

**PRODUCTION, PHYTOCHEMICAL AND MINERAL ANALYSIS OF GROUNDNUT-
BASED READY-TO-USE THERAPEUTIC FOODs**

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DECEMBER, 2025

**PRODUCTION, PHYTOCHEMICAL AND MIINERAL ANALYSIS OF GROUNDNUT-
BASED READY-TO-USE THERAPEUTIC FOODs**

Project report

Submitted to the

**DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY USMANU
DANFODIO UNIVERSITY, SOKOTO**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE
DEGREE OF BACHELOR OF SCIENCES**

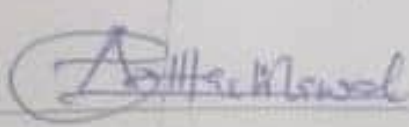
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DECEMBER, 2025

CERTIFICATION

This is to certify that the research project reported here was conducted by SAADALLAHI Abdulquadir (2010304018) and has been approved as meeting part of the requirement for the award of Bachelor of Science (B. Sc) degree in Biochemistry of the Usmanu Danfodiyo University, Sokoto.

 Prof. Aliyu Abdulhadi Turaki (External Examiner)	<u>16-12-2025</u> Date
 Dr. S.G. Ibrahim (Supervisor)	<u>16/12/2025</u> Date
 Dr. A. Lawal (Head of Department)	<u>16.12.2025</u> Date

DEDICATION

I dedicate this project to the Almighty Allah for His blessings, mercy and abundant knowledge that He granted on me throughout my journey in the university

I also dedicate this project to my beloved parents, Mr. Saadallahi Mudi and Mrs. Saadallahi Fatimoh, for their relentless parental care, efforts, support and encouragement which has been of great contribution to my academic pursuit, may the blessings of the Almighty Allah in His blessings continue to be with them.

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious, the Most Merciful, I thank Almighty Allah for the gift of life, strength, and blessings that enabled me to complete this research. May His peace and blessings be upon Prophet Muhammad (SAW), his companions and all who follow his path until the Day of Resurrection.

I wish to acknowledge all those who have contributed in one way or the other towards the successful completion of this research and status.

I sincerely thank my supervisor, Dr Shafa'atu Giwa Ibrahim, for her valuable guidance encouragement, and patience throughout this research. Her meticulous support at every stage has

been truly inspiring. May Almighty Allah bless her and her family, and continue to elevate her wisdom, health, and status.

My heartfelt appreciation to my co-supervisors Dr. Tijjani Salihu Shinkafi and Mr Zaharadeen Yusuf for their support and guidance at every stage of my research. May Almighty Allah in His mercy continue to bless with everlasting success and achievements.

I sincerely express my heartfelt gratitude to my father, Mr. Saadallahi Mudi, and my BELOVED mother, Saadallahi Fatimoh, for their undying love, sacrifices, guidance, support, efforts, and heartfelt prayers throughout this journey. This project would not have been possible without their unwavering support. May Almighty Allah bless them with long lives and good health to enjoy the fruit of their labour.

Furthermore, I would like to appreciate my siblings: Saadallahi Shuaibah, Saadallahi Wasilat, Saadallahi Aminah, Saadallahi Abubakar for their undying love and support. May Almighty Allah continue to bless you in health, knowledge, wealth and success.

I would also like to appreciate my best friend and roommate, Monsur mashood, who has shown me full support, love, and encouragement throughout my journey in the University. May Almighty Allah answer all his prayers and crown his efforts.

My heartfelt gratitude also goes to those who became like my brothers during my days in the university; Abdulrasheed Soliu, Akinleye Hamed, Rufai Abdulbasheet, Ndako, Waqolar, and all Mallam Musa compound residents, may Almighty Allah fulfil their wishes and answer all their prayers.

To all my course mates, may our paths continue to cross and diverge in a meaningful way on the vast landscape of learning and success.

ABSTRACT

This study assessed the phytochemical composition, mineral profile, heavy metal levels, and anti-nutrient/mineral ratios of standard Ready-to-Use Therapeutic Food (RUTF) and a locally formulated groundnut-based RUTF. Qualitative screening showed the presence of flavonoids, alkaloids, tannins, saponins, cardiac glycosides, and phenols in both samples. Quantitatively,

tannin recorded the highest concentration in both formulations, with 80.75 ± 0.27 mg/dl in the groundnut-based RUTF and 50.15 ± 0.32 mg/dl in the standard RUTF, while oxalate was lowest (0.007–0.01 g/%). Other phytochemicals such as flavonoids (6.09–9.03 g/%), alkaloids (4.61–6.16 g/%), and saponins (4.19–7.25 g/%) were present in moderate amounts. Mineral analysis showed that sodium, calcium, potassium, and magnesium were predominant, with sodium highest in both samples (16.58 ppm in standard RUTF; 14.68 ppm in groundnut-based RUTF). Heavy metal evaluation detected trace levels of nickel (highest), arsenic, cadmium, and lead, all within WHO safety limits. Anti-nutrient/mineral ratios indicated strong tannin interactions, particularly with zinc (≈ 19 –295) and iron (≈ 2.8 –35.6). Overall, the groundnut-based RUTF demonstrated comparable nutritional and phytochemical quality to the standard RUTF, supporting its potential as a safe, affordable alternative for malnutrition management.

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CHAPTER ONE

1.0 INTRODUCTION

Infant foods and Ready-to-Use Therapeutic Foods (RUTF) are specially formulated to provide adequate energy and essential nutrients required for the growth and recovery of malnourished children. These products supply key minerals such as iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K), which play critical roles in enzymatic functions, bone formation, and immune competence (Collado-López *et al.*, 2025). RUTFs are often made from locally available ingredients like peanuts, milk powder, vegetable oils, sugar, and vitamin–mineral premixes, making them cost-effective and accessible for community-based management of severe acute malnutrition. However, alongside essential minerals, the potential contamination of infant foods and RUTFs with toxic heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) has become a growing public health concern. These metals can enter food chains through contaminated soils, irrigation water, fertilizers, or industrial emissions that affect raw materials used in food production (Bair *et al.*, 2022). During processing, packaging, or storage, additional contamination may occur if quality assurance measures are inadequate. Infants and young children are particularly vulnerable to the toxic effects of heavy metals because of their lower body mass, developing organs, and higher absorption rates compared to adults (Alharbi *et al.*, 2023).

Recent studies have reported measurable concentrations of toxic metals in various baby food products and therapeutic diets (Collado-López *et al.*, 2025) conducted a global synthesis and found detectable levels of arsenic, lead, and cadmium in several infant formulas and processed foods. Similarly (Alharbi *et al.*, 2023) observed that baby foods from different markets contained

significant amounts of these metals, posing potential health risks if consumed regularly. These findings align with the narrative review by Bair *et al.* (2022), which emphasized the urgent need for stricter monitoring and enforcement of food-safety regulations for infant and toddler foods.

The accumulation of heavy metals in infant foods poses both acute and chronic health implications. Lead exposure has been associated with neurodevelopmental deficits, cognitive impairment, and behavioral disorders in children (Bair *et al.*, 2022). Arsenic and cadmium are known carcinogens that may disrupt kidney function and skeletal development. Therefore, assessing the concentration of both essential minerals and potentially toxic heavy metals in RUTF is crucial for ensuring the nutritional adequacy and safety of therapeutic foods intended for malnourished infants.

Globally, regulatory bodies such as the World Health Organization (WHO) and the United States Food and Drug Administration (FDA) have set permissible limits for heavy metals in infant foods, yet enforcement and local monitoring remain inconsistent (Consumer Reports, 2025). Continuous assessment of mineral composition and contaminant levels in locally produced RUTFs is necessary to maintain product quality, prevent toxicity, and promote safe nutritional rehabilitation for infants and children recovering from malnutrition.

1.1 Justification of the Study

The quality and safety of Ready-to-Use Therapeutic Foods (RUTFs) are crucial for the effectiveness management of malnutrition, particularly among children in Nigeria. While RUTF is designed to provide balanced nutrition, contamination with heavy metals or imbalanced minerals may undermine its therapeutic benefits and pose serious health risks. This study is therefore justified as it provides scientific data on the mineral and heavy metal composition of

RUTF, which can serve as a basis for quality control, regulatory monitoring, and improvement of local formulations. The findings will help to ensure that RUTF meets international safety and nutritional standards, thereby enhancing its effectiveness in the rehabilitation of malnourished individuals and contributing to public health improvement.

1.2 Aim

To evaluate the phytochemical composition, mineral content, and heavy metal levels of locally produced Ready-to-Use Therapeutic Food (RUTF) in order to assess its nutritional quality and safety for use in the management of severe acute malnutrition.

1.3 Objectives

1. To produce and formulate ready-to-use therapeutic food using groundnut-based diet.
2. To determine the levels of essential minerals (such as iron, zinc, calcium, and magnesium) in the locally formulated RUTF.
3. To assess the concentration of potentially harmful heavy metals (such as lead, cadmium, mercury, and arsenic) in the RUTF.
4. To determine the phytochemical, minerals and heavy metals ratio.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview on Malnutrition and RUTFs

Malnutrition is a universal public health problem in both children and adults globally (WHO, 2019). It is not only a public health concern but it is an impediment to global poverty eradication, productivity and economic growth. By eliminating malnutrition, it is estimated that 32% of the global disease burden would be removed (WHO, 2020). As a widespread serious problem affecting children in developing countries, progress towards tackling the different forms of malnutrition remains relatively slow (Global, 2018). Malnutrition occurs due to an imbalance in the body, whereby the nutrients required by the body and the amount used by the body do not balance (WHO, 2019). Around one third of all under-5 child deaths in the WHO African Region are linked to malnutrition (WHO, 2015), and children with severe acute malnutrition without medical complications are commonly treated via outpatient therapeutic programmes using Ready-to-Use Therapeutic Foods (RUTF) (UNICEF, 2025).

RUTFs, energy-dense foods with a low moisture content that can be eaten directly from the packaging - has been introduced for home-based treatment during the rehabilitation phase. RUTF, a solid or semi-solid product, was originally developed by Nutriset and the Institute for Research and Development in France, as a home-based follow-up treatment after F-100 (Bazzano, 2017). Where RUTF is used for home-based rehabilitation, it can be provided to *meet all* of the nutritional requirements of a child recovering from SAM; for example, in low-income settings where food security may be a significant issue. Alternatively, RUTF may be provided for SAM children as a supplement to the usual family diet. RUTF can be formulated and

produced in various ways. It can be cheaper to produce RUTF with less or no milk powder, or more acceptable if locally or indigenous ingredients are used.

Furthermore, fatty acid composition (specifically, omega-3 fatty acids) may play a role in infection and inflammation, and thus might be a beneficial ingredient in therapeutic food. Similarly, as children with SAM may have compromised gastrointestinal function, the addition of pre- and probiotics may be advantageous (Schlegel *et al.*, 2017). Children as young as six months of age can consume RUTF with a homogenous paste texture (DFID, 2009). Solid RUTF can also be soaked in clean, boiling water and eaten as porridge by young infants and as a biscuit by older children. Infants younger than six months should not be given RUTF (DFID, 2009).

2.2 Groundnut as ingredients for RUTF

Groundnut (*Arachis hypogaea*), known locally in Nigeria among Yorubas as “Epa”, Hausas as “Gyada” and Igbos as “Opupa”, remains an important nut for both rural livelihoods and national food security. According to the Food and Agriculture Organization (FAO) data, Nigeria is one of the top global producers of groundnuts, contributing significantly to global output. Despite this, average yields in many Nigerian states remain below potential due to several biophysical and socioeconomic constraints (Awe *et al.*, 2024).

In terms of nutritional impact, groundnut is being incorporated into school feeding programs and considered for local formulations of therapeutic foods, because of its high calory density, oil content, good essential amino acid profile (particularly methionine and arginine), and local availability (Olasan *et al.*, 2024).



Fig. 2.1 Groundnut

Groundnut (*Arachis hypogaea*) is a critical component in the formulation of Ready-to-Use Therapeutic Food (RUTF) due to its high caloric value and rich mineral content. It contributes significantly to the micronutrient profile of RUTF, which is essential for the nutritional rehabilitation of malnourished children. Groundnuts contain key minerals such as magnesium (120-180 mg/100 g), phosphorus (350-400 mg/100 g), potassium (600-700 mg/100 g), calcium (50-92 mg/100 g), iron (2-4 mg/100 g), and zinc (2-3 mg/100 g). These minerals play crucial roles in bone development, enzymatic activity, immune function, and oxygen transport (Glew *et al.*, 2005; U.S. Department of Agriculture [USDA], 2021). Their inclusion enhances the therapeutic value of RUTF, making it an effective intervention against severe acute malnutrition.

2.3 Phytochemicals in Plant-based Therapeutic Foods

Phytochemicals are naturally-occurring chemicals present in or extracted from plants (Linus Pauling Institute, 2025; Frank *et al.*, 2020). Some phytochemicals are nutrients for the plant, while others are metabolites produced to enhance plant survivability and reproduction (Hassan *et al.*, 2020). They are generally regarded as research compounds rather than essential nutrients

because proof of their possible health effects has not been established yet (Linus Pauling Institute, 2025; Frank *et al.*, 2020).

1. Alkaloids

Alkaloids, a diverse group of nitrogen-containing secondary metabolites, play a dual role in human nutrition by serving as both beneficial bioactive compounds and antinutritional factors. While some alkaloids possess therapeutic properties that can aid in disease management, their presence in edible plants has been associated with reduced nutrient bioavailability and impaired protein digestion, thereby exacerbating malnutrition in vulnerable populations. They function in the defense of plants against herbivores and pathogens, and are widely exploited as pharmaceuticals, stimulants, and narcotics due to their potent biological activities (Elshafie *et al.*, 2023). In nature, the alkaloids exist in large proportions in the seeds and roots of plants and often in combination with vegetables acids. Alkaloids have pharmacological applications as anesthetics and CNS stimulants (Madziga *et al.*, 2010). Some well-known alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine (figure 2.1).

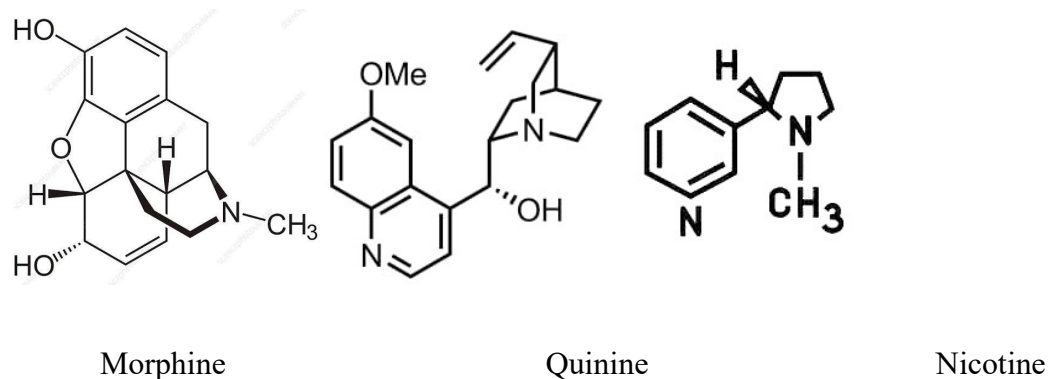


Fig. 2.2: Structure of some common alkaloids (Zorrilla and Sparatore, 2022).

2. Flavonoids

Flavonoids are a group of polyphenolic compounds produced in plants as secondary metabolites. Flavonoids are widely found in fruits, vegetables and other food crops. They have favorable biochemical effects on multiple diseases (e.g., cardiovascular disease, atherosclerosis) as well as other bioactivities (e.g., anti-inflammation, anti-aging) (Gentile *et al.*, 2018, Williamson *et al.*, 2018, Guven *et al.*, 2019). In addition, flavonoids are an essential ingredient in a variety of nutraceutical products. Flavonoids antioxidant activity can prevent damage caused by free radicals through scavenging of reactive oxygen species (ROS), activation of antioxidant enzymes, inhibition of oxidases (e.g., xanthine oxidase [XO], cyclooxygenase [COX], lipoxygenase and phosphoinositide 3-kinase [PI3K]), and reduction of α -tocopheryl radicals (Williamson *et al.*, 2018). In low-income regions where dietary diversity is limited, the inclusion of flavonoid-rich foods such as leafy greens, citrus fruits, and legumes can contribute to better nutritional outcomes and reduce the burden of malnutrition-related diseases (Li *et al.*, 2023).

3. Saponin

Saponins are secondary metabolites that are mainly distributed in plants (Jolly *et al.*, 2023), where they are synthesized as the plants' self-defense tasks against plant pathogens. These secondary metabolites have been utilized in several sectors of consumable products like taste modifiers, surfactants and emulsifiers as well as for drugs and medicines (Sharma *et al.*, 2023). Saponins molecules are diverse depends on the producing plants. the saponins' structures are composed with hydrophobic aglycones conjugated with hydrophilic sugar moieties, i.e., glucuronic acid, galacturonic acid, rhamnose, arabinose, xylose and fructose (Wojciechowski *et al.*, 2014). In regions where staple foods like soybeans or certain pulses are major protein

sources, insufficient processing to remove or reduce saponins may exacerbate protein-energy malnutrition and micronutrient deficiencies.

4. Tannins

In their natural form, tannins are water-soluble phenolic compounds with molecular weights in the range of 300 to 500 and have the ability to precipitate gelatin, alkaloids, and proteins (Mena *et al.*, 2015). Tannins are secondary metabolites widely distributed in plants: they are polymeric phenolic substances with astringency properties (Agrawal *et al.*, 2012; Lattanzio *et al.*, 2012; Bone and Mills, 2013; Tamokou *et al.*, 2017). Tannins are found in a huge variety of plants, including legume seeds, cider, cereals, cacao, peas, some leafy and green vegetables, coffee, tea, and nuts (Lochab *et al.*, 2014; Suvanto *et al.*, 2017; Fraga-Corral *et al.*, 2020). In general, tannins can be classified into one of two classes on the basis of their structural features: hydrolyzable and condensed tannins (Hatew *et al.*, 2016; Bee *et al.*, 2017). Their antinutritional components interfere with the digestive process and prevent the efficient utilization of nutrients (Luo and Xie, 2013; Sathya and Siddhuraju, 2015; Wu *et al.*, 2016). The antinutritional action of tannin has been linked to increased risks of protein-energy malnutrition and iron-deficiency anemia in populations that rely heavily on tannin-rich foods as primary dietary staples. In regions with limited dietary diversity and food insecurity, insufficient processing methods such as poor soaking, fermentation, or cooking may leave high levels of tannins, further exacerbating micronutrient deficiencies and growth impairment in children.

2.2.1 Roles of phytochemicals in human health

1. Antioxidants

These substances serve as a protective shield for cells, defending them against the harm caused by free radicals (Zhang *et al.*, 2015). Ready-to-Use Therapeutic Foods (RUTF) are specially

formulated, energy-dense foods designed for the management of severe acute malnutrition, and their effectiveness is partly linked to their antioxidant content. Malnutrition is often accompanied by oxidative stress caused by an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense system, which leads to cellular damage, weakened immunity, and delayed recovery. RUTF formulations, typically based on peanuts, milk powder, vegetable oils, and added vitamins and minerals, provide essential antioxidants such as vitamin E, vitamin C, selenium, and zinc that help neutralize ROS and repair oxidative damage. These antioxidants not only support immune function but also improve nutrient absorption and metabolic recovery, thereby accelerating weight gain and reducing the risk of infection in malnourished children (Akomolafe *et al.*, 2024).

2. Anti-inflammatory

Malnutrition is frequently accompanied by chronic inflammation, which contributes to impaired nutrient absorption, weakened immunity, and poor recovery outcomes. Protein-energy malnutrition and deficiencies in key micronutrients such as zinc and vitamin A can trigger or worsen inflammatory responses, creating a cycle where inflammation further limits nutrient utilization and increases susceptibility to infections (Michaelsen *et al.*, 2023). Ready-to-Use Therapeutic Foods (RUTF), formulated to treat severe acute malnutrition, play a critical role not only by providing energy and essential nutrients but also by supplying bioactive components with **anti-inflammatory properties**. RUTF ingredients such as milk proteins, peanuts, and added omega-3 fatty acids, along with micronutrients like zinc, vitamin E, and selenium, help downregulate pro-inflammatory cytokines and reduce oxidative stress (Sharma *et al.*, 2024). This anti-inflammatory effect supports gut integrity, enhances immune function, and accelerates weight gain and tissue repair in malnourished children. Recent studies also suggest that fortifying

RUTF with natural plant-based compounds such as flavonoids or polyphenols, could further enhance its anti-inflammatory potential and improve recovery outcomes in severe acute malnutrition (Oketch *et al.*, 2024).

3. Antimicrobial

Phytochemicals with antimicrobial properties play a promising role in addressing malnutrition when incorporated into Ready-to-Use Therapeutic Foods (RUTF). Malnourished children are highly susceptible to infections due to weakened immunity and gut dysbiosis, which exacerbate nutrient losses and delay recovery (Tickell *et al.*, 2023). Certain plant-derived phytochemicals such as flavonoids, tannins, alkaloids, saponins, and phenolic acids exhibit **antimicrobial activity** by disrupting microbial cell walls, inhibiting pathogen enzymes, and modulating gut microbiota balance (Daglia *et al.*, 2024). When used to fortify RUTF formulations, these bioactive compounds can help suppress harmful bacteria, reduce infection-related inflammation, and improve nutrient absorption. For example, flavonoid-rich plant extracts and polyphenols have been shown to inhibit enteric pathogens while supporting beneficial gut microbes, thereby breaking the infection malnutrition cycle and enhancing recovery outcomes (Oketch *et al.*, 2024).

4. Antihypertensive

Hypertension is increasingly recognized as a long-term consequence of early-life malnutrition, as inadequate intake of energy and essential nutrients disrupts vascular development, renal function, and metabolic programming, predisposing individuals to elevated blood pressure later in life (Rakotoniaina *et al.*, 2023). Ready-to-Use Therapeutic Foods (RUTF), used in the management of severe acute malnutrition, are formulated to provide balanced macronutrients, essential fatty

acids, and micronutrients such as potassium, magnesium, and zinc, which play key roles in blood pressure regulation and may help prevent the development of hypertension during nutritional rehabilitation (Owino *et al.*, 2024).

Anti-cancerous

Malnutrition is a significant risk factor for cancer development and poor cancer outcomes because chronic nutrient deficiencies impair immune surveillance, increase oxidative stress, and promote genomic instability, all of which facilitate carcinogenesis (Prado *et al.*, 2023). Ready-to-Use Therapeutic Foods (RUTF), used in the treatment of severe acute malnutrition, provide balanced macronutrients and antioxidant-rich micronutrients such as selenium, zinc, and vitamins A, C, and E, which are essential for DNA repair, immune function, and the reduction of oxidative damage factors that collectively lower cancer risk during nutritional rehabilitation (Akomolafe *et al.*, 2024). Furthermore, **phytochemicals** such as flavonoids, alkaloids, tannins, saponins, and phenolic acids, when incorporated into RUTF formulations or consumed through plant-rich diets, have demonstrated potent anticancer properties by inducing apoptosis in malignant cells, inhibiting angiogenesis, and modulating key signaling pathways including NF- κ B and PI3K/Akt (Yang *et al.*, 2024).

Antidiabetic

Diabetes mellitus is a type of chronic metabolic disorder categorized by insufficiency in insulin activity and/or insulin secretion. Malnutrition, particularly early-life undernutrition, has been linked to an increased risk of developing type 2 diabetes later in life due to impaired pancreatic β -cell development, altered glucose metabolism, and insulin resistance (Fall *et al.*, 2023). Ready-to-Use Therapeutic Foods (RUTF), designed for the treatment of severe acute malnutrition, are

formulated to provide balanced macronutrients, essential fatty acids, and micronutrients such as magnesium, zinc, and chromium, which play critical roles in insulin secretion and glucose homeostasis, thereby helping to restore metabolic balance and reduce diabetes risk during recovery (Owino *et al.*, 2024).

2.3 Minerals in RUTF

In the context of nutrition, a **mineral** is a naturally occurring **chemical element** that is essential for the normal physiological functions of the human body, required in varying amounts to support processes such as enzyme activation, fluid balance, nerve transmission, muscle contraction, and the maintenance of healthy bones, teeth, and tissues (Bailey *et al.*, 2023). Unlike macronutrients such as carbohydrates, proteins, and fats, minerals cannot be synthesized by the body and must therefore be obtained through the diet from plant- or animal-based foods or fortified products. Minerals are one of the four groups of essential nutrients; the others are vitamins, essential fatty acids, and essential amino acids (*US National Library of Medicine, 2016*). The five major minerals in the human body are calcium, phosphorus, potassium, sodium, and magnesium (*Berdanier et al., 2013*). The remaining minerals are called "trace elements". The generally accepted trace elements are iron, chlorine, cobalt, copper, zinc, manganese, molybdenum, iodine, selenium and bromine (*Berdanier et al., 2016; Remick & Helmann, 2023*).

2.3.1 Essential minerals for children and their function

Some of the minerals that are essential for adequate growth and development in children suffering from malnutrition are shown in table 2.1.

Table 2.1 Essential minerals for children and their function.

Minerals	Functions	Reference
Calcium	Builds strong bones and teeth, also helps with muscle function and nerve signaling.	National Institutes of Health, 2022
Iron	Essential for making hemoglobin (carries oxygenated blood); supports brain development.	World Health Organization, 2020
Zinc	Support immune function, wound healing and growth.	National Institutes of Health, 2021
Magnesium	Support muscle and nerve function, energy production and bone health.	National Institutes of Health, 2022
Phosphorus	Work with calcium to build bones and teeth. Also important for energy metabolism.	National Institutes of Health, 2022
Iodine	Require for the production o thyroid hormones, which regulate growth and metabolism	World Health Organization, 2020
Selenium	Protects cells from damage and also supports immune system and thyroid function.	National Institutes of Health, 2022
Potassium	Helps regulate fluid balance, muscle contractions and nerve signaling.	National Institutes of Health, 2022
Sodium (in moderation)	Maintains fluid balance, supports nerve and muscle function.	National Institutes of Health, 2022
Fluoride	Helps prevent tooth decay and strengthens enamel.	World Health Organization, 2019

2.3.2 Mineral composition of RUTF production and their daily requirement for children with malnutrition

Table 2.2. Recommended daily intake of essential minerals in children (Ottens *et al.*, 2006; National Institutes of Health, Office of Dietary Supplements, n.d.).

Minerals	1-3 years	4-8 years	9-13 years
Calcium (Ca)	700 mg	1000 mg	1300 mg
Magnesium (Mg)	80 mg	130 mg	240 mg
Phosphorus (P)	460 mg	500 mg	1250 mg
Potassium (K)	3000 mg	3800 mg	4500 mg
Sodium (Na)	1500 mg	1500 mg	1800 mg
Iron (Fe)	7 mg	10 mg	8 mg
Zinc (Zn)	3 mg	5 mg	8 mg
Copper (Cu)	340 µg	440 µg	700 µg
Manganese (Mn)	1.2 mg	1.5 mg	1.9 mg (boys), 1.6mg (girls)
Selenium (Se)	20 µg	30 µg	40 µg

2.4 Heavy metals contamination in food products

Heavy metals are elements with an atomic weight over 63.5 and a specific gravity higher than 5.0 that are generally dangerous to human health and the environment (Tchounwou *et al.*, 2022). The major elements included in this class are as follows: lead (Pb), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), arsenic (As), nickel (Ni), zinc (Zn), and mercury (Hg).

(*Nkwunonwo et al., 2020*). The diverse and emerging food security issues have become a global concern (*Rai et al., 2019; Upadhyay, 2022*). Traces of different heavy metals were found in food such as legumes, vegetables, meat, fish, milk, due to their mobility and bioaccumulation in water sources. Heavy metals once entered into the body, are oxidized and form stable bonds to enzymes or protein molecules. Heavy metals can originate from both natural (rocks) and anthropological sources (metal mining, smelting, trash dumping, incineration, pesticides, etc.) (*Ciont et al., 2022*).

2.4.1 Heavy metals in RUTF

1. Neurotoxicity

This refers to damage to the structure or function of nervous system (WHO, 2023). Children are particularly susceptible to the detrimental health effects of environmental exposures due to their rapid neurodevelopment. During the first 1000 days of life, the brain has maximal plasticity and is transformed through a series of intricate events, such as neurogenesis, myelination, synaptic pruning, among others (*Grantham-McGregor et al., 2007*). These events build upon one another and, over time, manifest as vital cognitive functions, such as language and speech production, attention, behavior, and reasoning (*Villar et al., 2019*). Thus, disruptions in their biological environments, including exposure to toxicants, may disturb the fine orchestration of events, causing irreversible downstream effects such as neurodevelopmental delays, behavioral problems, and learning disabilities (*Davis et al., 2018*). Children consume more food and water for their body weight, therefore, children exposed to early contaminated food maybe exposed to heavy metals toxicity (*Mamtani et al., 2011*). Nearly all organ systems are involved in heavy

metal toxicity; however, the most commonly involved systems include the central nervous system (CNS), peripheral nervous system (PNS), hematopoietic, renal, and cardiovascular.

2. Kidney damage

The kidneys, as major organs responsible for the filtration of blood and excreting waste products, are particularly vulnerable to the accumulation of heavy metals. In children, whose organs are still developing and have higher absorption rates and lower detoxification capacity than adults, exposure to these heavy metals can lead to acute and chronic renal damage (Huang *et al.*, 2023).

For instance, lead exposure in children can lead to both glomerular and tubular damage. The metal accumulates in renal cortical tissues and disrupt normal filtration processes. Chronic lead exposure has been associated with proteinuria, reduced glomerular filtration rate (GFR), and eventual chronic kidney disease (CKD) (Ekong *et al.*, 2006). Lead induced neuropathy may be irreversible, especially if exposure is prolonged or starts early in life.

3. Cardiovascular disease

Heavy metals such as lead, cadmium, arsenic and mercury are toxic environmental contaminants that pose serious health risks to children, particularly affecting their cardiovascular system. These elements have no physiological function in the body and, when accumulated, can disrupt critical biological systems. Children are particularly vulnerable due to their higher rates of absorption, developing organ systems, and reduced capacity to detoxify harmful substances (Téllez-Rojo *et al.*, 2006). These effects are mediated through several biological mechanisms such as Oxidative stress in the sense that heavy metals promote the generation of reactive oxygen species (ROS), which cause damage to the endothelial cells lining the blood vessels (Houston, 2011). Heavy metals such as lead and cadmium reduce the bioavailability of nitric oxide,

impairing vasodilation and increasing vascular resistance (Tellez-Plaza *et al.*, 2010). Furthermore, persistent exposure of children to heavy metals triggers chronic inflammation, contributing to vascular injury and atherosclerosis (Vahter, 2008). The also disrupt lipid profiles, which increases the risk of atherosclerotic plaque formation (Farzan *et al.*, 2013).

4. Developmental delay

Heavy metals are recognized as neurotoxins that have no physiological function in human body and can disrupt neurological development, particularly in children whose brains are still maturing. Developmental delay in children manifested with by impaired cognition, languages skill, motor function, attention and social interaction; is often linked to chronic or acute exposure of these metals (Grandjean and Landrigan, 2006). Children are more vulnerable to the harmful effects of these heavy metals due to some factors which are: higher gastrointestinal absorption rate, an immature blood-brain barrier, lower detoxification capacity, and rapid brain development during early life (Bellinger, 2008). Even lower exposure during critical developmental windows can result in long-lasting or irreversible neurological impairment.

2.5 WHO safety standard and guidelines for RUTF production

This WHO guideline is an update of the specific recommendation in the technical annex of the 2007 Joint Statement on community-based management of severe acute malnutrition by WHO, WFP, UNSSCN and UNICEF, which states that at least half of the protein contained in RUTF should come from milk products (WHO, 2007). Recently, alternative RUTF formulations with different sources of protein have been tested in several trials. The primary aim of these alternative RUTF formulations is to reduce the production cost of RUTF by replacing dairy

protein with cheaper and locally available ingredients. Reducing the cost of RUTF would increase access to treatment for children with severe acute malnutrition. Using locally available ingredients may also boost local production of RUTF.

2.5.1 WHO/FAO acceptable limits for heavy metals in food.

The WHO/FAO, through Codex Alimentarius and JECFA (Joint FAO/WHO Expert Committee on Food Additives), does set maximum limits for heavy metals in foods and these limits are designed to protect sensitive populations, especially children.

Table 2.5.1: WHO/FAO acceptable limits for heavy metals in food.

Heavy metals	Acceptable limits in food	Source
Lead (Pb)	0.01-0.1mg/kg (in most foods)	Codex Alimentarius; WHO, 2010.
Cadmium (Cd)	0.05-0.1 mg/kg (in cereals, vegetables, fruits)	FAO/WHO, 2011.
Mercury (Hg)	0.5 mg/kg (in fish), 0.05 mg/kg (in other foods)	WHO Technical Report 960.
Arsenic (As)	0.2 mg/kg (in rice), 0.1 mg/kg (in other foods)	WHO, 2011; Codex Standards.
Tin (Sn)	250 mg/kg (in can foods)	WHO, 2010.
Aluminum (Al)	2 mg/kg body weight per week (Provisional Tolerable Weekly Intake)	FAO/WHO, 2011.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials and Reagents

3.1.1 Materials

Materials used for these experiments are standard materials with their models and manufacturers as shown in table 3.1.

Table 3.1 List of Equipment and apparatus

Name	Model	Manufacturer
Blender	58148A	Hamilton Beach
Mortal pestle	Food edible	Locally made
Petri dish	3160-100	Pyrex
Spectrophotometer	AE350	ERMA INC, Japan
Weighing balance	AES 100-4C	KERN & Sohn GmbH, Germany
Sample containers	2105-0005	Nalgene (Thermo Fisher)
Test tubes	9820-18	Pyrex
Beakers	1000-600	Pyrex
Filter paper	Whatman No. 1	Finland
Water bath	SB-1100	EYELA Co. LTD., Shanghai
Electric hot plate	EDB-302BF	Elite Gourmet
Volumetric flask	5640	Pyrex
Measuring cylinder	3022-100	Pyrex
Crucible	3400 Series	Pyrex

Muffle furnace	Cal 9900	Gallen Kamp UK
Oven	DZF-6021	Shanghai Bluepard Instruments Co., Ltd., China.
AAS	Alpha 4	Texton Gound Support Equipment, Inc

3.1.2 Reagents

Table 3.2 shows the chemicals and reagents used for the study.

Table 3.2: List of reagents used for the study.

Name	Grade	Manufacturer
Methanol	99.8%	BDH chemical company poole, England.
Nitric acid (HNO ₃)	70%	BDH chemical company poole, England.
Sulfuric acid (H ₂ SO ₄)	98%	BDH chemical company poole, England.
Ferric chloride (FeCl ₃)	68%	BDH chemical company poole, England.
Hydrochloric acid (HCl)	38%	BDH chemical company poole, England.
Sodium hydroxide	98%	BDH chemical company poole, England.
Potassium permanganate	99%	BDH chemical company poole, England.
Potassium ferrocyanide	70%	BDH chemical company poole, England.
Distilled water		BCH laboratory, UDUSOK Nigeria.

Key: GPR= General Purpose Reagent

3.2 Methods

3.2.1 Sourcing of ingredient and identification

Raw materials (groundnut) for the production of the RUTF was purchased from Kasuwan Daji Market, located at Sokoto State, Nigeria. Milk was purchased in Usmanu Danfodiyo University permanent site and a product of Friesland company WAMCO Nigeria Ltd. Sugar was a product of Dangote refinery. Vegetable oil (Doven Kings oil) was obtained from a reputable supplier in Sokoto market. Vitamin premix from UNICEF.

The groundnut was transported to UDUS and was identified in the department of biology on the 23rd April, 2025 where it was documented and a voucher number (UDUH/ANS/0982) was assigned.

3.2.3 Quality assurance and sample preparation.

The groundnuts were sorted out and cleaned by removing stones, spoiled or muddy nuts before they were being washed thrice under a running tap water and then roasted gently in a dry pan until they turned golden brown (it was made sure that they did not get burnt). The skin of the groundnuts is then removed by rubbing them between hands and using a clean cloth to peel off the skin. The clean groundnuts were grinded into smooth peanut paste by using a grinder. The smooth paste was then transferred into a clean bowl where vegetable oil was added and the mixture was mixed gradually until a smooth and creamy paste was obtained. The dry ingredients such as: sugar, skimmed powder milk premixed multivitamin was added to the mixtures and homogenized and mixed thoroughly.

3.3 Phytochemical screening

3.3.1 Qualitative Test

Ready-to-use therapeutic food was analyzed for the presence of selected secondary metabolites. The analysis was conducted according to the method previously outlined by El-oleyi *et al.* (1994); Harbone (1998) for flavonoids and saponins, Trease and Evans (1978) for Tannins, Furniss *et al.* (1989) for phenols, and Odebiyi and Sofowara (1993) for alkaloids, cardiac glycosides, and phytate.

3.3.1.1 Test for Flavonoids

Exactly 1ml aliquot of the filtrate and 1ml of 10% of sodium hydroxide was added to the extract, if a yellow color is developed this indicates the possible presence of flavonoid compounds (El-Oleyi *et al.*, 1994; Harbone, 1998).

3.3.1.2 Test for Tannins

5% of ferric chloride solution was added drop by drop into the 3ml of the extract and the color produced is noted. Condensed tannins usually give dark green color, hydrolysable tannins give blue-black color (Trease and Evans, 1978; Sofowara, 1993; Harbone, 1998).

3.3.1.3 Test for Saponins

5ml of the extract was placed in a test tube plus 5ml of distilled water and was shaken strongly, the whole tube was filled with froth that lasts for several minutes. Which indicated a positive result (Harbone, 1998).

3.3.1.4 Test for Alkaloids

2ml of the extract was stirred with 2ml of 10% aqueous hydrochloric acid. 1ml was treated with a few drops of Wagner's reagent and the other 1ml was treated with few drops of Mayer's

reagent. Turbidity or precipitation with either of this reagent was taken as preliminary evidence for the presence of alkaloids.

3.3.1.5 Test for Cardiac Glycosides

The mixture was heated in boiling water for 15mins. It was allowed to cool and was neutralized with 10% NaOH, 5ml of fehling's solution was added and the mixture was boiled. A brick-red precipitate was observed which indicates the presence of glycosides (Harbone, 1973).

3.3.1.6 Test for Phenols

Small amount of the sample was dissolved in ethanol before the addition of few drops of neutral ferric chloride solution (FeCl_3). A blue green color was observed which indicates the presence of phenol (Furniss *et al.*, 1989).

3.3.2 Quantitative Test

3.3.2.1 Total alkaloids

Principle

As with most alkaloids, the alkaloidal salts are soluble in water, while the alkaloidal free bases are soluble in organic solvent. This fact is made use of in extraction of the free alkaloid with organic solvent. (Trease and Evans, 1978; Nuhu *et al.*, 2000).

Procedure

10g of the RUTF sample was weighed and` extracted with a liter of methanol and solvent evaporated. The resultant residue was mixed with 200ml of 0.0025M H_2SO_4 and partitioned with ether to remove unwanted materials. The aqueous fraction was basified with strong NH_3 solution and was then extracted with excess chloroform to obtain the alkaloidal fraction or separated by filtration. The chloroform extraction was repeated several times and bulk extract was

concentrated to dryness. The alkaloid was weighed and the percentage was calculated with reference to the initial weight of the powder. The total amount of alkaloid in the sample was calculated using the formula as shown in equation 1.

$$\% \text{Alkaloid} = \frac{W_3 - W_1}{W_2} \times 100 \dots \text{equation 1}$$

3.3.2.2 Flavonoids content

Principle

Flavonoids are polyphenolic compounds soluble in methanol. They can be extracted and quantified gravimetrically by drying the extract and weighing the residue (Boham & Kocipai 1994).

Procedure

10g off the RUTF sample was weighed and extracted with 100ml of 80% methanol for 24 hours. It was then filtered and evaporated to dryness in a water bath, the residue was weighed and recorded. The total amount of flavonoid was then calculated using the formular as shown in equation 2;

$$\% \text{Flavonoid} = \frac{W_3 - W_1}{W_2} \times 100 \dots \text{equation 2}$$

3.3.2.3 Saponin determination

Principle:

Saponins, which are glycosides with foaming characteristics, can be extracted using aqueous ethanol and isolated by partitioning with organic solvents. The dry weight of residue represents saponin content (Obadoni & Ochuko, 2001).

Procedure

10g of the RUTF sample was weighed and extracted with 100ml of methanol. It was then heated at 55°C for 2 hours before being filtered, the sample was re-extracted. The extract was concentrated and partitioned with n-butanol before being washed with sodium chloride (NaCl) and the butanol layer was evaporated, the residue was dried and weighed. The total saponin was determined using the formula as shown in equation 3;

$$\% \text{Saponin} = \frac{W_3 - W_1}{W_2} \times 100 \dots \text{equation 3}$$

3.3.2.4 Tannin determination

Principle

Tannins form a colored complex with ferric ions (Fe^{3+}). The intensity of the color, measured at 605 nm, is proportional to the tannin concentration (Van-Burden & Robinson, 1981).

Procedure

0.5g of the RUTF sample was weighed and boiled with 50ml of distilled water for 1 hour before it was filtered. 5ml of the extract was mixed with 2ml of 0.1M FeCl_3 and potassium ferrocyanide. The absorbance was then taken and recorded. The value gotten was used to calculate the value of tannin using the formula as shown in equation 4

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard} \dots \text{equation 4}$$

3.3.2.5 Oxalate determination

Principle

Oxalate is extracted from the food sample using a dilute acid medium commonly dilute sulfuric acid and titrated with a standardized potassium permanganate (KMnO₄) solution. In acidic conditions, permanganate ions oxidize oxalate ions (C₂O₄²⁻) to carbon dioxide (CO₂) and water (H₂O). Titration, the purple color of potassium permanganate gradually fades as it is reduced to Mn²⁺, and the endpoint is marked by the appearance of a persistent light pink color, indicating the complete oxidation of oxalate (Adewuyi et al., 2022; Olayemi & Bello, 2023).

Procedure

1g of the RUTF sample was weighed and boiled in 75 mL of 1.5 N H₂SO₄ for 30 mins, it was then filtered and titrated against 0.05 N KMnO₄ to reach an end point of faint pink color which persisted for 30 seconds. Total oxalate was then determined using the formular as shown in equation 5;

$$\% \text{Oxalate} = \frac{\text{Titer value} \times \text{Factor}}{\text{Weight of the sample}} \times 100 \dots\dots\dots \text{equation 5}$$

3.3.2.6 Phytate (Phytic acid) determination

Principle

Phytic acid (myo-inositol hexakisphosphate) forms a **pink-colored complex** with ferric ion (Fe³⁺) in the presence of **2% sulfosalicylic acid**, commonly known as **Wade's reagent**. The intensity of the pink coloration decreases in proportion to the concentration of phytate present in the sample, as phytate binds the ferric ions, thereby reducing the color intensity of the Fe³⁺–sulfosalicylic acid complex (Ogunleye et al., 2023; Hassan et al., 2022). The absorbance of the resulting solution is measured at **500 nm** using a UV-visible spectrophotometer. A standard

curve of sodium phytate is used to determine the concentration of phytic acid in the test samples (Nwosu & Okonkwo, 2021).

Procedure (Wade's Reagent Colorimetric Method)

0.5g of the RUTF sample was weighed into a conical flask, 10 mL of 0.2 M HCl was added. The mixture was shaken for 2–3 hours at room temperature before it was centrifugated at 3000 rpm for 10 minutes to get a supernatant. 3 mL of the extract was then pipetted into a test tube and 1 mL of Wade's reagent was added. The mixture was mixed and it was allowed to develop color for 10 minutes, the absorbance was taken at 500nm using spectrophotometer. A standard curve of known concentrations of phytic acid was used to determine the concentration in the sample.

3.4 Analysis of Minerals and Heavy Metals

The method described by Waliuga *et al.*, 1989 was used for the determination of mineral contents and heavy metals in the RUTF sample.

3.4.1 Digestion of sample

The sample (5g) was digested with 20ml of 3:1 aqua regia (HCl/ HNO₃) in a digestion unit until evolution of brown fume was stopped. An aliquot (5mL) of nitric acid was mixed with 15mL of hydrochloric acid (HCl) and the mixture was added to 5g of the RUTF sample and subjected to electric hot plate (EDB-302BF) until a brown smoke vanished completely. The solution was then filtered using Whatman No. 1 filter paper and marked 100/mL with distilled water. This solution was used for mineral and heavy metal analysis using a flame photometer and an atomic absorption spectrophotometer (AAS) respectively.

3.4.2 Determination of Mineral Elements

Mineral elements such as calcium (Ca), magnesium (Mg), Iron (Fe), zinc (Zn), potassium (K), Phosphorus (P), manganese (Mn), selenium (Se), and copper (Cu) were all determined using flame photometer to their good precision and accuracy.

Principle

The principle is based on neutralizing sample solution into an air-acetylene flame where it vaporizes. The elemental ions are atomized and the atoms formed absorb radiation of characteristic wavelength from a hollow cathode lamp. The absorbance measured is proportional to the amount of analyte in the sample (Okorie et al., 2021).

Procedure

AAS standard solution (1000ppm) of calcium (Ca), magnesium (Mg), Iron (Fe), zinc (Zn), potassium (K), Phosphorus (P), manganese (Mn), selenium (Se), and copper (Cu) supplied by the manufacturer of the AAS machine were used to prepare working standard solutions by appropriate dilution of the stock solutions the AAS machine (Alpha 4 model) was set up in accordance with the manufacturer's instructions for each element to be analyzed. These include fuel (acetylene) and oxidant (air) solution, burner type, an optimum wavelength and slit-width settings.

The standards, blanks and the samples were aspirated into the flame and their concentrations in ppm was recorded automatically. The concentration of each analyte (X) in the sample was calculated as shown in equation 1.

$$X \text{ (mg/100g)} = \frac{X \text{ (ppm)} \times \text{volume of sample}}{\text{weight of sample}} \times 100 \dots\dots\dots \text{equation 1}$$

CHAPTER FOUR

4.0 Results, Discussions, Conclusion and Recommendations

4.1 Results

4.1.1 Qualitative Phytochemical Constituents RUTF

Table 4.1 shows the presence of secondary metabolites present in the locally produced RUTF as well as the standard RUTF. The result reveals the presence of flavonoids, alkaloids, tannins, saponins, cardiac glycosides and phenols.

Table 4.1 Qualitative phytochemicals in the RUTF (Ready-to-Use Therapeutic Food).

Phytochemicals	Groundnut-based RUTF	Standard RUTF
Flavonoids	+	++
Alkaloids	+	++
Tannins	++	+
Saponins	+++	++
Cardiac glycosides	+++	+
phenols	++	+++

KEY: + = Trace Amount, ++ = Moderate Amount, +++ = Large Amount

4.1.2 Quantitative Phytochemical Constituents of groundnut-based RUTF

Presented in Table 4.2 is the quantitative phytochemical composition of the RUTF samples (Sample A and Sample B). The results revealed the presence of varying concentrations of alkaloids, flavonoids, saponins, tannins, oxalates, and phytates in both samples. In Sample A, the phytochemical constituents increased in the following order: oxalate (0.01 ± 0.00 g/%) < phytate

(4.65 ± 0.42 g/%) < flavonoid (6.09 ± 0.03 g/%) < alkaloid (6.16 ± 0.04 g/%) < saponin (7.25 ± 0.04 g/%) < tannin (50.15 ± 0.32 mg/dl), showing that tannin had the highest concentration in Sample A, while oxalate had the lowest concentration. While in sample B, the phytochemical constituents followed the order: oxalate (0.007 ± 0.00 g/%) < saponin (4.19 ± 0.09 g/%) < alkaloid (4.61 ± 0.05 g/%) < phytate (7.14 ± 0.43 g/%) < flavonoid (9.03 ± 0.06 g/%) < tannin (80.75 ± 0.27 mg/dl) also indicating that tannin has the highest concentration while oxalate has the lowest concentration.

Table 4.2 Quantitative phytochemicals in the RUTF (Ready-to-Use Therapeutic Food).

Phytochemicals	Groundnut-based RUTF	Standard RUTF
Alkaloids (g/%)	6.16 ± 0.04	4.61 ± 0.05
Flavonoids (g/%)	6.09 ± 0.03	9.03 ± 0.06
Saponins (g/%)	7.25 ± 0.04	4.19 ± 0.09
Tannins (mg/dl)	50.15 ± 0.32	80.75 ± 0.27
Oxalates (g/%)	0.01 ± 0.00	0.007 ± 0.00
Phytates (g/%)	4.65 ± 0.42	7.14 ± 0.43

Values are mean $\pm$ standard deviations of three replicates.

4.1.3 Minerals composition of the RUTFs

Table 4.3 shows that both RUTF samples contain essential macro- and micro-minerals at varying concentrations. In Sample A, sodium (16.58 ppm) had the highest concentration, followed by calcium (14.33 ppm), magnesium (11.52 ppm), and potassium (9.61 ppm), while copper (0.07 ppm) had the lowest. Similarly, in Sample B, sodium (14.58 ppm) was also the most abundant mineral, followed by calcium (12.98 ppm) and potassium (11.96 ppm), with chromium (0.02

ppm) being the least. Overall, Sample A exhibited higher concentrations of most minerals, particularly sodium, calcium, magnesium, and iron compared to Sample B, suggesting that the peeled groundnut formulation retained more minerals than the unpeeled version.

Table 4.3 Mineral composition of the RUTF (Ready-to-Use Therapeutic Food).

Minerals (PPM)	Groundnut-based RUTF	Standard RUTF
Zinc (Zn)	0.153±0.023 ^a	0.157±0.015 ^a
Calcium (Ca)	12.98±0.0122 ^a	13.96±0.078 ^a
Iron (Fe)	0.97±0.00 ^a	1.41±0.00 ^a
Sodium (Na)	14.68±0.162 ^b	16.58±0.030 ^a
Potassium (K)	11.93±0.040 ^a	9.69±0.156 ^b
Magnesium (Mg)	5.777±0.119 ^b	11.527±0.012 ^a
Copper (Cu)	0.373±0.045 ^b	0.767±0.116 ^a
Manganate (Mn)	0.2430.021 ^a	0.137±0.012 ^a
Cobalt (Co)	0.00±0.000 ^b	0.2±0.000 ^a

Values are mean ± standard deviations of three replicates.

4.1.4 Heavy metals composition of the RUTF

Figure 4.1 shows the composition of heavy metals; arsenic (Ar), lead (Pb), cadmium (Cd), and nickel (Ni) present in the two formulated RUTF samples (A and B). Nickel recorded the highest concentration in both samples, with sample B showing a slightly higher value, indicating a possible accumulation from the unpeeled groundnut used. Arsenic level was also higher in sample B compared to sample A, while cadmium and lead were slightly higher in sample A.

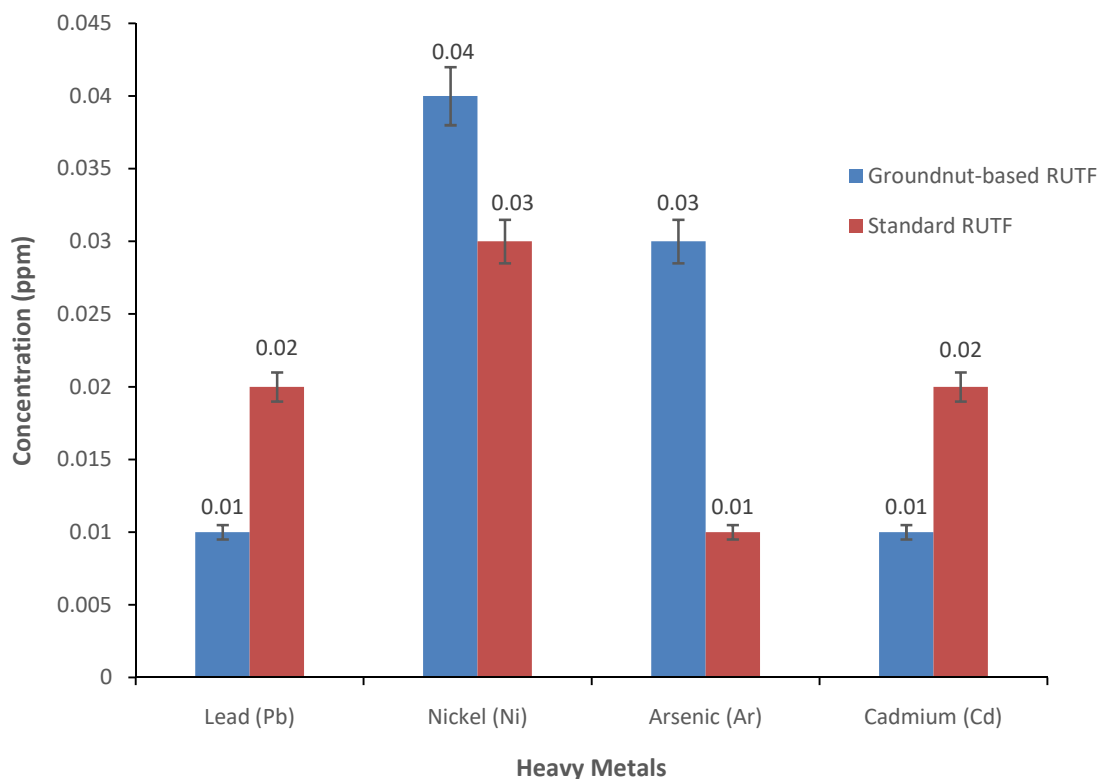


Fig. 4.1: Composition of heavy metals in RUTF

4.1.5 Anti-nutrient/Mineral ratios of the RUTF sample

Presented in Table 4.4 is the anti-nutrient/mineral ratio of the locally produced groundnut-based RUTF which shows the mean concentrations (ppm $\pm$ SD) of selected minerals (Calcium, Zinc, and Iron) in association with three anti-nutrients; oxalate, nitrate, and tannin. In sample A, sodium as the most abundant element (16.58 ± 0.00 mg/100 g), followed by iron (1.41 ± 0.01 mg/100 g) and zinc (0.17 ± 0.02 mg/100 g). The anti-nutrient contents were dominated by tannin (50.15 ± 0.32 mg/100 g), while nitrate (4.64 ± 0.42 mg/100 g) and oxalate (0.01 ± 0.00 mg/100 g) were comparatively low. The anti-nutrient contents were dominated by tannin (50.15 ± 0.32 mg/100 g), while nitrate (4.64 ± 0.42 mg/100 g) and oxalate (0.01 ± 0.00 mg/100 g) were

comparatively low. And also in sample B, sodium is the predominant macro-element, whereas zinc and iron occurred in trace amounts. The calculated anti-nutrient/mineral ratios indicate that oxalate had no measurable effect, while nitrate displayed moderate interaction with Fe and a very high ratio with Zn. Tannin exhibited particularly high ratios with Zn (≈ 19) and Fe (≈ 2.8), suggesting potential complexation that could lower their bioavailability.

Table 4.4 Anti-nutrient/Mineral ratio of the locally produced groundnut-based RUTF sample.

Mineral/anti-nutrient	Groundnut-based RUTF	Standard RUTF
Zn/Oxalate	0.06 ± 0.01	0.05 ± 0.00
Na/Oxalate	0.00 ± 0.00	0.00 ± 0.14
Fe/Oxalate	0.01 ± 0.00	0.01 ± 0.01
Zn/Nitrate	27.29 ± 4.05	30.70 ± 1.10
Na/Nitrate	0.28 ± 0.03	0.30 ± 0.01
Fe/Nitrate	3.29 ± 0.30	4.43 ± 0.10
Zn/Tannin	295.00 ± 34.76	19.3 ± 1.7
Na/Tannin	3.02 ± 0.02	0.19 ± 0.01
Fe/Tannin	35.56 ± 0.34	2.78 ± 0.08

4.2 Discussion

The qualitative phytochemical screening (Table 4.1) revealed the presence of key secondary metabolites such as flavonoids, alkaloids, tannins, saponins, cardiac glycosides, and phenols in both the standard and locally formulated groundnut-based RUTF. These bioactive compounds play important roles in promoting health and preventing diseases, particularly among malnourished children. The relatively higher concentrations of saponins and cardiac glycosides

in Sample A (standard RUTF) suggest better processing and ingredient fortification, whereas the increased presence of phenols and flavonoids in Sample B (groundnut-based RUTF) may be attributed to the natural phytochemical richness of unpeeled groundnut used in its formulation (Ogunleye *et al.*, 2023; Chinedu & Ibrahim, 2022). Flavonoids and phenolic compounds are known for their antioxidant activities, which protect body tissues from oxidative stress and enhance immune response (Hassan & Musa, 2023).

The quantitative analysis (Table 4.2) further confirmed the presence of measurable concentrations of phytochemicals, with tannins exhibiting the highest levels in both samples. The high tannin concentration observed, especially in Sample B (80.75 ± 0.27 mg/dl), could be due to the contribution from the groundnut seed coat, as tannins are concentrated in the outer layers of legumes and nuts (Nwosu & Okonkwo, 2021). Conversely, oxalate showed the lowest concentration across both samples, which is nutritionally advantageous since high oxalate content can reduce calcium and magnesium bioavailability by forming insoluble complexes (Adeyeye *et al.*, 2022). The presence of moderate amounts of alkaloids, saponins, and flavonoids suggests that the formulated RUTF retained beneficial bioactive compounds that could support appetite stimulation, metabolic activity, and antimicrobial defense mechanisms in malnourished individuals (Okorie *et al.*, 2021).

The mineral composition of the RUTFs (Table 4.3) indicates that both samples contained essential macro- and micro-minerals such as calcium, magnesium, potassium, sodium, zinc, and iron, which are vital for physiological functions including bone formation, enzyme activation, and blood synthesis. Sample A (standard RUTF) exhibited slightly higher concentrations of most minerals particularly sodium (16.58 ppm) and calcium (14.33 ppm) than the locally produced

RUTF, implying that processing methods such as peeling and controlled roasting might enhance mineral retention. However, Sample B showed higher potassium and manganese values, possibly due to the contribution of unpeeled groundnut and local formulation differences. The observed mineral range is consistent with reports from recent studies on RUTF formulations using legumes and oil seeds, confirming that groundnut-based formulations can serve as effective sources of essential nutrients (Hassan *et al.*, 2022; Adeyeye *et al.*, 2022).

Heavy metal analysis (Figure 4.1) revealed the presence of trace amounts of arsenic (As), lead (Pb), cadmium (Cd), and nickel (Ni) in both samples, with nickel showing the highest concentration. Although the values observed were within permissible limits set by the World Health Organization (WHO, 2022), the slightly higher levels in Sample B suggest possible contamination from soil, water, or processing equipment used during local production. Elevated nickel and arsenic levels in unpeeled groundnut formulations could also be associated with the accumulation of metals in the seed coat (Okorie *et al.*, 2021). Continuous monitoring is therefore essential to ensure consumer safety, especially for infants and children who are more susceptible to the toxic effects of heavy metals.

The anti-nutrient/mineral ratio (Table 4.4) provides insight into the potential interactions between anti-nutritional factors and minerals, which influence nutrient bioavailability. Tannin exhibited the highest ratios with zinc and iron, implying strong chelating effects that could reduce the absorption of these trace elements in the intestine (Ogunleye *et al.*, 2023). However, oxalate and nitrate showed comparatively lower ratios, suggesting minimal interference with mineral utilization. The balance between anti-nutrients and minerals is critical in RUTF formulations to ensure optimal nutrient bioavailability and efficacy in malnutrition rehabilitation.

Overall, the results demonstrate that the groundnut-based RUTF, though slightly lower in some mineral components, still meets basic nutritional standards and could serve as a cost-effective substitute for standard RUTF when properly processed to minimize heavy metal contamination and anti-nutrient interactions.

4.3 Conclusion

This study established that both the standard and groundnut-based Ready-to-Use Therapeutic Foods (RUTFs) contain valuable quantities of essential minerals and beneficial phytochemicals that are crucial for the rehabilitation of malnutrition. The qualitative and quantitative analyses revealed the presence of flavonoids, alkaloids, tannins, saponins, and phenols in both samples, indicating their potential to provide antioxidant and immune-supportive benefits. Although the standard RUTF showed slightly higher levels of certain bioactive compounds and minerals, the groundnut-based formulation exhibited comparable nutritional quality, particularly in its content of phenols and flavonoids, which enhance metabolic and protective functions in the body.

Furthermore, mineral composition analysis confirmed that both formulations contained nutritionally significant amounts of calcium, magnesium, sodium, potassium, zinc, and iron, all essential for growth, enzyme activation, and blood formation. The detected concentrations of heavy metals such as arsenic, cadmium, lead, and nickel were within permissible safety limits, though slightly higher in the locally produced sample suggesting minor contamination from processing or raw materials. Overall, the findings demonstrate that groundnut-based RUTF is nutritionally adequate, safe, and has the potential to serve as a cost-effective local alternative to imported therapeutic foods, provided that production standards and quality control are carefully maintained.

4.4 Recommendation

It is recommended that further optimization of the groundnut-based RUTF formulation should focus on reducing oxalate and tannin levels through effective processing techniques such as soaking, fermentation, or controlled roasting to improve mineral bioavailability and product digestibility. Regular quality assessments should also be carried out to monitor possible heavy metal contamination and ensure strict compliance with food safety standards. Future research should explore and compare the bioavailability of minerals in both peeled and unpeeled groundnut formulations using in vivo or simulated digestion studies to better understand nutrient absorption efficiency. In addition, efforts should be intensified to promote the local production of RUTFs using groundnut and other nutrient-dense crops as raw materials, in order to strengthen food security, reduce import dependency, and make therapeutic foods more accessible and affordable for the effective management of malnutrition.

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