DEVELOPMENT OF THE MINERAL AND ANTI NUTRIENT PROPERTIES FROM CARDABA BANANA, BAMBARA GROUNDNUT AND BEETROOT FOR BLOOD GLUCOSE REGULATION

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CERTIFICATION

This is to certify that this research project submitted to the department of Medical

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DEDICATION

This project is dedicated first and foremost to GOD ALMIGHTY, my source of life and Inspiration . Also ,special dedication to my parents Mr. And Mrs. OSAZUWA for their Immeasurable investment in my life.

ACKNOWLEDGEMENT

With a grateful heart , I want to express my gratitude to God Almighty who is my friend ,my

sustainer ,counsellor, guardian and my protector .My gratitude also goes to my parents

For providing the resources used in funding this project .With a deep sense of respect, joy

And appreciation, I wish to express my utmost gratitude to my ever supportive friendly,

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unwavering support ,motivation, bank of knowledge and patience with which she

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Big Thank!!

ABSTRACT

This study developed functional anti-diabetic snack bars using composite flours from Bambara groundnut, Cardaba banana, beetroot, guava leaf, and cinnamon, and assessed their mineral and antinutrient compositions in relation to blood glucose regulation. Three formulations (Samples A, B, and C) were analyzed for key minerals magnesium, zinc, calcium, iron, and Phosphorus and antinutrients such as phytates, oxalates, and cyanogenic compounds. Results revealed significant variations among samples. Sample B contained the most favourable mineral profile, with the highest levels of magnesium, zinc, and phosphorus, alongside low phytate content, indicating strong potential to support insulin activity and glucose metabolism. Sample A had high calcium but elevated antinutrients, while Sample C showed low antinutrient levels but reduced mineral density. Overall, Sample B demonstrated the best balance between nutrient richness and minimal antinutrient interference, highlighting the potential of indigenous crops in formulating functional foods for glycaemic management.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Diabetes mellitus, especially type 2 diabetes (T2DM), has become one of the biggest metabolic problems in the world. It is a long-term condition where the body has high levels of sugar in the blood, either because the body isn't making enough insulin or isn't using it properly (World Health Organization [WHO], 2022). The rising number of people with T2DM is a big concern for public health, and things like what people eat and how they live are major reasons for this trend. The International Diabetes Federation (IDF, 2025) estimates that around 589 million adults have diabetes now, and this number is expected to go up to 853 million by 2050. In Nigeria, recent data shows that about 7% of adults have diabetes, meaning millions are affected by the disease (Chukwuma *et al.,* 2024). This growing problem shows how important it is to find good ways to change diets to help control blood sugar levels. Dietary modification remains a cornerstone in the management and prevention of T2DM, with increasing emphasis on functional foods that possess low glycemic indices and high micronutrient densities (American Diabetes Association [ADA], 2023). Functional foods are those that provide health benefits beyond basic nutrition, including the modulation of blood glucose and lipid levels (Granato *et al.,* 2020). The development of composite flour-based products using locally available and underutilized crops such as Bambara groundnut (*Vigna subterranea*), Cardaba banana (*Musa ABB*), and beetroot (*Beta vulgaris L.*) represents a sustainable and nutritional approach to formulating functional foods targeted at glycemic control. These crops contain essential minerals that play key roles in glucose metabolism. For instance, magnesium acts as a cofactor in glucose oxidation, zinc supports insulin synthesis, and potassium contributes to cellular glucose uptake (Nielsen, 2018; Chimienti *et al.,* 2020). Incorporating these ingredients into biscuit formulations may provide a convenient and palatable vehicle for improving dietary mineral intake and glycemic control.

1.2 AIM OF THE STUDY

The aim of the study was to formulate and develop functional snack from the underutilized crop to assess their functionality in blood glucose regulation in lowering diabetic friendly rats.

1.3 OBJECTIVE OF THE STUDY

- Formulate composite flour blend from Bambara groundnut, Cardaba banana, and beetroot, cinnamon and guava leaf in varying proportions for suitable biscuits production.
- Determine the mineral composition (e.g magnesium, zinc iron, potassium and calcium) of the developed biscuits and assess their unique significance.
- Analyze the antinutrient contents (phytates tannins, oxalates and saponin of the biscuits.

1.4 JUSTIFICATION OF STUDY

Diabetes remains a major public health challenge in Nigeria and 8globally, contributing significantly to morbidity and mortality. Nigeria possesses a wide variety of indigenous plants rich in bioactive compounds with proven blood-glucose-lowering effects. Developing functional foods that incorporate these anti-diabetic ingredients offers a promising strategy for dietary management of diabetes. Although several studies have examined the properties of individual Nigerian plant species, there is still a need for more comprehensive research on formulating and evaluating functional foods made from combined ingredients. Promoting such functional snacks can enhance public awareness of diet-based diabetes management and empower individuals to make healthier nutritional choices.

CHAPTER TWO

LITERATURE REVIEW

2.1 FUNCTIONAL FOOD AND BIOACTIVE COMPOUNDS

Functional foods have gained increasing global attention due to their potential to promote health and reduce the risk of chronic diseases beyond their basic nutritional value. Unlike conventional foods that primarily provide macronutrients and micronutrients, functional foods contain additional biologically active components known as bioactive compounds. These compounds exert physiological effects that support metabolic balance, immune function, antioxidant defense, and chronic disease prevention. Recent advances in food science, biotechnology, and nutrition have expanded the formulation and application of functional foods across populations (Zainuddin *et al.,* 2024). Functional foods are defined as natural or processed foods that contain biologically active components which, when consumed in effective and safe amounts, provide health benefits beyond basic nutrition. This definition is widely supported by recent literature and functional food regulatory frameworks (Functional Food Center, 2024).

Functional foods may be classified into the following categories:

- Conventional Functional Foods

These are whole foods naturally rich in bioactive compounds. Examples include fruits, vegetables, nuts, legumes, grains, and fermented foods. They deliver health benefits through the synergistic presence of phytochemicals, fiber, vitamins, minerals, and antioxidants (Martínez-Álvarez *et al.,* 2023).

- Modified or Fortified Functional Foods

These foods are enriched with additional nutrients or beneficial microorganisms to enhance their physiological roles. Examples include omega-3 enriched eggs, vitamin-fortified cereals, and probiotic yogurts. Fortification enhances the health-promoting properties of such foods, supporting targeted nutritional needs (Singh and Verma, 2023).

- **Bioactive compounds**

Bioactive compounds are naturally occurring, non-essential components in foods that exert biological effects, promoting health and influencing metabolic functions as well as the development or progression of diseases (Kaur and Ahmed, 2024).Bioactive compounds are grouped into several key categories:

- Polyphenols and Flavonoids: Found in fruits, vegetables, tea, cocoa, and grains; exhibit strong antioxidant and anti-inflammatory activity (Zhang *et al.,* 2024).
- Carotenoids: Present in colored fruits and vegetables such as carrots, tomatoes, and leafy greens; support vision and oxidative stress reduction.
- Dietary Fiber and Prebiotics: Enhance digestive health, modulate gut microbiota, and improve glycemic control (Slavin, 2023).
- Fatty Acids (ω-3, ω-6): Found in nuts, seeds, and fish; promote cardiovascular and metabolic health.
- Probiotics and Fermented Products: Improve gut microbiota, immunity, and nutrient absorption (Nayak *et al.,* 2025).

2.1.1 IMPORTANCE OF FUNCTIONAL OF FOODS

Functional foods are foods that provide health benefits beyond basic nutrition because they contain bioactive compounds capable of promoting well-being and reducing the risk of chronic diseases (Rodriguez-Casado, 2021). Their importance lies in their ability to act as adjuncts to conventional therapy, especially in the prevention and management of non-communicable diseases such as type 2 diabetes mellitus (T2DM), cardiovascular diseases, obesity, and certain cancers. One of the most significant roles of functional foods is in metabolic regulation, particularly glucose homeostasis. Bioactive compounds such as polyphenols, flavonoids, saponins, and tannins modulate insulin sensitivity, inhibit carbohydrate-digesting enzymes, and reduce oxidative stress, thereby lowering blood glucose levels (Ojo *et al.,* 2021). Similarly, dietary fibers and resistant starches from legumes, fruits, and whole grains delay glucose absorption and blunt postprandial glucose spikes, which is vital for diabetes control (Birt *et al.,* 2020).Functional foods are also important in cardiovascular protection. Phytochemicals such as plant sterols and omega-3 fatty acids reduce serum cholesterol, lower blood pressure, and improve vascular health, thereby reducing cardiovascular risk (Esposito *et al.,* 2019). Furthermore, probiotic- and prebiotic-containing foods help maintain gut health, enhance nutrient absorption, and modulate immune response, which indirectly contributes to better metabolic control (Kellow *et al.,* 2021). In addition, functional foods provide preventive health benefits. Regular consumption of fruits, vegetables, legumes, and fortified foods is associated with reduced risk of chronic diseases and improved quality of life (Satija *et al.,* 2019). Their affordability and accessibility, especially in low and middle income countries, make them valuable alternatives to pharmacological interventions for populations with limited healthcare access.

2.1.2 BLOOD GLUCOSE REGULATION

The regulation of blood glucose is a vital physiological mechanism that ensures continuous energy availability for body tissues (Guyton *et al.,* 2021). Since the brain and red blood cells depend almost exclusively on glucose, maintaining its supply is essential for their normal function (Hall *et al.,* 2020). Typically, fasting blood glucose concentrations are tightly controlled within a range of 70–110 mg/dL (American Diabetes Association [ADA], 2023). When levels fall below or rise above this limit, resulting in hypoglycemia or hyperglycemia, serious metabolic complications can arise (World Health Organization [WHO], 2022). To safeguard against such disturbances, the body employs intricate regulatory systems involving hormones, target organs, and feedback loops to sustain glucose balance (Guyton *et al.,* 2021 The global prevalence of type 2 diabetes mellitus (T2DM) has risen sharply over the last three decades, establishing it as one of the most significant public health concerns of the 21st century (International Diabetes Federation [IDF], 2021; Saeedi *et al.,* 2019). Key drivers of this increase include urbanization, physical inactivity, dietary changes, and population aging, with the impact being particularly pronounced in low- and middle-income countries (LMICs) (WHO, 2022; IDF, 2021). Nigeria, the most populous nation in Africa, mirrors this global trend, with recent epidemiological evidence showing a higher-than-anticipated prevalence of T2DM (Uloko *et al.,* 2018; Adeloye *et al.,* 2021). Therefore, understanding both the worldwide burden and the Nigerian context is essential for developing effective prevention and management strategies. The Internationÿal Diabetes Federation (IDF) Diabetes Atlas (2025) reports that an estimated 589 million adults aged 20–79 years are currently living with diabetes worldwide, representing approximately 1 in every 9 adults (IDF, 2025). Projections indicate that this figure will rise to 853 million by 2050, which equates to 1 in 8 adults globally (IDF, 2025). Approximately 81% of these cases are concentrated in low- and middle-income countries (LMICs), highlighting the disproportionate impact of socioeconomic transitions and lifestyle changes on diabetes prevalence (IDF, 2025).In addition, nearly 43% of adults with diabetes, equivalent to 252 million people, remain undiagnosed, thereby delaying treatment initiation and increasing the likelihood of long-term complications (IDF, 2025). Data from the NCD Risk Factor Collaboration further reveal that the global prevalence of diabetes has doubled over the past three decades, with adult rates rising from about 7% in 1990 to nearly 14% in 2022 (NCD-RisC, 2023). This sharp escalation emphasizes the urgent necessity of implementing effective prevention and control strategies on a global scale (NCD-RisC, 2023; IDF, 2025). Recent evidence indicates that the prevalence of type 2 diabetes mellitus (T2DM) in Nigeria is considerably higher than earlier estimates suggested (Adeloye *et al.,* 2025). A comprehensive systematic review and meta-analysis covering studies from 1989 to 2024 reported a pooled prevalence of 7.0% (95% CI: 5.0–9.0%), corresponding to approximately 8.02 million Nigerian adults currently living with diabetes (Adeloye *et al.,* 2025). This prevalence is nearly twice the 3.7% reported by the International Diabetes Federation (IDF) in 2021, indicating that previous figures substantially underestimated the national diabetes burden (IDF, 2021). The prevalence of T2DM varies widely across Nigeria's geopolitical zones:

- South-South zone (e.g., Rivers, Akwa Ibom) has the highest prevalence (~11.3%).

- South-West zone (e.g., Lagos, Ogun) also records high levels (~10%).

•North-Central zone (e.g., Kwara) reports the lowest prevalence (~2.0%) (Chijioke *et al.,* 2014; Oguntade *et al.,* 2024).

2.2 FACTORS AFFECTING BLOOD GLUCOSE REGULATION LEVEL

2.2.1 DIETARY MODIFICATIONS

Blood glucose regulation is not determined solely by endogenous hormonal pathways; rather, dietary composition, micronutrient status, and bioactive non-nutrient compounds exert significant influence on glycemic responses. Understanding these external modifiers is therefore fundamental to developing nutritional strategies for preventing or managing hyperglycemia and related metabolic disorders (Guo et al., 2020). Carbohydrate quality and quantity remain among the most influential dietary determinants of post-prandial glucose response. Studies consistently show that diets rich in refined carbohydrates or simple sugars lead to rapid and high glucose excursions, whereas complex carbohydrates, particularly when coupled with fiber result in more gradual and moderated increases in blood glucose (Murarka and Singh, 2023; general evidence from dietary intervention literature).

Diet plays a central role in the regulation of blood glucose, and targeted dietary modifications are widely recognized as effective strategies for the prevention and management of hyperglycemia and type 2 diabetes mellitus (T2DM). Research shows that both the quantity and quality of dietary components , including macronutrients, fiber, micronutrients, and bioactive compounds influence glycemic control (Guo *et al.,* 2020; Sharma *et al.,* 2022).

i Carbohydrate Management

Glycemic Index (GI) and Glycemic Load (GL): Selecting foods with low GI/GL can slow postprandial glucose absorption and reduce spikes in blood glucose. Clinical studies have shown that low-GI diets improve postprandial glucose responses, reduce $HbA_{1}c$, and may assist with weight management in individuals with T2DM (Mishra *et al.,* 2021).Carbohydrate quantity and quality: Reducing intake of refined sugars and starches while emphasizing complex carbohydrates such as whole grains, legumes, and starchy vegetables can improve glycemic outcomes (Chauhan *et al.,* 2020). Resistant starches and slowly digestible carbohydrates further stabilize postprandial glucose levels.

ii. Dietary Fiber

Increased intake of soluble and insoluble fibers is strongly associated with improved glycemic control. Soluble fiber slows gastric emptying and glucose absorption, while insoluble fiber promotes intestinal health and may enhance insulin sensitivity (Guo *et al.,* 2020). Meta-analyses have shown that fiber supplementation reduces fasting plasma glucose, $HbA_{1}c$, and body mass index in individuals with T2DM.

iii Macronutrient Composition

Balanced intake of proteins and healthy fats along with carbohydrates modulates glucose response. Proteins and fats slow gastric emptying, thereby attenuating postprandial glucose spikes (Sharma *et al.,* 2022). Diets with appropriate macronutrient balance, particularly high-quality fats (monounsaturated and polyunsaturated fatty acids), contribute to improved insulin sensitivity and metabolic health.

Iv Micronutrients and Bioactive Compounds

Minerals such as magnesium, zinc, and chromium have been shown to influence insulin secretion, improve insulin sensitivity, and modulate glucose metabolism (Guo *et al.,* 2020).Polyphenols, flavonoids, and other plant-derived bioactive compounds can inhibit carbohydrate-digesting enzymes (α-amylase, α-glucosidase), reduce oxidative stress, and enhance insulin signaling pathways, contributing to better glycemic control (Chew *et al.,* 2023).

2.3 BISCUITS FOR ENRICHMENT NUTRIENT AND HEALTH PROMOTING INGREDIENT

Biscuits remain an attractive matrix for functional food development due to their widespread consumption, long shelf life, and relatively simple manufacturing process. Their low moisture content enables stability of added bioactives, and their formulation is flexible enough to allow for partial substitution of wheat flour with other nutrient-dense ingredients (e.g., plant proteins, vegetable powders, composite flours). Recent research has shown that using composite flours combined with non-traditional ingredients can greatly enhance the nutritional quality of biscuits. For example, Bakare et al. (2020) successfully formulated biscuits from a blend of breadfruit and wheat flour enriched with catfish meal, which led to significant increases in protein, calcium, iron, and phosphorus content key macro- and micronutrients beneficial for health. Additionally, Ingle et al. demonstrated that substituting approximately 25% of spelt flour with beetroot powder in biscuit formulations can substantially raise dietary fiber, beneficial pigments (likely antioxidants), and mineral levels, further improving the biscuits' nutritional profile. These approaches showcase how innovation in ingredient selection can provide nutrient-dense, functional bakery products beyond traditional formulations. Recent research has shown that using composite flours combined with non-traditional ingredients can greatly enhance the nutritional quality of biscuits. For example, Bakare et al. (2020) successfully formulated biscuits from a blend of breadfruit and wheat flour enriched with catfish meal, which led to significant increases in protein, calcium, iron, and phosphorus content key macro- and micronutrients beneficial for health. Additionally, Ingle et al. demonstrated that substituting approximately 25% of spelt

flour with beetroot powder in biscuit formulations can substantially raise dietary fiber, beneficial pigments (likely antioxidants), and mineral levels, further improving the biscuits' nutritional profile. These approaches showcase how innovation in ingredient selection can provide nutrient dense, functional bakery products beyond traditional formulations.

2.4. ANTINUTRIENT PROPERTIES IN PLANT BASED FOOD

Antinutrients are naturally occurring compounds found in many plant-based foods that can interfere with the digestion, absorption, or utilization of nutrients in the body. They are not harmful at normal dietary levels, and many even have beneficial health effects when consumed in moderation. Major Antinutrient include;

- **Phytates (phytic acid)**

  Phytates are naturally found in foods like legumes, cereals, seeds, and nuts. They have a strong ability to bind minerals such as iron, zinc, calcium, and magnesium, which can make it harder for the body to absorb these minerals. However, despite this downside, phytates also offer health benefits they act as antioxidants and have properties that may help fight cancer and reduce inflammation (MDPI,2025)

- **Oxalates**

  These compounds are found in foods like spinach, beetroot, nuts, and cocoa. They can bind with calcium to form substances that the body can’t easily absorb, which reduces how much calcium you actually get from these foods. Eating too much of these compounds might also increase the risk of developing kidney stones. (PMC, 2023)

- **Tannins**

Tannins are commonly found in foods like sorghum, tea, many legumes, and certain fruits. They can slow down the action of digestive enzymes such as amylase and trypsin, which may slightly reduce how well the body digest proteins. However, tannins also provide some benefits—they have natural antimicrobial effects and act as antioxidants, helping to protect the body from harmful free radicals. (MDPI 2023)

2.4.1 MINERALS PROPERTIES IN PLANT BASED FOOD

Minerals play an essential role in enhancing the nutritional and health value of functional food snacks. These snacks are often formulated using plant-based ingredients such as legumes, cereals, fruits, vegetable powders, and seeds, which naturally supply a wide range of macro- and micro-minerals. The presence of these minerals contributes not only to basic nutrition but also to physiological functions that support health promotion and disease prevention (Li *et al.,* 2023).

- **Iron(fe)**

Iron is vital for hemoglobin formation, oxygen transport, energy metabolism, and immune function. In functional snacks enriched with legumes, grains, or vegetable powders (e.g., beetroot), iron content helps reduce the risk of iron deficiency anemia. Although plant-based iron is non-heme and less bioavailable, processing methods like fermentation or germination can improve its absorption.

- **Calcium (ca)**

Calcium supports bone development, nerve transmission, muscle contraction, and enzyme activity. Functional snacks containing plantain, sesame, soybean, or leafy vegetable powders can significantly increase calcium levels. Adequate calcium intake through such snacks helps promote bone health and reduce the risk of osteoporosis. (MDPI, 2025)

- **ZINC (Zn)**

Zinc is essential for wound healing, immune function, DNA synthesis, and enzyme activity. Legumes, seeds, and composite flours used in functional snacks provide useful amounts of zinc. Zinc-enriched functional foods support immune strength and metabolic regulation.

- **Magnesium (Mg)**

Magnesium contributes to more than 300 enzymatic reactions in the body, including energy production, nerve function, and glucose regulation. Many functional snacks incorporate nuts, seeds, and whole grains, which enhance the magnesium content and support metabolic health. (ACLM,2023)

- **Potassium (K)**

Potassium helps regulate fluid balance, nerve impulses, and blood pressure. Ingredients such as banana flour, plantain flour, tubers, and vegetable powders make functional snacks rich in potassium. High-potassium snacks contribute to cardiovascular health by helping lower blood pressure.(MDPI,2025)

2.5 CARDABA BANANA (MUSA ABB)

Cardaba banana (*Musa ABB*) is a starchy cooking banana often processed into flour. According to Ayo-Omogie *et al.,* (2020), unripe Cardaba banana flour contains high levels of resistant starch, fiber, and antioxidants, which contribute to its low glycemic index. Although underutilized in Nigeria, it has significant potential in gluten-free and diabetic-friendly food formulations (Olorode *et al.,* 2021). Bioprocessing techniques such as blanching and fermentation can improve the nutritional quality of banana flour by increasing polyphenol content and enhancing enzyme-inhibitory activities. When used in composite flours or snacks, Cardaba banana flour contributes to reduced starch digestibility and improved postprandial glucose control (Abbas *et al.,* 2019).

2.5.1 STARCH COMPOSITION AND RESISTANCE

Cardaba banana, a member of the ABB genome group, is particularly rich in starch, especially in its unripe stage. Starch constitutes about 70–80% of the dry matter in unripe fruit, which gradually decreases as ripening progresses due to enzymatic conversion into simple sugars such as glucose, fructose, and sucrose (Zhang *et al.,* 2021). The starch is primarily composed of two fractions: amylose (21–24%) and amylopectin (76–79%). The relatively high amylose proportion in Cardaba starch compared to dessert bananas contributes to its lower digestibility and higher resistant starch content, which is nutritionally significant (Resurreccion *et al.,* 2019).Structurally, Cardaba starch granules exhibit a C-type crystalline arrangement (a mixture of A- and B-type), which reduces enzyme accessibility during digestion and favors resistant starch formation (Gonzales-Segovia *et al.,* 2021). The starch granules are polygonal to oval and relatively small, features that further enhance their resistance to enzymatic hydrolysis (Zhang *et al.,* 2005).Due to its starch composition and structural features, Cardaba banana provides a naturally high resistant starch (RS) content, which has been associated with lower glycemic response, improved gut health, and potential applications in functional foods (Resurreccion *et al.,* 2019; Gonzales-Segovia *et al.,* 2021).

2.5.2 MINERAL COMPOSITION OF CARDABA BANANA

Cardaba bananas are a good source of potassium (K), which is the most abundant mineral, contributing significantly to electrolyte balance and cardiovascular health. Unripe Cardaba contains ~300–400 mg K per 100 g fresh weight, similar to other plantains and cooking bananas (Wall, 2006; Aurore *et al.,* 2009). Calcium (Ca), magnesium (Mg), and phosphorus (P) are also present in moderate levels, supporting bone and muscle health. Cardaba banana also provides small but important amounts of iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu). These elements are essential for enzymatic reactions, haemoglobin formation, and antioxidant defense (Arinola and Ogunyemi, 2016).

- **POTASSIUM (K)**

Potassium (K) is one of the most abundant and nutritionally significant minerals in Cardaba banana (Musa ABB), contributing to both electrolyte balance and metabolic regulation. Potassium plays a critical role in nerve conduction, muscle contraction, and maintaining cellular osmotic balance, as well as in glucose metabolism, which is particularly relevant to

functional foods targeting glycemic control (Mohan *et al.,* 2020).

- **MAGNESIUM (Mg)**

Magnesium (Mg) is an essential macro-mineral involved in over 300 enzymatic reactions, including those regulating glucose metabolism, energy production, and protein synthesis. Its

role in insulin sensitivity and glycemic control makes it particularly relevant in functional foods derived from starchy crops such as Cardaba banana (Musa ABB) (Rosanoff *et al.,* 2012).

- **CALCIUM (Ca)**

Calcium (Ca) is an essential macro-mineral that plays a critical role in bone development, nerve transmission, muscle function, and enzymatic activity. In addition to these structural and physiological functions, calcium is involved in insulin secretion and intracellular signaling pathways, thereby influencing glucose metabolism and glycemic regulation (Fung *et al.,* 2017).

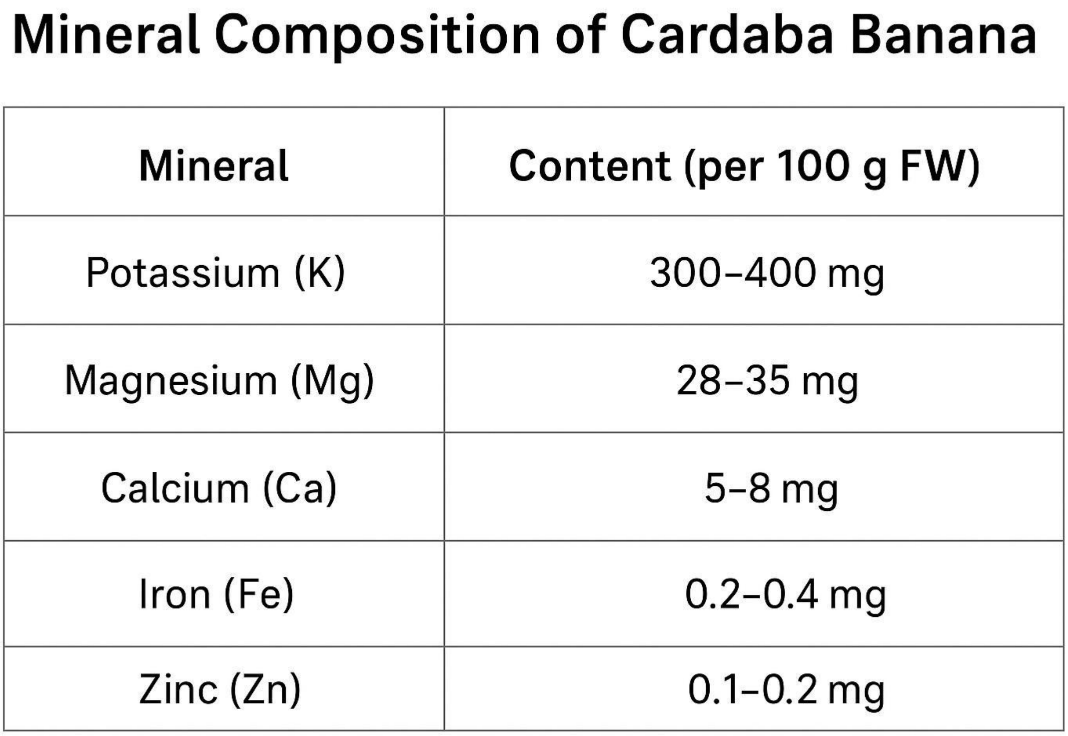
- **ZINC(Zn)**

Zinc (Zn) is a vital trace mineral involved in enzymatic activity, immune system function, protein synthesis, and carbohydrate metabolism. supports enzymatic activity, insulin function, and carbohydrate metabolism, reinforcing the crop’s potential in the development of nutrient-dense, functional foods.

- **IRON (Fe)**

Iron (Fe) is an essential trace mineral critical for oxygen transport, energy metabolism, and enzymatic reactions. It plays a central role in hemoglobin and myoglobin formation, as well as in various cellular redox reactions. Adequate iron intake is also associated with maintaining normal glucose metabolism, as iron is involved in cofactors for enzymes that regulate carbohydrate oxidation and energy production (Beard, 2019)

Table 2.1: Mineral composition of Cardaba banana



Mineral Composition of Cardaba Banana

Mineral	Content (per 100 g FW)
Potassium (K)	300–400 mg
Magnesium (Mg)	28–35 mg
Calcium (Ca)	5–8 mg
Iron (Fe)	0.2–0.4 mg
Zinc (Zn)	0.1–0.2 mg

2.5.3 CARDABA AND GLYCEMIC INDEX CONTROL

Cardaba banana is abundant in resistant starch (RS) and dietary fiber, both of which play important roles in regulating glycemic response. Resistant starch resists digestion in the small intestine and reaches the colon largely intact, where it is fermented by gut microbiota to produce short-chain fatty acids that confer various systemic metabolic benefits (Mohan *et al.,* 2020). Studies indicate that unripe Cardaba banana flour can contain RS levels of approximately 50–60% of total starch, contributing to a reduced predicted glycemic index when incorporated into food formulations (Osanbikan *et al.,* 2024). In contrast, as the banana ripens, sugar content increases while RS decreases, resulting in a higher GI, which underscores the significance of maturity stage in developing functional foods.

2.5.4 BIOACTIVE COMPOUNDS AND MECHANISM OF ACTION

Cardaba banana (*Musa balbisiana* × *Musa acuminata*) is a starchy banana variety consumed widely for cooking and processed foods. Beyond macronutrients, it contains bioactive compounds that have potential roles in glucose regulation and metabolic health.

Major Bioactive Compounds:

- Resistant Starch

Found predominantly in unripe Cardaba bananas, resistant starch (RS) resists digestion in the small intestine and reaches the colon, where it is fermented by gut microbiota.RS slows glucose absorption, reduces postprandial blood glucose peaks, and contributes to satiety (Borges *et al.,* 2020).

- Dietary Fiber

Cardaba banana contains soluble and insoluble fiber, which delays gastric emptying and moderates glucose absorption (Slavin, 2019).Fiber supports gut health and contributes to longer-term glycemic stability.

- Polyphenols

Includes flavonoids, tannins, and phenolic acids they Exhibits antioxidant and anti-inflammatory effects, reducing oxidative stress-induced β-cell dysfunction and improving insulin sensitivity (Kaur and Ahmed, 2024).

- Minerals

Magnesium, potassium, and zinc support insulin secretion, sensitivity, and glucose uptake in peripheral tissues (Guo *et al.,* 2020).

2.5.5 ANTINUTRITIONAL PROPERTIES

The Cardaba bananas (Musa ABB) are rich in resistant starch, essential minerals, and bioactive compounds, they also contain a range of anti-nutritional factors (ANFs). These compounds, while sometimes associated with adverse effects on nutrient absorption, may also contribute functional health benefits when consumed in moderate amounts. One of the major ANFs in Cardaba banana is tannins, which are abundant in the peel and unripe pulp, others include phytates, oxalates and saponin.

- **Tannins**

Tannins are polyphenolic compounds widely distributed in plants and are recognized as antinutritional factors due to their ability to bind proteins, digestive enzymes, and minerals, thereby reducing nutrient bioavailability (Oboh and Oladunjoye, 2021). In Cardaba banana (*Musa ABB*), tannins are present in small to moderate amounts, particularly in the peel and unripe pulp, contributing to astringency and impacting digestibility. The mechanism of tannin activity involves the formation of insoluble complexes with dietary proteins and starch, which can inhibit enzyme action and decrease protein digestibility (Sosulski *et al.,* 2019). Additionally, tannins can chelate minerals such as iron (Fe) and zinc [7](Zn), limiting their absorption in the gastrointestinal tract. While the tannin content in unripe Cardaba banana may negatively affect mineral bioavailability.

- **Phytates (phytic acid)**

This represent another class of anti-nutritional compounds in bananas. They chelate divalent minerals such as iron, zinc, calcium, and magnesium, reducing their bioavailability and potentially contributing to deficiencies in populations with banana-based diets. Despite this limitation, phytates also exhibit antioxidant properties (Ekesa *et al.,* 2012; Arinola and Ogunyemi, 2016).

● OXALATES

Similarly, **oxalates** have been detected in both pulp and peel, where they interfere with calcium absorption by forming insoluble complexes, occasionally contributing to kidney stone formation in sensitive individuals (Akinmoladun *et al.,* 2019). Minor ANFs such as saponins and alkaloids have also been identified. While they may hinder nutrient utilization at high concentrations, they are equally noted for beneficial effects, including cholesterol reduction, antimicrobial activity, and potential hypoglycemic effects (Arinola and Ogunyemi, 2016). In addition, protease inhibitors (trypsin inhibitors) present in cooking bananas can reduce protein digestibility, although recent evidence suggests that some protease inhibitors may also exert anticancer properties (Aurore *et al.,* 2009). Overall, the anti-nutritional factors in Cardaba bananas include tannins, phytates, oxalates, saponins, alkaloids, and protease inhibitors. While these compounds may reduce protein and mineral bioavailability, their bioactive functions ranging from antioxidant to hypoglycemic effects, highlight their dual role in nutrition and health. Importantly, traditional processing methods such as boiling, cooking, fermentation, or drying significantly reduce the levels of these anti-nutrients, thereby enhancing the nutritional value and safety of Cardaba banana as a functional food.

Fig 2.1: Cardaba banana (*Musa ABB*)

2.6 BEETROOT (*Beta vulgaris*)

Beetroot (*Beta vulgaris L.*), commonly known as the garden beet or red beet, is a root vegetable belonging to the family Amaranthaceae, subfamily Betoideae. It is one of the most economically and nutritionally significant root crops cultivated globally, valued for its edible taproot and leaves (Chhikara *et al.,* 2020). The species Beta vulgaris includes several cultivated forms such as sugar beet, fodder beet, and table beet (beetroot), all derived from the wild ancestor Beta vulgaris subsp. Maritima, commonly known as sea beet (Neelwarne, 2022). The domestication of Beta vulgaris is traced back to the Mediterranean region, particularly along the coasts of North Africa, Southern Europe, and Western Asia. Archaeological and historical evidence suggests that wild sea beet was first cultivated for its leafy greens around 2000 BCE, while the swollen, edible root was developed later during the classical Greek and Roman periods (Chawla *et al.,* 2021). Over centuries, selective breeding led to distinct beet types for sugar, fodder, and human consumption. Modern beetroot cultivation spread across Europe in the 16th century and later to Asia, Africa, and the Americas, adapting well to temperate and subtropical climates (Neelwarne, 2022). Today, beetroot is cultivated worldwide for food, colorant extraction (betacyanins), and nutraceutical uses due to its rich phytochemical profile. Beetroot is a biennial plant grown as an annual for its edible storage root. It develops a fleshy, bulbous taproot that varies in color from deep red to purple, yellow, or white depending on cultivar and pigment composition (betacyanins and betaxanthins) (Kujala *et al.,* 2021).

2.6.1 NUTRITIONAL AND FUNCTIONAL SIGNIFICANCE

Nutritionally, beetroot is a rich source of carbohydrates (mainly sucrose), fiber, folate, vitamin C, potassium, and iron. It also contains unique bioactive compounds such as betalains, polyphenols, and dietary nitrates, which confer multiple health benefits, including antioxidant, antihypertensive, and antidiabetic properties (Neelwarne, 2022; Kujala *et al.,* 2021). The dietary nitrate in beetroot is particularly valued for its role in nitric oxide synthesis, which improves vascular function and glucose metabolism.

2.6.2 MINERALS PROFILE AND DIABETIC CONTROL

Beetroot (*Beta vulgaris L.*) is not only valued for its bioactive pigments and nitrates but also for its rich mineral composition, which plays a significant role in glucose metabolism, insulin sensitivity, and overall diabetic regulation (Chhikara *et al.,* 2020; Neelwarne, 2022). The mineral profile of beetroot includes macrominerals such as potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), and phosphorus (P), as well as trace elements like iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) (Chawla *et al.,* 2021). Beetroot is particularly high in potassium (300–400 mg/100 g), which aids in maintaining normal cellular function and insulin signaling. Magnesium, present in significant amounts (25–35 mg/100 g), acts as a cofactor in more than 300 enzymatic reactions, including those involved in glucose utilization and energy metabolism (Adegoke *et al.,* 2023). Additionally, iron and zinc are essential for hemoglobin formation and insulin synthesis, respectively. Deficiencies in these minerals have been correlated with insulin resistance and oxidative stress in diabetic patients (Sadeghi et al). The mineral composition of beetroot contributes synergistically to glucose regulation through multiple biochemical mechanisms:

- **Magnesium** and **zinc** improve insulin receptor activity and enhance glucose uptake by facilitating phosphorylation pathways in muscle and adipose tissues (Sadeghi *et al.,* 2022).

- **Potassium** helps in reducing hyperglycemia-induced hypertension by maintaining electrolyte balance and vascular relaxation (Neelwarne, 2022).

- **Iron** and **copper** play a role in mitochondrial energy metabolism, while their antioxidant activity mitigates oxidative damage to pancreatic β-cells (Bamaiyi *et al.,* 2023).

- **Manganese** regulates gluconeogenic enzyme activity, thus influencing hepatic glucose output.

Collectively, these minerals enhance glycemic control, reduce insulin resistance, and protect pancreatic function, suggesting that regular consumption of beetroot can support metabolic health and diabetes prevention (Adegoke *et al.,* 2023; Bamaiyi *et al.,* 2023).

2.6.3 ANTINUTRITIONAL PROPERTIES OF BEETROOT (*BETA VULGARIS*)

Although beetroot is widely recognized for its rich nutrient composition and bioactive compounds, it also contains certain anti-nutritional factors (ANFs), naturally occurring plant metabolites that can reduce nutrient bioavailability or interfere with metabolic processes when consumed in large amounts. These compounds, however, often possess dual functional roles, acting as both inhibitors and bioactive agents depending on concentration and processing (Chhikara *et al.,* 2020; Al-Dhabi *et al.,* 2021).Several studies have identified phytates, oxalates, saponins, tannins, and nitrates as the primary anti-nutritional components in beetroot (Wootton-Beard *et al.,* 2021; Bhat *et al.,* 2022).

- **Oxalates** - Bind with calcium and magnesium to form insoluble salts, reducing mineral absorption and potentially contributing to kidney stone formation (Bhat *et al.,* 2022).
- **Phytates** -Form complexes with iron, zinc, and calcium, decreasing mineral bioavailability (Al-Dhabi *et al.,* 2021).
- **Tannins** -Inhibit digestive enzymes such as trypsin and amylase, reducing protein and carbohydrate digestibility (Chhikara *et al.,* 2020).
- **Saponins** - Can impair intestinal permeability, though moderate levels may have hypocholesterolemic and antidiabetic benefits (Kujala *et al.,* 2021).

- **Nitrates** -While high intake may pose risks of methemoglobinemia, dietary nitrates from beetroot are beneficial for nitric oxide production and vascular health .(Wootton-Beard *et al.,* 2021)

Figure 2.2: Beetroot (Beta vulgaris)

2.6.4 BIOACTIVE COMPOUNDS AND GLUCOSE SIGNIFICANCE

Beetroot is a functional food rich in bioactive compounds including betalains, polyphenols, and inorganic nitrates — which have been investigated for their potential to influence glucose homeostasis and metabolic health (Kaur and Ahmed, 2024; Campos *et al.,* 2020).

- **Betalains (trimethylglycine)**

Betalains are water-soluble nitrogen-containing pigments found predominantly in the order Caryophyllales, including beetroot (Beta vulgaris). They are subdivided into betacyanins (red-violet pigments, e.g., betanin, isobetanin) and betaxanthins (yellow-orange pigments, e.g., vulgaxanthin) (Kaur and Ahmed, 2024). Beyond providing color, betalains possess potent antioxidant, anti-inflammatory, and enzyme-modulating properties relevant to glucose metabolism.

● Inorganic Nitrate

Beetroot (Beta vulgaris) is a rich source of inorganic nitrate (NO_3^-), a bioactive compound that is metabolized in the body to nitric oxide (NO), a signaling molecule involved in vascular function, glucose metabolism, and insulin sensitivity (Kaur and Ahmed, 2024; Guo *et al.,* 2020). Inorganic nitrates contribute to beetroot's functional effects beyond its macronutrient content.

● Polyphenols and phenolic compounds

Beetroot (Beta vulgaris) is a rich source of polyphenolic compounds, including flavonoids, phenolic acids, and tannins, which contribute to its antioxidant, anti-inflammatory, and enzyme-modulating properties (Kaur and Ahmed, 2024; Campos *et al.,* 2020). Polyphenols are key bioactive molecules that may modulate glucose metabolism through multiple mechanisms. Polyphenols in beetroot reduce oxidative stress and chronic low-grade inflammation, both of which are implicated in insulin resistance and β-cell dysfunction (Chew *et al.,* 2023).

2.7 BAMBARA GROUNDNUT

Bambara groundnut, also known as Bambara bean or earth pea, is a leguminous crop belonging to the Fabaceae family. It is one of Africa's indigenous and underutilized legumes, primarily cultivated in West, Central, and Southern Africa, including Nigeria, Ghana, and Cameroon (Bamshaiye *et al.,* 2021). The plant's name is derived from the Bambara ethnic group of Mali, where it has been cultivated for centuries as a staple food and a traditional source of plant-based protein.Bambara groundnut is a self-pollinating annual plant that produces its pods underground, similar to groundnuts. The seeds are round, smooth, and occur in various colors including cream, brown, black, and red, often with characteristic patterns on the seed coat (Oyeyinka and Oyeyinka, 2018). It is recognized as a "complete food" because it contains a balanced composition of carbohydrates, proteins, and lipids, as well as essential minerals and vitamins (Azam-Ali *et al.,* 2023).

2.7.1 NUTRITIONAL PROFILE COMPOSITION

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is recognized as a nutritionally balanced legume, often referred to as a "complete food" due to its combination of macronutrients and micronutrients essential for human health (Oyeyinka and Oyeyinka, 2018; Azam-Ali *et al.,* 2023). The seed's composition varies slightly with variety and growing conditions but remains consistently rich in carbohydrates, proteins, lipids, minerals, and dietary fiber, making it an important crop for combating malnutrition and enhancing food security across Africa.Bambara groundnut seeds typically contain 63–68% carbohydrates, 18–25% protein, and 4–7% fat (Hillocks *et al.,* 2012; Adegboyega *et al.,* 2022). The carbohydrate fraction is dominated by starch and resistant starch, which provide a slow energy release and contribute to its low glycemic index (GI) (Abioye *et al.,* 2023). This property makes Bambara groundnut particularly beneficial for individuals with diabetes or those at risk of metabolic syndrome.

The protein quality of Bambara groundnut is high compared to other legumes, containing all essential amino acids—especially lysine, leucine, and valine—though sulfur-containing amino acids (methionine and cysteine) are present in smaller amounts (Mohammed *et al.,* 2021; Chinedu *et al.,* 2022). This balanced amino acid profile makes it an excellent supplement to cereal-based diets that are often deficient in lysine.The lipid fraction of Bambara groundnut consists mainly of unsaturated fatty acids, particularly linoleic and oleic acids, which support cardiovascular health by reducing low-density lipoprotein (LDL) cholesterol levels (Oyeyinka *et al.,* 2020). Bambara groundnut is also a good source of essential minerals such as iron, calcium, magnesium, potassium, zinc, and phosphorus (Hassan *et al.,* 2021; Olaleye *et al.,* 2023).

- Iron and zinc contribute to hemoglobin synthesis and immune function.
- Magnesium and potassium play key roles in insulin sensitivity and glucose homeostasis, which may explain part of the legume's antidiabetic potential (Adegboyega *et al.,* 2022).
- Calcium and phosphorus support bone metabolism and cellular signaling.

2.7.2 ROLE IN MANAGEMENT OF BLOOD GLUCOSE REGULATION

Bambara groundnut has gained increasing attention as a functional food with significant potential in glycemic regulation and the management of type 2 diabetes mellitus (T2DM). Its unique nutritional composition—characterized by low glycemic carbohydrates, resistant starch, high dietary fiber, and bioactive phytochemicals—collectively contributes to its ability to maintain optimal blood glucose levels (Abioye *et al.,* 2023; Adegboyega *et al.,* 2022).

The carbohydrate fraction of Bambara groundnut is dominated by starch and resistant starch, which undergo slow enzymatic hydrolysis during digestion (Hillocks *et al.,* 2012;

Abioye *et al.,* 2023). Resistant starch resists digestion in the small intestine and undergoes fermentation in the colon, producing short-chain fatty acids (SCFAs) such as butyrate and propionate. These SCFAs enhance insulin sensitivity and regulate hepatic glucose production (Raji *et al.,* 2021).

Studies have shown that Bambara groundnut-based meals elicit lower postprandial glucose responses compared to high-GI foods such as white rice or yam flour, confirming its low glycemic index (Abioye *et al.,* 2023). Thus, incorporating Bambara groundnut into daily diets can contribute to steady blood glucose levels and prevent hyperglycemic spikes.

2.7.3 ANTINUTRITIONAL PROPERTIES OF BAMBARA GROUNDNUT

Bambara groundnut is rich in phytate, flavonoids, tannins, and saponins, which exert antioxidant and anti-inflammatory effects (Oyeyinka *et al.,* 2020; Adegboyega *et al.,* 2022). These compounds reduce oxidative stress, a major contributor to insulin resistance and pancreatic β-cell dysfunction in diabetes (Olaleye *et al.,* 2023).

- **Phytates**

Phytates, particularly phytic acid represent one of the key antinutritional compounds in Bambara groundnut (Vigna subterranea). Phytic acid has a high affinity for essential minerals such as iron, zinc, calcium, and magnesium, forming insoluble complexes that limit their absorption in the gastrointestinal tract. This strong mineral-binding capacity is the primary reason phytates are associated with reduced nutrient bioavailability in legume-based foods (Reddy *et al.,* 2020).

- **Tannins**

Tannins are polyphenolic compounds present in varying quantities in Bambara groundnut (Vigna subterranea) and are regarded as a significant antinutritional factor. They can bind to proteins, digestive enzymes, and minerals, forming complexes that impair protein digestibility and may restrict the availability of certain nutrients. This ability to bind proteins is why high tannin levels are frequently linked to lower nutritional quality in legume foods (Agbor *et al.,* 2019), they also possess functional advantages, including antioxidant, antimicrobial, and anti-inflammatory activities. These bioactive properties contribute positively to health when tannins are present in moderate amounts (Dambe *et al.,* 2020).

- **Saponins**

Saponins are bioactive compounds that occur naturally in Bambara groundnut and other legumes. They have a soap-like foaming ability, and from a nutrition standpoint they are viewed as antinutritional: they can hinder the absorption of some nutrients, add bitterness, and form complexes with proteins and minerals, lowering digestibility and bioavailability. Yet, in moderate amounts they also provide possible health advantages, such as antioxidant, cholesterol-lowering, and anti-inflammatory activities (Oladunmoye *et al.,* 2020).

- **Flavonoids**

Flavonoids are a diverse class of polyphenolic compounds naturally occurring in Bambara groundnut (Vigna subterranea), contributing to its functional and biochemical characteristics. Although not typically considered among the most potent antinutritional factors, flavonoids can interact with proteins and minerals, forming complexes that may modestly reduce protein digestibility or limit the absorption of certain micronutrients. These mild antinutritional effects are consistent with observations in other legumes containing phenolic compounds (Udeh *et al.,* 2020).Flavonoids are naturally occurring polyphenolic compounds found in Bambara groundnut (Vigna subterranea), and they play an important role in shaping the legume's functional and biochemical qualities. Although they are not among the strongest antinutritional factors, flavonoids can still interact with proteins and minerals, forming complexes that may slightly reduce how well these nutrients are digested or absorbed. Such mild antinutritional effects have also been noted in other legumes rich in phenolic compounds (Udeh *et al.,* 2020).

2.7.4 MINERAL COMPOSITION TO DIABETIC CONTROL

Minerals play a fundamental role in maintaining hyperglycemic condition, they are essential micronutrients required for the proper functioning of metabolic pathways involved in blood-glucose control. They support insulin production, enhance insulin sensitivity, aid glucose transport, and protect pancreatic β-cells from oxidative damage. Because glucose regulation depends on enzymatic reactions and hormone signaling, minerals act as indispensable cofactors and structural components in these processes (Dubey, Thakur and Chattopadhyay, 2020).

2.7.5 MAGNESIUM AS A COFACTOR IMPROVES INSULIN SENSITIVITY

Magnesium plays a crucial role in glucose metabolism because it functions as a cofactor for numerous enzymes involved in insulin signaling pathways. It is required for ATP activation, which is essential for insulin receptor phosphorylation—a key step that allows insulin to bind effectively and trigger glucose uptake in cells. When magnesium levels are adequate, insulin can interact more efficiently with its receptors, promoting improved insulin sensitivity. Conversely, magnesium deficiency impairs insulin action, reduces glucose transport, and increases the risk of insulin resistance. Therefore, optimal magnesium status is essential for maintaining normal blood-glucose control and preventing metabolic disorders such as type 2 diabetes (Dubey, Thakur and Chattopadhyay, 2020). •**Zinc** supports insulin storage in pancreatic β-cells and protects them against oxidative damage.

2.7.6 CALCIUM AND INSULIN SECRETION

Calcium plays a fundamental role in the regulation of insulin secretion from pancreatic β-cells. When blood glucose levels rise, glucose enters β-cells through GLUT2 transporters and undergoes metabolism, leading to increased ATP production. This rise in ATP causes the closure of ATP-sensitive potassium channels, resulting in membrane depolarization. The depolarized membrane then opens voltage-dependent calcium channels, allowing calcium ions (Ca^{2+}) to flow into the β-cells, which ultimately triggers insulin exocytosis (García-Hermoso *et al.,* 2020).

2.7.7 ZINC MINERALS AND OXIDATIVE STRESS

Zinc is an essential trace mineral that plays a critical role in protecting cells, including pancreatic β-cells, from oxidative stress. It functions as a structural and catalytic component of many antioxidant enzymes, most notably superoxide dismutase (SOD), which neutralizes harmful reactive oxygen species (ROS). Zinc also stabilizes cell membranes and reduces lipid peroxidation, thereby preventing oxidative that can impair insulin secretion and glucose metabolism. In pancreatic β-cells which have naturally low antioxidant defenses,adequate zinc levels are essential for maintaining cellular integrity, supporting insulin synthesis, and preventing oxidative stress–induced dysfunction (Chasapis *et al.,* 2020).

Fig 2.4 Bambara groundnut (vigna subterranea) source; Wikipedia

2.8 RESEARCH GAP IDENTIFIED

Despite increasing global efforts toward developing functional foods for diabetes management, significant gaps persist in the utilization of indigenous African crops such as Bambara groundnut, Cardaba banana, and beetroot in composite food formulations.Existing literature on Bambara groundnut primarily focuses on its nutritional composition, protein quality, and processing characteristics (Oyeyinka and Oyeyinka, 2020; Chai *et al.,* 2021). However, limited studies have examined its integration into baked products, particularly biscuits designed for glycemic control. Furthermore, there is insufficient data on how Bambara groundnut flour influences postprandial glucose response when combined with other low-glycemic ingredients. Similarly, while Cardaba banana is recognized for its high resistant starch content and low glycemic index (Santiago *et al.,* 2021; Rafiq *et al.,* 2023), its functional application in composite flour formulations remains underexplored. Most studies have examined its physicochemical and textural properties, but few have evaluated its synergistic nutritional and metabolic effects when combined with legumes or root vegetables.

In the case of beetroot (Beta vulgaris), although its bioactive compounds (betalains, nitrates, and polyphenols) have demonstrated antidiabetic potential (Kapadia *et al.,* 2020; Clifford *et al.,* 2021), its use as a functional ingredient in bakery products aimed at blood glucose regulation has received minimal attention. Existing research has mostly concentrated on its juice or extract rather than its flour form in composite biscuits. Moreover, limited studies have simultaneously investigated the mineral composition and antinutrient interactions among these three food sources. The presence of phytates, tannins, and oxalates in Bambara groundnut and beetroot may influence mineral bioavailability, yet no comprehensive analysis has addressed how processing and formulation could minimize antinutrient effects while enhancing nutritional value.

Therefore, there is a clear gap in the scientific literature concerning:

- The development and characterization of composite biscuits from Bambara groundnut, Cardaba banana, and beetroot flours.
- The evaluation of their mineral composition and antinutrient levels in relation to bioavailability.
- The assessment of their glycemic regulatory potential as a functional food for diabetic and prediabetic populations.

Addressing this gap could provide a foundation for novel, culturally relevant, and nutritionally balanced food products that support metabolic health and contribute to sustainable food systems in sub-Saharan Africa and beyond.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

- Bambara groundnut (*VIgna subterranea*)
- Cardaba banana (*Musa Abb*)
- Beetroot (*Beta vulgaris*)
- Guava leaves (*Psidium guajava*)
- Cinnamon leaves
- Vegetable oil
- Skimmed milk
- Date paste
- Egg/ Albumin

3.1.1 APPARATUS AND EQUIPMENT

- Blending machine (Kenwood KCB -239K)
- Microwave oven (COV_8320_B)
- Digital scale (Model SBS -TWS -500/10)
- Dehydrator
- Refrigerator
- Measuring cylinder
- Sieves

- Towel
- Bowl
- knives
- Masking tape
- Sponge
- Scissors
- Foil paper
- Bucket
- Gloves
- Weighing scales
- Baking paper
- Air tight container for storage

3.1.2 CHEMICALS/REAGENT

Sodium Metabisulphite ($Na_2S_2O_5$)

3.2 METHODS

3.2.1 PREPARATION OF RAW MATERIALS

3.2.1.1 PREPARATION OF CARDABA BANANA FLOUR

The Cardaba banana (*Musa ABB*) at the mature unripe stage were obtained from Efeyi market located at New Benin, Edo State. The fruits were sorted to remove damaged or overripe samples, thoroughly washed with distilled water to eliminate dirt and surface contaminants, and then peeled. The banana pulp was sliced into thin pieces (approximately 5 mm thickness) to facilitate uniform drying. Thereafter, we introduced a solution (containing 5g of Sodium Metabisulphite into a 10L of distilled water and stirred properly for uniform mixture, we placed the already sliced cardaba banana inside the solution for 30 minutes inside the sieve). The solution (Sodium Metabisulphite acts as a preservative to retain its color of cardaba banana). The slices were oven-dried at 60°C until a constant weight was achieved. The dried samples were ground into fine powder using a laboratory electric blender and sieved through a mesh to obtain uniform particle size. The resulting Cardaba banana extract was stored in airtight containers, and kept in a cool, dry place until further analysis. The prepared extract was used for biochemical and antinutritional analyses, including assessment of phytates, tannins, oxalates, and Saponins.

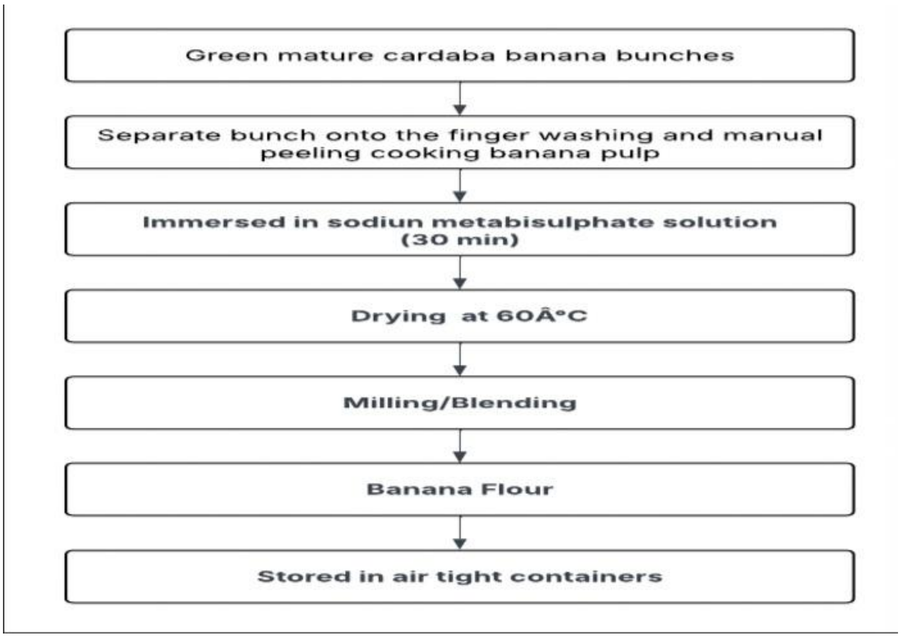


Fig.3.1 Flowchart of the preparation of Cardaba flour

3.2.1.2 PREPARATION OF BEETROOT (*BETA VULGARIS*) FLOUR

Fresh beetroot tubers (*Beta vulgaris L.*) were obtained from Hausa market in Benin Nigeria. The beetroots were carefully sorted to remove any diseased, bruised, or damaged ones, ensuring only healthy, firm, and mature roots were selected for processing. The selected tubers were thoroughly washed under running tap water to eliminate adhering soil particles and surface contaminants. After cleaning, the beetroots were peeled using a sterile stainless-steel knife to remove the outer skin and then sliced into thin pieces to facilitate uniform drying. The slices were spread evenly on stainless-steel trays and dried in a hot-air oven at 50°C for until a constant weight was achieved, Once fully dried, the beetroot slices were allowed to cool to room temperature and then milled into fine powder using a laboratory blending machine. The resulting flour was sieved to obtain a uniform particle size. The beetroot flour was then packaged in airtight containers and stored at room temperature until further use for formulation and analysis.

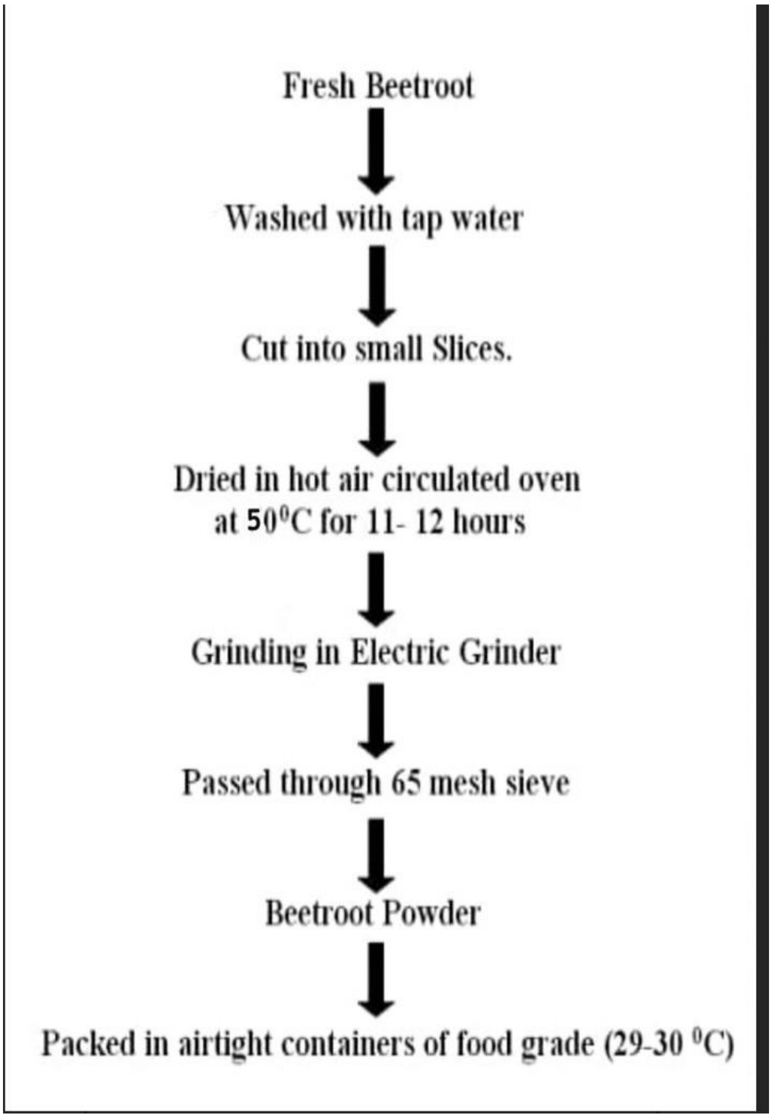


Fig 3.2: Flowchart of Preparation of Beetroot flour

3.2.1.3 PREPARATION OF GUAVA LEAVES POWDER

The guava leaves (*Psidium guajava*) were obtained from a healthy, pesticide-free plant within the vicinity of Uselu, Benin city. The leaves were carefully hand-picked, sorted to remove diseased, insect-damaged, or discoloured samples, and then thoroughly washed under running tap water to eliminate dirt, dust, and surface contaminants. The cleaned leaves were spread out on clean trays and shade-dried for 14 days in a well ventilated area away from direct sunlight to prevent degradation of heat- and light-sensitive phytochemicals. Once dried, the leaves were milled into a fine powder using a laboratory blender. The powdered leaves were passed through a mesh sieve to ensure uniform particle size. The resulting guava leaf powder was stored in airtight glass jars and kept in a cool, dry place until required for extraction and further analysis.

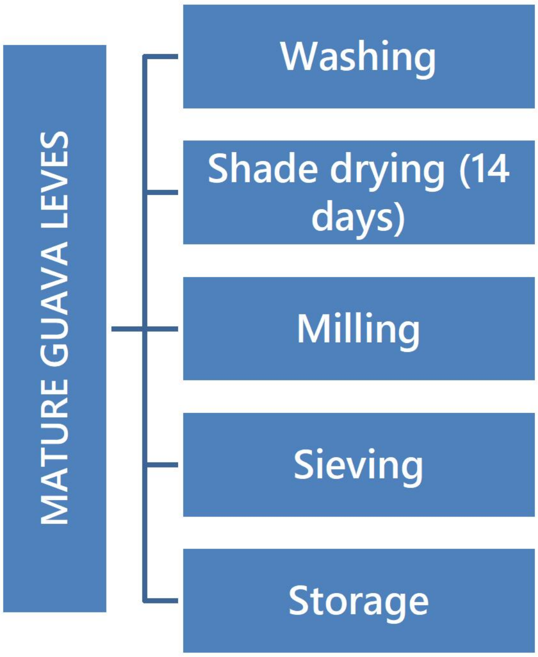


Fig 3.3: Flowchart Of preparation of guava leaf powder

3.2.1.4 PREPARATION OF BAMBARA GROUNDNUT FLOUR

The Bambara groundnut seeds were sourced from Oba market in Benin City, Nigeria. The seeds were first carefully sorted by hand to remove stones, broken seeds, and other unwanted materials. They were then washed thoroughly with water to remove any traces of dust or surface impurities. After cleaning, the seeds were soaked in clean water at room temperature for 12 hours to soften the seed coats and make dehulling easier and more efficient. After soaking, the Bambara groundnut seeds were spread evenly on clean trays and oven-dried at 50°C until they reached a constant weight, ensuring all moisture was removed. Once dried, the cotyledons were ground into a fine flour using a laboratory blender and sieved to achieve a smooth, uniform texture. The resulting flour was carefully packed in airtight, moisture proof containers and stored at room temperature to maintain its quality until further analysis.

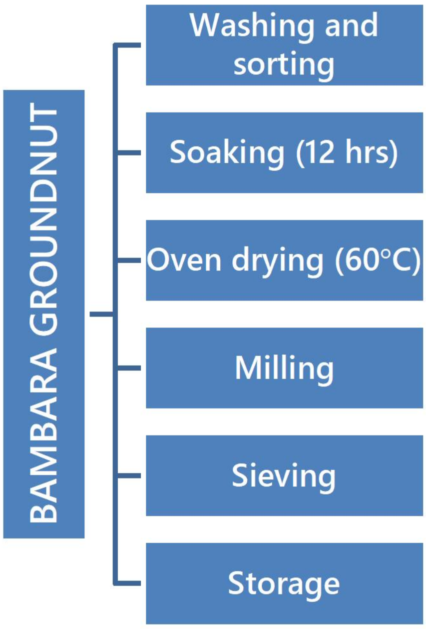


Fig 3.4: Flowchart of Preparation of Bambara groundnut flour

3.3 PREPARATION OF THE SNACK BAR FORMULATION

3.3.1 SAMPLE FORMULATION

The various snack bar formulations were developed using composite flours and powders derived from beetroot, Cardaba banana, bambara groundnut, guava and cinnamon. All ingredients were precisely measured and mixed in different ratios to produce snack bars with distinct nutritional characteristics and sensory attributes. Each formulation varied in composition, weight, and size, allowing for a comprehensive comparison of product quality and consumer acceptability.

Table 3.1: Detailed table showing the precise amount of each ingredient needed in various samples

Ingredient	Cont	Sample A	Sample	Sample C

	rol (%)	**(%)**	**B (%)**	**(%)**
Wheat flour	100	-	-	-
Bambara groundnut flour	-	35	40	30
Cardaba banana flour	-	35	30	40
Beetroot powder	-	10	15	8
Guava leaf powder	-	5	3	7
Cinnamon powder	-	2	1	3
Other ingredients	-	13	11	12
Total	-	100	100	100

The snack formulation process is shown below:

Ingredients Weighing & Sorting

↓

↓

Mix Dry Ingredients

(Banana flour, beetroot, bambara flour, guava leaf powder + cinnamon + baking powder)

↓

Add Wet Ingredients

(Egg white + butter + Date paste + skimmed milk)

↓

Knead into Dough

↓

Roll Out Dough & Cut into Shapes

↓

Preheat the oven before baking

Bake at 160°C for 15–20 mins

↓

Cool to Room Temperature

↓

Packaging (airtight bags or jars)

↓

Storage

3.4 MINERAL AND ANTI NUTRIENT ANALYSIS

1. DETERMINATION OF CYANIDE CONTENT[ALKALINE PICRATE METHOD,(Onwuka 2005)]

Materials needed:

*Whatmann No 1filter paper.

*Potassium cyanide (KCN)

*Alkaline picrate solution-Dissolve 1g of picrate and 5g of Na_2Co_3 in 200ml of dist. H_2O.

Procedure

*Weigh 5g of ground sample and dissolve in 50ml of dist. H_2O. Allow to stay overnight and then filter with Whatmann No1 filter paper.

- Prepare different concentration of KCN solution containing 0.1-1.0mg/ml.

4ml of Alkaline picrate was added into 1ml of extract solution, for 15mins incubated @ 50◦C, take Absorbance reading @ 490nm.

2. DETERMINATION OF OXALATE CONTENT.

Materials needed:

*3m H_2SO_4

- Magnetic stirrer
- Whatmann No1 filter paper

*0.05M $KMnO_4$

Procedure

*Weigh 1g of sample into 100ml conical flask.

- Add 75ml of Conc H_2So_4.
- Stir for 1hour with a magnetic stirrer.
- Filter using a Whatmann No 1 filter paper.
- Take 25ml of the filtrate and titrate.

While hot against 0.05M $KNnO_4$ Solution until a faint pink color persist for at least 30 seconds.

*The oxalate content is then calculated by taking 1ml of 0.05M $KMnO_4$ as equivalent to 2.2mg oxalate (Chinma&Igyor 2007; Ihekoronye & Ngoddy, 1985)

1ml=2.2mg oxalate

Oxalate content= titre value x 2.2mg.

3. DETERMINATION OF PHYTATE CONTENT

Materials Needed:

*2%HCL

*0.3% Ammonium thiocyanate

*Ferric chloride (0.00195g/ml)-To prepared

1000ml=1.95g in 1000ml diluted H_2O.

Procedure (Lucas&Markakas, 1975)

*2g of sample is weighed into different 250ml conical flasks.

*100ml of 2% HCL is added to soak sample for 3hours.

*Filter with a Whatmann No 1 filter paper

*50ml of each filtrate is taken into a 250ml beaker and 10ml of diluted H_2O is added to each sample to give proper acidity.

*10ml of 0.3% to ammonium thiocyanate solution is added as indicator.

*Titrate with standard iron (III) chloride solution containing 0.00195g Iron/ml.

*Endpoint is observed to be yellow which persists for 5mins.

%Phytic acid= Titre value X 0.00195g X 1.19 X 100.

3.5 STATISTICAL ANALYSIS

All data regarding mineral and antinutrient composition were evaluated statistically using SPSS (Version 25.0). The results are expressed as the mean ± standard deviation (SD). Differences among sample means were assessed using one-way Analysis of Variance (ANOVA). Significant differences were identified using Duncan's Multiple Range Test (DMRT) at a 5% probability level ($p < 0.05$).

CHAPTER FOUR

RESULTS

Table 4.1: Quantitative anti-nutritional composition of various anti-diabetic snack bar formulations (Mean ± SD)

Samples	Oxalate (mg/100g)	Phytate (%)	Cyanide (µg/mL)
A	1.247 ± 0.127	0.804 ± 0.197	9.604 ± 0.958
B	1.833 ± 0.336	0.472 ± 0.035	8.396 ± 0.989
C	0.880 ± 0.220	0.704 ± 0.035	6.894 ± 0.386

Table 4.2 Mineral composition of various anti-diabetic snack bar formulations

Element	A (mg/L)	B (mg/L)	C (mg/L)
Ca	6.8	1.1	2.5
Mg	8.26	10.26	7.82
Zn	0.29	2.25	0.25
Fe	9.3	10.1	9.7
P	2.16	3.26	2.54

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study examined the mineral and antinutrient composition of Samples A, B, and C to determine their potential contributions to blood glucose regulation. The findings revealed notable differences in essential minerals magnesium, zinc, calcium, iron, and phosphorus, as well as in antinutrients such as phytates, oxalates, and cyanogenic compounds, all of which collectively influence glucose metabolism and insulin activity. Among the samples, Sample B demonstrated the most favourable mineral profile, containing the highest levels of magnesium (10.26 mg/L), zinc (2.25 mg/L), and phosphorus (3.26 mg/L). These minerals play critical roles in glucose homeostasis: magnesium supports insulin receptor sensitivity and ATP-dependent glucose transport, while zinc is required for insulin synthesis, structural stabilization, and the protection of pancreatic β-cells from oxidative stress. The elevated levels of these minerals in Sample B therefore indicate a strong potential to enhance insulin secretion, improve insulin responsiveness, and support antioxidant defences, which are the three essential mechanisms in maintaining normal glycemic balance. Sample A, although rich in calcium (6.8 mg/L), was limited by its high phytate (0.804%) and cyanide (9.604 µg/mL) concentrations. Phytates reduce the bioavailability of divalent minerals such as calcium, magnesium, and zinc by forming insoluble complexes in the gut. Cyanogenic compounds impair cellular respiration and ATP generation, thereby disrupting energy-dependent processes fundamental to glucose uptake and metabolism. As a result, despite its mineral content, Sample A's high antinutrient load significantly diminishes its contribution to effective glucose regulation. Sample C exhibited the lowest levels of oxalate (0.880 mg/100 g) and cyanide (6.894 µg/mL), suggesting minimal inhibition of mineral absorption and limited interference with cellular function. However, its lower magnesium (7.82 mg/L) and zinc (0.25 mg/L) levels weaken its functional potential, since these minerals are central to carbohydrate metabolism, insulin signalling, and protection against oxidative stress. Thus, while Sample C possesses a favourable antinutrient profile, its reduced mineral density limits its effectiveness in supporting glycemic control. The overall nutrient–antinutrient interaction patterns reinforce Sample B as the most promising option. Its low phytate content (0.472%) enhances mineral bioavailability, allowing magnesium and zinc to remain physiologically accessible even in the presence of higher oxalate levels. Samples A and C do not achieve this balance: Sample A is hindered by high phytate and cyanide, while Sample C lacks sufficient mineral density.

5.2 CONCLUSION

This research set out to evaluate the mineral and antinutrient composition of three formulated samples in relation to their potential role in blood glucose regulation. The findings clearly indicate that variations in nutrient density and antinutrient levels significantly influence their metabolic usefulness. Among the samples analysed, Sample B demonstrates the most favorable balance, combining high concentrations of glucose-regulating minerals—particularly magnesium, zinc, and phosphorus, with comparatively low levels of phytate, thereby enhancing mineral bioavailability, followed by Sample C, while Sample A offers the lowest functional benefit.

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