BIOPROCESSING AND MINERAL PROPERTIES OF AN ANTI-ANAEMIC SNACK BAR FORMULATED FROM *JUSTICIA CARNEA*, BAMBARA GROUNDNUT, GROUNDNUT, MILLET, SOYABEANS AND DATES

BY

Ella Osaruemen OKHIONS-SCOTT

(BMS2209290)

A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE, B.SC. (HONS.) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY.

NOVEMBER 2025

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CERTIFICATION

We the undersigned hereby certify that Ella Osaruemen OKHIONS-SCOTT (BMS2209290) carried out this research work in the Department of Medical Biochemistry, University of Benin, Benin City, and we approve same as adequate in scope and quality for the reward of Bachelors of Science Degree (B.Sc.) in Medical Biochemistry.

Signed

……………………………	**………………………**
Prof (Mrs.) Henrietta A. Oboh	**Date**
(Project Supervisor)	
…………………………........	**………………………**
Dr. N. Bobby Aguebor-Ogie	**Date**
(Ag. Head of Department)	
……………………………...	**………………………**
External Examiner	**Date**

DEDICATION

This project is dedicated to Almighty God, the giver of life who has made it possible to complete my Bachelor of Science Degree (B. Sc) program in the Department of Medical biochemistry.

ACKNOWLEDGEMENTS

I give all glory to God Almighty for His grace, guidance, and protection throughout this endeavour. His mercy and faithfulness have accompanied me every step of the way. I would like to express my heartfelt appreciation to my parents, Mr and Mrs Scott Okhionkpamwonyi, for their unwavering love, prayers, and constant encouragement. Your support has been invaluable to me.

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My thanks also go to the Head of Department, Dr N. Bobby Aguebor-Ogie, and the entire staff of the Department of Medical Biochemistry for their efforts, patience, and dedication throughout this journey.

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ABSTRACT

Anaemia remains a major nutritional and public health concern, particularly in developing countries, where limited dietary diversity contributes to inadequate micronutrient intake. This study aimed to formulate and evaluate an anti-anaemic snack bar produced from *Justicia carnea* (blood leaf), Bambara groundnut, (*Vigna subterranea*), millet (*Pennisetum glaucum*), soyabeans (*Glycine max)* and dates (*Phoenix dactylifera*), focusing on its mineral composition. The raw materials were cleaned, processed and incorporated into a composite flour, after which snack bar samples were prepared using standard baking procedures. The formulations underwent mineral analysis to determine levels of iron, calcium, magnesium, zinc and other relevant micronutrients using established analytical methods. Results showed that the snack bar samples contained appreciable amounts of essential minerals, particularly iron and calcium, indicating their potential contribution to improving haematological status. The inclusion of *Justicia carnea* further enhanced the micronutrient quality due to its naturally high iron content. Based on these findings, the formulated snack bar demonstrates strong potential as an affordable, functional food product capable of supporting dietary strategies for anaemia prevention, especially in low-resource communities. The study concludes that locally sourced ingredients can be effectively combined to produce nutritionally beneficial snacks, while recommending further work on sensory evaluation, shelf-life determination, and clinical assessment of the product's impact on anaemic individuals.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Anaemia remains a major global health concern, particularly in developing countries such as Nigeria, where it affects children, pregnant women and individuals in low-income communities. It arises from a reduction in the number of red blood cells or inadequate haemoglobin levels, resulting in impaired oxygen transport and symptoms such as fatigue, weakness, dizziness and reduced immunity (Kassebaum *et al.,* 2023; Adebayo *et al*., 2021). In severe cases, it may contribute to developmental delays, maternal complications and increased morbidity.

Many cases of anaemia stem from inadequate iron intake, infections and diets lacking in essential micronutrients. While iron supplementation remains a standard intervention, side effects, inconsistent use and limited availability particularly in rural or low-resource settings often reduce its effectiveness (Lopez *et al.,* 2021). These barriers have encouraged a shift toward food-based approaches that naturally enhance dietary nutrient intake (Afolabi and Babajide, 2021). Functional foods have become notable within this strategy because they provide physiological benefits beyond basic nourishment. Snack products, in particular, are attractive due to their popularity, affordability and ease of distribution, making them suitable vehicles for delivering key nutrients to vulnerable groups (Ijarotimi and Keshinro, 2022). Incorporating locally accessible, nutrient-rich crops into such foods offers a culturally appropriate and cost-effective means of addressing anaemia.

Justicia carnea (“blood leaf”) is traditionally used in Nigeria to support blood health and contains iron, vitamin C and antioxidants (Ejiofor et al., 2025). When combined with cereals and legumes such as millet, soyabeans, groundnut and Bambara groundnut, it forms a composite flour that provides balanced proportions of protein, fibre, healthy fats and essential minerals (Adebowale et al., 2023; Enujiugha et al., 2022). Incorporating these ingredients into a nutrient-enriched snack bar may offer a practical way to improve mineral intake in communities with limited access to conventional health interventions. This study therefore focuses on formulating such a snack bar and assessing its mineral properties.

1.2 Aim of the Study

The aim of this study was to evaluate the mineral properties of an anti-anaemic snack bar formulated from Bambara groundnut, *Justicia carnea*, millet, soyabeans and dates.

1.3 Objective of the Study

The specific objective of the study were to:

1. To formulate a functional anti-anaemic snack bar incorporating Bambara groundnut, *Justicia carnea*, millet, soyabeans and dates.
2. To evaluate the mineral composition of the developed snack bar .

1.4 Justification of The Study

Anaemia remains prevalent in communities with low intake of bioavailable iron and limited access to nutrient-rich foods. Developing a snack bar enriched with *Justicia carnea*, millet, soyabeans, Bambara groundnut and dates provides a simple, affordable and culturally acceptable means of improving mineral intake. This food-based intervention offers a practical strategy for supporting better iron status among vulnerable populations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Anaemia and Nutritional Deficiency

Anaemia is one of the most prevalent nutritional and public health challenges globally, particularly in low- and middle-income countries. It occurs when the blood contains an insufficient number of red blood cells or inadequate haemoglobin concentration, resulting in a diminished capacity to transport oxygen to tissues. This leads to fatigue, weakness, dizziness, and shortness of breath. According to the World Health Organization (WHO, 2024), approximately one-third of the global population is affected by anaemia, with significantly higher rates in developing countries. In Nigeria, it remains a major health concern, especially among women of reproductive age and children under five years.

In many African countries, the high prevalence of anaemia is strongly linked to inadequate dietary iron intake, infectious diseases, and parasitic infestations. Many households rely heavily on cereal-based diets that are low in bioavailable iron and other essential micronutrients (FAO, 2021). In rural communities, access to fortified foods, iron supplements, and diverse diets is limited, making nutritional adequacy difficult to achieve (Ayogu and Okafor, 2022). Socio-economic constraints, limited healthcare access, and low awareness further contribute to persistent anaemia.

2.1.1 Causes and Prevalence of Anaemia

Iron deficiency remains the leading cause of anaemia globally. Iron is essential for haemoglobin synthesis, and inadequate dietary intake leads to reduced red blood cell production. Other causes include deficiencies in vitamin B_{12}, folate, and vitamin C, which are also important for erythropoiesis. Infectious diseases such as malaria, HIV, and helminth infections contribute to anaemia by destroying red blood cells or increasing iron loss (Uchegbu and Ijeh, 2022).

In developing countries, women and children are disproportionately affected because of increased iron requirements associated with menstruation, pregnancy, and rapid growth. Studies indicate that low intake of iron-rich foods, poor nutrient absorption, and disease burden significantly increase anaemia prevalence (Afolabi *et al.,* 2021). The World Health Organization (2023) advocates the use of food-based strategies to prevent and reduce anaemia, particularly through consumption of locally available nutrient-dense foods.

Table 2.1: Major Causes of Anaemia, Their Nutritional Impact and Health Outcomes (Ayogu and Okafor, 2022; Afolabi *et al.*, 2021)

Causes of Anaemia	**Nutritional Impact**	**Outcome on Health**
Iron deficiency	Decreased haemoglobin synthesis	Fatigue, weakness, shortness of breath
Poor diet	Reduced nutrient intake	Impaired immunity and growth
Infections	Increased red cell destruction	Chronic anaemia
Poor absorption	Limited iron bioavailability	Persistent deficiency
Poverty and poor diet diversity	Low intake of functional foods	High anaemia prevalence

2.1.2 Role of Diet and Micronutrients

Diet plays a fundamental role in preventing and managing anaemia. Iron, vitamin B_{12}, folate, and vitamin C are particularly important for haemoglobin formation and red blood cell production. Although plant-based foods provide iron, this non-heme form is less bioavailable; however, absorption improves when consumed with vitamin C-rich foods (Gibson *et al*., 2020; Bello and Olatunji, 2023).

Food-based approaches are now recognised as sustainable methods of addressing anaemia in vulnerable communities. *Justicia carnea*, commonly called "blood leaf," is naturally rich in iron, vitamin C, and antioxidants that support erythropoiesis (Kehinde and Olajide, 2023; Adeoye *et al*., 2021). Legumes such as *Glycine max* (soya beans), *Vigna subterranea* (Bambara groundnut), and *Arachis hypogaea* (groundnut) provide essential proteins, minerals, and healthy fats beneficial for blood health (Olapade and Adetuyi, 2020; FAO, 2021). The relationship between micronutrient intake and haemoglobin production is illustrated in Figure 2.1.

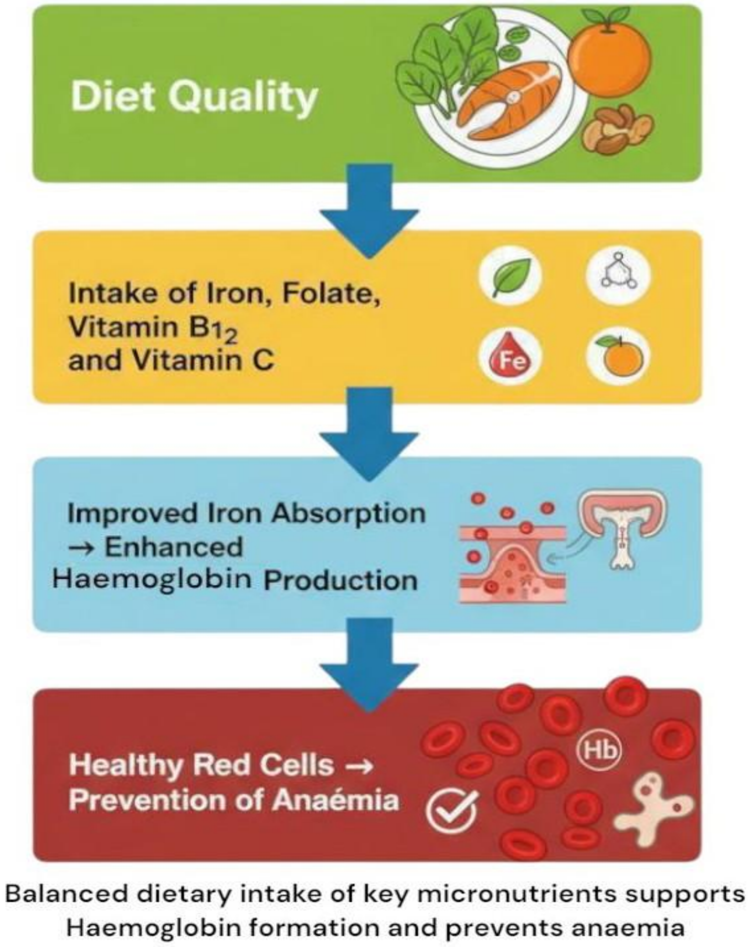

Figure 2.1: Diagram Showing the Relationship Between Diet, Micronutrient Intake and Haemoglobin Synthesis (Bello and Olatunji, 2023).

2.2 Functional Foods and Anti-Anaemic Potential

Functional foods are conventional foods that provide additional health benefits beyond basic nutrition. They may be naturally rich in bioactive compounds or formulated to include nutrients that support the prevention or management of disease. Their growing relevance in public health is linked to their affordability, accessibility, and suitability for daily dietary inclusion (Oluwafemi and Bello, 2022).

Cereals, legumes, fruits and vegetables are common functional food sources as they naturally contain vitamins, minerals, antioxidants and other beneficial phytochemicals (Martirosyan and Singh, 2021; Domínguez Díaz *et al.,* 2020). With recent advances in food science, several fortified or enhanced products such as protein-rich snack bar s, iron-fortified flours, and nutrient-dense snack bars have been developed to improve micronutrient intake, particularly in populations where anaemia remains prevalent (Oloruntola *et al*., 2023; Majola *et al*., 2023). Such functional products are especially useful in low-resource communities where poor dietary diversity contributes significantly to iron deficiency and other micronutrient-related disorders (Kitadi *et al.*, 2022).

The incorporation of locally available functional plants such as *Justicia carnea* has become increasingly important in functional food formulation. *Justicia carnea,* commonly referred to as "blood leaf," contains iron, vitamin C and antioxidant compounds that support erythropoiesis and enhance haemoglobin concentration (Kehinde and Olajide, 2023; Adeoye *et al.*, 2021). Its addition to snack bars and snack bar provides a natural, culturally acceptable, and inexpensive means of improving iron intake in vulnerable populations.

Cereals and legumes including millet, Bambara groundnut (*Vigna subterranea*), soyabeans (*Glycine max*) and groundnut (*Arachis hypogaea*) also contribute significantly to functional food development. They are rich in plant protein, dietary fibre and essential minerals that promote blood health (Haruna and Ibrahim, 2022; Singh, 2021). Although millet contains micronutrients such as iron and zinc, its bioavailability may be reduced by anti-nutritional factors like phytates. However, combining millet with legumes and applying bioprocessing methods such as fermentation or germination enhances the release and absorption of these nutrients (Haruna and Ibrahim, 2022; Al-Juhaimi *et al.*, 2020).

Together, these ingredients form a nutrient-dense functional food matrix capable of supporting improved haemoglobin synthesis and reducing the risk of anaemia.

Table 2.2: Functional Food Components and Their Roles in Supporting Blood Health (Bello and Olatunji, 2023)

Functional Component	**Source Ingredient**	**Role in Blood Health**
Iron	*Justiciacarnea,* millet, soyabeans, dates	Promotes haemoglobin synthesis
Protein	Bambara groundnut, soyabeans, groundnut	Builds red blood cells
Vitamin C	*Justicia carnea*, dates	Enhances iron absorption
Antioxidants	*Justicia carnea,* soyabeans	Protects red blood cells from oxidative damage
Energy (Carbohydrates)	Millet, dates, groundnut	Supports metabolism and oxygen transport

2.3 Bioprocessing in Cereal and Legume-Based Foods

Bioprocessing is widely applied in food science to enhance the nutritional value, digestibility, and safety of food products. It involves the use of biological processes such as fermentation, germination, and enzymatic modification to convert raw agricultural materials into foods with improved nutrient availability. This is particularly important for cereal- and legume-based foods, which naturally contain anti-nutritional factors that limit the absorption of minerals such as iron, zinc, and calcium. Through bioprocessing, the nutritional quality and functional properties of ingredients including millet, Bambara groundnut, soyabeans, and groundnut can be significantly improved (Olawale and Abiodun, 2023; Haruna and Ibrahim, 2022).

Bioprocessing has been recognised as a sustainable strategy for increasing the bioavailability of essential minerals in plant-based diets. As plant foods predominantly supply non-haem iron which has lower absorption, methods such as fermentation and germination are used to improve iron solubility while reducing anti-nutritional compounds (Balogun and Olayinka, 2021; Al-Juhaimi *et al*., 2020). These improvements make bioprocessed ingredients particularly valuable for developing functional snacks and anti-anaemic foods aimed at reducing iron deficiency (Martirosyan and Singh, 2021; Kitadi *et al.*, 2022).

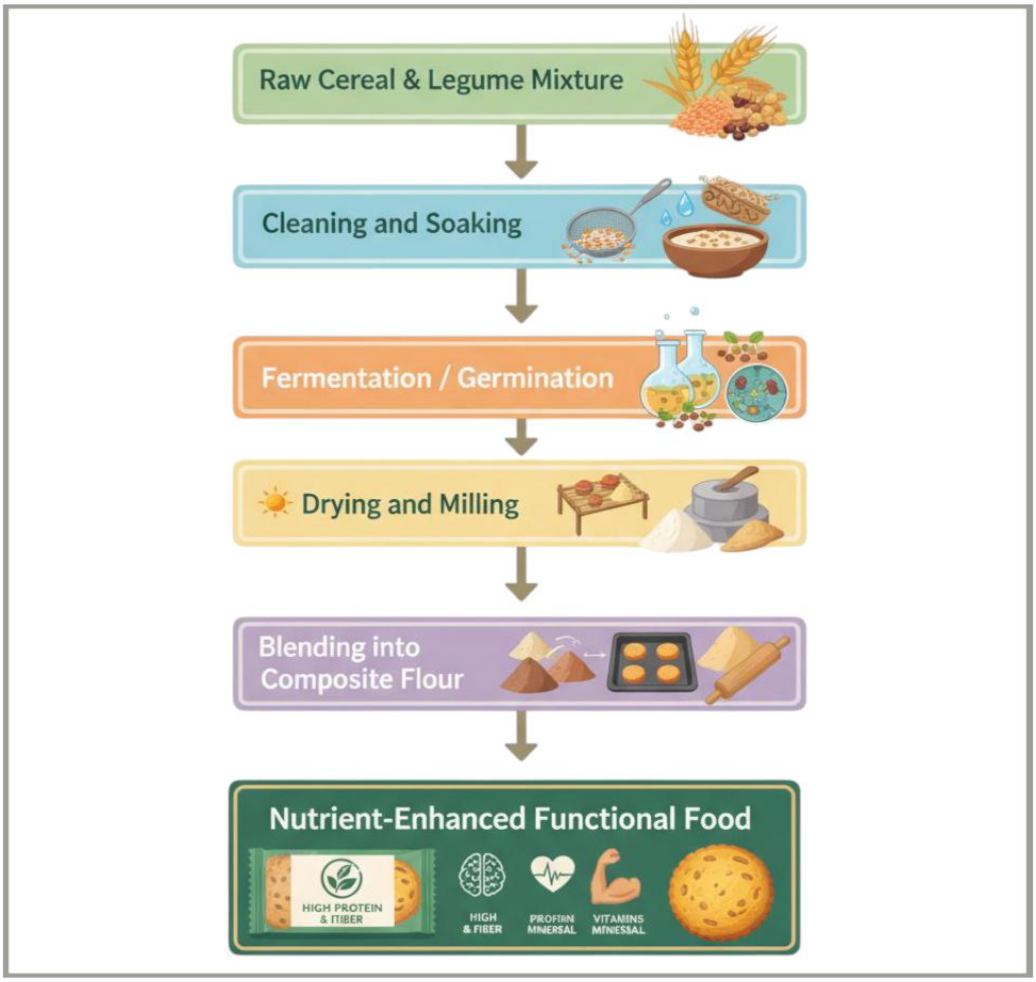

Figure 2.2: Overview of bioprocessing stages in cereal–legume food systems (Oluwafemi and Bello, 2022)

2.3.1 Definition and Principles of Bioprocessing

Bioprocessing refers to the application of biological or biochemical systems—often involving controlled microbial activity, enzymatic reactions, or germination to modify food materials and enhance their nutritional and functional attributes. These processes help release bound nutrients, reduce anti-nutritional compounds such as phytates, tannins, and oxalates, and improve the flavour, texture, and stability of foods (Bello and Olatunji, 2023).

In cereal–legume blends, bioprocessing improves protein digestibility, increases mineral bioavailability, and enhances sensory attributes. For the formulation of anti-anaemic snack bar s, bioprocessing improves nutrient release from millet, soyabeans, Bambara groundnut, and groundnut, thereby improving their contribution to iron intake and overall nutritional quality (Oluwafemi and Bello, 2022).

2.3.2 Traditional Bioprocessing (Fermentation and Germination)

Fermentation and germination are traditional techniques widely used to improve food quality, nutrient utilisation, and safety. These methods remain fundamental in indigenous food systems across developing regions (Aremu and Ibrahim, 2021).

Fermentation involves beneficial microorganisms such as yeasts and lactic acid bacteria, which break down complex food components, increase vitamin content, and reduce anti-nutritional factors. This improves mineral availability and enhances flavour (Haruna and Ibrahim, 2022).

Germination, also known as sprouting, activates endogenous enzymes that enhance nutrient composition (Chavan and Kadam, 2021). It reduces anti-nutritional compounds and increases vitamin C levels, which support iron absorption (Kaur and Kapoor, 2022). Research shows that germination improves iron and protein digestibility in millet, soyabeans, and Bambara groundnut by up to 20% (Akinola *et al*., 2022). When applied prior to snack bar production, these processes improve nutritional quality, sensory characteristics, and overall acceptability (Olapade and Aworh, 2021).

2.3.4 Effect of Bioprocessing on Nutritional Quality

Bioprocessing has consistently been shown to enhance the nutritional quality of cereal–legume foods. By reducing anti-nutritional factors such as phytates, oxalates, and tannins, bioprocessing increases the bioavailability of essential minerals including iron, zinc, and calcium (Balogun and Olayinka, 2021). It also enhances protein digestibility, improves amino acid balance, and increases overall nutrient density.

Furthermore, bioprocessing significantly enhances sensory attributes such as aroma, colour, taste, and texture. Studies report that snack bar produced from bioprocessed blends of millet, Bambara groundnut, soyabeans, and groundnut show improved iron content, better protein digestibility, and superior sensory ratings compared with non-bioprocessed samples (Edeh and Omodamiro, 2020).

Overall, bioprocessing serves as an essential link between nutrition and food technology in the development of functional foods aimed at managing anaemia. It provides a practical and sustainable means of improving the nutritional benefits of indigenous plant-based ingredients without relying solely on synthetic fortification.

2.4 Functional Foods

Functional foods are dietary components that provide health benefits beyond basic nutrition because of their bioactive compounds, micronutrients, and regulatory effects on metabolic processes. Martirosyan and Singh (2021) note that functional foods contribute to improved immunity, enhanced antioxidant defence, and better metabolic function. Domínguez Díaz *et al.* (2020) further reported that these foods contain bioactive substances such as flavonoids, polyphenols, carotenoids, probiotics, dietary fibres, and essential fatty acids that promote health and physiological stability.

Plant-based functional foods are particularly effective in addressing micronutrient deficiencies and reducing inflammation owing to their high antioxidant and mineral content (Mohammed *et al.,* 2023). International bodies such as the Food and Agriculture Organization (FAO, 2021) and the World Health Organization (WHO, 2023) recommend functional food strategies as sustainable and culturally acceptable approaches to combating iron deficiency, zinc deficiency, and anaemia.

Research further indicates that combining cereals and legumes improves amino acid profiles, increases mineral concentration, and enhances nutrient bioavailability through synergistic interactions (Tan *et al*., 2020; Adebayo *et al*., 2024). Therefore, cereal–legume composite foods serve as a practical foundation for developing functional foods aimed at reducing micronutrient deficiencies.

2.4.1 Snack Bar Production as a Functional Food Carrier

Snack bar are widely consumed baked products suitable for all age groups. Their affordability, ease of production, and long shelf life make them ideal vehicles for delivering nutrients in functional food formulations. For this reason, snack bar are increasingly being utilised to address micronutrient deficiencies, including anaemia.

In this study, the snack bar formulation incorporates Justicia carnea, Bambara groundnut, groundnut, millet, soyabeans, and dates. Each ingredient contributes important nutrients: *Justicia carnea* supplies iron and vitamin C essential for blood formation; Bambara groundnut and soyabeans provide plant-based protein; groundnut offers healthy fats and energy; millet contributes fibre and minerals; and dates serve as a natural sweetener rich in iron and potassium (Akinola *et al.,* 2022; Kehinde and Olajide, 2023).

The snack bar -making process involves cleaning and preparing raw materials, applying bioprocessing techniques such as fermentation or germination to cereals and legumes, drying and milling them into flour, and mixing them with fat, water, and baking agents to produce dough. The dough is then shaped and baked at moderate temperatures. Bioprocessing enhances digestibility and increases iron bioavailability by reducing anti-nutritional compounds such as phytates and tannins (Haruna and Ibrahim, 2022).

These functional snack bar offer a feasible and culturally appropriate approach to alleviating anaemia in communities with limited access to supplements or fortified foods. Their appealing flavour, natural colour derived from *Justicia carnea,* and enhanced nutrient density make them suitable for vulnerable populations such as children, pregnant women, and individuals with limited dietary diversity (Elkatry *Justicia carnea* ., 2024).

2.5 Nutritional and Mineral Properties of Functional Food

The nutritional and mineral composition of the ingredients used in developing anti-anaemic snack bar is central to their functional performance. Each ingredient offers a unique profile of macro- and micronutrients that help improve iron status, antioxidant capacity, and overall dietary quality. The combination of *Justicia carnea*, Bambara groundnut, groundnut, millet, soyabeans, and dates provides a balanced mix of proteins, minerals, vitamins, healthy fats, and bioactive compounds needed for blood health and energy metabolism. This composite formulation supports synergistic nutrient interactions that enhance mineral bioavailability and functional effectiveness (Adebayo *Justicia carnea* ., 2024).

2.5.1 *Justicia carnea*

Justicia carnea ("blood of Jesus leaf") contains substantial levels of iron, calcium, magnesium, and potassium that are essential for red blood cell development and electrolyte balance (Oloruntola *Justicia carna* ., 2023; Majola *et al.*, 2023). Its rich flavonoid and anthocyanin content contributes to strong antioxidant activity, which helps protect erythrocytes from oxidative damage and supports haemoglobin synthesis. The presence of vitamin C in the leaf further improves the absorption of non-haem iron through reduction mechanisms (Kitadi *et al*., 2020). Recent findings show that *Justicia carnea* supplementation in anaemic models improves haemoglobin, packed cell volume, and red cell count, confirming its haematinic potential (Majola *et al.*, 2025). In addition, its phytochemicals may modulate inflammatory pathways linked to nutritional anaemia (Udochukwu *et al*., 2023), making it valuable in functional food applications.

Figure 2.3: *Justicia carnea* Leaves

Source: Bustle / Getty Images (2020).

2.5.2 Bambara Groundnut (*Vigna subterranea*)

Bambara groundnut is a nutrient-dense legume containing 14–24% protein, carbohydrates, dietary fibre, and minerals including iron, zinc, magnesium, and potassium (Tan *et al.,* 2020). Its protein complements cereal proteins by supplying essential amino acids such as lysine, which millet lacks (Tamangwa *et al.*, 2023). Bioprocessing techniques such as germination and fermentation significantly reduce phytates and tannins, increasing the availability of iron and zinc (Adebayo *et al.*, 2024). Mild heat treatment has also been shown to improve its protein digestibility and mineral solubility (Oyeyinka *et al.*, 2022). These properties make Bambara groundnut a valuable component in functional snacks intended to enhance mineral intake and satiety.

Figure 2.4: Bambara Groundnut Seeds (*Vigna subterranea*)

Source: Dreamstime

2.5.3 Groundnut (*Arachis hypogaea*)

Groundnut contains 20–30% protein and up to 55% oil, predominantly monounsaturated fatty acids that support cardiovascular health (Çiftçi *et al*., 2022). It also provides minerals such as magnesium, phosphorus, potassium, and trace iron, together with vitamin E and phenolic antioxidants that enhance oxidative stability and nutritional value (Sanni *et al.,* 2024). Roasting improves flavour, increases antioxidant activity, and enhances its suitability for bakery products (Ayalew *et al*., 2022). The combination of healthy fats and micronutrients supports energy metabolism and contributes to the improved texture and palatability of functional snack bar s.

Figure 2.5: Groundnut (*Arachis hypogaea*)

Source: ResearchGate

2.5.4 Millet (*Pennisetum glaucum*)

Pearl millet is rich in iron, zinc, phosphorus, magnesium, and dietary fibre, making it a favourable cereal for addressing mineral deficiencies (Satyavathi *et al.*, 2021). It also contains polyphenols with antioxidant properties that help protect cells from oxidative stress (Jacob *et al.*, 2024). Although natural antinutrients can reduce mineral bioavailability, germination and fermentation significantly decrease phytate levels and enhance iron and zinc absorption (Almeida *et al.*, 2022). When used in composite flour with legumes, millet improves nutrient balance and supports sustained energy release.

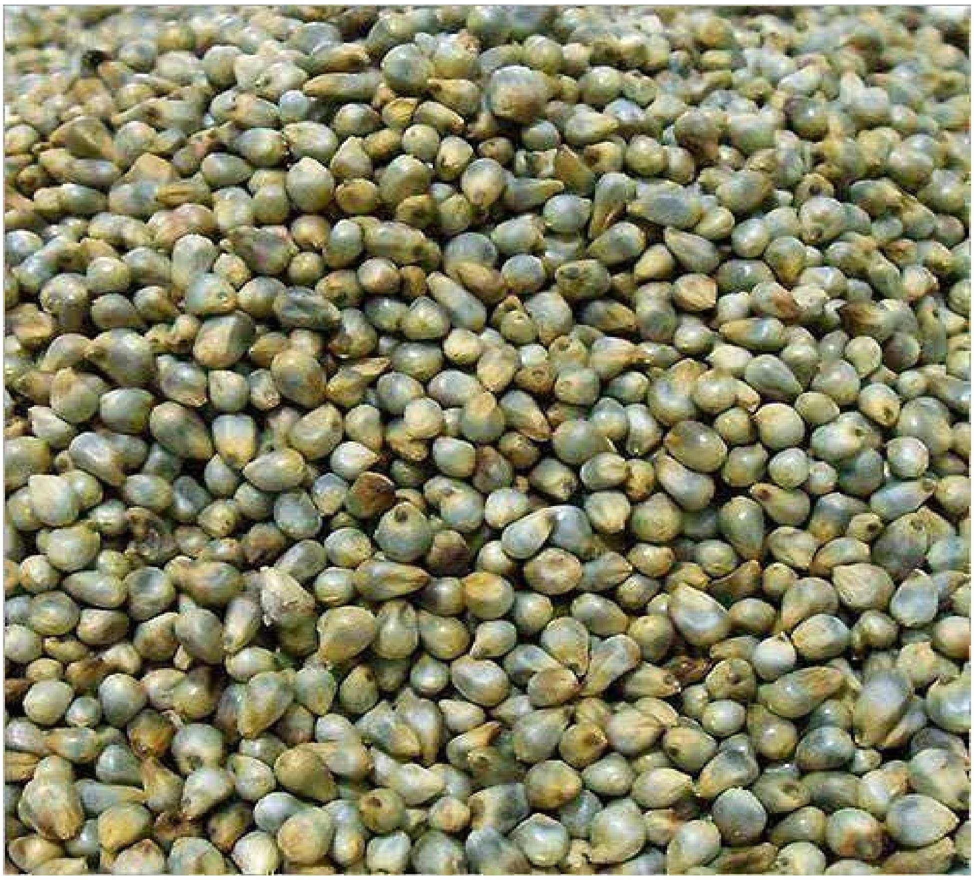

Figure 2.6: Millet Grains (*Pennisetum glaucum*)

Source: iStockPhoto

2.5.5 Soyabean (*Glycine max*)

Soyabean provides 30–40% high-quality protein and significant levels of iron, calcium, magnesium, phosphorus, zinc, and antioxidant isoflavones (Messina, 2022). Processing techniques such as heat treatment and enzyme-assisted methods reduce trypsin inhibitors and phytates, improving nutrient digestibility (Spréa *et al.,* 2024). In bakery applications, soy flour enhances moisture retention and protein density while contributing to the overall mineral content of functional foods (Tao *et al.*, 2023).

Figure 2.7: Soyabean Seeds (*Glycine max*)

Source: ResearchGate

2.5.6 Date Palm (*Phoenix dactylifera*)

Dates are composed of 60–75% carbohydrates, mainly natural sugars, along with dietary fibre, potassium, magnesium, and moderate iron levels (Idowu *et al*., 2020; Barakat *et al.,* 2023). They are rich in polyphenols and flavonoids, providing antioxidant protection that supports cell integrity and product shelf stability (Ibrahim *et al.,* 2024). Their natural sweetness reduces the need for refined sugar while contributing energy and improving the texture of baked products. The fibre content also assists digestive health and can moderate post-prandial glucose response.

Figure 2.8: Date Fruits (*Phoenix dactylifera*)

Source: Dreamstime

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

- Bambara groundnut flour
- Groundnut flour
- Soyabeans flour
- Millet flour
- *Justicia carnea* leaves
- Whole wheat flour
- Vegetable oil
- Cinnamon
- Date paste
- Egg/albumin
- Skimmed milk
- Gloves
- Whatman No. 1 filter paper
- Foil paper
- Towel
- Buckets & bowls

3.1.1 Apparatus and Equipments

- Oven (Model COV-8320-B)
- Blender (Kenwood Model KCB-239K)
- Weighing balance (Camry)

- Digital table scale (Model SBS-TW-500/10)
- Refrigerator
- Trays
- Mechanical sieve
- Knife
- Scissors
- Airtight containers

3.1.2 Chemicals and Reagents

- Nitric acid (HNO_3)
- Perchloric acid ($HClO_4$)
- Hydrochloric acid (HCl)
- Distilled water
- Deionised water.

3.2 Methods

The methods used in this study included the preparation of raw materials, formulation of composite flour blends, snack bar production, and analytical procedures for mineral determination.

3.2.1 Preparation of Raw Materials (Bioprocessing Procedures)

All raw materials were prepared under hygienic laboratory conditions to enhance quality, improve nutrient bioavailability, and ensure sample safety.

3.2.1.1 Preparation of *Justicia carnea* Leaf Powder

Fresh Justicia carnea leaves were obtained from a local garden in Benin City, Edo State Nigeria. The leaves were plucked, washed thoroughly, and air-dried at room temperature for four weeks, with light dampening each morning to prevent mould. Once constant weight was achieved, the dried leaves were milled, sieved, and stored in sealed glass containers wrapped in aluminium foil to protect them from light and moisture.

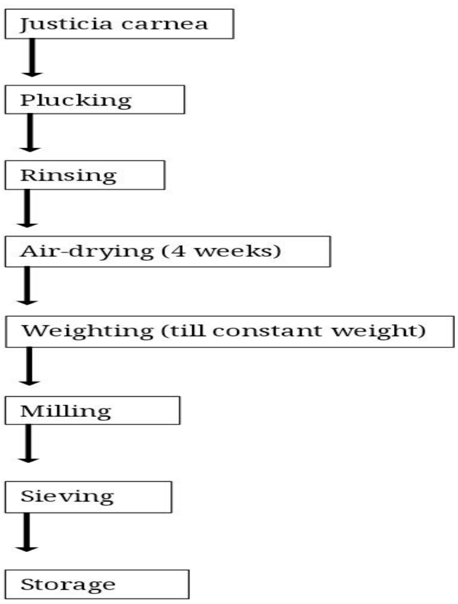

Figure 3.1: Flow diagram for the preparation of Justicia carnea leave powder

3.2.1.2 Preparation of Bambara Groundnut Flour

Bambara groundnuts were obtained from Oba Market, Benin City, Edo State, Nigeria. The seeds were sorted to remove discoloured, broken, and other defective grains, then washed and soaked in water at a 1:3 seed-to-water ratio for 12 hours at room temperature. After soaking, the water was completely drained, and the hydrated seeds were spread evenly on a clean, wet jute bag, covered, and kept in a dark, well-ventilated room to facilitate germination for 72 hours. Water was sprinkled every 12 hours to maintain adequate moisture and prevent dryness during rootlet development.

After germination, the seeds were oven-dried at 60 °C for 12 hours until brittle, then milled and sieved. The resulting flour was packaged in sealed plastic containers, labelled, and stored until required for further use.

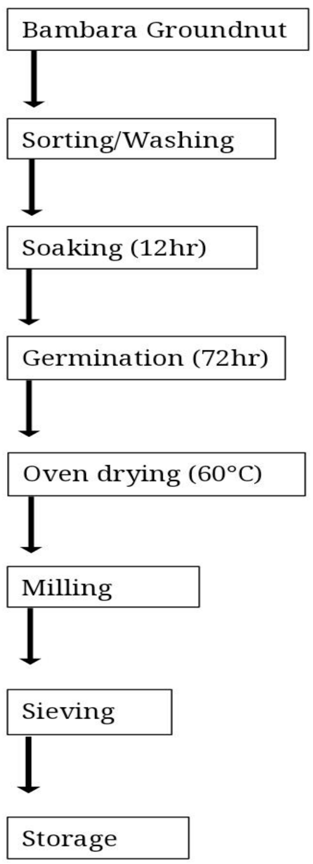

Figure 3.2: Flowchart for the preparation of Bambara Groundnut flour

3.2.1.3 Preparation of Groundnut Flour

Raw groundnuts were purchased from Ring Road Market, Benin City, Edo State, and manually sorted to remove bad seeds, stones, and other foreign materials. The clean nuts were roasted at 120°C for 30 minutes. After roasting, the nuts were cooled to room temperature, dehulled manually, and milled into flour. The groundnut flour was sieved packaged in airtight containers, labelled, and stored until needed.

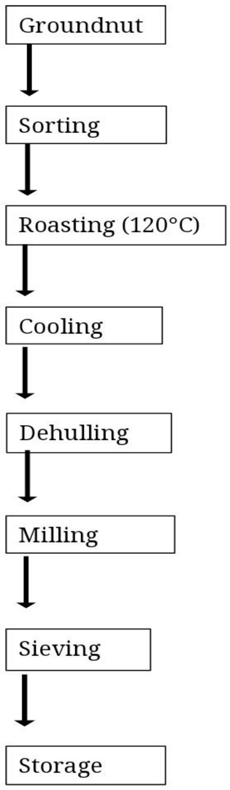

Figure 3.3: Flowchart for the preparation of Groundnut flour

3.2.1.4 Preparation of Millet Flour

Millet grains were purchased from New Benin Market, Benin City, Edo State, Nigeria and manually cleaned to remove dirt and stones. The grains were soaked for 30 minutes in clean water, rinsed, and boiled for 30 minutes. After boiling, the millet was drained and oven-dried at 80°C until a constant weight was achieved. The dried millet was milled, sieved, and stored in airtight containers until required.

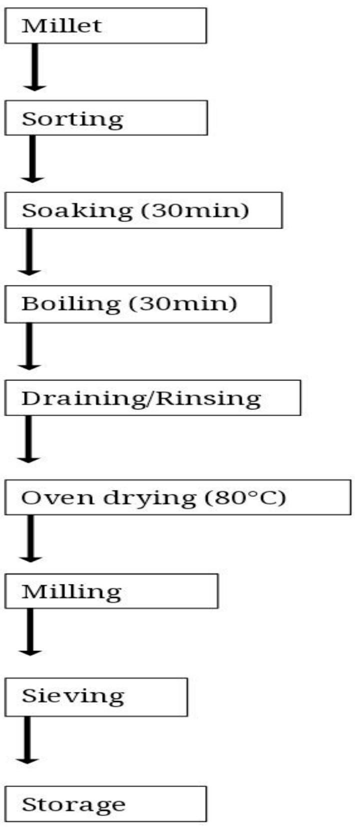

Figure 3.4: Flowchart for the preparation of Millet flour

3.2.1.5 Preparation of Soyabean Flour

Raw soyabeans were purchased from New Benin Market, Benin City, Edo State, Nigeria. The soyabean were sorted manually to remove stones, chaff, and other foreign materials. The clean beans were soaked in clean water for 24 hours at room temperature, then boiled for 25 minutes, drained, and rinsed. The boiled beans were dried in a hot air oven at 80°C for 3 days until a constant weight was obtained. The dried beans were milled, sieved, and stored in a labelled airtight container until use.

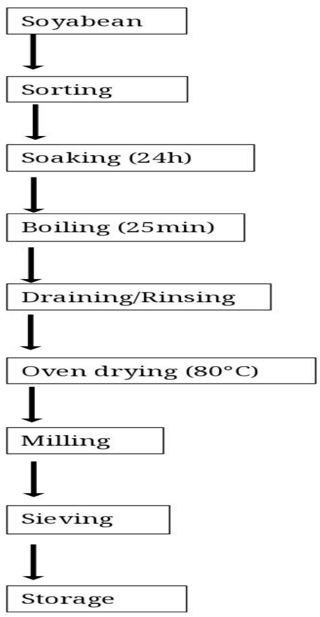

Figure 3.: Flow diagram for the preparation of Soyabean flour

3.2.1.6 Preparation of Date Paste

Date fruits were purchased from Ring Road Market, Benin City, Edo State, Nigeria. The fruits were washed, pitted (seeds removed), and soaked in clean water for 12 hours at room temperature to soften the pulp. The soaked dates were blended with the soaking water into a smooth paste, and stored under refrigeration until required.

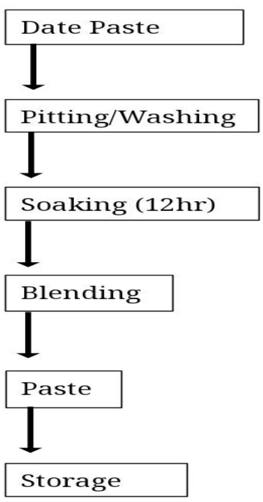

Figure 3.6: Flowchart for the preparation of Date Paste

3.2.2 Formulation and Preparation of the Anti-Anaemic Snack bar

The Anti-Anaemic Snack were formulated based on a 200 g total batch weight as shown in Table 3.1. Ingredients were weighed according to their respective proportions, and all formulations contained *Justicia carnea* as a functional additive (0.5%).

Table 3.1: Formulation of the Functional Snack Samples

Ingredient	**Control (%)**	**Sample A (%)**	**Sample B (%)**	**Sample C (%)**
All-purpose flour	55.0	–	–	–
Soyabean flour	–	10.0	15.0	10.0
Millet flour	–	5.0	7.5	10.0
Bambara groundnut flour	–	25.0	20.0	20.0
Groundnut flour	–	10.0	5.0	5.0
Date syrup	–	2.5	2.5	2.5
Justicia carnea powder	–	0.5	0.5	0.5
Baking powder	0.5	0.5	0.5	0.5
Liquid egg white	17.5	17.5	17.5	17.5
Vegetable oil	5.0	5.0	5.0	5.0
Skim milk powder	22.0	22.0	22.0	22.0
Cinnamon	~0.0	~0.0	~0.0	~0.0
Total	**100%**	**100%**	**100%**	**100%**

3.2.3 Anti-Anaemic Snack bar Preparation Procedure

Dry ingredients (All-purpose flour/composite flours, skimmed milk powder, baking powder, *Justicia carnea* powder, and cinnamon) were mixed thoroughly. Wet ingredients (liquid egg white, vegetable oil, and date paste) were blended separately until smooth. The wet mixture was gradually incorporated into the dry mixture and kneaded into a uniform dough. The dough was moulded, shaped, and baked at 120°C for 20 minutes. Baked snack bar were cooled, packaged in airtight containers, and stored for analysis.

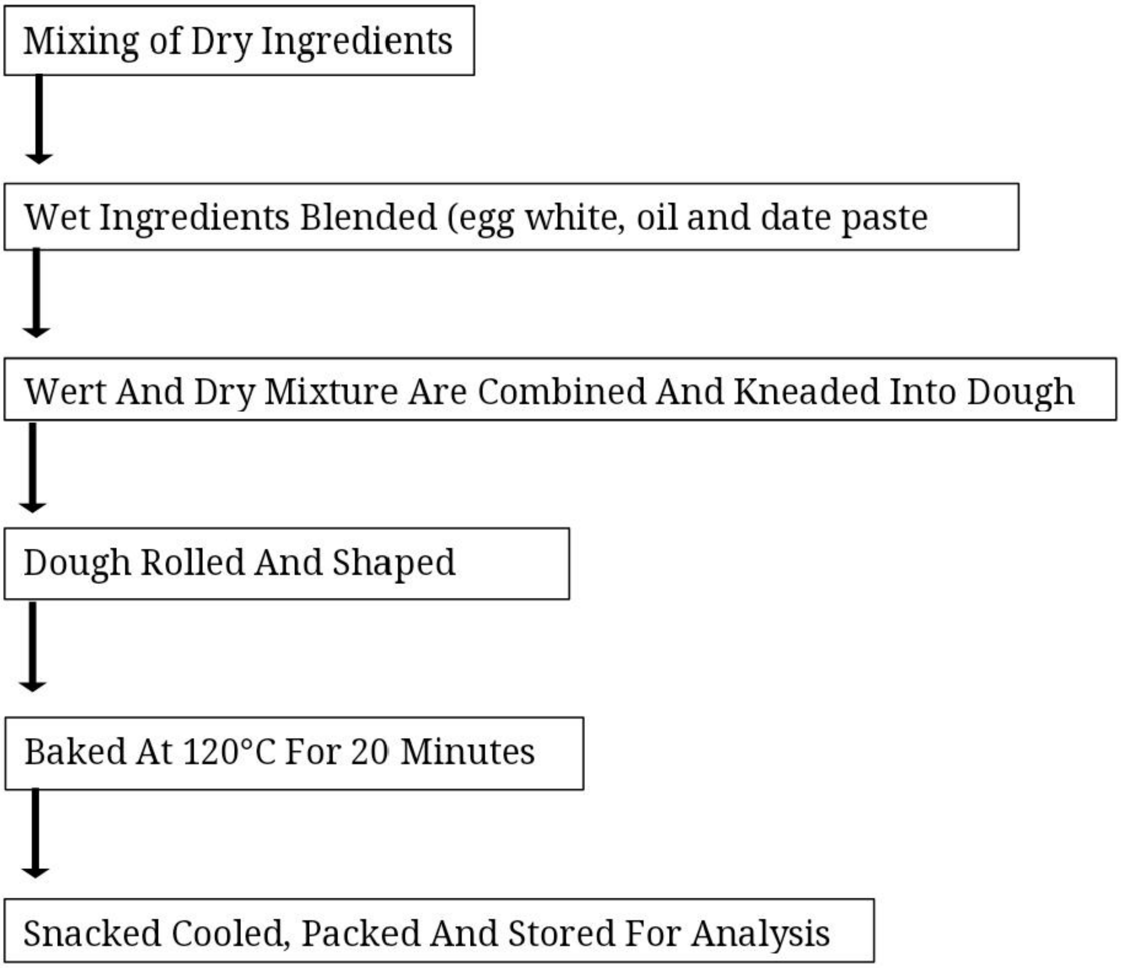

Figure 3.7: Flowchart for the preparation of Snack bar .

3.3 Elemental (Mineral) Analysis

The elemental composition of the snack bar samples (Fe, Zn, Ca, Mg, K, Na, P, Mn, Cr, and Pb) was determined using the method of AOAC (2016). Approximately 2 g of each dried, homogenized snack sample was digested with a 3:1 mixture of nitric acid (HNO_3) and perchloric acid ($HclO_4$) on a hot plate until a clear solution was obtained. The digest was filtered through Whatman No. 1 filter paper and diluted to 50 mL with deionized water.

All glassware used was pre-soaked overnight in a 1:1 hydrochloric acid-water solution, rinsed with distilled and deionized water, and oven-dried before use.A reagent blank was prepared alongside the samples.The mineral concentrations were determined using an Atomic Absorption Spectrophotometer (AAS, Buck Scientific 210 VGP, USA), and results were expressed in mg/kg of sample using the relationship:

$$\text{Concentration (mg/kg)} = \frac{A \times V}{W}$$

Where:

A = Concentration from AAS reading (mg/L)

V = Final volume of digest (mL)

W = Weight of sample digested (g)

3.4 Statistical Analysis

Data generated from mineral analysis were expressed as mean ± standard deviation (SD). Significant differences among means were determined using one-way Analysis of Variance (ANOVA), and where significant, Duncan's Multiple Range Test (DMRT) was used for mean separation at $p < 0.05$. Statistical analysis was carried out using SPSS statistical software (Version 25.0).

CHAPTER FOUR

RESULTS

4.1 Mineral Composition of the Snack bar Samples

The table presents the mineral composition of snack bar samples formulated with composite flours containing *Justicia carnea*, Bambara groundnut, groundnut, millet, soyabeans, and dates, with values expressed in mg/kg.

Table 4.1: Mineral Composition of the Snack bar Samples (mg/kg)

Minerals	**Sample A**	**Sample B**	**Sample C**
P	1.150 ± 0.001^{b}	1.107 ± 0.012^{c}	2.151 ± 0.001^{a}
Zn	0.181 ± 0.002^{b}	0.210 ± 0.001^{a}	0.151 ± 0.001^{c}
Mn	0.111 ± 0.001^{c}	0.170 ± 0.001^{a}	0.131 ± 0.001^{b}
Fe	0.103 ± 0.006^{c}	0.203 ± 0.006^{a}	0.105 ± 0.007^{b}
Cr	0.203 ± 0.006^{b}	0.150 ± 0.001^{c}	0.471 ± 0.002^{a}
Pb	0.100 ± 0.010^{b}	0.130 ± 0.010^{a}	0.080 ± 0.000^{c}
Ca	0.507 ± 0.012^{b}	1.010 ± 0.017^{a}	0.205 ± 0.007^{c}
Mg	0.971 ± 0.002^{a}	1.080 ± 0.000^{a}	0.615 ± 0.007^{b}
K	3.307 ± 0.012^{a}	3.307 ± 0.012^{a}	2.515 ± 0.021^{b}
Na	9.203 ± 0.006^{b}	9.603 ± 0.006^{a}	8.810 ± 0.014^{c}

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

This study assessed the mineral composition of anti-anaemic snack bar formulated from composite flours comprising *Justicia carnea,* millet, soyabeans, Bambara groundnut, groundnut and dates. The mineral concentrations presented in Table 4.1 show notable variations across the formulated samples, indicating the influence of ingredient proportions and bioprocessing on mineral retention and availability.

Iron (Fe), the primary mineral of interest in evaluating anti-anaemic potential, was highest in Sample B, followed by Sample C, as shown in Table 4.1. This observation aligns with Akinola *et al*., (2022), who reported that legumes and leafy vegetables enhance iron content due to their naturally elevated mineral levels. The superior iron composition in Sample B may be attributed to the complementary nutrient interaction between soyabean and Bambara groundnut, which supports improved mineral preservation during processing.

Zinc (Zn) levels also varied significantly across the snack bar samples, with Sample B presenting the highest concentration (Table 4.1). This trend is consistent with the findings of Tan *et al*., (2020), who noted that cereal–legume combinations enhance zinc density through synergistic nutrient interactions. Given its essential role in immune regulation and iron metabolism, the elevated zinc level strengthens the haematinic potential of the snack bar s.

Calcium (Ca) and magnesium (Mg) concentrations were notably higher in Samples A and B compared to Sample C, as indicated in Table 4.1. This supports Adebayo *et al*., (2024), who highlighted that legumes substantially enrich food matrices with divalent minerals. The inclusion of *Justicia carnea* further contributed to improved calcium and magnesium levels, consistent with its documented mineral richness (Majola *et al*., 2023).

Potassium (K) and sodium (Na) were highest in Samples A and B, reflecting the natural electrolyte contributions of dates and groundnut included in the formulations. Idowu *et al*., (2020) similarly noted that date fruits provide considerable potassium content, contributing to cardiovascular and metabolic support.

Chromium (Cr) and manganese (Mn), although required only in trace amounts, showed characteristic variations among samples, with Sample C recording the highest chromium value (Table 4.1). Their presence highlights the nutritional breadth offered by the composite blends. Lead (Pb) concentrations were minimal and within acceptable safety limits across all samples, indicating proper handling and preparation.

Overall, the results in Table 4.1 illustrate that Sample B consistently exhibited the most favourable mineral profile. The enhanced mineral density of Sample B suggests that combining millet, soyabeans and Bambara groundnut supports improved nutrient availability and functional food quality. This observation is in agreement with Haruna and Ibrahim (2022), who reported that cereal–legume composite flours enhance the nutritional performance of baked products.

5.2 Conclusion

This study demonstrated that snack bar formulated from composite flours enriched with *Justicia carnea,* millet, soyabeans, Bambara groundnut, groundnut and dates possess favourable mineral compositions capable of supporting improved micronutrient intake. Among the formulated snack bar samples, Sample B contained the highest concentrations of key minerals such as iron, zinc, calcium and magnesium, indicating that its specific ingredient combination provided the most effective nutritional enhancement. These findings confirm that the incorporation of cereal–legume blends and *Justicia carnea* can significantly improve the mineral density of baked products and serve as a practical, food-based strategy for reducing micronutrient deficiencies such as iron deficiency and anaemia.

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APPENDIX

Appendix A: Formulation Tables for Anti-Anaemic Snack Bar Samples

This appendix presents the formulation details of the anti-anaemic snack bar samples used in this study.

Table A1: Ingredient Composition of Snack Bar Samples (% w/w)

Ingredient	**Control (%)**	**Sample A (%)**	**Sample B (%)**	**Sample C (%)**
All-purpose flour	55.0	–	–	–
Soyabean flour	–	10.0	15.0	10.0
Millet flour	–	5.0	7.5	10.0
Bambara groundnut flour	–	25.0	20.0	20.0
Groundnut flour	–	10.0	5.0	5.0
Date syrup	–	2.5	2.5	2.5
Justicia carnea powder	–	0.5	0.5	0.5
Baking powder	0.5	0.5	0.5	0.5
Liquid egg white	17.5	17.5	17.5	17.5
Vegetable oil	5.0	5.0	5.0	5.0
Skim milk powder	22.0	22.0	22.0	22.0
Cinnamon	~0.0	~0.0	~0.0	~0.0
Total	**100%**	**100%**	**100%**	**100%**

Appendix B: Images of Final Formulated Anti-Anaemic Snack Bar Samples

Figure B1: Sample A snack bar during cooling

Figure B2: Sample B snack bar during cooling

Figure B3: Sample C snack bar during cooling

Appendix C: Raw Mineral Analysis Data Tables

This appendix presents the raw mineral analysis data obtained from Atomic Absorption Spectrophotometric (AAS) measurements of the snack bar samples prior to statistical analysis.

Table C1: Raw Mineral Concentrations of Snack Bar Samples (mg/kg)

SAMPLE	P	Zn	Mn	Fe	Cr	Pb	Ca	Mg	K	Na
A1	1.15	0.18	0.11	0.10	0.20	0.11	0.50	0.97	3.30	9.20
A2	1.151	0.183	0.112	0.11	0.21	0.10	0.52	0.973	3.32	9.21
A3	1.15	0.18	0.11	0.10	0.20	0.09	0.50	0.97	3.30	9.20
B1	1.10	0.21	0.17	0.20	0.15	0.14	1.00	1.08	3.30	9.60
B2	1.12	0.211	0.171	0.21	0.151	0.13	1.03	1.08	3.32	9.61
B3	1.10	0.21	0.17	0.20	0.15	0.12	1.00	1.08	3.30	9.60
C1	2.15	0.15	0.13	0.10	0.47	0.08	0.20	0.61	2.50	8.80
C2	2.151	0.151	0.132	0.11	0.472	0.08	0.21	0.62	2.53	8.82