

ASSESSMENT OF THE IMPACT OF THERAPEUTIC FOOD
FORMULATED FROM LOCALLY SOURCED FOODSTUFF ON
ANTHROPOMETRIC INDICES OF SEVERELY ACUTE
MALNOURISHED CHILDREN

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DECLARATION

I hereby declare that this work is the product of my research efforts undertaken under the supervision of Dr. Salisu Maiwada Abubakar and has not been presented and will not be presented anywhere for the award of degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

I certify that the research work for this dissertation titled ‘Assessment of the Impact of Therapeutic Food Formulated from Locally Sourced Foodstuff on Anthropometric Indices of Severely Acute Malnourished Children’ was carried out by Ahmad Alhassan Siddan (SPS/15/MBC/00002) under my supervision.

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DEDICATION

This research work is dedicated to my late parents; Alh. Ahmad A. Siddan and Hajiya Hannatu Usman. May Allah (S.W.T) grant them Al-Jannatul Firdaus, Ameen. And to my wife Hauwa'u Muhammad Sani for her support and motivation throughout the completion of this research work.

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The responsibility of this work is mine, if any credit, I share with all those mentioned. May Allah bless them all?

ABSTRACT

One of the key strategies to tackle the menace of severe acute malnutrition (SAM) is the use of therapeutic foods. However, management of patients with SAM is usually challenged as result of therapeutic food shortages. This is partly due to lack of production from locally available foodstuff. The aim of this research was to formulate a therapeutic food for the management of SAM in children under five using foodstuff available in Nigeria. Three different therapeutic foods were formulated using *Phoenix dactylifera*, *Moringa oleifera* leaves, *Glycine max*, *Sorghum bicolor*, and *Arachis hypogaea* at ratio of 12.5:2.5:35.0:30.0:20.0 (Diet A), 12.5:2.50:30.0:25.0:20.0 (Diet B) and 13.0:1.0:30.0:17.5:38.5 (Diet C) respectively. Proximate, mineral, vitamins, amino acids and phytochemicals contents of the individual food samples and the formulated food were analysed. Twenty (20) children (ten boys and ten girls aged between 09-36 months) (15 with Severe Acute Malnutrition, 05 with Moderate Acute Malnutrition) were fed three times daily with diet C for 14 days (150g/day; 420-480kcal/d). Anthropometric measurement (weight, height, head circumference, Mid-Upper-Arm circumference MUAC) were taken at day 0 and 14th day after administration of the food. The results of the proximate analyses showed significant differences ($p<0.05$) in proximate composition of food samples and the formulated food. There is a significant difference ($p<0.05$) in minerals content of food samples and the formulated foods. Vitamins A, C and E were also obtained in all food samples with the diet C having the highest amounts. Analyses have shown the presence of 18 amino acids with glutamic acid, aspartic acid, leucine, arginine and valine having the highest amount among all the samples. Phytochemicals like saponins, tannins, alkaloids, glycosides, steroids, flavonoids and phytates were found in significant amount in all the samples. Approximately, 19 % increase in weight of children was observed after administration of Diet C for 14 days. The results of this study suggest that diet C could be used in the management of SAM. However, more robust clinical trials may be required to confirm.

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CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

Good nutrition and well-being of children are determined by status of their nutrition. Children with available and healthy diet have greater chances of survival to reach their potential growth and prospects. The available resources at home, community, and the country at large affect the children's nutrition. The place they survive, the parent's income and their gender also plays a pivotal role (Popkin *et al.*, 2012). Mother's illiteracy and the level of awareness and access to locally produced food also contribute on how the food is allocated within a family (Popkin *et al.*, 2012).

In most developing countries, protein-energy malnutrition (PEM) is a major public health concern (Muller and Krawinkel, 2005). In poor countries, it accounts for the death of almost 50-60% of children between 0-59 months (Faruque *et al.*, 2008; Gernaat and Voorhoeve, 2000) and a myriad of morbidities. Protein Energy Malnutrition is classified by various anthropometric measurements (Gernaat and Voorhoeve, 2000). Weight for height or bilateral edema is used to measure acute malnutrition while height for age is used to measure chronic malnutrition (Thailand Burma Border Consortium, 2007). Severe acute malnutrition was defined by WHO as a very low weight for height (below -3Z score of the median WHO growth standards), visible severe wasting or the presence of nutritional edema (WHO, 2015). Therefore, severe acute malnutrition include wasting (marasmus) and various forms of kwashiorkor. Deficiencies of iron, iodine, zinc, and vitamin A are the main symptoms and signs of malnutrition in developing countries, together with deficiency of macronutrients and other detrimental factors such as very

high level of ignorance, poverty, unemployment and overpopulation also contribute immensely to the proliferation of PEM (Muller and Krawinkel, 2005).

Low weight for age (underweight), low height for age (stunting) and low weight for height (wasting) are the signs and symptoms of PEM which exposed the child to a very high risk when it worsens and constitute a threat to the survival of the child. The prevalence of low height for age (stunting) in Nigeria between 1990 and 2013 from 45% to 37% has changed marginally (NPC and ICF International, 2014). Also, the challenges of acute malnutrition increased at that particular period. In 1990, it was estimated at 9% and 18%. The prevalence of acute malnutrition remains as high as 27% in the North West region. Also, the level of severe acute malnutrition increases from 2% in 1990 to 9% in 2013 (NPC and ICF International, 2014). Severe acute malnutrition is a major critical challenge to health and to clinicians (Rytter *et al.*, 2015). About 1.7 million children are suffering from severe acute malnutrition in Nigeria, and other number constitute of about one tenth of all severely acutely malnourished children in the world. Also according to the United Nation office of the coordination of Humanitarian Affairs (2014), Nigeria is the second highest of high burden of malnutrition in the world with an estimate of about 3.78 million children suffering from wasting.

Prevalence of wasting, stunting and underweight is generally lower in the Southern states than the Northern States of Nigeria (NPC and ICF International, 2014). For instance, states in the North like Bauchi, Jigawa, Kaduna, Katsina, Kebbi, Sokoto and Zamfara have the highest proportions of malnourished children than the other states. The prevalence of stunting in all these states exceeds 50%. While other states like Gombe, Kano, Taraba and Yobe, the prevalence exceeds 40%. The prevalence of moderate wasting among children under five exhibits similar patterns. Except for Jigawa and Zamfara, all states in the northwest show higher prevalence of

acute malnutrition (wasting) than the national average. Also, North-Eastern states like Bauchi, Borno and Yobe have a disproportionately high burden of wasting. Kaduna and Kano are States with high prevalence of severe acute malnutrition with 27.6% and 25.1% respectively while Bayelsa state has the lowest burden with 3.1%. Analyses based on the seasonal effects in the northern states have shown that the children were mostly admitted in to Community Based Management of Acute malnutrition (CMAM) programs in June, August and September, and the number of admitted children is constantly raised since the year 2009 (NPC and ICF International, 2014).

Several policies and programs have been put in place by the Government of Nigeria to address the issue of children malnutrition. These include, The National Policy on Food and Nutrition (2016), The Food Security Bill (2015), the National Plan Action on Food and Nutrition in Nigeria (2004), the National Strategic Plan of Action (the health sector response), the Micronutrient Control Programme, the Baby-Friendly Hospital Initiative, and the School Feeding programme. The Government has also enacted laws requiring the fortification of massed consumed foods with vitamin A, Iron and salt iodization.

The term ‘Ready-To-Use-Therapeutic Foods’ (RUTF) is a subset of therapeutic foods, refers to several varieties of ready to eat foods, ranging from those prepared from locally available foods by village women in their own self-help groups for the malnourished children in their village, to those prepared according to specific formulas in factories to be shipped all over the world. The latter is specifically a peanut and milk powder based spread with specific amount of micronutrients, providing energy equivalent to WHO requirement i.e 520-550 Kcal/100gm (Dubey and Bhattacharya, 2011; Kapil, 2009)

Children with severe acute malnutrition need safe, palatable foods with high energy content and adequate amount of vitamins and minerals. RUTF is energy-dense, micronutrient-enriched which are soft or crushable or drinkable foods that can be directly consumed easily by children from the age of six month without cooking. RUTF has a similar nutrient composition with F100, which is the therapeutic diet used in hospital settings. But unlike F100, RUTF is not water-based, meaning that bacteria cannot grow in them. Therefore, these foods can be used safely at home without refrigeration and even in areas where hygiene conditions are not optimal.

Locally available foods like Date palm, Moringa leaves, soybean, sorghum and groundnuts if formulated in the right proportions, could be used to produce therapeutics foods for the management of severe acute malnutrition in children. Date palms (*Phoenix dactylifera* L.) are considered a complete diet and a very important item of food. With plenty of vitamins, minerals and fiber, dates have 25% more potassium than bananas and being free from fat, sodium and cholesterol, besides having certain medicinal properties (Vayalil, 2002; Al-Farsi and Lee, 2008). Besides its high nutritional value and long life, the date palm has been mentioned as the ‘tree of life’ (Augstburger *et al.*, 2002)

Groundnut is the second most important leguminous crop in the world after soybeans as it provides foods for humans and form valuable dietary protein components in the absence of meat (Redden *et al.*, 2005 cited in Sim, 2011).

Moringa oleifera has been proven to be effective for relief of malnutrition. Due to the presence of available essential phytochemicals present in its leaves, pods and seeds, moringa is very rich in nutrients. It is said to provide seven times more vitamin C than oranges, ten times more vitamin A than carrots, seventeen times more calcium than milk, nine times more protein than

yoghurt, fifteen times more potassium than bananas and twenty-five times more iron than spinach (Rockwood *et al.*, 2013). The leaves are also rich in magnesium, zinc and copper (Kasolo *et al.*, 2010). Also, vitamins such as B complex, folic acid, pyridoxine and nicotinic acid, vitamin D and E are also present (Mbikay, 2012). Phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins and alkaloids were also present (Mbikay, 2012).

Generally soybean seeds contained 5.6-11.5% of water, ranges for crude protein is from 32 to 43.6%, for fat from 15.5 to 24.7%, for crude ash from 4.5 to 6.4%, for neutral detergent fiber (NDF) from 10 to 14.9%, acid detergent fiber (ADF) from 9 to 11.1%, carbohydrates content from 31.7 to 31.85% on a dry matter basis (Ensminger *et al.*, 1990; NRC, 1998; Poultry Feeding Standards, 2005).

Sorghum (*Sorghum bicolor* L.) Moench), is among the world most important food crops followed by wheat, rice, maize and barley (FAO, 2006), which provides staple food for a large number of people in Africa, India and in the semi-arid parts of the tropics. It is a major source of protein and calories in the diet which is consumed by poor masses of many countries. It also acts as a principal source of energy, proteins, vitamins as well as minerals for vast number of the poorest people of Africa, Asia and the semi-arid tropics worldwide (Mauder, 2006). The processed sorghum seeds or flour were found to be important source of calories and proteins to the millions of the population as well as for the poultry and livestock (FAO, 2006).

1.2 STATEMENT OF THE PROBLEM

Globally, SAM affects nearly twenty million children under five years, causing up to one million deaths each year by increasing susceptibility to death from severe infection (Black *et al.*, 2013). Despite concerted efforts to curb the menace, Nigeria has the highest number of children under

five years with chronic malnutrition (in sub-Saharan Africa) at more than 11.7 million, according to Demographic Health Survey (NPC & ICF International, 2014). A new survey carried out in 2016/2017 by National Bureau of Statistics, (NBC) in collaboration with the National Primary Healthcare Development Agency (NPHCDA) and National Agency for the Control of AIDS (NACA) as part of the global Multiple Indicator Cluster Survey (MICS) program has revealed that the prevalence of underweight increased from 24.2% to 31.5%, prevalence of stunting increased from 34.8% to 43.6% while wasting prevalence increased marginally from 10.2% to 10.8%.

1.3 JUSTIFICATION

Ready-To-Use Therapeutic Foods (RUTF) is effective in the management of Severe Acute Malnutrition among children because of its essential nutrients. UNICEF collaborates with government to procure RUTF for CMAM around the world. However, a lot of children die due to shortage of RUTF in Northern Nigeria. Therefore, researches to increase availability of RUTF and its access to children with SAM through local production can fill in the RUTF shortages and potential to reduce child mortality. If carefully selected, locally available foods can be used to produce RUTF and could be available than those procured by the UNICEF. Also, UNICEF encourages the production of therapeutic foods using locally available foodstuff to reduce the number of mortality as a result of malnutrition in the world.

1.4 AIM AND OBJECTIVES

Aim:

This research aims to evaluate the impact of locally produced therapeutic foods on nutritional status of severely acute malnourished children

Specific Objectives:

- i. To determine the proximate (Carbohydrate, Protein, Ash, Crude fat/oil, Crude fibre and Moisture), amino acid composition of the individual components and the formulated therapeutic foods.
- ii. To determine the minerals (Na, K, Ca, Mg, Fe, Cu) and vitamin (A, C and E) contents of the individual component and the formulated therapeutic foods.
- iii. To determine the phytochemicals content of the individual components and the formulated therapeutic foods.
- iv. To assess the impact of consumption of the formulated therapeutic food on the anthropometric indices of children under 5 years.

CHAPTER TWO

LITERATURE REVIEW

2.1 MALNUTRITION IN NIGERIA

Global Acute Malnutrition has been re-estimated by the nutrition cluster in 2016 that more than 7 million children under five are suffering from acute malnutrition nationally; of these 2.5 million children suffer the most severe form of malnutrition (SAM). At least 89% of children with SAM-meaning more than 2.2 million-live in the 12 northern states of Nigeria, which are accessible by aids, actors, excepts certain areas of Borno.

According to the Food and Agricultural Organization of the United Nations (FAO, 2012), more than 14% of the populations in the developing countries were undernourished in the period between 2011 and 2013. Malnutrition includes both deficiencies in nutrients and excesses. It's defined by the world food program 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 bodily performance processes such as growth, pregnancy, lactation, physical work, and resistance to and recovering from disease' (2005). It results in disability, morbidity, and mortality, especially among infants and young children (Pelletier, 1994). Malnutrition often begins at conception, and child malnutrition is linked to poverty, low levels of education, and poor access to health services, including reproductive health and family planning (IFPRI, 2014). Undernutrition is mostly associated with developing countries like Nigeria (NDHS, 2013).

The prevalence of low height for age (Stunting) in Nigeria between 1990 and 2013 has drastically reduced from 43% 37%, but the burden of acute malnutrition increased which was estimated at 9% in 1990 and at 2013 estimated at 18%. The prevalence of acute malnutrition in

the North-West region remains at high as 27%. While the level of severe acute malnutrition rises from 2% in 1990 to 9% in 2013 (NCP and ICF International, 2014). Severe acute malnutrition is a major health challenge (Rytter *et al.*, 2015). There are about 1.7 million affected by severe acute malnutrition which constitutes of about one tenth of all children suffering from severe acute malnutrition in the world (UNICEF, 2015). Also, UN office for the coordination of Humanitarian Affairs in 2014 reported that Nigeria is the second with highest prevalence of acute malnutrition in the world with an estimated of 3.78 million children suffering from wasting.

The burden of underweight, stunting and wasting is more pronounced in the Northern part of Nigeria than their counterpart (Southern part). Some states in the north like Bauchi, Jigawa, Kaduna, Katsina, Kebbi, Sokoto and Zamfara have the highest proportions of malnourished children. In all these states, the prevalence of stunting exceeds 50%. The prevalence of stunting in other states such as Gombe, Taraba, Yobe and Kano exceeds 40%. Also, the prevalence of moderate wasting among children under five exhibits similar pattern. Except for Jigawa and Zamfara in the North West, all states in the regions have higher prevalence of wasting than the national average. Other states in the northeast like Bauchi, Borno and Yobe have a disproportionately high burden of wasting, while Kano state have more than twice the national average (39.7%). Kaduna and Kano states have the highest number of severely acute malnourished children with (27.6 and 25.1% respectively) while Bayelsa is the state with the lowest number (1.3%).

2.1.1 Types of Malnutrition in Nigeria

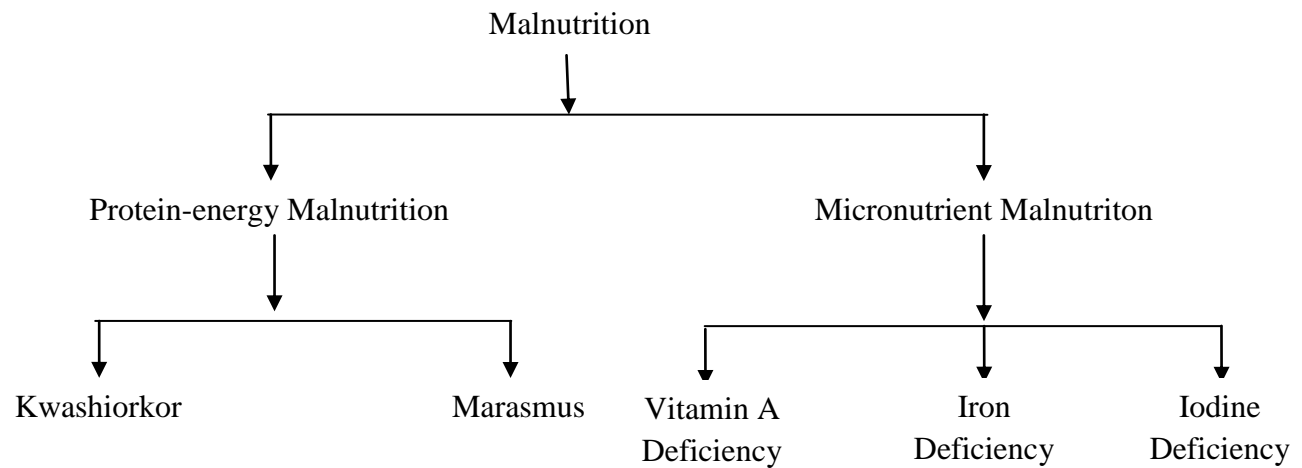


Figure 1: Types of Malnutrition

There are two main types of malnutrition in Nigeria. These are:

1. Protein- Energy Malnutrition and
2. Micronutrient Malnutrition

2.1.2 Protein-Energy Malnutrition

Protein-energy malnutrition is characterized by group of related diseases which result from inadequate and/or under-utilization of food protein, which is characterized by loss of weight, decline in linear growth, changes in the behavior (irritability, apathy, decreased social responsiveness, and anxiety and attention deficits). Apart from the deficiency of micronutrient, deficiencies in iron, iodine and vitamin A and zinc are the main manifestation of malnutrition in developing countries. The lack of an easier source of proteinous foods of high quality for the overpopulated developing countries like Nigeria is still a problem till date (Akinpelu, 2015).

2.2.1 Causes of Protein-Energy Malnutrition

The primary type of PEM is purely due to deficiency in diet. It begins at the early (fetal) stage and continues into infancy and childhood. The secondary malnutrition arises due to a serious illness such as tuberculosis, cancer, and inability of the body to absorb nutrients. Dietary factors that leads to PEM are inadequate breastfeeding by the mother due to inability of mothers' body to make milk due to inadequate nutrition, ignorance of weaning and weaning food, inverted or cracked nipples in mother causing difficulty in breastfeeding (Shettigar *et al.*, 2013; Ejaz and Latif, 2010). Studies have shown that the growth and development of children of 0-5 years is affected by protein energy malnutrition.

The causes of PEM can either be directly or indirectly. The direct factors may include inadequate food intake as a result of limited access to food in terms of quality and quantity, and diseases notably malaria and measles which lead to loss of appetite, increased rate of metabolism due to fevers thereby increasing the need of nutrient by the body. Diarrhea also reduces the absorption of food nutrients, while vomiting decreases intake of food. Intestinal parasites compete for nutrient with the body e.g. hookworm competes for iron.

The indirect causes of protein energy malnutrition include the following:

- (i) Limited access to foodstuffs and food insecurity which results in:
 - Families are not capable of acquiring or produce enough food to cater for their needs
 - Limited access or lack of land or agriculture inputs, marketing and distribution of foods
 - The vandalisation of foods by pests, fungi, rodents, birds and wild animals
 - Soil erosion, often resulting from overstocking, deforestation and discriminate burning

- Poor farming practices often due to lack of knowledge, money, time and equipments
- Poor weather conditions like failure of rains, floods etc
- Urbanization and rapid migration to the larger towns resulting in unemployment and low income

(ii) Poor water/sanitation and inadequate health services which includes:

- Health services may be of low quality expensive, non-existent or unfriendly
- Lack of pre-natal and child health care
- Inadequate management of sick children
- Inadequate water and sanitation facilities

(iii) Inadequate maternal and childcare practice which comprises of the following:

- Families do not give adequate time and resources for women and children's health, dietary and emotional needs
- Poor caring practices, including the inappropriate care of sick children
- Not utilizing health care facilities for special needs of pregnant mothers or adolescent girls
- Not supporting mothers to breastfeed adequately
- Inadequate diets for women including food taboos during and after pregnancy

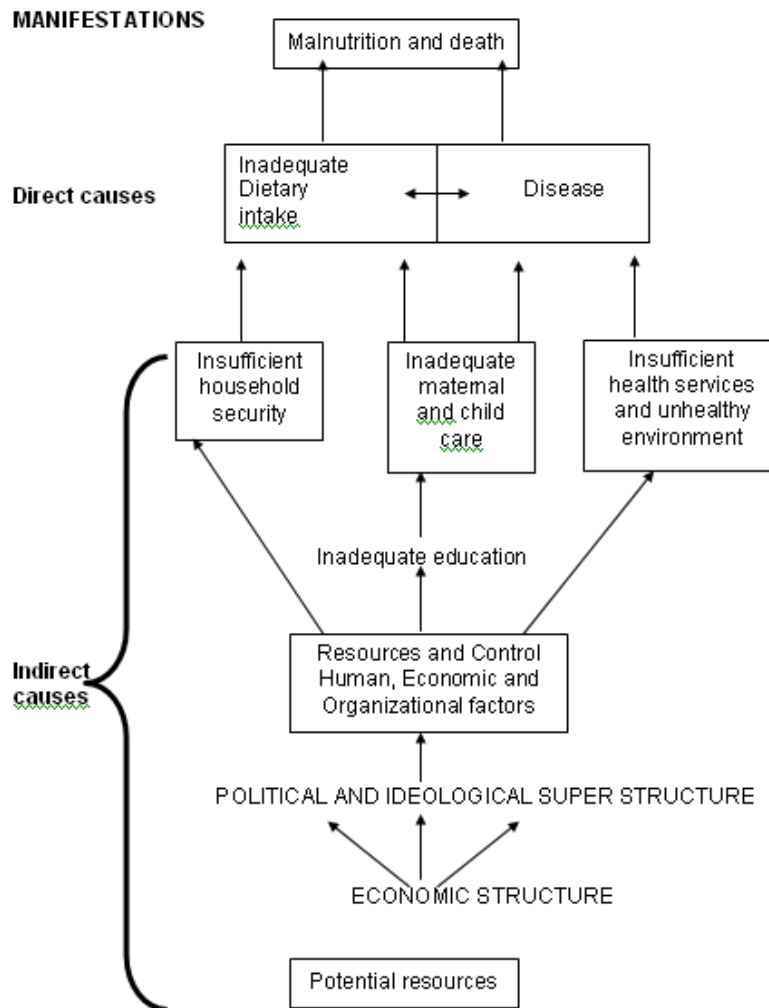


Figure 2: Causes of Manutrition

2.2.2 Types of Protein-Energy Malnutrition

PEM has been divided into different types. The two most important ones are; Kwashiorkor and Marasmus.

2.2.3 Kwashiorkor

The term kwashiorkor is derived from the Ga language of Ghana and means '*the sickness of weaning*'. It is a form of malnutrition most often found in children, which occurs as a result of not eating protein despite the intake of a reasonable amount of calories. It is mostly found in areas of

drought and famine. The term was first used in 1933 to refer to an inadequate protein intake with reasonable caloric intake (Shashidhar, 2016). Symptoms may include irritability and fatigue followed by slowed growth, weight loss and muscles wasting, generalized swelling, skin change, enlargement of liver and abdomen and weakening of the immune system, leading to frequent infection. Children are mostly affected by kwashiorkor than adults.

Signs of kwashiorkor

(i) Failure of growth: Growth failure is also a sign of kwashiorkor: Whereby the height of the child is shorter than normal. Except in cases of gross edema, the child will be lighter in weight than normal, usually between 60 and 80 % of standard.

(ii) Edema: Edema causes swelling owing to the fluid in the tissue, and is always present. It usually starts with a slight swelling of the feet and often spreads up the legs. Later, the hands, the scrotum and the face may also swell.

(iii) Irritable child: The child is usually miserable and apathetic. When being moved or disturbed, the child is irritable and prefers to remain in one position and is nearly always miserable and has no appetite and is difficult to feed.

(iv) Changes in hair: In kwashiorkor, the texture often changes, with the hair becoming silker and losing its tight curl. At the same time it lacks luster, is dull and lifeless, and may change colour to brown or reddish-brown or grey. It becomes sparse, and is easily pulled out.

(v) Skin changes: The skin, especially of the face, may be considerably lighter in color than that of either parent. In some cases a dermatosis develops; this tends to occur first in areas of friction or of pressure, for example, the groin, behind the knees and at the elbow. Darkly pigmented

patches appear which may peel off or desquamate, rather like old sunbaked, blistered paint. This observation has given rise to the term “flaky paint dermatosis” particularly around the large joints like the elbows. Underneath these flakes are atrophic depigmented areas that may resemble a healing burn. These areas may become secondarily infected. Skin cracks leading to ulcerations may also occur.

(vi) Diarrhea: Stools are frequently loose and contain undigested particles of food. Sometimes they are offensive, watery, or blood-stained.

Causes of kwashiorkor

The following are some of the factors responsible for causing kwashiorkor in children:

(i) Kwashiorkor occurs most frequently in children between 1 and 3 years of age, after they have been taken off the breast. The immediate cause for stopping breastfeeding is frequently the realization by the mother that she is pregnant again. She may often believe that her milk will now poison the child, who is therefore taken away from the breast. There are of course other reasons why mothers stop breast-feeding their babies early, including the need for some mothers to work or live away from home. The young child may then be put on a diet of gruel or porridge of whatever is the staple food of the family. This may be maize, cassava, banana, millet, rice, sorghum, or sometimes potatoes. The child is sometimes sent away to live with a relative, frequently a grandmother, in order to make the process of complete weaning easier.

(ii) Short intervals between births. If a mother becomes pregnant and when her previous baby is only 6 months old, she may neglect the first child. Her breast milk will get less and her time and attention will be concentrated more on the new baby.

(iii) Other diseases may sometimes play an important role in precipitating the onset of true kwashiorkor in an already poorly nourished child. Among the most important of these are gastrointestinal infections, which cause diarrhoea and may hinder proper absorption of nutrients. They may also result in vomiting, and thus loss of food. Intestinal worms and other parasitic infections may be important, as well as measles, whooping cough and other infectious diseases. Nearly all infectious diseases lead to an increased loss of nitrogen from the body. This can only be replaced by protein in the diet.

2.2.4 Marasmus

Marasmus is a severe form of malnutrition that consists of the chronic wasting away of fat, muscle, and other tissues in the body which occurs as a result of inadequate intake of both amounts of protein and calories resulting in a deficit in the body. Treatment is based on nutritious, well-balanced diet with lots of fresh fruits and vegetables, grains and protein (Victor, 2010; Reuters, 2009)

Causes of marasmus

The possible underlying causes of marasmus include:

- (i) Infectious and parasitic diseases of childhood: These include measles, whooping cough, diarrhea, malaria and other parasitic diseases. Chronic infection such as tuberculosis also leads to marasmus.
- (ii) Premature birth, mental deficiency and digestive upset (malabsorption, vomiting, etc.).

(iii) Early cessation of breast-feeding: This may be due to death of the mother, pregnancy, failure of lactation, separation of the mother from the infant because of family problems or because she is a working mother, etc.

(v) The family having insufficient income: The family having insufficient income to buy enough milk to feed a baby properly. The tendency therefore is to over dilute a purchased mixture. Similarly, few households have running water or the other items in their homes that facilitate the sterile preparation of milk bottles for an infant. As a result of improperly cleaned feeding bottles, the child commonly develops gastrointestinal infections, which starts the vicious circle leading to marasmus.

(vi) Prolonged breastfeeding without introduction of other foods: Another cause of marasmus found in certain parts of Africa is prolonged breast-feeding without the introduction of other foods, or enough of other foods. It is unusual for a mother to be able to produce sufficient breast milk to supply all the energy and other nutrients necessary for an infant over 6 months of age.

Signs of Marasmus

A child with marasmus presents with the following signs:

(i) The child fails to grow properly: The child has severely retarded growth. If the age is known, the weight will be found to be extremely low by normal standards (below 60 percent of the standard).

(ii) In severe cases the loss of flesh is obvious: The ribs are prominent, the belly, in contrast to the rest of the body, may be protuberant, the face looks like that of an old person, and the limbs are very emaciated. The child is “skin and bones”.

(iii) The muscles are always extremely wasted: There is little if any subcutaneous fat left. The skin is loose and seems to be too big for the body. It hangs in wrinkles, especially around the thighs and buttocks where the muscles should be thick. When taken between forefinger and thumb will reveal the absence of the usual layer of adipose tissue.

(iv) Children with marasmus are quite often not disinterested like those with kwashiorkor. Instead the deep sunken eyes have a rather wide-awake appearance. Similarly, the child may be less miserable and less irritable.

(v) The child usually has a good appetite. In fact, like any starving being, he may be ravenous. He often violently sucks his hands or clothing or anything else available. Sometimes he is heard making sucking noises.

(vi) Loose stools. Stools may be loose, but this is not a constant feature of the disease. Diarrhoea of an infective nature, as mentioned earlier, may commonly have been a precipitating factor.

(vii) Anaemia due to iron, protein and other deficiencies is usually present.

(ix) In contrast to kwashiorkor, there is no oedema and no flaky-paint dermatosis in marasmus. There may be pressure sores, but these are usually over bony prominences, not in areas of friction. Hair changes similar to those in kwashiorkor can occur. There is more frequently a change of texture than of colour. Dehydration, although not a feature of the disease itself, is a frequent accompaniment of the disease, and results from severe diarrhea and sometimes vomiting.

2.2.5 Poverty as a Cause of Malnutrition in Nigeria

Poverty has proven to be a major determinant of child undernutrition (Ubesie & Ibeziakor, 2012; Van de Poel *et al.*, 2007). Among the factors, they affects the nutritional status of children in Nigeria suggest that economic status of the parents is associated with their nutrition. 74% of the total population are very poor and cannot be able to buy the nutritious diets to cater for their needs (NPC and ICF International, 2014). They survive on foods with low calories and therefore, the acute and chronic malnutrition are common among children.

In Nigeria, almost three times children from poor background are more likely to be stunted and 4.3 times likely to be severely stunted compared to the children from the wealthiest family. The prevalence of malnutrition among the poorest groups reveals that the prevalence of stunting for the poorest children increased from 48.8% in 2003 to 53.8% in 2013. The poorest children are also 3.7 times more likely to be wasted and 3.2 times more likely to be severely wasted compared to children living in richest family (NPC and ICF International, 2014). Also, the prevalence of malnutrition in Nigeria among children of the rural areas is considerably higher than children of the urban areas. It has been estimated that the rate of stunting in rural areas at 43.2%, compared to 26% for children surviving in urban areas.

2.2.6 Prevention of Protein-Energy Malnutrition

2.2.7 Household Food Security

By improving the method of preservation and storage of different food items during the period of harvesting in the country, the level of PEM can be reduced. The Government of Nigeria must ensure food security household all over the year. This can be achieved in partnership with private sectors and non-governmental organizations through a number of collaborative strategies. The introduction of small-scale start up businesses such as farming, fishery, poultry, and tailoring etc

can provides jobs to our teaming unemployed youth in the country. The Government of Nigeria must provide an alternative way apart from the oil by investing large amount of resources on agriculture for feeding as the country is fast growing population. Industries responsible for processing and preservation of food must be established which also, in turn, create job opportunities to our unemployed graduates. The irrigation processes should also be improved by providing efficient varieties such as genetically modified seeds and simple technological tools in order to get all year round farming to reduced PEM and hunger in the country. Moreover, high priority should be given to low-income households by creating more poverty alleviation programs and loans. Nigeria is the third country with highest number of poor in the world after China and India while being the six exporters of crude oil in the world. Few elites have accumulated the wealth leaving the poor with starving and death daily. Nigeria is among the 20 countries in the world with the widest gap between the rich and the poor (Igbuzor, 2015)

2.2.8 Safety Nets

The less privilege must be provided with safety nets as the PEM is mostly found in children with low socioeconomic background which lives in low purchasing power. Even in the developed nations of the world, safety nets are still being created in different ways for their vulnerable populations. Beyond therapeutic foods, supplementary feeding, which is the provision of food rations (either local staples or specialized foods) to vulnerable or malnourished persons to supplement the local diet and provide balanced and/or adequate daily energy intake, should be encouraged (Koethe *et al.*, 2009). A good example of such specialized food is the high-energy Ready-to-Use Therapeutic Foods (RUFT) which can be consumed at home.

2.2.9 Community-Level Strategies

An organization in the community can play a pivotal role to household food security. As the government is providing high-energy dense therapeutic foods to the community for the management of PEM, the community should not wait for such intervention rather than to proceeds with local preparation usually done in hospital settings such as high-energy mixture, kwash pap. The mothers should thought on how to prepare such mixtures for their children (Eckhoff *et al.*, 2011). Sadly, according to a UNICEF report, between 2005 and 2009, only 13% of babies less than 6 months were exclusively breastfed and 32% were still breastfeeding at age 20–23 months in Nigeria (UNICEF, 2010). A recent study put the average exclusive breastfeeding rate in Nigeria for infants during their fifth month of age at 7.1% (Agho *et al.*, 2011). The study also noted that the odds of EBF were higher in rich households than in poor households (Agho *et al.*, 2011). This is very disturbing considering that PEM is commoner among the low socioeconomic households. There is an urgent need to mobilize the available resources to step up our exclusive breastfeeding rate and encourage breastfeeding into the second year of child's life with timely and appropriate complementary feeding.

2.3 MICRONUTRIENT MALNUTRITION

Deficiency of micronutrient or ‘hidden hunger’ occurs as a result of limited amount of minerals and/or vitamins in the diet; it is a very serious public health concern in most developing countries that has negative consequences and usually affects a vast group of people including children under the age of five, pregnant and lactating women (WFP, 2007). According to WHO, one-third of people in developing countries are affected by micronutrients deficiencies; in Nigeria, these deficiencies are primarily in iodine, iron and vitamin A. These deficiencies if left untreated, may lead to irreversible physical damage, that is why they are considered a major health concern

that deserve attention internationally. Micronutrient malnutrition is responsible mortality in infant (Bryce *et al.*, 2003)

2.3.1 Deficiency of Iodine

Iodine has a significant role in the growth and development of the human body. It is responsible for the production of thyroid hormones which are essential for the normal development of the brain (WHO/UNICEF/ICCIDD, 2007). Iodine Deficiency Disorder (IDD) is health problems that occurs as a result of low intake of iodine in the diet. The health consequences of IDD includes mental retardation,, goiters, retarding growth and increased neonatal and post-natal mortality. Deficiencies of iodine at the first instances leads to maternal hypothyroidism, which has negative consequences for the fetus, including severe and irreversible brain damage. It is estimated that 2 Billion people, or 30.6% of the global population, have inadequate iodine intake, including 59.7 million school-aged children in Africa (UNICEF 2007; De-Bonoist *et al.*, 2007)

From 2001-2003, NFCNS data revealed that a total of 25.5% of children suffered various degrees of iodine deficiency, while 46.5% had more than adequate levels (Maziya-Dixon, 2004). The deficiency was severe in 4.2%, moderate in 8.7% and mild in 14.6% of children. More than 20% of the total population suffered from goiter, the abnormal enlargement of the thyroid gland, which is the most severe form of iodine deficiency. Endemic iodine deficiency reduces the IQ by 3.5%, permanently affecting intellectual development.

2.3.2 Deficiency of Iron

Iron is essential for physical activity in all humans and for cognitive and motor development in childhood. Nutritional deficiency of iron is a major health obstacle in many developing countries, often coexisting with iodine deficiency in the same population. Iron Deficiency

Anemia (IDA) is the most common and widespread nutritional disorder in the world today, which affects population in both developed and developing countries. When affected, this disease reduces the work capacity of an individual as well as the entire population at large, resulting in to serious economic consequences that inhibit natural development.

Low intake of iron can lead to increased maternal death, reduced motor skills development and learning capacity, lethargy, and reduced immunity to diseases. The iron profile from the Maziya-Dixon *et al.* (2004) survey showed that almost 25% of children were iron deficient and another 80% had depleted iron stores. With more than 25% of children under five iron deficient, it is critical that the global community pay attention to adequate dietary intake of iron. The level of deficiency of iron may be due to poor dietary sources of iron, or sources in which the iron is not in absorption form. Vegetables are the major sources of iron subject to chelation by oxalates, phytates, and other anti-nutrients, making it unavailable for absorption by the body. Other nutrients enhances iron absorption particularly vitamin C. Animal source of iron are most desirable (UNICEF, 2007).

2.3.3 Deficiency of vitamin A

Deficiency of vitamin A constitutes of about 25% infant, child and maternal death in Nigeria because of reduced resistance to protein-energy malnutrition, acute respiratory infection, measles, malaria and diarrhea (WHO, 2009; UNICEF, 2013). Vitamin A, a fat soluble vitamin, essential for vision in dim light; cellular bone and tooth growth, formation and maintenance of healthy skin, hair and mucus membranes; reproduction; and immunity boosting. Vitamin A is also important in embryological development that without it, the fertilized egg cannot develop in to a fetus (Brody, 2007). The deficiency of vitamin A leads to night blindness or impaired adaptation to the dark; lowered immunity to infections such as measles, diarrhea, chicken pox,

and respiratory infections; anemia; poor growth; slowed bone development; blindness and death. All these have dangerous effects on the healthy growth and intellectual performance of a child.

At the national level, indicators of micronutrient deficiencies reveal that 4.7% of children under five had serum retinol concentration ($< 10 \mu\text{g/dl}$) and were vitamin A deficient; and 71.5% of children were normal. If those who were marginally deficient are combined with those who were clinically deficient, 29.5% of children under five were suffering from vitamin- A deficiency (Maziya-Dixon *et al.*, 2006; MI, 2014).

2.3.4 Prevention and Control of Micronutrient Malnutrition

Several policies and strategies have been put in place in many developing countries to reduce the burden of macronutrient malnutrition. This includes food fortification, supplementation and dietary diversification. In all these three strategies, food fortification is mostly used because of its accessibility, cost-effective and direct intervention to increase the intake of micronutrient without changes in cultural and dietary patterns across various groups and levels of income.

2.3.5 Prevention of vitamin A deficiency In Nigeria

The strategies to reduce the incidence of micronutrient deficiency disorder in Nigeria have been put in place since 1990. The government in the year 2002 approved a new strategy: vitamin A fortified in staple foods so that the children will consumes it naturally in their food. A mandatory standard for vitamin A fortification in vegetable oil, sugar and flour was published by the Standard Organization of Nigeria in 2002. By 2004, 55% of vegetable oil, 70% of sugar and 100% of wheat flour in the market were fortified with vitamin A. Wheat flour is also being fortified with iron to improve general physical and mental health.

In recognition of the effectiveness of food fortification to control vitamin A deficiency, a national vitamin A Food Fortification Consultative group was established in 1996 which charged with the responsibility of creating the minimum level of vitamin A to be fortified in staple foods and they conclude at a certain amount of the fortification as: flour, sugar and edible vegetable oil at 30,000, 25,000 and 20,000 I μ of vitamin A/kg respectively, which was launched in the flour and vegetable oil industries in September 2002 (Akunyili, 2005)

Supplementation of vitamin A in Nigeria was achieved through the primary health care system, which is the primary level of health care delivery in the country. The National Immunization Days (NID) has also been used for effective coverage since NID targets the population of children under five.

2.3.6 Biofortification

Biofortification is one of the potential solutions of deficiency of micronutrient. It involves the breeding and delivering staple food crops with higher content of micronutrient (Qaim *et al.*, 2007; Bouis *et al.*, 2011; Saltzman *et al.*, 2013). In order to remove deficiency of micronutrient in rural areas of developing countries, the process of biofortification could be sustainable strategy and cost-effective. In those countries, the diet of the poor's households of staple foods and where access to food supplement and commercially marketed fortified foods is limited. Since 2003, breeders across the Consultative Group on plus program to develop varieties of seven staple crops (Cassava, maize, sweet potato, beans, pearl millet, rice and wheat) that contain significant amount of bioavailable, critical micronutrients. The targeted micronutrient are iron, zinc and vitamin A, which are-part from iodine, which can be fairly addressed by the iodization of table salt-recognized by the international nutrition community as most limiting in diets (Black *et al.*, 2013).

Since 1990s, the HarvestPlus has excited several projects when these are in need in increasing the level of micronutrients in major African staple foods. Cassava and maize fortified with vitamin A, has been developed and introduced by the HarvestPlus through the National Root Crops Research Institute (NRCRI) and the International Institute for Tropical Agriculture (IITA). An additional three new varieties of cassava were introduced recently by NRCRI and IITA which are richer in vitamin A than those biofortified earlier. These cassava varieties are 25% richer in beta-carotene than the first set of vitamin A cassava varieties released in 2011, which are being grown by more than 250,000 Nigerian farmers. In addition to their higher beta-carotene content, the vitamin A cassava varieties boast of improved pest-and disease- resistant traits and are high yielding.

2.3.7 School Feeding

School feeding simply means the provision of food to school children (state of school feeding worldwide, 2013). It is a program organized to reduce micronutrient malnutrition while supporting education, health and agriculture community development (WFP, 2007). Many countries have engaged in these programs based on their own objectives. The programs are classified based on their modalities, including in-school feeding; where foods are given to children attend school (WFP, 2013). Like the other developing countries, Nigeria has engaged in the program that provides children with breakfast, lunch, or a combination of the two.

2.4 READY-TO-USE THERAPEUTIC FOODS (RUTF)

RUTFs are energy-dense, micronutrients rich, homogenous lipid pastes. Depending on the capacity of the industries, and available raw materials, the content of RUTF differs across geographical regions. They are prepared according to the F-100 formula (the standard therapeutic foods deployed for the treatment of severe acute malnutrition in children) with

addition of peanut butter. The standard RUTFs consists of peanut butter, milk powder, oil, sugar, vitamins and minerals (Ndekha *et al.*, 2005). The mineral supplemented in the diet is iron, zinc, magnesium, copper, potassium, selenium and iodine. While the micronutrients include vitamin B such as folate and niacin (Manary, 2006). The content of calories in most of the RUTF is between 520 and 550Kcal/100g. Out of that, 45% to 60% is provided in the form of lipids; another 10% to 125 is in the form of proteins (USDA, 2012). The diet is administered to the malnourished children based on the body weight and caloric needs. Due to low water content in the RUTF, most do not provide a favorable environment for the growth of bacteria and have shelf life of about two years. Also, they do not require cooking and have taste that is accepted by children which facilitate their use in remote and rural regions.

There are different types of RUTF which are different commercial products. The plumpy nuts® is most widely used which was formulated since 1990s, manufactured by Nutriset, a French firm. It is produced in 92gram foil sachets, each providing 500 kilocalories. UNICEF has stated that this nutritional paste (peanuts, powdered milk, vegetable oil, sugar, vitamin and mineral mixture) contain the right mixture of nutrients to treat children with severe acute malnutrition, and in a form that is easy to consume and safe. It is used in health facilities and in the community. Nutriset also included 'ready-to-use supplementary food (RUSF) that is used to tackle malnutrition at the early stage (moderate acute malnutrition, or in prevention of acute or chronic malnutrition). In addition to breastfeeding, these ready-to-used supplementary foods are used for young children above 6 month of age, and traditional complementary foods.'

Table 2.1: Nutritional composition of commonly used, Specially Formulated Foods for the Prevention and Treatment of Acute Malnutrition

	F75 (100g milk powder)	F100 (100g milk powder)	Plump'Sup (100g)	Plumpy;Doz (100g)	Plumpy'Nut (100g)	Supercereal Plus (100g dry matter)
Used for	SAM	SAM	MAM	MAM	MAM or SAM	Prevention of MAM
Recommended serving size (kcal/kg/d)	80-100	200	75	46.3g/day	SAM:200 MAM:75	200g/day
<i>Macronutrients</i>						
Energy (kcal)	446	520	520-550	534-587	520-550	410
Protein (g)	5.9	>13	12.6-15.4	13.4-17.7	13-16	>16.4
Lipid (g)	15.6	>26	31.5-38.6	26.7-39.1	26-36	>4.1
<i>Minerals</i>						
Potassium (mg)	775	1,100	980-1,210	660-870	1,100-1,400	140
Calcium (mg)	560	300	300-350	800-980	300-500	452
Phosphorus (mg)	330	300	300-350	530-660	300-600	232
Magnesium(mg)	50	80	80-100	115-140	80-100	-
Zinc (mg)	12.2	11	12-15	8.7	11-14	5

Note: --- = not available; d = day; g = gram; kcal = kilocalorie; kg = kilogram; MAM = moderate acute malnutrition; mg = milligram; SAM = severe acute malnutrition.

Nutriset catalogs; Supercereal Plus from USAID specifications

2.5 DATE PALM (*PHOENIX DACTYLEFERA* L.)

Phoenix dactylifera (dates in English, Dabino in Hausa and Tamr in Arabic) is a palm plant which belongs to the family Aracaceae. It is one of the oldest cultivated plants in the world (Al-

Shahib *et al.*, 2003a; 2003b). It is mostly found in Northern Africa, Arabia, Canary Island and southwest of Asia to Pakistan and India, also cultivated in most other parts of the world. Most of the world especially Arabs are consuming date fruits as one of the important components of their staple food and diet. (Al-Farsi and Lee, 2008) because of its high level of nutrients, good yield, and long life, the date palm has been named as the ‘tree of life’ (Augstburger *et al.*, 2002)

Date has become a major source of energy and nutrition among the Muslims and non-Muslims in northern part of Nigeria. For Muslims, it’s being used as a meal to break fasting during the holy month of Ramadan (Ahmad *et al.*, 2013). Despite the important role played by the date to human life, the Nigerian date palm industry is beleaguered with lack of awareness of the nutritional importance of the date (AbdulQadir *et al.*, 2011)

2.5.1 Nutritional Composition of Date palm

The health benefits and the level of nutrition of the date are well known all over the world because of its high nutrient profile and health-promoting properties. *Phoenix dactylifera* is a multipurpose tree packed with an impressive list of essential nutrients, vitamins and mineral required for normal growth, development and well-being of individuals. It predominantly contain large amount of carbohydrates like glucose and fructose (Ahmed *et al.*, 1995; Al-Hooti *et al.*, 1997; Myhera *et al.*, 1999) and sucrose (Guizani *et al.*, 2010). The protein in dates contains 23 types of amino acids, some of which are not present in most popular fruits such as oranges, apples, and bananas (Al-Shahib *et al.*, 2003a; 2003b). Dates is also a good source of dietary fiber and many important minerals like iron, selenium, calcium, potassium and also important vitamins like vitamin C, B1, B2, a riboflavin and niacin but very low in protein and fat contents (Myhera *et al.*, 1999; Sawaya *et al.*, 1983). The date fruit has also been recommended in folk

remedies for the treatments of various infections, diseases, cancer and heart diseases (Lambiote, 1982; Duke, 1992)

2.6 MORINGA OLEIFERA

Moringa oleifera is plants which belong to the family of moringaceae and is an effective remedy for malnutrition. It is usually called a miracle plants due to its numerous important in a field of nutrition and medicine. Among the 13 different species of Moringaceae family, *M. oleifera* is most widely cultivated because of its adverse uses and vital nutrients. Almost all the parts of the tree are very useful. For example, the leaves are used for forage, tree trunk for making gums, flower nectar in honey and powdered seed for water purification (Fuglie, 1999). It is also been used for the treatment of malnutrition in children younger than three years of age. In northern part of the Nigeria, it is mostly grown locally and cultivated and locally called ‘Zogale’ by the Hausa speaking people. It can be grown in different soil conditions such as well-drained sandy or slightly alkaline loamy soil (Abdul, 2007; Anjorin, 2010).

2.6.1 Nutritional Composition of *Moringa oleifera*

Moringa oleifera is an important tree in which the leaves have been reported to contain substantial amounts of nutrients such as vitamins (Hekmat *et al.*, 2015), proteins, fibre and minerals (Hekmat *et al.*, 2015) and also a good sources of phytonutrients like carotenoids, tocopherols ascorbate (Saini *et al.*, 2014b; 2014d) that play an important role in human nutrition. It consists of minerals such as calcium, potassium, zinc, magnesium, iron and copper (Kasolo *et al.*, 2010). Vitamins such as beta-carotene of vitamin A, vitamin B like folate, nicotinic acid and pyridoxine, vitamin C, D and E are also present (Mbikay, 2012). Phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars present along with anti cancerous agents like glucosinolates, isothiocyanates, glycosides compounds

(Berkovich *et al.*, 2013). Research shows that immature pods contain around 46.78% fiber and around 20.60% protein content. Pods have 30% of amino acid content; leaves have 44% and flower have 31% (Sanchez-Machado *et al.*, 2011). Based on the source and cultivars, the level of nutrient in *M. oleifera* may vary. Jongrungruangchok *et al.* (2014) observed a variation in the protein (approx. 19-29%) and fiber (16-24%) content of *M. oleifera* leaves grown in different provinces in Thailand. Also the protein, mineral and vitamin content of the leaves vary. (Teixeira *et al.*, 2014)

2.7 THE SOYBEAN (*GLYCINE MAX*)

The soybean (*Glycine max* L.) Merrill family Leguminosae) undoubtedly originated in the orient, probably in china is among the most important human diets in many eastern countries because of its high nutritional value and very low in cost. The relationship between human health and soybean consumptions has been extensively known because of its high nutritional value including its high protein content, dietary fibre, oligosaccharides, phytochemicals (especially isoflavones), significant amount of minerals, very little amount of saturated fat, and absence of cholesterol (Silva *et al.*, 2006). Sulfur-containing amino acids in soybean protein is low have significant amount of lysine which is absent in most of the cereals (Mateos-Aparicio *et al.*, 2008)

2.7.1 Nutritional Composition of Soybean

Soybean is one of the most highly nutritious leguminous seed and substitutes to meat and milk in food value. The soybean seeds contain high amount of protein and the amino acid composition is approximate to composition of animal proteins, and often used a replacement component of meat protein. It contains a reasonable amount of non-saturated fatty acids but lack vitamin A and C, of most minerals, and of starch. Except for methionine, the soybean contains all the essential amino acids (Synder and Kwon, 1987)

2.7.2 The Protein Content of Soybeans

Protein in soybean is valuable because of its amino acids composition that complements that of cereals. Sulfur-containing amino acids are very limited in most animal species, including humans, but has adequate amount of lysine to overcome the lysine deficiency of cereals (Potter and Hotchkiss, 1995). The amount of protein in soybeans, 38%-44%, is larger than that of cereals 8-15% (Synder and Kwon, 1987). This large amount of protein in soybeans along with the high biological value increases their value as feedstuff and is one reason for the economic advantage that soybeans have over other oil seeds.

2.7.3 The Soybean Lipids

Oil in soybeans provides the essential fatty acids, calories and vitamin A and E, and also contributes insignificant amounts of vitamins D and K (Bates and Matthews, 1975). Among other oilseeds, soybean has the highest iodine value (a value of 134), which is similar to that of sunflower; peanut, maize and palm oils which have values of 101, 127 and 51 respectively indicating lower unsaturation compared to soybean oil (Weiss, 1983). In soybean oil, the fatty acids, linoleic, oleic, palmitic and linolenic, make up 54, 24, 12 and 8 respectively (Bates and Matthews, 1975). Soy oil can serve as a good source of oleic and essential fatty acid (EFA) linoleic acid, with even with the partially hydrogenated soy oil containing 25% linoleic and 3% linolenic acid (Potter and Hotchkiss, 1995). Soy oil is a good source of vitamin E, but is a poor source of beta carotene, a precursor of vitamin A (Synder and Kwon, 1987)

2.7.4 Soybeans Minerals

The ash content in dry soybean is almost 5%. Sulfates, phosphates, and carbonates are the major minerals in soybean. Soybeans contain high amount of potassium, followed by phosphorus, magnesium, sulfur, calcium, chloride, and sodium (O'Dell, 1979). Other minerals present in

minute amounts are silicon, iron, zinc, manganese, molybdenum, fluoride, chromium, selenium, cobalt, cadmium, lead, arsenic, mercury and iodine (O'Dell, 1979).

2.7.5 Soybean Vitamins

The soybean contains water soluble vitamins such as riboflavin, thiamin, pantothenic acid, niacin and folic acid. While the vitamin C content in soybean is present but very negligible in mature beans and present in measurable amount in the immature and the germinating beans (Bates and Matthews, 1975). It also contains fat soluble vitamins like vitamin A (Retinol) that exists as the provitamin a-carotene and vitamin E (tocopherol). Like the vitamin C, the vitamin D and K are also present in a minute amount (Bates and Matthews, 1975)

2.8 SORGHUM BICOLOR

Sorghum (*Sorghum bicolor* L.) Moench), among the world most important food crops followed by wheat, rice, maize and barley (FAO, 2006), provides staple food of a large number of people in Africa, India and in the semi-arid parts of the tropics. It is a major source of protein and calories in the diet which is consumed by poor masses of many countries. It also acts as a principal source of energy, proteins, vitamins as well as minerals for vast number of the poorest people of Africa, Asia and the semi-arid tropics worldwide (Mauder, 2006). The processed sorghum seeds or flour were found to be important source of calories and proteins to the millions of the population as well as for the poultry and livestock (FAO, 2006). The cereal used as whole grain as well as ground flour because it is free of gluten as it is an important source of protein, vitamins, minerals, energy and nutraceuticals such as antioxidant phenolics and cholesterol-lowering waxes (Taylor *et al.*, 2006).

2.8.1 Chemical Composition of Sorghum

Sorghum has similar chemical composition with corn (*Zea mays*)

2.8.2 Carbohydrates in Sorghum

Starch is the predominant carbohydrates in sorghum, while soluble sugars, pentosans, cellulose and hemicelluloses are present in minute amount. The regular type of endosperm sorghum contains 23-30% amylase while the waxy type consists of less than 5% amylase. Also, sorghum consists largely of insoluble fiber (86.2%) which may decrease transit time and prevent gastrointestinal problems.

2.8.3 Proteins in Sorghum

Due to certain factors such as genotype, temperature, availability of water, soil fertility and environmental conditions during grain development, the protein content and composition of sorghum varies, which is usually 11-13% but sometimes higher values are reported (David, 1995). Sorghum contains a higher amount of prolamins as proteins, followed by glutelins. There is variability in the composition of essential amino acids in sorghum protein. Lysine content was reported to vary from 71-212mg per gram of nitrogen while the tryptophan and methionine content on average are 63 and 87 mg per gram of nitrogen. These deficiencies arise from the amino acid composition of the grain storage proteins, called kafirins, which account for up to 80% of the total grain proteins (Taylor and Schosster, 1989).

2.8.4 Lipids in Sorghum

Sorghum consists of average of 3% crude fat, which is higher than that of wheat and rice. The composition of fatty acids is similar to that of corn oil; linoleic has the higher concentration (49%), followed by oleic (31%) and palmitic acids (14%). Like maize, the energy content of

sorghum is high. It also consists of about 1.5ppm total carotenoids. Apart from maize and durum wheat, sorghum is the only cereal which contains a significant amount of β -carotene, the provitamin of vitamin A, which is very important in human physiology.

2.9 GROUNDNUT (*ARACHIS HYPOGAEA*)

Groundnut (*Arachis hypogaea* L.) is a plant of the Fabaceae family, which is the second most important leguminous crop in the world after soybean that contain essential foods for human and livestock consumption and has a component of dietary protein in the absence of meat (Redden *et al.*, 2005). The nut has high nutritive value which is affordable and can be used in variety of ways such as con factory products and in supplementary feeding programs such as in weaning food formulation in combination with other cereals and pulses in many developing countries. The vegetable oil has contributed immensely in many countries in their diet, which serve as an important source of proteins, lipids and fatty acids for human nutrition including the repair of worn-out tissue, formation of new cells as well as good source of energy (Gaydou *et al.*, 1983; Grosso and Guzman, 1995; Grosso *et al.*, 1997,1999). Preparation of food that involves groundnut to improves the level of protein has help in several ways to reduced malnutrition in many developing countries (Asibuo *et al.*, 2008)

2.9.1 Chemical Composition of *Arachis hypogaea*

Nutritionally, the seeds of groundnut provide an extensive source of high quality of dietary protein, oil, niacin, fiber and rich in sources of minerals such as phosphorus, calcium, magnesium, potassium and manganese, and vitamins (E, K and B complexes) (Savage and Keenan, 1994). The seeds of groundnut are reported to contain 44-56% oil and 22-30% protein on a dry seed basis and also contain 9.5-19.0% total carbohydrates as both soluble and insoluble carbohydrates (Crocker and Barton, 1957; Rao *et al.*, 1965). They are also naturally free from

trans fatty acids and sodium (Savage and Keenan 1994). Generally, the chemical composition of groundnut seeds has been evaluated in relation to protein, amino acid composition and fatty acids composition (Young and Hammons 1973, Young *et al.*, 1973, Grosso and Guzman 1993). Both the raw and roasted form of groundnut has digestibility of consumption, and the amount of energy is higher in the roasted form than the raw form (Nagaraj, 1988).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

All laboratory glassware and equipments used in this study are listed in appendix I.

3.1.2 Reagents

The list of all the reagents used in the study is listed in the appendix II.

3.2 Methods

3.2.1 Ethical Approval

Ethical approval was obtained from Ethics Sub-Committee of Health Operational Research unit of the Kano State Ministry of Health under Hospital Management Board Kano State to conduct the research at Hasiya Bayero Paediatric Hospital Kano with the approval number (MOH/Off/797/T.I/360).

3.2.2 Volunteers Recruitment

Twenty children (between 0-59 months of age) whose parents/caregivers assented to their participation were recruited in the study. They were given detailed explanation about the terms and conditions and the aim of carrying out the study. Most of them agreed as the research will immensely contribute to tackling the menace of severe acute malnutrition in children less than five years. Apart from that, a well structured questionnaire was filled in by each of the parents/Guardians to know their age, status, educational back ground, level of income, occupation and also the child feeding practices (different types of foods consumed by the children during breakfast, dinner and lunch).

3.2.3 Food Samples Collection and Identification

Groundnut, Soybeans, Sorghum, Date palm and Moringa leaves were purchased from Rimi main market and carefully selected and labeled properly in polythene bags. The samples were taken to the Botany unit of Bayero University Kano and identified as follows:

1. Species :*Phoenix dactylifera*

Kingdom: Plantae

Division: Magnoliophyta

Order: Arecales

Family: Arecaceae

Genus: *Phoenix*

Common name: Date palm

Local name: Dabino

BUK Herbarium Accession Number: BUKHAN 0207

2. Species: *Moringa oleifera* Lam.

Kingdom: Plantae

Division: Magnoliophyta

Order: Brassicales

Family: Moringaceae

Genus: *Moringa*

Common name: Drumstick tree

Local name: Zogale

BUK Herbarium Accession Number: BUKHAN 0011

3. Species: *Glycine max*

Kingdom: Plantae

Division: Magnoliophyta

Order: Fabales

Family: Fabaceae

Genus: *Glycine*

Common name: Soybean

Local name: Waken suya

BUK Herbarium Accession Number: BUKHAN 0138

4. Species: *Sorghum bicolor* (L.) Moench

Kingdom: Plantae

Division: Magnoliophyta

Order: Poales

Family: Poaceae

Genus: *Sorghum*

Common name: Sorghum

Local name: Jar Dawa

BUK Herbarium Accession Number: BUKHAN 0475

5. Species: *Arachis hypogaea*

Kingdom: Plantae

Division: Magnoliophyta

Order: Fabales

Family: Fabaceae

Genus: *Arachis*

Common name: Peanut

Local name: Gyada

BUK Herbarium Accession Number: BUKHAN 0405

3.2.4 Sample Preparation and Preservation

The samples were thoroughly washed with distilled water and air-dried at room temperature. Seeds of the date palm were removed; the moringa leaves were shade dried. The dried samples were then ground mechanically using mortar and pestle and stored in air-tight containers in the laboratory prior to the commencement of the analysis.

3.2.5 Preparations of the Locally Prepared Therapeutic Food

The preparation of therapeutic foods includes washing, drying, grinding and packaging. The groundnut and soybean were slightly heated at a temperature of about 60⁰C for 20-30 minutes to remove the husk. This was followed by grinding them into smaller particle sizes in a grinding machine.

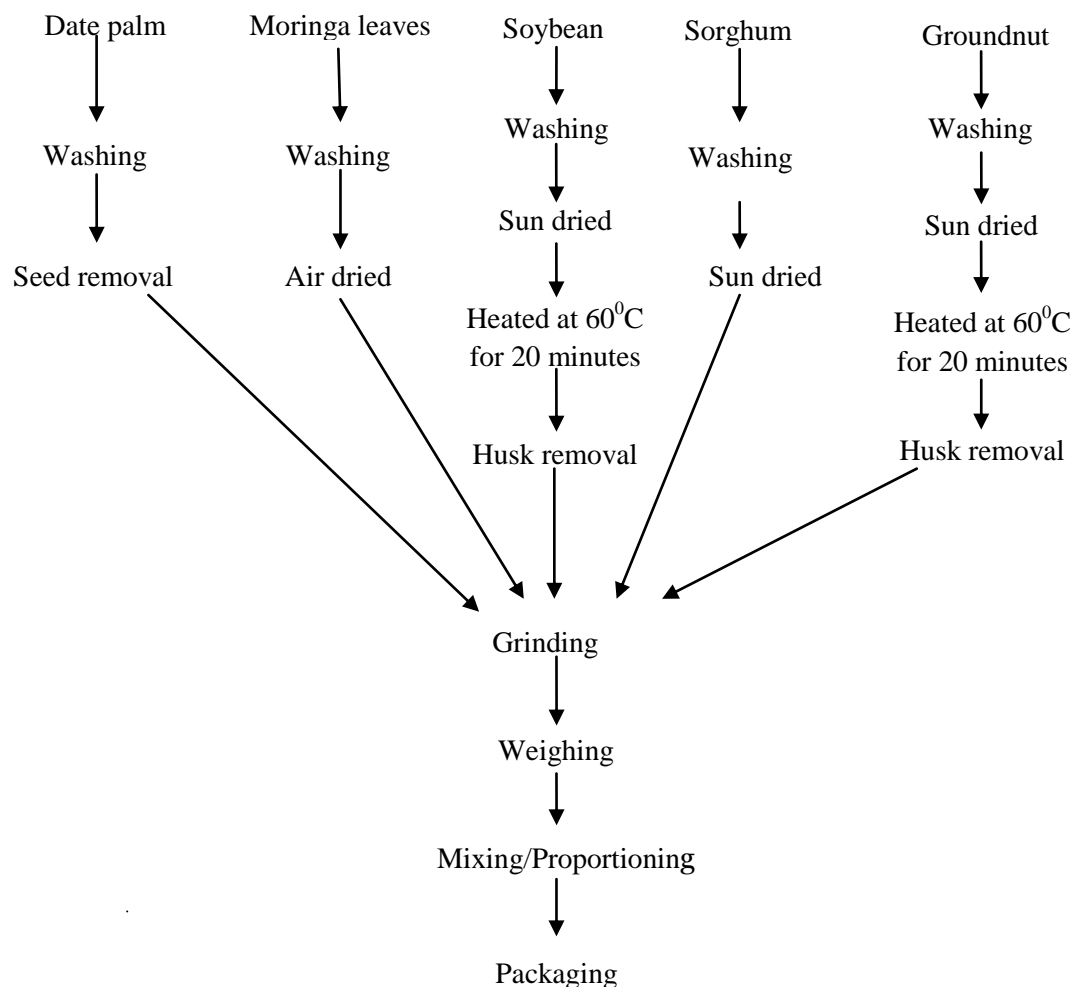


Figure 3: Flowcharts for the Preparations of Formulated Diets

3.2.6 Diet Formulation

The food samples (Date palm, Moringa leaves, Soybeans, Sorghum and Groundnut) were mixed in three different ratios and designated as A, B and C. The formulation of the samples is as shown in Table 3.1 below.

Table 3.1: Percentage Compositions of the Formulated Diets

SAMPLES	Date palm	Moringa	Soybean	Sorghum	Groundnut	Total	Energy
	(%)	(%)	(%)	(%)	(%)	(100%)	Kcal/100g
A	12.50	2.50	35.00	30.00	20.00	100	475.28
B	12.50	2.50	30.00	25.00	30.00	100	469.43
C	13.00	1.00	30.00	17.50	38.50	100	489.16

3.2.7 Study Area

The study area of the research was Hasiya Bayero Pediatric Hospital Kano (a specialized service hospital) in Kano metropolitan, located besides Emir's palace Kano State, North Westren Nigeria, situated in the Sahelian geographic region, south of the Sahara of about 499 square kilometers (193 square miles) with a population of 9,401,288 as of 2006 Nigerian census, with coordinates of 12⁰00'N8⁰31'E (Illife, 2007). Kano is Hausa-Fulani dominated city and is the nerve center of the Northern Nigeria and second largest city in Nigeria (Barau, 2007).

3.2.8 Appetite Test

One of the criteria for hospitalization and inpatient treatment was poor appetite test. A measured quantity of about 5/kg of the prepared therapeutic food was used to measure the appetite. The idea of doing the appetite test is that any child who passes appetite test means that he is able to take one fourth of his maintenance calories at a time, and thus if four or five equal amounts of feeds are given at home, child will not further lose weight.

3.2.9 Diet Selection and Administration

Formulated diet C was selected for the research based on the analysis of three different diets formulated. Diet C was found to contain highest amount proximate composition (protein lipid and fiber) as well as highest energy content, minerals compositions (Na, Mg, K, Fe and Ca), vitamins contents (vitamin C and E) as well as amino acid compositions equivalent or higher than the reference RUTF. Also, based on the appetite test carried out, diet C was consumed at a faster rate than the other diets.

The therapeutic food was prepared in lots daily (150g/day; 420-480kcal), a quantity of which was determined by number of children with SAM admitted at particular time. It was prepared in the hospital kitchen based on the number of children admitted at a particular time by the nutritionist under all hygienic and aseptic precautions at 8:00 am, 2:00 pm and 8:00 pm. Malnourished children were fed with the therapeutic food for 14 days, while those discharged before the stated days were given the food and taught how to prepare it at home in the same quantity as in the hospital and report after 14 days where their various weight, MUAC gain were measured.

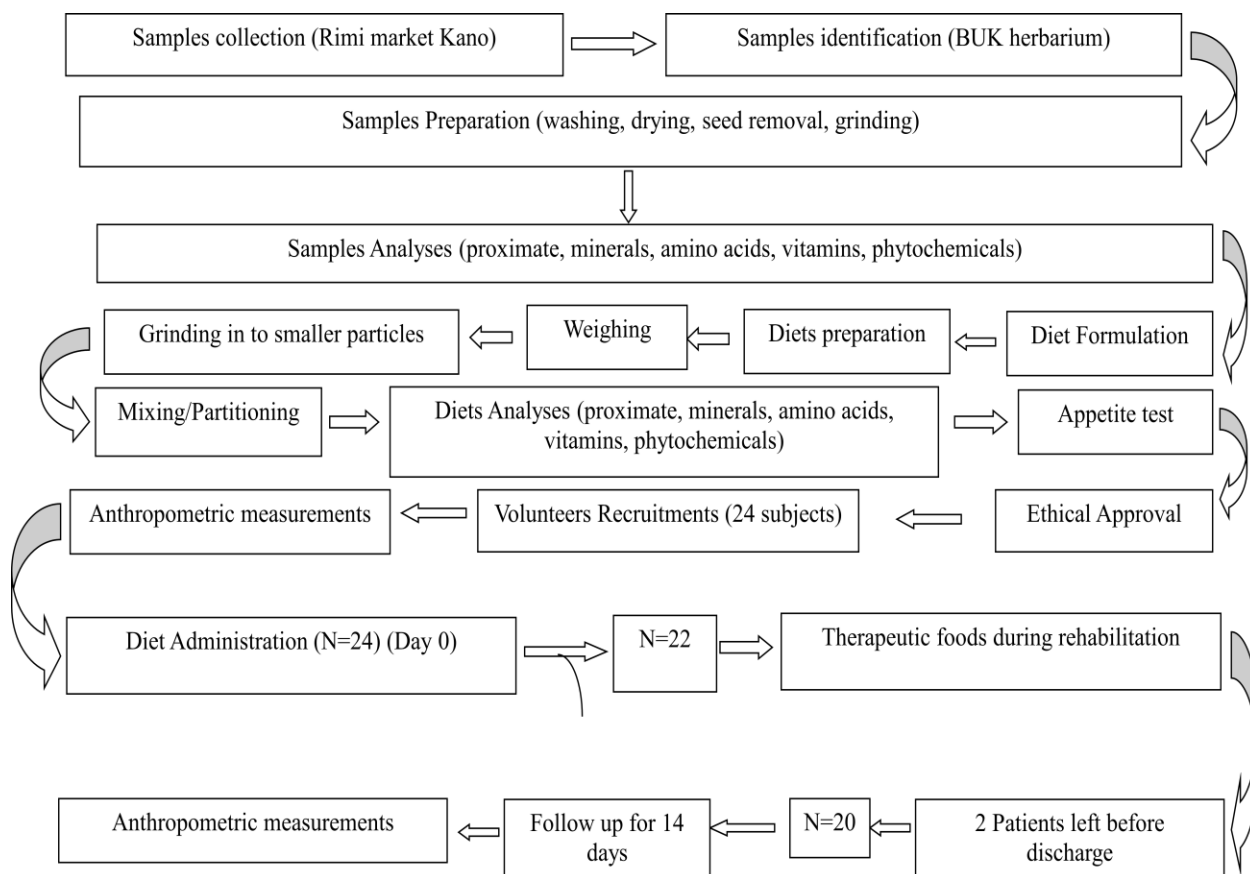


Figure 4: General Scheme of Research Design

3.3 DETERMINATION OF PROXIMATE COMPOSITION

This method partitioned nutrients in feed into 6 components: water, ash, crude protein, lipids/fats, crude fibre and carbohydrate.

3.3.2 Determination of moisture

The method employed for the determination of moisture content of the sample was based on the measurement of the loss in weight due to drying at a temperature of about 105⁰C as described (AOAC, 1990)

Six watch glasses were washed and dried in an oven at about 105⁰C after which they were cooled and weighed empty. Two grams of each sample were weighed into their respective watch

glasses. The watch glasses and their contents were dried in an air circulated oven at about 105⁰C to a constant weight. The watch glasses and their contents was cooled in desiccators and reweighed.

The percentage moisture content of each sample were calculated using the expression

$$\% \text{ moisture} = \frac{\text{Loss of weight on drying}(g)}{\text{Initial sample weight}} \times 100$$

3.3.3 Ash Content Determination

The term ash refers to the residue left after the combustion of the dried sample in oven and is a measure of the total mineral content. The determination of ash content was done according to the method described by AOAC (1990).

Six crucibles were preheated in a muffle furnace at about 550⁰C. Each crucible was cooled in a desiccator and weighed. Approximately 1g of each sample was weigh into the different crucibles. The crucibles and their contents were transferred into the muffle furnace at 550⁰C and allowed to stay for 5 hours. The weights of the crucible contents were taken and recorded.

Percentage ash was calculated using the expression below

$$\% \text{ ash} = \frac{\text{Weight of ash}}{\text{Weight of dry sample}} \times 100$$

3.3.4 Determination of Crude Lipid Content

The lipid content of each sample was determined by the procedure described by AOAC (1990). A clean dry round bottom flask containing anti bumping granules was used. Exactly 210cm³ of petroleum ether (60 – 80⁰C) 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 switched on and the heating rate adjusted until the solvent is refluxed at a steady rate. Extraction was carried out for 8hours.

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

$$\% \text{ lipid} = \frac{\text{Weight of lipid extracted}}{\text{Weight of dry sample}} \times 100$$

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.

3.3.5 Determination of Crude Fibre

The crude fiber was determined by the method of AOAC (1990). Two grams of ground sample was placed in a round bottom flask. 100ml of 0.25M H₂SO₄ was added and 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 was washed with hot water until it is base free (C2). It was then ashed in a furnace at 550⁰C for 2 hours. The furnace was then put off and allowed to cool down. The sample was then removed and cooled in a desiccator and weighed (C3). The crude fiber content was then calculated as loss of weight in ashing. Weight of original sample was used as W.

$$\% \text{ crude fiber} = \frac{C2 - C3}{W} \times 100$$

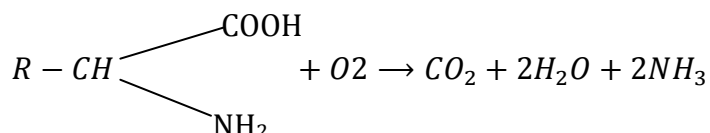
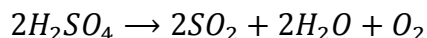
3.3.6 Determination of Nitrogen Content and Crude Protein

Principle

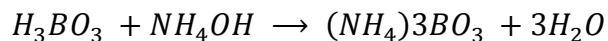
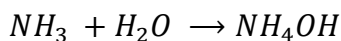
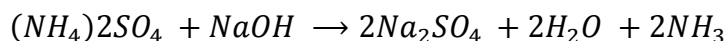
Proteins are major compounds containing nitrogen primarily in the form of amino acids which are their building blocks. Nitrogen is used as an index termed crude protein as distinct from true protein. The Kjeldahl method of AOAC (1990) was used for the crude protein determination.

Steps for determination

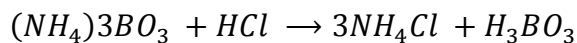
A. Mineralization steps of organic substance in boiling sulphuric acid.



B. Distillation Steps of Ammonium Sulphate after Alkalisiation of the Boric Acid Solution



C. Titration of ammonium with hydrochloric acid of standardizing concentration



Method:

Exactly 2.0g of each sample was weighed in to 100ml Kjeldahl flask and a few anti bumping granules were 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 were added. The flask was placed on a Kjeldahl 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. Ten milliliters of the digest were pipetted into Markham 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% boric acid and a drop of methyl blue indicator until the color changed 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 color change from green to pink and the volume (v) of the acid for each distillate was noted. Percentage nitrogen per sample was calculated using the expression below

$$\% \text{Nitrogen} = \frac{M \times v \times 14 \times 100 \times 100}{\text{Weight of the sample} \times 1000 \times 10}$$

Where, M = Molarity of HCl

V= volume of HCl used

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

$$\% \text{ Protein} = 6.25 \times \% \text{ Nitrogen.}$$

3.3.7 Determination of Carbohydrate Content

The percentage carbohydrate was obtained by difference thus;

$$\text{Percentage carbohydrate} = 100 - (\% \text{ ash} + \% \text{ crude fibre} + \% \text{ crude fat} + \% \text{ moisture} + \% \text{ crude protein}).$$

3.3.8 Determination of Energy content

The amounts of energy in the samples were calculated using the expression below:

$$\text{Energy (Kcal)} = (\% \text{ Carbohydrate} \times 4) + (\% \text{ Fat} \times 9) + (\% \text{ Protein} \times 4) \text{ (FAO, 2002).}$$

3.4 VITAMINS ANALYSIS

The vitamins in the samples were determined by the official methods of the Association of Official Analytical Chemists (AOAC, 1990).

3.4.1 Determination of Vitamin A (Retinol)

One gram of the sample was weighed and macerated with 20mls of n- hexane in a test tube for 10 minutes. Then 3mls of the upper hexane extract was transferred into a dry test tube in duplicates and evaporated to dryness. Following this, 0.2ml of acetic anhydride chloroform reagent was added and 2ml of 50% trichloroacetic acid (TCA) in chloroform was also added. The absorbance was taken at 15 seconds and 30 seconds intervals at 620nm.

3.4.2 Determination of Vitamin C (Ascorbic acid)

A 0.5g of the sample was weighed, macerated with 10mls of 0.4% oxalic acid in a test tube for 10 minutes, centrifuged for 5 minutes and the solution filtered. One milligram of the filtrate was transferred into a dry test tube in duplicates, 9mls of 2,6- dichlorophenol indophenol was added and absorbance was taken at 15 seconds and 30 seconds interval at 520nm.

3.4.3 Determination of Vitamin E (Tocopherol)

One gram (1g) of the original sample was weighed, macerated with 20mls of n- hexane in a test tube for 10 minutes and centrifuged for 10 minutes. The solution was filtered; 3mls of the filtrate was transferred into a dry test tube in duplicates and evaporated to dryness in a boiling water bath. Following this, 2mls of 0.5N alcoholic potassium hydroxide was added and boiled for 30 minutes in a water bath. Then 3mls of n-hexane was added and was shaken vigorously. The n-hexane was transferred into another set of test tubes and evaporated to dryness. A volume, 2mls, of ethanol was added to the residue. Another volume, 1ml of 0.2% ferric chloride in ethanol was added. Then 1ml of 0.5% $\alpha^1 \alpha^1$ -dipyridyl in ethanol was added followed by the addition of 1ml of ethanol to make it up to 5mls. The solution was mixed and absorbance was taken at 520nm against the blank test tube in duplicate. The standard solution of vitamin E of 100ppm was also prepared in the above manner. Then 3mls of calcium working reagent was added and absorbance at 512nm was read against the blank.

3.5 MINERALS ANALYSIS

3.5.1 Determination of Iron, Copper, and Magnesium using Atomic absorption spectrophotometer

Principle:

Atomic Absorption Spectroscopy is much more accurate and sensitive method of analyzing a wide ranges of metals. In the most common implementation of AAS, an aqueous sample is atomized in a hot flame generated by a highly combustive gas mixture (air-actylene) at temperature of 2300°C . This breaks the metal compounds in the sample in to free metal atoms or free radicals. Radiation emanating from the cathode lamp made to pass through the free atoms will be absorbed by the atoms where the lap produces radiation of an appropriate wavelength while passing through the flame. The absorbed energy is measured by a photo-detector read-out system and the extent of this absorption is a measure of the concentration of the metal in the solution. The concentration of sample is usually determined from a standard calibration curve.

Sample preparation: By design, analyte sample is aspires only as liquid into the sample compartment of the AAS instrument that uses the burner system. Many materials whose samples are to be analyzed such as tissues, plants, soil, and other minerals are not in liquid form. Thus, there is need for pre-treatment of samples. This is achieved through:

- i. Digestion: This involves heating 1g of the sample with *aqua regia* (a mixture of HNO_3 and HCl in 1:3 ratio) in a fume cupboard until the color of the acid changes pale yellow from yellow-orange fuming liquid. Allow to cool, filter and make up to 100ml with deionized water

- ii. Ashing: This involves heating 1g of the sample in a muffle furnace at about 700-800°C for 4-6 hours (make sure all trace of carbon are completely burnt). Allow to cool, add drops of concentrated HCl to dissolve, filter and make up to 100ml with deionized water.

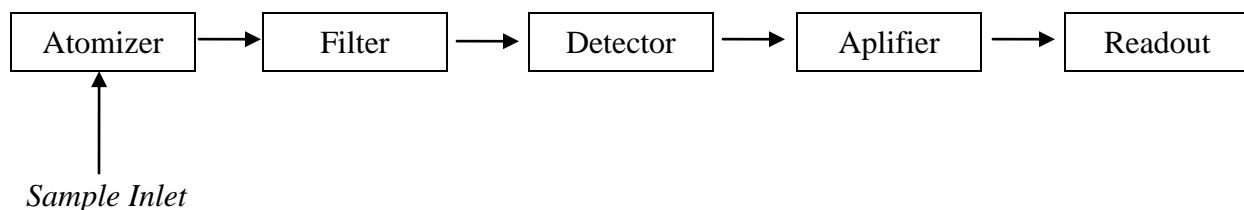
Method:

About 1.0g of each sample was first digested with 20ml of an acid mixture (650ml conc. HNO₃; 80ml perchloric acid; 20ml conc. H₂SO₄) in the mixture ratio of 3:1. The samples were taken into the digesting flask and heated until the sample was completely digested. Then it was filtered and made to 100ml with deionized water. A blank solution was also prepared using a mixture of the acid for the analysis. Copper, Magnesium, and Iron were investigated using BUCK 205 Atomic Absorption Spectrophotometer.

3.5.2 Determination of Sodium, Potassium, and Calcium using Flame photometer

Principle:

When atoms of alkali metals are heated sufficiently to high temperature, they absorb energy from the source of heat and become easily excited. The electrons move from lower energy level to a higher energy level. To maintain stability, the electrons are forced to return to their original state as they cool to the original state. The atoms will therefore emit some radiant energy equivalent in magnitude to the amount of energy absorbed during excitation. The wavelength of the emitted energy has a direct relationship with the electronic transition that occurred. As every element has its peculiar electronic configuration, the wavelength of light emitted is unique for such a particular element.



Method:

About 1.0g of each sample was first digested with 20ml of acid mixture (650ml conc. HNO_3 ; 80ml perchloric acid; 20ml conc. H_2SO_4) in the mixture ratio of 3:1. The samples were taken into the digesting flask and heated until the sample is completely digested. Then it was filtered and made to 100ml with deionized water. A blank solution was also prepared using a mixture of the acid for the analysis. Calcium, Potassium, and Sodium were investigated using Flame Photometer respectively.

3.6 DETERMINATION OF AMINO ACID PROFILE

The Amino Acid profile in the sample was determined using methods described by Benitez (1989). The following procedures were followed for the amino acid determination: The sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

3.6.1 Defatting Sample:

The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 500mg of the sample was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus (AOAC, 2006).

3.6.2 Nitrogen Determination:

A small amount (115mg) of ground sample was weighed, wrapped in whatman filter paper (No.1) and put in the Kjeldhal 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. Six pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham 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 standardize 0.01 N hydrochloric acid to grey coloured end point.

$$\text{Percentage Nitrogen} = \frac{(a - b) \times 0.01 \times 14 \times v \times 100}{W \times C}$$

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.

3.6.3 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 is 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 $105^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. It should be noted that tryptophan is destroyed by 6N HCl during hydrolysis.

The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

3.6.4 Loading of the Hydrolysate into Analyzer

The amount loaded was 60 microlitres. This 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.

3.6.5 Method of Calculating Amino Acid Values

An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

3.7 QUALITATIVE ANALYSIS OF THE PHYTOCHEMICALS OF THE SAMPLES

3.7.1 Test for Tannins

The method reported by Ejikeme *et al.* (2014) was used for Tannins determination. Powdered sample (0.3g) was dissolved with 30cm³ of distilled water in a test tube and boiled in a water bath at temperature of a 100⁰C for 10minutes. After the boiling, the sample was filtered using Whatman filter paper (125mm). To 5cm³ of the filtrate, 3 drops of ferric chloride were added. A blue black colouration indicates a positive test.

3.7.2 Test for Saponin

Saponin was determined by the method reported by Ejikeme *et al.* (2014). Each powdered sample (0.3g) was dissolved in 30cm³ of distilled water and boiled for 10minutes in a water bath. The sample was filtered using Whatman filter paper (125mm). The mixture of the sample was

agitated vigorously for a stable persistent froth. The formation of emulsion on addition of three drops of olive oil showed a positive result.

3.7.3 Test for Steroid

Steroid was determined by the method reported by Ejikeme *et al.* (2014). Each powdered sample (0.3g) was mixed with 20cm³ of ethanol in a beaker, and the component was extracted for 2hours. 2cm³acetic anhydride was added to each extract followed by the addition of 2cm³concentrated tetraoxosulphate IV acid. A green colour change indicates the presence of steroids.

3.7.4 Test for Flavonoids

Flavonoids determination was carried out according to the method reported by Sofowara, 1993 and Harborne, 1973. Each of the powdered samples (0.30g) was extracted with 30cm³of distilled water for 2hours. The solution was filtered with Whatman filter paper (125mm). 5cm³of 1.0M dilute ammonia was added to about 10cm³of aqueous filtrate of each sample followed by the addition of 5cm³concentrated tetraoxosulphate (IV) acid. Appearance of yellow colouration which disappeared on standing indicate the presence of flavonoids

3.7.5 Test for Alkaloids

Alkaloid was determined according to method reported by Hikino *et al.* (1984). Each of the sample (2g) was extracted with the addition of 20cm³ tetraoxosulphate VI acid in 50% ethanol and boiled for 2 minutes. The solution was filtered using filter paper. 5cm³ of ammonia solution was added in to the filtrate to make it alkaline in a separate funnel. Equal volume of chloroform (5.0 cm³) was used in further solution extraction in which chloroform solution was extracted with two 5 cm³ portions of 1.0 M dilute tetraoxosulphate (VI) acid. This final acid extract was

then used to carry out the following test: 0.5 cm³ of Dragendorff's reagent (Bismuth potassium iodide solution) was mixed with 2 cm³ of acid extract and precipitated orange colour indicate the presence of alkaloid.

3.7.6 Test for Glycoside

Glycoside was determined according to the method reported by Hikino *et al.* (1984). For each of the samples, 20cm³ of distilled water was added to 2.00g and heated for 5 minutes in a water bath. The solution was filtered with a Gem filter paper (12.5cm).The following tests were carried out with the filtrate:

(a) 0.2 cm³ of Fehling's solutions A and B was mixed with 5 cm³ of the filtrate until it became alkaline (tested with litmus paper). A brick-red colouration on heating showed a positive result.

(b) Instead of water, 15 cm³ of 1.0 M sulphuric acid was used to repeat the above test and the quantity of precipitate obtained compared with that of (a) above. High precipitate content indicates the presence of glycoside while low content shows the absence of glycoside.

3.8 QUANTITATIVE DETERMINATION OF PHYTOCHEMICAL CONSTITUENTS OF THE SAMPLES

3.8.1 Tannin Determination

An analytical method for quantitative determination of tannin was according to Amadi *et al.* (2004); Ejikeme *et al.* (2014). By dissolving 50 g of sodium tungstate (Na₂WO₄) in 37 cm³ of distilled water, Folin-Denis reagent was made. To the reagent prepared above, 10 g of phosphomolybdic acid (H₃PMo₁₂O₄₀) and 25 cm³ of orthophosphoric acid (H₃PO₄) were added. Two-hour reflux of the mixture was carried out, cooled, and diluted to 500 cm³ with distilled

water. One gram of each powdered sample in a conical flask was added to 100 cm³ of distilled water. This was boiled gently for 1 hour on an electric hot plate and filtered using number 42 (125 mm) Whatman filter paper in a 100 cm³ volumetric flask. Addition of 5.0 cm³ Folin-Denis reagent and 10 cm³ of saturated Na₂CO₃ solution into 50 cm³ of distilled water and 10 cm³ of diluted extract (aliquot volume) was carried out after being pipetted into a 100 cm³ conical flask for color development. The solution was allowed to stand for 30 minutes in a water bath at a temperature of 25⁰C after thorough agitation. With the aid of a Spectrum Lab 23A spectrophotometer optical density was measured at 700 nm and compared on a standard tannic acid curve. Dissolution of 0.20 g of tannic acid in distilled water and dilution to 200 cm³ mark (1 mg/cm³) was used to obtain tannic acid standard curve. Varying concentrations (0.2–1.0 mg/cm³) of the standard tannic acid solution were pipetted into five different test tubes to which Folin-Denis reagent (5 cm³) and saturated Na₂CO₃ (10 cm³) solution were added and made up to the 100 cm³ mark with distilled water. The solution was left to stand for 30 minutes in a water bath at 25⁰C. Optical density was ascertained at 700 nm with the aid of a Spectrum Lab 23A spectrophotometer. Optical density (absorbance) versus tannic acid concentration was plotted. The following formula was used in the calculation:

$$Tannic\ acid\ \left(\frac{mg}{100g}\right) = \frac{C \times extract\ volume \times 100}{Aliquot\ volume \times weight\ of\ sample}$$

where *C* is concentration of tannic acid read off the graph.

3.8.2 Determination of Alkaloids

The quantitative determination of alkaloid was according to the method of Harborne (1973). Exactly 200 cm³ of 10% acetic acid in ethanol was added to each powdered sample (2.50 g) in a 250 cm³ beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to

one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide dropwise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 cm³ of 0.1 M of ammonium hydroxide and then filtered using Gem filter paper (12.5 cm). Using electronic weighing balance Model B- 218, the residue was dried in an oven and the percentage of alkaloid is expressed mathematically as

$$\% \text{ Alkaloid} = \frac{\text{weight of alkaloid}}{\text{weight of sample}} \times 100$$

3.8.3 Determination of Flavonoid

Flavonoid determination was by the method reported by Ejikeme *et al.* (2014); Boham and Kocipai, (1994). Exactly 50 cm³ of 80% aqueous methanol added was added to 2.50 g of sample in a 250 cm³ beaker, covered, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re-extracted (three times) with the same volume of ethanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of each sample. Each powdered sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a dessicator and weighed until constant weight was obtained.

The percentage of flavonoid was calculated as

$$\% \text{ Flavonoid} = \frac{\text{weight of flavonoids}}{\text{weight of sample}} \times 100$$

3.8.4 Determination of Saponin

Saponin quantitative determination was carried out using the method reported by Ejikeme *et al.* (2014); Obadoni and Ochuko, (2002). Exactly 100 cm³ of 20% aqueous ethanol was added to 5 grams of each powder sample in a 250 cm³ conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55⁰C. The residue of the mixture was re-extracted with another 100 cm³ of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55⁰C with constant stirring. The combined extract was evaporated to 40 cm³ over water bath at 90⁰C. 20 cm³ of diethyl ether was added to the concentrate in a 250 cm³ separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. 60 cm³ of n-butanol was added and extracted twice with 10 cm³ of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight.

The saponin content was calculated as a percentage:

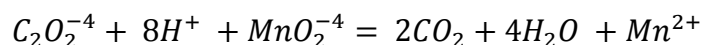
$$\% \text{ Saponin} = \frac{\text{weight of saponin}}{\text{weight of sample}} \times 100$$

3.8.5 Determination of Oxalate

Quantitative determination of Oxalate was carried out using the method reported by Ejikeme *et al.* (2014) and Munro and Bassir, (1969). Exactly 20cm³ of 0.3M HCl in each powder sample (2.50 g) was extracted three (3) times by warming at a temperature of 50⁰C for 1 hour with constant stirring using a magnetic stirrer.

For oxalate estimation, 1.0 cm³ of 5M ammonium hydroxide was added to 5.0 cm³ of extract to ensure alkalinity. Addition of 2 drops of phenolphthalein indicator, 3 drops of glacial acetic acid, and 1.0 cm³ of 5% calcium chloride to make the mixture acidic before standing for 3 hours were followed by centrifugation at 3000 rpm for 15 minutes. After discarding the supernatant, the precipitate was washed three times using hot water by mixing thoroughly each time centrifugation was run. Then, to each tube, 2.0 cm³ of 3M tetraoxosulphate (VI) acid was added and the precipitate dissolved by warming in a water bath at 70°C. Freshly prepared 0.01M potassium permanganate (KMnO₄) was titrated against the content of each tube at room temperature until the first pink colour appears throughout the solution. The solution was allowed to stand until it returned colourless, after which it was warmed on an electric hot plate at 70°C for 3 minutes, and retitrated again until a pink colour appears and persists for at least 30 seconds.

Titration reaction of oxalate in sample was calculated as:



Ratio of reacting ions = 1:1

From $M_1V_1 = M_2V_2$

Where M_1 is molarity of KMnO₄, M_2 is molarity of extract (oxalate), V_1 is volume of extract (oxalate), and V_2 is volume of KMnO₄ (Titre Value).

Molecular Weight of CaCO₃ = 100

Weight of oxalate in titre = $M_2 \times \text{molecular weight} = Xg$

Weight of oxalate in titrand 2cm³ = $\frac{Xg}{1000} \times 12 = Y$

$$100\text{cm}^3 \text{ of oxalate extract will contain } = \frac{Y}{2.5} \times 100g = W$$

$$\% \text{ oxalate composition g/100 g} = \frac{W}{2.5} \times \frac{100}{1}$$

3.8.6 Determination of Glycoside

Glycoside quantitative determination of glycosides was carried out according to the method of Amadi *et al.* (2004) as reported by Ejikeme *et al.* (2014). It was weighed into a 250cm³ round bottom flask and about 200 cm³ of distilled water was added to one gram of each dry powder sample and allowed to stand for 2 hours for autolysis to occur. Full distillation was carried out in a 250 cm³ conical flask containing 20 cm³ of 2.5% NaOH (sodium hydroxide) in the sample after adding an antifoaming agent (tannic acid). Glycoside (100 cm³), 8 cm³ of 6M NH₄OH (ammonium hydroxide), and 2 cm³ of 5% KI (potassium iodide) were added to the distillate(s), mixed, and titrated with 0.02M AgNO₃ (silver nitrate) using a microburette against a black background.

Turbidity which was continuous indicates the end point.

Content of glycoside in the sample was calculated as:

$$\text{Glycoside} \left(\frac{\text{mg}}{100g} \right) = \frac{\text{Titre value (cm}^3\text{)} \times 1.08 \times \text{exact volume}}{\text{Aliquot volume (cm}^3\text{)} \times \text{sample weight (g)}} \times 100$$

3.9 ANTHROPOMETRIC INDICES

Anthropometrics are a set of non-invasive, quantitative body measurements used to assess growth, development, and health parameters. Anthropometric measurements, including length or height, weight, mid-arm and head circumference, help providers determine if a child is growing properly and can indicate when the child's health and well-being are at risk. Additionally,

anthropometric measurements assist providers in selecting appropriate treatment options for children and adolescents.

- Head circumference: The largest circumference of the head was measured by extending a non-stretchable measuring tape around the most prominent part of the occiput to the middle of the forehead. The tape was pulled snugly to compress the hair and underlying soft tissue. The measurement was read to the nearest 0.1 cm and repeated twice, and average of the two closest measurements was recorded.
- Height (stature): The height of the children aged two years and older wearing light clothing and without shoes that can stand using a standard measuring board with stadiometer attached to a flat wall were measured to the nearest 1 cm. The measurement was repeated twice or until two measurements agree within 1 cm. the average of the two closest measurements was recorded.
- Weight: Each of the children without clothes was weighed using a digital infant scale. Child was position in the center of the scale tray and the measurement was read to the nearest 0.1kg.
- The mid-arm circumference was measured by placing the tape round the left mid-arm of the child and recorded to the nearest 1cm.

The height, weights, mid-arm and head circumference were compared with WHO Standards.

3.10 Statistical Analysis

Each experimental analysis was done in triplicate. Data obtained from experiments were analyzed by U-ANOVA (Univariate Analysis of Variance) using INSTAT statistics software. Significance was accepted at 0.05 level of probability ($p < 0.05$). The analysis was used to

compare the proximate, elemental composition, vitamins, phytochemicals and formulated diets of all the samples.

CHAPTER FOUR

4.0 RESULTS

4.1 PROXIMATE COMPOSITION OF FOOD SAMPLES STUDIED

The results of proximate composition of *P. dactylifera*, *M. oleifera*, *G. max*, *S. bicolor*, *A. hypogaea* and formulated diets A, B and C were presented in Table 1. The average moisture content ranged from 1.29% to 9.42% in the sample. Higher content (9.42%) was recorded in formulated diet C while *P. dactylifera* has the lowest content at 1.29%. The total ash content ranged between 1.57% to 9.44% with *S. bicolor* having the highest amount 9.44% while *A. hypogaea* have the lowest amount. The percentage of crude fiber in the sample ranged between 2.13% to 9.14%. *M. oleifera* has the highest content 9.14% with *P. dactylifera* with the lowest amount of 2.15%. The crude fat in the sample ranged from 2.11% to 37.80%. The higher crude fat was recorded from *A. hypogaea* 37.80% and lower fat content from *M. oleifera*. The protein content ranged between 1.59% to 41.25% with *Glycine max* having the highest amount 41.25% while *P. dactylifera* with the lowest amount 1.59%. The carbohydrate content in the sample ranged from 30.17 to 87.61%. Higher carbohydrate content was recorded from *P. dactylifera* 87.61% while formulated diet C with the lowest content among the samples.

Table 1: Proximate Composition of *Phoenix dactylifera*, *Moringa oleifera*, *Glycine max*, *Sorghum bicolor*, *Arachis hypogaea* and Formulated Diets A, B and C

Proximate Composition (%)							
SAMPLES	Moisture	Ash	Crude Fibre	Crude Fat	Protein	Carbohydrate	Energy Kcal/g
<i>Phoenix dactylifera</i>	1.29±0.02 ^a	3.87±0.04 ^a	2.13±0.13 ^a	3.49±0.21 ^c	1.59±0.21 ^b	87.61±0.34 ^g	388.21±0.48 ^a
<i>Moringa oleifera</i>	8.94±0.08 ^{bi}	9.04±0.08 ^b	9.14±0.08 ^f	2.11±0.18 ^{cd}	23.88±0.18 ^a	46.75±0.20 ^a	301.51±0.36 ^b
<i>Glycine max</i>	5.31±0.13 ^{cj}	5.75±0.17 ^{ab}	5.14±0.14 ^g	9.66±0.19 ^f	41.25±0.27 ^f	32.88±0.28 ^b	383.46±0.86 ^c
<i>Sorghum bicolor</i>	6.44±0.16 ^d	9.44±0.19 ^b	3.35±0.17 ^b	3.07±0.08 ^{cd}	33.24±0.74 ⁱ	44.45±1.02 ^{ad}	289.43±0.52 ^d
<i>Arachis hypogaea</i>	4.81±0.09 ^{ejk}	1.57±0.24 ^a	2.50±0.20 ^{ac}	37.80±0.40 ^g	21.10±0.07 ^c	32.21±0.50 ^{be}	610.24±0.67 ^e
<i>Formulated Diet A</i>	4.62±0.17 ^{fk}	3.45±0.18 ^{abc}	2.30±0.11 ^{ac}	23.36±0.31 ^a	19.85±0.78 ^c	46.41±0.60 ^{ad}	475.28±0.21 ^f
<i>Formulated Diet B</i>	7.36±0.14 ^g	4.73±0.90 ^{abc}	2.66±0.14 ^{ac}	25.71±1.32 ^a	21.10±0.38 ^c	38.41±1.06 ⁱ	469.43±0.42 ^g
<i>Formulated Diet C</i>	9.42±0.19 ^{hi}	3.48±0.24 ^{abc}	3.23±0.08 ^{bc}	30.80±0.48 ^e	22.82±0.50 ^{ac}	30.17±0.55 ^{ce}	489.16±0.18 ^h

All values are expressed in triplicate as mean ± SEM, values with the same alphabet in the same column are not significantly different at p<0.05

4.2 MINERAL COMPOSITION OF THE FOOD SAMPLE STUDIED

The results of the mineral composition of *P. dactylifera*, *M. oleifera*, *G. max*, *S. bicolor*, *A. hypogaea* and formulated diets A, B and C were presented in table 2. The average Na (mg/100g) content ranged from 0.48 to 8.60mg/100g in the sample. Higher content 8.60mg/100g was recorded in *M. oleifera* while formulated diet B has the lowest content of 0.48mg/100g. The total Mg content ranged from 2.03 to 22.01mg/100g with formulated diet C having the highest amount 22.01mg/100g while *G. max* has the lowest amount, 2.03mg/100g. The amount of K (mg/100g) in the sample ranged from 3.10 to 8.08mg/100g. Formulated diet C has the highest content 8.08 mg/100g while *G. max* with the lowest amount of 3.10mg/100g. The amount of Fe (mg/100g) in the sample ranged from 0.52 to 3.93mg/100g. The higher crude fat was recorded from formulated diet C 3.93mg/100g and lower fat content from *P. dactylifera*. The Ca (mg/100g) content ranged from 0.37 to 1.20mg/100g with formulated diet C having the highest amount of 1.20mg/100g while *P. dactylifera* with the lowest amount 0.37mg/100g. The amount of Cu (mg/100g) in the sample ranged from 0.10 to 5.40mg/100g. Higher Cu content was recorded from formulated diet B while Cu was not detected in *P. dactylifera* and *S. bicolor* in the samples.

Table 2: Mineral Composition (mg/100g) of *Phoenix dactylifera*, *Moringa oleifera*, *Glycine max*, *Sorghum bicolor*, *Arachis hypogaea* and Formulated Diets A, B and C

PARAMETERS						
SAMPLES	Na	Mg	K	Fe	Ca	Cu
<i>Phoenix dactylifera</i>	6.04±0.35 ^a	2.14±0.25 ^a	6.20±0.22 ^{cd}	0.52±0.11 ^a	0.37±0.07 ^b	ND
<i>Moringa oleifera</i>	8.60±0.37 ^b	3.99±0.22 ^{ab}	6.56±0.28 ^{cd}	0.75±0.21 ^{ab}	0.64±0.14 ^b	0.03±0.03 ^{bc}
<i>Glycine max</i>	4.60±0.22 ^{ac}	2.03±0.15 ^{ad}	3.10±0.13 ^{ab}	0.85±0.18 ^{abc}	0.44±0.10 ^b	0.10±0.08 ^{bc}
<i>Sorghum bicolor</i>	7.02±0.64 ^{abd}	2.40±0.07 ^{abdc}	3.72±0.20 ^{ab}	0.91±0.09 ^{abc}	0.55±0.11 ^b	ND
<i>Arachis hypogaea</i>	6.58±0.45 ^{acd}	2.49±0.05 ^{abdc}	5.74±0.38 ^{cd}	0.96±0.14 ^{abc}	0.47±0.13 ^b	0.72±0.15 ^{bc}
Formulated Diet A	0.65±0.53 ^g	20.60±0.87 ^f	6.71±0.45 ^{cd}	3.20±0.54 ^d	1.01±0.23 ^b	4.55±0.12 ^a
Formulated Diet B	0.48±0.32 ^g	19.60±0.38 ^f	5.92±0.92 ^{cd}	3.39±0.21 ^d	0.90±0.48 ^b	5.40±0.32 ^d
Formulated Diet C	0.73±0.20 ^g	22.01±0.46 ^f	8.08±0.37 ^{cd}	3.93±0.18 ^d	1.20±0.32 ^b	5.03±0.18 ^{ad}

All values are expressed in triplicate as mean ± SEM, ND = Not detected, values with the same alphabet in the same column are not significantly different at p<0.05

4.3 QUANTITATIVE PHYTOCHEMICALS OF THE SAMPLES

The results of phytochemicals of *P. dactylifera*, *M. oleifera*, *G. max*, *S. bicolor*, *A. hypogaea* and formulated diets A, B and C were presented in table 3. The saponin content (mg/100g) in the sample ranged from 1.14 to 47.61. Higher content 47.61mg/100g was recorded in *G. max* while *P. dactylifera* has the lowest content 1.14mg/100g. The amount of tannins ranged between 0.10 to 12.13mg/100g with formulated diet C having the highest amount 12.13mg/100g while *S. bicolor* has the lowest amount, 0.10mg/100g and not detected in *G. max*. The amount of Glycosides (mg/100g) in the sample ranged from 0.53 to 31.94. Formulated diet C has the highest content 31.94 mg/100g while *M. oleifera* with the lowest amount 0.53mg/100g. The amount of alkaloids (mg/100g) in the sample ranged from 0.32 to 32.13. The higher alkaloid was recorded from formulated diet C 32.13mg/100g while alkaloid was not detected in *G. max*. The steroids (mg/100g) content ranged between 1.94 to 20.86 with *A. hypogaea* having the highest amount 20.86mg/100g while formulated diet A with the lowest amount 1.94mg/100g. The amount of flavonoids (mg/100g) in the sample ranged from 1.72 to 84.83. Higher flavonoid content was recorded from *P. dactylifera*, 84.83mg/100g while *S. bicolor* has the lowest amount in the samples, 1.72mg/100g. The amount of oxalates ranges from 2.87 to 21.20mg/100g in which formulated diet C recorded a highest amount, 21.20mg/100g, while *A. hypogaea* have the lowest content in the sample, 2.87mg/100g. The amount of phytate in the sample ranged from 1.78 to 32.17mg/100g with formulated diet C recorded the highest amount 32.17mg/100g while *A. hypogaea* have the lowest amount.

Table 3: Phytochemical Contents (mg/100g) of *Phoenix dactylifera*, *Moringa oleifera*, *Glycine max*, *Sorghum bicolor*, *Arachis hypogaea* and Formulated Diets A, B and C

SAMPLES	PARAMETERS mg/100g sample							
	Saponins	Tannins	Glycosides	Alkaloids	Steroids	Flavonoids	Oxalates	Phytate
<i>Phoenix dactylifera</i>	1.14±0.13 ^a	11.12±0.18 ^a	13.87±0.28 ^c	8.69±0.83 ^a	3.40±0.07 ^a	84.83±0.23 ^b	8.53±0.48 ^a	4.82±0.41 ^c
<i>Moringa oleifera</i>	2.52±0.54 ^a	9.67±1.02 ^{ab}	0.53±0.08 ^d	20.53±0.15 ^b	5.66±0.24 ^c	7.33±0.54 ^c	9.84±0.06 ^a	14.30±0.13 ^d
<i>Glycine max</i>	47.61±0.46 ^f	ND	21.56±0.38 ^e	ND	8.73±0.02 ^d	72.58±0.37 ^d	3.34±0.47 ^b	2.13±0.28 ^a
<i>Sorghum bicolor</i>	12.53±0.08 ^b	0.10±0.67 ^{cd}	23.34±0.32 ^f	9.23±0.43 ^{ab}	1.98±0.28 ^{ab}	1.72±0.13 ^e	14.02±0.41 ^c	11.01±0.38 ^e
<i>Arachis hypogea</i>	11.42±0.21 ^b	0.21±0.02 ^{cd}	10.56±0.38 ^a	0.32±0.24 ^b	20.86±0.12 ^f	31.64±0.58 ^f	2.87±0.03 ^b	1.78±0.02 ^a
<i>Formulated Diet A</i>	30.92±0.35 ^d	0.89±0.98 ^{cd}	19.63±0.45 ^g	7.33±0.54 ^{ab}	1.94±0.45 ^{ab}	18.93±0.39 ^a	12.10±0.38 ^d	9.03±0.13 ^b
<i>Formulated Diet B</i>	39.00±0.72 ^c	4.39±0.49 ^f	11.28±0.43 ^a	23.63±0.58 ^d	12.15±0.84 ^g	20.61±0.28 ^a	19.39±0.21 ^e	32.17±0.54 ^f
<i>Formulated Diet C</i>	40.76±0.56 ^c	12.13±0.48 ^{ab}	31.94±0.18 ^h	32.13±0.23 ^e	17.20±0.29 ⁱ	29.82±0.19 ^g	21.20±0.21 ^f	21.73±0.43 ^h

All data expressed in triplicate as mean ± SEM, ND= Not Detected, values with the same alphabet in the same column are not significantly different at p<0.05

4.4 VITAMINS CONTENT OF THE SAMPLES

The results of the vitamins content of *P. dactylifera*, *M. oleifera*, *G. max*, *S. bicolor*, *A. hypogaea* and formulated diets A, B and C were presented in table 4. The vitamin A content ranged from 3.40 to 92.03mg/100g in the sample. Higher content, 92.03mg/100 was recorded in *G. max* while formulated diet A has the lowest content 3.40mg/100g. The amount of vitamin C in the sample ranges between 16.05 to 110.009mg/100g with formulated diet C having the highest amount 110.00mg/100g while *A. hypogaea* has the lowest amount 16.05mg/100g. The amount of vitamin E in the sample ranged between 0.49 to 22.11mg/100g. Formulated diet A content 22.11mg/100g with *P. dactylifera* having the lowest amount, 0.49mg/100g.

Table 4: Vitamins Contents (mg/100g) of the *Phoenix dactylifera*, *Moringa oleifera*, *Glycine max*, *Sorghum bicolor*, *Arachis hypogaea* and Formulated Diets A, B and C

PARAMETERS			
SAMPLES	Vitamin A	Vitamin C	Vitamin E
<i>Phoenix dactylifera</i>	8.71±0.52 ^a	96.66±0.06 ^a	0.49±0.43 ^g
<i>Moringa olifera</i>	73.21±0.38 ^d	52.82±0.19 ^b	16.94±0.18 ^b
<i>Glycine max</i>	92.03±0.45 ^e	78.71±0.20 ^c	19.00±0.26 ^a
<i>Sorghum bicolor</i>	16.00±0.76 ^f	21.15±0.62 ^d	7.53±0.34 ^c
<i>Arachis hypogeal</i>	11.01±0.09 ^g	16.05±0.65 ^e	15.00±0.09 ^d
<i>Formulated Diet A</i>	3.40±0.43 ^b	94.58±0.33 ^f	22.11±0.65 ^e
<i>Formulated Diet B</i>	7.00±0.54 ^{ac}	102.39±0.56 ^g	19.75±0.49 ^a
<i>Formulated Diet C</i>	5.42±0.26 ^{bc}	110.00±0.34 ^h	25.01±0.36 ^f

All data expressed in triplicate as mean ± SEM, values with the same alphabet in the same coloumn are not significantly different at p<0.05

4.5 AMINO ACIDS COMPOSITION OF THE SAMPLES

The results of the amino acid composition of *P. dactylifera*, *M. oleifera*, *G. max*, *S. bicolor*, *A. hypogaea* and formulated diets A, B and C were presented in table 5. The amount of leucine ranged from 3.21 to 9.86g/100g in the sample. Higher content 9.86g/100g was recorded in *S. bicolor* while *P. dactylifera* has the lowest content 3.21g/100g. The lysine content ranged between 1.01 to 5.89g/100g with *G. max* having the highest amount of 5.89g/100g while *P. dactylifera* has the lowest amount 1.01g/100g. The amount of isoleucine in the sample ranged from 0.78 to 4.58g/100g. *Glycine max* has the highest content 4.58g/100g with *P. dactylifera* with the lowest amount 0.78g/100g. The amount of Phenylalanine in the sample ranged from 1.06 to 4.97g/100g. The higher value was recorded from *G. max* and *S. bicolor*, 4.58g/100g and lower amount from *P. dactylifera*, 1.06g/100g. The tryptophan content ranged between 0.42 to 1.89g/100g with *S. bicolor* having the highest amount 1.89g/100g while *P. dactylifera* with the lowest amount 0.42g/100g. The amount of valine in the sample ranged from 0.70 to 5.32g/100g. Higher valine content was recorded from *S. bicolor* 5.3g/100g while *P. dactylifera* with the lowest content among the samples, 0.70g/100g. The amount of methionine in the sample ranges between 0.80 to 2.40g/100g. *S. bicolor* has the highest amount 2.40g/100g with *P. dactylifera*, 0.71 with the lowest amount. The amount of proline in the sample ranges from 0.71 to 5.89g/100g. *S. bicolor* was recorded the highest amount of 5.89g/100g while *P. dactylifera* has the lowest amount 0.71g/100g among the sample. The amount of arginine ranged between 1.38 to 8.77g/100g. *A. hypogaea* has the highest amount of 8.77 while *P. dactylifera* has the lowest amount of 1.38g/100g. The amount of tyrosine in the sample ranges from 0.69 to 3.44g/100g with *G. max* having the highest amount of 3.44 while *P. dactylifera* have the lowest amount, 0.69g/100g. The amount of histidine in the samples ranges between 0.83 to 2.43g/100g with *G.*

max having the highest amount of 2.43 while lower amount was recorded from *P. dactylifera*, 0.83g/100g. Values ranges from 0.48 to 2.12 were recorded for cysteine with *S. bicolor* with the highest amount, 2.12 while *P. dactylifera* has the lowest amount, 0.48g/100g. The amount of alanine in the sample ranges from 1.14 to 7.36g/100g with *S. bicolor* having the highest amount 7.36, while *P. dactylifera* has the lowest amount, 1.14g/100g. Values ranges from 4.39 to 17.56 were recorded for glutamic acid with *S. bicolor* having the highest amount, 17.56 while *P. dactylifera* recorded a lowest amount of 4.39g/100g. The amount of glycine in the sample ranges between 0.81 to 4.37g/100g with *G. max* having the highest amount, 4.37 while *P. dactylifera* recorded a lowest amount, 0.81g/100g. Values ranging from 1.00 to 4.00 were recorded for threonine with *S. bicolor* having the highest amount, 4.00 while *P. dactylifera* has the lowest amount, 1.00g/100g. Amount of serine ranges from 0.86 to 4.70g/100g with *S. bicolor* having the highest amount, 4.70g/100g while *P. dactylifera* has the lowest amount, 0.86g/100g. The amount of aspartic acid in the samples ranges from 2.96 to 9.86 with *G. max* recorded a higher value of 9.86 while *P. dactylifera* has the lowest amount of 2.96g/100g.

Table 5: Amino Acids Composition of *Phoenix dactylifera*, *Moringa oleifera*, *Glycine max*, *Sorghum bicolor*, *Arachis hypogaea* and Formulated Diets C

AMINO ACIDS	SAMPLES Concentrations (g/100g protein)					
	<i>Phoenix dactylifera</i>	<i>Moringa oleifera</i>	<i>Glycine max</i>	<i>Sorghum bicolor</i>	<i>Arachis hypogaea</i>	<i>Formulated Diet</i>
Leucine*	3.21	8.64	7.59	9.86	6.48	8.81
Lysine*	1.01	4.35	5.89	3.29	3.39	5.46
Isoleucine*	0.78	3.93	4.58	4.39	3.54	4.06
Phenylalanine*	1.06	4.61	4.97	4.97	4.43	4.61
Tryptophan*	0.42	0.95	1.31	1.89	1.21	1.21
Valine*	0.70	4.34	4.97	5.32	4.33	4.27
Methionine*	0.80	1.17	1.33	2.40	1.76	1.87
Proline	0.71	3.05	3.35	5.89	3.45	3.65
Arginine	1.38	4.82	7.31	4.99	8.77	6.71
Tyrosine	0.69	3.10	3.44	3.27	2.41	3.10
Histidine*	0.83	2.36	2.43	2.17	2.36	2.30
Cysteine	0.48	0.97	1.51	2.12	1.33	1.33
Alanine	1.14	4.25	3.41	7.36	3.34	4.63
Glutamic acid	4.39	11.05	12.41	17.56	15.29	14.69
Glycine	0.81	3.94	4.37	3.70	3.52	3.75
Threonine*	1.00	3.11	3.72	4.00	3.66	3.33
Serine	0.86	2.86	3.89	4.70	3.62	3.08
Aspartic acid	2.98	9.06	9.86	8.25	9.68	8.74

*Essential amino acids

4.6 ANTHROPOMETRIC INDICES OF CHILDREN UNDER 5 YEARS THAT PARTICIPATED IN THE STUDY

Table 6 shows the results of the anthropometric indices (mean value of weight and MUAC for children participated in the study) before and after the administration of the therapeutic food for 14 days. The results revealed that the mean value for the initial mean weight and MUAC for children participated in the study before the administration of the diets are 5.76 ± 3.14 , and 10.64 ± 3.31 , while the final mean weight and MUAC are 6.70 ± 3.24 , and 11.08 ± 3.21 respectively. The mean height and head circumference of the children were presented in Table 9.

Table 6: Anthropometric indices (Initial and Final Mean of Weight and MUAC after Administration of the Diet for 14 days

Measurements	SUBJECTS	
	Weight (kg)	MUAC (cm)
Initial (Day 0)	5.76 ± 3.14^a	10.64 ± 3.31^b
Final (Day 14)	6.70 ± 3.24^a	11.08 ± 3.21^b

Values are expressed as mean \pm SEM, MUAC= Mid-upper-arm circumference, N=20, value with the same superscript on the same column are not significantly different at $p < 0.05$.

Table 7: Mean Height and Head Circumference of the Children that Participated in the Study

Measurements	SUBJECTS
Height (cm)	70.75±1.53 ^a
Head circumferences (cm)	44.28±0.58 ^b

Values are expressed as mean±SEM, N=20, value with different superscript on the same row are significantly different at $p<0.05$

4.7 RESULTS OF THE APPETITE TEST

A total of five children were recruited for the appetite test. About 150g of each of the three formulated therapeutic foods (A, B and C) were prepared for each of the children where they consumed at various time intervals. Most of them consumed formulated food C at faster rate than the other food. This is the basis for the choice of food C for the study, and at the same time has the highest energy contents among the other foods. The amount and the time for consumption are presented in the table below:

Table 8: Appetite Test for the Control Subjects

DIET(S)	Amount (gram)	Time (minutes)
DIET A	150	10.84±0.70
DIET B	150	9.45±0.99
DIET C	150	8.28±0.89

Values are expressed as mean ±SEM, N=5

4.7 DISCUSSION

The result of the proximate compositions of the food samples and the formulated diets is presented in table 3. The results revealed that the percentage of moisture content in the sample was found to vary considerably. It was high in the diet C (9.42 ± 0.19), followed by *Moringa oleifera* (8.94 ± 0.08), diet B (7.36 ± 0.14), and *Sorghum bicolor* (6.44 ± 0.16) and *Glycine max* (5.31 ± 0.13) in decreasing order. The value for *M. oleifera* agrees with the findings of Okiki *et al.* (2015), (7.88 ± 0.29). Ape *et al.* (2016) reported a value similar to the present study for *S. bicolor* (6.36%) and lower than that of the FAO composition table (10.1%). The percentage of moisture of *G. max* in the present study is lower compared to the FAO food composition table (9.3%) (FAO, 2012). Siulapwa and Mwambungu. (2014) reported a value for *G. max* similar to the present study (5.30 ± 0.20). Moisture content of food is a good parameter for food spoilage and acceptability.

Low moisture content was found in *A. hypogaea* (4.81 ± 0.09), diet A (4.62 ± 0.17) with *P. dactylifera* having the lowest amount (1.29 ± 0.02). This amount is lower compared to the FAO composition table (6.3%) for *A. hypogaea* and (18.7%) for *P. dactylifera* (FAO, 2012). The low moisture content is evidence that these samples may not be more inclined to decay (Fennema and Tannenbaum, 1996).

The percentage of ash was found to be higher in *S. bicolor* (9.44 ± 0.19) followed by *M. oleifera* (9.04 ± 0.08), *G. max* (5.75 ± 0.17), diet B (4.73 ± 0.09) and *P. dactylifera* (3.87 ± 0.04). The percentage of ash in the present study is lower compared to (2.4%) according to the FAO food composition table (FAO, 2012). Okiki *et al.* (2015) reported a value for ash in *M. oleifera* similar to the present study (9.82 ± 0.32). Also, the % of ash corresponds to that of the FAO table for *G.*

max (FAO, 2012). The high ash content in the sample may indicate that date palm fruit would likely contain very high qualitative essential minerals (Oloyede, 2005). This result is within the acceptable ash content mean values of legumes of 2.4 to 5.0% recommended by FAO (1989).

A low amount of ash was found in diet C (3.48 ± 0.24), followed by diet B (3.45 ± 0.18) while *A. hypogaea* has the lowest % (1.57 ± 0.24). FAO table reported a value of (2.6%) for ash content (FAO, 2012).

Dietary fiber serves as a useful tool in the control of oxidative processes in food products and as functional food ingredient. *Arachis hypogaea* (9.14 ± 0.08) was found to contain the highest value for the crude fiber, followed by *G. max* (5.14 ± 0.14) and *S. bicolor* (3.35 ± 0.17). FAO table reported a value of crude fiber (8.5%, 9.3% and 9.9%) for these three samples respectively (FAO, 2012).

All the other samples; diet A, B, C, *A. hypogaea* and *P. dactylifera* contain a low amount of crude fiber. Kumar *et al.* (2013) and Shaba *et al.* (2015) reported a value of crude fiber for *A. hypogaea* (2.91%) and *P. dactylifera* ($2.17 \pm 0.44\%$, $2.00 \pm 0.50\%$ and $2.26 \pm 0.07\%$) similar to the present study.

The amount of lipid/fat found in *A. hypogaea* was highest (37.80 ± 0.40) followed by the diet C (30.80 ± 0.48), diet B (25.71 ± 1.32) and diet A (23.36 ± 0.31). This amount in the diets corresponds to the nutritional composition of F75 and F100 for the management of SAM. Also, Kumar *et al.* (2013), reported a value of *A. hypogaea* (39.10%) similar to the present study.

Glycine max contains low amount of fat (9.66 ± 0.19), followed by *P. dactylifera* (3.49 ± 0.21) while *M. oleifera* has the lowest amount (2.11 ± 0.18). Uba *et al.* (2015) reported lower value for fat in *P. dactylifera* (1.00 ± 0.00) and Bamishaiye *et al.* (2011) (2.50 ± 1.21) for *M. oleifera* than

the present study. The low level of fat in the samples indicates that the samples have little amount of oil.

The amount of proteins in all the samples was found to be high with *G. max* (41.25 ± 0.27), *S. bicolor* (33.24 ± 0.74), *M. oleifera* (23.88 ± 0.18), diet C (22.82 ± 0.50), diet B and *A. hypogaea* (21.10 ± 0.38) and diet A (19.85 ± 0.78) while the *P. dactylifera* has the lowest amount of protein in all the samples (1.59 ± 0.2). Siulapwa and Mwambungu, (2014) reported a protein value of 39.80 and 37.60 ± 0.08 for *G. max*, also Bamishaiye *et al.* (2011) reported similar amount of protein for *M. oleifera* (23.7 ± 0.12) similar to the present study. The amounts of proteins in the diets are higher than that of the F75 and F100 for the management of SAM. Uba *et al.* (2015) and Shaba *et al.* (2015) reported similar amount of protein (1.53 ± 0.04) and (1.21 ± 0.02) for *P. dactylifera*. Legumes are good source of protein and other nutrient for humans and animal consumption and that their utilization in infant formula and other food products has been significantly solving nutrition problems in the community (Asibuo *et al.*, 2008).

The percentage of carbohydrate composition was found to be high in *P. dactylifera* (87.61 ± 0.34) followed by *M. oleifera* (46.75 ± 1.02), diet B (38.41 ± 1.06), *G. max* (32.88 ± 0.28), *A. hypogaea* (32.21 ± 0.50) and diet C (30.17 ± 0.55) in decreasing order. Uba *et al.* (2015) reported similar value for *P. dactylifera* (89.99 ± 1.07). Also, Bordingnon and Mandarino, (1994) reported a value of (34%) for *G. max* which is similar to the present study. Generally, carbohydrates add to the bulk of the diets, they play a pivotal role as they provide primary energy to cells such as brain, muscles, and blood (Asibuo *et al.*, 2008).

The amount of energy (Kcal/100g) in all the samples shows that *A. hypogaea* has the highest amount of energy (610.24 ± 0.67) followed by diet C (489.16 ± 0.18), diet B (469.43 ± 0.42), *P. dactylifera* (388.21 ± 0.48), *G. max* (383.46 ± 0.86) and *M. oleifera* (301.51 ± 0.36), while *S. bicolor* has the lowest amount of energy in all the samples (289.43 ± 0.52) respectively.

The mineral composition (mg/100g) of the food samples and the formulated diets are presented in table 2. The results revealed that the amount of sodium (Na) in the samples are within the range of 8.00 ± 0.37 for *M. oleifera*, (7.02 ± 0.64) for *S. bicolor*, (6.58 ± 0.45) for *A. Hypogaea*, (6.04 ± 0.35) for *P. dactylifera* and (4.60 ± 0.22) for *G. max*. These results are within the range of FAO composition table; (14.0) for *S. bicolor*, (6.0) for *A. hypogaea*, (4.0) for *P. dactylifera* and (5.0) for *G. max* (FAO, 2012). The amount of (Na) in all the three diets was considerably low ranging from (0.48 ± 0.32) to (0.73 ± 0.20)

The amount of Magnesium (mg/100g) was considerably high in all the three diets; diet C has the highest amount (22.01 ± 0.46), followed by diet A (20.60 ± 0.87) and diet B (19.60 ± 0.38).

The amount of Mg was low in all the food samples (3.99 ± 0.22) for *M. oleifera*, (2.49 ± 0.05) for *A. hypogaea*, (2.40 ± 0.07) for *S. bicolor* and (2.14 ± 0.25) for *P. dactylifera* respectively. Also, these results were very low compared to that of the FAO food composition table for all the food samples (FAO, 2012). Magnesium is required in over 300 enzymes that use adenosine triphosphate and contribute to DNA and RNA synthesis during cell proliferation (Asuk, 2015).

The amount of Potassium (mg/100g) was found to be higher in diet C (8.08 ± 0.37) followed by diet A (6.71 ± 0.45), *M. oleifera* (6.56 ± 0.28), *P. dactylifera* (6.20 ± 0.22), diet B (5.92 ± 0.92) and *A. hypogaea* (5.74 ± 0.38). All these amount were very low compared to the FAO food composition table and that of F75 and F100 for the management of SAM. The amount of (K) was found to be

low in *S. bicolor* (3.72 ± 0.20) and *G. max* (3.10 ± 0.13). Potassium is very important in the regulation of water and electrolyte balance and acid-base balance in the body as well as responsible for nerve action and functioning of the muscles. Deficiency of potassium leads to muscles paralysis (Akpabio, 2013).

The amount of Iron (Fe) (mg/100g) was found only in the three diets. Diet C (3.93 ± 0.18), diet B (3.39 ± 0.21) and diet A (3.20 ± 0.54). All the food samples contain a negligible amount of Fe in the range of 0.52 ± 0.11 , 0.75 ± 0.21 , 0.85 ± 0.18 , 0.91 ± 0.09 and 0.96 ± 0.14 for *P. dactylifera*, *M. oleifera*, *G. max*, *S. bicolor* and *A. hypogaea* respectively. These values were low compared to FAO food composition table. Iron is essential for the synthesis of hemoglobin and myoglobin, its deficiency results in anaemia (Asuk, 2015).

The result of this study showed that the amount of Calcium (mg/100g) were roughly in the same range of (0.37 ± 0.07) in *P. dactylifera*, (0.64 ± 0.14) in *M. oleifera*, (0.44 ± 0.10) in *G. max*, (0.55 ± 0.11) in *S. bicolor*, (0.47 ± 0.13) in *A. hypogaea*, (0.90 ± 0.48) in diet B, (1.01 ± 0.23) in diet A and (1.20 ± 0.32) in diet C which is the highest in all the samples. Uba *et al.* (2015); Amir *et al.* (2015) and Atasie *et al.* (2009) reported a value of 1.33mg/100g, 5.98 ± 0.01 and 2.28 ± 1.94 mg/100g for *P. dactylifera*, *S. bicolor* and *A. hypogaea* respectively. Calcium along with phosphorus is required for formation and maintenance of bones and teeth and also required in blood clotting and muscle contraction (Asuk, 2015).

The amount of copper (mg/100g) in the samples showed that diet A, B and C contains the higher amount of copper in the same range of (5.40 ± 0.32) in diet B, (5.03 ± 0.18) in diet C and (4.55 ± 0.12) in diet A.

A very little amount of copper was found in *M. oleifera* (0.03 ± 0.03), *G. max* (0.10 ± 0.08) and *A. hypogaea* (0.72 ± 0.15), while copper was not detected in *P. dactylifera* and *S. bicolor*. These findings are in close agreement with that of FAO food composition table; (0.28) for *P. dactylifera*, (0.26) for *S. bicolor* (1.48) for *G. max* and (0.86) for *A. hypogaea* respectively. Copper is an essential mineral required for the proper functioning of organs and metabolic processes and synthesis of haemoglobin (Sadhra *et al.*, 2007).

Vitamins are essential micronutrients for organism's multiple biochemical reactions as their deficiencies affect metabolism in the body. The results of the vitamins content for all the samples and formulated diets were presented in table 4. The results of vitamin A showed that *G. max* (92.03 ± 0.45), *M. oleifera* (73.21 ± 0.38) contains the highest amounts of vitamin A.

All other samples were found to contain appreciable amount of vitamin A. *S. bicolor* (16.00 ± 0.76), *A. hypogaea* (11.01 ± 0.09), *P. dactylifera* (8.71 ± 0.52), diet B (7.00 ± 0.54) diet C (5.42 ± 0.26), and diet A (3.40 ± 0.43) with the lowest amount. Vitamin A plays an important role in bone growth, tooth development, reproduction, cell division, gene expression, and regulation of the immune system (Gafar, 2011).

The results of vitamin C (mg/100g) in all the samples showed that formulated diet C (110.00 ± 0.34) has the highest amounts; followed by diet B (102.39 ± 0.56), *P. dactylifera* (96.66 ± 0.06), diet A (94.58 ± 0.33), *G. max* (78.71 ± 0.20) and *M. oleifera* has (52.82 ± 0.19).

Only *A. hypogaea* and *S. bicolor* contains the lowest amount of vitamin C (16.05 ± 0.65) and (21.15 ± 0.62) respectively. All the food samples contains significant amount of vitamin C than the FAO composition table which reported no or trace amount of vitamin C. Vitamin C also aids in wound healing, bone and tooth formation, strengthening blood vessel walls, improving

immune system function, increasing absorption and utilization of iron, and acting as an antioxidant (Gafar, 2011).

The results of vitamin E (mg/100g) in the samples showed that diet C, diet A, diet A, diet B, *G. max*, *M. oleifera* and *A. hypogaea* contains higher amount of (25.01±0.36), (22.11±0.65), (19.75±0.49), (19.00±0.26), (16.94±0.18) and (15.00±0.09) respectively. While *S. bicolor* (7.53±0.34) and *P. dactylifera* (0.49±0.43) contains the lowest amount of vitamin E. Vitamin E benefits the body by acting as an antioxidant, and protecting vitamins A and C, red blood cells, and essential fatty acids from destruction (Gafar, 2011).

Phytochemicals posses biological functions which include anti-inflammatory, antioxidative, antiviral and anti-carcinogenic properties (flavonoids), some acts as pain relievers and tranquilizers (alkaloids) while some confer protection against platelet aggregation and oxidative damage as a results of free radicals (Fahey, 2005; Okwu, 2005; Adesuyi, 2012). The results of the phytochemical contents (mg/100g) of all the samples were presented in table 3. The results revealed that the *G. max* (47.61±0.46), diet C (40.76±0.56), diet B (39.00±0.72), diet A (30.92±0.35), *S. bicolor* (12.53±0.08), and *A. hypogaea* (11.42±0.21) contain the highest amount of saponins in all the samples.

Moringa oleifera (2.52±0.54) and *P. dactylifera* (1.14±0.13) contains lowest amount of saponins. Shaba *et al.* (2015) reported a value of (1.89±0.12) for *P. dactylifera* similar to the present study. The value for saponins falls within the WHO permissible limit of 48.50mg/100g as recommended, (WHO, 2003). The hypoglycemic effect of saponins is believed to be due to stimulation of pancreatic β -cells, inhibition of glucose transport across the brush border cells of the small intestines and suppression of transfer of glucose from the stomach to the small intestines (Fahey, 2005).

The amount of tannins in the sample show that diet C (12.13 ± 0.48), *P. dactylifera* (11.12 ± 0.18), *M. oleifera* (9.67 ± 1.02) and diet B (4.39 ± 0.49) contain the highest amount of tannins in the samples. *Sorghum bicolor*, *A. hypogaea* and diet A contains negligible amount while tannins are not detected in *G. max*.

The amount of glycosides in the sample showed that all the samples contain significant amount of glycosides with the formulated diet C having the highest amount (31.94 ± 0.18), followed by *S. bicolor* (23.34 ± 0.32), *G. max* (21.56 ± 0.38), diet A (19.63 ± 0.45), *P. dactylifera* (13.87 ± 0.28), diet B (11.28 ± 0.43) and *A. hypogaea* (10.56 ± 0.38). *Moringa oleifera* was found to contain lowest amount of glycosides among all the samples studied (0.53 ± 0.08).

The amount of alkaloids (mg/100g) was found to be considerably high in diet C (32.13 ± 0.23), followed by diet B (23.63 ± 0.58) and *M. oleifera* (20.53 ± 0.15). The amount was found to be low within the same range in *S. bicolor* (9.23 ± 0.43), *P. dactylifera* (8.69 ± 0.83) and diet A (7.33 ± 0.54) respectively. Alkaloid was not detected in *G. max* and a minute amount found in *A. hypogaea* (0.32 ± 0.24). Alkaloids have antimicrobial properties due to their ability to intercalate with DNA of the microorganisms (Kasolo *et al.*, 2010).

A higher amount of steroids (mg/100g) was found in *A. hypogaea* (20.86 ± 0.12) followed by diet C (17.20 ± 0.29) and diet B (12.15 ± 0.84). The amount of steroids was found to be low in *G. max* (8.73 ± 0.02), followed by *M. oleifera* (5.66 ± 0.24), *P. dactylifera* (3.40 ± 0.07) while lowest amount in diet A (1.94 ± 0.45) and *S. bicolor* (1.98 ± 0.28) respectively. Steroids regulate permeability and fluidity of cell membranes and also act as biogenic precursors of growth factors (Kasolo *et al.*, 2010).

The amount of flavonoids were found to be high in all the samples with *P. dactylifera* (84.83 ± 0.23), *G. max* (72.58 ± 0.37) having the highest amount, followed by *A. hypogaea* (31.64 ± 0.58), diet C (29.82 ± 0.19), diet B (20.61 ± 0.28), diet A (18.93 ± 0.39) and *M. oleifera* (7.33 ± 0.54). *S. bicolor* is the only sample that has the lowest amount of flavonoids in all the samples (1.72 ± 0.13). Flavonoids are known to improve cardiac function, decrease anginas and lowers cholesterol levels (Adesuyi, 2012).

The amount of oxalates in the samples was found to be considerably high in diet C (21.20 ± 0.21), diet B (19.39 ± 0.21), *S. bicolor* (14.02 ± 0.41), diet A (12.10 ± 0.38). The remaining samples, *M. oleifera* (9.84 ± 0.06), *P. dactylifera* (8.53 ± 0.48) and *G. max* (3.34 ± 0.47) contain lowest amount in all the samples. Oxalate has been accounted to have negative impact on accessibility of mineral which will prompts assimilation of fundamental minerals in the body particularly calcium by framing insoluble salts (Onyeike and Omubo-Dede, 2002).

The amount of phytate (mg/100g) in the samples was found to be high in diet B (32.17 ± 0.54), followed by diet C (21.73 ± 0.43), *M. oleifera* (14.30 ± 0.13), *S. bicolor* (11.01 ± 0.38) and diet A (9.03 ± 0.13), while the other samples, *P. dactylifera* (4.82 ± 0.41), *G. max* (2.13 ± 0.28) and *A. hypogaea* (1.78 ± 0.02) contain the lowest amount of phytate in all the samples. Phytate in diets decrease the availability of calcium, phosphorus and zinc and decrease the activity of protein, amino acids, and energy (Sebastian *et al.*, 1998).

Amino acids play central roles both as building blocks of protein and as intermediates in metabolism. The results of the amino acids composition of all the samples and the formulated diets were presented in table 5. The results showed that essential amino acids content (g/100g) had higher leucine in *S. bicolor* (9.86), followed by diet C (8.81), *M. oleifera* (8.64), *G. max*

(7.59) and *A. hypogaea* (6.48), while *P. dactylifera* has the lowest amount (3.21) among the samples. Leucine is a great energy source especially during intense athletic performance and other extreme physical activities. It protects against fatigue and regulates the body's glucose levels (Jillian, 2018). The amount of lysine (g/100g) shows that *G. max* (5.89) has the highest lysine content, followed by formulated diet C (5.46), *M. oleifera* (4.35), *A. hypogaea* (3.39) and *S. bicolor* (3.29) while *P. dactylifera* contain the lowest amount of lysine in the samples (1.01). Lysine is a key component in the production of hormones and enzymes and plays an important role in the production of collagen, a substance that is critical in bone, muscle, cartilage, and skin formation. The amount of isoleucine in the samples shows that *G. Max* has the highest amount of (4.58), followed by *S. bicolor* (4.39), diet C (4.06), *M. oleifera* (3.93) and *A. hypogaea* (3.54) while *P. dactylifera* has the lowest amount of isoleucine in the samples. Isoleucine plays a key role in the transport of oxygen from the lungs to the various parts of the body and the production of hemoglobin, which contains iron (Jillian, 2018). The amount of phenylalanine in the samples shows that *G. max* and *S. bicolor* contains highest amount of (4.97), followed by *M. oleifera* and diet C (4.61), *A. hypogaea* (4.43), while *P. dactylifera* has the lowest amount of Phenylalanine (1.06). The amount of tryptophan in the samples shows that *S. bicolor* has the highest amount (1.89), followed by *G. max* (1.31), *A. hypogaea* and diet C (1.21), *M. oleifera* (0.95), while *P. dactylifera* has the lowest amount of tryptophan in the samples. Research studies show that excessive tryptophan, combined with a vitamin B6 deficiency, may lead to the onset and development of bladder cancer (Jillian, 2018). The amount of valine (g/100g) in the samples shows that *S. bicolor* has the highest amount (5.32), followed by *G. max* (4.97), *M. oleifera* (4.34), *A. hypogaea* (4.33) and diet C (4.27), while *P. dactylifera* has the lowest amount of valine (0.70) in the samples. The amount of methionine in the samples shows that *S. bicolor* has the highest amount (2.40) followed by diet C (1.87), *A. hypogaea* (1.76), *G. max* (1.33) and *M. oleifera* (1.17), while *P. dactylifera* contain the

lowest amount of methionine in all the samples (0.80). Methionine is a perfect scavenging agent against oxidative stress, due to its ability to be converted to methionine sulfoxide (Jillian, 2018). The amount of histidine in the samples show that *G. max* has the highest amount (2.43), followed by *M. oleifera* and *A. hypogaea* (2.36), diet C (2.30) and *S. bicolor* (2.17) while *P. dactylifera* contain lowest amount of histidine in the samples (0.83). Histidine is an essential amino acid that is used to form active sites of enzymes (Jillian, 2018). The amount of threonine (g/100g) in the sample show that *S. bicolor* contain the higher amount of threonine (4.00) followed by *G. max* (3.72), *A. hypogaea* (3.66), diet C (3.33) and *M. oleifera* (3.11), while *P. dactylifera* has the lowest amount of threonine in all the samples (1.00). Threonine and Valine presence is an indication that the plant can help in the generation of cells, red and white blood corpuscles, involved in the functioning of the mammary glands and ovaries (Jillian, 2018).

The results of the non essential amino acids (g/100g) in the samples shows that *S. bicolor* has the highest amount of proline (5.89) followed by diet C (3.65), *A. hypogaea* (3.45), *G. max* (3.35) and *M. oleifera* (3.05), while *P. dactylifera* contain the lowest amount of proline in all the samples (0.71). Proline is a most important amino acid as a natural moisturizing factor that brings moisture to the skin (Jillian, 2018). The amount of arginine (g/100g) in the sample shows that *A. hypogaea* has the highest amount of arginine (8.77), followed by *G. max* (7.31), diet C (6.71), *S. bicolor* (4.99) and *M. oleifera* (4.82), while *P. dactylifera* has the lowest amount of arginine in all the samples (1.38). Arginine is an amino acid needed to maintain normal functions of blood vessels and other organs (Jillian, 2018). The amount of tyrosine in the sample shows that *G. max* has the highest amount of tyrosine (3.44), followed by *S. bicolor* (3.27), *M. oleifera* and diet C (3.10) and *A. hypogaea* (2.41) while *P. dactylifera* contains the lowest amount of tyrosine (0.69) in the samples. The amount of cysteine (g/100g) in the sample shows that *S.*

bicolor has the highest amount of cysteine in the sample (2.12), followed by *G. max* (1.51), *A. hypogaea* and diet C (1.33) and *M. oleifera* (0.97), while *P. dactylifera* contain the lowest amount of cysteine in the samples (0.48). Cysteine is easy to be deficient in the infants and synthesized from methionine in the human body (Jillian, 2018).

The amount of alanine in the samples shows that *S. bicolor* contain the highest amount of alanine (7.36), followed by diet C (4.63), *M. oleifera* (4.25), *G. max* (3.41) and *A. hypogaea* (3.34), while *P. dactylifera* contain the lowest amount of alanine in the samples (1.14). Alanine is an important amino acid as it is an energy source for the liver (Jillian, 2018). The amount of glutamic acid in the samples shows that *S. bicolor* contain the highest amount (17.56), followed by *A. hypogaea* (15.29), diet C (14.69), *G. max* (12.41) and *M. oleifera* (11.05), while *P. dactylifera* contain the lowest amount of glutamic acid in the samples (4.39). Glutamate is an amino acid which is most easily used as energy source and contained in various natural foods (Jillian, 2018). The amount of glycine in the samples shows that *G. max* contain the highest amount (4.37), followed by *M. oleifera* (3.94), diet C (3.75), *S. bicolor* (3.70) and *A. hypogaea* (3.52) while *P. dactylifera* contain the lowest amount of glycine (1.00) in the samples. The amount of serine in the samples shows that *S. bicolor* contain the highest amount (4.70), followed by *G. max* (3.89), *A. hypogaea* (3.62), diet C (3.08) and *M. oleifera* (2.86), while *P. dactylifera* contain the lowest amount of serine in the samples (0.86). The amount of aspartic acid in the samples shows that *G. max* contain the highest amount (9.86), followed by *A. hypogaea* (9.68), *M. oleifera* (9.06), diet C (8.74) and *S. bicolor* (8.25), while *P. dactylifera* contain the lowest amount of aspartic acid in the samples (2.98). Aspartic acid is an amino acid which is most easily used as an energy source.

The result of the anthropometric measurement (initial and final weight and MUAC) of children participated in the study is presented in table 6. The result shows that there is an average increase in weight by 0.94 kg and MUAC increase of 0.44, which is an indication that the formulated therapeutic diet has the essential nutrients, vitamins, mineral and phytochemicals essential for proper growth and development of children which is effective in treating or management of severe acute malnutrition in children.

The mean average of the height and head circumference of the children that participated in the study are presented in table 7. The mean height of all the 20 children was $(70.75 \pm 1.53 \text{ cm})$, while the mean head circumference was (44.28 ± 0.58) .

Based on correlation analysis between the diet used in the study and the parameters analyzed, high amount of proximate parameters (protein, lipid and carbohydrates), minerals (Ca, Mg and Na), vitamins (vitamin C and E) may be responsible for an increase in weight and MUAC of children that participated in the study.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 SUMMARY

The research conducted brings out the following significant findings:

1. The formulated therapeutic foods contained substantial amounts of proteins, minerals, essential amino acids, vitamins required for proper growth and developments of individuals.
2. The amount of lipid (30.80) and proteins (22.82) in the formulated therapeutic food C is higher than that of the reference F-75, F-100, Plumpy' Nut RUTF procured by UNICEF.
3. The amount of energy found in the food (489KCal) is less than that of the RUTF (520-550KCal).
4. There is a strong correlation between weight/MUAC and levels of lipid, minerals and vitamins which may be responsible for an increase in weight/MUAC after consumption of the therapeutic foods for 14days.

5.2 CONCLUSION

This study has shown that our locally available foodstuffs like date palm, moringa leaves, soybean, sorghum and groundnut formulated in different ratios (specifically formulated diet C) contained the essential nutrients, vitamins, proteins, minerals, amino acids and phytochemicals and may have a potential for use in the management of severe acute malnutrition.

5.3 RECOMMENDATIONS

1. Government should encourage establishment of industries for the production of therapeutic foods in Nigeria using locally available foodstuffs to overcome the incidence of malnutrition in the country.
2. Government and the community should create awareness to mothers especially in rural areas on how to prepare therapeutic foods at home using the locally foods for their children under aseptic and hygienic condition.
3. More effort should be made on poverty alleviation, means of production of food can be made available and also mothers should be properly educated on the importance of exclusive breastfeeding, also an acceptable and affordable health service should be provided to the mothers and children.
4. Another area is, government should provide equipment and facilities in our clinics, health centers, and hospital for more effective treatment of children with protein energy malnutrition.
5. Maternal education should be given priority because education has a lot of role to play as educated mother not only could take better care of child nutrition but could also help prevent infant morbidity and mortality to a large extent.
6. The hospital managements should allow and give the necessary support for the conduct of such type of research so as to find the lasting solution for this serious condition that is taking lives of children on daily basis.
7. Further research should be conducted on other available foodstuff and extended to other paediatric hospitals within the northern part of the country.

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APPENDICES

APPENDIX I: List of Equipment

S/N	EQUIPMENTS	MANUFACTURERS
1	Atomic Absorption Spectrophotometer (AAS)	Bulk Scientific (Model 205) USA
2	Flame Photometer (PFPT)	JENWAY UK (Model 8515)
3	Applied Biosystems PTH Amino acid Analyzer	Applied Biosystems Inc. Foster City CA. 94404. USA. Model I20A
4	Genlab Incubator	Genlab Limited Tenhouse Lane Widnes, Cheshire.
5	Electronic weighing balance	(Adams equipment)
6	Flask shaker (SFL)	Bibby Scientific Limited UK
7	Spectrophotometer 6300 JENWAY	Bibby Scientific Limited UK
8	Orbital shaker (SSLI)	Bibby Scientific Limited UK
9	Water bath	Nickel Electro Limited England (clifton)
10	Murple Furnance	Vecstar Ltd (Chesterfield UK)
11	Vortex Mixer (SAT)	Biocote Bibby Scientific Limited UK
12	Centurion Scientific K3 Series.	(UK) Model: K241
13	Recirculating Cooler (SRC4)	Bibby Scientific Limited UK

APPENDIX II: List of Reagents

S/N	REAGENTS	MANUFACTURERS
1	Ammonium Hydroxide	Griffints George Lab. Equipments UK
2	Hydrochloric acid	BDH Limited Poole England
3	Sodium Tungsten	BURGOYNE Laboratory reagent India
4	Ferric chloride	BURGOYNE Laboratory reagent India
5	Olive oil	LOBA Chemie Pvt Ltd. Mumbai
6	Ethanol	BDH Limited Poole England
7	Concentrated Tetraoxosulphate VI acid	BDH Limited Poole England
8	Chloroform	Prolabo Pairs Limited UK
9	Acetic anhydride	BDH Chemical Poole England
10	Bismuth potassium iodide	BDH Limited Poole England
11	Fehling solution	Nino Scientific Company Ltd
12	Phosphomolybdic acid	KEM Light Laboratory Pvt Ltd. India
13	Orthophosphoric acid	Guangdong Guanghua Sci. Tech Co., Ltd China
14	Acetic acid	BDH Limited Poole England
15	Sodium bicarbonate	LOBA Chemie Pvt Ltd. Mumbai
16	Methanol	Sigma Aldrich France
17	Diethyl ether	LOBA Chemie Pvt LTD Mumbai
18	n-butanol	BDH Limited Poole England
19	Sodium chloride	LOBA Chemie Pvt LTD Mumbai
20	Phenolphthalein	Nino Scientific Company Ltd

21	Calcium chloride	Guangdong Guanghua Sci. Tech Co., Ltd China
22	Potassium per manganate	KEM Light Laboratory Pvt Ltd. India
23	n- hexane	LOBA Chemie Pvt Ltd. Mumbai
24	Trichloroacetic acid (TCA)	LOBA Chemie Pvt Ltd. Mumbai
25	Oxalic acid	LOBA Chemie Pvt Ltd. Mumbai
26	Dichlorophenol indophenols	KEM Light Laboratory Pvt Ltd. India
27	Potassium hydroxide	Scientific Ltd Northampton UK
28	Nitric acid	Qualikems Laboratory Reagents
29	Sodium hydroxide	BDH Limited Poole England
30	Copper II teraoxosulphate VI	Nino Scientific Company Ltd
31	Potassium tetraoxosulphate VI	Scientific Ltd Northampton UK
32	Methyl blue indicator	LOBA Chemie Pvt Ltd. Mumbai
33	Methyl red indicator	Scientific Ltd Northampton UK
34	Bromocresol green indicator	Nino Scientific Company Ltd
35	Sodium tetraoxosulphate VI	KEM Light Laboratory Pvt Ltd. India
36	Selenium oxide	LOBA Chemie Pvt Ltd. Mumbai

APPENDIX III: Consent Form

Kindly go through the following information and sign on an agreed statement to consent the participation of your child for the research “Production of therapeutic food from available foodstuff in Nigeria: A strategy for tackling the menace of acute malnutrition in children under five years”. Having been selected as one of the participant in the research, you will be required to fill the questionnaire. You may wish to participate fully in the study and at any point you may withdraw freely with no reservation what so ever.

I agree that my child will participate in the study. Agree ☐ Disagree ☐

I agree that my child will be given Ready to Use Therapeutic Diet (DAMA RUTF) once in a Day. Agree ☐ Disagree ☐

I agree that blood and urine will be taken from my child for analysis and also his weight. Agree ☐ Disagree ☐

Parent/Guardian -----

Witness -----

Researcher -----

APPENDIX IV: Questionnaire

Please answer each of the questions below to help the researcher have a better understanding of your needs.

Serial Number:

1. Age:
2. Sex:
3. Marital status:
Single married divorced
4. Income:
Rate: Low Moderate High
5. Educational information of the parent:
Dad: None Primary Secondary Tertiary Others
Mom: None Primary Secondary Tertiary Others

FOODS	NEVER	<1/WK	1 TO 3x/WK	4 TO 6x/WK	1x/DY	2 TO 3x/DY	4 or >/DY
KununTsamiya							
KununGyada							
Koko(pap)							
Beanscake/ Moimoi							
Diary							
Tea							
Bread							
Waina							
Plantain							
Chips							
Egg							
Noodles							
Beans porridge							
TuwonMasara							
TuwonDawa							
Jolop Rice							
Fried Rice							
Yam & egg sauce							
Yam & stew							

Vegetables							
Drinks							
Rice & Stew							
Couscous							
Spaghetti							
TuwonShinkafa							
Fruits							
Gari							

6. Disease condition: Acute Severe
7. Have you ever take any RUTF: YES NO
8. If Yes for how long:

APPENDIX V: Ethical Approval



KANO STATE OF NIGERIA
MINISTRY OF HEALTH
2nd & 3rd Floor, Post Office Road,
P.M.B. 3066, Kano.

Commissioner: 08023337417
Permanent Secretary: 08096619985
website: www.kanostateministryofhealth.gov.ng

Ref: _____

MOH/Off/797/T.I/360

Date: _____

2nd May 2017

*Abdulrazaq Sani Yahaya, Dalaram Muhammad,
Ahmad Alhassan, Mariya Yarima Abubakar*
Department of Biochemistry,
Faculty of Basic Medical Sciences,
Bayero University,
Kano.

RE: APPLICATION FOR ETHICAL APPROVAL

Reference to your letter dated 26th April 2017 on the above request addressed to the Chairman Ethics Sub-Committee of Health Operational Research Unit of the Ministry requesting for ethical approval to conduct MSc research at Murtala Muhammad Specialist Hospital, Hasiya Bayero Paediatric Hospital and Sharada Paediatric Clinic, Kano.

2. The research entitled "*Potentials of Locally Produced Ready-To-Use Therapeutic Food on Management of Malnutrition in Children*" is for the award of Masters of Science Degree in Biochemistry (MSc Biochemistry).

3. In view of the foregoing, I wish to convey the Ministry's approval for you to conduct the research at the above mentioned hospitals in Kano.

4. You are also requested to share your findings with the Ministry of Health, Kano.

5. Best Regards

Abdullahi Liti Gwarzo

DPRS

Secretary (ORAC)

For: Honourable Commissioner

APPENDIX VI: Weight measurements before and after the diet administration for 14 days.

SUBJECT	BOYS				GIRLS			
	Years (month)	Initial Weight (Kg)	Final Weight (Kg)	Percentage of Weight Gain (%)	Years (month)	Initial Weight (Kg)	Final Weight (Kg)	Percentage of Weight Gain (%)
A	24	7.00	7.80	11.43	24	6.80	7.00	2.94
B	12	6.50	7.20	10.77	09	2.10	3.50	66.67
C	18	6.50	7.60	16.92	14	5.30	6.10	15.09
D	13	4.00	4.30	7.50	14	6.00	7.30	21.67
E	24	5.70	7.20	26.32	30	7.00	7.80	11.40
F	18	3.20	4.30	34.40	20	8.00	9.20	15.00
G	18	5.00	6.20	24.00	14	5.00	6.20	24.00
H	21	7.50	8.00	6.67	12	5.70	5.90	3.51
I	21	6.00	7.40	23.33	30	6.50	7.10	9.23
J	30	5.40	6.80	25.93	36	5.92	7.10	19.93
	TOTAL			18.73				18.95

APPENDIX VII: Mid-Upper-Arm circumferences measurements before and after the diet administration for 14 days.

SUBJECT	BOYS				GIRLS			
	Years (month)	Initial MUAC (cm)	Final MUAC (cm)	Percentage of MUAC Gain (%)	Years (month)	Initial MUAC (cm)	Final MUAC (cm)	Percentage of MUAC Gain (%)
A	24	12.50	13.10	4.80	24	11.00	11.50	4.55
B	12	12.00	12.00	0.00	09	8.50	9.00	5.88
C	18	10.80	11.10	2.78	14	11.00	11.30	2.72
D	13	9.60	9.80	2.08	14	10.50	11.20	6.67
E	24	10.00	10.50	5.00	30	11.00	11.00	0.00
F	18	7.80	8.10	3.85	20	13.00	13.40	3.08
G	18	10.00	10.20	2.00	14	11.00	12.80	16.36
H	21	12.00	12.20	1.67	12	8.50	9.00	5.88
I	21	12.00	12.40	3.33	30	10.00	11.00	10.00
J	30	10.50	10.80	2.86	36	11.00	11.20	1.8
	TOTAL			2.83				5.69