NUTRITIONAL EVALUATION OF A POTENTIAL READY-TO-USE THERAPEUTIC FOOD (RUTF) FORMULATED FROM SESAME, WHEAT AND SOYA BEANS BLEND

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ZARIA, NIGERIA

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ZARIA, NIGERIA

SEPTEMBER, 2017

DECLARATION

I declare that the work in this Dissertation entitled "**Nutritional Evaluation of a Potential Ready-to-use Therapeutic Food Formulated from Sesame, Wheat and Soya Beans Blend"** has been carried out by me in the Department of Biochemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree or diploma at this or any other Institution.

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Signature

Date

CERTIFICATION

This Dissertation entitled "**NUTRITIONAL EVALUATION OF A POTENTIAL READY-TO-USE THERAPEUTIC FOOD FORMULATED FROM SESAME, WHEAT AND SOYA BEANS BLEND"** by **ALIYU BABANGIDA JIBRIL** meets the regulations governing the award of the degree of Master of Science in Nutrition of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. I.A. Umar — Signature _____
Chairman, Supervisory Committee — Date _____

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Member, Supervisory Committee — Date _____

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Head of Department — Date _____

Prof. S. Z. Abubakar — Signature _____
Dean, School of Postgraduate Studies — Date _____

DEDICATION

This work is dedicated to my parent in persons of Alh. Jibo A. Dango and Haj. Tayyaba Abubakar, and my late sisters (Farida and Khadija) may their souls rest in peace, Amen.

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ABSTRACT

Ready to-use-therapeutic food (RUTF) is a specially designed product for the nutritional rehabilitation of severe acute malnutrition in children over 6 months of age. The potential of Sesame-Wheat-Soya beans blend (SWS-RUTF) as a candidate for local formulation of RUTF was determined. Three products were formed with varying ratios; SWS-RUTF1 (sesame 25%, Wheat 10%, soya beans 30%), SWS-RUTF2 (sesame 25%, wheat 15%, soya beans 25%), and SWS-RUTF3 (sesame 20%, wheat 10%, soya beans 35%), while soya oil, sugar, and mineral/vitamin mix constitutes the remaining 35%. Statistically significant difference ($P<0.05$) was observed in the proximate composition of the products; carbohydrate ranged between 49% - 54%, crude protein (24% to 28%), lipid (9% to 11%), and energy value 404kcal to 411kcal. The three formulations had Amino Acid scores of 72, 65 and 75, PDCAAS; 62, 55, and 67, and Protein efficiency ratio; 1.08, 1.07, and 1.10, respectively. The percentage change in body weight of weanling albino rats used in the research show that all the animal groups fed with SWS-RUTF had values that were not significantly different from each other, but significantly ($P<0.05$) lower than animal group fed with Pea nut-based RUTF, and greater than animal group fed with normal feed. Impact of the product on serum total protein was found to be higher in groups fed with SWS-RUTF, but the difference was not statistically significant ($P< 0.05$). Thus, the formulation made in this study may serve as candidate for RUTF formulation if improved.

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Abbreviations

CMAM	Community Management of Acute Malnutrition
CSB	Corn-Soy-Blend
FBF	Fortified Blended Foods
LNS	Lipid Based Nutrients
MNP	Micronutrient Powder
MUAC	Mid-Upper Arm Circumference
NDHS	National Demographic and Health Survey
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PER	Protein Efficiency Ratio
RDA	Recommended Daily Allowance
RUCF	Ready-to-Use Complimentary Food
RUSF	Ready-to-Use Supplementary Food
RUTF	Ready-to-Use Therapeutic Food
SAM	Severe Acute Malnutrition
SMS	Soya bean-Maize-Sorghum
SWS	Sesame-Wheat-Soya beans
UNICEF	United Nations International Children Emergency Fund
UNSSCN	United Nation Systems Standing Committee on Nutrition
UNU	United Nations University
WFP	World Food Programme
WHO	World Health Organisation

CHAPTERONE

1.0 Introduction

Malnutrition is one of the major problems faced by most countries in the world. Malnutrition has been defined by WFP (2000), as "a state in which the physical function of an individual is impaired to the point where he or she can no longer maintain adequate performance process such as growth, pregnancy, lactation, physical work and resisting and recovering from disease." The term malnutrition is a broad term which literally means bad nutrition, and technically refers to both over nutrition and under nutrition.The problem of over nutrition which is associated with excessive nutrients intakes is mostly found in advanced or developed countries. Whereas under nutrition is more prevalent in developing countries particularly in sub-Saharan Africa and Southeast Asia. It occurs when people do not consume food consistently, or the food consumed is not adequately absorbed by the body, or the food is lacking in essential nutrients required by the body for its normal functions.

In Nigeria, malnutrition (referring to under nutrition in this case)among children is a very serious problem, accounting for about fifty percent (50%) of one million (1 Million)deaths of children under five annually (NDHS, 2013).About 2 out of every 5 Nigerian children are stunted, with rates of stunting varying from one region to another across the country. Almost 30% of Nigerian children are underweight, meaning that their weight is less than the expected weight for their age. Wasting (i.e. being too thin for their height) constitute about 18%(NDHS,2013). Severe acute malnutrition (SAM) remains a major killer of children under five years of age. It is defined by a very low weight for height (below -3z-scores of the median WHO growth standards), by visible severe wasting, or by the presence of nutritional (bilateral pitting) oedema (WHO*et al.*, 2006). Children with SAM need to be treated with specialized therapeutic diets in combination with diagnosis and management of infections and other complications.(WHO*et al.*,2007).

Ready-to-Use Therapeutic Food (RUTF) is a key component for the treatment of Severe Acute Malnutrition, which is linked to dramatically increased childhood mortality**.** The term therapeutic food is used generally to refer to foods or food products that are improved nutritionally so as to have high energy and nutrients content, which are used in the treatment of malnutrition. Ready-to-use therapeutic foods, from the name are therapeutic foods that are consumed as given (without any form of cooking, or preparation) for the management of malnutrition. RUTF contains all the energy and nutrients required for rapid catch-up growth and are used particularly in the treatment of children over 6 months of age with severe acute malnutrition without medical complications (Manary, 2006).Some alternative RUTF formulations have been proposed by SteveandJeya (2004), and are based on four main ingredients: a cereal as the main ingredient, a protein source that can be of vegetal origin (beans, legumes, etc.) or animal origin (milk, red or white meat, fish meat, egg, etc.), a mineral and vitamin supplement (derived from vegetables, fruits, or a mixture of both), and an energetic supplement (e.g. lipids, oil, sugar, etc.).

Sesame (*Sesamumindicum*) is a flowering plant in the genus *Sesamum*. It occurs mostly in Africa and a smaller number in India. It is widely naturalized in tropical regions around the world and is cultivated for its edible seeds, which grow in pods. Sesame is one of the highest oil containing seed plants. It has a rich nutty flavor and it is a common ingredient in cuisines across the world (Ray,2011).

The **soybean** in the United State (U.S.), also called the **soya bean** in Europe (*Glycine max*), is a specie of legume native to East Asia, widely grown for its edible bean which has numerous uses. It is a leguminous plant with hairy pod that grows in clusters of three to five; each pod is 3–8 cm long and usually contains two to four or more seeds. Soy flour has 50% protein and 5% fiber. It

has higher levels of protein, thiamine, riboflavin, phosphorus, calcium, and iron than wheat flour (Lim, 2012).

Wheat (*Triticumspp*.) is a cereal grain, originally from the Levant region of the Near East but now cultivated worldwide. Wheat is a major ingredient in many foods such foods as bread, crackers, biscuits, pancakes, pies, pastries, cakes, cookies, and doughnuts. In 100 grams, wheat provides 327 calories and is an excellent sourceof multiple essential nutrients, such as protein, dietary fiber, manganese, phosphorus and niacin. Several B vitamins and other dietary minerals are in significant content (Zuzana*et al*., 2009).

1.1 Statement of Research Problem

According to United Nations International Children Emergency Fund(UNICEF) Country Representative, "There are approximately 1.7 million acutely malnourished children under five in Nigeria – accounting for a tenth (10^{th}) of the global figure. Nearly about one thousand Nigerian children die of malnutrition-related causes every day, reaching a total of 361,000 each year(Obi, 2015).However, the effectiveness of RUTF is limited since they are not produced in Nigeria. Because the malnutrition management programs are anchored on the availability of RUTF, without RUTF there is no effective malnutrition management at the community level.

The cost for Community Management of Acute Malnutrition(CMAM) is about US$160 for each child treated, including $76 for the RUTF; the remaining $84 covers all other costs, including staff time and training, transport and storage of supplies, and basic medicines (Obi, 2015). The commercially produced RUTF, which are mainly bought and distributed by UN agencies and non-governmental aid organizations, is a totally unaffordable option for most parents of children with SAM.

1.2 Justification

Demand for RUTF has extensively increased. The endorsement of a community based management approach to acute malnutrition in 2007 by World Health Organization(WHO), World Food Programme(WFP), UNICEF and the United Nations System Standing Committee on Nutrition (UNSSCN), resulted in the demand for RUTF increasing to nearly 29,000 MT. The increased quantity represents the treatment of more than 2 million children in 47 countries including Nigeria, and has been driven by recent emergencies and greater programmatic acceptance (WHO *et al.*,2007).

Only 20% of the global production of RUTF occurs in program countries where therapeutic foods are used to treat malnutrition. Management of SAM would be more effective if programmatic nations cannot continue to depend on therapeutic foods manufactured in other countries (usually developed nations) for their supply. As Nigeria is a country with a high burden of malnutrition, the local production of RUTF is a logical next step; it will increase availability, acceptability, access, and efficiency in supply, and lower costs (Ojo, 2013).

A breakthrough in the RUTF production to reduce the cost down could be the replacement of the milk powder in the RUTF recipe as suggested by UNICEF.RUTF can be safely and easily produced in small or large quantities in most settings worldwide. The local availability of the necessary ingredients limits its use in some settings, and further investigation into alternative ingredients is needed to overcome this limitation (Manary, 2006).

1.3 Aim

The aim of this study was to formulate and nutritionally evaluate a potential ready to use therapeutic food formulated from sesame, wheat and soya beans blend.

1.4 Objectives

- To formulate a potential RUTF using sesame, wheat and soya beans (SWS-RUTF).
- To determine the proximate composition, minerals, andantinutrient contents of the potential SWS-RUTF.
- To assess protein quality of the potential SWS-RUTF
- To determine the effect of consumption of the potentialSWS-RUTF on growth and levels of serum proteins of weanlings albino rats.

CHAPTER TWO

2.0 Literature Review

The management of acute malnutrition has now been revolutionized. The approach is now through innovations in nutrition products, which are specifically designed to treat severe acute malnutrition. There is now an expanding range used to prevent acute malnutrition, treat moderate acute malnutrition, and tackle stunting and micronutrient deficiencies (Philip, 2011). The growing options can present opportunities to improve nutrition programming, but also introduce potential confusion and challenges related to which product to use, cost-effectiveness, local production, sustainability, patents, ethics and the evidence-base for impact (Philip, 2011).

Ready-to-use therapeutic foods have been recognized to be successfully used in the treatment of severe acute malnutrition, including in large scale programmes. There are different types of RUTF. Almost all are commercial products. Plumpy'nut is the type that is most widely used. It is a patented branded product which was originally formulated in the late 1990s; it is manufactured by Nutriset,Malaunay, France. It is available in a 92 gram foil sachets, each providing 500 kilocalories. In 2009,Nutriset manufactured 14,000tonnes, mostly purchased and distributed by the UN Children's Fund (UNICEF), to be given to over half a million children, amounting to $US 66 million in sales. UNICEF has stated that this 'nutritional paste (peanuts, powdered milk, vegetable oil, sugar, vitamin and mineral mix) contains the right mixture of nutrients to treat a child with severe acute malnutrition, and in a form that is easy to consume and safe' (Latham *et al.*, 2011).

2.1 Standard Pea Nut-Based RUTF

2.1.1 Ingredients in standard P-RUTF

The formulation of RUTF was derived from F-100 and uses the same ingredients with the addition of peanut butter. Peanut butter changes the physical properties of the food to a viscous liquid product instead of a powder (Manary, 2006). A typical recipe for RUTF is given in the Table in appendix IIA.

2.1.1.1 Milk powder

Milk powder is supplied locally throughout the world, however the milk itself is often imported. Standard commercial techniques to produce milk powder yield a product that is suitable for RUTF production (Manary, 2006).

2.1.1.2 Vegetable oil

Different types of oil produced by standard commercial methods may be used for RUTF formulations, including soya oil, cottonseed oil, rapeseed oil and corn oil. Rapeseed oil and soybean oil have advantage over the other oils, as they provide a good balance of essential fatty acids (Manary, 2006).

2.1.1.3 Sugar

Granulated brown or white sugar can be used to make RUTF. The sugar must be ground into a fine powder, to reduce the particle size to less than 200 microns (Manary, 2006).

2.1.1.4 Peanut butter

This is simply peanuts that have been roasted and ground, without added oil, salt or preservatives (Manary, 2006).

2.1.1.5 Powdered vitamins and minerals

This is a mixture of vitamins and minerals formulated to provide the same amount of micronutrients to the malnourished child as F-100, the standard therapeutic food (Manary, 2006). Currently it is available from a commercial supplier Nutriset (Malaunay, France).

2.2 Categories of Ready-to-Use Foods

The food products are broadly classified as dietary supplements, functional food and Beverages.

2.2.1 Ready-to-use food (RUF)

This encompasses any food that is designed to be eaten straight from the packet, without the need for cooking or any other form of preparation. RUF is therefore a general term to include the RUTF, RUSF and RUCF categories (Philip, 2011).

2.2.2 Therapeutic milk

These are manufactured milk powders used to treat children with severe acute malnutrition who require inpatient care (hospital based management) due to medical complications. F-75 milk is used in the initial stabilization stage, and F-100 milk is used in the next stages as the child begins to recover, after which the child is normally discharged and treated through outpatient facilities (community based management) with Ready-to-Use Therapeutic Food. For the rehabilitation of severely acute malnourished children under 6 months of age, diluted F100 is used, through a 'supplementary suckling technique', until breastfeeding can be fully re-established and the child is gaining weight (Philip, 2011).

2.2.3 Ready-to-use supplementary foods (RUSF)

RUSF aredesigned to provide part of the daily energy and nutrient requirements. These are commonly in the form of pastes (e.g. Plumpy'sup®) and provide about 500 kcal per day in doses

of 92g (Philip, 2011). RUSF is used in the treatment of ModerateAcute Malnutrition (MAM), and are similar in design to RUTF.

2.2.4 Ready-to-use complementary foods (RUCF)

These are also similar to RUTF and RUSF. They use an ingredient mix that is similar to RUTF and RUSF. These products are more likely to be used for targeting of young children (6-23 months) to avoid deterioration of nutritional status and therefore preventing acute malnutrition, products given in doses of around 50g per day (e.g. Plumpy'doz®). Products are also given in doses of around 20g a day to be used for the prevention of micronutrient deficiencies (e.g. Nutributter®) (Philip, 2011).

2.2.5 Fortified blended foods (FBF)

These are food products used in the treatment and prevention of moderate acute malnutrition, and used as a supplementary food in food assistance programme. They are relatively low cost products with good micronutrients and protein content (Saskia and Martin, 2008)

2.2.6 Micronutrient powders (MNP)

These are used at the household level and are designed to provide the recommended nutrient intake for at least 15 minerals and vitamins (e.g. MixMeTM, SprinklesTM). They are sprinkled onto food and then eaten to ensure an adequate intake of micronutrients essential for bodily functions (Saskia and Martin, 2008)

2.2.7 Lipid-based nutrient supplements (LNS)

Theseare products in which the majority of energy supplied is derived from fats. They typically contain varying amounts of vegetable fat, milk powder, ground nuts, sugar and micronutrient mixes. LNS therefore comprise some RUTF, RUSF and RUCF products (Philip, 2011).

2.2.8 High-energy biscuits

These are high energy nutrient supplements compressed in the form of bar. They can be used during the first phase of an emergency, particularly when people are displaced with no access to a general ration or local food. They can be eaten directly, and some products can also be crumbled into porridge. Sometimes these biscuits are added to other rations in the management of moderate malnutrition (Philip, 2011). Examples include BP-5, NGR-5 and high protein biscuits.

2.2.9 Ready-to-use infant formula (RUIF)

This is used as a breast milk substitute for infants who cannot be breast-fed. Infant formula is not used for treatment of acute malnutrition, and is only used in well-nourished children if it is appropriate and safe to do so (Philip, 2011).

2.2.10 Ready-to-use therapeutic food (RUTF)

RUTF contains all the energy and nutrients necessary to allow for rapid catch-up growth and is used particularly in the treatment of children over 6 months of age with severe acute malnutrition without medical complications. The majority are lipid-based products, based on a paste of peanuts, sugar, milk powder and micronutrient mix, with low risk of contamination and a long shelf-life (e.g. Plumpy'nut®, Eezeepaste-NUT, Nutty butta). RUTF can also be found in the form of biscuits (e.g. BP-100) where ingredients are compressed into a bar (Saskia and Martin, 2008).

2.3 Alternative RUTF

Numerous cereal, legume and oilseed mixtures were evaluated for RUTF formulation. So many efforts were made to combine the various cereal, legume and seedoil mixtures to maximise the protein quality. This process led to a list of some products that had reasonable theoretical properties. After so many products development trials, the list was reduced to three potential alternatives. The foods were prepared fromroasted or processed ingredients with total exclusion of water. The foods were prepared in such a way that they had low dietary bulk, low potential for bacterial contamination and were ready to eat without cooking (Steve and Jeya, 2004).

The three most suitable recipes were:

2.3.1 Rice - sesame' RUTF 1

Ingredients: Roasted rice flour, roasted sesame seeds paste, Soyamin 90, sunflower oil, icing sugar, vitamin and mineral premix (CMV therapeutique, Nutriset) (see appendix IIB)(Steve and Jeya, 2004).

2.3.2 Barley - sesame' RUTF 2

Ingredients: Roasted pearl barley flour, roasted sesame seeds paste, Soyamin 90, sunflower oil, icing sugar, vitamin and mineral premix (CMV therapeutique, Nutriset) (see appendix IIC)(Steve and Jeya, 2004).

2.3.3 Maize - sesame' RUTF 3

Ingredients: Roasted sesame seeds paste, roasted maize flour, roasted chickpeas flour, sunflower oil, icing sugar, vitamin and mineral premix (CMV therapeutique, Nutriset) (see appendix IID)(Steve and Jeya, 2004).

Many trials have been conducted to produce RUTF locally in different parts of the world, particularly in countries where severe acute malnutrition is prevalent (see appendix IIE). According to a study carried out in Zambia to compare the effectiveness of two different RUTFs (a milk-free soy-maize-sorghum-based ready-to-use therapeutic food (SMS-RUTF) and a standard ready-to-use therapeutic food (P-RUTF) with 25% milk) in the treatment of SAM without complications. The study shows that overall recovery rates in children treated with P-RUTF was higher than those treated with the SMS-RUTF, but the study was inconclusive. However, there was evidence of a possible heterogeneity of treatment effect (HTE) between children <24 months and those >24 months, with a suggestion that although the SMS-RUTF might be inferior to P-RUTF for children <24 months, this might not apply to children aged >24 months (Abel *et al.,* 2015).

Another study was conducted in Malawi, Project Peanut Butter that produces a peanut/soy paste from 25% whole roasted soy (not dehulled), 20% soy oil, 26% peanut paste, 27% sugar and 2% micronutrients (providing 1 RDA per daily dose of 125g). The product was compared to Fortified Blended Foods (FBF) with additional fish powder, but there was no difference in linear growth observed (Lin *et al.,* 2008). The absence of milk in the spread and the addition of fish powder to the FBF may explain why there was no difference (Phuka*et al.,* 2008). However, another study that compared Milk/peanut spread, soy/peanut spread and Corn Soy Blend (CSB), found that recovery from moderate wasting was higher in both spreads groups compared to the CSB group (80% vs 72% recovery) (Matilsky*et al.,* 2009).

Concerns about the acceptability of peanut-based Ready-to-Use-TherapeuticFoods for the treatment of severe acute malnutrition in South East Asia, lead to the development of an

alternative, culturally acceptable RUTF. It was made in the form of a rectangular bar, from the locally available ingredients; Rice, Soya beans and green beans (Nga *et al.,* 2013).

Although some of these products are likely less effective than RUTF, they are presumably better than fortified blended foods which is the main product that is currently provided to moderate acutely malnourished children (Saskia and Martin, 2008).

2.4 Proximate Analysis

This refers to the determination of the major constituents of feed and it is used to assess if a feed is within its normal compositional parameters or somehow been adulterated. This method partitioned nutrients in feed into 6 components(Karoly*et al.,* 2011):

- moisture,
- Ash
- crude protein,
- crude lipid,
- crude fibre and
- Carbohydrate by difference.

The moisture content is determined as the loss in weight that results from drying a known weight of food to constant weight. This method is satisfactory for most foods, but with a few, such as silage, significant losses of volatile material may take place (Karoly*et al.,* 2011).

The ash content is determined by ignition of a known weight of the food at 550^{o}C until all carbon has been removed. The residue is the ash and is taken to represent the inorganic constituents of the food. The ash may, however, contain material of organic origin such as sulphur and

phosphorus from proteins, and some loss of volatile material in the form of sodium, chloride, potassium, phosphorus and sulphur will take place during ignition (Karoly*et al.,* 2011).

The crude protein (CP) content is calculated from the nitrogen content of the food, determined by Kjeldahl method. In this method the food is digested with sulphuric acid, which convertsall nitrogen presentto ammonia. The ammonia is liberated by adding sodium hydroxide to the digest, distilled off and collected in standard acid; the quantity so collected is then subjected totitration. It is assumed that the nitrogen is derived from protein containing 16 per cent nitrogen, and by multiplying the nitrogen value by 6.25 (i.e. 100/16) an approximate protein value is obtained (Karoly*et al.,* 2011).

The carbohydrate of the food is contained in two fractions, the crude fibre (CF) and the nitrogen-free extractives (NFE). The former is determined by subjecting the residual food from ether extraction to successive treatments with boiling acid and alkali of defined concentration; the organic residue is the crude fibre.When the sum of the amounts of moisture, ash, crude protein, ether extract and crude fibre (expressed in %) is subtracted from 100, the difference is designated the nitrogen-free extractives (Karoly*et al.,* 2011).

2.4.1 Calorie

A calorie is the unit used to measure the energy-producing value of food. Technically, a calorie is defined as the amount of heat necessary to raise the temperature of one gram of water by one degree centigrade. The major sources of energy in food are: carbohydrate, protein, fat, dietary fibre and alcohol. When burned (metabolized), they provide different amounts of energy (FAO, 2003):

Carbohydrate = 4 calories per gram

Protein = 3.2 calories per gram

Alcohol = 7 calories per gram

Fat = 9 calories per gram

Dietary Fibre = 1.9 calories per gram

The calorie content of food depends on the amount of carbohydrate, protein, fat, and alcohol. Fat is the most concentrated source of energy and yields more than twice as many calories per unit weight as carbohydrate and protein.

2.5 Minerals

Knowledge of the concentration and type of specific minerals present in food products is often important in the food industry. The major physicochemical characteristics of minerals that are used to distinguish them from the surrounding matrix are: their low volatility; their ability to react with specific chemical reagents to give measurable changes; and their unique electromagnetic spectra. The most effective means of determining the type and concentration of specific minerals in foods is to use atomic absorption or emission spectroscopy. Instruments based on this principle can be used to quantify the entire range of minerals in foods, often to concentrations as low as a few ppm (McClements, 2005).

2.6 Antinutrients

Plants commonly synthesize a range of secondary metabolites as part of their protection against attack by herbivorous, insects and pathogens or as means to survive in adverse growing conditions. If humans consume these plants, these compounds may cause adverse physiological effects (Santosh and Richard, 2003). The term anti-nutrients refers to defense metabolites, having specific biological effects depending upon the structure of specific compounds which

range from high molecular weight proteins to simple amino acids and oligosaccharides. Antinutrients are natural or synthetic compounds found in a variety of foods especially grains, beans, legumes and nuts. They interfere with the absorption of vitamins, minerals and other nutrients. They can even affect the performance of the digestive enzymes, which are keys for proper absorption. Anti-nutrient substances from nutritional point of view, interferes with normal growth, reproduction and health, when consumed regularly in amount above their tolerable levels (Josh, 2016).

2.6.1 Trypsin inhibitors

Trypsin inhibitors are found in most grain-containing products, including cereals, porridge, breads and even baby foods. They seem to be degraded well by heat processing and cooking but can still cause problems like mineral deficiencies for young infants, children and anyone with reduced pancreatic function. The LD_{50} for rats was found to be 200mg/kg (Irvin, 1951)

2.6.2 Phyticacid (Phytate)

This is a well-known antinutrient that's found in grains and legumes and interferes with the absorption of minerals. Phytic acid can lock up high percentages of phosphorus, calcium, copper, iron, magnesium and zinc. Some research shows that up to 80 percent of phosphorous found in high-phosphorus foods like pumpkin or sunflower seeds, along with 80 percent of zinc found in high-zinc foods like cashews and chickpeas, might be blocked by phytate. The same can be said for about 40 percent of magnesium-rich foods (Josh, 2016).

At the same time, it interferes with calcium and iron absorption, which increases the risk for problems like anemia (resulting from an iron deficiency) and bone loss. Another very problematic component to phytic acid is that it inhibits certain essential digestive enzymes

likeamylase, trypsin and pepsin. Amylase breaks down starch, while both pepsin and trypsin are needed to break down protein. For good health, phytate intake should be kept below 25mg/100g (Ramiel, 2010).

2.6.3 Tannins

Tannins are also antinutrients that act by inhibiting enzyme activity, thereby preventing adequate digestion and can cause protein deficiency and gastrointestinal problems. Because the body needs enzymes to properly metabolize food and distribute nutrients to our cells, molecules that inhibit enzymes can cause bloating, diarrhea, constipation and other gastrointestinal issues. Ingestion of tannic acid at a dose of 45mg/100g of body weight per day was found to be toxic (Samantha *et al.*, 2004)

2.6.4 Oxalates

Similar to tannins, oxalates are found in the highest quantities in sesame seeds, soya beans, and black and brown varieties of millet. The presence of these antinutrients makes plants (especially legumes) proteins of poor quality(Josh, 2016).

2.6.5 Saponins

Saponins are group of natural products possessing the property of producing lather or foam when shaken with water. These are glycosides of high molecular weight. Occur widely in plant species and exhibit a range of biological properties. They have been reported in soyabean, sword bean and jack bean. Toxicity includes nausea and vomiting. Saponins affect the gastrointestinal lining, contributing to leaky gut syndrome and autoimmune disorders. They're particularly resistant to digestion by humans and have the ability to enter the bloodstream and trigger immune responses. Wieslaw*et al.,* (1999) found out that crude saponin fraction of *Amaranthuscruentus* Seeds

containing 70% of pure saponins in the matrix, showed some toxicity; the approximate lethal dose was calculated as 1100 mg/kg of body weight.

2.7 Protein Quality Evaluation

Numerous claims exist stating that different proteins vary in quality and that some are more beneficial than others. There are a number of factors which may affect the protein quality from food, including (FAO, 1990):

1. The amino acid profile of the protein
2. The structure of the protein
3. The amount of protein consumed in one meal
4. Other nutrients and food constituents present in the meal
5. How the foods been prepared
6. The metabolic state of the individual, e.g. illness, exercise, sleep

Protein quality evaluation aims to determine the capacity of food protein sources and diets to satisfy the metabolic demand for amino acids and nitrogen. Thus any measure of the overall quality of dietary protein, if correctly determined, should predict the overall efficiency of protein utilization (FAO *et al.*, 2007).

2.7.1 Methods of determining protein quality

There are different methods of analysis for assessing quality of proteins that look at the amino acid profile and how readily the protein is digested and absorbed.

2.7.1.1 Amino acid score (AA score)

Humans require certain minimal quantities of essential amino acids from a biologically available source as part of a larger protein/nitrogen intake (FAO, 1990). The required amounts of these amino acids vary with age, physiological condition and state of health. Of the 20 amino acids used by the human body, nine are considered indispensable and must be sourced from the human diet. Food marketed as protein-rich may be rich in amino acids, but it doesn't necessarily mean that the protein has a high content of indispensable amino acids; they are in the amounts that humans require, or are well-absorbed (FAO *et al.,* 2007). The amino acid score determines the effectiveness with which absorbed dietary nitrogen can meet the essential amino acid requirement at the safe level of protein intake. This is achieved by a comparison of the content of the limiting amino acid in the protein or diet with its content in the requirement pattern. It measures the essential amino acids present in a protein and compares the values with a reference protein. The rating of the protein being tested is based upon the most limiting essential amino acid. The score is between 0 to 100%, with 100 being the highest percentage. Any score above 100 is rounded down. The better the score, the better the protein meets our body's needs. Recognizing the need for amino acid scoring patterns which can be used to assess quality of food protein sources and diets in all age groups, the FAO, WHO and UNU Consultation suggested that the scoring pattern proposed for the preschool children (considered to be nutritionally the most demanding group), which is based on various criteria of amino acid adequacy is robust and represents the best available estimates of Indispensable amino acid requirements for this age group (FAO and WHO, 1990). (See appendix IC).

2.7.1.2 Protein digestibility corrected amino acid score (PDCAAS)

PDCAAS is a method for determining the quality of protein in food. It is used to determine the protein grams. The value may be determined by a laboratory, or may be determined mathematically. PDCAAS is determined mathematically by multiplying the amino acid score of the recipe by the protein digestibility of the recipe. PDCAAS takes into account the profile of essential amino acids of the protein in question, as well as its digestibility in humans; it is the amino acid score with an added digestibility component. Scores are from 0.1 to 1.0, with 1.0 being a high quality protein. The PDCAAS is the current accepted measure of protein quality, and is the method adopted by the World Health Organisation / Food and Agriculture Organisation (WHO/FAO) and the US Food and Drug Administration (FDA). All proteins of a score greater than 1.0 are rounded down as scores above 1.0 are considered to indicate the protein contains EAAs in excess of the human requirements (WHO and FAO, 1990).

PDCAAS = (amino acid score x recipe protein digestibility)

2.7.1.3 Protein efficiency ratio (PER)

Protein Efficiency Ratio is a bioassay which measures the ability of a protein to support growth in young, rapidly growing rats. It is determined by calculating the average weight gain of animals which will then be divided by the amount of protein intake from diet. The score is a value between 0 and 2.5 (FAO and WHO, 1990).

2.8 Body Weight

Changes in body dimensions reflect the overall health and welfare of individuals and populations. Anthropometry is used to assess and predict performance, health and survival of individuals and reflect the economic and social well-being of populations. The four

anthropometric indicators of human nutritional status are age, weight, height, and length. Each of these variables provides one piece of information about a person. When they are used together they can provide important information about a person's nutritional status (Cogill, 2001).

In this study, body weight was used to obtain information about the nutritional status of animals. This was because the anthropometric indexes are standardized for human subject, and as such not applicable to animal subjects (Cogill, 2001).

2.9 Serum Proteins

Blood is a complex mixture of cells suspended in a fluid medium called plasma. 92-93% of this fluid medium is water and the remaining 8% is dissolved proteins, minerals, glucose, etc. The largest amount of the total solutes is the plasma proteins, collectively referred to as Total Protein. Serum proteins serve a number of different functions. They constitute a portion of the amino acid pool of the body. They can be de-aminated to give ketoacids which can provide caloric energy, or be transformed into carbohydrates and lipids. Some serum proteins are also transport agents, carrying many vital metabolites, metal ions, carbohydrates and lipids. Nutritional status is the key determinant of serum protein. When the diet is consistently low in protein, it leads to a drop in serum protein levels. Oedematous forms of malnutrition are caused by dietary protein deficiency. This is the main reason why various high protein diets have been used as therapeutic means (Veronika and Peter, 2000).

CHAPTER THREE

3.0 Materials andMethods

3.1.0 Materials

- Sesame seeds
- Wheat
- Soya beans
- Soya oil
- Sugar
- Mineral and Vitamins mix

3.1.1 Sesame seeds, wheat, and soya beans

Sesame seeds, wheat, soya beans, and soya oil samples were obtained from Gusau central market, Gusau local government area of Zamfara State. Vitamins and mineral mix were obtained from Bio-organics nutrient systems LTD. The seeds and grains were identified at the herbarium of Department of Biological Sciences, Ahmadu Bello University Zaria, Kaduna state, Nigeria, and voucher numbers (Sesame 931, Wheat 1365, Soya beans 2117) were allocated.

3.1.2 Equipments used

PTH analyzer, Vacuum oven, Muffle furnace, Soxhlet extractor, Weighing Scale, Hot plate, Centrifuge, Spectrophotometre, Water bath, pH meter,

3.1.3Chemicals/ Reagents

Sodium Sulphate (Na_2SO_4), Copper Sulphate ($CuSO_4$), Selenium Oxide (SeO_2), Hydrochloric acid (HCl), Sodium Potassium Tartrate (NaK), Potassium Iodide (KI), etc.

All chemicals and reagents used are of analytical grade.

3.1.4Experimental animals

Twenty five weanling (3 weeks old) albino rats bred in the animal house of the department of Biochemistry Ahmadu Bello University Zaria were used for the research.

3.2 Methods

3.2.1 Processing of raw materials:

The materials were washed with clean water to remove dust particles, and then sun dried in a dust free area. The washed raw materials were roasted by heating at a temperature of about 157°C for 15 minutes. Materials were stirred throughout the cooking time so as to achieve even cooking. After roasting, the hull of soya beans was removed using a clean local grinding stone. It was ground gently and slowly so as to allow the removal of the hulls from the seeds. The seeds were then separated from the hulls. The samples were ground using a hammer mill. The powdered (ground) materials were sieved so as to remove particles with larger size. This was to ensure that the particle sizes were minimal, which is an important requirement in the production of RUTF.

3.2.2Formulation of SWS-RUTF

Table 3.1 IngredientsUsed for the Formulation of the Sesame-Wheat-Soya bean RUTF

Ingredients	Formulations(%)		
	SWS-RUTF1	SWS-RUTF2	SWS-RUTF3
Sesame	25	25	20
Soya bean	30	25	35
Wheat	10	15	10
Soya oil	20.4	20.4	20.4
Sugar	13	13	13
Mineral/vitamin Premix	1.6	1.6	1.6

Modification of Steve and Jeya(2004)

3.2.3 Production of RUTF

Firstly, the soya oil was fried at a temperature of 70^{o}C for 2 minutes. The powdered (ground) ingredients (sesame seeds, soya beans wheat, sugar and mineral/vitamin premix) were added slowly into the fried oil. As the powdered ingredients were being added, it was also simultaneously stirred. After all the powdered ingredients were added, the entire mixture was stirred vigorously for 15 minutes. This allows the mixture to stick together.Although a mechanical mixer is required for RUTF production, hand mixing of the ingredients is possible for small quantities (Manary, 2006).The products were packaged in nylon bags, and stored in a carton at ambient temperature.

3.2.4 Proximate analysis

The product was analyzed for moisture, crude proteins, crude lipids, ash, crude fiber and available carbohydrate according to the method recommended by Association of Official Analytical Chemists (1984).

3.2.4.1 Determination of moisture

The method described by AOAC (1984) was used for the determination of moisture content of the product. This was done by the measurement of loss in weight that occurs due to drying at a temperature of 105^{o}C.

Watch glass was washed and dried in an oven at 105^{o}C, after which it was cooled and weighed empty. Two grams of the sample was weighed into the watch glass. The watch glass and thecontent were dried in an air circulated oven at a temperature of 105^{o}C to a constant weight. The watch glass together with the content was cooled in desiccator and reweighed.

The percentage moisture content of the sample was calculated using the expression below

$$\%\ moisture\ =\ (Loss\ of\ weight\ on\ drying(g) \times 100)/(Initial\ sample\ weight)$$

3.2.4.2 Ash content determination

The term ash refers to the residue left after the combustion of the oven dried sample, and is a measure of the total mineral content. Determination of ash content was carried out according to the method described by AOAC (1984).

Crucible was preheated in a muffle furnace at about 550^{o}C. The crucible was cooled in a desiccator and weighed. Approximately 1g of the sample was weighed into the different crucibles. The crucible and its content was then transferred into the muffle furnace at 550^{o}C and allowed for 5 hours. The weights of the crucible contents was taken and recorded. Percentage ash was calculated using the expression below:

Ash (%) = Weight of Ash x 100
Weight of dry sample

3.2.4.3 Determination of crude lipid content

The lipid content of the sample was determined by using the procedure described by of AOAC (1990). A clean dry round bottom flask containing anti bumping granules was used. Then 210ml of petroleum ether (60 – 80^{o}C) was put into a flask fitted with soxhlet extraction unit. The weighed sample was transferred into a thimble already fixed into the Soxhlet extraction unit. Cold water was put into circulation. The heating mantle was then switched on and the heating rate adjusted until the solvent was refluxed at a steady rate. The extraction was carried out for 6 hours.

The sample was removed and dried to a constant weight in an oven, cooled in a dessicator and reweighed and the percentage crude lipid content was determined;

$$\%\ lipid\ = (Weight\ of\ lipid\ extracted\ \times 100)/(Weight\ of\ dry\ sample)$$

Where the weight of lipid extracted was the loss in weight of the sample after extraction, drying in an oven and cooling in a desiccator.

3.2.4.4 Determination of crude fiber

Crude fibre was determined by the method of AOAC (1984). Two grams of the sample was placed in a round bottom flask. Exactly 100ml of 0.25M H_2SO_4 was added and the mixture was boiled under reflux for 30minutes. The insoluble matter was washed several times with hot water until it was acid free (C1). It was then transferred into a flask containing 100ml of 0.25M NaOH solution. The mixture was boiled again under reflux for 30 minutes and filtered under suction. The insoluble residue waswashed with hot water until it was base free (C2). It was thenashed in a furnace at 550^{o}C for 2 hours. The furnace was then put off and allowed to cool down. The sample was removed and cooled in a desiccator and weighed (C3). The crude fibre content was calculated as loss of weight in ashing. Weight of original sample was used as W.

$$\%\ crude\ fiber\ =\ (C2 - C3\ \times 100)/W$$

3.2.4.5Determination of nitrogen content and crude protein

Nitrogen is used as an index termed crude protein as distinct from true protein. The Kjedahl method of AOAC (1984) was used for the crude protein determination.

Steps for determination

A. Mineralization step: The sample was first digested in strong sulfuric acid in the presence of catalyst. This converts the amine nitrogen to ammonium ions.

B. Distillation Step: The ammonium ions are then converted into ammonia gas after addition of alkali, which was then heated and distilled.

C. Titration step: The ammonia gas was led into a trapping solution with hydrochloric acid of standardized concentration where it dissolves and become ammonium ion once again.

Procedure

Exactly 2.0g of the sample was weighed into 100ml Kjedahl flask and a few anti bumping granules werealso added. One gram of the mixed catalyst ($CuSO_4$ and K_2SO_4 in the ratio 8:1 respectively) and 15ml of concentrated sulphuric acid was added. The flask was placed on a Kjedahl digestion rack and heated until a clear solution was obtained. At the end of the digestion, the flask was cooled and the sample was quantitatively transferred to a 100ml volumetric flask and made up to the mark with distilled water. Then 10ml of the digest was pipetted into semi micro nitrogen steel tube, 10ml of 40% NaOH solution was then added cautiously. The sample was then steam distilled liberating ammonia into a 100ml conical flask containing 10ml of 4% 0.1M boric acidusing methyl blue indicator until the colour changes from pink to green. Exactly 30ml of sample volume was then collected. The content of the conical flask was then titrated with 0.1M HCl. The end point was indicated by a colour change from green to pink and the volume (v) of the acid for each distillate was taken. Percentage nitrogen per sample was calculated using the expression below;

$$\%\, nitrogen = (M \times v \times 14 \times 100 \times 100)/(Weight\ of\ sample \times 1000 \times 10)$$

Where, M = Molarity of HCl

14 = Atomic weight of Nitrogen.

100 = Total volume of digest.

100 = % conversion.

10 = Volume of the digest taken.

1000 = Conversion to litre.

The crude protein was calculated as:

% Protein = 6.25 x % nitrogen.

3.2.4.6 Determination of carbohydrate content

The percentage carbohydrate was obtained by difference as follows;

Percentage carbohydrate = 100 - (%ash + %crude fibre + %crude fat + %moisture + %crudeprotein).

3.2.4.7 Determination of dietary fibre

Dietary fiber was determined by gravimetricenzymatic method (AOAC, 1992). Samples were defatted and gelatinized in the presence of heat stable alpha amylase, and then enzymatically digested with protease and amyloglucosidase to remove digestible protein and starch. Four volumes of ethanol was added to precipitate soluble dietary fibre. Total residue was filtered off and washed with ethanol and acetone. The residue was weighed after drying. The remaining material was analyzed for protein and ash content respectively. Subtracting the amounts measured for protein, ash and a blank control from the dry weight of the filtered residue yields a value for total dietary fiber content.

Procedure

About 1g of the sample was weighed inside a 400ml beaker; 40ml of the buffer solution was added and stirred with a magnetic stirrer to prevent lump formation. The solution was then incubated with 50µl of heat stable α- amylase solution while stirring at low speed and incubated in water bath at (95-100^{o}C) for 35mins with continuous stirring. Then, the solution was allowed to cool before adding 100ml protease solution and incubated with shaking water bath at 60^{o}C for 30mins. The pH was checked by dispensing about 5ml of 0.5N HCl solution, and adjusted if necessary with 5%NaOH or 5%HCl solution. The mixture was further incubated with 200µl amyglogucosidase solution, while stirring and also incubated at the same temperature of 60^{o}C for 30mins with constant agitation.

The solution was then filtered, the residue was washed twice with 10ml each of distilled water, 95% ethanol and acetone, and then dried in the crucible for protein/ash determination for insoluble fibre analyses .The filtrate preserved was used for soluble fibre analysis, by precipitation using 4 vols of 95% extract preheated to 60^{o}C and allowed at room temperature for 60mins. The precipitate wasfiltered. The residue was washed successively with two 15ml eachof 78% ethanol, 95% ethanol, and acetone. The residue was later dried, weighed,and then ash and protein content were determined.

Dietary Fibre (%) =(weight of residue – protein – ash – blank) x100 / weight of sample

3.2.5Mineral analysis

Zinc (Zn), Iron (Fe), Calcium (Ca), and Magnesium (Mg),as well as the presence of heavy metals Cadmium (Cd) and Lead (Pb)were determined by Atomic Absorption Spectrometry; while Sodium (Na) and Potassium (K) were determined by flame photometry according to the method of AOAC (2003).

3.2.5.1 Wet digestion of sample

Exactly 1.0g ofthe powdered sample was weighed in a digesting glass tube. Twelve milliliters (12ml) of HNO_3 was added to the sample and the mixture was kept overnight at room temperature. Then 4.0ml perchloric acid ($HClO_4$) was added to this mixture and was kept in the fume hood for digestion. The temperature was increased gradually starting from 50^oC and increasing up to 250 – 300^oC. The digestion was completed in about 70- 85 minutes by the appearance of white fumes. The mixture was left to cool down and the content of the tubes was transferred to 100ml volumetric flasks and the volume of the content was made to 100ml with distilled water. The wet digested solution was transferred to plastic bottles labeled accurately and stored for mineral determination.

Procedure

The digested samples were analyzed for mineral contents. The absorption measurement of the elements for the samples was read out. Different electrode lamps were used for each mineral. The equipment was run for standard solutions of each of the mineral, before and during determination to ensure that it was working properly. The dilution factor for all minerals except Mg was 100.Further dilution of the original solution was done for determination of Mg, using 0.5ml original solution and sufficient quantity of distilled water was added to make the volume up to 100ml. For the determination of Calcium, 1.0ml lithium oxide solution was added to the

original solution to unmask Ca from Mg. The concentration of minerals was determined using the formula below:

Mw= absorbance × dry wt. × D/wt. of sample × 100

Where Mw = Conc. of minerals

D = Dilution factor

Determination of Sodium (Na) and Potassium (K) was done by the method of flame photometry. The same wet digested sample solutions used in AAS was used for the determination of Na and K. Standard solutions of 20, 40, 60, 80 and 100milli equivalent/L was used both for Na and K. The calculations for both mineral involved the same procedure as given in AAS.

3.2.6 Determination of anti nutritional factors

3.2.6.1 Determination of trypsin inhibition (onwuka, 2005)

Extraction of sample: Exactly 1g of ground sample was dispensed into 50cm^3 of 0.5M NaCl solution. The mixture was stirred for 30mins at room temperature and centrifuged at 4000rpm for 5mins. The supernatant was filtered through whattman filter paper. The filtrate was used for the assay (Onwuka, 2005).

Procedure

A 1mg/ml solution of trypsin in 0.1M HCl was prepared, 1% casein substrate in 0.1M phosphate buffer pH 7.7 was also prepared. To 2mloftrypsin standard solution, 1ml of trypsin inhibitor, and 5ml of substrate was added and incubated for 10mins at 37^0C. A blank of 5cm^3 substrate and 2ml trypsin standard solution was prepared in a test-tube (with no trypsin inhibitor extract added). The content in the test-tube was left for 10mins and the reaction was stopped by adding 3ml 5%

TCA. It was then filtered and measured spectrophotometrically at 410nm. The trypsin inhibitor activity was expressed as the number of trypsin unit inhibited (TUI) per unit weight of the sample that wasanalyzed.

Calculation: TUI/mg = (b-a)/0.1 X F

Where b = absorbance of test sample solution

a =absorbance of the blank

F= (1 x Vf/Va x D)/W

Where W = weight of sample

Vf = total volume of extract used in the assay

D = dilution factor

Va = volume of standard

3.2.6.2 Determination of phytic acid

The phytic acid was determined using the procedure described by Lucas and Markakas (1975). Exactly 2.0g of the sample powder was weighed into 250ml conical flask, 100ml of 2%HCl was added to the sample in the conical flask and allowed for 3hrs. It was then filtered through a double layer of harden filter paper, 50ml of each filtrate in 250ml beaker and 107ml of distilled water was added in each.

Ten milliliters of 0.3% Ammonium thiocyanidesolution was added into the sample and titrated with standard iron chloride solution which contains 1.95mg of iron/ml. The end point was by

appearance of brownish colour persisting for about 5minutes. The percentage of phytic acid was calculated using the formula:

$$\%\ phytic\ acid\ (mg/100g) = (Y \times 1.19 \times 100)/(Sample\ weight)$$

Where;

Y = titre value x 1.95mg.

3.2.6.3 Determination of oxalates

Oxalate was determined using method of Oke (1969). The total oxalic acid of the powdered sample was determined by weighing 2g of sample into 250ml conical flask. 190ml of distilled water and 10ml of 6M HCl acid was then added. The mixture was incubated for 1hr on a boiling water bath, cooled, transferred into a 250ml volumetric flask, diluted to volume and filtered. Four drops of methyl red indicator was added, followed by addition of concentrated ammonia till the solution turned faint yellow. It was then heated to 100^{o}C, allowed to cool and filtered to remove precipitate containing ferrous irons. The filtrate was then boiled, and 10ml of 5% $CaCl_2$was added with a constant stirring. It was then allowed to stand overnight.

The mixture was filtered through Whatman No. 4 filter paper. The precipitate was then washed several times with distilled water and transferred to a beaker. 5ml of 25% sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80^{o}C and titrated against 0.5% potassium permanganate until the pink colour persists for approximately one minute.

A blank wasalsorun. From the amount of potassium permanganate to be used, the oxalate content of the unknown sample was calculated using the equation below:

1ml potassium permanganate = 2.24mg oxalate.

3.2.6.4 Determination of tannins

Tannin was estimated according to the method described by Makkar *et al.*,(1993).

Sample preparation and extraction of tannins

Tannin extraction was done using 400mg of ground sample in a conical flask with 40ml of diethyl ether containing 1% acetic acid (v/v) to remove the pigment material. The supernatant was carefully discarded after 5minutes and 20ml of 70% aquaeous acetone was added and the flask sealed with cotton plug covered with aluminium foil and kept in an electrical shaker for 2hours for extraction. It was then filtered through Whatmann No. 1 filter paper and the sample was kept in a refrigerator at 4^{o}C until analysis.

Standard calibration curve preparation

From the stock solution of tannic acid (0.5mg/ml) 0, 10, 20, 30, 40 and 50µL was placed in a test tube and their volumes made up to 1ml. After which 0.5ml of folin reagent and 2.5ml of sodium carbonate was added and the whole content mixed peoperly and after 40minutes, absorbance was taken at 725nm using a spectrophotometer.

Procedure

Fifty microlitres of tannin extracts for each sample wastaken in a test tube and the volume was made up to 1ml with distilled water. Then 0.5ml of Folin Ciocalteu reagent was added and mixed properly. Then 2.5ml of 20% sodium carbonate solution was added, mixed and kept for 40 minutes at room temperature. Optical density was then taken at 725nm on the spectrophotometer and the concenterations was obtained from the standard curve. Percentage concenteration of tannin was determined using the expression:

$$\%\ Tannin\ (mg/100g) = (An \times C \times Df)/(As \times W) \times 100$$

Where;

An = Absorbance of test sample

As = Absorbance of standard tannic acid

C = Concenteration of standard tannic acid (mg/ml)

Df = Dilution factor V_{ex}/V_a

W = Weight of test sample (mg)

V_{ex} = Volume of extract

V_a = Volume of extract analysed

3.2.6.5 Determination of saponin

Saponin content was determined by the modified method of Fenwick and Oakenfull (1981). Saponin was extracted for 2 hours in a reflux condenser containing pure acetone. Exhaustive re-extraction over heating mantle with methanol in the soxhlet apparatus was done for 2 hours. The extract was weighed after allowing the methanol to evaporate. The saponin content was calculated as a percentage of the sample.

% Saponin = Weight of exract x100/ Weight of sample

3.2.7 Protein quality evaluation

Protein quality evaluation aims to determine the capacity of food protein sources and diets to satisfy the metabolic demand for amino acids and nitrogen. Thus any measure of the overall

quality of dietary protein, if correctly determined, should predict the overall efficiency of protein utilization. Safe or recommended intakes can then be adjusted according to the quality measure, so that demands can be met (FAO *et al*., 2007).

3.2.7.1 Determination of amino acid profile

Amino Acid profile of the samples was determined using methods described by Benitez (1989). The sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Biosystems PTH Amino Acid Analyzer.

Defatting Sample

The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 4g of the sample was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus (AOAC, 2006).

Nitrogen determination:

A small amount (200mg) of the sample was weighed, wrapped in whatman No.1 filter paper and put in the Kjedhal digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules was added.

The flask was then put in Kjedhal digestion apparatus for 3 hours. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10m1) of the diluted solution with 10ml of 45% sodium hydroxide was put into the distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected.

The distillate was then titrated with 0.01N hydrochloric acid.

$$Percentage\ Nitrogen\ =\ ((a-b)\times 0.01\times 14\times V\times 100)/(W\times C)$$

Where:

a. = Titre value of the digested sample

b. = Titre value of blank sample

v. = Volume after dilution (100ml)

W. = Weight of dried sample (mg)

C. = Aliquot of the sample used (10ml)

14. = Nitrogen constant in mg.

Hydrolysis of the sample

A known weight of the defatted sample was weighed into glass ampoule. 7ml of 6NHCl was added and oxygen was expelled by passing nitrogen into the ampoule (this was to avoid possible oxidation of some amino acids during hydrolysis (e.g Methionine and Cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105^{o}C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered. It should be noted that tryptophan was destroyed by 6NHCl during hydrolysis.

The filtrate was then evaporated to dryness at 40^{o}C under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0).

Loading of the hydrolysate into analyzer

About 60 microlitre was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The Analysis last for 76 minutes.An integrator attached to the Analyzer automatically calculates the peak area proportional to the concentration of each of the amino acids.

3.2.7.2 Determination of tryptophan

The tryptophan in the known sample was hydrolyzed with 4.2M Sodium hydroxide (Robel, 1984). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

All other procedures remain the same as above except hydrolysis.

Hydrolysis of the Sample for Tryptophan Determination

A known weight of the defatted sample was weighed into glass ampoule. Then 10ml of 4.2M NaOH was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^{o}C \pm 5^{o}C$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was neutralized with 6N HCl and evaporated to dryness at $40^{o}C$ under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 7.0) and stored in plastic specimen bottles, which were kept in the freezer.

3.2.7.3 Amino acid score(FAO, 1990)

This is achieved by a comparison of the content of the limiting amino acid in the protein or diet with its content in the requirement pattern.

The amino acid score was calculated using the ratio of a gram of the limiting amino acid in the food to the same amount of the corresponding amino acid in the reference diet multiplied by 100.

$$\text{Amino acid score} = \frac{\text{mg of amino acid in 1 g test protein}}{\text{mg of amino acid in requirement pattern}} \times 100$$

3.2.7.4 Protein digestibility corrected amino acid score (PDCAAS) (FAO, 1990)

The PDCAAS was determined mathematically by multiplying the amino acid score of the recipe by the protein digestibility of the recipe.

PDCAAS = (amino acid score x recipe protein digestibility)

Protein digestibility

For this research, protein digestibility was calculated using tables according to FAO (1990). The weighted protein digestibility of each recipe ingredient was added together to create the recipe total.

Weighted Average Protein Digestibility =Sum of (protein x ingredient protein digestibility)divided by protein total

3.2.7.5Protein efficiency ratio (FAO, 1990)

This is based on the weight gain of a test subject divided by its intake of a particular food protein during the test period. PER was determined by calculating the average weight gain of animals which was then divided by the amount of protein intake from the diet.

3.2.8 Experimental animals

Twenty fiveweanling (3 weeks old) albino rats bred in the animal house of the Department of Biochemistry Ahmadu Bello University Zariawere used for the research. The animals were fed with the different formulations for a period of four weeks. Animals were allowed to have free

access to food and water *ad libitum.* Weekly body weight of the animals and feed intakes were monitored.

3.2.8.1 Animal groupings

The animals were grouped into five groups consisting of five rats each.

Group 1: Control group fed with normal rat feed.

Group 2: Animals were fed withSWS-RUTF**1 (**sesame seeds 25%, soya beans 30%, wheat 20%, soya oil 13.4%, sugar 10%, and mineral/vitamin mix 1.6%).

Group 3: Animals were fed withSWS-RUTF**2 (**sesame seeds 25%, soya beans 25%, wheat 25%, soya oil 13.4%, sugar 10%, and mineral/vitamin mix 1.6%).

Group 4: Animals were fed withSWS-RUTF**3 (**sesame seeds 20%, soya beans 35%, wheat 20%, soya oil 13.4%, sugar 10%, and mineral/vitamin mix 1.6%).

Group 5: Animals were fed with the standard P-RUTF (Nutty Butta, by USDA).

At the end of the four weeks of feeding, animals from each group were anesthetized and decapitated. Blood samples were collected in labeled sample containers, and serum was collected.

3.2.8.2 Determination of serum protein

Preparation of the Biuret Reagent.

The Biuret reagent is based on a reaction between copper and molecules with at least 2 peptide bonds. The color intensity caused by this reaction is directly proportional to the number of peptide bonds, and therefore the amount of protein.

Biuret reagent.

The Biuret reagent was prepared by dissolving 3g of copper sulphatein500ml of distilled water, 9g of Sodium Potassium(NaK)tartrate and 5g of potassium iodide were added to the solution. The solution was made to 1000ml by addition of 0.2M NaOH.

Reference solution

The standard solution was prepared by preparing 10mg/ml of egg albumin stock solution. A set of six test tubes was arranged, and serial dilution was done with distilled water. 4ml of biuret reagent was dispensed into each test tube. Into the first test tube, 1ml of the stock solution was added, 0.8ml of stock solution and 0.2ml distilled water was added into the second test tube, 0.6ml stock solution and 0.4ml distilled water was added into the third test tube, test tube no.4 contains 0.4ml stock solution and 0.6ml distilled water, test tube no.5 contains 0.2ml stock solution and 0.8ml distilled water, and the sixth test tube contains 1ml distilled water in addition to the biuret reagent. These were allowed for 30mins at room temperature. Absorbance was taken at 540nm.

Procedure

To 0.5ml of the test sample,2ml of biuret reagent was added and mixed. It was allowed for 30mins at room temperature. Absorbance was determined at 540 nm.

Calculations

The relationship of absorbance to protein concentration is approximately linear within the indicated range of protein concentrations for the reference solutions.

The absorbance of the reference solutions was plotted against protein concentrations and linear regression was used to establish the standard curve.From the standard curve and the absorbance of the test solution, the concentration of protein in the test solution was determined.

3.2.9 Statistical analysis

All values were expressed as mean ± SD. Data analysis was carried out using Statistical package for social sciences (SPSS) version 20. Data was subjected to analysis of variance (ANOVA), and Bonferroni post hoc test was carried outto establish where the difference in mean lies. P value < 0.05 was considered significant.

CHAPTER FOUR

4.0 Results

The result of proximate analysis carried out on the formulated sesame-wheat-soya beans based potential Ready-to-use therapeutic food is presented in Table 4.1. SWS-RUTF1 had values for protein, dietary fibre and moisture as well as the caloric value significantly greater ($P < 0.05$) than that of SWS-RUTF2 and 3. SWS-RUTF3 had values that were significantly lower for all parameters determined, except for lipids. Highest value of carbohydrate was found in SWS-RUTF2. However, P-RUTF had energy and fat composition that are significantly greater than all other formulations.

The result of mineral analysis for the SWS-RUTF is shown in Table 4.2. SWS-RUTF2had the highest levels of Zn, Ca, Fe and Mn, with Fe having the highest value. Higher levels of Na and K were found in SWS-RUTF3. All minerals determined were lower in SWS-RUTF1except for Mg which was found to be higher. High level of the heavy metal Pb was found in SWS-RUTF2 while SWS-RUTF3 has high level of Cd. P-RUTF had concentrations of all minerals determined significantly greater than all the other formulations.

Results ofantinutrients present in the SWS-RUTF are depicted in Table 4.3. Significantly lower levels of Trypsin inhibitor, Tannin, Phytate, and Saponin were found in SWS-RUTF1. Lowest concentrationof oxalate was found in SWS-RUTF3.

The Amino Acid profile of the SWS-RUTF is presented in Table 4.4. All the three formulations had values of the essential amino acids Phenylalanine, Threonine,Histidine and Valine within the same range. SWS-RUTF2 had the lowest values for all the Amino Acids, with most of the Amino Acids being significantly ($P<0.05$) lower as compared with the other two formulations.

Glutamate, Aspartate, Leucine, and Arginine had the highest values in all the three formulations. While Tryptophan, Methionine, and Cystein had the lowest concentration in all the three formulations,

The Amino Acid score, Protein Digestibility Corrected Amino Acid Score, and Protein Efficiency Ratio of the formulation as presented in Table 4.5, showed that;higher values of the three determinants of protein quality were found in SWS-RUTF3, followed by SWS-RUTF1.Lowest values were observed in SWS-RUTF2. However, it was found that P-RUTF had levels of all these parameters higher than all other formulations.

Table 4.1:Proximate Composition of the Formulated PotentialSWS-RUTF

Nutrient	SWS-RUTF1	SWS-RUTF2	SWS-RUTF3P-RUTF
Energy (kcal)	411.56±0.25^{b}	407±0.12^{c}	404.24±0.07^{d} 530.00^{a}
Protein (%)	28.88±0.03^{a}	24.25±0.21^{c}	26.19±0.14^{b} 14.50^{d}
Carbohydrate (%)	49.47±0.23^{b}	55.31±0.10^{a}	54.91±0.21^{c} 43.00^{d}
Fat (%)	11.16±0.02^{b}	9.86±0.20^{b}	9.01±0.02^{b} 33.50^{a}
Moisture (%)	4.69±0.10^{a}	4.12±0.24^{b}	4.14±0.28^{b} <5.00
Ash (%)	1.57±0.20^{d}	2.62±0.12^{c}	2.67±0.32^{b} 4.00^{a}
D/Fibre (%)	16.68±0.63^{a}	15.47±0.88^{b}	15.24±0.23^{c}
C/Fibre (%)	4.24±0.24^{b}	3.84±0.12^{c}	3.07±0.05^{d} <5.00^{a}

Values are mean ±SD for three determinations with the exception of P-RUTF
Different superscripts in rows signify statistically significant difference ($P< 0.05$)
D/fibre = Dietaryfibre, C/fibre = Crude fibre.

Table 4.2: Concentration of Some Minerals in the Potential SWS-RUTF

Minerals	SWS-RUTF1	SWSRUTF2	SWS-RUTF3	P-RUTF (10^4)
	(mg/kg)			
Zn	0.56 ± 0.12^{d}	0.60 ± 0.03^{b}	0.59 ± 0.25^{c}	13.00^{a}
Ca	1.77 ± 0.23^{d}	3.48 ± 0.42^{b}	2.85 ± 0.74^{c}	310.00^{a}
Na	4.55 ± 0.32^{d}	4.99 ± 0.12^{c}	5.21 ± 0.16^{b}	$<290.00^{a}$
Mg	1.08 ± 0.10^{b}	0.90 ± 0.28^{c}	0.90 ± 0.73^{c}	86.00^{a}
Fe	7.43 ± 0.01^{c}	8.43 ± 0.03^{b}	7.25 ± 0.14^{d}	12.45^{a}
K	2.45 ± 0.13^{d}	2.58 ± 0.52^{b}	3.06 ± 0.26^{c}	1100.00^{a}
Cd (10^{-4})	□ □	7.00 ± 0.12^{d}	26.00 ± 0.10^{c}	34.00 ± 0.14^{b} $<0.03^{a}$
Pb(10^{-3})	□ □	4.20 ± 0.20^{c}	125.10 ± 0.03^{b}	26.10 ± 0.32^{d} $<0.1^{a}$

Values are mean ±SD for three determinations
Different superscripts in rows signify statistically significant difference ($P< 0.05$)
□ □ (not applicable to P-RUTF)

Table 4.3: Levels of SelectedAntinutrients in the Potential SWS-RUTF

AntinutrientsSWS-RUTF1	SWS-RUTF2	SWS-RUTF3	
Trypsin inhibitor(TUI/mg)	6.00 ± 0.02^{a}	6.40 ± 0.02^{b}	6.66 ± 0.05^{b}
Tannin (%)	3.58 ± 0.03^{a}	4.39 ± 0.24^{b}	3.25 ± 0.25^{a}
Oxalate (mg/100g)	5.44 ± 0.23^{c}	4.32 ± 0.33^{b}	2.30 ± 0.12^{a}
Phytate (mg/100g $x10^{-4}$)	1.70 ± 0.20^{a}	3.90 ± 0.14^{b}	7.90 ± 0.02^{c}
Saponin (%)	17.00 ± 2.00^{a}	24.00 ± 1.00^{b}	23.00 ± 0.21^{b}

Values are mean ±SD for three determinations
Different superscripts in rows signify statistically significant difference ($P<0.05$)

Table 4.4: Amino Acid(g/100g) Profile of the Potential SWS-RUTF

Amino acidsSWS-RUTF1	SWS-RUTF2	SWS-RUTF3	
Leucine	6.74± 2.26[b]	5.90±0.14[a]	7.24±0.28[b]
Lysine	3.71±1.83[ab]	3.32±0.35[a]	4.03±0.04[b]
Isoleucine	3.24±0.42[ab]	3.01±0.01[a]	3.60±1.41[b]
Phenylalanine	4.35±0.28[a]	4.08±1.41[a]	4.52±1.13[a]
Tryptophan	1.42±0.14[b]	1.21±0.28[a]	1.58±0.14[c]
Valine	4.03±0.18[a]	3.89±1.41[a]	4.24±0.23[a]
Methionine	1.39±2.14[b]	1.20±0.10[a]	1.42±1.40[b]
Histidine	2.49±1.42[a]	2.33±0.30[a]	2.81±1.40[a]
Threonine	2.39±0.41[a]	2.28±0.14[a]	2.61±0.01[a]
Total EAA	29.76	27.22	32.05
Arginine	5.49±0.09[a]	4.99±1.42[a]	6.19±0.40[b]
Proline	3.45±1.91[a]	3.25±0.42[a]	3.65±0.42[a]
Tyrosine	2.92±0.28[b]	2.41±0.20[a]	2.92±1.20[b]
Cystein	1.33±0.14[b]	1.21±0.01[a]	1.45±0.01[c]
Alanine	3.83±1.40[b]	3.19±0.01[a]	4.32±0.14[c]
Glutamic acid	13.32±2.14[b]	12.87±0.07[a]	13.85±0.42[b]
Glycine	3.56±0.28[a]	3.37±1.41[a]	4.04±0.56[b]
Serine	3.51±1.27[b]	3.03±0.04[a]	3.78±1.41[b]
Aspartic acid	9.55±0.35[b]	9.12±1.83[a]	9.80±0.01[b]
Total NEAA46.96	43.44	50.00	

Values are mean ±SD for two determinations.
EAA = Essential Amino Acid, NEAA = Non-Essential Amino Acid.
Different superscripts in rows signify statistically significant difference (P<0.05)

Table 4.5: Amino Acid Score, Protein Digestibility Corrected Amino Acid Score, and Protein Efficiency Ratio of the Potential SWS-RUTF

	SWS-RUTF1	SWS-RUTF2	SWS-RUTF3	P-RUTF
AA Score (%)	72	65	79	100
PDCAAS (%)	62	55	67	100
PER	1.08	1.07	1.10	2.18

The result of average weekly food consumptions of animals fed the various diets is shown in Figure 4.1. Animals in the normal control group had the highest consumption level which was statistically significant ($p<0.5$) as compared with the food intake of all other groups. Groups fed with SWS-RUTF1, 2, 3, and P-RUTF had food consumption levels occurring within a similar range. Although there were slight variations between the groups, the difference was not statistically significant.

As presented in Figures 4.2, the result of average weeklybody weightof animals fed with the various diets for the period of four weeks showed that;animals fed P-RUTF had body weight that was significantly ($P<0.05$) higher than normal control group and all the SWS-RUTF treatment groups which had values that were within the same range.

The percentage change in body weight of animals is presented in Figure 4.3. Animal groups fed SWS-1, 2, and 3 had percentage change in body weight within a similar range without any statistically significant difference. The animal group fed P-RUTF had percentage change in body weight that was statistically ($P<0.05$) higher than other animal groups, and animals in the normal control group had value that is significantly ($P<0.05$) lower.

Result of Serum total protein of animals fed with the different food formulations is presented in Figure 4.4. Groups fed with normal rat mash, and P-RUTF had low levels of serum protein in comparison with other groups. Groups fed with the different SWS-RUTF had serum protein concentrations at similar level. The differences between groups were not statistically significant.

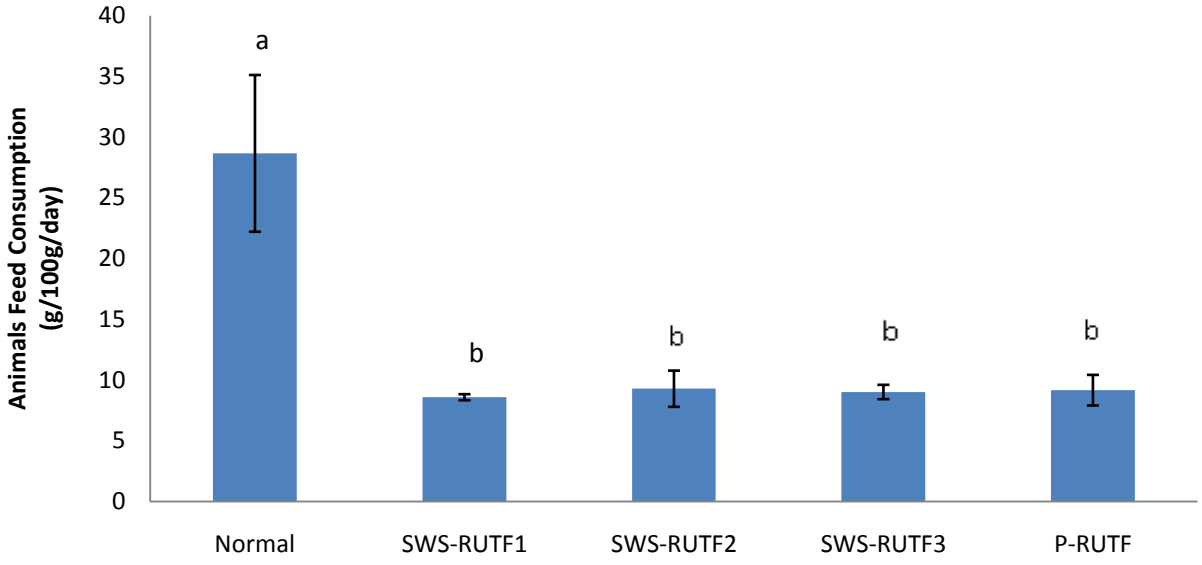


Figure 4.1: Average Feed Consumption of Animals Fed with the Different Diets
Values are Mean±SD
Different superscripts signify statistically significant difference ($P<0.05$)

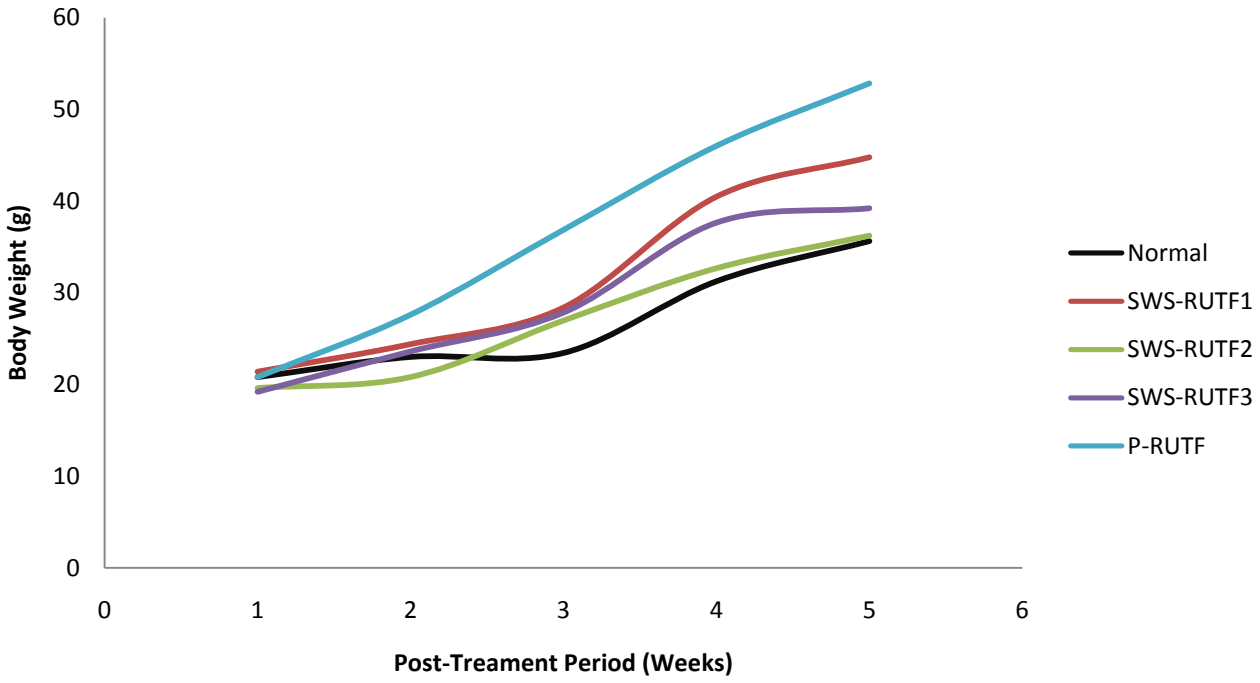


Figure4.2:Weekly Body Weights (grams) of Animals Fed with the Different Diets Values are Mean±SD

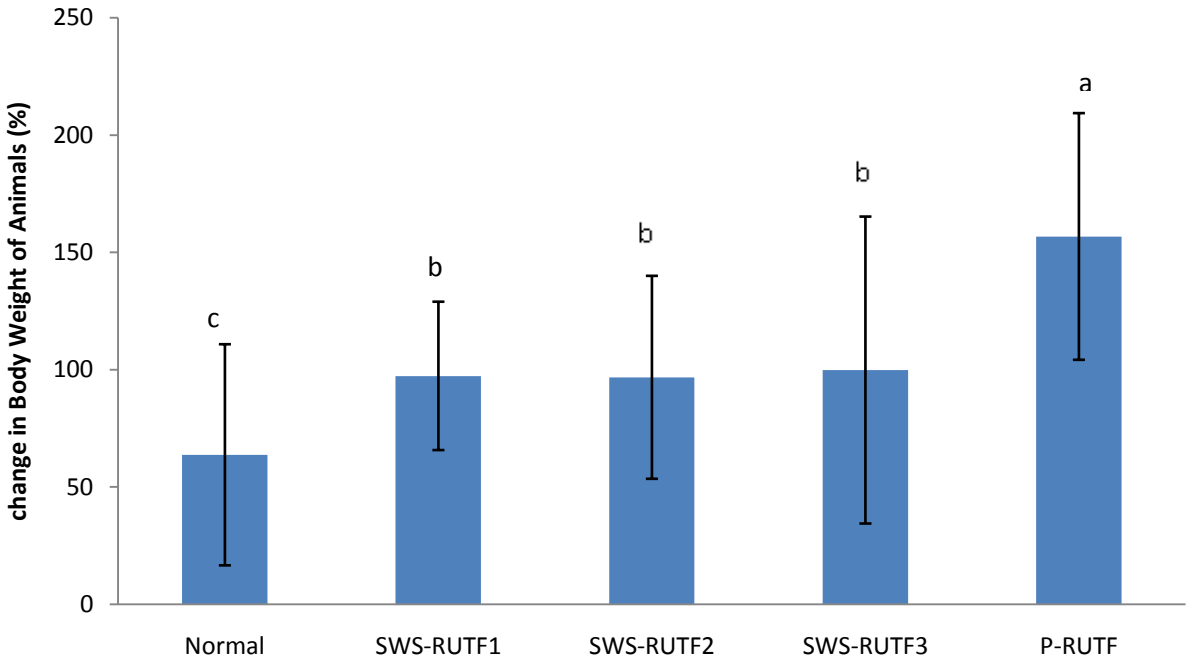


Figure 4.3: Percentage Change in Body Weight of Animals after Four Weeks
Values are Mean ±SD
Different superscripts signify statistically significant difference ($P<0.05$)

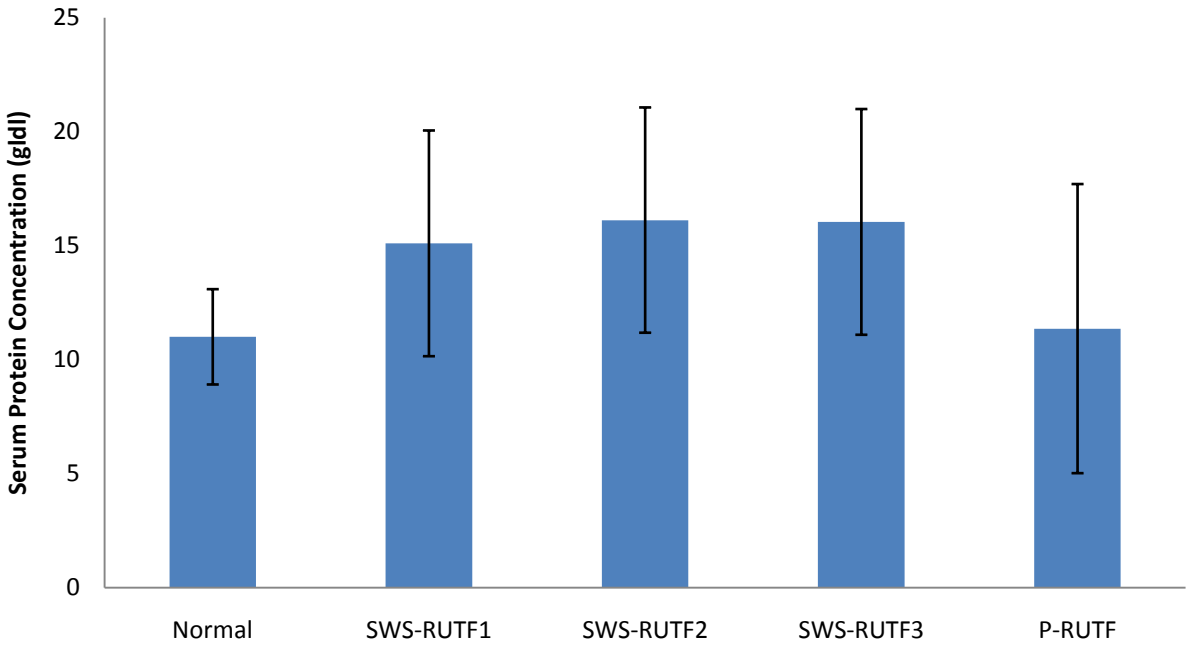


Figure 4.4: Serum Total Protein (g/dl) of Rats Fed with the Different Diets
Values are Mean ±SD

CHAPTER FIVE

5.0 Discussion

The basic requirement to reduce child malnutrition is availability of nutritious food, improved hygiene, health services and adequate care. Poverty and food insecurity seriously affect the accessibility to nutritious diets, including high protein quality, adequate micronutrient content and bioavailability, low anti-nutrient content, and high nutrient density. These conditions predispose children to severe acute malnutrition. Guidelines provided by WHO to manage children with severe acute malnutrition without any medical complication (e.g. anorexia, diarrhoeaetc.), suggested the use of Ready-to-use therapeutic food. RUTF can be safely and easily produced in small or large quantities. The local availability of the necessary ingredients limits its use in some settings, and further investigation into alternative ingredients is needed to overcome the limitation (Manary, 2006).

The caloric values of the SWS-RUTF were found to be significantly lower than the minimum caloric requirement (520 – 550 kcal/100g) of RUTF formulation (Vijay and Bhawesh, 2014). This is attributed to lower calorie contribution from fats, which contribute only about 25% to the total calorie below the recommended requirement of 45% - 60% (UNICEF, 2010).Unlike Corn-Soy-Blend a supplementary food having a caloric value of 380Kcal and 15g protein per 100g (Saskia and Martin, 2008), SWS-RUTF however, contained significantly high amounts of protein which was above the recommended levels of 10 – 12% of total energy (Vijay and Bhawesh, 2014). Therefore,adjusting the balance between protein and fat source foods in the SWS-RUTF recipe may yield a product that can meet these requirements.

Minerals are substances found in food which are required by the body for normal growth and function. Mineral contents of the SWS-RUTF appeared to be significantly lower than the recommended value(Caron, 2012 and UNICEF, 2010). This was due to the poor micronutrient content of plant based diets (Solomon, 2005), and type of mineral/vitamin premix used in the formulation which contains low amounts of the micronutrients as compared to the Nutriset Mineral/vitamin premix recommended for RUTF formulations. A lack of sufficient micronutrients in the diet affects the health and development of children and results in potentially life-threatening deficiency diseases such as anemia and vitamin A deficiency (FAO, 2001). This feature makes SWS-RUTF unsuitable for management of SAM. Lead and Cadmium are heavy metals of public health concern, these metals were found to be below the acceptable limits <0.1mg/100g and <0.03mg/100g respectively for RUTF formulation (Caron, 2012).

Antinutrients are plant compounds that reduce ability of the body to absorb essential nutrients.The presence of antinutritional factors such as phytates, oxalates and tannins in foods has been reported to affect the effective utilization of its nutrients in human nutrition (Mbofung*et al*., 1990;Ijarotimi and Keshinro, 2012). Iron and Zinc Most cereal-based diets have poor bioavailability of nutrients as a result of the presence of antinutritional factors (Anigo*et al.,*2009).The concentrations of phytate were low and less than 1% that was reported to interfere with mineral availability (Erdman, 1979) and also below the concentration that may cause danger in diets (Ogunyinka*et al.,* 2016).Levels of tannin were below the ingestion dose of 45mg/100g of body weight per day that was reported by Samantha *et al., (*2004) to cause toxicity.The high concentration of saponin in the formulations may be deleterious when a high amount of the product is consumed. Saponin of 1mg/100g inrats' diet was found to decrease the plasma cholesterol and increase bile acid production(Oakenfull and Sidhu, 1990). Oxalate and trypsin

inhibitor were also below the safe level of consumptions (Oly-Alawuba and Obiakor-Okeke, 2014). Although traces of these antinutrients were found in the formulations, the concentrations are at safe level that could pose no danger in diets (Ogunyinka*et al.,* 2016)

All the values obtained for essential amino acids fell below the recommended value for RUTF formulation.Short-fall in amino acid can limit growth and brain development of children due to unavailability of these amino acids for use in the metabolic processes of the body (Solomon, 2005).Furthermore, these low levels of amino acids affect the protein quality, of the diet making it significantly lower than that of P-RUTF which contains milk (animal source protein). Replacing milk proteins with proteins derived from other sources is likely to have an impact on protein quality (Andre *et al.,* 2015).Protein quality determines the capacity of food protein sources and diets to satisfy the metabolic demand for amino acids and nitrogen. Thus any measure of the overall quality of dietary protein,should predict the overall efficiency of protein utilization (FAO *et al.*, 2007).

Animals in the normal control group had the highest consumption level which is statistically significant ($P<0.05$) as compared with the food intake of all other groups. This may be attributable to low energy and nutrient density of the feed given to the animals.Animals in the other groups consumed less feed because of high protein and fat contentof the feed which makes it nutrient dense. As such they don't have to consume high amount to meet their nutrient requirement.

One important characteristics of RUTF is its ability to facilitate rapid catch-up growth. Anthropometric indices are the standard indicators used in monitoring nutritional status of malnourished children. The indicators are; weight-for-height, height-for-age, and weight-for-age.

These standards are only applicable to human subjects, but not animals (Cogill, 2001). In this research, therefore, weight was used as the indicator. Animals in the P-RUTF control group had percentage change in body weight significantly ($P<0.05$) higher than that of SWS-RUTF treatment groups. This may be as result of variation in protein quality, energy and nutrient density between the feeds (Solomon, 2005). According to a study carried out in Zambia to compare the effectiveness of a milk-free soy-maize-sorghum-based ready-to-use therapeutic food (SMS-RUTF) and a standard ready-to-use therapeutic food (P-RUTF) in the treatment of SAM without complications. The study showed that overall recovery rates in children treated with P-RUTF was higher than those treated with the SMS-RUTF, but the study was inconclusive (Abel *et al.,* 2015).

Blood proteins, also termed serum/plasma proteins, are protein present in blood plasma. They serve many different functions within the body. Serum protein has often been used as laboratory indicator of nutritional status (Aaron and Vikram, 2015). It is also used to find out if a diet contains enough protein. Groups fed with normal rat mash and P-RUTF had low levels of serum protein as compared with groups fed SWS-RUTF. Although the serum protein levels are higher in SWS-RUTF, animals fed with the product still had lower growth rate due poor quality of the food protein.

CHAPTERSIX

6.0 Summary, Conclusion and Recommendation

6.1 Summary

The SWS-RUTF had caloric value that fell below the 520 – 550 kcal/100g recommended for RUTF formulation, which was likely to be as a result of lower calorie contribution from fats. Ithowever, contains high amounts of protein.

Due to the poor micronutrient content of plant based diets, and type of mineral/vitamin premix used in the formulation, which contains low amounts of the micronutrients, Mineral contents of the products appeared to be significantly lower than the recommended value.

All the values obtained for essential amino acids fell below the recommended value for RUTF formulation. This short-fall can limit growth and brain development of the children due to unavailability of these amino acids for use in the metabolic processes of the body. Also, these low levels of amino acids can affect the protein quality of the diet. These along with some other factors could explain the reason animals in the P-RUTF control group had percentage change in body weight significantly ($P<0.05$) higher than that of SWS-RUTF treatment groups.

Although the serum protein levels are higher in SWS-RUTF, animals fed with the product still had lower growth rate due poor quality of the food protein.

6.2 Conclusion

The Sesame-Wheat-Soya beans based RUTF formulated had energy and nutrient density below the standard recommended for RUTF formulations. However, it contains high level of protein but with low quality. High quality protein is a fundamental requirement for RUTF formulations. Therefore, the SWS-RUTF if improved in terms of protein quality, energy and nutrient density may serve as alternative to P-RUTF or can be a very good candidate for supplementary food.

6.3 Recommendations

Further studies should be done on improving the quality of the formulation, particularly in terms of its energy value and mineral composition.

Studies should also be carried out on malnourished subjects in order to know the actual potential of the formulation.

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APPENDIX

Appendix I

A: Chemical Score of the SWS-RUTF

Amino acid	SWS-RUTF1	SWS-RUTF2	SWS-RUTF3
Leucine	100	100	100
Lysine	72	65	79
Isoleucine	100	100	100
Phe+Tyr	100	100	100
Tryptophan	100	100	100
Valine	100	100	100
Methionine	100	96	100
Histidine	100	100	100
Threonine	88	84	96

B:Amino Acid(g/100g) Profile (based on wet weight) of the Potential SWS-RUTF

Amino acid	SWS-RUTF1	SWS-RUTF2	SWS-RUTF3
Leucine	6.42	5.66	6.94
Lysine	3.54	3.18	3.86
Isoleucine	3.09	2.89	3.45
Phenylalanine	4.15	3.91	4.33
Tryptophan	1.35	1.16	1.51
Valine	3.84	3.73	4.06
Methionine	1.32	1.15	1.36
Proline	3.29	3.12	3.49
Arginine	5.23	4.78	5.93
Tyrosine	2.78	2.31	2.79
Histidine	2.73	2.23	2.69
Cysteine	1.27	1.16	1.39
Alanine	3.65	3.06	4.14
Glutamate	12.69	12.33	13.28
Glycine	3.39	3.23	3.87
Threonine	2.28	2.18	2.50
Serine	3.34	2.91	3.62
Aspartate	9.10	8.74	9.39

C: Amino Acid Scoring Pattern for Use in Children > 1 Year of Age and in All Other Older Age Groups (FNB/IOM 2002)

Amino Acid	mg/g proteins	mg/g N
Histidine	18	114
Isoleucine	25	156
Leucine	55	341
Lysine	51	320
Methionine + cysteine	25	156
Phenylalanine + tyrosine	47	291
Threonine	27	170
Tryptophan	7	43
Valine	32	199

D: Protein Digestibility-Corrected Amino Acid Score, calculated using the method proposed by FAO/WHO/UNU

Table 6
Calculation of PDCAAS value for a mixture of wheat, chickpea and milk powder[a]

	Analytical data									Digestible quantities in mixture[b]				
	Weight	Protein	Lysine	Sulfur amino acids	Threonine	Tryptophan	Digestibility	Protein	Lysine	Sulfur amino acids	Threonine	Tryptophan
	(g)	(g/100 g)	(mg/g protein)					(g)	(mg)			
	A	B	C	D	E	F	G	AxBxG/100 =P	PxC	PxD	PxE	PxF
Wheat	400	13	25	35	30	11	0.85	44	1105	1547	1326	486
Chickpea	100	22	70	25	42	13	0.8	18	1232	440	739	229
Milk powder	35	34	80	30	37	12	0.95	11	904	339	418	136
Totals								73	3241	2326	2483	851
Amino acids: mg/g protein (total for each amino acid/total protein)									44	32	34	12
Weighted average digestibility: sum of digestible protein/ total protein							0.85					

Age group (years)	Reference patterns: mg/g protein[c]				Amino acid score for mixture: amino acids/g protein per reference pattern				PDCAAS value: lowest score x digestibility
	Lysine	Sulfur amino acids	Threonine	Tryptophan	Lysine	Sulfur amino acids	Threonine	Tryptophan	
Infants (0–5 years)	57	28	31	8.5	0.78	1.14	1.10	1.98	0.67
Preschool children (1–2 years)	52	26	27	7.4	0.85	1.22	1.26	1.58	0.72
Older children and adolescents (4–18 years)[d]	48	23	25	6.5	0.93	1.38	1.36	1.80	0.79
Adults (>18 years)	45	22	23	6.0	0.99	1.45	1.48	1.94	0.84

[a] Values for protein, amino acid content and digestibility from reference 4.
[b] Protein and amino acids calculated as digestible amounts.
[c] Reference patterns from section 9.
[d] Based on the scoring pattern derived for the 2–10-year age group.

The Weighted Average Protein Digestibility of the three products was calculated to be 0.85

Appendix II

A: Common ingredients in RUTF (Peanut-based RUTF (P-RUTF)(Collins, 2004)

Ingredients	Percentage (%)
Full fat milk	30
Sugar	28
Vegetable oil	15
Peanut	25
Mineral /Vitamin mix	1.6

B: Ingredients used Rice-Sesame RUTF1 (Collins, 2004)

INGREDIENTS	PERENTAGE (%)
Roasted rice flour	20.0
Soyamin 90	8.0
Roasted sesame seeds paste	29.0
Sunflower oil	19.4
Icing sugar	22.0
Mineral/vitamin Mix	1.6
Total	100.0

C:Ingredients used in Barley-Sesame RUTF2 (Collins, 2004)

INGREDIENTS	**PERENTAGE (%)**
Roasted pearl barley flour	15.0
Soyamin 90	9.0
Roasted sesame seeds paste	27.0
Sunflower oil	24.0
Icing sugar	23.4
Premix	1.6
Total	100.0

D:Ingredients used in Maize-Sesame RUTF3 (Collins, 2004)

INGREDIENTS	PERENTAGE (%)
Roasted maize flour	33.4
Roasted sesame seeds paste	27.0
Roasted chick peas flour	25.0
Sunflower oil	12.0
Icing sugar	15.0
Premix	1.6
Total	100.0

E:Nutritional Composition per 100g and Percentage Contribution to Energy RUTF-1, RUTF-2, RUTF-3 and NutrisetPlumpy'nut® (Collin, 2004)

Nutrients	RUTF 1	RUTF 2	RUTF 3	Plumpy nut
Energy (kcal)	551	567	512	530
Protein (g)	13.8	14.1	13.4	14.5
Carbohydrate (g)	43	39.9	50.2	43
Fat (g)	36	39	28.6	33.5
Ash (g)	4.3	3.9	4.9	4
Moisture (g)	2.9	3.1	2.9	<5

F: Mineral Composition for RUTF Products (Collins, 2004)

Minerals	RUTF 1 (mg/kg)	RUTF 2 (mg/kg)	RUTF 3 (mg/kg)	Plumpy nut (mg/kg)
Cu	2.1	2.1	1.8	1.7
Zn	10.9	10.9	10.2	13
Ca	338.1	338.1	209.8	310
Na	256.5	256.5	189.9	<290
Mg	118.4	118.4	119.1	86
Fe	5.6	5.6	4.4	12.45