenols) and tannins are increasingly explored in legumes because of their functional food potential, antioxidant capacity, and contribution to health-promoting properties. Evaluating both the proximate composition and phenolic/tannin content provides a more holistic view of legume nutritional-functional quality.

2.3.1 Definitions

- **Total Phenolic Content (TPC):** This refers to the aggregate content of phenolic compounds in a sample, encompassing free (extractable), conjugated (esterified or glycosylated), and bound (linked to cell-wall materials) forms. TPC is commonly expressed in milligrams of gallic acid equivalents (GAE) per gram or per 100 g of dry weight.
- **Tannins:** These are a subclass of polyphenolic compounds distinguished by their large molecular size and ability to form complexes with proteins and other macromolecules. They are often classified into hydrolysable tannins (which can break down into phenolic acids) and condensed tannins (proanthocyanidins).

2.3.2 Phenolics and Tannins in Soybeans

Soybeans are well studied for their phenolic composition, particularly isoflavones (a subgroup of flavonoids) such as genistein and daidzein, which contribute significantly to their functional properties. Soybeans also contain phenolic acids (e.g., caffeic acid, ferulic acid), flavonols, and some tannins, although the content of tannins is typically lower compared to darker-seeded legumes. These phenolic compounds are associated with antioxidant capacity and have been linked to beneficial health effects such as improved cardiovascular profiles.

2.3.3 Velvet Beans(*Mucuna pruriens)*

Velvet bean seed studies report substantial levels of phenolics and tannins. For example, total phenolic content (free + bound) in one variety of *M. pruriens* was estimated at about 7.09 % (i.e., 7.09 g per 100 g), and tannin content around 0.27–0.37 % in white germ plasms (Siddhuraju et al, 2020). Processing methods (such as soaking, autoclaving, germination) were found to significantly reduce phenolic and tannin contents. The high phenolic content in this under-utilised legume emphasizes its potential as a functional food, especially if anti nutritional factors can be managed.

2.3.4 Jack Beans(*Canavalia ensiformis)*

Jack bean flour was reported to have relatively low total phenolic content (0.13%) in a comparative study (Praseptiangga et al., 2022). This indicates that among the four legumes under study, jack beans may contain lower phenolic/tannin levels or have seed-traits (pigmentation, composition) that inhibit accumulation or extractability of these compounds. Nonetheless, the paucity of data mandates further investigation.

2.3.5 Black Gram(*Vigna mungo)l*

Black gram, like many pigmented-seed legumes, tends to display higher phenolic content particularly in its seed coat. Comparative studies have shown that pigmented beans (such as black-seed or red-seed varieties) often have higher total phenolic content than lighter-seed counterparts. Although specific values for black gram vary widely by variety

and region, the trend holds: seed coat color is a strong determinant of phenolic and tannin content in legumes.

2.3.6 Factors Influencing Phenolic and Tannin Levels

Various factors influence the concentration of phenolics and tannins in legumes:

- **Seed-coat colour and structure:** Darker, thicker seed coats often correlate with higher phenolic/tannin concentrations because these compounds tend to localise in the seed coat.
- **Genotype or variety:** Genetic differences influence phenolic biosynthesis pathways and accumulation.
- **Environmental and agronomic factors:** Soil fertility, rainfall, temperature, solar exposure, and nutrient availability affect phenolic synthesis.
- **Processing methods:** Soaking, germination, dehulling, cooking, autoclaving, fermentation, and other treatments can significantly reduce phenolic and tannin levels, sometimes by over 90 % (for example in M. pruriens) when certain processing regimes are applied.
- **Free vs. bound phenolics:** Many phenolics are linked to cell-wall polysaccharides or proteins (bound form) and are less extractable unless hydrolysis is applied. Bound phenolics may contribute significantly but are often underestimated in simple extraction protocols.

2.4 Distribution of Phenolics and Tannins within Legume Seeds

The internal distribution of phenolic compounds and tannins in legume seeds is non-uniform and depends on seed anatomy. Understanding this distribution is important because it influences extraction efficiency, bioavailability and functional properties.

- **Seed coat (testa or hull):** This layer frequently carries the highest concentration of phenolic compounds and tannins. In many legumes, such as black beans and red kidney beans, the seed coat has been shown to contain elevated levels of proanthocyanidins (condensed tannins) and flavonoids.
- **Cotyledons (the main seed body):** Although phenolic concentration tends to be lower here than in the seed coat, cotyledons still contain relevant amounts of flavonols, phenolic acids and other secondary metabolites.
- **Embryo, germ, integument and pods/leaves (adjacent tissues):** While less significant in the context of seed nutrient composition for food legumes, these tissues may contain flavones, isoflavones (especially in soybeans) and other phenolic metabolites.

In the context of the four selected legumes: black gram (particularly pigmented-seed varieties) is likely to have high seed-coat phenolic contents; velvet bean and jack bean may have lower seed-coat contributions but still meaningful levels; soybean exhibits a more distributed phenolic profile across seed coat and cotyledon, especially due to isoflavones located in the cotyledons and germ.

2.5 Chemical Characteristics and Functional Properties of Phenolics and Tannins

Phenolic compounds are chemically defined by one or more aromatic rings bearing hydroxyl (-OH) groups. Their structural complexity varies widely: simple phenolic acids (e.g., gallic acid, caffeic acid) represent the lower end of complexity, while polymers or oligomers such as condensed tannins (proanthocyanidins) and hydrolysable tannins occupy the higher end (Scalbert et al.,2005). The major classes relevant to legumes include: flavonoids (sub-classes such as flavonols, flavones, isoflavones, anthocyanins), phenolic acids (hydroxybenzoic and hydroxycinnamic acids), stilbenes, lignans and tannins.

Key functional properties of phenolics and tannins include:

- **Antioxidant capability:** They can donate electrons or hydrogen atoms to neutralise free radicals, chelate transition metals (iron, copper) that catalyse oxidative reactions, and interrupt oxidative chain reactions.
- **Anti-inflammatory action:** Some phenolic compounds modulate enzyme systems (such as cyclooxygenase (COX) and lipoxygenase (LOX)), suppress pro-inflammatory cytokines (e.g., TNF-α and IL-6) and thereby reduce inflammatory processes.
- **Antimicrobial activity:** Certain phenolics can inhibit bacterial, fungal or viral growth by disrupting microbial membranes or interfering with key microbial enzymes.

- **Protein- binding / anti-nutritional effect:** Tannins, due to their large molecular size and propensity to bind proteins and digestive enzymes, may reduce protein digestibility and nutrient availability this is especially relevant in legumes. (Pandey & Rizvi,2009).
- **Metal- chelating activities:** By binding to metals, phenolics reduce metal- catalysed oxidative damage.

In food legumes, the presence of phenolics and tannins influences not just health- promoting properties (antioxidant, anti- inflammatory) but also functional and anti-nutritional aspects such as changes in protein digestibility, flavour, astringency, color, and interactions with other nutrients. Thus, assessing tannin levels alongside total phenolics is particularly relevant when evaluating legume seed quality.

2.6 Biological and Health- Promoting Effects of Phenolics and Tannins in Legumes

Legumes rich in phenolic compounds and tannins have been linked to numerous beneficial physiological and biochemical effects:

- **Cardiovascular benefits:** Phenolic compounds improve endothelial function (for example via increased nitric oxide bioavailability), reduce LDL cholesterol oxidation and inhibit platelet aggregation—thereby reducing risks of atherosclerosis and thrombosis.

- **Antioxidant and anti-inflammatory benefits:** Phenolics neutralise reactive oxygen species (ROS), inhibit lipid peroxidation, protect DNA, proteins and lipids from oxidative damage, and regulate inflammatory mediators (such as TNF-α, IL-6) (Manach et al, 2004).
- **Anticancer potential:** Some legume-derived flavonoids and tannins have been shown to affect cellular signalling pathways (such as MAPK or PI3K/Akt), induce apoptosis in cancer cells, inhibit tumour angiogenesis, and suppress tumour growth.
- **Metabolic health benefits:** Phenolics improve insulin sensitivity, promote beneficial lipid metabolism, regulate carbohydrate-metabolising enzymes (e.g., HMG-CoA reductase), contribute to weight management and reduce markers of metabolic syndrome.
- **Gut health and microbiome modulation:** Phenolic compounds in legumes may act as prebiotic substrates for beneficial microbiota, reduce gut inflammation, improve intestinal barrier integrity and thereby enhance gut health.
- **Neuroprotective effects:** Some studies suggest phenolics reduce neuroinflammation, oxidative stress in neural tissues, modulate brain signalling pathways (e.g., BDNF, ERK/MAPK) and could support cognitive function and delay neurodegenerative disease.

When applied to the four legumes under study: soybeans, for instance, being rich in isoflavones, have documented health benefits; velvet beans and black gram, depending on their phenolic/tannin levels, may offer significant functional-food potential; jack beans while less documented may nonetheless contribute phytochemicals worth exploration.

2.7 Factors Affecting Proximate, Phenolic and Tannin Content in Legumes

The nutrient and phytochemical composition of legume seeds is shaped by a combination of genetic, environmental, anatomical and processing-related factors:

- **Genetic variety / cultivar:** Differences in genotype influence storage protein profiles, seed-coat pigmentation (which is related to phenolic/tannin accumulation), enzyme systems for phenolic biosynthesis, and overall nutrient allocation.
- **Environmental and agronomic conditions:** Soil type and fertility, rainfall distribution, solar radiation, temperature, plant spacing, pesticide/fertiliser use, and harvest maturity all influence seed composition and phytochemical content.
- **Seed-coat colour and texture:** Darker and thicker seed coats tend to correlate with higher phenolic and tannin concentrations; seed coat thickness and the presence of pigments (anthocyanins, proanthocyanidins) influence both nutrient and phytochemical content, as well as extractability (e.g., darker-seed legumes show higher TPC) (Praseptiangga et al., 2022).

- **Post-harvest processing and storage conditions:** Drying method and temperature, storage period, maize moisture, microbial action or oxidation can degrade nutrients and phytochemicals over time.
- **Pre-treatment and processing methods (soaking, dehulling, germination, cooking, autoclaving, fermentation):** These processes can significantly modify proximate composition (for example reducing antinutritional factors, altering digestibility), and can reduce phenolic/tannin levels (sometimes drastically). In *Mucuna pruriens*, for example, some processing treatments reduced phenolics and tannins by more than 90 % (Ezegbe et al., 2023; Mugendi et al., 2020).
- **Analytical extraction and measurement protocols:** The form of phenolic (free vs bound), the particle size of the sample, solvent type and concentration, extraction time and temperature, pH, hydrolysis (for bound phenolics) and detection method all influence measured values. Without standardisation, comparisons across studies may be problematic.

2.8 Analytical Methods for Determining Proximate Composition, Total Phenols and Tannin Content in Legumes

2.8.1 Proximate Composition

Proximate analysis typically follows standardised procedures for example those specified by the Association of Official Analytical Chemists (AOAC). Key methods include:

- Moisture determination: oven- drying at a specified temperature and duration.
- Ash determination: incineration of a known sample at high temperature until mineral residue remains.
- Crude protein: Kjeldahl method (nitrogen determination × conversion factor) or Dumas combustion method.
- Crude fat (ether extract): extraction of lipids using ether or suitable solvent.
- Crude fibre: sequential acid and base digestion of ash- free residue to isolate fibre fraction.
- Carbohydrate: often calculated by difference (100 % minus sum of moisture, ash, protein, fat and fibre), or alternately by measurement of nitrogen- free extract (NFE).

2.8.2 Total Phenolic Content (TPC) and General Phenolics

I. The Folin–Ciocalteu assay remains the most common method for estimating total phenolics. It is based on the reduction of the reagent by phenolic compounds and yields results expressed as gallic acid equivalents (GAE). Although rapid and relatively simple, this assay is non- specific—other reducing substances may interfere (Singleton et al.1999).

II. Chromatographic methods such as High- Performance Liquid Chromatography (HPLC) (often with UV/Vis detection or diode array detector (DAD)) or HPLC coupled with Mass Spectrometry (MS) enable separation, identification and

quantification of individual phenolic compounds (Ignat, Volf & Popa, 2011). Ultra-High Performance Liquid Chromatography (UHPLC) is increasingly used for higher throughput and sensitivity.

2.8.3 Tannin Determination

I. Condensed tannins (proanthocyanidins) are frequently measured by the vanillin-HCl assay; this colorimetric method is relatively simple.

II. Hydrolysable tannins may be estimated with modified Folin-Ciocalteu or Folin-Denis methods, or by protein-precipitation assays (e.g., bovine serum albumin precipitation) to assess their biological activity.

III. More advanced techniques such as HPLC or HPLC-MS enable detection and quantification of monomeric or oligomeric flavan-3-ols, proanthocyanidins or tannin oligomers.

2.8.4 Extraction Protocols for Phenolics and Tannins

I. **Sample preparation:** Legume seeds must be cleaned, dried (to constant weight), de-hulled (if applicable), defatted (especially for oil-rich seeds, using solvents such as hexane), and ground into fine powder to enhance extraction efficiency. For example, in one jack bean study, flour was produced with a particle size < 500 μm. (Praseptiangga et al, 2022)

II. **Solvent extraction:** Aqueous organic solvents—commonly ethanol, methanol, acetone (often in 50–80 % v/v with water)—with or without acidification (e.g., 0.5 % acetic acid) are broadly used. For instance, one study reported that 70 % acetone with 0.5% acetic acid was optimal for extracting TPC from black beans and lentils (Xu & Chang, 2007).

III. **Hydrolysis of bound phenolics:** Because many phenolic compounds are bound to cell-wall components (polysaccharides, proteins) they may not be extracted readily by conventional solvents. Acid or alkaline hydrolysis (or enzymatic treatment) is often required to release bound phenolics.

IV. **Advanced extraction techniques:** To improve efficiency, reduce solvent usage and shorten extraction times, techniques such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) and pressurised liquid extraction (PLE) are increasingly employed (Sasidharan et al.2020).

V. Proper control of extraction variables (solvent polarity, temperature, time, pH, particle size, solvent-to-solid ratio) is essential to ensure repeatable and meaningful results.

2.9 Gaps in the Literature and Relevance to the Current Study

A review of available literature reveals that while some legumes (particularly soybeans) are well researched in terms of their proximate composition and phenolic/tannin profiles,

significant gaps remain especially for lesser-utilized legumes such as jack beans, velvet beans and black gram. Notable gaps include:

- **Jack beans:** There are very few comprehensive studies reporting proximate, total phenolic and tannin content concurrently. The limited data that exist (e.g., total phenolics of 0.13 % in one jack bean flour study) suggest low phenolic content, but the breadth of data is insufficient for robust conclusions (Praseptiangga et al, 2022).
- **Velvet beans:** While some studies have reported proximate composition and anti-nutritional factors, fewer investigations have systematically reported total phenolic and tannin contents under standardized conditions across varieties and processing methods. For example, one study reports TPC 7.09 % and tannins 0.27–0.37 % in *M. pruriens* (Siddhuraju et al, 2020).
- **Black gram:** Although widely consumed, especially in Asian countries, there is limited literature reporting detailed phenolic/tannin quantification across varieties, seed-coat colours, processing states and agro-ecological contexts.
- **Comparative studies across the four legumes under identical conditions:** Few studies have simultaneously compared proximate composition, total phenolic content and tannin content in jack beans, soybeans, velvet beans and black gram under consistent analytical protocols, seed-preparation and extraction methods.

In this chapter, the definitions and classification of legumes were presented, followed by an in-depth examination of proximate composition for the four selected legumes (soybeans, velvet beans, jack beans and black gram). The biological significance, chemical characteristics, and distribution of phenolic compounds and tannins in legumes were discussed, along with analytical methodologies for measurement. Factors influencing nutrient and phytochemical content were outlined, and gaps in the existing literature were identified particularly the lack of comprehensive data for under-utilized legumes and lack of comparative studies across legume types. This literature review establishes a solid foundation for your subsequent chapters, which will detail the methodology, present empirical data, analyze results and situate findings within the broader scientific context.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SOURCES OF MATERIALS

Raw sample of jack bean (*Canavalia ensiformis*), velvet bean (*Mucuna pruriens*), soya beans*(Glycine max*), black gram(*Vigna mungo*) and faba beans(*Vicia faba*) 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 laboratory blender, and sieved through a 60-mesh sieve. The flour was stored in airtight containers at 4°C until used for proximate and polyphenol analyses.

3.3 PROXIMATE ANALYSIS

The proximate composition of the bean samples was determined following the procedures of the Association of Official Analytical Chemists (AOAC, 2019)**.**The parameters analyzed included moisture, ash, crude protein, crude fat, 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 casein (1% w/v), phosphate buffer (0.1 M, pH 7.6), trichloroacetic acid (TCA, 5%), hydrochloric acid (HCl), sodium hydroxide (NaOH), petroleum ether, sulphuric acid, boric acid, copper sulphateand selenium catalyst mixture, and distilled water.

3.3.2 APPARATUS AND EQUIPMENTS

Analytical weighing balance, laboratory blender, 60-mesh sieve, Soxhlet extractor, Kjeldahl digestion and distillation unit, muffle furnace, centrifuge, water bath (37°C), UV–Visible spectrophotometer (280 nm), and standard.

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 103°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;

(W0) = weight of empty crucible

(W1) = weight of crucible + sample before drying

(W2) = 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. 2-5g 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 1hour30mins then cooled in a desiccator and weighed.

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

3.3.6: Crude Lipid (Ether Extract) Determination: The soxhlet equipment model 520 with heating module, condenser and evaporation systems was the soxhlet extractor used for crude lipid analysis. Bean flour (10 g) was weighed into the soxhlet extraction thimble. Ether (200ml) was used for the extraction process for a period of 6 hours. Following extraction and evaporation the residue was removed from soxhlet system, cooled, weighed and the oil content of each sample was computed by calculation: 6 Ether Extraction weight of lipid x 100 weight of sample.

3.3.7: 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 Whatmanfilter 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

3.3.8: CRUDE PROTEIN DETERMINATION

The conventional Kjedahl procedure in which sample was digested, distilled and titrated was used for all crude protein determination for each sample analyzed. 1g was weighed

into kjedahl flask. Ig of selenium catalyst was added followed by 10ml of H,504. The flask was placed in gestion system and heated until solution became colorless or light green. Next, flask was moved from heating module and cooled. Using the Tecator nitrogen analyzer fitted with provision for sodium hydroxide and boric acid the solution was distilled and titrated and the Instrument reading was recorded as nitrogen content per gram of sample. Crude protein was thus obtained by multiplying instrument reading with the recommended factor 6.25 (A.O.A.C. 1990, 795).

3.3.9 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: DETERMINATION OF TOTAL PHENOLIC CONTENT

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.

3.4.2: 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).

3.4.3: 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 2.5mLof 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.

3.4.4: 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 1 hr 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.

3.4.5: 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

3.4.6: OXALATE DETERMINATION

About 1g of the sample was added to 75 ml of 1.5N H2SO4, 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 KMnO4 solution to a faint pink colour endpoint. Oxalate = (titre value × 0.9004) mg/g

3.5: STATISTICAL ANALYSIS

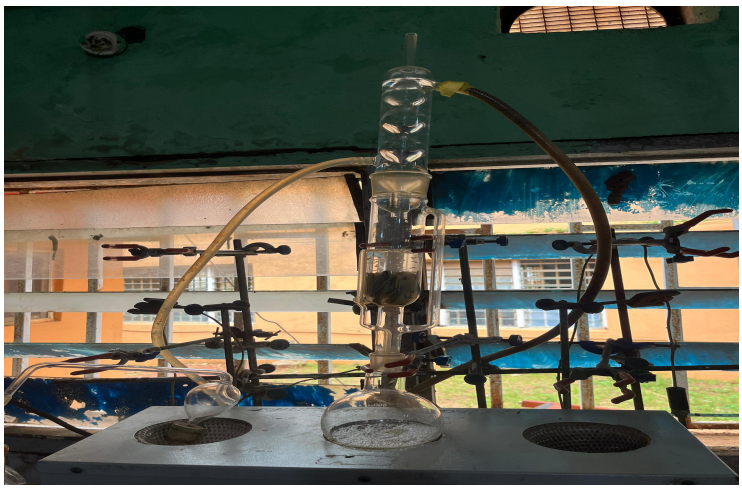

Plate 5: Soxhlet Extractor

Plate 6: Process of protein distillation

Plate 7: Process of protein Digestion

CHAPTER FOUR

RESULTS

This chapter deals with ***Results,*** which deals with the presentation of results obtained from the study.

Table 4.1: The Result of the Proximate Analysis of various legume seed.

Sample	Moisture content	CP	EE	CF	ASH	NFE
SB	3.61	42.0	15.68	5.75	3.03	28.43
FB	5.87	25.74	8.96	9.00	4.53	47.46
BG	4.79	20.00	6.10	5.47	1.60	62.04
JB	5.82	17.33	9.71	10.00	1.92	55.22
VB	1.30	25.74	5.65	4.69	3.79	58.83

CODE:

JB- Jack bean

FB- Faba bean

WB- White bean

SB- Soya bean

VB- Velvet bean

BG- Black gram

SEM- Standard error mean.

This table compares the basic nutritional composition of five different types of beans. The values are percentages (%), meaning that for every 100 grams of dry bean, this is the breakdown of its content.

MC% (Moisture Content):

The represents amount of water present in the sample. Lower moisture is generally better for storage, as it prevents microbial growth and spoilage. All samples have relatively low moisture, with Velvet Bean (VB) being the driest (1.30%) and Faba Bean (FB) having the highest (5.87%).

CP% (Crude Protein):

This estimates the total protein content. Soybean (SB) has the highest protein content (42.0%), making it an exceptional source. The others, especially Black Gram (20.00%), Jack Bean (17.33%), and Velvet Bean (25.74%), have significantly lower protein content.

EE% (Ether Extract / Crude Fat):

This estimates the total fat or lipid content present in the sample. Faba Bean (FB) and Jack Bean (JB) have the highest fat content (9-10%), which contributes to their energy density. Soybean (SB) has a moderate amount (15.68%), while Velvet Bean (VB) is very low (5.65%).

CF% (Crude Fiber):

This measures the indigestible cellulose, lignin, and other fibrous components. It gives a rough estimate of dietary fiber. Jack Bean (JB) has the highest fiber content (10.00%), followed by Faba Bean (FB) at 9.00%. High fiber is good for digestive health but can lower the overall digestibility of the feed for monogastric animals (like poultry and pigs).

ASH% (Ash Content):

This represents the total mineral content, the inorganic residue left after the sample is completely burned. Faba Bean (FB) has the highest ash content (4.53%), suggesting a richer mineral profile. Black Gram (1.60%) and Jack Bean (1.92%) have the lowest.

NFE% (Nitrogen-Free Extract):

This is not measured directly but calculated. It represents the easily digestible carbohydrates, primarily starches and sugars.

Calculation: `NFE% = 100% - (MC% + CP% + EE% + CF% + Ash %)

This is the main energy source in most feeds. Black Gram (BG) has the highest NFE (62.04%), meaning it is predominantly starchy. Soybean (SB) has the lowest (28.43%) because its composition is dominated by protein and fat instead.

Table 4.2: The Result of the phytochemical contents of various legume seed extracts.

Sample	1	2	3	4	5	6	SEM
JB	163.4^{bc}	111.5^{cd}	22.4^{de}	4.8^{e}	248.7^{b}	2593.3^{a}	32.3
FB	87.5^{b}	56.3^{bc}	32.7^{cd}	3.5^{d}	13.1^{d}	465.2^{a}	10.6
WB	97.1^{c}	57.0^{d}	21.6^{e}	4.6^{f}	340.1^{b}	1808.0^{a}	3.4
SB	150.1^{c}	84.8^{d}	50.5^{e}	5.6^{f}	1791.1^{a}	225.1^{b}	2.4
VB	55.89^{b}	15.04^{d}	27.17^{c}	5.67^{e}	3.79^{e}	91.16^{a}	1.3

Code

1 = Total phenolic content (g TAE/kg)

2 = Flavonoid content (g QE/kg)

3 = Total Tannins content (g TEA/kg)

4 = Saponin (g/kg)

5 = Oxalate (mg/100g)

6 = Phytate (mg/100mg).

4.2: Phytochemical Contents of Various Legume Seed Extracts

This table presents the quantitative results of the phytochemical contents of different legume seed extracts, namely JB (Jack Bean), FB (Fava Bean), WB (White Bean), SB (Soy Bean), and VB (Velvet Bean). The analyzed parameters include total phenolic content, flavonoid content, total tannin content, saponin, oxalate, and phytate levels.

4.2.1: Total Phenolic Content (g TAE/kg)

Total phenolic compounds are important antioxidants that help in scavenging free radicals. Among the samples, Jack Bean (JB) exhibited the highest phenolic content (163.4 g TAE/kg), followed closely by Soy Bean (SB) (150.1 g TAE/kg), while Velvet Bean (VB) had the lowest (55.89 g TAE/kg). This suggests that JB and SB may possess stronger antioxidant properties compared to the others.

4.2.2: Flavonoid Content (g QE/kg)

Flavonoids contribute to anti-inflammatory and antioxidant activities in legumes. The highest flavonoid content was recorded in Jack Bean (111.5g QE/kg), whereas Velvet Bean (15.04g QE/kg) contained the least. This indicates a wide variation in flavonoid accumulation among the legumes.

4.2.3: Total Tannin Content (g TEA/kg)

Tannins are poly-phenolic compounds that can influence the taste and nutritional value of legumes. The Soy Bean (50.5g TEA/kg) showed the highest tannin content, while White Bean (21.6g TEA/kg) had the lowest. High tannin levels may reduce protein digestibility but can also provide health-promoting antioxidant effects.

4.2.4: Saponin (g/kg)

Saponins are bioactive compounds known for their cholesterol-lowering and immune-boosting properties. The saponin content across all samples was generally low, ranging from 3.5 g/kg in Fava Bean (FB) to 5.67 g/kg in Velvet Bean (VB).

4.2.5: Oxalate (mg/100 g)

Oxalate levels were notably high in some legume species, with Soy Bean (1791.1 mg/100 g) and White Bean (340.1 mg/100 g) recording the highest values. High oxalate concentration can interfere with calcium absorption, hence excessive consumption should be moderated.

4.2.6: Phytate (mg/100 mg)

Phytate content, which can act as an anti-nutritional factor by binding essential minerals, varied widely among the samples. The Jack Bean (2593.3 mg/100 mg) exhibited the highest phytate concentration, followed by White Bean (1808.0 mg/100 mg), while Velvet Bean (91.16 mg/100 mg) had the lowest.

CHAPTER FIVE

5.0: DISCUSSION

5.1 Proximate Composition of Some Legumes

This chapter interprets the results of the proximate and phytochemical analyses of the selected legume seeds (Soya Bean, Faba Bean, Black Gram, Jack Bean, White Bean, and Velvet Bean) presented in Chapter Four. The discussion focuses on the nutritional implications of the major components and the health or anti-nutritional consequences of the detected phytochemicals, relating these findings to the suitability of each legume for food and feed applications.

Moisture Content (MC):

The moisture content of all tested legume seeds was observed to be relatively low, ranging from 1.30% (Velvet Bean, VB) to 5.87% (Faba Bean, FB). Low moisture content is a crucial factor for ensuring the preservation and prolonging the shelf life of stored seeds. A low MC reduces water activity, thereby inhibiting the proliferation of microbes (fungi and bacteria) and minimizing enzymatic activity, which causes spoilage. The very low MC of the VB suggests superior storage stability compared to the other samples under similar conditions.

Crude protein (CP):

The crude protein (CP) content exhibited the widest variation, ranging from 17.33% (Jack Bean, JB) to an exceptional 42.0% (Soya Bean, SB). The SB results confirm its global standing as a superior source of plant-based protein, making it an invaluable component for formulating high-protein human diets and commercial livestock feed. Conversely, legumes like JB and Black Gram (BG) (with 17.33% and 20.00% CP, respectively) would primarily serve as supplementary protein sources, or as energy feeds where protein fortification is necessary. The moderate protein content in FB, VB, and BG (25.74% and 20.00% CP) positions them well for general nutritional roles, often replacing cereals in traditional diets.

Crude Fat (EE):

Soya Bean (SB) recorded a high Ether Extract (EE) of 15.68%, which contributes significantly to its high energy density. This justifies its industrial use for edible oil extraction. Other legumes, like JB and FB, show moderate fat levels (around 9-10%). The variation in EE content influences both the caloric value and the potential for lipid oxidation during storage.

Crude Fibre (CF):

In terms of Crude Fibre (CF), Jack Bean (JB) and Faba Bean (FB) showed the highest values (10.00% and 9.00%, respectively). While a high CF is beneficial for digestive

health in humans and ruminants, providing bulk and promoting satiety, it may reduce the overall digestibility and nutrient utilization efficiency in mono gastric animals (e.g., poultry and swine).

Ash content (Ash):

The Ash content, an indicator of total mineral composition, was highest in Faba Bean (FB) at 4.53%. This suggests that FB has a richer macro- and micro-mineral profile, potentially making it a valuable source of essential dietary minerals.

Nitrogen free extract (NFE):

Conversely, the Nitrogen-Free Extract (NFE), which primarily represents easily digestible carbohydrates (starches and sugars), was highest in Black Gram (BG) at 62.04% and lowest in Soya Bean (SB) at 28.43%. This confirms the distinct nutritional roles of the legumes: BG functions predominantly as an energy source, while SB is primarily a protein and fat source.

5.2 Discussion of Phytochemical Contents (Table 4.2)

The phytochemical analysis reveals significant variations in bioactive and anti-nutritional factors (ANFs) among the legume species, which are critical determinants of their consumption safety and processing requirements.

Phenolic content of some legumes:

Jack Bean (JB) showed the highest concentrations of both Total Phenolic Content (163.4g TAE/kg). These compounds are renowned for their antioxidant, anti-inflammatory, and cardio protective properties. The high levels found in JB and, to a lesser extent, SB (Total Phenolics: 150.1gTAE/kg) suggest that these legumes possess superior potential as functional foods compared to Velvet Bean (VB) and White Bean (WB).

Tannin content of some legumes:

The highest Total Tannin content was observed in Soya Bean (50.5 g TEA/kg). Tannins, although they contribute to antioxidant activity, are considered ANFs because they can form indigestible complexes with proteins, thereby reducing protein absorption.

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CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Based on the proximate and phytochemical analyses conducted on the five legume seed samples, the following conclusions are drawn:

6.1.1 Proximate Composition

The study confirmed the diverse nutritional profiles of the legumes, affirming their varying suitability for dietary and feed formulations:

- Soya Bean (SB) is confirmed as an outstanding protein and lipid source, exhibiting the highest Crude Protein (42.0%) and Crude Fat (15.68%) content, making it a valuable candidate for commercial protein concentrates and oil production.
- Black Gram (BG) emerged as the primary energy source, possessing the highest Nitrogen-Free Extract (NFE) content (62.04%), signifying its high easily digestible carbohydrate content.
- Velvet Bean (VB) demonstrated the best storage potential with the lowest Moisture Content (1.30%).
- Faba Bean (FB) showed the richest mineral profile, indicated by the highest Ash content (4.53%).

6.1.2 Phytochemical Content

The analysis of anti-nutritional factors (ANFs) and bioactive compounds provided critical insights into processing needs:

- Jack Bean (JB) contained the highest levels of beneficial Total Phenolics and Flavonoids, suggesting potent antioxidant properties. However, its nutritional utility is severely compromised by the highest Phytate content (2593.3 mg/100 mg), which restricts mineral bioavailability.
- Soya Bean (SB), despite its superior macronutrient profile, presents significant ANF challenges due to its exceptionally high Oxalate content (1791.1 mg/100 g) and high Tannin levels, necessitating aggressive processing to ensure safe consumption and high nutrient utilization.
- Velvet Bean (VB) is the most bioavailable option, recording the lowest levels of the key ANFs, Oxalate and Phytate, which suggests a lower inhibitory effect on mineral absorption compared to the other samples.

In summary, while Soya Bean offers the highest macronutrient density, Jack Bean and Soya Bean require mandatory and intense processing to mitigate the effects of their high anti-nutritional compounds.

6.2 Recommendations

Based on the observed nutritional value, anti-nutritional factors, and potential applications, the following recommendations are put forth:

6.2.1 Recommendations for Processing and Utilization

1. Mandatory ANF Reduction for Jack Bean and Soya Bean: It is strongly recommended that Jack Bean and Soya Bean undergo rigorous processing, such as soaking, germination, and fermentation, before incorporation into human or animal diets. These techniques are proven to significantly reduce Phytate and Oxalate levels, thereby increasing protein and mineral bioavailability.
2. Strategic Application based on Profile:
    - Soya Bean should be prioritized for high-protein applications (e.g., weaning diets, protein supplements) only after certified ANF reduction.
    - Black Gram is best utilized as a primary energy feed component, especially when paired with a protein supplement.
    - Velvet Bean should be promoted in regions where minimal processing capacity exists, given its inherently low ANF levels, offering a nutritionally safer and more accessible option.
3. Optimal Handling of Fibre-Rich Samples: Due to the high Crude Fibre in Jack Bean and Faba Bean, their inclusion in monogastric animal feeds (e.g., pigs or

poultry) should be managed carefully to avoid reducing the overall digestibility of the feed.

6.2.2 Recommendations for Future Research

1. **Digestibility and Bioavailability Trials:** Future studies should focus on in vivo digestibility trials (using suitable animal models) to precisely quantify the true protein and energy utilization rates, especially after ANF reduction processes are applied to Jack Bean and Soya Bean.
2. **Mineral Profiling and Bioavailability:** A detailed analysis of the mineral content (e.g., iron, zinc, calcium) of the most mineral-rich sample (Faba Bean) and subsequent mineral bioavailability studies are required to confirm the benefits suggested by the high Ash content.
3. **Long-term Storage and Quality Assessment:** Investigate the long-term storage stability of Soya Bean, given its high fat content and susceptibility to rancidity, versus the highly stable Velvet Bean, under various environmental conditions.

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