# EFFECT OF SPROUTING ON THE FORMULATION OF WEANING DIETS FROM BAMBARA NUT (*Vigna. subterranean*) AND SORGHUM (*Sorghum bicolor*)


## ABSTRACT

Protein energy malnutrition (PEM) accounts for over 50 % mortality among weaning children globally. Fortification and formulation had been used to supply some of the nutritionally deficient nutrients in diets of malnourished children. Sorghum is generally used as weaning foods in developing countries but is deficient in protein while Bambara nut is rich in protein and can provide the nutrients lacking in sorghum. This study focused on the effect of sprouting on formulation of weaning food from sorghum and Bambara nut flour blends. The sorghum and the Bambara nut were sprouted separately for seven days and sprouts of each day were obtained. The sprouts with maximum nutrients and lowest antinutrients were selected for diet formulation. Proximate and anti-nutrient compositions of the sprouts were determined using the AOAC methods and amino acid composition was carried out using amino acid analyser (Model 120 A). The functional properties of the blends were determined using standard analytical methods. Forty (40) Wister strain weaning albino rats of both sexes weighing between 34 – 55 g were randomly distributed into eight (8) groups (A to H) of 5 rats each. The animals were administered formulated diets and water ad libitum for 28days. Group A rats were fed only sorghum (control) and Group B only Bambara, while groups C, D, E and F were fed sorghum supplemented with 5 %, 10 %, 15%, and 20% Bambara nut, respectively. Group G was fed normal rat pellets and H was fed cerelac. The weight of the rats was measured weekly. Haematological parameters were determined using auto-haematological analyzer (Mindray BC-5300). Liver and kidney function tests were carried out using Teco and Agape diagnostic assay kits. Nutrient compositions were highest on day 5 for sorghum and on day 4 for Bambara nut which also coincided with the days when the anti-nutrients were found to be lowest. The group fed on 80 % sorghum + 20 % sprouted Bambara nut had significantly higher ($p<0.05$) weight (15.28 %) compared to the other groups (15 % supplemented (13 %), 10 % supplemented (11.33 %), 5 % supplementation (6.23 %) 100 % sorghum (3.44 %), 100 % Bambara (5.35 %), rat chow (12.44 %) and ceralac (15.09 %). Haematological analysis showed that all parameters were within normal range. However, PCV, Hb, MCV and MCH were significantly ($p<0.05$) higher in the group fed with cerelac compared to other groups while there was no significant difference ($P>0.05$) in the RBC of the groups fed 20 % Bambara nut supplemented diet and cerelac. The serum enzyme activities (AST, ALT and ALP) determined were higher in rats placed only on sorghum and Bambara nut flours and lowest in the rats placed on cerelac diet but were all within normal range. The renal function indices were significantly ($p<0.05$) higher in the cerelac group. Based on the nutrient content and growth performance outcome there was no significant difference ($p<0.05$) between cerelac and the 20 % Bambara nut supplemented diet. Hence, this work suggests that blend of 80 % sprouted sorghum and 20 % sprouted Bambara nut may be good for the formulation of weaning diets.

# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1 Background to the study

Nutrition is the study of nutrient in food/diet and the relationship between diet/food, health and diseases (Khan *et al.,* 2017). The energy needed by the body for growth and maintenance and for its proper functions is provided from the food. The US national library of medicine (2017) defines nutrition as the science that interprets the interaction of nutrients and other substances in food in relation to maintenance, growth, reproduction, health and disease of an organism. It includes food intake, absorption, assimilation, biosynthesis, catabolism and excretion". Adequate energy and nutrient (nutrition) empower the cell for its function (Rodriguez *et al.,* 2016). On the other hand, the science of nutrition also deals with the abnormalities and diseases associated with nutritional deficiency (Pradhan *et al.,* 2019).

A good nutrition should contain all the nutrients in the right amount (proteins, carbohydrate, fats and oil, minerals and vitamins). There is a correlation between nutrition and infectious diseases, when the essential nutrients required in a diet are lacking the immune system is compromised.

According to World Health Organization (2020b), malnutrition denotes insufficiencies, excesses or imbalances in a person's intake of nutrients. Malnutrition can be categorised into three major groups or forms, namely undernutrition, overnutrition (overweight and obesity) and bad eating habit. The first form undernutrition deals with inadequate intake of nutrient which can lead to conditions such as stunting (low height for age) when growth is permanently impeded, wasting (low weight for height) dangerously thin for their height, underweight (low weight for age) and micronutrient deficiencies (a lack of important vitamins and minerals). The second form of malnutrition is overweight and obesity

(consumption of diets rich in excess calories than the body's metabolic needs) or diets rich in excess fat, salt and sugar may trigger the risk of diseases such as (heart disease, stroke, diabetes and cancer) (Hawkes *et al.,* 2017). The third form is bad eating habit majorly concerned with eating junk foods or eating at an inappropriate time. It can also be considered as unhealthy eating habits with health consequences.

Protein-energy malnutrition (PEM) is a form of malnutrition defined as a state of consistent inadequacy of food and nutrient intake especially protein to meet body requirements, leading to alterations in body weight and composition hence, compromised functioning of the body (Adejumo *et al*., 2019; Skipper, 2012 and Marshall, 2016). The state of malnutrition are in varying degrees which could be mild, moderate or severe (Verhagen *et al.,* 2013). It is a major threat to childhood health in developing countries world-wide (Cavuilati, 2018). In Nigeria it has shown that over 50 % of children and infant mortality is as a result of malnutrition (National Bureau of Statistics (2018). The statistics is higher in developing countries like Nigeria, Ghana, Mali, Kenya, Togo and Cote d ivoire (James *et al*., 2018).

Weaning period is a crucial time of transition (6-24 months) during which the diets of infant is changed gradually from liquid breast-milk to semi solid food (Bintu *et al*., 2017). In Nigeria, traditional weaning foods are basically pap made from sorghum, millet and corn which are mono-cereal grain products lacking in the essential amino acids (Bintu *et al*., 2017). Often, in African countries, weaning foods are characterized by bulk and indigestibility and the young infants do not have the ability to digest a wide variety of foods due to their digestive and excretory systems (Van der Merwe *et al.,* 2007). As they grow, they gradually develop the ability to chew, swallow and digest a wide range of foods (Oyeyinka, 2016). The ability to transfer solid foods from the front of the tongue to the pharynx is a milestone developed at about the age of 6 months (Pridham, 1990).

In addition, locally processed weaning foods are inadequate in essential nutrients hence even when taken are unable to protect the body from infection and diseases (Nnam, 2001). Sorghum like maize and millet is one of the major grains used in the preparation of traditional weaning food in Nigeria. It is rated very high on the staple food table as one of the most important food crops after wheat, rice, maize and barley especially for the poor people in Africa (Raihanatu *et al.,* 2011). Sorghum is a major source of carbohydrate, vitamins and minerals. However, it is sad to say that sorghum products contain very low protein and are deficient in basic amino acids such as (lysine, threonine, tryptophan) required for weaning diets (Wakil and Kazeem 2012, Bello *et al.,* 2017).

Sorghum-based foods contain tannins and phytates which interact with proteins, vitamins, and minerals thereby making food indigestible and limiting the bio-availability of their nutrients (Wakil and Kaseem 2012 and Modu *et al*., 2014).

Bambara nut is leguminous seed rich in nutrients such as protein, carbohydrate, fibre, vitamins and minerals. It does not require fortification and has been described as a complete food (Mbata *et al.*, 2009, Ibrahin and Ugunwusi, 2016). The cultivar has a potential for attainment of food security and poverty alleviation (Ibrahin and Ugunwusi 2016). However, its bulky nature and high anti-nutrient factors such as tannins, phytic acid and enzyme inhibitors have made it inadequate for weaning food formulation (Murevanhema and Jideani, 2013). In order of importance Bambara nut has been ranked as the third most important grain legume, after groundnut (*Arachis hypogaea L*.) and cowpea (*Vigna unguiculata*) in semi-arid Africa, but has been underutilized for decades (Rachie and Silvester, 1977). Some of the factors accounting for the underutilization of Bambara include long cooking time which places high demand on fuel consumption. For instance, a time lag of 45-60 minutes is required to boil fresh Bambara nut and about 3-4hrs is required to boil the dried seed when compared to other legumes like cowpea and

pigeon pea that are cooked within 40minutes to 1hour: 30 minutes (Ibrahin and Ogunwusi 2016). In addition to the above, the beany flavour and poor de-hulling capabilities are other factors responsible for its underutilization.

The name Bambara nut was derived from the Bambara tribe of Mali (Yao *et al.,* 2015). It is a popular cultivar in Nigeria known by different names. The Igbo and Igala tribes call it 'okpa', the Hausas call it 'gurjiya'. In Yoruba it is called 'epa-roro', the Nupe people call it 'kpere' and the Idomas call it 'ipeyi'. It is widely cultivated due to its drought resistance and ability to thrive in any kind of soil. Bambara is eaten by many people in Nigeria. The seeds are either boiled or roasted and eaten as snacks, the flour is made into cake, porridges and 'okpa' which are common delicacies eaten by those in the eastern part and middle belt of Nigeria.

In view of the high cost of milk from animals, plant seeds (legumes) are mostly used as substitute for high protein (Bello *et al.,* 2017). A number of studies have shown the prospects of combining plant proteins with cereal flour to produce composite for example, sprouted sorghum and sprouted pumpkin seeds have been used for formulation to solve protein –energy malnutrition (Bello *et al.,* 2017). Also, Bambara nut has been used in feed formulation with soya beans, sweet potato and cray fish (Akaninwor and Okechukwu, 2006).

**Plate I: *Sorghum bicolor* (Source: Anonymous)**

Sprouting is the process by which seeds or spores are induced to germinate or put out shoots mainly for the purpose of consumption or commercialization. Some seeds are food sources but are not easily digested when consumed because they contain some anti-nutritive factors like tannins, phytates, oxalates and others which inhibit nutrient availability. Sprouting reduces these anti-nutrient factors and improve bioavailability of the nutrient, sprouting also improve polyphenols and phenolic compounds of grains and legumes which on consumption provides the body with antioxidants that protect the system against reactive oxygen species (Nyau *et al*., 2017). By sprouting, vitamins (B-complex) and sugars are released, minerals are liberated, dietary fibre, free amino acids and proteins are generated. In the same vein, bulk is reduced, digestibility is improved, flavour is improved and cooking time is reduced (James *et al*., 2018). It will be of interest to explore sprouted Bambara nut and sprouted sorghum in the formulation of weaning diets.

## 1.2 Statement of Research Problem

Protein-energy malnutrition (PEM) has been identified to occur mostly in weaning children and infants especially those under the age of five years. This is the case in Nigeria, where many children whether in the villages or townships are vulnerable to diseases due to inability to meet with their nutrient needs. One of the main factors responsible for protein- energy malnutrition is poor weaning practice and inability of parents and caregivers to afford the commercially available weaning formula. Most of the locally processed weaning foods are deficient in micronutrients such as vitamins, iron, potassium, copper, sodium etc. The commercially processed weaning foods which contain most of the required nutrients are very expensive and the cost is beyond the reach of a low-average income earner. The global economic recession, insurgency and pandemics such as COVID-19 and others are some of the factors that have exacerbated the plight of the general populace and in particular those of low socio-economic status as most of the affected persons are unable to meet their basic needs. Leguminous seeds generally are high in proteins but most often are neglected and are poorly utilized or underutilized. This is because their bulky nature makes them indigestible and therefore inadequate for weaning food formulation. The presence of anti-nutrients like oxalates, phytate and tannins is a major constraint to use of plant seeds in infant food formulation. Another major challenge of the locally prepared weaning foods is that they are low in proteins and micro nutrients and hence do not meet the nutritional needs of the weaning child.

### 1.3 Justification for the Study

Bambara nut is cheap and can be easily grown. It is a complete food composed of (24-34 %) protein, (30-50 %) carbohydrate. The protein content of sorghum and its products is low and deficient in some amino acids especially (lysine and methionine) and therefore needing fortification which can be provided by Bambara nut which is rich in protein and these essential amino acids. Supplementation with protein from Bambara nut will reduce

the dependence on commercially produced weaning foods and increase the use of underutilized local staples. Soaking, sprouting and cooking of Bambara nut and sorghum will improve their qualities. Sprouting is a physical modification method generally acceptable in food processing unlike most chemical methods. Sprouting will be advantageous in the weaning food processing as it reduces the problem of bulk, removes anti-nutrient and increases protein content and so their qualities is improved. Knowledge on the use of sprouted Bambara and sorghum as a potential weaning food is very low. This therefore has made this study necessary.

## 1.4 Aim and Objectives of the study

### 1.4.1 Aim

The aim of this study is to evaluate the effect of sprouting on the formulation of weaning diets from Bambara nut and sorghum.

### 1.4.2 Objectives of the study

The specific objectives of this study were to determine:

i. the best sprouting duration for Bambara nut and sorghum to be used for formulating the blend.
ii. the proximate composition of sprouted Bambara nut and sorghum
iii. the functional properties of sprouted Bambara nut and sorghum flour
iv. the ratio of sprouted Bambara nut/sorghum flour blend for weaning;
v. the effects of feeding the formulated diet on the growth performance, biochemical and haematological indices in rats.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Weaning and Malnutrition in infants and children

Weaning period is a crucial time of transition (6-24 months) during which the diets of infant is changed gradually from liquid breast-milk to semi solid food (Bintu *et al*., 2017). According to WHO, (1998), weaning is the mechanism by which a baby gradually becomes used to consuming family or adult foods while becoming less reliant on breast milk. The process varies by culture and is often regulated by the individual requirements of the child. Since healthy babies of weaning age grow and develop at a rapid rate, it's critical to ensure that they get enough of the right kind of food (Cushing, 1989). Weaning may be mother-led (planned) or infant-led (unplanned) which is natural. When it is mother-led or planned the child is stopped from breastfeeding. Situations like mastitis, breast engorgement, not having enough milk, baby's growth concern, working women, new conception, teething causing sore nipples and so on can cause planned weaning. While in natural weaning an infant naturally starts accepting complementary feeds due to the increase in demand of nutrition. This process of natural weaning takes between 2–4 years (Sugarman and Kendall-Tackett, 1995).

Weaning practice is a major factor responsible for malnutrition in infants and weaning children. Maintenance and enhancement of healthy living standard among infants and weaning children entails proper weaning practice. According to WHO, (2020) infant and young child feeding is a key area to improve child survival and promote healthy growth and development. The first 2 years of a child's life are particularly important, as optimal nutrition during this period lowers morbidity and mortality, reduces the risk of chronic disease, and fosters better development overall. Improper practices that affect children health include introducing weaning at less than two months of age, mother being pregnant,

insufficient breast milk, wish of husband, mother and relations advice (Ezenduka *et al.*, 2018). Research conducted by Uwaegbute, (1991) showed that the Yoruba, Ibos and Hausa groups used cereals, legumes, roots and tubers as weaning foods but the Hausas used more cereal products. The cereal paps offered to children by Hausa mothers had higher protein values than those given by Yoruba and Ibo mothers, probably because of better processing procedures. Most of the weaning foods are nutritionally deficient and do not meet the nutrient requirement of weaning children.

Survey conducted in Ibadan South-West Nigeria revealed that pap was the most frequently given weaning food to the infants. Chocolate beverages, natural fruit juice and soft drinks were the most commonly given drinks. Over 57 % of mothers sweetened pap with sugar. Forty seven percent of mothers added glucose to children's drinking water. Over two-third (64.9 %) of children ate biscuits several times a day. Soft drinks, commercial fruit juices and squash were consumed by 16.1 %, 9.6 % and 7.7 % of the infants respectively on a daily basis. This practice exposes many infants to highly cacinogenic diet at an early age which has harmful effect on the body and teeth of these children (Bankole *et al.*, 2006).

During the important time of weaning, improper practices such as these lead to malnutrition in infants and weaning children especially in the developing countries like Nigeria, India, Somalia and a host of other low-income countries (Rathore *et al.*, 2016).

Malnutrition in all its forms generally depicts three broad groups of conditions; which are undernutrition, overnutrition and ‘’micro-nutrient deficiencies’’. It is a deviation from adequate and optimum nutritional status which can occur in infants, children and adults (WHO, 2020). The first type of malnutrition which is undernutrition or undernourishment are of two main types; protein-energy malnutrition and dietary deficiencies. Protein-energy malnutrition are of three severe types; kwashiorkor (a state of lack of protein), marasmus (a lack of protein and calories) and an intermediate state known as marasmic-

kwashiokor. The second type of undernutrition is micronutrient deficiencies; a condition arising from a lack of minerals (iron, iodine etc) and vitamins like vitamin A (Awuchi *et al.,* 2020). There are 4 broad sub-forms of undernutrition; stunting, wasting, underweight and micronuorient deficiencies (WHO, 2020).

It is estimated in 2018 that 21.9 percent or 149 million children under age 5 were stunted, 7.3 per cent or 49 million children under age 5 were affected by wasting, and 5.9 per cent or 40 million children were underweighted globally (World Health Organization. (2020). UNICEF/WHO/The World Bank Group joint child malnutrition estimates). Childhood malnutrition has been a major concern in public health world-wide (UNICEF 2018, de Groot *et al.,* 2017). According to WHO (2019), Good nutrition allows children to survive, grow, develop, learn, play, participate and contribute – while malnutrition robs children of their futures and leaves young lives hanging in the balance. The implication therefore is that good nutrition is advantageous to childhood growth while malnutrition is disadvantageous to childhood growth. The World Health Organisation (WHO) has used some indicators to characterise childhood malnutrition, these indicators include underweight, stunting, wasting and overweight. The indicators are used as tools to measure the nutritional imbalance emanating from undernutrition (underweight, stunting, wasting) and overweight (De Onis *et al.,* 2019).

Stunting for instance is a resultant effect of protracted periods of malnutrition and exposure to contagious diseases; it is evident that over 70 % of stunting commences before 2 years of age (Leroy and Frongillo, 2019). Generally, children at early childhood have the same growth rate potential globally if the right conditions for growth such as adequate feeding and protection from infectious diseases are met (UNICEF 2018, WHO, 2019). According to Dewey and Begum 2011, deficits in growth are a strong pointer of a living standard of a people. When such early life deficits occur, the later effect could pose challenges leading

to poor achievement and performance in their later life. The consequences may be low living standards. For instance; children who are stunted in early life are known to have reduced mental growth as their brains may never develop to their full cognitive potential, other effects may be difficulty in learning and comprehending what is being thought leading to poor school achievement and in general lower output and therefore their financial income at adulthood may be meagre, hence they are unable to compete with their counterparts in the society they find themselves.


**Plate II: Stunted Children in India (Source: Anonymous)**

Wasting in children is also a lethal case of malnutrition in early childhood which originates from poor nutrition and/or disease. The brunt of wasting is weakened immunity in children, susceptibility to long term developmental interruptions, and greater risk of mortality, predominantly in severe wasting conditions survival of these children is dependent on quick feeding interventions, urgent treatment and care. Statistically, about 49 million children were affected by wasting and close to 17 million were severely wasted worldwide (UNICEF, WHO 2018).

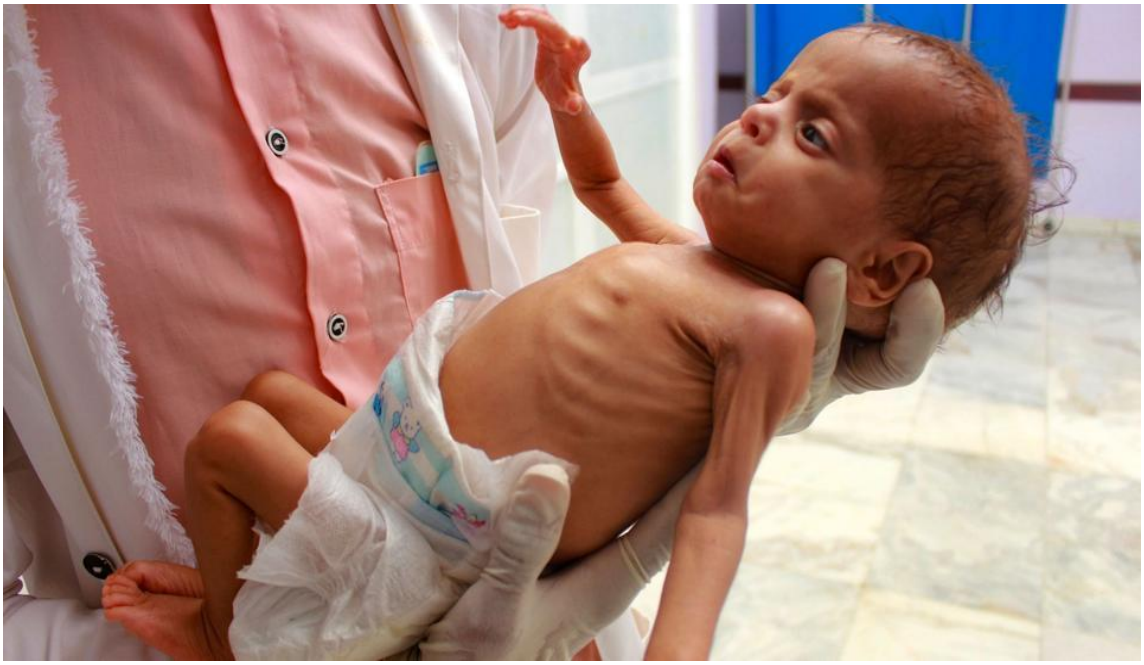

**Plate III: Yemeni child suffering from wasting during the covid 19 pandemic. Source; Anonymous**

Underweight is a type of undernutrition characterized by low weight compared to the age of an individual. According to UNICEF, (2018) underweight is an acute and chronic malnutrition which is a measure of a combination that can occur as a result of stunting or wasting or both.


**Plate iv; underweight child (Source; Anonymous)**

Undernutrition can also result in micronutrient deficiencies; this is defined as a lack of essential vitamins and minerals required in small amounts by the body for proper growth and development (Ritchie and Roser, 2017).

Some of the essential micronutrients include, iron, zinc, calcium, iodine, vitamin A B-vitamins, and vitamin C. The adverse effect of micronutrient deficiency includes poor physical and mental development in children, vulnerability or aggravation of disease, mental retardation and general loses in productivity and potential.

Micronutrient deficiency is often termed hidden hunger, because the effect is not always visible. Iron is critical in the development of motor and cognitive function. The consequences of iron deficiency are more among children and pregnant women and it is the major cause of anemia (low hemoglobin concentration). It is estimated that 43 % of children under age 5 and 38 % of pregnant women are affected by anemia (Stevens *et al.*, 2013). Deficiency in zinc can lead to loss of appetite, stunted growth, and delayed healing of wounds and diarrhea. Also, Iodine deficiency can result in Enlarged thyroid glands (goiters), decreased production of thyroid hormone, growth and development issues. The role of vitamins in human nutrition cannot be overemphasised. Vitamin A is very important to the eye. Dry eyes, night blindness and increased risk of infection has been linked to Vitamin A deficiency (WHO, 2011). According to the US Department of Health and Human Services (2011), another vitamin that is very important especially to children and pregnant women is Vitamin D. It is a nutrient required for good health. It helps the body to absorb calcium, one of the main building blocks for strong bones. Together with calcium, vitamin D helps protect body from developing osteoporosis, a disease that trigger leakage of calcium from bones and as well weakens the bones leading to bone breakage. Furthermore, vitamin D is needed for other functions like boosting the immune system to fight invading bacteria and viruses, movement of muscles and transmission of messages

from nerves to the brain. Other vitamins that are very important in human nutrition in which their absence has an adverse effect on pre-school children includes vitamins B and C.

**Plate V: Micronutrient Deficient Child (Source; Anonymous)**

Another type of malnutrition is over-nutrition which is characterised by overweight and obesity. It is defined as overconsumption of nutrients and food to the point at which health is adversely affected.

Overnutrition can develop into obesity which increases some risks health conditions, such as cardiovascular diseases, hypertension, cancer, and type-2 diabetes. Reports from UNICEF and WHO 2019, indicates that in 2018 more than 40 million children were over-weight globally heightening the population by 10 million from what it was in year 2000. Children suffering from overweight and obesity a case of (overnutrition) is shown in plate 5.0.


**Plate VI: Children Suffering from Overweight and obesity.**
**Source: (Anonymous)**

### 2.1.1 Protein-energy malnutrition

The word protein-energy malnutrition (PEM) relates to a group of associated disorders including marasmus, kwashiorkor, and an intermediary state of marasmus-kwashiorkor (Atassi *et al.,* 2019). PEM is noted mostly during the crucial phase of transition when children are weaned from liquid breast milk to semi-solid food. It is important to supply nutritionally balanced foods to the children at this stage in addition to the breast milk to enable the body meet its nutritional demands (Arise *et al.*, 2014). PEM at infancy is characterised by diverse deficiency diseases and all the developing countries are at risk of having more children with PEM (Ahmed *et al.,* 2019).

Patients suffering from PEM may be deficient in vitamins, essential fatty acids and trace elements all of which culminate to disease condition (Saadat *et al.,* 2020 and Ahmed *et al.*, 2019). For instance, the essential fatty acids, (linoleic and linolenic acid) synthesis are impaired. This is because the metabolism of lipid is generally altered (Santos *et al.*, 2012). Other negative effects of PEM are low serum zinc level in pre-school children, and

susceptibility to infection as a result of immature immune system (Batool *et al.*, 2012). When diets of children are low in protein kidney development is affected and that in the same vein can impair formation of blood vessels. Manifestation of PEM can be either moderate acute malnutrition (MAM) or severe acute malnutrition (SAM).

Marasmus is a serious type of protein-energy malnutrition that occurs when an individual consumes insufficient protein and calories. Because of the low nutrient and energy supply, vital functions either stops or slows down. The drastic drop in nutrient level leads to severe wasting, significant loss of body fat and muscle tissues, leading to a remarkably low body mass index (BMI) and in some cases reduced growth (Mehta, 2018). Marasmus affects both adult and children. In children, the major symptom of marasmus is stunting while for adults and older children is wasting or loss of muscle tissues. Children suffering from marasmus may be very hungry to the extent of sucking their cloths or hands excessively, others may have anorexia and may lack appetite. Long-term effect of marasmus in children are stunting and repeated infections. Some serious complications in children with marasmus are Diarrhea, measles, or a respiratory infection and can be fatal. Some children have been reported to have displayed complications such as bradycardia, hypotension, and hypothermia (Basheir and Hamza, 2015).

Generally, people with marasmus will lose body tissue and fat in their face. Equally, their bones become visible under their skin, and development of folds of skin from the loss of body mass. Their eyes may appear sunken. Also, patients experience persistent dizziness, lack of energy, dry skin and brittle hair (Mehta, 2018).

**Plate VII: Severely Wasted Child Suffering from Marasmus an Extreme Case of Protein-Energy Malnutrition (Source: Anonymous)**

Kwashiorkor is a severe case of protein- energy malnutrition characterized majorly by extreme loss or deficiency in protein and pitting (edema). The name "kwashiorkor" was introduced by Williams in 1935 in a classic description of her observations of the Ga tribe on the Gold Coast of Africa (currently Ghana). "Kwashiorkor" in the Ga language is interpreted as "the disease of the deposed child (deposed from the breast) when the next baby is born." It mostly affects infants and children, with the majority of cases occurring between the ages of weaning and age five. The disease is found in extreme cases of malnutrition and poverty-stricken areas globally (Benjamin and Lappin, 2019). Kwashiorkor is characterized by hypoproteinemia, pitting edema, varying degrees of wasting and/or stunting, dermatosis, and fatty infiltration of the liver and is caused by relative protein deficiency in the presence of sufficient energy intake (Schwartz, 2012). Other physical findings of kwashiorkor may include rounded cheeks, pursed lips, dry peeling skin, sparse hair, hepatomegaly, bradycardia, and hypotension in which if left

untreated can lead to significant morbidity and mortality due to a greater susceptibility to and severity of infections (Kamaruzaman, 2020).

Children with kwashiorkor generally have a very low plasma albumin concentration due to lack of protein (Marcus *et al.,* 2017 and Diamanti *et al.*, 2011). The proper treatment for kwashiorkor is the gradual introduction of enteral feeds (Kamaruzaman, 2020).


**Plate VIII : Children Suffering from Kwashiokor (Source: Anonymous)**

The third type of protein-energy malnutrition is the intermediate state known as marasmic kwashiorkor. This is caused by an acute or chronic protein deficiency and chronic energy deficit that is characterized by edema, wasting, stunting, and mild hepatomegaly. The distinction between kwashiorkor and marasmus is frequently blurred, and many children present with features of both conditions (Swartz, 2012). People with marasmic-kwashiokor are extremely thin, they show signs of wasting in certain areas of the body, and they have excessive fluid build-up in other parts. In children with marasmus kwashiorkor, their weight is usually 60 % less than the standard weight for their age. Hence, mmediate

medical treatment is essential. In children suffering from marasmic-kwashiorkor it has shown that as the condition progresses, recovery becomes more difficult, and the chances of survival reduces (Mehta, 2018).

## 2.2 Complementary Feeding

Fitness and general wellbeing (Health), growth and development of infants and pre-school children is exclusively a function of adequate nutrition and good nutritional practice. Hence, good feeding habits is a remedy for malnutrition and growth impedance in early childhood and infancy (Michaelsen, 2000). According to the WHO (2015), breast milk can only be sufficient for infants and weaning children in the first six months of life and the breastfeeding must be exclusive to meet their nutritional needs. This is supported by Dukuzumuremyi *et al*. (2020) who reported that the World Health Organization recommends that infants and weaning children must be breastfed exclusively for at least six months after birth to enable them meet the complete nutritional requirements in their early stage of life. It is evident that children at infancy that are not exclusively breastfed are at a high risk for both short and the long-term effects of retarded growth and impaired intellectual ability (Ogbo *et al.*, 2019). Despite the recommendations that exclusive breastfeeding is required for infants and weaning children, efforts to persuade mothers to engage in exclusive breastfeeding remains a bottleneck (Oyelana *et al.*, 2020). After the first 6 months, breastfeeding of infants and weaning children should last for 2-3 years in addition to solids or semi-solid food to attain the desired growth, development as well as maintain a healthy status (WHO, 2019).

The process of introducing infants and children to solid foods and liquids in addition to the breast milk when breast milk becomes insufficient to meet nutritional needs of children is called complementary feeding (World Health Organization, 2015). During complementary feeding, the food of the infant is gradually changed to solid and semi-solid

foods of different variety with varying texture, flavor, aroma and appearance (WHO 2019; Michaelsen *et al.,* 2000). These Solids and semi-solid foods are required to provide the nutrients which the breast milk is unable to supply (Oyeyinka, 2016). At this transition phase, the feeding of infants and the young should be timely, suitable, safe, sufficient, and regular because it is stage or phase where optimal growth and development is required and children are also vulnerable (Andualem *et al.,* 2020). Bimpong *et al.* (2020) reported that infants and young children from developing countries do not meet these conditions for proper complementary feeding. The reasons could be due to mother's attitude in the feeding time, suitability of the food (whether it is the required food), safety of the food, (is it harmless?) adequacy of the food (is it complete and enough to satisfy?) and regularity (is it administered as and when due?). It is necessary to give adequate training to mothers and care givers to educate them on initiation and proper attitude on complementary feeding as most mothers in developing countries still lack the proper knowledge (Bimpong *et al.,* 2020) According to Chiampo and Chiampo (2020), infants require nutrients in sufficient quantity and quality during his growth and development, which is dependent on the system's capability to receive, metabolize, absorb and excrete the waste from the diet.

## 2.3 Complementary Foods

Complementary foods are foods other than breast milk or infant formula (liquids, semi-solids, and solids) introduced to an infant to provide nutrients (Abeshu *et al.,* 2016). Protein energy malnutrition among infants and pre-school children has been attributed majorly to poor feeding habits in developing countries. For instance, in sub-Saharan region like Nigeria, most of the complementary foods that are fed to the at-risk infants are low in both macro and micro nutrients (Onoja *et al.,* 2014). Sufficient nutrition is therefore key to promotion of optimum growth, development and health during infancy and childhood

particularly at age (0-2 years) otherwise referred to as ‘’critical window’ this is because stunting at this stage is irreversible.

Onoja *et al.* (2014) produced a complementary food for infants by fortifying local staples (sorghum; soybean and plantain) with foods rich in micronutrients. Results obtained showed that fortification increased energy and nutrient content of the complementary food produced from the staples, also the anti-nutrient were at safe levels and the formulated food was generally acceptable. If proper growth for children and infants must be met, it is necessary to complement the usual breast milk with weaning foods as the breast milk administered to these children is no longer sufficient for them after six months of age. Most of the complementary foods are processed from local staples such as sorghum, corn, millet, cassava and yam which are generally starchy (Kent, 2017). These foods are expected to be liquids or semi-solid foods which should meet the standard requirements of conventional weaning foods globally. Good complementary foods should be energy rich, adequate in protein and micronutrients (particularly iron, zinc, calcium, vitamin A, vitamin C and folate); they should be easily digestible and not bulky, spicy or salty; it should be easily consumed, attractive and likable, affordable and locally available (WHO, 2019 & Ezeji and Ojimelukwe, 1993). Unfortunately, most of our locally made weaning foods do not meet the requirements of complementary food probably due to processing procedures (Aloysius and Ajawubu, 2013).

## 2.4 Sorghum

Sorghum (*Sorghum bicolor (L.) Moench*) has been ranked behind wheat *(Triticum aestivum, L.*), maize (Zea mays, L.), paddy rice *(Oryza sativa, L),* and barley *(Hordeum vulgare L.)* as the fifth most important cereal grain globally (Chisi and Peterson, 2019). The food and agricultural organization statistics (FAOSTAT) stated that roughly 45 million hectares of sorghum was produced in 2014. The origin and evolution of farmed

sorghum has been a long argument for decades. The staple is documented as one of the products of the oldest grassland on the earth. Sorghum is known to thrive very well in the African Savannas, serving as the lifeblood for millions of low-income earners as well as resource-limited populations in the poor areas of Africa and Asia (Ananda *et al.*, 2020).

Since sorghum flour is a gluten-free food, it is a safe alternative for those with celiac disease (Ratnavathi and Komala, 2016). In developing countries like Nigeria, sorghum like maize, millet, yam and cassava is one of the major staples used in the preparation of weaning foods such as pap (Akamu), beverages like kunu a non-alcoholic drink and alcoholic drinks such as burukutu and pitto (Ukwuru *et al.,* 2018). Others include porridges, fura and ogi, sorghum rice and sorghum flour that is used for different purposes (Kiranmayee *et al.*, 2020).

Like maize, millet and other staples, sorghum has been used in the formulation of weaning food with plant proteins like soya beans, groundnut, cowpeas, and pumpkin seeds. It was reported that sorghum has been blended with soya beans as weaning food and its physicochemical properties investigated. Results obtained showed that the water holding capacity of the weaning food blends ranged between 1.52 and 3.81 g/mL while the viscosity ranged between 14.32 and 33.61 entipoise at 10 % (w/v) flour concentration. The least gelation concentration ranged between 8.02 and 20.21 g/mL. The inclusion of sorghum malt in the blends reduced both water holding capacity and viscosity but increased the least gelation concentration (Usman *et al.,* 2016).

Adebo, (2020) also reported that sorghum when blended with groundnut, sesame seeds, chickpeas, and skim milk powder had a composition and properties comparable with Cerelac a commercially produced weaning food. Furthermore, Asma *et al.,* (2006) investigated the composition of instant weaning food (flakes) formulated from sorghum. The blends were found to contain 16.6 % to 19.3 % protein, 68.7 % to 72.7 % carbohydrate,

0.9 % to 1.3 % fiber, and 405.8 to 413.2 kcal of energy per 100 g. The iron content of the blends ranged from 5.3 to 9.1 mg/100 g, and the calcium content ranged from 150 to 220 mg/100 g. All blends reconstituted well and formed a soft paste when stirred with hot or cold water.

Nutritionally, Sorghum is a rich source of various phytochemicals including tannins, phenolic acids, anti-oxidants, anthocyanins, phytosterols, and policosanols in addition to proximate composition, sorghum contains vitamins, and minerals. These phytochemicals have significant impact on human health (Ratnavathi and Komala, 2016). Sorghum is a major source of food in in several countries in South Asia, Africa, and Central America (Reddy and Patil, 2015). All over sub-Saharan Africa, sorghum is a grain of choice because of its many uses. Apart from its conventional use in the production of traditional hazy and cloudy (sorghum) beers, malted sorghum is very useful in the brewing industries for beer production. In Nigeria most non-alcoholic beverages such as 'Malta, malt, cocoa' are Guinness products from sorghum. Also, Milo is a sorghum powder-based drink produced by Nestle Company (Taylor and Taylor, 2002).

The capability of sorghum for its malt and lager was studied concurrently with alpha amylase activity and diastatic movement of various cultivars examined. Result showed that Sorghum demonstrated most elevated amylase exercises at 96h germination. Ale lager is delivered from 100% sorghum utilizing exogenous compounds. The ale lager from sorghum malt and adjunct demonstrated prevalence in piece, for example, expanded free alpha amino nitrogen and shading units in sorghum malt and aide brew was observed (Ratnavathi and Chavan, 2016).

Apart from the various usefulness of sorghum as food, the species sorghum bicolor (L.) Pers (*Gramineae, Poaceae*) is also a variety of plant having its various parts broadly utilized in traditional medicine. Reports on ethno-organic studies have shown that

decoction from Sorghum *bicolor* seed had demulcent, diuretic, emollient properties (Adzu *et al*., 2015 & Erah *et al.,* 2003). Sorghum *bicolor* leaves have been used to develop (NIPRISAN®) (an anti-sickling drug) by National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria (Adzu *et al*., 2015) and is additionally one of the three segments of Jubi Formular®, a business home grown hematinic fabricated by Health Forever Products Ltd., Lagos, Nigeria (Erah *et al.,* 2003).

## 2.5 Bambara Nut

Bambara nut (*Vigna subterranean*) is predominantly grown for human consumption (Mbata *et al.,* 2009). It is a dicotyledonous plant and a member of the *Fabaceae* family and sub family of *Faboidea* (Bamshaiye *et al.,* 2011). The staple is grown primarily for its edible seeds (Mbata *et al.,* 2009). It has been reported that the seeds grown in Côte d'Ivoire showed a 19 % protein, containing all the essential amino acids with tryptophan as the restricting amino acid, dietary fibre level of 10 %, a fat content of 1.4 %, with a high content of total unsaturated fats (61 %) of which 36 % of the fats were n-6 unsaturated fats. This nut contains phosphorus, as the significant mineral, then, magnesium and calcium, followed by trace elements such as (iron, copper and zinc). It also contains α-tocopherol and some cancer prevention agents. The high content of amino acids in some species Bambara nut shows that this legume has a possibility in supplementary diet formulation. (Yao *et al.,* 2015).

## 2.6 Sprouting

Sprouting has an effect on the nutrient quality of seeds with respect to macro and micronutrients, anti-nutrient and functional properties. For instance, cowpea (*vigna unguiculata*) was investigated and the effect of sprouting time (0 hr, 24 hr, 48 hr, 72 hr, 96 hr, and 120 hr at 25 $^{o}$c) on the nutritional and anti-nutrient properties was determined. Results showed that sprouting increased most of the nutrients in cowpea seeds with respect

to time for proteins, ash, fibre, moisture, and minerals (potassium, sodium, calcium, magnesium and phosphorus) while fats and carbohydrates contents decreased as sprouting time increased. Consequently, all the investigated anti-nutrients (alkaloids, flavonoids and phytates) decreased as sprouting time increased (Ehirim *et al.,* 2018)

Onwuka *et al.* (2009) investigated the effect of sprouting on the proximate composition of African yam bean (*Sphenostylis stenocarpa*) and fluted pumpkin (*Telferia Occidentalis*). Results obtained indicated that unsprouted African yam bean seeds have lower moisture and lipid content ($P<0.05$) than the sprouted. While nutrients such as crude protein, total carbohydrate, ash and metabolizable energy content was higher. For the fluted pumpkin seeds the unsprouted had significantly lower moisture, crude protein, ash and total lipid ($P<0.05$) and a higher carbohydrate content.

### 2.6.1 Effect of sprouting on protein content of seeds.

It is documented that sprouting increases the protein content of seeds especially legumes. This increase may be due to enzymatic hydrolysis of insoluble proteins to soluble proteins thereby increasing the protein that is available (Echendu *et al.*, 2009). Other reasons why protein content is increased during sprouting are that free amino acids are released after enzymatic hydrolysis for the synthesis of new proteins and proteases (Odedeji, 2020). The increase observed in protein may be as a result of loss in dry weight, predominantly carbohydrate by respiration during germination (Uppal and Bains, 2012). Nonogaki *et al*. (2010) reported that hormonal changes occur during germination and these changes play a key role in the synthesis of proteins. In addition, this protein synthesis takes place as a result of inhibition. Work done on sprouted sorghum and defatted fluted pumpkin seed (*Telfairia occidentalis*) showed that sprouting increased crude protein content of seeds (Bello *et al.,* 2017).

### 2.6.2 Effect of sprouting on the fat content of seeds

When seeds are sprouted, most times there is a decrease in the fat content as sprouting time is increased. This is linked to some metabolic activities that occur in the processes involved. Devi *et al.* (2015) reported that decrease in fat content may be due to depletion of the fat stored that contributed to the catabolic activities of the seeds during sprouting. Also, during sprouting energy is required for germination. The fat is used as a source of energy for germination to take place and so the fat present in the seeds reduces as germination progresses (El-Adawy, 2002).

The decrease observed in fat content of seeds has also been linked to increased activities of the lipolytic enzymes in the course of germination. Fats are hydrolysed to fatty acids and glycerol which become an energy source for the development of the embryo. Elegbede (1998) made similar observation when he sprouted Bambara nut for formulation of diet. In a similar study conducted on fluted pumpkin seed, result showed that sprouting increased the fat content of fluted pumpkin seed as against the decrease observed by El-Adawy (2002) and Elegbede (1998) and therefore disagree with the earlier reports that sprouting increases fat content of seeds (Onwuka *et al.*, 2009). Low or decrease fat content is indicative to increase shelf-life for germinated seeds when compared to the ungerminated (Inyang and Zakari, 2008).

### 2.6.3 Effect of sprouting on the fibre content of seeds

Studies have shown that fibre content of seeds is increase upon sprouting. In an experiment conducted by Ehirim *et al.* (2018) on cowpea (*Vigna unguiculata*), results obtained showed that the Crude fibre content of cowpea was significantly increased from 4.14 % to 5.08 % ($p<0.05$). The increase observed as a result of sprouting may be due rise in plumule and free radicals during germination of seeds (Berry *et al.,* 1988 and Echendu *et al.,* 2009). In different study conducted by Shah *et al.* (2011) on barley, results showed that sprouting significantly increased the crude fibre

content of barley from 3.75 % in non-sprouted to 6 % after sprouting for 5 days. This increment may be associated with the synthesis of structural carbohydrates such as cellulose and hemicelluloses which are major constituents of cell walls.

### 2.6.4 Effect of sprouting on the ash content of seeds

Sprouting has been shown to increase the ash content of seeds (Echendu *et al.,* 2009). Ash contents, calculated on moisture free basis, increased with increase in sprouting time for cowpea (*Vigna unguiculata*) (Ehirim *et al.,* 2018). The ash content slightly increased but they were not significantly difference ($p<0.05$) and maximum ash content was reached after 120 hr germination. In the same vein, El-Adawy (2002) reported that a significant increase in ash content was observed during sprouting in mungbean, pea and lentil seed. The relative increase in ash content and other chemical components may be as a result of the decrease observed in crude fat and carbohydrate content during sprouting and majorly due to endogenous enzyme hydrolysis of complex organic compounds thereby releasing more nutrients and leaving the anti-nutrients to leach into the germination medium (Echendu *et al.,* 2009)

### 2.6.5 Effect of sprouting on the carbohydrate

Generally, many studies show that carbohydrate content of legumes and seeds decreases as the sprouting time increases. Work done by Ehirim *et al*. (2018) revealed that Carbohydrate content of sprouted cowpea decreased from 64.01 to 47.87 %. In similar experiment conducted by Uppal and Bains (2012) it was reported that carbohydrate content of cowpea decreased by 5.6 % and Jirapa *et al.* (2001) also reported 2.34 % decrease in carbohydrate content after 24 h of sprouting in cowpea. An explanation by Vidal-Valverde *et al*. (2002) elucidated that in sprouting; carbohydrate is used as a predominant energy source for the growth of the seed embryo. This explanation accounts for the observable changes that occur with respect to carbohydrate content after sprouting.

Furthermore, the activity of the enzyme β-amylase that hydrolyses the starch into simple carbohydrate is increased during sprouting (Suda *et al.,* 1986). In addition, the Starch content in cotyledon of the seed is broken down into smaller molecules such as glucose and fructose so as to provide the energy required for cell division as the seeds mature and grow (Ehirim *et al.,* 2018, Nonogaki *et al.,* 2010 and Vidal-Valverde *et al*., 2002).

### 2.6.6 Effect of sprouting on the moisture content of seeds

Usually increase in moisture content of seed is notable during sprouting. This may be as a result of the processing techniques adopted for the sprouting which include soaking of seeds. When seeds are soaked, they absorb more water in addition to the one contained in such seeds to create an environment for sprouting, this on the overall increases the total moisture content of the seeds (Ehirim *et al*., 2018). Additionally, as sprouting progresses, the germinating seeds continue to absorb water by a process known as imbibition (Nonogaki *et al.,* 2010; Sampath *et al.,* 2008). Experiment conducted on nutrient composition of sprouted cow pea (*Vigna unguiculata*) showed that the moisture content increased significantly from 5.63 to 6.72 % after sprouting for 120 hrs (Ehirim *et al.,* 2018). In a similar experiment Murugkar and Jha (2009) also observed that moisture content of soybeans increased from 5.4 to 56.1 % after 48 h of sprouting time. Therefore, it can be said that moisture content of seeds increases via sprouting in most cases.

### 2.6.7 Effect of sprouting on the functional properties of seeds

WHO (1998) recommends that weaning food should be concentrated in energy, nutritious, soft and easy to swallow. The family's food on the contrary is often filling and bulky thereby making it difficult for weaning children to digest and hence absorption of nutrient is impaired. In an attempt to address this bottleneck seeds used in formulation of baby foods are being sprouted to improve functional properties of children meals.

Ocheme *et al.* (2015) reported that sprouting of sorghum for 24, 48 and 72 hours improved the functional properties and degree of starch gelatinization of the flour using non-germinated sorghum as control. The flours were treated separately. Germination of sorghum grains for 48 hours or more essentially ($p<0.05$) diminished both free and bulk densities from 0.59 g/mL and 0.77 g/mL to 0.56 g/mL and 0.70 g/mL separately. The water ingestion limit of the 72 hours sprouted sample was 1.38 g/g which was significantly ($p<0.05$) higher than the other samples. The oil retention limit of the 48- and 72-hours samples were (1.16 and 1.18 g/g separately) were essentially ($p<0.05$) higher than those of the control test (non-germinated) and 24-hour germination (1.03 and 1.0 4 g/g respectively). Germination likewise significantly ($p<0.05$) expanded the growing control (22-23.2 g/mL), frothing limit (14-16.2 %) and emulsion limit (58.6-65.5 %).

The level of starch gelatinization increased with increasing germination time. However, there was a decrease in gelatinization with increasing temperature. In general, germination improved the properties of sorghum. In a similar study conducted on millet grain and Bambara nut, results obtained showed that 48hrs sprouting improved the functional and pasting properties of both samples (James *et al.*, 2018). Further studies conducted on the effect of sprouting on the functional properties of maize (*zeamays*) showed that the pH decreased significantly ($p\leq0.05$) as sprouting time increased with 72 hrs with sprouted samples having the lowest pH value of 5.67. Also, significant difference ($p\leq0.05$) was observed with bulk density between ungerminated sample and germinated samples when germination was allowed to progress for 48 and 72 hours.

A significant difference ($p\leq0.05$) was observed with the samples with respect to water absorption capacity. Also, there was variation among the samples with respect to oil absorption capacity ($p\leq0.05$) significantly different. In addition, Emulsion capacity of the flour samples also differ significantly ($p\leq0.05$) with germination for 72 hrs having the

highest value. There was a significant difference ($p \leq 0.05$) in swelling power of the samples with the ungerminated staple displaying highest value. The foaming capacity also showed significant difference ($p \leq 0.05$) with the 48 hours sprout having the least value (Ocheme *et al.,* 2015).

## 2.8 Nutrient needs of infants

The supply and usage of nutrients are of more prominent biological significance during early childhood than during some other time of life. In adults, the nutrient supply must be available for maintenance and meet the body's requirements for physical action. In infants and growing children, energy requirement is for growth and development. The amount and nature of nutrient supply during early life regulates the separation of tissues and organs and has an immediate and long-term effect on the wellbeing of an individual (Koletzko, 2008).

According to WHO, (2018) few children receive nutritionally adequate and safe complementary foods; in many countries less than a fourth of infants 6–23 months of age meet the criteria of dietary diversity and feeding frequency that are appropriate for their age. After 6 months of breast feeding, the capability of breast milk to meet supplies for macronutrients and micronutrients (energy, protein, iron, zinc, and some fat-soluble vitamins) gradually turn out to be low especially as the age of the infant increases (Isabelle and Chan, 2011). There are some nutritional gaps that need to be covered by the complementary food being administered to the growing infants. A good complementary food should offer adequate energy, protein and micronutrients to fill up nutritional gaps and meet the infant's needs in addition to breast milk. Figure. 2 shows the nutritional gaps to be covered by complementary food for a breastfed child of 12-23 months (WHO, 2009).

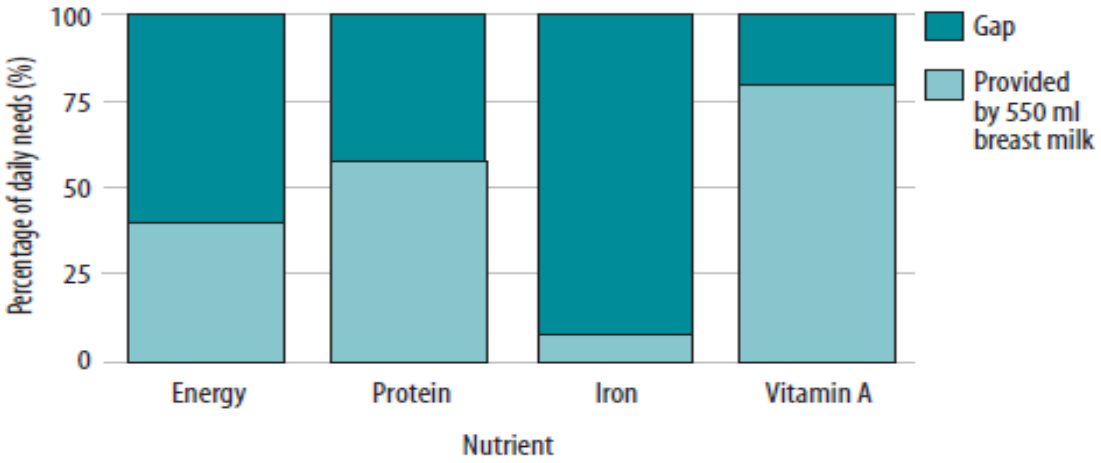


**Figure 2.1: Gaps to be filled by Complementary Foods for a Breastfed Child 12–23 Months**.

**Source: WHO, (2009)**

From the figure above, the largest gap to be covered is for iron, therefore complementary food is expected to be fortified with much iron which can either come from animal source or plant source (legumes such as cowpea, Bambara nut, soya beans, groundnut among others) to fill up the gaps which the breast milk is not able to meet.

### 2.8.1 Energy needs of infants

Infants and pre-school children require energy for both physiological and biological activities such as, growth, and normal development. This energy is obtained from foods containing carbohydrate, protein, or fat and expressed in (Kcal) (WHO, 2009). According to WHO (2009), infants have the ability to regulate their intake of food to consume the quantity of kilocalories they need. Therefore,

The World Health Organization's expert report on energy and protein requirements states that; The energy requirement of an individual is a level of energy intake from food that will balance energy expenditure when the individual has a body size and composition and level of physical activity, consistent with long-term good health; and that would allow for

the maintenance of economically necessary and socially desirable physical activity *(*WHO, 2020) According to the guiding principle of complementary feeding of breastfed child, the energy needs from complementary foods for infants with "average" breast milk intake in developing countries is approximately 200 kcal per day at 6-8 months of age, 300 kcal per day at 9-11 months of age, and 550 kcal per day at 12-23 months of age. In developed countries there is a deviation due to the differences in average intake of breast milk (WHO, 2020). However, the total energy requirements of a breastfed infant that is healthy is roughly 615 kcal/d at 6-8 months, 686 kcal/d at 9-11 months, and 894 kcal/d at 12-23 months of age (Dewey and brown, 2003). Energy needs from complementary foods are estimated by subtracting average breast milk energy intake from total energy requirements at each age (WHO, 2003).

### 2.8.2 Protein need of infants

Protein is a highly complex substance made up of amino acids that is present in all living organisms. They function directly in chemical processes essential to life (Rakhshani, *et al.*, 2019). About 20-22 amino acids are the building blocks of proteins, these amino acids are classified as either essential or non-essential amino acids. About nine of the amino acids must be supplied in the diets because the human body is not able to manufacture them and are referred to as essential or indispensable amino acids. They include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine while the others are manufactured by the body and are called non-essential amino acids.

According to Table (2005), cystine and tyrosine are essential for infants and weaning children, this is because enzyme activities for their synthesis are immature in the young children. Importantly, active infants need high quality protein from the human breastmilk as well as commercial baby food or locally formulated baby formula for different cellular processes such as development, maintenance, and repair of new tissues. The different

tissues of the heart, lungs, skin, eyes, muscles, heart, brain and several organs as well as development of some important enzymes, hormones, antibodies required for special regulatory functions are a product of proteins. These proteins from the Human breast milk are reported to be about (0.9 g/mL) which is lower than in other animal milks but sufficient for infants. Breast milk from other animals is conversely higher and is heavy for the human infants and can overload their immature kidneys with nitrogenous waste products.

In addition, the human breastmilk contains less protein casein that is softer, easily digestible and having a different molecular structure from protein of other sources. More so, human milk is reported to contain better alpha-lactalbumin as against the beta-lactoglobulin in other animal breast milk like cow which can cause intolerance for human infants (WHO, 2009; Riordan, 2004). Because plant proteins contain less amino acid as compared to animal proteins, supplementation of plant Proteins can help provide all the necessary essential amino acids required to meet infant protein. For instance, plant foods that are deficient in one essential amino acid are complemented with other plant foods that are high in that amino acid (example of legumes such as pureed kidney beans, Bambara nut, groundnut (low in methionine but high in lysine) and grain products such as sorghum, mashed rice (high in methionine, low in lysine), thereby providing the sufficient amounts of all the essential amino acids that are needed by the body (Young and Pellet, 1994).

In as much as the protein content of diet is important in diets, a balance is expected to be maintained because excess protein administered to children can result in adverse effect such as stimulation of secretion of insulin-like growth factor (IGF-1) and this can result in cell proliferation leading to accelerated growth and increased adipose tissue. The adverse effect of excess protein has been supported by the European obesity project experimentally by comparing moderate protein diet with excess protein diet (Koletzko *et al*., 2008). Weber *et al.* (2014) reported that infants fed with high protein formula at age 2–12 months gained

more weight at 12 months of age but not greater length, suggesting increased adiposity. The average intake protein for infants between ages 0-6 months is 9.1g/day and the recommended daily allowance (RDA) for older infants between ages 7-12months is 11g/day (WHO, 2009)

## 2.9 Anti-nutrients found in seeds

Anti – nutrients have been described as compounds or substances that act to decrease nutrient availability by inhibiting digestion, absorption and utilization of the nutrient and may produce harmful outcomes when found in the system (Akande *et al.,* 2010). Conversely, some antinutrients such as phytic acid and tannins found in grains have been indicated to be of potential health benefits to the system (Oyeyinka *et al.,* 2017 and Boateng *et al.,* 2008). Leguminous seeds and other plant sources in their raw forms contain toxic diverse varieties of antinutrients. Chiefly, toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins (phyto-haemagglutinins), protease inhibitors, chlorogenic acid and amylase inhibitors are the anti-nutrients found in plant. It has been reported that processing method like sprouting, fermentation, dehaulling, and soaking influences the chemical, anti-nutrient and nutritional composition of grains (Ojha *et al.,* 2018)**.**

Studies conducted by Oyeyinka *et al.,* 2017 showed that anti-nutrients probably had an adverse effect on the bioavailability of Bambara nut protein as in vitro protein digestibility of the different species improved as their (anti-nutritional factors) levels reduced upon processing. The total phenolic content (TPC) and antioxidant activity of the samples decreased roughly by 83% and 18% respectively, while tannin (approx. 36%) decreased after dehulling. Also, the reports by Mohapatra *et al.,* 2019 on sorghum showed that indigestibility of protein was in connection to presence of anti-nutrients. The unavailability

of nutrient in the system is as a result of nutrient to anti-nutrient interaction hampering the release of the needed nutrient (Petroski *et al.,* 2020)

### 2.9.1 Tannins

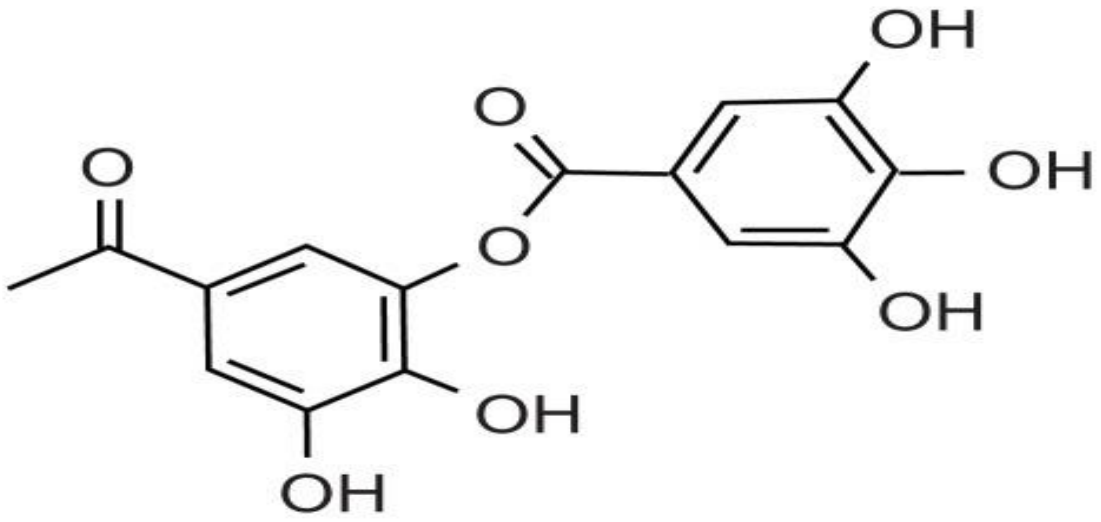


**Figure 2.2: Chemical Structure of Tannins**

Tannins are high molecular weight water soluble phenolic compounds (greater than 500 Daltons) which can bind to proteins, sugar and starches, they are capable of forming strong chemical complexes and are stable at pH 3.5 to 7 (Akande *et al*., 2010, and Strabel and Cieslak, 2012). According to Baoling *et al.,* 2007 anti-nutrients including tannins are found in Bambara nut and the level of anti-nutrients found in the legume has a correlation with the colour of the seed. For instance, the brown and red colour Bambara nut had higher tannin contents than the cream. Oyeyinka *et al.,* 2017 reported that the tannin content of dehulled red and brown Bambara nut ranges from 3.04 to 5.26 mg/100 g while that of common bean was (541.45 mg/100g). In a similar work carried out by Mohapatra *et al.,* 2019, results indicated that tannin content of sorghum decreased by 30–39 %, after processing.

### 2.9.2 Phytic acid

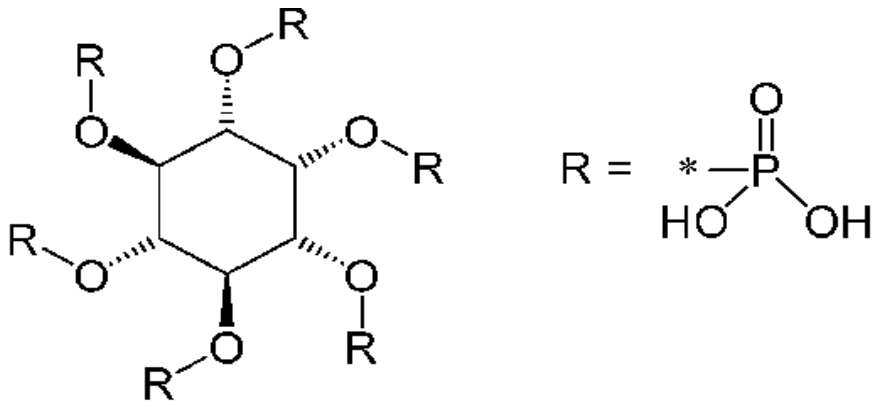

**Fig. 2.3: Chemical Structure of Phytic Acid**

Phytic acid has been defined as food inhibitors stored in a wide variety of food crops in the form of phosphorus which function majorly in the formation of structural elements of the cell such as cell membranes and in the production of energy. They are anti-nutrients found in grains (sorghum, millet, maize) legumes (including peanut, soybeans, and Bambara nut), and some other seeds (Nissar *et al.,* 2017).

Phytic acid chelates micronutrients hence reduce nutrient bioavailability (Chhikara *et al.,* 2019). Studies by Afify *et al.* (2011) reported that Phytate content of sorghum was significantly reduced from 23.59 to 32.40 % for soaking and 24.92 to 35.27 % for germination treatments, respectively. Also, phytic acid has been implicated in the reduction of the absorption of zinc and iron. It has also been reported that in developing countries pre-school children are deficient in iron and zinc because most of their foods are prepared from cereals with high concentration of phytates (Afify *et al.,* 2011).

### 2.9.3 Oxalates

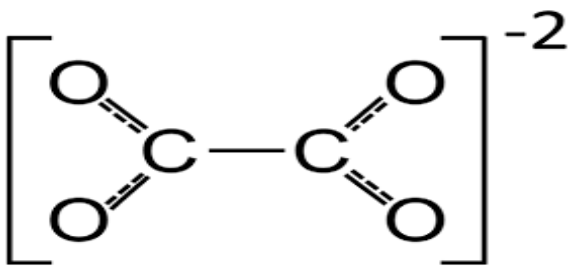

**Fig. 2.4 Chemical Structure of Oxalate**

Oxalates (oxalic acids) and their salts (calcium and magnesium) are products present in many plant tissues including sorghum and Bambara nut. The content of oxalates in the sorghum flour was found to be 1.12 mg/g (Chhikara, *et al.,* 2018) higher than that reported for Bambara nut total oxalates (0.6 mg/100 g) (Fungo *et al.,* 2015). Oxalates in combination with calcium form a calcium oxalate complex in the intestinal lumen which makes calcium inaccessible for absorption. When calcium oxalates accumulate in the system it may result to kidney stones when the acid is excreted through urine.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Materials

#### 3.1.1 Plant seeds

(a) Bambara nut (*Vigna subterranean*)

(b) Sorghum (*Sorghum bicolor*).

Bambara nut and sorghum were purchased from Kure Ultra-Modern Market Minna, Niger State.

#### 3.1.2 Standard baby food

Cerelac (commercial weaning Baby formula produced by Nestle Nigeria PLC) was purchased from Garima pharmacy Bosso Minna, Niger State.

#### 3.1.3 Rat feed

Rat chow was purchased from Vital feeds Kpakungu Minna, Niger State.

#### 3.1.4 Feed Supplements

(a) Sucrose

(b) Vitamin premix,

(c) Salt

(d) Oil.

Sucrose was purchased from Sim best scientific world Bosso, Minna, Niger State. Vitamin premix was purchased from Jumra farms and Animal feed, Block C shop 1 Awwal Ibrahim Shopping complex Minna, Niger State. Salt and Oil were purchased from Kure Ultra-Modern Market Minna, Niger State.

### 3.2.1 Reagents and chemicals

All reagents and chemicals used were of analytical grade manucfactured by British Drug House (BDH) Limited, England and Sigma Aldrich Chemical Company Incorporation, Milwaukee, Wisconson. USA. Assay kits for alanine transminase, aspartate transaminase, alkaline phosphatase, bilirubin, total protein, creatinine, urea and albumin were products of Randox Laboratory Limited, County Antrim, United Kingdom purchased from Sim best Scientific and Chemicals Minna, Niger State.

### 3.2.2 Equipment

Atomic absorption spectrophotometer -6800 Shimadzu, Technicon sequential Multi-Sample Amino Acid Analyser (TSM), Spectrophotometer (Metrohm Spectronic 21D Model, Genway model 6000 electronic spectrophotometer), Mindray Auto Hematology analyser BC- 5300 model, Eppendorf centrifuge 5702, Cobas C111 Roche Autoanalyser, weighing balance (model AR223CN Ohaus Adventurer), High speed refrigerated centrifuge LR 16- A, Shimadzu UV- spectrophotometer, UV -1800, Automated Haematologic Analyser (Abacus Junior haematology autoanalyser) Water bath (Light water, Surrey GU 185TA,UK), hot plate (Biotec), Microscope (Olympus CX21).

### 3.2.3 Experimental animals

Weaning Albino rats (3 weeks old) weighing between 34-55 g were purchased from Olatunji Farm Ilorin, Kwara State.

## 3.3 Methods

### 3.3.1 Sample preparation

**S**amples of sorghum and Bambara nut were sorted, cleaned, washed and soaked in water separately for 12 and 15 hrs respectively. The water was changed at intervals of 3hrs during soaking to prevent fermentation and then the water was drained. Samples were spread in wet trays for sprouting. (Modu *et al.,* 2014 and Jammes *et al.*, 2018).

### 3.3.2 Sprouting /germination

Exactly 20 g of soaked Bambara nut was weighed in 8 different Petri dishes each for different germination periods (0, 1, 2, 3, 4, 5, 6 and 8 days). The same treatment was carried out for sorghum. The samples were covered with paper to create an enabling sprouting environment for the samples. The sprouted Bambara nut was oven dried to a constant weight at 60 $^{o}$C for 12hrs while sorghum was dried to constant weight at 40 $^{o}$C for 8hrs respectively. The dried sample were milled and sieved with a 2 mm mesh size sieve.

## 3.4 Proximate Analysis

The proximate composition of the flours (sprouted Bambara nut and sorghum) and their corresponding supplemented diets were carried out in triplicate. Methods used are described below.

### 3.4.1 Determination of moisture content.

The method described by Onwuka (2005) was used to determine the moisture content. Moisture content is determined through a thermo-gravimetric method that is by loss on drying, a principle in which the sample is heated and the weight loss due to evaporation of moisture is recorded. This was carried out by oven drying method. Two grams (2 g) of well-mixed samples was accurately weighed in clean, dried crucible ($W_1$). The crucible was allowed in an oven at 100-105 $^{o}$C for 6-12 hours until a constant weight was obtained. The crucible was then cooled for 30 min. After cooling it was weighed again ($W_2$), the percentage moisture was calculated by following formula.

$$Moisture\ Content\ (\%) = \frac{W1 - W2}{Weight\ of\ the\ Sample} \times 100 \qquad \textbf{Equation 1}$$

Where $W_1$ = Initial weight of crucible + Sample 1 and $W_2$ = Final weight of crucible + Sample 2.

### 3.4.2 Determination of fat content

Crude fat was determined by the method described by (Onwuka, 2005). The principle is based on based on solvent extraction. Fat is extracted based on the fact that it is soluble in diethyl ether and petroleum ether and hence can precipitate into the solvent. This method involves extraction of liquid (ether) using Soxhlet apparatus. Exactly 2 g of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. A weighed, cleaned and dried receiving flask was filled with petroleum ether and fitted into the apparatus. The Soxhlet apparatus was assembled and allowed refluxing for 6 hrs; extract was transferred into clean glass dish with washing which was evaporated on water bath. Then the dish was placed in an oven at 105 -110 $^{o}$C for 1hr and cooled in a desiccator. The percentage crude fat was determined using the following formula:

$$Crude\ Fat\ Content\ (\%) = \frac{Weight\ of\ Extract}{Weight\ of\ the\ Sample} \times 100 \qquad \textbf{Equation 2}$$

### 3.4.3 Determination of carbohydrate content

The nitrogen free method described by AOAC (1990) was used. Carbohydrate content can be measured by hydrolyzing polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharide. The carbohydrate was calculated as weight by difference between 100 and summation of other proximate parameter as Nitrogen free extract (NFE) percentage carbohydrate (NFE) = 100-(M+P+F+A+$F_2$) where M = moisture, P= protein, $F_1$=Fat, A=ash, $F_2$=crude fibre.

### 3.4.4 Determination of Crude Protein

Protein in the sample was determined by Kjeldahl method. The principle is based on digestion of organic matter with sulfuric acid in the presence of a catalyst, rendering the reaction product alkaline the distillation and titration of the liberated ammonia, calculation

of the nitrogen content, multiplication of the result by the conventional factor 6.25 to obtain the crude protein content.

Exactly 0.25 g of dried flour samples were taken in digestion flask, with 6 mL of concentrated $H_2SO_4$ and a speck of Kjeldah1 catalyst (mixture of 10 g $Na_2SO_4$+5 g CuSO4+ 0.05 g selenium). The flask was swirled in order to mix the contents thoroughly then digested on the digestion block till the mixtures became clear (colourless or greenish in color). The digest was cooled and transferred to 100 mL volumetric flask and volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markham Distillation Apparatus. Ten millilitres of digest was introduced in the distillation tube then 10 mL of 40 % NaOH was gradually added through the same way. Distillation was continued for at least 10 min and $NH_3$ produced was collected as $NH_4OH$ in conical flask containing 5 mL of 4 % boric acid solution with few drops of methyl red indicator. During distillation yellowish colour appeared due to the $NH_4OH$. The distillate was then titrated against standard 0.1 N HCI solutions till the appearance of pink colour. A blank was also run through all steps as above. Percentage crude protein content (% Crude Protein) = 6.25* x %N

$$Protein\ Content\ (\%) = \frac{(S-B) \times N\ \times 0.014\ \times D}{Weight\ of\ the\ Sample\ \times V} \times 100 \qquad \textbf{Equation 3}$$

Where S and B = Sample and blank titration values, N = HCl Normality, D = Dilution of sample after digestion, V = Volume taken for distillation, 0.014 – Milli equivalent weight of Nitrogen and * = Correction factor.

### 3.4.5 Determination crude fibre

The method described by (Onwuka, 2005) was used in the determination of the crude fibre. Crude fiber is determined gravimetrically after chemical digestion and solubilization of test sample. The principle is based on acid/alkali treatment, oxidative hydrolytic degradation of cellulose and lignin and filtration. The residue obtained after final filtration

is weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fiber content. Two grams (2 g) of sample was defatted with petroleum ether; boiled under reflux for 30min with 200 mL of a solution containing 1.25 g of $H_2SO_4$ per 100 mL of solution. The solution was filtered through several layers of cheese cloth on fluted funnel, washed with boiling water until the washings are no longer acidic then the residue was transferred into a beaker and boiled for 30 min with 200 mL of solution containing 1.25 g of carbonate free NaOH per 100 mL, the final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible, then dried in an electric oven at 105 $^oC$ and weighed after which it was incinerated at 550 $^oC$ for 30 mins, it was then cooled and reweighed. The loss in weight after incineration x 100 is the percentage crude fibre.

### 3.4.6 Determination of ash content

The principle of ashing is based on burning off of the organic matter and to determine the inorganic matter remaining. It requires two stages of heating; the first involves removal of the water present and to char the sample thoroughly; and finally, ashing at 550 $^oC$ in a muffle furnace, the weight of ash thus obtained is expressed in terms of percentage. The method of (Onwuka, 2005) was used for the determination of the ash content. Clean empty crucible was placed in a muffle furnace at 550 $^oC$ for an hour, cooled in desiccator and then weight of empty crucible was noted ($W_1$). Two grams of each of the samples was taken in crucible ($W_2$) and was charred over a burner, until it was charred. Then the crucible was placed in muffle furnace for ashing at 550 $^oC$ for 2-4 h. The appearance for gray white ash which indicated complete oxidation of all organic matter in the sample. After ashing the crucible was cooled and weighed ($W_3$). Percentage ash was calculated by the following formula.

$$Ash\ Content\ (\%) = \frac{Difference\ in\ Weight\ of\ Ash}{Weight\ of\ the\ Sample} \times 100 \qquad \textbf{Equation 4}$$

Difference in weight of ash = $W_3 - W_1$

### 3.5 Amino Acid Profile

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

#### 3.5.1 Defatting of Sample

The sample was defatted using chloroform/methanol mixture of ratio 2:1. Exactly 500 mg of the sample was put in extraction thimble and extracted for 15 hours in Soxhlet extraction apparatus.

#### 3.5.2 Hydrolysis of the sample for tryptophan determination

Two grams (2 g) of the defatted Bambara nut sample was weighed into glass ampoule. Exactly 7 mL 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 example methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 $^{o}$C± 5 $^{o}$C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was neutralized to pH 7.00 and evaporated to dryness at 40 $^{o}$C under vacuum in a rotary evaporator. The residue was dissolved with 5 mL of borate buffer (pH 9.0) and stored in plastic specimen bottle for further analysis.

### 3.5.3 Nitrogen determination

Exactly 115 mg of ground sample was weighed, wrapped in Whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10 mL) was added. Catalyst mixture (0.5 g) 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 100 mL in standard volumetric flask. Aliquot (10 mL) of the diluted solution with 10 mL of 45 % sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 mL of 2 % boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 mL of distillate was collected. The distillate was then titrated with standardize (0.01 N) hydrochloric acid until a grey coloured end point was obtained.

$$Ash\ Content\ (\%) = \frac{(a-b) \times 0.01 \times 14\ \times V}{W\ \times C} \times 100 \qquad \textbf{Equation 5}$$

Where: a = Titre value of the digested sample, b = Titre value of blank sample, v = Volume after dilution (100 mL), W =Weight of dried sample (mg), C = Aliquot of the sample used (10 mL) and 14 = Nitrogen constant in mg.

### 3.5.4 Sample loading

Five microlitre of sample was dispensed into the cartridge of the analyser. The TSM analyser is designed to separate and analyse free acidic, neutral and basic amino acids of the hydrolysate. The period of the analysis lasted for 76 minutes.

### 3.5.5 Method of calculating amino acid values from chromatogram peaks

The system is an automated one in which an integrator is attached to the Analyzer which calculates the peak area proportional to the concentration of each of the amino acids.

## 3.6 Functional Properties

### 3.6.1 Determination of bulk density.

The method reported by James *et al.* (2018) was used to determine the bulk density. A 10-mL graduated measuring cylinder was weighed and filled with the Bambara nut and sorghum flours, and the bottom of the cylinder was gently tapped on the laboratory bench several times until there was no further decrease of the sample level after filling to the 10 mL mark. This was calculated using the formula;

$$Bulk\ Density = \frac{Weight\ of\ Sample\ (g)}{Volume\ of\ Sample\ (mL)} \qquad \textbf{Equation 6}$$

### 3.6.2 Swelling capacity and solubility index

The method described by AOAC (2000) was adopted in the determination of swelling capacity and solubility index. One gram of the sample was accurately weighed and transferred into a clean dried test tube and weighed ($W_1$), and then it was dispersed into 30 $cm^3$ distilled water using blender. The resultant slurry was heated at temperatures of 60, 70, 80, and 90 °C, respectively, for 30 min in a regulated water bath. The mixture was then cooled to room temperature and centrifuged at 500 rpm for 15 min. 5 mL of the supernatant was withdrawn and the residue was the amount solubilized in water.

$$Solubility = \frac{Weight\ after\ Drying\ of\ Supernatant}{Weight\ of\ Sample\ after\ Drying} \times 100 \qquad \textbf{Equation 7}$$

### 3.6.3 Water absorption capacity

Water absorption capacity was determined using the method of Sathe and Salunkhe (1981) with slight modifications. 10 mL of distilled water was added to 1.0 g of the sample in a beaker. The suspension was stirred using a magnetic stirrer for 5 min. The suspension obtained was thereafter centrifuged at 3555 rpm for 30 min and the supernatant measured in a 10 mL graduated cylinder. The density of water was taken as 1.0 g/cm 3. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant.

### 3.6.4 Foaming capacity

The foam capacity and stability were studied by the method of Coffman and Garcia (1977). A known weight of sorghum and Bambara nut flour each sample was dispersed in 100 mL distilled water. The resulting solution was homogenized for 5 min at high speed. The volume of foam separated was noted. The total volume remaining at interval of 0.00, 0.30, 1, 2, 3, 4 up to 24 h was noted for the study of foaming stability.

$$Foaming\ Capacity\ (\%) = \frac{Volume\ after - Volume\ before\ Homonisation}{Volume\ before\ Homogenization} \times 100$$

***Equation* 8**

The effect of pH on foaming properties was carried out by adjusting 2% (w/v) dispersion to the desired pH range from 2 to 11 using either 1 M HCl or NaOH followed by vigorous whipping as described above.

### 3.6.5 Determination of gelation capacity

Method described by Onwuka (2005) was adopted in the determination of gelation capacity. Bambara nut and sorghum flour sample each of 5 % (w/v) in 5 mL of distilled water was prepared in test tubes. The samples in the test tubes were then heated for 1 hr in a boiling water bath followed by rapid cooling under running cold tap water. The test tubes

were further cooled for 2 hr at 4 °C. The least gelation concentration determined is the concentration when the sample from the inverted test tube did not fall or slip.

### 3.7 Anti-nutritional factors

Tannin, phytates, oxalate, cyanide, saponin and alkaloid contents of sprouted Bambara nut and sorghum were determined using spectrophotometric methods described by Arntfield *et al.* (1985).

#### 3.7.1 Determination of phytates

Phytate content of the samples was determined using the procedure of Lolas and Markakis (1975) as described by Essien and Akpan (2014). Two grams (2 g) of sample was weighed into a 250 mL conical flask. Exactly 100 mL of 2 % concentrated HCl was used to soak the sample in the conical flask for 3 hours. The mixture was filtered and 50 mL of filtrate was placed in a 250 mL beaker and 107 mL of distilled water was added. A 0.3 % ammonium thiocyanate (10 mL) was added to the sample as indicator and titrated with iron III chloride solution which contained 1.95 mg iron per mL. Titration continued until a brownish yellow colour that persisted for 5 minutes was observed.

#### 3.7.2 Determination of tannins

The Folin Denis Spectrophotometric method was employed as described by Onwuka (2005) to determine the tannin. One gram (1 g) of each sample was dispersed in 10 mL distilled water and shaken. The mixture was allowed to stand for 30 minutes at room temperature. At the end of 30 minutes, the mixture was centrifuged and the extract obtained. (2.5 mL) of the supernatant (extract) was transferred into a 50 mL volumetric flask. Similarly, 2.5 mL of standard tannic acid solution was transferred into a separate 50 mL flask. One millitre (1 mL) of Folin – Denis reagent was measured into each flask, followed by 2.5 mL of saturated $Na_2CO_3$ solution. The mixture was diluted to mark in the

flask (50 mL) and incubated for 90minutes at room temperature. The absorbance was measured at 250 nm using Jenway model 6000 Electronic Spectrophotometer. The tannin content was calculated from: % Tannin = An/As×C×100/W×Vf.

Where An = absorbance of test sample, As = absorbance of standard solution. C= concentration of standard solution, W = weight of sample, Vf = total volume of extract.

### 3.7.3 Determination of oxalates

The titration method described by Day and Underwood (1986) was employed in the determination of oxalate content of the sample. One gram (1 g) of each sample was weighed into a 100 mL volumetric flask, where 75 mL of 3N $H_2SO_4$ was added and stirred for 1 hour. The mixture was then filtered with Whatman No 1 filter paper. From the filtrate, 25 mL was taken and titrated against 0.1N KMnO4 solution, until a pink colour persisted for at least 30 seconds. The oxalate content was calculated as follows:

$$Oxalate\ (mg/100g) = \frac{T\ \times Vme\ (DF) \times 105}{Me\ \times Mf} \qquad \textbf{Equation 9}$$

Where: T = Titre of $KmNO_4$ (mL), Vme is the volume mass equivalent (1 $cm^3$ of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid), Df is the dilution factor (Vt/A =75/25 =3) where Vt is the total volume of filtrate (75 mL) and A is the aliquot used for titration (25 mL) and Me is the molar equivalent of $KMnO_4$ in oxalate and Weight of sample.

## 3.8 Feed Formulation

Sprouted sorghum flour and Bambara nut flour were used for weaning food formulation in the different ratios as shown in Table 3.1 (Arise *et al.*, 2014 and James *et al*., 2018) Rat chow was used as the control diet and Cerelac Baby formula was the standard.

**Table 3.1: Sprouted Sorghum and Sprouted Bambara Nut Blends**

| Sample codes | Sprouted Sorghum (%) | Sprouted Bambara nut (%) |
|---|---|---|
| F1 | 100 | 0 |
| F2 | 0 | 100 |
| F3 | 95 | 5 |
| F4 | 90 | 10 |
| F5 | 85 | 15 |
| F6 | 80 | 20 |

### 3.9 Experimental Designs

#### 3.9.1 Animal grouping and experimentation

Forty (40) Wister-strain weaning albino rats (3 weeks old) of both sexes weighing between 34 – 55 g were purchased from animal House, Ahmadu Bello University Zaria. The rats were randomly distributed into eight (8) groups of 5 rats each (1-8 groups). They were distributed randomly in separate cages in the animal House of Biochemistry department Federal University of Technology Minna, Niger State. The rats were allowed access to their corresponding diets and water *ad libitum* for 28 days. Initial weights of animals were taken prior to the commencement of each experiment and weights of animals were taken on a weekly basis. Feed intake of the animals was measured on a daily basis.

**Group A:** were fed with 100 % of sprouted sorghum (control)

**Group B:** were fed with 100 % of sprouted Bambara nut (control)

**Group C:** were fed with sprouted sorghum and sprouted Bambara nut blend (95 %: 5 %)

**Group D:** were fed with sprouted sorghum and sprouted Bambara nut blend (90 %: 10 %).

**Group E:** were fed with sprouted sorghum sprouted Bambara nut blend (85 %: 15 %)

**Group F**: were fed with sprouted sorghum and sprouted Bambara nut blend (80 %: 20 %)

**Group G:** were fed with 100 % rat chow and they served as the negative control

**Group H:** The group were fed with Standard weaning formula (Cerelac). Animals in this group served as the standard.

### 3.9.2 Determination of daily feed intake

The daily feed intake was determined by weighing the remnant of the feed and subtracting from the amount of feed (in grams) given to the rats on a daily basis.

### 3.9.3 Determination of percentage body weight gain

The rats were weighed using electronic compact scale (Labtech BL10001). The weight of the rats was monitored on a weekly basis. Body weight gain was determined using the formula

$$\text{Body weight gain} = \text{Final body weight} - \text{Initial body weight}$$

## 3.10. Data Analysis

Statistical analysis was carried out on the duplicate or triplicate value of the data obtained using Statistical Package for Social Sciences (SPSS) version 21. Analysis of variance (ANOVA) was used to test for significance level using Duncan Multiple Range Test (DMRT) at $p < 0.05$ confidence level.

# CHAPTER FOUR

# 4.0 RESULTS AND DISCUSSION

## 4.1 Results

Sprouted sorghum and Bambara nut is shown in plate IX. The sorghum displayed in the plate is the sprout obtained on day 5 while the Bambara nut is the sprout obtained on day 3.

**Sprouted Sorghum** **Sprouted Bambara Nut**

**Plate IX: Photograph of Sprouted Sorghum and Bambara nut**

### 4.1.1 Proximate composition of sprouted and unsprouted sorghum and Bambara nut flours

The proximate composition of the sprouted and un-sprouted sorghum flour is shown in Table 4.1. There was a significant difference ($p<0.05$) in the ash content for sprouted and un- sprouted sorghum. The crude fat content of the un-sprouted is 1.80±0.176 % and that of the sprouted ranged from 2.03±0.045 % to 3.27±0.081 % with the highest fat content on day 5. There was a significant difference ($p<0.05$) in the fat content of the sprouted and un-sprouted sorghum.

The moisture content of the un-sprouted sorghum is 10.35±0.338 % and that of the sprouted ranged from 10.58±0.304 % to 13.40±0.070 % with day 5 having the highest moisture content. The moisture content of the sprouted was significantly different ($p<0$, 05) compared to the un-sprouted. The protein content of the sprouted sorghum ranged between 8.58±0.253 % to 10.99±0.014 % with the highest protein content on day 5 and that of the un-sprouted is 7.73±0.303 %. The fibre content of the un-sprouted sorghum is 9.98±0.152 % and that of the sprouted ranged from 8.78±0.058 % to 10.08±0.012 % with day 7 sprouts having the highest fibre content. There was no significant difference ($p<0.05$) in the fibre content of sprouted and un-sprouted sorghum. The carbohydrate content of the un-sprouted sorghum is 69.82±0.197 % and that of the sprouted ranged from 62.04±0.55 % to 68.17±0.69 %. The un-sprouted had a significantly ($p<0.05$) higher carbohydrate content than the sprouted. Ash content of the un-sprouted was $0.29 \pm 0.029^{a}$ % while that of the sprouted ranged between $0.29 \pm 0.009^{a}$ -$1.10 \pm 0.702^{e}$. The sprouted was significantly ($p<0.05$) higher than the un-sprouted.

**Table 4.1: Proximate Composition of Un-Sprouted and Sprouted Sorghum Flour (%)**

| | Ash | Fat | Moisture | Protein | Fibre | Carbohydrate |
|---|---|---|---|---|---|---|
| Un-sprouted | 0.29 ±0.029$^{a}$ | 1.80±0.176$^{a}$ | 10.35 ±0.338$^{a}$ | 7.73 ±0.303$^{a}$ | 9.98 ±0.152$^{ef}$ | ***69.82 ±0.197$^{f}$** |
| Day 1 sprouted | 0.29 ±0.009$^{a}$ | 2.03±0.045$^{b}$ | 10.58±0.304$^{b}$ | 8.58±0.253$^{bc}$ | 10.02 ±0.062$^{ef}$ | 68.17 ±0.685$^{ef}$ |
| Day 2 sprouted | 0.82±0.020$^{c}$ | 2.10±0.058$^{b}$ | 11.33±0.381$^{b}$ | 8.97 ±0.171$^{cd}$ | 9.59 ±0.262$^{cd}$ | 66.54 ±1.771$^{cde}$ |
| Day 3 sprouted | 0.95 ±0.015$^{d}$ | 3.03 ±0.040$^{c}$ | 12.22 ±0.006$^{c}$ | 10.05 ±0.012$^{d}$ | 9.28 ±0.023$^{bc}$ | 64.32 ±0.283$^{bcd}$ |
| Day 4 sprouted | 0.98 ±0.009$^{de}$ | 3.15 ±0.038$^{c}$ | 13.29 ±0.032$^{c}$ | 10.33 ±0.061$^{d}$ | 9.06 ±0.012$^{ab}$ | 63.02 ±0.033$^{bc}$ |
| Day 5 sprouted | ***1.10 ±0.702$^{e}$** | ***3.27 ±0.081$^{c}$** | ***13.40 ±0.070$^{c}$** | ***10.99 ±0.014$^{e}$** | 8.78 ±0.058$^{a}$ | 62.04 ±0.549$^{a}$ |
| Day 6 sprouted | 0.72 ±0.044$^{b}$ | 3.21 ±0.063$^{c}$ | 13.14 ±0.038$^{c}$ | 9.19 ±0.034$^{b}$ | 9.68 ±0.018$^{de}$ | 63.81 ±0.201$^{ab}$ |
| Day 7 sprouted | 0.33 ±0.009$^{a}$ | 2.18 ±0.015$^{b}$ | 13.23 ±0.058$^{c}$ | 8.65 ±0.018$^{a}$ | ***10.08 ±0.012$^{f}$** | 65.52 ±0.493$^{def}$ |

Values are reported as mean ± standard error of means. Values with the same letter along column are not significantly different at $p \leq 0.05$ while values on the same column with different alphabetic superscript are significantly different at ($p \leq 0.05$)

The result in Table 4.2: shows the proximate composition of the un-sprouted and sprouted Bambara nut flour. The ash content of the un-sprouted is 2.21±0.061 % and that of the sprouted ranged from 2.24±0.064 % to 4.13±0.075 % with maximum ash content on day 4. There was a significant difference ($p<0.05$) in the ash content of the sprouted and un-sprouted Bambara nut flour. Crude fat of the sprouted Bambara nut flour ranged from 2.73±0.264 % to 4.22±0.064% and for the un-sprouted 3.06±0.104 %. There was a significant difference ($p<0.05$) in the crude fat of sprouted and un-sprouted Bambara nut flour. The protein content of un-sprouted Bambara nut flour is 19.49±0.572 % and the sprouted ranged from 21.15±0.570 % to 25.67±0.403 % with the maximum protein yield on day 4. The protein content on day 4 sprout was significantly higher ($p<0.05$) than the un-sprouted and the sprouts of the other days. The moisture content of the un-sprouted is 4.18±0.105 % and that of the sprouted ranged from 4.33±0.064 % to 5.73±0.042 % with day 4 having the maximum moisture content. Fibre content of the un-sprouted Bambara nut flour is 7.17±0.131% and that the sprouted ranged from 5.15±0.287 % to 9.04±0.073 % with day 7 sprouts having the maximum fibre content. There was a significant difference in the fibre content of the sprouted and un-sprouted Bambara nut flour, the carbohydrate content of the un-sprouted Bambara nut flour is 62.33±0.587 % and that of the sprouted ranged from 53.93±0.386 % to 61.31±0.554 %. The un-spouted Bambara nut had significantly higher ($p<0.05$) carbohydrate content than the sprouted.

**Table 4.2: Proximate Composition of Un-sprouted and Sprouted Bambara Nut Flour (%)**

| Parameters | Ash | Fat | Protein | Moisture | Fibre | Carbohydrate |
|---|---|---|---|---|---|---|
| Un-sprouted | 2.21±0.061$^{a}$ | 3.06±0.104$^{a}$ | 19.49±0.572$^{a}$ | 4.18±0.105$^{a}$ | 7.17±0.131$^{b}$ | ***62.33±0.587$^{d}$** |
| Day 1 sprouted | 2.24±0.064$^{a}$ | 3.13±0.068$^{a}$ | 21.15±0.570$^{a}$ | 5.47±0.009$^{bc}$ | 5.15±0.287$^{a}$ | 61.31±0.554$^{d}$ |
| Day 2 sprouted | 3.08±0.078$^{b}$ | 3.90±0.131$^{de}$ | 22.62±0.349$^{ab}$ | 5.53±0.214$^{bc}$ | 5.24±0.307$^{a}$ | 58.32±0.235$^{c}$ |
| Day 3 sprouted | 3.09±0.074$^{b}$ | 3.70±0.248$^{cd}$ | 23.74±0.370$^{cd}$ | 5.53±0.205$^{bc}$ | 5.24±0.065$^{a}$ | 57.41±0.336$^{bc}$ |
| Day 4 sprouted | ***4.13±0.075$^{d}$** | ***4.22±0.064$^{e}$** | ***25.67±0.403$^{d}$** | ***5.73±0.042$^{c}$** | 5.34±0.006$^{a}$ | 53.93±0.386$^{a}$ |
| Day 5 sprouted | 3.77±0.058$^{c}$ | 3.42±0.098$^{bc}$ | 23.33±0.593$^{bc}$ | 5.31±0.061$^{b}$ | 7.68±0.242$^{b}$ | 55.31±0.133$^{b}$ |
| Day 6 sprouted | 3.73±0.052$^{c}$ | 3.05±0.085$^{a}$ | 22.46±0.634$^{ab}$ | 5.20±0.101$^{b}$ | 8.51±0.163$^{c}$ | 55.72±0.289$^{b}$ |
| Day 7 sprouted | 2.95±0.046$^{b}$ | 2.73±0.264$^{a}$ | 22.12±0.626$^{ab}$ | 4.33±0.064$^{a}$ | ***9.04±0.073$^{d}$** | 57.19±0.573$^{bc}$ |

Values are reported as mean ± standard error of means. Values with the same letter along column are not significantly different at $p \leq 0.05$ while values on the same column with different alphabetic superscript are significantly different at $p \leq 0.05$.

### 4.1.2 Amino acid composition of sorghum and bambara nut

The result presented in Table 4.3 shows that *S. bicolor and V. subterranean* seeds contain both essential (leucine, lysine, isoleucine, phenylalanine, threonine, valine, histidine and methionine) and non-essential amino acids (proline, tyrosine, arginine, glutamic acid, cysteine, aspartic acid, alanine and serine). The seeds of *V. subterranean* contained a higher concentration of most of the amino acids except for isoleucine and threonine that were higher in the *S. bicolor*. Glutamate was the most abundant amino acid in both seeds. Methionine had the lowest concentration of amino acid in sorghum while in Bambara cysteine had the lowest concentration of amino acid.

**Table 4.3 Amino Acid Composition (g/100g Crude Protein) of Sorghum and Bambara nut**

| Amino acid | Sorghum | Bambara nut |
|---|---|---|
| Leucine* | 1.08 | 5.7 |
| Lysine * | 1.60 | 2.90 |
| Isoleucine * | 4.62 | 3.6 |
| Phenylalanine * | 2.16 | 3.88 |
| Norleucine | - | - |
| Valine * | 1.85 | 3.30 |
| Methionine* | 0.62 | 1.87 |
| Proline | 0.89 | 2.06 |
| Arginine | 3.19 | 3.20 |
| Tyrosine | 1.69 | 3.30 |
| Histidine * | 1.65 | 2.20 |
| Cysteine | 0.89 | 0.90 |
| Alanine | 1.29 | 4.02 |
| Glutamic acid | 6.05 | 12.50 |
| Glycine | 1.39 | 2.75 |
| Threonine* | 2.24 | 2.05 |
| Serine | 2.00 | 2.2 |
| Aspartic acid | 3.64 | 4.20 |

***Essential amino acid

The result in Table 4.4 shows the classification of the amino acid composition of sorghum and Bambara nut flours. The total amino acid content of the Bambara nut flour (61.67±0.015 g/100g) is significantly higher ($p<0.05$) than that of the sorghum flour (37.86±0.035 g/100g). This pattern was also seen in the essential amino acid with histidine for Bambara nut flour (26.55±0.050 g/100g) while sorghum flour (16.83±0.010 g/100g), the amino acid without histidine for Bambara (24.36±0.055 g/100g) for sorghum flour

(15.18±0.010 g/100g) and the total non-essential amino acid in Bambara flour (35.16±0.010 g/100g) and in sorghum flour (21.05±050 g/100g). Also, this trend was maintained for total sulphur amino acid found in Bambara nut flour (2.77±0.001 g/100g) for sorghum (1.51±0.001 g/100g), total non-sulphur amino acid in Bambara (58.87±0.001 g/100g) while sorghum (36.20±0.001 g/100g), total aromatic amino acid in Bambara nut flour (10.38±0.001 g/100g) while sorghum flour (6.50±0.001 g/100g). total non-aromatic amino acid in Bambara nut flour (52.27±0.001 g/100g) while sorghum (31.32±0.001 g/100g). Bambara nut flour contained significantly higher ($p<0.05$) the various amino acid than sorghum flour. There was no significant difference ($p>0.05$) in the ratios of total essential amino acid to that of non-essential amino acid in Bambara (0.76±0.001 g/100g) while sorghum flour (0.75±0.001 g/100g) but there was a significant difference ($p<0.05$) in the ratio of total aromatic amino acid to total non-aromatic amino acid in Bambara nut flour (0.20±0.001 g/100g) and sorghum flour (0.21±0.001 g/100g).

**Table 4.4: Amino Acid Classes of Sorghum and Bambara Nut Flour**

| Amino acid class | Sorghum (g/100g) | Bambara (g/100g) |
|---|---|---|
| Total AA | 37.86±0.035[a] | 61.67±0.015[b] |
| TEAA with HISTIDINE | 16.83±0.010[a] | 26.55±0.050[b] |
| TEAA WITHOUT HIS | 15.18±0.010[a] | 24.36±0.055[b] |
| TNEAA | 21.05±050[a] | 35.16±0.010[b] |
| TEAA:TNEAA | 0.75±0.001[a] | 0.76±0.001[a] |
| TSAA | 1.51±0.001[a] | 2.77±0.001[b] |
| TNSAA | 36.20±0.001[a] | 58.87±0.001[b] |
| TArAA | 6.50±0.001[a] | 10.38±0.001[b] |
| TNArAA | 31.32±0.001[a] | 52.27±0.001[b] |
| TArAA:TNArAA | 0.21±0.001[b] | 0.20±0.001[a] |

Values are reported as mean ± standard error of means. Values with the same letter along column are not significantly different at $p \leq 0.05$ while values on the same column with different alphabetic superscript are significant at $p \leq 0.05$.

AA-Amino acid, TEAA-Total essential amino acid, TNEAA-Total non-essential amino acid, TSAA-Total sulphur amino acid, TNSAA-Total non-sulphur amino acid, TArAA-Total aromatic amino acid, and Total non-aromatic amino acid.

### 4.1.3 Anti-nutrient composition of sprouted and unsprouted sorghum and Bambara nut flours

Table 4.5 shows the anti-nutrient content of the un-sprouted and sprouted sorghum flour. Sprouting decreased the anti-nutrient contents of sorghum flour but was later increased as sprouting progressed. The phytate, alkaloid and tannin contents of sorghum were decreased upon sprouting for 5 days. Phytate values; un-sprouted (28.33 ±0.334 mg/g) for sprouted sorghum (25.80±0.122), Alkaloid content of the un-sprouted (8.27±0.187 mg/g) sprouted (6.68±0.091), tannin content was found to be (165.24±0.576) for un-sprouted and (140.52±0.414) for the sprouted. However, sprouting had a faster effect on saponins and oxalate as they were both decreased on day 4. Saponins un-sprouted was (427.82 ± 3.179 mg/g) and the sprouted (385.53±3.003), oxalate un-sprouted (6.44±0.129 mg/g) while the sprouted (3.90±0.122mg/g). Sprouting decreased significantly ($p<0.05$) the anti-nutrient of sorghum flour.

**Table 4.5: Anti-nutrient Composition of Un-sprouted and Sprouted Sorghum Flour**

| Parameters | Phytate (mg/g) | Alkaloids (mg/g) | Saponins (mg/100g) | Oxalate (mg/g) | Tannins (mg/100g) |
|---|---|---|---|---|---|
| Un-sprouted | 28.33±0.334$^{e}$ | 8.27±0.187$^{e}$ | 427.82±3.179$^{d}$ | 6.44±0.129$^{g}$ | 165.24±0.576$^{e}$ |
| DAY1sprouted | 27.80±0.489$^{de}$ | 7.66±0.038$^{d}$ | 414.61±2.811$^{c}$ | 5.73±0.115$^{f}$ | 151.52±0.576$^{d}$ |
| DAY2sprouted | 26.80±0.263$^{bc}$ | 7.27±0.033$^{c}$ | 402.54±0.797$^{b}$ | 5.09±0.064$^{e}$ | 148.81±0.795$^{c}$ |
| DAY3sprouted | 26.10±0.124$^{ab}$ | 7.00±0.009$^{b}$ | 397.67±1.570$^{b}$ | 4.18±0.094$^{b}$ | 146.12±0.617$^{b}$ |
| DAY4sprouted | 26.21±0.165$^{ab}$ | 7.00±0.053$^{b}$ | *385.53±3.003$^{a}$ | *3.90±0.122$^{a}$ | 141.58±0.240$^{a}$ |
| DAY5sprouted | *25.80±0.122$^{a}$ | *6.68±0.091$^{a}$ | 401.09±1.031$^{b}$ | 4.53±0.462$^{c}$ | *140.52±0.414$^{a}$ |
| DAY6sprouted | 27.16±0.095$^{cd}$ | 7.28±0.033$^{c}$ | 417.93±3.007$^{c}$ | 4.75±0.046$^{cd}$ | 145.35±0.513$^{b}$ |
| DAY7sprouted | 27.68±0.116$^{de}$ | 7.80±0.670$^{d}$ | 430.51±0.407$^{d}$ | 4.99±0.054$^{de}$ | 149.26±0.329$^{c}$ |

Values are reported as mean ± standard error of means. Values with the same letter along column are not significantly different at $p\leq0.05$ while values on the same column with different alphabetic superscript are significantly different at $p\leq0.05$

The result in Table 4.6 shows the anti-nutrient content of un-sprouted and sprouted Bambara nut flours. The anti-nutrients in Bambara nut flour were generally decreased via sprouting but increased later with the progression of sprouting. The phytate content of Bambara flour was decreased upon sprouting on day 5. The values for un-sprouted (21.76±0.235 mg/g) while for sprouted (16.89±0.061). There was no significant difference ($p<0.05$) in the values obtained for phytate on days 4 and 5 respectively. However, alkaloids, saponins, oxalates and tannin contents in Bambara nut flour were decreased upon sprouting for 4 days. Alkaloids un-sprouted (7.33±0.181 mg/g) for sprouted (6.33±0.058 mg/g), saponin un-sprouted (327.82±3.179 mg/g) for sprouted (285.53±3.003), oxalate content of the un-sprouted Bambara (6.37±0.097mg/g) while the sprouted (3.90±0.101). The highest tannin content was found in the un-sprouted with the value of (151.91±2.873 mg/g) and the sprouted (137.19±2.954). The un-sprouted had significantly ($p<0.05$) higher anti-nutrient than the sprouted.

**Table 4.6: Anti-nutrient Composition of Un-sprouted and Sprouted Bambara Nut Flour**

| Parameters | Phytate (mg/g) | Alkaloids (mg/g) | Saponins (mg/100g) | Oxalate (mg/g) | Tanins (mg/100g) |
|---|---|---|---|---|---|
| Un-sprouted | 21.76±0.235$^{e}$ | 7.33±0.181$^{e}$ | 327.82±3.179$^{d}$ | 6.37±0.097$^{f}$ | 151.91±2.873$^{b}$ |
| DAY1sprouted | 20.01±0.124$^{d}$ | 7.03±0.034$^{d}$ | 314.61±2.811$^{c}$ | 5.60±0.107$^{e}$ | 141.52±1.107a |
| DAY2sprouted | 18.72±0.177$^{c}$ | 6.86±0.040$^{cd}$ | 302.54±0.797$^{b}$ | 4.99±0.107$^{d}$ | 142.14±3.576$^{a}$ |
| DAY3sprouted | 17.92±0.055$^{b}$ | 6.54±0.047$^{ab}$ | 297.67±1.570$^{b}$ | 4.11±0.064$^{a}$ | 143.12±3.460$^{a}$ |
| DAY4sprouted | 17.12±0.047$^{a}$ | ***6.33±0.058$^{a}$** | ***285.53±3.003$^{a}$** | ***3.90±0.101$^{a}$** | ***137.19±2.954$^{a}$** |
| **DAY5sprouted** | ***16.89±0.061$^{a}$** | 6.64±0.043$^{bc}$ | 301.09±1.031$^{b}$ | 4.43±0.046$^{b}$ | 137.58±2.820$^{a}$ |
| DAY6sprouted | 17.12±0.090$^{a}$ | 6.85±0.032$^{cd}$ | 317.93±3.007$^{c}$ | 4.68±0.057$^{bc}$ | 140.02±3.083$^{a}$ |
| DAY7sprouted | 18.10±0.064$^{b}$ | 7.07±0.070$^{d}$ | 330.51±0.407$^{d}$ | 4.91±0.095$^{cd}$ | 141.26±1.414$^{a}$ |

Values are reported as mean ± standard error of means. Values with the same letter along column are not significantly different at $p\leq0.05$ while values on the same column with different alphabetic superscript are significantly different at $p\leq0.05$.

*days where the analysed sample had the least composition of anti-nutrients.

### 4.1.4 Functional properties of sprouted sorghum, unsprouted sorghum, sprouted unsprouted Bambara nut flours and formulated diets.

Figure 4.1 presents the functional properties of unsprouted and sprouted Bambara nut flour while Figure 4.2 shows the functional properties of unsprouted and sprouted sorghum flour. The gelatinization temperatures for Bambara nut and sorghum flours decreased significantly ($p<0.05$) after sprouting the samples. Conversely, sprouting increased the gelatinization time for Bambara nut but had no effect on the gelatinization time of sorghum flour. Sprouting had no effect on swelling capacity on the two seeds, either sprouted or unsprouted. The same trend was observed for water absorption capacity of the flours. On the other hand, sprouting reduced the bulk densities of Bambara and sorghum flours significantly ($p<0.05$). There was no observable effect in the foam capacity of sorghum after sprouting, but that of Bambara nut was significantly ($p<0.05$) reduced upon sprouting.

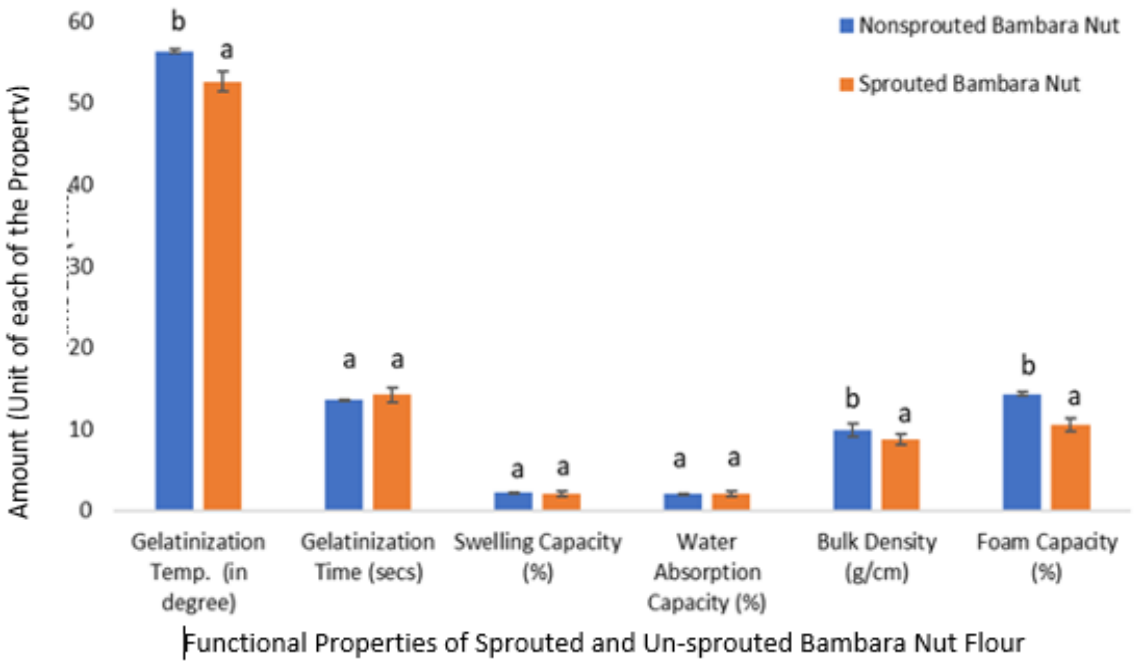


**Figure 4.1: Functional Properties of Un-sprouted and Sprouted Bambara Nut Flour**

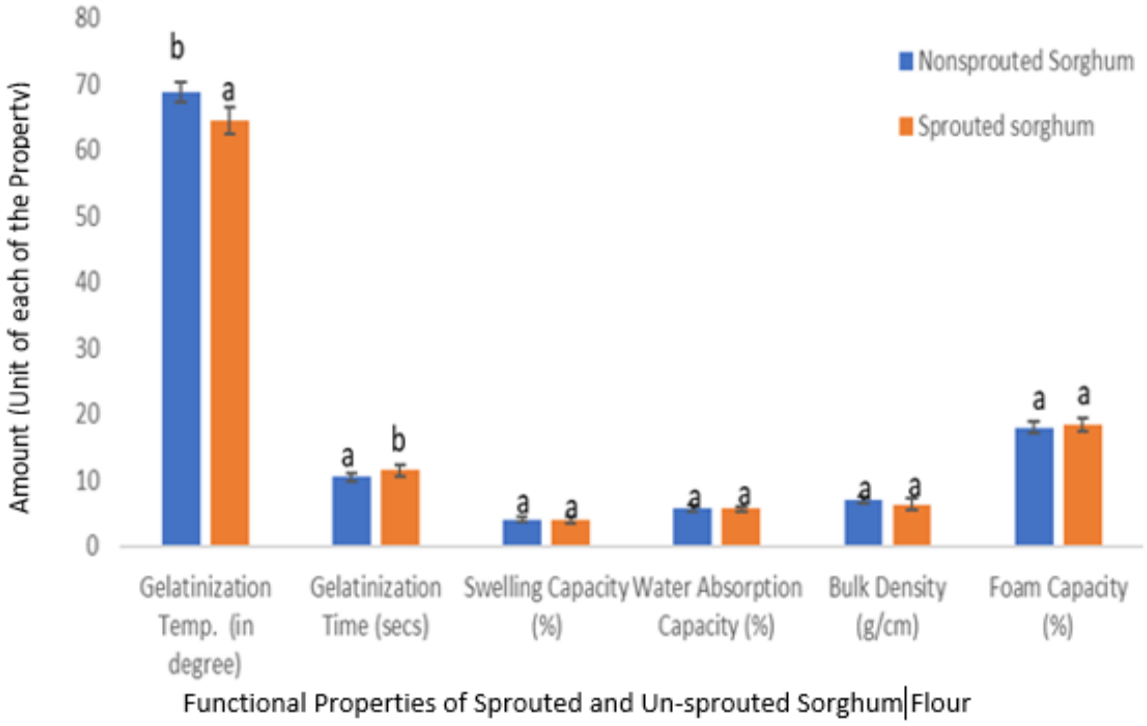


**Figure 4.2: Functional Properties of Un-sprouted and Sprouted Sorghum Flour**

The result in Figure 4.3 presents the functional properties of sprouted Bambara nut and sprouted sorghum formulated diets. The gelatinization temperatures of the different diets reduced significantly ($p<0.05$) as the supplementation with Bambara was increased. Also, gelatinization time increased significantly ($p<0.05$) when supplementation was increased. There was no significant difference ($p>0.05$) in the swelling capacity of the formulated diets, except for the (80 % sorghum + 20 % Bambara). The water absorption capacity reduced significantly ($p<0.05$) in the formulated diets as supplementation with Bambara increased. There was no significant difference ($p>0.05$) in the bulk densities of the formulated diets. Foaming capacity was increased significantly ($p<0.05$) with a corresponding stability as supplementation with Bambara was increased above 5 %.

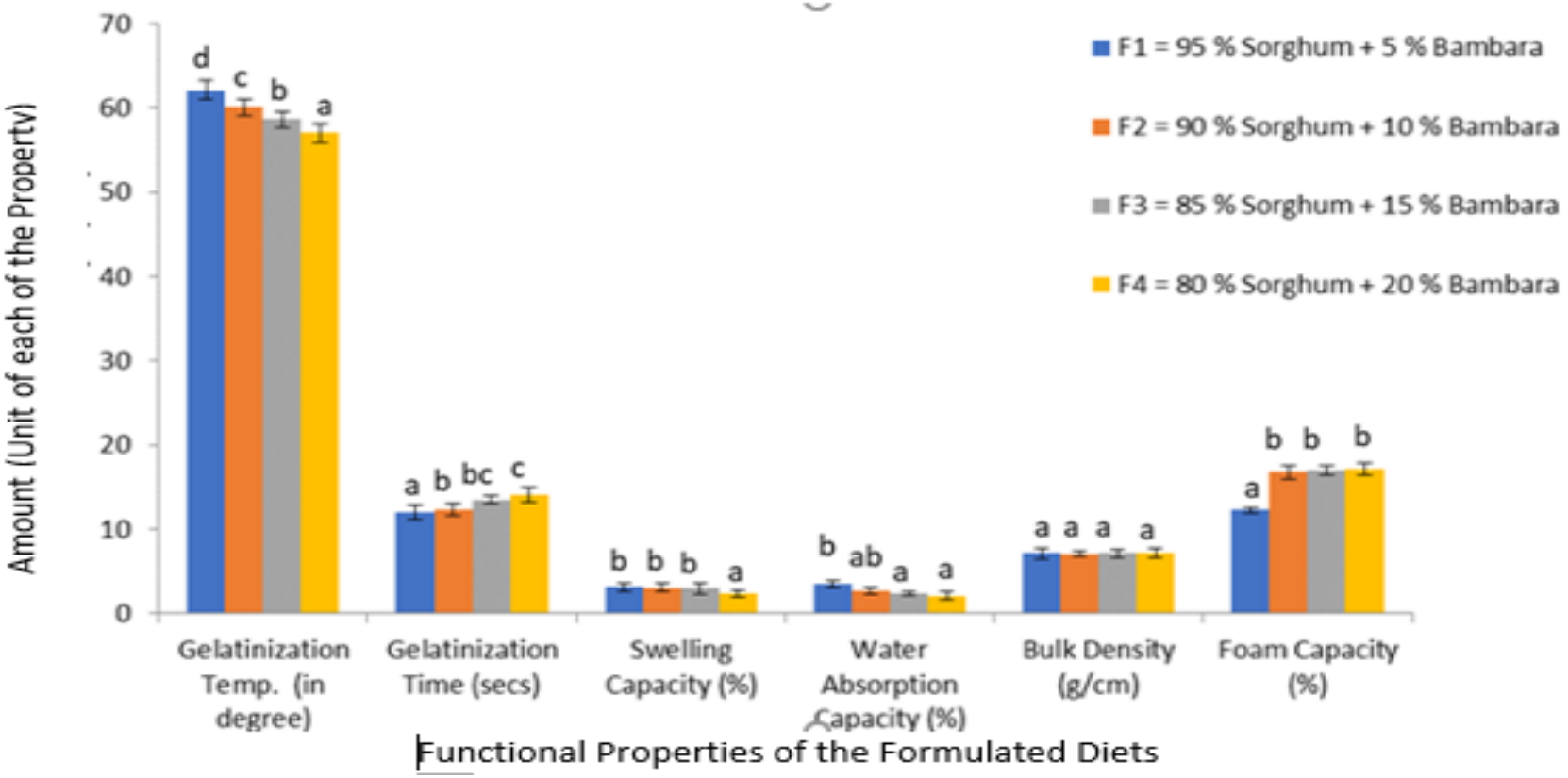


**Figure 4.3: Functional Properties of Sprouted Sorghum and Bambara Nut Flour Formulated Diets**

### 4.1.5 Proximate composition of formulated diets

The proximate composition of the formulated diets (Table 4.7) showed that there was a significant difference ($p<0.05$) in the ash contents of the different diets when supplementation with Bambara flour was increased. There was a statistically significant ($p<0.05$) increase in the fat content of the diet supplemented with 20 %. Bambara. In all the diets the 100 % un-sprouted sorghum diet had the lowest fat content. Protein content of the diets increased with corresponding increase in supplementation with Bambara nut flour. Moisture content in all the diets was found to be generally low; however, the 100 % sprouted sorghum diet had the highest moisture content while the un-sprouted Bambara diet had the lowest. All the diets had significantly different moisture content ($p<0.05$). The crude fibre was found to be lowest in the sprouted Bambara nut diet (100 %) and highest in the un-sprouted sorghum diet. There was no significant difference ($p>0.05$) in the fibre contents of the 10 % and 15 % Bambara supplemented diets. The carbohydrate content of the un-sprouted sorghum diet was significantly ($p<0.05$) higher compared to all the other diets.

**Table 4.7: Proximate Composition of Sprouted, Un-sprouted Bambara Nut and Sorghum Formulated diets (%)**

| Diet | Ash | Fat | Protein | Moisture | Fibre | Carbohydrate |
|---|---|---|---|---|---|---|
| Un-sprouted Bambara Nut | 2.21±0.061$^{c}$ | 3.06±0.104$^{b}$ | 19.49±0.572$^{f}$ | 4.18±0.105$^{a}$ | 7.17±0.131$^{b}$ | 62.33±0.587$^{e}$ |
| Un-sprouted Sorghum | 0.28±0.009$^{a}$ | 1.67±0.176$^{a}$ | 7.72±0.303$^{a}$ | 10.32±0.338$^{f}$ | 9.98±0.152$^{f}$ | 68.82±0.197$^{f}$ |
| Sprouted Bambara nut day 4 | 4.13±0.075$^{g}$ | 4.22±0.064$^{e}$ | 25.67±0.403$^{g}$ | 5.73±0.042$^{b}$ | 5.34±0.006$^{a}$ | 53.93±0.386$^{a}$ |
| Sprouted Sorghum day 5 | 1.06±0.702$^{b}$ | 3.27±0.081$^{d}$ | 10.98±0.014$^{b}$ | 13.40±0.070$^{g}$ | 8.78±0.058$^{c}$ | 61.04±0.549$^{de}$ |
| 95% Sorghum + 5% Bambara | 2.35±0.087$^{c}$ | 3.18±0.015$^{c}$ | 12.72±0.637$^{c}$ | 10.35±0.088$^{f}$ | 9.72±0.026$^{e}$ | 60.56±0.277$^{d}$ |
| 90% Sorghum + 10 % Bambara | 2.79±0.015$^{d}$ | 3.33±0.046$^{c}$ | 14.64±0.317$^{d}$ | 9.22±0.055$^{e}$ | 9.53±0.056$^{d}$ | 59.73±0.273$^{d}$ |
| 85% Sorghum + 15% Bambara | 2.94±0.033$^{e}$ | 3.61±0.026$^{c}$ | 16.25±0.214$^{e}$ | 8.18±0.102$^{d}$ | 9.43±0.226$^{d}$ | 58.67±0.320$^{c}$ |
| 80% Sorghum + 20% Bambara | 3.25±0.077$^{f}$ | 3.87±0.027$^{d}$ | 20.31±0.226$^{f}$ | 6.48±0.091$^{c}$ | 8.38±0.159$^{c}$ | 57.04±0.093$^{b}$ |

Values are reported as mean ± standard error of means. Values with the same letter on the column are not significantly different at $p \leq 0.05$

Figure 4.4 shows that the animals fed on cerelac had the highest ($p<0.05$) feed intake compared to all the other groups. The group of rats fed 20% Bambara supplemented diet was next, while those fed 100 % Bambara diet had the lowest feed intake.

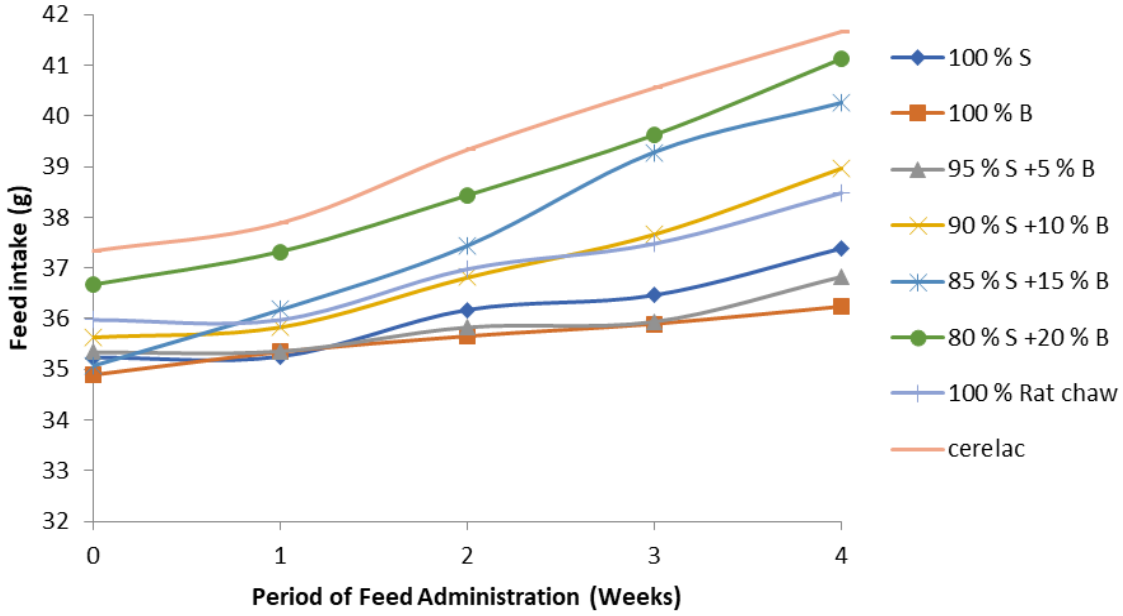


**Figure 4.4 Feed Intake of Rats fed on Experimental Diets Formulated from Sprouted Sorghum and Bambara Nut Flours.**

In the experiment, (Figure 4.4) all the animals fed with the different formulated diets gained weight over the four weeks period of the experiment. Though, irregularities were noticed in the weight gain pattern of the animals fed 15 % Bambara supplemented diets, 100 % Bambara supplemented and 100 % sorghum supplemented diets. All the other groups had a consistent increase in weight gain with the group of animals that fed on cerelac having the most consistent weight gain. In week 1, the animals fed on 20 % Bambara supplemented diet had higher weight gain than all the other groups. The animals fed on 15 % Bambara supplemented diet gained higher weight in week 2 while those fed on cerelac had a higher weight gain in week 3. In the 4$^{th}$ week where the experiment terminated, the animals fed on the 20 % Bambara had the highest weight gain while those fed on 100 % sorghum had the lowest weight gain.

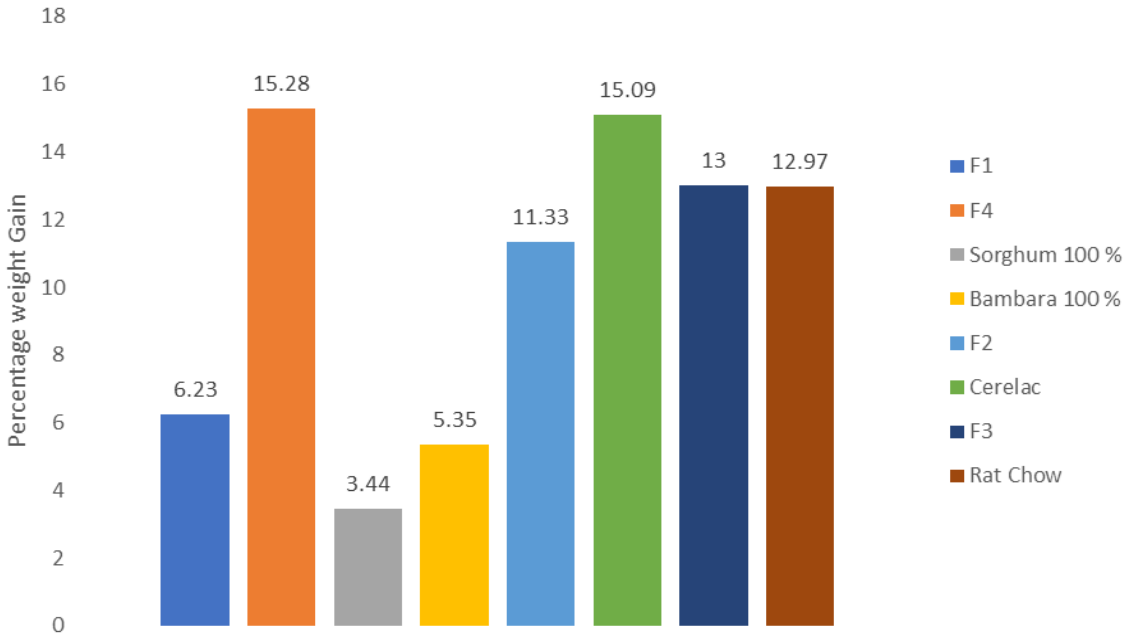


**Fig 4.5: Percentage Weight Gain in Rats Fed on Bambara Nut and Sorghum Flours Formulated Diets.**

100%S = 100 % sorghum, 100 % B = 100 % Bambara nut, F1 = 95 % Sorghum + 5 % Bambara, F2 = 90 % Sorghum + 10 % Bambara, F3 = 85 % Sorghum + 15 % Bambara and F4 = 80 % Sorghum + 20 % Bambara respectively.

### 4.1.6 Hematological Parameters in Rats Fed on Sprouted Bambara Nut and Sorghum Formulated Diets.

The hematological parameters in the experimental rats as shown in Table 4.8 were within normal range. The group of animals fed on cerelac had a significantly higher ($p<0.05$) value in the Hb (15.85±0.06), PCV (47.50±0.65), MCV (83.00±0.91) and MCH (28.00±0.41) than the other groups while the group of animals fed 20 % Bambara nut supplemented diet was next and highest in the group of rats fed different supplemented diets. The group fed on 100 % sorghum had the lowest value for these indices, Hb (10.28±0.06), PCV (30.00±0.41), MCV (71.50±0.65), MCH (20.00±0.41). The group fed on rat pellets had the highest MCHC (34.50±0.65) while the group of animals fed on 100 % Bambara diet had the lowest MCHC (30.00±0.41). There was no significant difference ($p>0.05$) in the RBC of the animals fed 20 % Bambara supplemented diet and cerelac. Those fed 100 % Bambara diet had the highest PLC value (274.75±0.48), while those that were fed on 100 % sorghum diet had the lowest PLC (121.50±0.65). The group fed on 100 % sorghum had the highest TWBC (14.55±1.85) while those fed on cerelac had the lowest TWBC (8.40±0.04).

**Table 4.8: Hematological Parameters in Rats Fed on Bambara Nut and Sorghum Flour Formulated Diets**

| Parameters | 100%Sorghum | 100%Bambara | 95%Sorghum + 5%Bambara | 90%Sorghum + 10%Bamabara | 85%Sorghum +15%Bambara | 80%Sorghum+ 20%Bambara | 100%Rat chow | Cerelac |
|---|---|---|---|---|---|---|---|---|
| Hb (g/di) | 10.28±0.06$^{a}$ | 10.33±0.04$^{a}$ | 10.60±0.05$^{b}$ | 11.65±0.06$^{c}$ | 13.35±0.06$^{d}$ | 14.40±0.04$^{f}$ | 14.12±0.09$^{e}$ | 15.85±0.06$^{g}$ |
| PCV (%) | 30.00±0.41$^{a}$ | 30.25±0.48$^{a}$ | 30.30±0.41$^{a}$ | 33.00±0.41$^{b}$ | 34.00±0.41$^{b}$ | 40.25±0.48$^{d}$ | 36.00±0.41$^{c}$ | 47.50±0.65$^{e}$ |
| MCV (Fi) | 71.50±0.65$^{a}$ | 72.00±0.41$^{a}$ | 71.50±0.65$^{a}$ | 75.00±0.41$^{bc}$ | 76.00±0.41$^{c}$ | 77.00±1.08$^{c}$ | 73.50±0.65$^{ab}$ | 83.00±0.91$^{d}$ |
| MCH (pg) | 20.00±0.41$^{a}$ | 22.00±0.41$^{ab}$ | 23.25±1.25$^{bc}$ | 24.25±1.11$^{bc}$ | 24.50±0.65$^{c}$ | 27.50±0.65$^{d}$ | 26.75±0.48$^{d}$ | 28.00±0.41$^{d}$ |
| MCHC (g/di) | 33.00±0.41$^{bcd}$ | 30.00±0.41$^{a}$ | 31.50±0.65$^{ab}$ | 33.75±1.31$^{cd}$ | 33.00±0.41$^{bcd}$ | 34.00±0.41$^{cd}$ | 34.50±0.65$^{d}$ | 32.25±0.48$^{bc}$ |
| RBC (g/L) | 4.00±0.04$^{a}$ | 4.33±0.05$^{b}$ | 4.43±0.13$^{bc}$ | 4.48±0.17$^{bc}$ | 4.65±0.06$^{c}$ | 4.70±0.04$^{c}$ | 4.45±0.16$^{bc}$ | 4.70±0.04$^{c}$ |
| PLC (g/L) | 121.50±0.65$^{a}$ | 274.75±0.48$^{g}$ | 121.75±0.48$^{a}$ | 133.25±0.48$^{b}$ | 239.50±0.65$^{f}$ | 143.75±0.48$^{c}$ | 224.00±1.08$^{e}$ | 150.00±1.47$^{d}$ |
| TWBC (g/L) | 14.55±1.85$^{d}$ | 13.08±0.06$^{cd}$ | 10.10±0.04$^{ab}$ | 10.25±0.06$^{ab}$ | 11.15±0.06$^{bc}$ | 11.90±0.27$^{bc}$ | 11.95±0.06$^{bc}$ | 8.40±0.04$^{a}$ |

Values are reported as mean ± standard error of means. Values with the same letter on the row are not significantly different at $p \leq 0$ while values on the same row with different alphabetic superscript are significant at $p \leq 0.05$.

The results in Figures 4.6, 4.7, 4.8 and 4.9 show the Neutrophils, Lymphocytes, Monocytes, and Eosinophils values respectively. Results obtained showed that all the parameters were within normal range. The neutrophils in rats fed on 20 % Bambara supplemented diets was significantly ($p<0.05$) higher than all other groups, while the group that was placed on rat pellets had significantly ($P<0.05$) lower neutrophils compared to the other groups. Lymphocytes in the group that fed on 100 % Bambara was significantly ($p<0.05$) higher than the other groups. The groups that fed on 20 % Bambara supplementation had the lowest value for lymphocyte compared with the other groups. Monocytes in rats fed 10 % supplemented Bambara was significantly ($p<0.05$) higher compared with other groups while those fed on 20 % supplementation was significantly ($p<0.05$) lower. There was a significant difference ($p<0.05$) in the eosinophils of the different groups fed with the diets, though the group that fed on rat pellets had higher eosinophils than the other groups while the group fed on 20 % supplementation was the lowest.

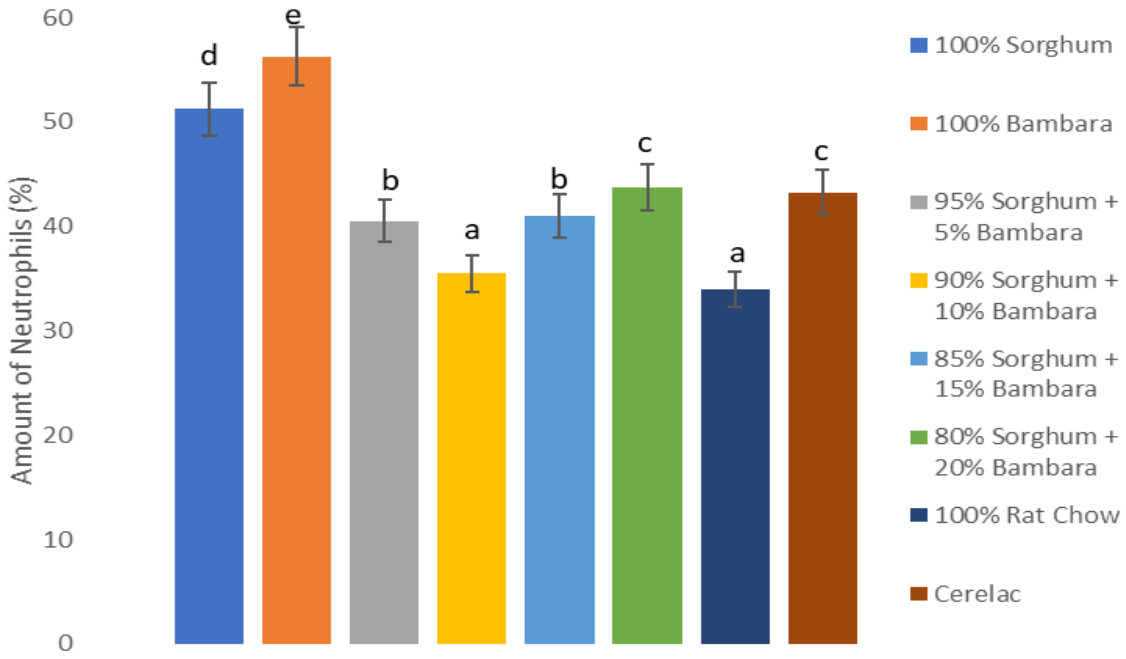


**Figure 4.6: Neutrophils in Rats Fed with Sorghum and Bambara Nut Flour Formulated Diets**

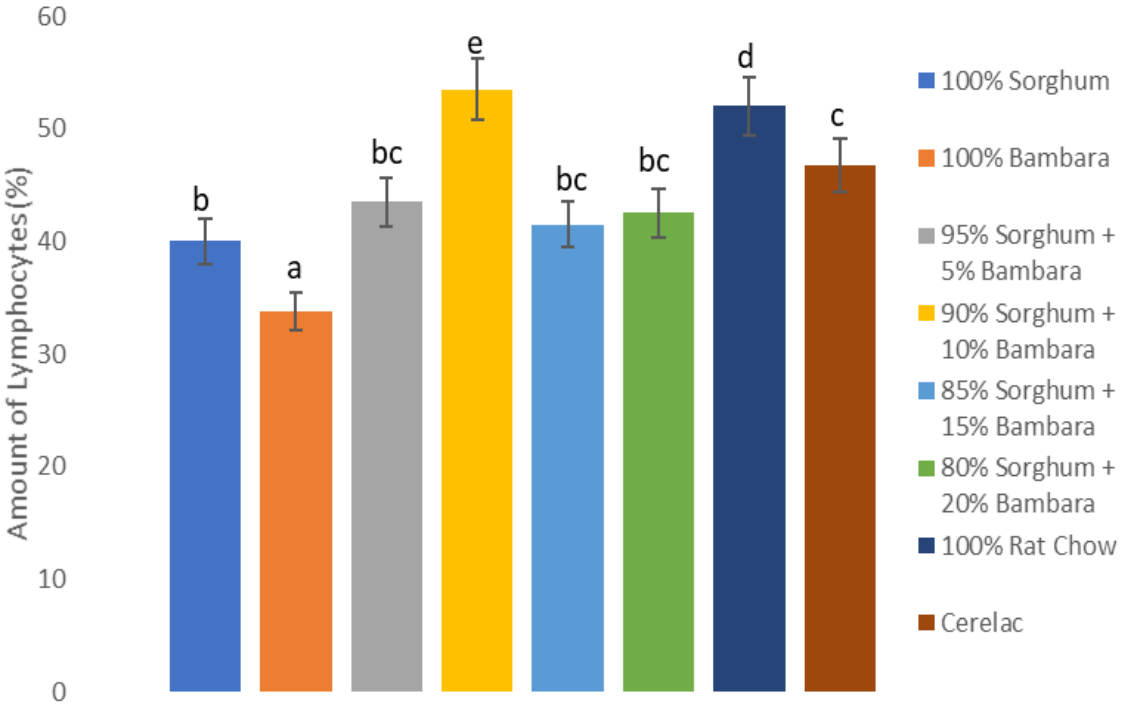


**Figure 4.7: Lymphocytes in Rats Fed with Sorghum and Bambara Nut Flour Formulated Diets**

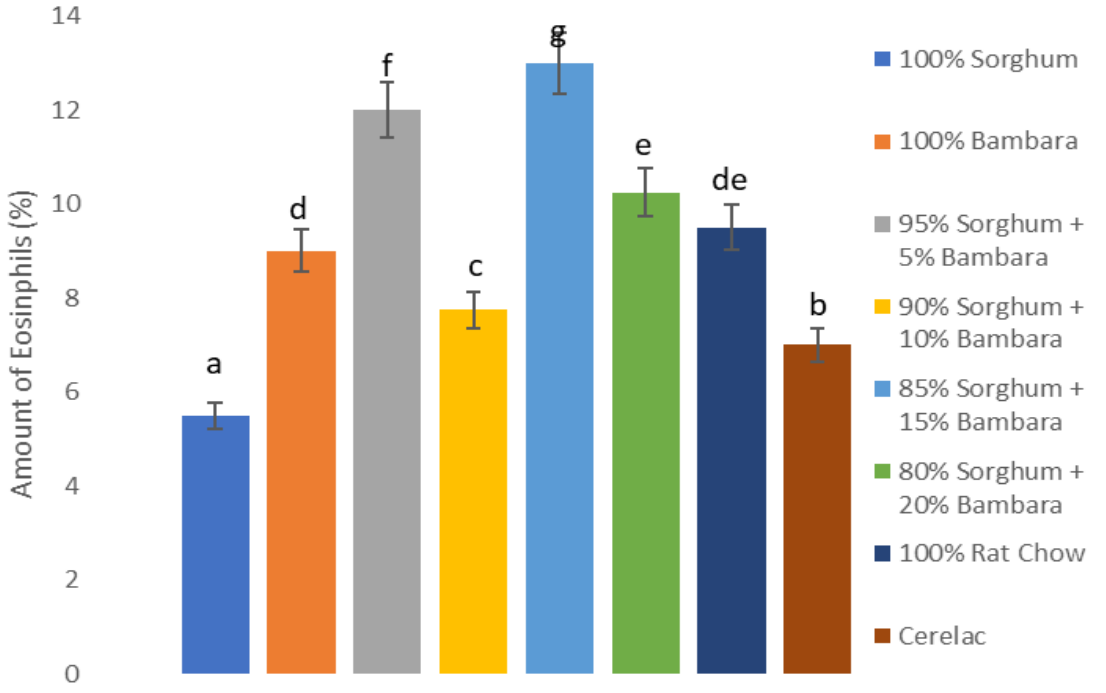


**Figure 4.8: Eosinophils in Rats Fed with Sorghum and Bambara Nut Flour Formulated Diets**

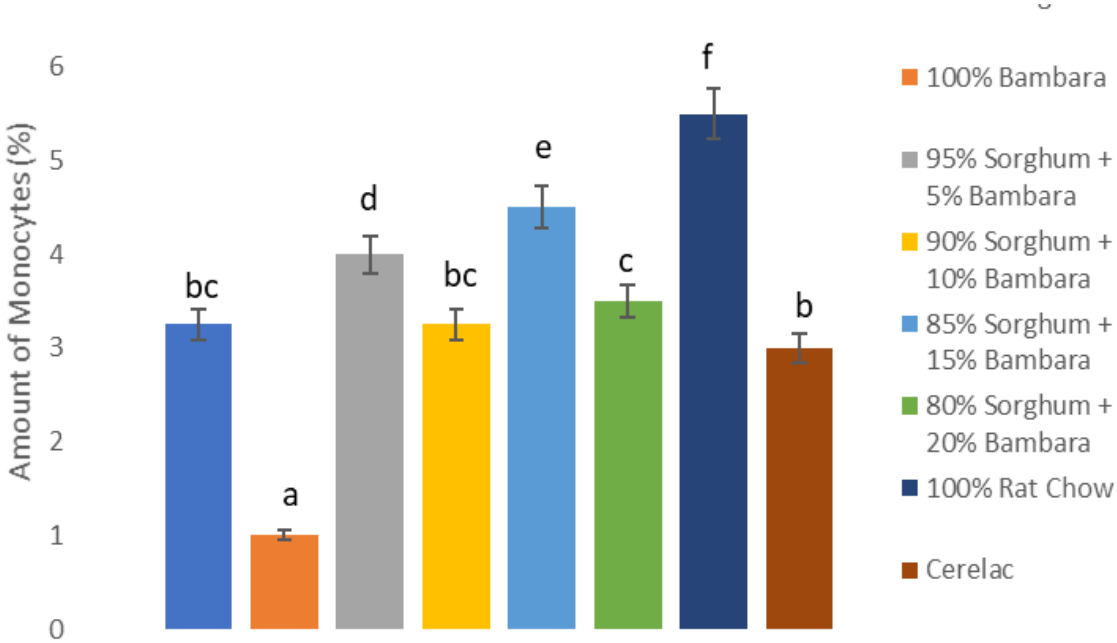


**Figure 4.9: Monocytes in Rats Fed with Sorghum and Bambara Nut Flour Formulated Diets**

### 4.1.7 Serum AST, ALT and ALP level of Rats Fed on Sprouted Bambara Nut and Sorghum Formulated Diets.

Figure 4.10 showed that the levels of aspartate transaminase activity (AST) in the serum of the animals were within normal range. However, there was significant difference ($p<0.05$) in the concentration of AST of the different groups. The AST values in the groups fed 100 % sorghum were significantly ($p<0.05$) higher than the other groups. Those fed on cerelac had the lowest AST values.

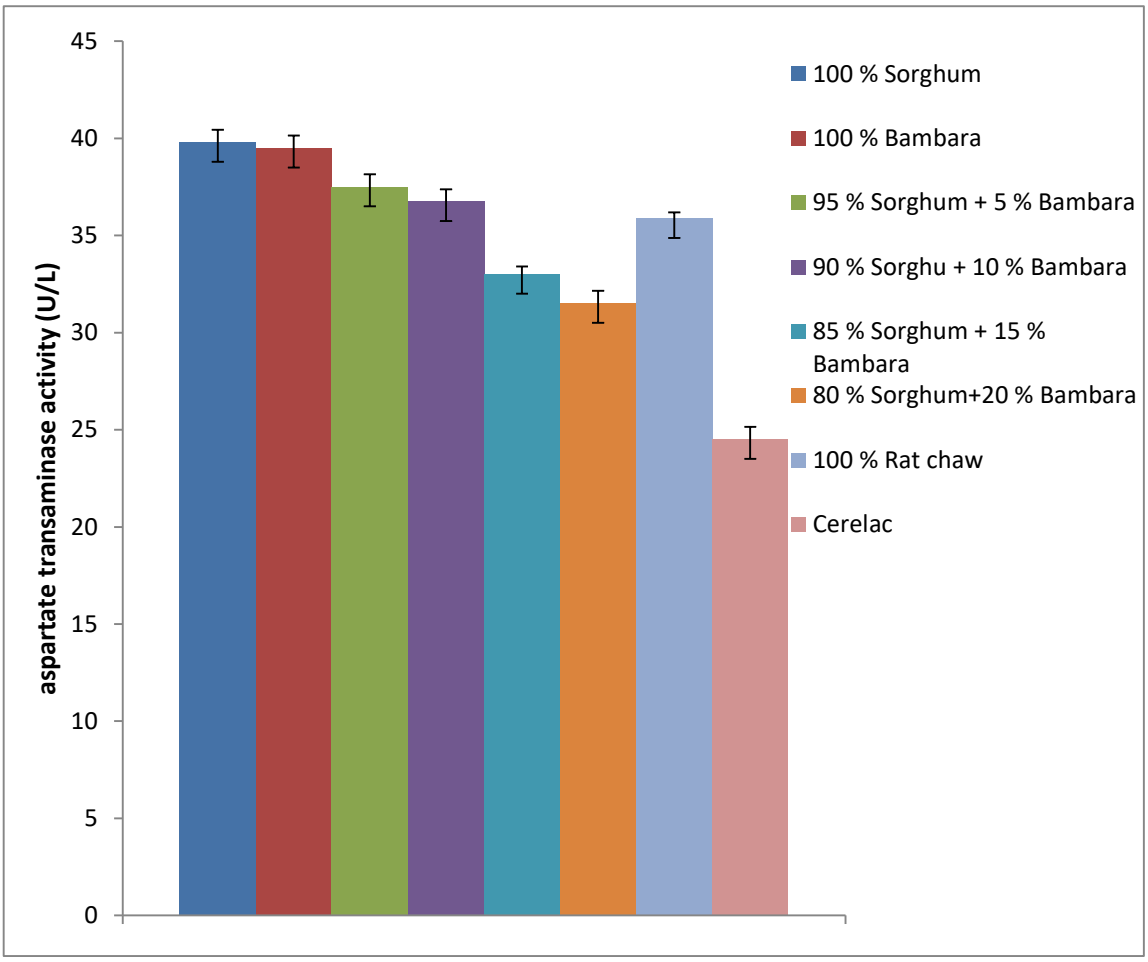


**Figure 4.10: Aspartate Transaminase Activity (U/L) in Rats Fed with Sprouted Sorghum and Bambara Nut Formulated Diets.**

Alanine transaminase activity (ALT) (Figure 4.11) in rats fed the different diets showed that rats fed on 100 % sorghum and 100 % Bambara had significantly ($p<0.05$) higher

values than the other groups. Also, the ALT value was lowest in the group placed on cerelac. Generally, the serum ALT levels in the rats were within normal range.

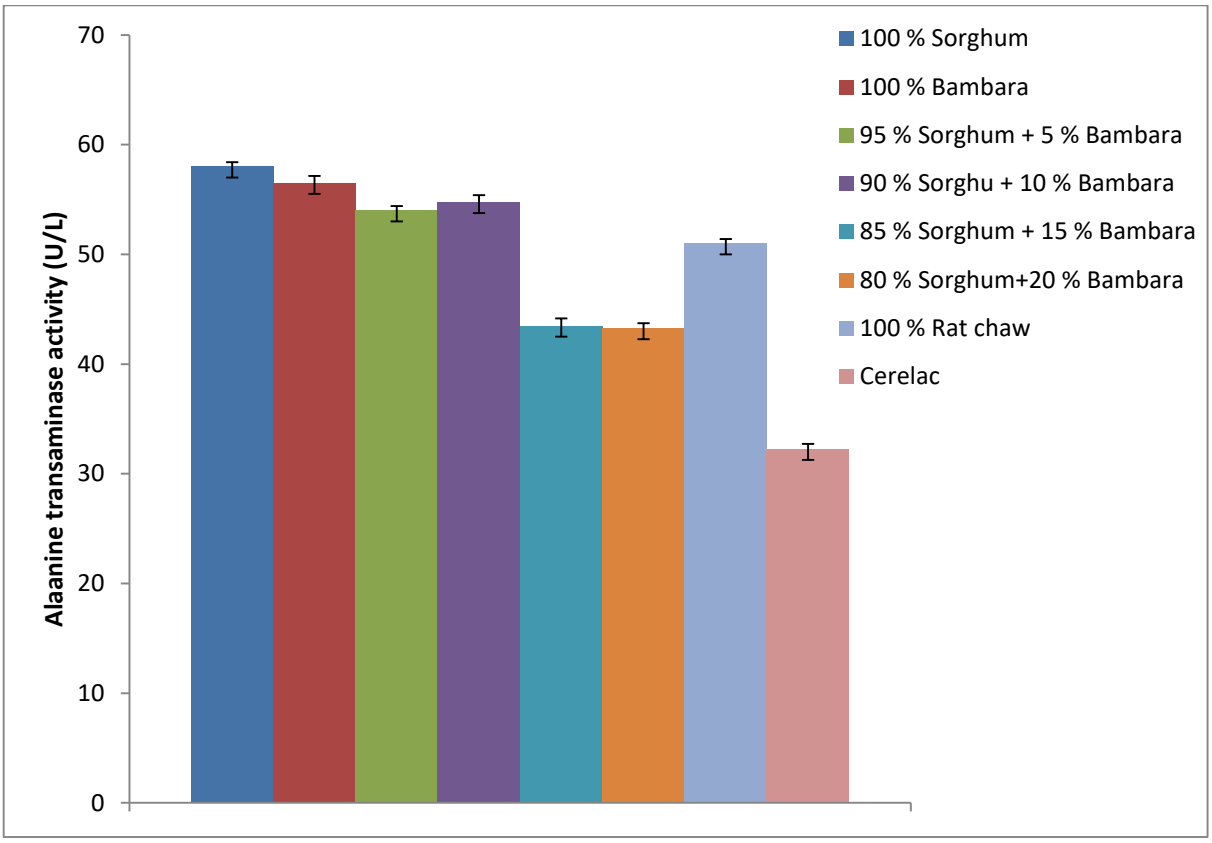


**Figure 4.11: Alanine Transaminase Activity U/L) in Rats Fed with Sprouted Sorghum and Bambara Nut Formulated Diets.**

The alkaline phosphatase activity (ALP) in rats fed the various diets (Figure 4.12) shows that the values obtained were within normal range. Rats fed on 100 % sorghum had significantly ($p<0.05$) higher serum ALP values compared to the other groups while the group that were fed on rat chow had significantly ($p<0.05$) lower ALP serum compared to every other group.

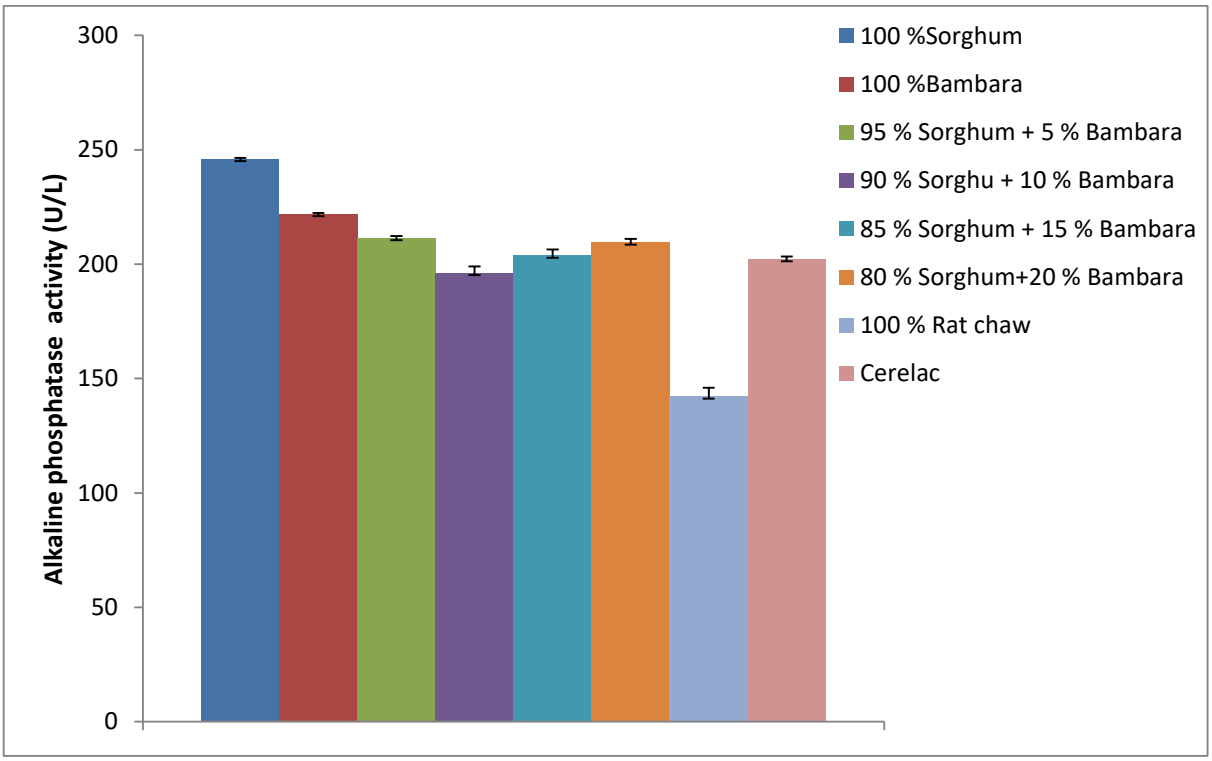


**Figure 4.12: Alkaline Phosphatase Activity (U/L) in Rats Fed with Sprouted Sorghum and Bambara Nut Formulated Diets.**

Table 4.9: shows the kidney function indices in the rats**.** The rats fed on cerelac had significantly ($p<0.05$) higher concentrations of urea, potassium, sodium, chloride and creatinine compared to the rats placed on the other diets. On the other hand, the rats that were fed on 100 % sorghum had significantly ($p<0.05$) lower concentrations of these indices compared to the other groups. It was observed that all the parameters found in the kidney function indices in the different groups of rats fed different diets were within normal range.

**Table 4.9: Renal Function Indices of Rats Fed with Sprouted Bambara Nut and Sorghum Flour Formulated Diets.**

| Parameters | 100%Sorghum | 100%Bambara | 95%Sorghum + 5%Bambara | 90%Sorghum + 10%Bambara | 85%Sorghum + 15%Bambara | 80%Sorghum + 20%Bambara | 100%Rat chaw | Cerelac |
|---|---|---|---|---|---|---|---|---|
| Urea (mmol/L) | 2.50±0.04$^{a}$ | 3.85±0.03$^{e}$ | 2.70±0.04$^{b}$ | 2.85±0.03$^{c}$ | 4.18±0.05$^{f}$ | 4.55±0.06$^{g}$ | 3.55±0.06$^{d}$ | 4.88±0.05$^{h}$ |
| $K^+$ (mmol/L) | 3.60±0.04$^{bc}$ | 3.63±0.09$^{c}$ | 3.13±0.10$^{a}$ | 3.30±0.09$^{ab}$ | 4.40±0.11$^{d}$ | 4.50±0.07$^{d}$ | 3.88±0.09$^{c}$ | 4.71±0.19$^{e}$ |
| $Na^+$ (mmol/L) | 87.00±0.35$^{a}$ | 112.28±1.14$^{c}$ | 94.00±2.04$^{b}$ | 87.88±0.42$^{a}$ | 94.70±0.26$^{b}$ | 93.40±0.22$^{b}$ | 117.00±1.96$^{d}$ | 148.88±0.43$^{e}$ |
| $Cl^-$ (mmol/L) | 97.13±0.43$^{a}$ | 97.70±1.08$^{b}$ | 99.65±0.24$^{d}$ | 99.50±0.65$^{d}$ | 98.50±0.65$^{c}$ | 99.00±0.41$^{cd}$ | 100.00±1.03$^{e}$ | 102.13±0.43$^{f}$ |
| Creatinine (mg/dL) | 0.78±0.02$^{a}$ | 0.82±0.02$^{b}$ | 0.81±0.01$^{b}$ | 0.82±0.01$^{b}$ | 0.92±0.02$^{c}$ | 0.92±0.00$^{c}$ | 1.21±0.03$^{d}$ | 1.34±0.05$^{e}$ |

Values are reported as mean ± standard error of means. Values with the same letter on the row are not significantly different at $p \leq 0.05$ while values on the same row with different alphabetic superscript are significantly different at $p \leq 0$.

## 4.2 Discussion

The proximate compositions of sprouted sorghum and Bambara nut as shown in Tables 4.1 and 4.2 indicate that sprouting improved some of their nutrient's content. The significant increase ($p<0.05$) in ash, fat, moisture and protein contents from 4-5 days is in agreement with the report of Lemmens *et al.* (2019) that sprouting times of between 4-5 days are needed to maximize nutrient availability in grains and legumes. Nutrient availability during sprouting is attributable to many factors. Metabolic enzymes such as proteinases are activated leading to release of some amino acids and peptides synthesis and utilization of these amino acids may form new proteins (Devi *et al.,* 2015). Sugars and vitamin B-complex are released, minerals are liberated, phenols and polyphenolic constituents in the food which provides anti-oxidants that helps to protect the body against reactive oxygen species are increased. Also, anti-nutritional factors and inhibitors such as phytate, oxalate etc. are reduced remarkably by the action of soaking and sprouting (Nyau *et al.,* 2017 and James *et al.,* 2018).

Ash content in food is a measure of its mineral content (Afifi *et al.,* 2011). The range obtained in this study for sprouted sorghum ($0.28 \pm 0.009^{a}$ - $1.06 \pm 0.702^{e}$) is lower than that reported by (Rainhanatu *et al.,* 2011) (1.00 – 2.50) and that obtained for Bambara nut ($2.24 \pm 0.061$ – $4.13 \pm 0.075$) is higher than that reported by (Okafor *et al.,* 2014) ($3.64 \pm 0.92^{a}$ – $3.85 \pm 1.11^{a}$). This difference could be attributed to the source of seeds, soil type, type of fertilizers and climatic condition (Sharif *et al.,* 2014). The observable increase in the ash contents of sorghum and Bambara with sprouting is in agreement with the report of El-Adawy (2002) that sprouting increased the ash content of mungbean, pea and lentil seeds significantly ($p<0.05$).

The increase in the fat content of the sprouts (sorghum and Bambara nut) is in agreement with the findings of Onwuka *et al.* (2009) that sprouting increased the fat content of fluted

pumpkin seeds significantly. This however, disagrees with the reports of El-Adawy (2002) and Elegbede (1998) that sprouting decreased the fat content of seeds.

The observable increase in the moisture contents of sorghum and Bambara nut agrees with the work of (Ehirim *et al.,* 2018) that sprouting of *Vigna. unguiculata* for 120 hours increased the moisture content significantly from 5.63 to 6.72%. Murugkar and Jha (2009) also observed that moisture content of soybeans increased from 5.4 to 56.1 % after 48 h of sprouting time. The reason for the increase in moisture content may be hinged on long duration of soaking which allows seeds to absorb much water needed for sprouting and the prolonged water intake as sprouting progresses by the process of imbibition (Ehirim *et al.,* 2018 and Nonogaki *et al.* 2010).

Proteins are critical to normal functioning of life. The increase obtained in the protein contents of sorghum and Bambara corroborates with the work of (Bello *et al.,* 2017) who reported a significant increase in the protein content of fluted pumpkin and sorghum after sprouting. The significant increase in the protein content could be ascribed to enzymatic hydrolysis of insoluble proteins to soluble forms (Echendu *et al.,* 2009), and the release of free amino acids after enzymatic hydrolysis for the synthesis of new proteins and enzyme protein (proteases) (Bau *et al.,* 1997 and Bliss, 1975).

The initial decrease in the crude fibre in the sprout suggests that fibre could be a source of energy for the enzymatic process of sprouting. However, the later increase obtained in fibre content as sprouting progressed could be attributed to the development of structural carbohydrates, celluloses and hemicelluloses which are major constituents of the cell walls (Shah *et al.,* 2011). This is supported by the report of (Ehirim *et al.,* 2018) that the fibre content of Bambara nut increased significantly ($p<0.05$) after sprouting for 5 days.

The reduction in the carbohydrate content is expected because during sprouting carbohydrate serve as the predominant source of energy for the enzymatic processes and for growth of the embryo (Vidal-Valverde *et al*., 2002). The activity of the enzyme β-amylase that hydrolyses the starch into simple carbohydrate is increased during sprouting (Suda *et al.*, 1986). Starch content in cotyledon of the seed is also broken down into smaller molecules such as glucose and fructose so as to provide the energy required for cell division as the seeds mature and grow (Ehirim *et al.*, 2009, Nonogaki *et al.*, 2010 and Vidal-Valverde *et al*., 2002). The reduction in the carbohydrate agrees with that reported by Ehirim *et al.* (2018) that sprouting reduced the carbohydrate content of cowpea from 64.01 to 47.87 %.

Sorghum and Bambara nut contain 18 amino acids respectively. Bambara nut contain both essential and non-essential amino acids to varying level. The richer content of Bambara nut is supported by the reports of Maphosa and Jideani (2017) that legumes have higher amino acid and protein compared to cereal crops and hence can serve as substitutes for meat, milk and egg. Suleiman *et al.*, (2016) reported that Bambara nut is rich in amino acid and could be used to supplement sorghum in the formulation of weaning food. The total amino acid obtained for Bambara nut in this study (61.67±0.015 g/protein) ranged within the recommended dietary allowance (RDA) reported by Ijarotimi and Keshinro (2013) for complementary diets.

Glutamate was found to be the most abundant non-essential amino acid in Bambara nut while cysteine was the least. This is in agreement with the report of Suleiman *et al.* (2016). The composition of the non-essential amino acid in the Bambara flour in this study is more than that reported by Oyeyinka (2016) for Bambara nut flour used in the formulation of complementary food. The reason for this difference may be as a result of the sprouting employed in this study.

Essential amino acids, also known as indispensable amino acids cannot be synthesized by humans and vertebrates, hence must be taken in diets (Lopez and Mohiuddin 2020). The quality of dietary protein is a measure of the required essential amino acids that it can provide for growth and maintenance of tissues. The total essential amino acid found in this study for Bambara nut flour (43.05 %) is in the same range with that reported by Hussin *et al.* (2020) for Bambara nut flour (42.6 %). The reason for this similarity, may be as a result of the same species of seed used or the seed were gotten from the same locality. The concentration of leucine, isoleucine, valine, histidine and threonine found in Bambara nut in the present study is lower than that reported by Suleiman (2016). Methionine was found to be the least essential amino acid which is also in agreement with that reported by Hussin *et al.* (2020). Huang *et al.* (2012) reported that llysine is an essential amino acid that is primarily used for protein synthesis and it is a limiting amino acid in most cereals. Deficiency of lysine limits protein synthesis leading to weight loss in infants and children. The concentration of lysine in the Bambara nut used in this study suggests that it can aid protein synthesis and support growth of infants and children.

The sulphur containing amino acids (methionine and cysteine) found in the Bambara nut used in this study is higher than that reported by Suleiman (2016) for Bambara nut flour. Sprouting may have enhanced the sulphur amino acid of Bambara nut in this study. The sulphur containing amino acids are important sources of sulphur in the body. Methionine in the form S- adenosyl methionine is required for transmethylation reactions (Rubin *et al*., 2007) while cysteine is also important detoxicants of specific substances and an important component of gluthathione an important antioxidant in cells. Methionine is essential for protein synthesis, synthesis of antioxidants and lipotropic compounds like taurine, glutathione, choline, carnitine and S –adenosyl methionine (Kiruthikajothi *et al*., 2014).

Aromatic amino acid (phenylalanine and tyrosine) was found to be high in Bambara nut. Tyrosine is required for the synthesis of certain hormones such as thyroid hormone, epinephrine and norepinephrine and a pigment, melanin (Fernstrom and Fernstrom, 2007). Bambara nut contained sufficient amount of phenylalanine and tyrosine which can be used to supplement sorghum in the formulation of weaning diet.

Bambara nut was also found to be a good source of semi essential amino acid, histidine and arginine. These amino acids are referred to as growth promoting factors because they are not synthesised in sufficient amounts during growth, therefore they are crucial in growth of children, pregnant women and lactating mothers (Chatterjea and Shinde, 2007).

Methionine and cysteine were the limiting amino acid found in Bambara nut. This agrees with Suleiman (2016). When the essential amino acid provided in a diet is low, protein synthesis is limited to the rate of the essential amino acid. Hence the essential amino acid is termed limiting and this can reduce growth and maintenance of the body. Though methionine and cysteine were the limiting amino acids in Bambara nut. Bambara has a higher amount of these amino acid that can be used in the supplement sorghum in the formulation of weaning diet.

The significantly lower anti-nutrient levels obtained in the sprouts compared with the values in the unsprouted in this study as shown in Tables 4.5 and 4.6 is an indication that sprouting played a significant role in the reduction of anti-nutrients in sorghum and Bambara nut. Processing especially sprouting has been reported to reduce anti-nutrient contents of foods (Nwadi *et al.,* 2019). The low levels of anti-nutrients suggest that food formulated from these sprouts would be safe for human consumption (Sharma, 2020). The reduction in anti-nutrients for sprouted sorghum and Bambara nut is in agreement with that reported by Modu *et al.* (2014) who observed a remarkable reduction in anti-nutrient contents of corn flour, Bambara nut, cowpea and groundnut nut.

Physical modifications such as (sprouting, fermentation, cooking among others) have been shown to change and enhance functional properties of food (James *et al.,* 2018). Figure 4.1 has shown that sprouting decreased the gelatinization temperatures of sorghum and Bambara nut resulting in increase in gelatinization time. This is in agreement with the works of Ocheme *et al.* (2015) who reported decrease in gelatinization temperatures and increase in gelatinization time of sorghum flour upon 3-5 days sprouting. This decrease in gelatinization temperatures leading to increase in gelatinization time of sorghum and Bambara nut in the present study may be attributed to the disruption of the starch crystalline making it require low temperature for gelatinization and concentration of the crystalline extending the gelatinization time (Ocheme *et al.,* 2015). The process by which intermolecular bonds of starch molecules is broken down upon application of heat and water giving room for hydrogen bonding sites to absorb more water is known as gelatinization (Ubwa *et al.,* 2012). The decrease in gelatinization temperatures observed in this study suggests that infant formula prepared from sprouted sorghum and Bambara nut will require less heat to attain gelation (James *et al.,* 2018) and this translates to low energy cost in cooking (Owuamanan *et al.,* 2014). Low cooking temperatures helps retain some nutrients in food. Water-soluble vitamins like vitamin C and the B vitamins thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folic acid (B9), and cobalamin (B12) and fat-soluble vitamins like vitamins A, D, E, and K are retained at low cooking temperature. Also, minerals: primarily potassium, magnesium, sodium, and calcium are retained at low temperature (Spritzer, 2017). The decrease in gelatinization temperature of the formulated diets indicates that these nutrients are retained upon cooking. Low temperature and high exposure time have been implicated to promote the availability of the free polyphenols and sugars in food (Alfeo *et al.,* 2020). Gelatinization

temperature obtained with extension of cooking time in the preparation of the formulated diets suggests that free polyphenols and sugars in the food will be made available.

In this study sprouting had no effect on swelling capacity of the samples and this is not in agreement with previous reports by Ocheme *et al.* (2015) and James *et al.* (2018) that sprouting increased the swelling capacity of sorghum, pearl millet and Bambara nut on day 3. The reason for this variation may be as a result of additional fermentation methods used by these researchers.

The significant reduction in bulk densities of sorghum and Bambara nut is in agreement with the reports of (Ocheme *et al.*, 2015, Otutu *et al.*, 2015, Abd Elmoneim and Bernhardt, 2010) that bulk densities of seeds reduced significantly after sprouting. The reason for this reduction in bulk is due to the breakdown of the complex compounds such as starch, proteins, and fibres to simple molecule in the course of sprouting (Ocheme *et al.*, 2015). The reduction in bulk observed in this study is advantageous to preparation of weaning foods as foods low in bulk density can be easily digested by infants and weaning children owing to the fact that their digestive system is not fully developed to accommodate bulky foods. Abd Elmoneim and Bernhardt (2010) reported that sprouting is one of the most useful traditional technologies implored in the reduction of bulk in weaning foods.

The decrease in gelatinization temperature and increase in gelatinization time observed for the formulated diets is the same trend as it is for the effect of sprouting on sorghum and Bambara nut flours. The decrease in gelatinization temperature may be an indication that the formulated diets can easily form gel when stirred with boiling water within a short period. Umerah *et al.* (2020) reported that Gelation capacity indicates the solubility of the native proteins in the continuous phase (water) in the formulated sample.

Water absorption capacity is the index of water absorbed and retained in a sample. The reduction in water absorption capacity obtained upon supplementation of sprouted sorghum with sprouted Bambara in this study is in agreement with the reports of James *et al.* (2018) that water absorption capacity reduced when sprouted Bambara nut and pearl millet were blended. Low water absorption capacity is required in the preparation of thinner gruels with high caloric density per unit volume. This enhances the absorption of nutrients by infants and reduction in microbial activities as a result of the low water activity leading to extension of the shelf life of the product (Gomez & Aguilera, 1983).

The ability of a formula to foam when water and heat is added is called foaming capacity (James *et al.,* 2018). The increase in foaming capacity may be as a result of supplementation with Bambara nut that is rich in protein. Awuchi *et al.* (2020) reported that foaming of flours and related foods is attributed to the protein present in the food. Generally, foaming capacity and stability is dependent on the interfacial film formed by the proteins, which maintains the suspension of air bubbles and slows down the coalescence rate.

The observed increase in foam capacity in this study supports the work of James *et al.* (2018) that reported increase in foam capacity in complementary diet prepared from Bambara nut, sorghum and pearl millets.

The significant increase obtained in the ash content of the formulated diets is due to supplementation with Bambara nut which suggests that the weaning diets are rich in mineral. Afify *et al.* (2011) reported that ash content is a measure of mineral present in food. The ash content obtained in this study is higher compared to that reported by Suleiman *et al.* (2016), and Baba *et al.* (2012) for sorghum fortified with Bambara nut flour. The discrepancies may be due to the quantity and source of the Bambara nut flour used in supplementation in the different studies.

The increase in fat content in this study is in agreement with the work of Suleiman *et al*, (2016), who observed increase in fat content upon fortification of sorghum with Bambara. The similarity is expected as the same processing method and the same specie of Bambara nut was used.

The protein enrichment obtained in the formulation of 20 % Bambara supplemented diet is higher than the (RDA) requirement reported by Modu *et al.* (2014). The remarkable enhancement in the protein content suggests that it can serve as a supportive diet for growing infants and weaning children. Maphosa and Jideani (2017) reported that legumes are excellent source of protein and the use of legumes in supplementation is preferred to animal protein because they contain less fat. Similar report has been published by Oyeyinka (2016) that supplementation with Bambara nut increased the protein of complementary foods.

Low moisture content in food is advantageous to its storage (shelf-life) and also prevent it from microbial attack that can quicken its spoilage (Danso *et al.,* 2019). The moisture content obtained in this study for the formulated diets were lower than that reported by Ijarotimi and Keshinro, (2013) for complementary food fortified with African locust bean and Bambara nut but higher than that reported by Suleiman *et al.* (2016) for sorghum fortified with Bambara nut flour. The reason for this difference may be due to the processing techniques used by the individual researchers.

The fibre content of the supplemented diets was higher compared to the 100 % sorghum flour. This is in agreement with the work of Mesfin and Shimeli (2013) who observed progressive increase in fibre following incorporation of soybean flour into cereal based bread. The increase in fibre content of the blend in the present study is due to the incorporation of Bambara flour that is high in fibre content. High fibre content in food have been implicated to enhance food quality. Belluco *et al.* (2013) reported that high fibre

content in food is advantageous to bowel movement of food and aid promotion of gut microbes. Anderson *et al. (*2009) also reported that fibre plays a critical role in the prevention of overweight, constipation in adult and children, cardiovascular disease, diabetes and colon cancer.

The reports above suggests that the high fibre content obtained in the present study for the formulated diets may play potential role in bowel movement of food during digestion and would enhance the growth of gut friendly microbes.

The decrease in carbohydrate content of the blends with increase in proportion of Bambara flour supplementation is expected because it follows the trend reported in several studies that supplementation of cereals with legumes reduced carbohydrate content of the cereals. For instance, Bintu *et al.* (2017) reported a decrease in carbohydrate when he fortified cereals with cowpea and Bambara. Baba *et al.* (2012) also found a reduction in carbohydrate upon fortification of cereal-based food with cowpea and groundnut. However, the carbohydrate content of the diets found in this study is in the range of the recommended dietary allowance (RDA) reported by Bintu *et al.* (2017). The high carbohydrate content observed in the present study suggests that the prepared diets are rich in energy and are able to satisfy the energy requirement for infants and weaning children.

The significantly higher ($p<0.05$) level of feed intake by the rats fed on cerelac compared to the other groups (Figure 4.4) may be due to palatability and balance of nutrients in the diets. This is in agreement with the reports of Mariam (2005) that in a weaning food experiment rats fed on cerelac as control diet had significantly higher feed intake compared to other rats subjected to different weaning foods. Similar reports by Idoko *et al.* (2015) and Oibiokpa *et al.* (2018) have shown that high levels of anti-nutrient, low protein and unpalatability affected food intake of rats fed on different formulated diets.

The significantly lower ($p<0.05$) weight gain in rats fed on 100 % sorghum compared to the other rats fed higher protein is expected because sorghum is low in protein which makes the diet inadequate to support growth of the rats. Protein is also required for building and maintainance of muscle mass which in turn contribute to weight gain. This is lacking in the diet composed of 100 % sorghum. The lower weight gain observed in this study is in line with the findings of Kamau *et al. (*2017) who reported similar trend upon administration of 100 % maize diet to weaning albino rats. Mosha and Vicent (2005) reported that such diets low in protein pre-disposes children to PEM. This suggests that 100 % sorghum is insufficient to support growth of infants and weaning children.

The low weight gain observed in rats fed 100 % Bambara may be attributable to the poor level of feed intake by the rats while that noticed in the rats fed on 5 % Bambara supplementation may be due to the low level of protein composition of the diet. The higher weight gain observed in the rats fed on 10 %, 15 % and 20 % Bambara supplementation suggests that the increased protein enhanced the growth of the rats. It is clear that the consistent weight gain by rats fed on cerelac compared to the other groups is due to higher feed intake by the animal as well as the rich nutrient content of the control diet. The significant weight gained by the animals fed 20 % Bambara over all the other supplemented diets groups of rats is mainly due to the increased protein supplementation from Bambara. Also, another reason is that the feed intake is higher (Figure 4.4). This is in agreement with Bintu *et al,* (2017) and Akeredolu *et al.* (2005), who reported that growth rate in experimental rats was influenced by their feed intake.

Animals' physiological, nutritional, and pathological status may be determined by haematological parameters. These haematological parameters are also useful in monitoring feed toxicity (Olafedehan *et al.,* 2010 and NseAbasi *et al.*, 2014). The haematological parameters Hb, PCV, MCV, MCH, MCHC, RBC, PLC and TWBC (Table

4.8) in the group of rats fed Bambara supplemented diets were within normal range. This suggests that the formulated diets did not have any cytotoxic effect on the rats. The significantly higher ($p<0.05$) in the Hb, PCV, MCV, MCH and RBC in the rats fed 20 % Bambara supplemented diets, rat pellets and cerelac than the other group of animals fed different supplemented diet is an indication that the diet had good nutritional quality. The reason for this higher value may be due to the supplementation with higher protein from Bambara nut and the higher feed intake by the animals in these groups. This is in agreement with Adejuwon *et al.* (2021) who reported high Hb, PCV, MCV, RBC in rats fed high protein supplemented diets and cerelac. Roberts *et al.* (2000) also reported that diets containing quality protein usually enhance production of hemoglobin and enhanced immunity in animals. The lower values of these indices in the rats fed on 100 % sorghum diets indicates that the diet was deficient in protein. White blood cell (WBC) are immune cells that defend the body against infections (Al-Dulaimi *et al.,* 2018). The WBC of the rats fed different formulated diets rat pellets and crelac were within normal range. This shows that the animals had high immunity against infection. The significantly higher ($p < 0.05$) white blood cells in rats fed on 100 % sorghum may imply that these rats had increased immunity against infection. Animals with low white blood cell and white blood cell differentials (lymphocytes, eosinophils, basophils, monocytes and neutrophils) are exposed to high risk of infection while those with high white blood cell counts are known to be capable of generating antibodies and have a high degree of resistance (Soetan *et al*., 2013). The non-significant difference ($p<0.05$) in the RBC of rat fed 20 % Bambara supplemented diet and cerelac suggests that the formulated diet was not toxic to the red blood cells of the animals. This agrees with the report of Oibiokpa *et al.* (2018) that there was no significant difference in the RBC of rats fed insect supplemented diet and Casein.

Serum enzymes found in tissues and body fluids are essential diagnostic tools or biomarkers for evaluating pathological state of animals (Akhigbe, 2014). Elevated levels of the amino transferases which are aspartate transferase (AST) and alanine transferase (ALT) are indicators of tissue and cellular damage with AST specific to the liver and ALT specific to the skeletal muscle (Ige *et al.,* 2011). However, there are situations where elevated levels of these enzymes may not necessarily be due to organ damage or injury. Presence of pyridoxal -5- phosphate (PLP) (active vitamin B6) as co- enzymes in unhealthy rats, low protein diet and stimulation of other enzymes can lead to elevated levels of these enzymes (Rossouw *et al.,* 1978). The AST and ALT of the rats fed on formulated diets, cerelac and rat pellets (Figures 4.10. and 4.11) were within normal range. This suggests that there was no damage to the liver and so no outflow of enzymes into the plasma or blood stream. This shows that the formulated food is safe for consumption. This finding is in agreement with the report of Mariam (2005) that cereal-based food supplemented with Bambara and other legumes do not negatively affect the activity of liver enzymes. However, the higher levels of AST and ALT in the group of rats fed 100 % sorghum may be as a result of the low protein content of the diet and presence of vitamin B6 in the diet. Low protein diet has shown to elevate levels of AST and ALT (Sulaiman *et al.,* 2016a). Also, presence of vitamin B6 as co-enzymes has shown to elevate AST and ALT activities (Rossouw *et al.,* 1978). This indicates that the animals in this group were malnourished leading to elevated AST and ALT activities as suggested by Oibiokpa *et al.* (2018) and Ekpo, (2011) who fed rats with insect supplemented diets low in protein and reported elevated levels of AST and ALT.

In animals there is usually a high concentration of alkaline phosphatase (ALP) in bone, kidney, liver and mucosa of the small intestine. Injuries to these organs can result in outflow of the ALP enzymes into the plasma. Akanya *et al.* (2014) reported that Alkaline

Phosphatase (ALP) is a biomarker enzyme for assessing the integrity of plasma membrane. Increase in the activities of Alkaline phosphatase is an indication that there could be damage due to cytotoxic effect. Therefore; it is used as a diagnostic tool for the assay of the integrity of the plasma membrane. Nevertheless, elevated ALP is connected to growth of bone and may not necessarily be as a result of liver damage. The values obtained for ALP in the present study are within normal range. This therefore suggests that the formulated food did not alter the integrity of the organs and tissues. The significantly higher ($p<0.05$) ALP levels in the rats fed 100 % sorghum compared to the other rats may be an indication of growth of bone.

The rats fed on cerelac had significantly ($p<0.05$) higher concentrations of urea, potassium, sodium, chloride and creatinine compared to the rats placed on the other diets. The reason for this higher value may be due to the concentration of the cerelac as well as the higher level of feed intake by the animals fed cerelac. On the other hand, the rats that were fed on 100 % sorghum had significantly ($p<0.05$) lower concentrations of these parameters compared to the other groups. This may be as a result of the low concentration of protein in the diet and the low level of feed intake. It was observed that all the parameters found in the entire groups of rats fed different diets were within normal range. This is in agreement with the report of Adejuwon *et al.* (2020) that low feed intake by rats fed complementary diet resulted in lower haematological parameters and kidney indices of the rats fed the complementary diet.

# CHAPTER FIVE

## 5.0 CONCLUSION AND RECOMMENDATIONS

### 5.1 Conclusion

Bambara nut and sorghum were found to contain essential and non-essential amino acids, fats and carbohydrate. But Bambara nut had richer % of protein than sorghum.

Methionine was the limiting amino acid in sorghum while Bambara nut was cysteine.

The remarkable enhancement in the protein content of sorghum obtained upon supplementation with 20 % Bambara suggests that it can serve as a supportive diet for growing infants and weaning children.

The findings in this study shows that the best sprout for sorghum was obtained on day 5 while that of Bambara nut was obtained on day 4.

The nutritional composition of sorghum and Bambara nut were enhanced upon sprouting with respect to protein, ash, fibre and fats.

Functional properties such as bulk density and gelatinization temperature were decreased upon sprouting.

Antinutrients in Bambara and sorghum were reduced below permissible limit upon sprouting.

Feed intake by the animals showed that the rats liked the 20 % Bambara supplemented diet and cerelac than the other diets. However, all the animals gained weight during the period of the experiment which suggests that the formulated diet supported the growth of the animals.

Supplementation of sorghum with Bambara nut improved the protein quality of the formulated diets. This is an indication that supplementation of our local weaning diet can be cheaply carried out.

The formulated diets did not have any adverse effect on serum and tissue levels of AST, ALT, ALP, Urea, creatinine, electrolytes and haematological indices of the rats and hence safe.

## 5.2 Recommendation

This work has established the fact that Bambara nut, an underutilized legume can be included in weaning diets, however the following are recommended.

i. More research work should be carried out on sprouting procedures for Bambara nut as to optimize the production of the flour for commercial purposes.

ii. Supplementation of other weaning base foods (rice, maize, millet) with Bambara nut should also be evaluated at different levels of inclusion.

## REFERENCES


Abd Elmoneim, O. E., & Bernhardt, R. (2010). Influence of grain germination on functional properties of sorghum flour. *Food Chemistry*, 121(2), 387-392.

Abeshu, M. A., Lelisa, A., & Geleta, B. (2016). Complementary feeding: review of recommendations, feeding practices, and adequacy of homemade complementary food preparations in developing countries–lessons from Ethiopia. *Frontiers in nutrition*, 3, 41.

Adebo, O. A. (2020). African sorghum-based fermented foods: past, current and future prospects. *Nutrients*, 12(4), 1111.

Adegbanke, O. R., Dada, T. A., Akinola, S. A., & Akintuyi, T. (2017). Physicochemical and sensory qualities of complementary meal made from sprouted and unsprouted sorghum, Irish potato and groundnut. *Food Science & Nutrition*, 6(2), 307–317

Adejumo, A. C., Adejumo, K. L., Adegbala, O. M., Enwerem, N., Ofosu, A., Akanbi, O. & Adeboye, A. (2019). Inferior Outcomes of Patients with Acute Myocardial Infarction and Comorbid Protein-Energy Malnutrition. *Journal of Parenteral and Enteral Nutrition.* 8 (5), 34-37.

Adejuwon, K. P., Osundahunsi, O. F., Akinola, S. A., Oluwamukomi, M. O., & Mwanza, M. (2021). Effect of Fermentation on Nutritional Quality, Growth and Hematological Parameters of Rats Fed Sorghum-Soybean-Orange flesh Sweet Potato Complementary Diet. *Food science & nutrition*, 9 (2), 639-650.

Adzu, B., Masimirembwa, C., Mustapha, K. B., Thelingwani, R., Kirim, R. A., & Gamaniel, K. S. (2015). Effect of NIPRISAN® on CYP3A4 activity in vitro. *European journal of drug metabolism and pharmacokinetics*, *40*(1), 115-118.

Afify, A. E. M. M., El-Beltagi, H. S., Abd El-Salam, S. M., & Omran, A. A. (2011). Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. *Plos one*, *6*(10), e25512.

Agrahar-Murugkar, D., & Jha, K. (2010). Effect of drying on nutritional and functional quality and electrophoretic pattern of soyflour from sprouted soybean (Glycine max). *Journal of food science and technology*, 47(5), 482-487.

Ahmed, M. A. A., Massaad, S. O., Abdalla, K. O., Omar, S. M., & Adam, G. K. (2019). Protein Energy Malnutrition in Children at Gadarif Eastern Sudan. *Journal Nutrition and food science*, *9*, 758.

Akande, K. E., Doma, U. D., Agu, H. O., & Adamu, H. M. (2010). Major antinutrients found in plant protein sources: their effect on nutrition. *Pakistan journal of nutrition*, 9(8), 827-832.

Akaninwor, J.O., and Okechukwu P.N. (2006) Evaluation of processed sweet potato, crayfish, soya beans and sweet potato, crayfish and Bambara nut weaning mixtures. *Journal of applied sciences and environmental management.* 10 (1) 55-61

Akanya, H. O., Peter, S., Ossamulu, I. F., Oibiokpa, F. I., & Adeyemi, H. Y. (2015). Evaluation of the changes in some liver function and haematological parameters in MSG fed rats. *International Journal of Biochemistry Research & Review*, 6(3), 113.

Akeredolu, I. A., Addo, A. A., & Akeredolu, O. A. (2005). Clinical evaluation of pearl millet conophor weaning mix as supplementary food for Nigerian children. *Brazilian archives of biology and technology*, *48*(4), 531-536.

Akhigbe, R. E. (2014). Discordant results in plant toxicity studies in Africa: attempt of standardization. In *Toxicological survey of African medicinal plants* (pp. 53-61). Elsevier.

Alamgir, K., Salahuddin, Khan., Syed Z., Abdul, M. T. & Manzoor K. (2017) Causes, sign and symptoms of malnutrition among the children. *Journal of Nutrition Human Health.* 1 (1), 24-27.

Al-Dulaimi, K. A. K., Banks, J., Chandran, V., Tomeo-Reyes, I., & Nguyen Thanh, K. (2018). Classification of white blood cell types from microscope images: Techniques and challenges. *Microscopy science: Last approaches on educational programs and applied research (Microscopy Book Series, 8)*, 17-25.

Alfeo, V., Bravi, E., Ceccaroni, D., Sileoni, V., Perretti, G., & Marconi, O. (2020). Effect of baking time and temperature on nutrients and phenolic compounds content of fresh sprouts bread-like product. *Foods*, *9*(10), 1447.

Allen, L. H. (2012). Adequacy of family foods for complementary feeding.

Aloysius, M. T., & Ajawubu, I. O. (2013). Formulation of infant weaning food from sorghum and bambara-ground nut. *International Journal of Advancement in Biological Sciences*, 6, 89-98.

Ananda, G. K., Myrans, H., Norton, S. L., Gleadow, R., Furtado, A., & Henry, R. J. (2020). Wild sorghum as a promising resource for crop improvement. *Frontiers in Plant Science*, 11, 1108.

Anderson, J. W., Baird, P., Davis, R. H., Ferreri, S., Knudtson, M., Koraym, A., & Williams, C. L. (2009). Health benefits of dietary fiber. *Nutrition reviews*, 67(4), 188-205.

Andualem, A., Edmealem, A., Tegegne, B., Tilahun, L., & Damtie, Y. (2020). Timely Initiation of Complementary Feeding and Associated Factors among Mothers of Children Aged 6–24 Months in Dessie Referral Hospital, Northeast Ethiopia, *Journal of Nutrition and Metabolism*, 2020, 1–7.

AOAC (1990). Official method of Analysis 15$^{th}$ edition, Association of Official analytical Chemists, Washington D.C.

AOAC (2006). Official method of Analysis of Association of Official analytical Chemists, 18$^{th}$ edition, W. Horwitz editor, Washington D.C.

AOAC, 2000. Association of Official Analytical Chemists. 17th Edition., Official Method of Analysis, Washington D.C., USA.

Arise, A. K., Amonsou, E. O. & Ijabadeniyi, O. A. (2015) Influence of extraction methods on functional properties of protein concentrates prepared from South African Bambara groundnut landraces. *International Journal of Food Science & Technology,* 50 (1), 70-76.

Arise, A. K., Arise, R. O., Sanusi, M. O., Esan, O. T. & Oyeyinka, S. O. (2014). Effect of *Moringer oleifera* flower fortification on the nutritional quality and sensory properties of weaning food. *Journal of food science and technology,* 6 (2), 65-71.

Armtfiled, S. D., Ismond M. A. H., Murray E. D. (1985). The fates of antinutritional factors during the preparation of the faba bean protein isolate using micellization techniques. *Food Science. Journal.*, 18, 137-147

Asma, M. A., El Fadil, E. B., & El Tinay, A. H. (2006). Development of weaning food from sorghum supplemented with legumes and oil seeds. *Food and Nutrition Bulletin*, 27(1), 26-34.

Atassi, H., & Romesh, K. (2019). Protein-Energy Malnutrition, *A critical review*, 14-17.

Awuchi, C. G., Igwe, V. S., & Amagwula, I. O. (2020). Ready-to-Use Therapeutic Foods (RUTFs) for Remedying Malnutrition and Preventable Nutritional Diseases. *International Journal of Advanced Academic Research*, 6(1), 47-81.

Baba, G. M., Modus, S., Falmata, A. S., Hajjagannah, L., & Ibrahim, Z. (2012). Evaluation of the nutritional value of sprouted sorghum fortified with cowpea and groundnut. *Journal of Agricultural Science*, 2(11), 292-296.

Bamshaiye O, Adegbola J, Bamshaiye E (2011). Bambara groundnut: An under-utilized nut in Africa. *Advances in Agricultural Biotechnology,* 1, 60-72.

Bankole, O. O., Aderinokun, G. A., Odenloye, O., & Adeyemi, A. T. (2006). Weaning practices among some Nigerian women: implication on oral health. *Odonto-stomatologie tropicale Tropical Dental Journal*, *29*(113), 15-21.

Baoling, H., Chengqun, L., Bo, W., & Liqin, F. (2007). A rhizobia strain isolated from root nodule of gymnosperm Podocarpus macrophyllus. Science in China Series C-Life Science, 50, 1-6

Basheir, H. M., & Hamza, K. M. (2015). Hematological parameters of malnourished Sudanese children under 5 years–Khartoum state–2011. *Journal of Clinical Medicine*, 1(4), 152-156.

Batool, Z., Zafar, M. I., Anwar, S., Bano, S., Gul, M., & Nazir, F. (2012). An investigation into socio-economic factors explaining dietary intake in pregnant women: A study in urban areas of District Faisalabad, Punjab, Pakistan. *Pakistan Journal of Nutrition*, 11(7), 568.

Bau, H. M., Villaume, C., Nicolas, J. P., & Méjean, L. (1997). Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya

bean (Glycine max) seeds. *Journal of the Science of Food and Agriculture*, 73(1), 1-9.

Bello, F. A., Udo, I. I., & Mbak, D. L. (2017). Proximate composition and functional properties of sprouted sorghum (Sorghum bicolor) and defatted fluted pumpkin seed (Telfairia occidentalis) flour blends. *American Journal of Innovative Research and Applied Sciences*, 5(4), 254-259.

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C. C., Paoletti, M. G. & Ricci, A. (2013). Edible insects in food safety and nutritional perspective. A critical Review. *Comprehensive Reviews in Food Science and Food Safety*, 12, 296-313.

Benincasa, P., Falcinelli, B., Lutts, S., Stagnari, F., & Galieni, A. (2019). Sprouted Grains*: A Comprehensive Review. Nutrients,* 11(2), 421.

Benitez, L.V. (1989). Amino acid and fatty acid profiles in aquaculture nutrition studies. In: S.S. De Silva (ed.). Fish Nutrition Research in Asia. *Proceedings of the Third Asia Fish Nutrition Network meeting*. Asian Fish Society Special Publication, 4, 166.

Benjamin, O., & Lappin, S. L. (2019). Kwashiorkor. *StatPearls [Internet]*.

Berry, S. K., Rokiah, H. I. & Karin, N.A. (1988). Effect of germination on the biological quality of winged beans (P. tetragonolobus) *Nutrition. Report Inernational*, 37(6), 1238-1245.

Bimpong, K. A., Cheyuo, E. K.-E., Abdul-Mumin, A., Ayanore, M. A., Kubuga, C. K., & Mogre, V. (2020). Mothers' knowledge and attitudes regarding child feeding recommendations, complementary feeding practices and determinants of adequate diet. *BMC Nutrition,* 6(1), 1-8.

Bintu B. P., Falmata A. S., Maryam, B. K., Zainab, M. A. & Modu, S. (2017) Effect of feeding complementary diet blends formulated from *Zeamays* (maize), *Vigna unguiculata* (cowpea), *Vigna subterranean* (Bambara nuts) *Arachis hypogea* (groundnut) on weaning rats. *African Journal of Food Science and Technology*, 8 (5), 099-107.

Bliss, F.A. (1975). Cowpeas in Nigeria. In: Nutritional Improvement of Food Legumes by Breeding, Milner, M. (Ed.). John Wiley and Sons, New York, pp: 151-158.

Brugaillères, P., Issanchou, S., Nicklaus, S., Chabanet, C., & Schwartz, C. (2019). Caloric compensation in infants: *American Journal of Clinical Nutrition*, 9 (3) 22-28.

Cavuilati, V. (2018). Climate change and human health: an ecological study on climate variability and malnutrition in Fiji.

Chatterjea M.N., & Shinde R. (2007). Textbook *of Medical Biochemistry* (7th ed.). New Delhi, India; Jaypee Brothers Medical Publishers (P) Ltd, pp 73 -91.

Chhikara, N., Abdulahi, B., Munezero, C., Kaur, R., Singh, G., & Panghal, A. (2019). Exploring the nutritional and phytochemical potential of sorghum in food processing for food security. *Nutrition & Food Science*.

Chiampo, L. A. D., & Chiampo, I. R. L. D. (2020). Anatomical and Physiological Characteristics of the Digestive Tract in Childhood. *Archives of Current Research International*, 20 (3), 50-56.

Chisi, M., & Peterson, G. (2019). Breeding and agronomy. In *Sorghum and millets* (pp. 23-50). AACC International Press.

Cieslak, A., Zmora, P., Matkowski, A., Nawrot-Hadzik, I., Pers-Kamczyc, E., El-Sherbiny, M. & Szumacher-Strabel, M. (2016). Tannins from Sanguisorba officinalis affect in vitro rumen methane production and fermentation. *JAPS: Journal of Animal & Plant Sciences*, 26(1).

Cieslak, A., Zmora, P., Pers-Kamczyc, E., & Szumacher-Strabel, M. (2012). Effects of tannins source (Vaccinium vitis idaea L.) on rumen microbial fermentation in vivo. *Animal Feed Science and Technology*, 176(1-4), 102-106.

Coffman, C. W. & Garcia, V. V. (1977). Functional properties and amino acid content of protein isolate from mung bean flour. *Journal of Food Technology*, 12, 473-484.

Cushing, J. (1989). Weaning-from breast-Milk to family food. 151-151.

Danso, J., Alemawor, F., Boateng, R., Barimah, J., & Kumah, D. B. (2019). Effect of drying on the nutrient and anti-nutrient composition of Bombax buonopozense sepals. *African Journal of Food Science*, 13(1), 21-29.

Day, R.A., Underwood A.L (1986). Quantitive analysis 5th ed. Prentice. Hall publication p. 701.

De Groot, R., Palermo, T., Handa, S., Ragno, L. P., & Peterman, A. (2017). Cash transfers and child nutrition: pathways and impacts. *Development Policy Review*, 35(5), 621-643.

De Onis, M., Blössner, M., Borghi, E., Frongillo., E. A., & Morris, R. (2015). Estimates of global prevalence of childhood underweight. *Journal of the American Medical Association,* 291 (21); 2600-2606.

De Onis, M., Borghi, E., Arimond, M., Webb, P., Croft, T., Saha, K., & Flores-Ayala, R. (2019). Prevalence thresholds for wasting, overweight and stunting in children under 5 years. *Public health nutrition*, *22*(1), 175-179.

Devi, C. B., Kushwaha, A., & Kumar, A. (2015). Sprouting characteristics and associated changes in nutritional composition of cowpea (Vigna unguiculata). *Journal of Food Science and Technology*, *52*(10), 6821-6827.

Dewey, K. G., & Begum, K. (2011). Long-term consequences of stunting in early life. *Maternal & Child Nutrition,* 7, 5-18.

Dewey, K. G., & Brown, K. H. (2003). Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food and Nutrition Bulletin*, *24*(1), 5-28.

Diamanti, A., Pedicelli, S., D'Argenio, P., Panetta, F., Alterio, A., & Torre, G. (2011). Iatrogenic Kwashiorkor in three infants on a diet of rice beverages. *Pediatric Allergy and Immunology*, 22 (8), 878-879.

Dukuzumuremyi, J. P. C., Acheampong, K., Abesig, J., & Luo, J. (2020). Knowledge, attitude, and practice of exclusive breastfeeding among mothers in East Africa: a systematic review. *International Breastfeeding Journal*, 15(1), 1-17.

Echendu, C. A., Obizoba, I. C., & Anyika, J. U. (2009). Effects of germination on chemical composition of ground bean (*Kerstingiella geocarpa harm*) seeds. *Pakistan Journal of Nutrition*, 8(12), 1849-1854.

Ehirim, F.N., Ezeji, C.N., Onugha., F.C., & Nwogu, O. G. (2018). Effect of Sprouting Time on the Nutrient and Anti-Nutrient Properties of Cowpea (*Vigna Unguiculata*). *Journal of Environmental Science, Toxicology and Food Technology*, (12), 01-08.

Ekpo, K.E (2011). Nutritional and Biochemical Evaluation of the Protein Quality of Four Popular Insects Consumed in Southern Nigeria. *Archives of Applied Science Research*, 3(5), 428 – 444.

El-Adawy, T. A. (2002). Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum L*.) undergoing different cooking methods and germination. *Plant Foods for Human Nutrition*, 57(1), 83-97.

Elegbede, J. A. (1998). Legumes. *Nutritional Quality of Plant Foods*, (1) 120-133.

Erah, P. O., Asonye, C. C., & Okhamafe, A. O. (2003). Response of Tripanosoma brucei brucei–induced anaemia to a commercial herbal preparation. *African Journal of Biotechnology*, 2(9), 307-311.

Essien, E., & Akpan, S. (2014). Phytochemical and anti-nutrient evaluation of some wild fruiting polypore microfungi. *Journal of Chemical and Physical Research*, 6(12), 280-283.

Ezeji, C., & Ojimelukwe, P. C. (1993). Effect of fermentation on the nutritional quality and functional properties of infant food formulations prepared from Bambara-groundnut, fluted-pumkin and millet seeds. *Plant Foods for Human Nutrition*, 44(3), 267-276.

Ezenduka, P. O., Ndie, E. C., & Nwankwo, C. U. (2018). Weaning practices among breastfeeding mothers' local communities of Enugu State Nigeria. *Clinics Mother Child Health*, *15*(293), 2.

Fernstrom, J.D. & Fernstrom, H.M. (2007). Tyrosine, Phenylalanine and Catecholamine synthesis and Function in the Brain. *Journal of Nutrition*, 137(6), 1539S – 1547S.

Fungo, R., Muyonga, J., Kaaya, A., Okia, C., Tieguhong, J. C., & Baidu-Forson, J. J. (2015). Nutrients and bioactive compounds content of Baillonella toxisperma, Trichoscypha abut and Pentaclethra macrophylla from Cameroon. *Food science & Nutrition*, *3*(4), 292-301.

Gomez, M. H.,& Aguilera, J. M. (1983). Changes in the starch fraction during extrusion-cooking of corn. *Journal of Food Science*, 48, 378–381.

Greiner, T. (1996). The concept of weaning: definitions and their implications. *Journal of Human Lactation*, *12*(2), 123-128.

Hawkes, C., Demaio, A. R., & Branca, F. (2017). Double-duty actions for ending malnutrition within a decade. *The Lancet Global Health, 5(8),* e745–e746.

Huang, L. (2012). The Essential Amino Acid Requirements of Infants.

Hussin, H., Gregory, P. J., Julkifle, A. L., Sethuraman, G., Tan, X. L., Razi, F., & Azam-Ali, S. N. (2020). Enhancing the nutritional profile of noodles with Bambara groundnut (*Vigna subterranea*) and Moringa (*Moringa oleifera*): a food system approach. *Frontier Sustainabe Food System*, 4 (59), 44.

Ibrahin H.D & Ogunwusi A. A. (2016) Industrial Potentials of Bambara Nut. *Journal of Poverty, Investment and Development* 22, 12-18.

Idoko, A. S., Zaharadeen, S. A., Ilounu, E., Nura, L., Sabin, U. A., Alao, S. O & Saidu, M. (2019). Mixed-Spices Supplementation Suppresses Fat Deposition and Causes no Alterations in Lipid and Red Blood Cell profiles in Broiler Chickens. *FUDMA Journal of Sciences*, 3(2), 288-294.

Ige SF, Akhigbe RE, Edeogho O, Ajao FO, & Owolabi, O.Q. (2011) Hepatoprotective activities of Allium cepa in Cadmium-Treated Rats. *International Journal of Pharmaceutical Science*, 3, 60-63.

Ijarotimi, O. S., & Keshinro, O. O. (2013). Determination of nutrient composition and protein quality of potential complementary foods formulated from the combination of fermented popcorn, African locust and Bambara groundnut seed flour. *Polish Journal of Food and Nutrition Sciences*, 63(3), 155-166.

Inyang, C. U., & Zakari, U. M. (2008). Effect of germination and fermentation of pearl millet on proximate, chemical and sensory properties of instant "Fura"-a Nigerian cereal food. *Pakistan Journal of Nutrition*, 7(1), 9-12.

Isabelle, M., & Chan, P. (2011). Seminar on young child nutrition: improving nutrition and health status of young children in Indonesia. *Asia Pacific Journal of Clinical Nutrition*, 20 (1), 141.

Jackson, J. E., & Hallam, J. (2020). Against all odds—why UK mothers' breastfeeding beyond infancy are turning to their international peers for emotional and informative support. *Health Care for Women International*, 1–17.

James, S., Akosu, N. I., Maina, Y. C., Baba, A. I., Nwokocha, L., Amuga, S. J & Omeiza, M. Y. M. (2018). Effect of addition of processed Bambara nut on the functional and sensory acceptability of millet-based infant formula. *Food Science & Nutrition*, 6 (4), 783-790.

Jirapa, P., Normah, H., Zamaliah, M. M., Asmah, R., & Mohamad, K. (2001). Nutritional quality of germinated cowpea flour (*Vigna unguiculata*) and its application in

home prepared powdered weaning foods. *Plant Foods for Human Nutrition*, 56 (3), 203-216.

Kamaruzaman, N. A., Jamani, N. A., & Said, A. H. (2020). An infant with kwashiorkor: The forgotten disease. *Malaysian family physician: the official Journal of the Academy of Family Physicians of Malaysia*, 15(2), 46.

Kamau, E. H., Serrem, C. A., & Wamunga, F. W. (2017). Rat bioassay for evaluation of protein quality of soy-fortified complementary foods. *Journal of Food Research*, 6(6), 35-44.

Kent, G. (2017). *Governments Push Infant Formula*. Lulu. com.

Khan, A., Khan S., & Zia-ul-Islam S. (2017) Causes, signs and symptoms of malnutrition among the children. *Journal of Nutrition Human Health*, 1(1), 24-27

Kiranmayee, K. N. S., Hash, C. T., Sivasubramani, S., Ramu, P., Amindala, B. P., Rathore, A., & Deshpande, S. P. (2020). Fine-Mapping of Sorghum Stay-Green QTL on Chromosome10 Revealed Genes Associated with Delayed Senescence. *Genes*, 11(9), 1026.

Kiruthikajothi, K., Chandramohan G., & Muniyappan K. (2014). Human Body Function Regulatory Amino Acids Cysteine and Methionine Containing Plants. *International Journal of Current Microbiology and Applied Sciences*, 3(10), 373 - 378.

Koletzko, B., Bhatia, J., Bhutta, Z. A., Cooper, P., Makrides, M., Uauy, R., & Wang, W. (Eds.). (2015). Pediatric nutrition in practice. *Karger Medical and Scientific Publishers, pp. 45.*

Kumar, V., Sinha, A. K., Makkar, H. P., & Becker, K. (2010). Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry*, 120 (4), 945-959.

Lemmens, E., Moroni, A. V., Pagand, J., Heirbaut, P., Ritala, A., Karlen, Y., & Delcour, J. A. (2019). Impact of cereal seed sprouting on its nutritional and technological properties: a critical review. *Comprehensive Reviews in Food Science and Food Safety*, 18 (1), 305-328.

Leroy, J. L., & Frongillo, E. A. (2019). Perspective: what does stunting really mean? A critical review of the evidence. *Advances in Nutrition*, 10 (2), 196-204.

Lolas, G. M., & Markakis, P. (1975). Phytic acid and other phosphorus compounds of beans (Phaseolus vulgaris L.). *Journal of Agricultural and Food Chemistry*, *23*(1), 13-15.

Lopez, M. J., & Mohiuddin, S. S. (2020). Biochemistry, Essential Amino Acids. *StatPearls [Internet]*.

Maphosa, Y., & Jideani, V. A. (2017). The role of legumes in human nutrition. *Functional Food-Improve Health Through Adequate Food*, 1, 13.

Marcus, H. D. S. X., Gilmar, F. O., Mariana, K. M., Prates de Almeida, E., & Natália, B. O. (2017). A child with kwashiorkor misdiagnosed as atopic dermatitis. 5 (4) 2-5.

Mariam, S. (2005). Nutritive value of three potential complementary foods based on cereals and legumes. *African Journal of Food, Agriculture, Nutrition and Development*, *5*(2).

Marshall, S. (2016). Protein-energy malnutrition in the rehabilitation setting: Evidence to improve identification. *Maturitas*, 86, 77-85.

Mbata, T. I., Ikenebomeh, M. J., & Ezeibe, S. (2009). Evaluation of mineral content and functional properties of fermented maize flour blended with Bambara groundnut (*Vigna subterranean L)*. *African Journal of Food Science*, *3*(4), 107-112.

Mehta, F. (2018). Marasmus: A type of malnutrition. *Medical News Today*.

Mesfin, W., & Shimelis, A. (2013). Effect of Soybean/Cassava Flour Blend in the Proximate Composition of Ethiopian Traditional Bread Prepared from Quality Protein Maize. *African Journal of Food, Agriculture, Nutrition and Development*, 13(4), 7985-8003.

Michaelsen, K. F. (2000). *Feeding and nutrition of infants and young children: guidelines for the WHO European region, with emphasis on the former Soviet countries* (87).

Modu S., Babagana, M., Chamba, G., Falmata F. S., & Sanda S.K., (2014) Rheological and Microbiological Assessment of Complementary meal produced from Sprouted and Fermented Sorghum (*S. bicolor*) blended with Cowpea (*Vignia unguiculata*) and Groundnut (*Arachis hypogea) Merit Research Journal of Food Science and Technology* 2(3), 031-037.

Mohapatra, D., Patel, A.S., Kar, A., Deshpande, S.S. & Kumar. T M. (2018) Effect of Different Processing Conditions on Proximate Composition, Anti-oxidants, Anti-nutrients and Amino Acid Profile of Grain Sorghum. *Journal of Food Chemistry*, (10), 11-15.

Mosha, T. C., & Vicent, M. M. (2005). Nutritional quality, storage stability and acceptability of home-processed ready-to-eat composite foods for rehabilitating undernourished preschool children in low-income countries. *Journal of Food Processing and Preservation*, 29 (5-6), 331-356.

Murevanhema, Y. Y., & Jideani, V. A. (2013). Potential of Bambara groundnut (*Vigna subterranea (L.) Verdc*) milk as a probiotic beverage—a review. *Critical Reviews in Food Science and Nutrition*, *53*(9), 954-967.

Murugkar, D. A., & Jha, K. (2009). Effect of sprouting on nutritional and functional characteristics of soybean. *Journal of Food Science Technology*, *46*(3), 240-243.

National Bureau of Statistics (2018). National nutrition and health survey (NNHS) (2018).

Nissar, J., Ahad, T., Naik, H. R., & Hussain, S. Z. (2017). A review phytic acid: As antinutrient or nutraceutical. *Journal of Pharmacognosy and Phytochemistry*, 6 (6), 1554-1560.

Nnam, N.M (2001) Chemical, sensory and rheological properties of porridges from processed sorghum (Sorghum bicolor), bambara groundnut (*Vigna subterranea L.*

*Verdc*) and sweet potato (*Zpomoea batatas*) flours. *International Journal of Plant Foods for Human Nutrition* 56: 25 1-264.

Nonogaki, H; Bassel, GW; Bewley, JW (2010). Germination still a Mystery. Plant Science. 179: 574 – 581.

NseAbasi, N.E. Williams, M.E., Akpabio, U., & Offiong, E.E.A. (2014). Haematological parameters and factors affecting their values. *Agricultural Science*, 2(1), 37- 47.

Nwadi, O. M. M., Uchegbu, N., & Okonkwo, T. M. (2019). Effect of processing methods on the antinutrient reduction of Bambara groundnut: A review, *Sky Journal of Food Science*, 7(3), 037-041.

Nyau, V., Prakash, S., Rodrigues, J., & Farrant, J. (2017). Profiling of Phenolic Compounds in Sprouted Common Beans and Bambara Groundnuts. *Journal of Food Research*; 6, (6) 74.

Ocheme, O. B., Adedeji, O. E., Lawal, G., & Zakari, U. M. (2015). Effect of germination on functional properties and degree of starch gelatinization of sorghum flour. *Journal of Food Research*, 4 (2), 159.

Odedeji, J. O., Akande, E. A., Ayinde, L. A., & Alade, O. A. (2020). Influence of Sprouting on Proximate and Anti-nutritional Factors of Jacbean (*Canavalia ensiformis*) Flour. *Journal of Applied Sciences and Environmental Management*, 24(8), 1455-1461.

Ogbo, F. A., Okoro, A., Olusanya, B. O., Olusanya, J., Ifegwu, I. K., Awosemo, A. O., & Page, A. (2019). Diarrhoea deaths and disability-adjusted life years attributable to suboptimal breastfeeding practices in Nigeria: findings from the global burden of disease study 2016. *International Breastfeeding Journal*, 14(1), 1-9.

Oibiokpa, F. I., Akanya, H. O., Jigam, A. A., Saidu, A. N., & Egwim, E. C. (2018). Protein quality of four indigenous edible insect species in Nigeria. *Food Science and Human Wellness*, *7*(2), 175-183.

Ojha, P., Adhikari, R., Karki, R., Mishra, A., Subedi, U., & Karki, T. B. (2018). Malting and fermentation effects on antinutritional components and functional characteristics of sorghum flour. *Food Science & Nutrition*, *6*(1), 47-53.

Okafor, J. N. C., Ani, J. C., & Okafor, G. I. (2014). Effect of processing methods on qualities of Bambara groundnut (Voandzeia subterranea (L.) Thouars) flour and their acceptability in extruded snacks. *American Journal of Food Technology*, 9 (7), 350-359.

Oladiran, D. A., & Emmambux, N. M. (2020). *Locally Available African Complementary Foods: Nutritional Limitations and Processing Technologies to Improve Nutritional Quality—A Review. Food Reviews International, 1–31.*

Olafedehan, C.O., Obun, A.M., Yusuf, M.K., Adewumi, O.O., Olafedehan, A.O., Awofolaji A.O. & Adeniji A.A. (2010). Effects of residual cyanide in processed cassava peal meals on haematological and biochemical indices of growing rabbits.

*Proceedings of 35th Annual Conference of Nigerian Society of Animal Production*, 212.

Onoja, U. S., Akubor, P. I., Gernar, D. I., & Chinmma, C. E. (2014). Evaluation of complementary food formulated from local staples and fortified with calcium, iron and zinc. *Journal of Nutrition and Food Sciences*, 4 (6), 1-6.

Onwuka, C. F., Ikewuchi, C. C., Ikewuchi, C. J., & Ayalogu, O. E. (2009). Investigation on the effect of germination on the proximate composition of African Yam bean (*Sphenostylis stenocarpa*) and fluted Pumpkin (*Telferia occidentalis*). *Journal of Applied Sciences and Environmental Management*, 13(2), 59-61.

Onwuka, G.I. (2005). *Food analysis and Instrumentation theory and practice (1st ed.).* Nigeria: Napththali prints, pp 64 – 67, 98, 140 – 145.

Otutu, O. L., Ikuomola, D. S., & Oloruntoba, R. O. (2014). Effect of sprouting days on the chemical and physicochemical properties of sorghum starch. *American Journal of Food and Nutrition*, *4*(1), 11-20.

Owuamanam, C.I, Edom T.A, Ogueke, C.C, Iwuoha, J.O, & Olawuni, I.A. (2014). Quality characteristics of some tropical legumes flours sprouted paste (Moi Moi) Production as affected by seed sprouting. *Asian Journal of Biological Sciences;* 6(8),347-355.

Oyelana, O., Kamanzi, J., & Richter, S. (2020). A critical look at exclusive breastfeeding in Africa: through the lens of diffusion of innovation theory. *International Journal of Africa Nursing Sciences,* 100267.

Oyeyinka, A. T. (2016). Nutritional, sensory and functional properties of Bambara groundnut complimentary food (Doctoral dissertation).

Oyeyinka, A. T., Pillay, K., Tesfay, S. & Siwela, M. (2017). Physical, nutritional and antioxidant properties of Zimbabwean Bambara groundnut and effects of processing methods on their chemical properties. *International Journal of Food Science and Technology*, 52 (10), 2238–2247.

Petroski, W., & Minich, D. M. (2020). Is There Such a Thing as "Anti-Nutrients"? A Narrative Review of Perceived Problematic Plant Compounds. *Nutrients*, 12 (10), 2929.

Pradhan, M., Guru, P. & Paital, B. (2019). Daily Dietary Nutrition and Nutraceutical Intake in Agricultural Laborers of Hirakud. *Journal of Drug Delivery and Therapeutics,* (5), 56-61.

Prasad, P., Kochhar, A., & Javed, M. (2016). Development of ready-to-eat supplementary foods using germinated cereal-pulse mix, potato flour and green leafy vegetable for malnourished children. *Nutrition & Food Science*, 46 (1), 30-42.

Pridham, K. F. (1990). Feeding behavior of 6-to 12-month-old infants: assessment and sources of parental information. *The Journal of Pediatrics*, 117 (2), S174-S180.

Rachie, K. O. & Sylvester, P. (1977). Grain legumes. In: Leakey CLA, Wills JB (eds), Food crops of the low land tropics (pp. 41–74). Oxford University Press.

Raihanatu, M. B., Modu, S., Falmata, A. S., Shettima, Y. A., & Heman, M. (2011). Effect of processing (sprouting and fermentation) of five local varieties of sorghum on some biochemical parameters. *Biokemistri*, 23 (2), 45.

Rakhshani, H., Idoumghar, L., Lepagnot, J., & Brévilliers, M. (2019). Speed up differential evolution for computationally expensive protein structure prediction problems. *Swarm and Evolutionary Computation*, 50, 100493.

Rao, B. D., & Kiranmai, E. (2020). Novel Processes, Value Chain, and Products for Food, Feed, and Industrial Uses in Sorghum in the 21st Century. *Food–Fodder–Feed–Fuel for a Rapidly Changing World*, 859-887.

Rathore, A. S., Joshi, N. H., & Deshpande, A. (2016). Various Aspects of Weaning. *Research & Reviews: Journal of Medical Science and Technology*, *5*(1), 13-16.

Ratnavathi, C. V., & Chavan, U. D. (2016). Malting and brewing of sorghum. In *Sorghum Biochemistry*, 63-105. Academic Press.

Reddy, S., & Patil, J. V. (2015). Genetic enhancement of rabi sorghum: *Adapting the Indian Durras*. Academic Press.

Rico, D., Peñas, E., García, M. del C., Martínez-Villaluenga, C., Rai, D. K., Birsan, R. I., Martín-Diana, A. B. (2020). *Sprouted Barley Flour as a Nutritious and Functional Ingredient. Foods,* 9 (3), 296.

Riordan, C. G. (2004). Acetyl coenzyme A synthase: New insights into one of nature's bioorganometallic catalysts.

Ritchie, H., & Roser, M. (2017). Micronutrient deficiency. *Our World in data*.

Roberts, K. M., Daryl, K. G., Peter, A. M., & Victor, W. R. (2000). Mayer's Biochemistry, 25. (pp. 763–765).

Rodriguez-Llanes, J. M., Ranjan-Dash, S., Mukhopadhyay, A., & Guha-Sapir, D. (2016). Looking upstream: enhancers of child nutritional status in post-flood rural settings. *Peer Journal*, *4*, e1741.

Rossouw, JE, Labadarios, D., Davis, M. & Williams, R. (1978). Vitamin B6 and aspartate aminotransferase activity in chronic liver disease. *South African Medical Journal*, 53 (12), 436-438.

Rubin, L.L., Canal C.W., Ribeiro, A.L.M., Kessler, A., Silva, I., Trevizan L., Viola, T., Raber, M., Goncalves T.A., & Kras R. (2007). Effects of methionine and arginine dietary levels on the immunity of broiler chickens submitted to immunological stimuli. *Brazilian Journal of Poultry Science*, 9(4), 241 -247.

Saadat, S., Akhtar, S., Ismail, T., Sharif, M. K., Shabbir, U., Ahmad, N., & Ali, A. (2020). Multilegume bar prepared from extruded legumes flour to address protein energy malnutrition. *Italian Journal of Food Science*, *32*(1).

Sampath, S., Rao, M. T., Reddy, K. K., Arun, K., & Reddy, P. V. M. (2008). Effect of germination on oligosaccharides in cereals and pulses. *Journal of Food Science and Technology-Mysore*, 45(2), 196-198.

Sanchez-Siles, L. M., Bernal, M. J., Gil, D., Bodenstab, S., Haro-Vicente, J. F., Klerks, M., & Gil, Á. (2020). Are Sugar-Reduced and Whole Grain Infant Cereals Sensorially Accepted at Weaning? *A Randomized Controlled Cross-Over Trial. Nutrients*, 12(6), 1883.

Santos, C. R., & Schulze, A. (2012). Lipid metabolism in cancer. *The FEBS journal*, 279 (15), 2610-2623.

Sathe SK, Deshpande SS, Salunkhe DK (1981). Functional properties of black gram (Phaseolus mango, L) proteins. Lebensm-wiss. U. Technol. 16: 69-74.

Schwartz, M. W. (Ed.). (2012). *The 5-minute Pediatric Consult*. Lippincott Williams & Wilkins.

Shah, S. A., Zeb, A., Masood, T., Noreen, N., Abbas, S. J., Samiullah, M., & Muhammad, A. (2011). Effects of sprouting time on biochemical and nutritional qualities of Mungbean varieties. *African Journal of Agricultural Research*, 6 (22), 5091-5098.

Sharif, M. K., Butt, M. S., Anjum, F. M., & Khan, S. H. (2014). Rice bran: A novel functional ingredient. *Critical Reviews in Food Science and Nutrition*, 54(6), 807-816.

Sharma, A. (2020). A review on traditional technology and safety challenges with regard to antinutrients in legume foods. *Journal of Food Science and Technology*, 1-21.

Skipper, A. (2012). Agreement on defining malnutrition. *Journal of Parenteral and Enteral Nutrition*, 36 (3), 261-262.

Soetan, K.O., Akinrinde, A.S., & Ajibade, T.O. (2013). Preliminary Studies on the haematological parameters of cockerels fed raw and processed guinea corn (*Sorgum bicolors*). *Proceedings of the 38th Annual Conference of the Nigerian Society of Animal Production*, pp 49 -52.

Soeters, P., Bozzetti, F., Cynober, L., Forbes, A., Shenkin, A., & Sobotka, L. (2017). Defining malnutrition: A plea to rethink. *Clinical Nutrition*, 36(3), 896–901.

Spritzler, F. (2017). How cooking affects the nutrient content of foods.

Stevens, G. A., Finucane, M. M., De-Regil, L. M., Paciorek, C. J., Flaxman, S. R., & Branca, F. (2013). Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: a systematic analysis of population-representative data. *The Lancet Global Health*, *1*(1), e16-e25.

Suda, M., Watanabe, T., Kobayashi, M., & Matsuda, K. (1986). Changes in starch content and related enzyme activities during the growth of germinating soybeans. *Agricultural and Biological Chemistry*, 50 (12), 3195-3196.

Sugarman, M., & Kendall-Tackett, K. A. (1995). Weaning ages in a sample of American women who practice extended breastfeeding. *Clinical Pediatrics*, *34*(12), 642-647.

Suleiman, A. E. (2016). Chemical composition and in vitro protein digestibility of hulled and de-hulled Bambara groundnut] Vigna subterranea (L.) Verdc. seeds.

Suleiman, H., Akani, A., & Olayanju, A. (2016). Impact of Hypoproteic Diet on Liver Function and Thrombopoiesis in New Zealand White Rabbits. *Hematology & Transfusion International Journal*, *3*(1).

Table, M. (2005). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids 5, pp 589-768. National Academy Press: Washington, DC, USA.

Taylor, J., & Taylor, J. R. (2002). Alleviation of the adverse effect of cooking on sorghum protein digestibility through fermentation in traditional African porridges. *International Journal of Food Science & Technology*, 37 (2), 129-137.

Temba, M. C., Njobeh, P. B., Adebo, O. A., Olugbile, A. O., & Kayitesi, E. (2016). *The role of compositing cereals with legumes to alleviate protein energy malnutrition in Africa. International Journal of Food Science & Technology, 51(3), 543–554.*

Temesgen, M. (2013). Nutritional status of Ethiopian weaning and complementary foods: a review. *Open Access Sci Rep*, *2*(2), 1-9.

Ubwa, S. T., Abah, J., Asemave, K., & Shambe, T. (2012). Studies on the gelatinization temperature of some cereal starches. *International Journal of Chemistry*, 4 (6), 22.

Ukwuru, M. U., Muritala, A., & Iheofor, A. O. (2018). Cereal utilization in Nigeria. *Research Journal of Food and Nutrition*, 2 (3), 1-12.

Umerah, N. N., Oly-Alawuba, N. M., Asouzu, A. I., & Oluah, C. G. U. (2020). Rheology and Functional Properties of Complementary Food Made from Maize (Zea mays) Supplemented with Crayfish (Euastacus spp) and Carrot (Daucus carota) Flour. *Asian Journal of Biotechnology and Genetic Engineering*, 41-48.

UNICEF (2018). Levels and trends in child malnutrition. *eSocial Sciences.*

Uppal, V. & Bains, K. (2012). Effect of germination periods and hydrothermal treatments on in-vitro protein and starch digestibility of germinated legumes. *Journal of Food Science and Technology*, 49 (2), 184-191.

US Department of Health and Human Services. (2011). Strengthening knowledge and understanding of dietary supplements. *National Health Institute: Office of Dietary Supplements*.

US National Library of Medicine. Joint Collection Development Policy: Human Nutrition and Food. Accessed in March 04, 2021.

Usman, M. A., Bolade, M. K., & James, S. (2016). Functional properties of weaning food blends from selected sorghum (Sorghum bicolor (L.) Moench) varieties and soybean (Glycine max). *African Journal of Food Science*, 10 (8), 112-121.

Uwaegbute, A. C. (1991). Weaning practices and weaning foods of the Hausas, Yorubas and Ibos of Nigeria. *Ecology of Food and Nutrition*, *26*(2), 139-153.

Van Der Merwe, J., Kluyts, M., Bowley, N., & Marais, D. (2007). Optimizing the introduction of complementary foods in the infant's diet: a unique challenge in developing countries. *Maternal & Child Nutrition*, 3 (4), 259-270.

Verhagen, L. M., Incani, R. N., Franco, C. R., Ugarte, A., Cadenas, Y., Ruiz, C. I. S., & Pinelli, E. (2013). High malnutrition rate in Venezuelan Yanomami compared to Warao Amerindians and Creoles: significant associations with intestinal parasites and anemia. *PLoS One*, *8*(10), e77581.

Vidal-Valverde, C., Frias, J., Sierra, I., Blazquez, I., Lambein, F., & Kuo, Y. H. (2002). New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology*, 215 (6), 472-477.

Wakil, S.M & Kazeem, M.O., (2012). Quality assessment of weaning food produced from fermented Cereal-legume blends using starters. *International Food Research Journal*, 19 (4), 1679-1685.

Weber, M., Grote, V., Closa-Monasterolo, R., Escribano, J., Langhendries, J. P., Dain, E. & European Childhood Obesity Trial Study Group. (2014). Lower protein content in infant formula reduces BMI and obesity risk at school age: follow-up of a randomized trial. *The American Journal of Clinical Nutrition*, *99*(5), 1041-1051.

Williams, C. D. (1935). Kwashiorkor. A nutritional disease of children associated with a maize diet. *Lancet*, *229*, 1151-1152.

World Health Organization (2015). e-library of evidence for nutrition actions (eLENA) *Avaialbe from http://www. who. int/elena/titles/exclusive_breastfeeding/en*.

World Health Organization (WHO), e-library of evidence for nutrition actions (eLENA). 2015. Available from http://www.who.int/ elena/titles/exclusive_breastfeeding/en.

World Health Organization. (2003). Complementary feeding: *Report of the Global Consultation, and summary of Guiding Principles for Complementary Feeding of the Breastfed Child*.

World Health Organization. (2009). *Infant and young child feeding: model chapter for Textbooks for Medical Students and Allied Health Professionals*..

World Health Organization. (2011). Guideline: vitamin A supplementation in infants and children 6-59 months of age.

World Health Organization. (2019). Nutrition Landscape Information System (NLIS) country profile indicators: *Interpretation Guide*.

World Health Organization. (2020a). Infant and young child feeding Model Chapter for textbooks for medical students and allied health professionals, Geneva (Switzerland): 2016-2020.

World Health Organization. (2020b). UNICEF/WHO/The World Bank Group joint child malnutrition estimates: levels and trends in child malnutrition: *Key Findings of the 2020 Edition.*

Yao, D. N., Kouassi, K. N., Erba, D., Scazzina, F., Pellegrini, N., & Casiraghi, M. C. (2015). Nutritive evaluation of the bambara groundnut Ci12 landrace [Vigna subterranea (L.) Verdc. (Fabaceae)] produced in Côte d'Ivoire. *International Journal of Molecular Sciences*, 16 (9), 21428-21441.

Young, V. R., & Pellett, P. L. (1994). Plant proteins in relation to human protein and amino acid nutrition. *The American Journal of Clinical Nutrition*, 59 (5), 1203S-1212S.