CHAPTER ONE

INTRODUCTION

1.1. Background

Adequate nutrition, which is achieved through qualitative breastfeeding and complementary feeding practices, is vital for the health and survival of the children. The breastfeeding and complementary feeding practices and access to appropriate quality and quantity of foods are essential components of optimal nutrition for infants and young children (Lutter and Rivera, 2003; Lutter, 2003). Complementary feeding period, that is, the period when family diets or infant formula (liquids, semisolids and solids) are introduced to infant at the age of 6 months when breastmilk is no longer adequate to provide nutrients for the normal growth and development (WHO/OMS, 2000). Complementary period is the stage at which the nutritional requirements of many infants are not met, thus leading to the onset of malnutrition among the children of under 5 years of age worldwide (Daelmans and Saadeh, 2003; Anigo *et al*., 2009).

In Nigeria, scientific studies have reported that prevalence of malnutrition among children is high due to early introduction of complementary foods, low nutritional quality of complementary foods and insufficient intakes of complementary foods (Villapando, 2000; WHO, 2002; Anigo *et al*., 2007, 2009). Poor knowledge of complementary feeding practices and poverty among nursing mothers are the most important direct factors responsible for nutrition problem amongst children in Nigeria (Solomon, 2005). For instance, high cost of fortified nutritious proprietary complementary foods is beyond the reach of most Nigerian families (Mosha *et al*. 2000; Amankwah *et al*., 2009; Bruyeron *et al*., 2010; Muhimbula *et al*., 2011); hence many depend on low quality traditional complementary foods, like ‘Ogi’ (a corn gruel). Scientific investigation had reported that over dependence on traditional complementary foods,

such as Ogi and other family diets, without adequate supplementation with high quality protein sources is the main attributable factor for the widespread of protein-energy malnutrition in Nigeria and other developing countries (Kikafunda *et al*. 2006; Aremu *et al*., 2011)

There are three basic conditions to prevent malnutrition in children: adequate food availability and consumption; good health and access to medical care; and adequate feeding practices; if any one of these is absent, protein-energy malnutrition is a likely outcome (de Onis *et. al*., 2000). Caregivers might not make the best use of these available resources, because of lack of knowledge of optimal feeding practices and inappropriate cultural beliefs and practices regarding complementary feeding (Allen and Gillespie, 2001; Engle, 2002; Caulfield *et al*., 2004; Moore, *et al*., 2006).

In most cultures in Nigeria, the first line complementary food is cereal gruel, which are bulky, low in energy and nutrient density (Ibe 2008). Plant-based complementary foods are usually insufficient to meet the protein and certain micronutrients requirements of growing children (Ibe 2008; Nwamarah and Amadi 2009). Traditional complementary foods usually consist mainly of un-supplemented cereal porridges (Mosha, *et al*. 2000; Nnam, 2002; Eka *et al*., 2010), which are often fail to meet the nutritional needs of the infant due to poor nutritive values (Fernandez *et al,* 2002). Cereal-based complementary foods have been implicated in the aetiology of protein–energy malnutrition especially in the community where it is solely used as the complementary food (Appoh and Krekling, 2005; Kikafunda *et al*. 2006; Ukegbu and Anyika, 2012). For instance, a number of scientific studies have reported that ogi (a corn gell and traditional complementary food) is characterized by low protein (e.g., lysine and tryptophan) (Ikujenlola and Adurotoye, 2014), low energy density and bulky (meaning, food of high viscosity but low energy density) (Fernandez *et al,* 2002; Kikafunda *et al.,* 2006; Ukegbu and Anyika 2012). These factors limit energy and essential intake of an infant weaned on such low

energy-dense foods is due to the volume the child can consume at a time and the frequency of feeding (Walker and Pavitt, 2007). In order to improve the nutrient content of cereal based complementary diets, combination of varieties of food materials such as staple starchy roots, tubers and cereals in combination with plant proteins like soybean and groundnut are considered the appropriate food groups for weaning children (Nnam 2002; Ibe 2008; Ugwu, 2009).

In view of these, several researchers have worked extensively in combining two or more food materials to formulate complementary foods with a hope to solve infant and young child nutrition problems (Ijarotimi and Olopade, 2009; Oyarekua and Adeyeye, 2009; Eka *et al*., 2010; Md. Ariful *et al*., 2013). However, despite all these efforts the problem of protein-energy malnutrition still persist due to problems of complementation and the technology involved in the complementary food production. Low-income mothers therefore depend on available low-cost foods to wean their infants (Eka *et al.*, 2010).

Cereals are widely utilized as food in African countries than in the developed world (Makinde and Ladipo, 2012). Traditional cereal foods play an important role in the diet of the people of Africa, particularly in cereal producing zones (Assohoun *et al.*, 2013**)**. For instance, cereals account for as much as 77% of the total caloric consumption and the primary basis for most of the traditional complementary foods in Africa countries (Taiwo, 2009; Malunga *et al*., 2014; Oyarekua, 2014). Compositionally, cereals consist of carbohydrate and less in amount of protein and vital minerals. Among the cereals, popcorn maize contains appreciable amount of protein and minerals when compared with other maize species (Iken and Amusa, 2010). Popcorn, which is grown solely for human consumption in the developed countries, is now becoming popular in Nigeria (Iken and Amusa, 2010).

Legumes (Cowpea, African locust beans, Bambara groundnut, etc.) are the most important food crops grown throughout the tropics. The legumes have little vitamin and more

than human requirements of thiamin and nicotinic acid. Methionine is the most limiting amino acid. The legume provides a significant level of dietary protein and lysine in regions of chronic protein shortage like Nigeria. African locust beans and Bambara groundnut are used extensively as flavouring and nutrition additives to soups and stews; some research has been carried out on the production of fermented condiments-*iru*-from African locust bean (Achi, 2005). Nutritionally, African locust bean and Bambara groundnut contain appreciable amount of vital nutrient like essential fatty acids, amino acids and micronutrients (Amarteifio *et al*., 2002; Abdulsalami and Sheriff, 2010; Mahala and Mohammed 2010; Mune-Mune *et al*., 2011).

1.2. Statement of Problem of the Study

Malnutrition describes a state of imbalance between the dietary needs of the body and the type of diet provided to the body. It affects brain development, behavioral development, cognitive development and perhaps most visibly growth development. More specifically, the numbers of underweight (low weight-for-age), stunted (low height-for-age) and wasted (low weight-for-height) children are common measures for the number of malnourished children in a particular community. Prevalence of child malnutrition in Nigeria is high due to low protein and energy-dense of local complementary foods (Anigo *et al*., 2007, 2009). High cost of fortified nutritious proprietary complementary foods is always beyond the reach of most Nigerian families; hence, there is increased in protein-energy malnutrition.

One of the best ways to reduce malnutrition is to produce quality complementary foods that are affordable to majority of low-income mothers. Unfortunately in Nigeria, The traditional complementary foods are cereal based (e.g, Ogi) (Nnam, 2002) and other family diets (cassava, yam, rice, amala, etc); and these plant-based complementary foods are not beneficial to the growth and development of the children (Nemer *et al.*, 2001; Müller *et al*., 2003; Black *et al*., 2003; FAO 2004; NPC/ICFM, 2009; Eka *et al.*, 2010). Scientific investigations have shown that

traditional complementary foods, particularly 'Ogi' and other family diets often fail to meet the nutritional needs of the infant due to poor nutritive values (Fernandez *et al*, 2002; Solomon 2005). These local complementary foods have been implicated in the aetiology of protein–energy malnutrition in the community where they are solely used as the complementary food (Kikafunda *et al*. 2006; Ikujenlola and Adurotoye, 2014). Considering these barriers (i.e. low quality of local complementary foods and high cost of fortified nutritious proprietary complementary foods) therefore, there is a need to produce qualitative complementary foods using locally available and affordable raw food materials.

1.3. Justification for the Study

Poor child complementary feeding practices hold a central role in child malnutrition in Nigeria and other developing countries (Kothari and Noureddine, 2010). In Nigeria, It is evident that the traditional complementary foods consists mainly of un-supplemented starchy staples food materials like sorghum, maize, millet, yams, cassavas, coco yams and sweet potatoes (Nnam, 2002). These food materials are poor in protein and limited in other important nutrients. Hence, there is increased in protein-energy malnutrition, particularly among the children that depend solely on these low quality food materials as complementary foods.

In view of inadequate nutrient composition of local complementary foods, couple with high prevalence of protein-energy malnutrition among weaning aged children in Nigeria, several complementary foods have been produced from cereal and legumes. However, popcorn, African locust beans and Bambara groundnut combinations have not been used in the formulation of infant foods. The present study, therefore, produces and determine nutritional efficacy of potential complementary foods from these locally available food materials using familiar home-based processing methods like germination and fermentation. Several studies have validated the uses of two or more plant-based food materials such as cereal and legume for the production of

complementary foods in order to improve the nutritional quality of the food combinations in terms of essential amino acid profile (Solomon, 2005; Osman 2007; Oyarekua and Adeyeye, 2009; Ukegbu and anyika, 2012; Oyarekua, 2013).

1.4. General and specific objectives of the study

The general objective of this study is to produce and evaluate the nutritional qualities of complementary foods produced from locally available food materials such as popcorn, African locust bean and bambara groundnuts using traditional processing methods.

The specific objectives of the study are to

1. evaluate nutrient compositions (i.e., proximate, minerals, amino acids and fatty acids) and ant-nutritional factors of popcorn, Bambara groundnut, African locust bean flour, composite blends and control food samples (Cerelac and ogi),
2. determine functional properties of infant foods based on popcorn, African locust beans and bambara groundnut flour and control food samples (Cerelac and ogi),
3. assess sensory properties of the novel infant foods formulated from popcorn, Bambara groundnut and African locust bean flour and control food samples (*Cerelac* and Ogi) using nursing-mothers as the panelists and,
4. evaluate microbial status of the popcorn, African locust beans and bambara groundnut flour samples used in infant food formulations
5. determine protein digestibility, heamatological properties and growth patterns of albino rats fed with the formulated diets and control food samples (*Cerelac* and Ogi).

1.5. Realization of the specific objectives

The objectives outlined above were achieved through

1. the use of standard chemical methods to evaluate the proximate nutrients, minerals and anti-nutritional factors of the formulations,
2. the use of Atomic Absorption Spectrophotometer and ion chromatographic analyzer to determine the mineral components,
3. the use of Technicon Amino Acid Analyzer to determine the amino acid profiles
4. animal studies to assess the nutritional efficacy of the formulated diets and compared with those rats fed with proprietary formula (Cerelac),
5. the use of nursing mothers as panelist to evaluate sensory attributes of the formulated complementary foods and
6. data analysis to warrant recommendations on the use of the local diets.

CHAPTER TWO

LITERATURE REVIEW

2.1. Food and Nutrition

Food is any substance, whether processed, semi-processed or raw, consumed to provide nutritional support for the body (FAO/WHO, 1999). Food is usually of plant or animal origin, and it contains combination of essential nutrients, such as carbohydrates, fats, proteins, vitamins, or minerals and non-nutrient. No single food can supply every nutrient (with the exception of breast-milk for young infants). Food is a necessity of healthy life and it is the "vehicles" for taking nutrients like carbohydrates, protein, fats/oil, vitamins and minerals into the body (Omonoma and Agoi, 2007) and bringing about human pleasure, hence, the need for food to be taken in the right quantity and quality.

Food utilization, which is typically reflected in the nutritional status of an individual, is determined by the quantity and quality of dietary intake, general childcare and feeding practices, along with health status of individuals (Omonoma and Agoi, 2007). Poor infant care and feeding practices, inadequate access to, or poor quality of health services are also major determinants of poor health and nutrition. The attainment of good nutrition depends on and encompasses the entire food supply. Nutrition is vital, not only in the growth and in development of humans and animals but also in the prevention and treatment of disease. Nutrition is fundamental to the maintenance of good health and functionality. Several factors like availability, economy, cultural and social habits, physiological and psychological attributes, marketing methods, and nutritional knowledge, among others influence the choice of the food consumed by individuals (Popkin *et al*., 2005).

2.2 Nutrient Compositions of Foods

Nutrition is the various processes involved ingestion, digestion, absorption, assimilation and the utilization of food substances by which growth, repair and maintenance of the body are accomplished (Tuladhar *et al*., 2013). Nutrients are stored by the body in various forms and drawn upon when the food intake is not sufficient (Tuladhar *et al*., 2013).

The nutrient consists of essential and non-essential, the essential nutrients are those nutrients that must be provided through the food, because the body cannot synthesize them. The nutrient classes can be categorized as either macronutrients (needed in relatively large amounts) or micronutrients (needed in smaller quantities). The macronutrients are carbohydrates, fats, fiber, proteins, and water, while the micronutrients are minerals and vitamins. Both the macronutrients and micronutrients must be provided in the diet, as they are absolutely essential for body growth and functioning. Failure of the body to receive the various food nutrients in adequate amounts makes them unable to perform their functions properly (Tuladhar *et al*., 2013). Excess availability of nutrient in the body can also lead to serious health problem. It is therefore necessary to consume nutrient at the levels compatible with maintenance of normal health.

Carbohydrates: Carbohydrates are classified as monosaccharide, disaccharides, or polysaccharides by the number of monomer (sugar) units they contain. They constitute a large proportion of foods such as cereals, tubers and other grain-based products. Complex carbohydrates take longer to digest and absorb than monosaccharide or disaccharide, since their sugar units are processed one-by-one off the ends of the chains. Simple sugars form a greater part of modern diets, leading to more obesity disease in populations. Simple carbohydrates are absorbed quickly, and therefore raise blood-sugar levels more rapidly.

***Fat*:** Fat consists of several fatty acids (containing long chains of carbon and hydrogen atoms), bonded to a glycerol, and they are classified as saturated or unsaturated. Saturated fats have all the carbon atoms in their fatty acid chains bonded to hydrogen atoms, whereas unsaturated fats have some of these carbon atoms double-bonded, so their molecules have relatively few hydrogen atoms. Unsaturated fats are further classified as monounsaturated (one double bond) or polyunsaturated (many double bonds). Saturated fatty acids are typically solid at room temperature (such as butter or lard), while unsaturated fats are typically liquids, such as olive oil flaxseed oil.

***Essential fatty acids*:** Most fatty acids are non-essential, meaning the body can produce them as needed. However, in humans at least two fatty acids are essential and must be included in the diet. An appropriate balance of essential fatty acids—omega-3 and omega-6 fatty acids—is important for health. Both of these "omega" long-chain polyunsaturated fatty acids are substrates for a class of eicosanoids known as prostaglandins, which have roles throughout the human body. Good sources of essential fatty acids include most vegetables, nuts, seeds, marine oils, fish, flax seed oils, soy beans, pumpkin seeds, sunflower seeds and walnuts (Barker, 2002).

***Fiber*:** Fiber is a carbohydrate (or a polysaccharide), which is not digested or absorbed by the endogenous secretions of the human digestive tracts (Komal and Avinash. 1992; AACC, 2001). Like other carbohydrates, when fiber is metabolized it produces four calories (kilocalories) of energy per gram. Dietary fiber consists mainly of cellulose, a large carbohydrate polymer that is indigestible, because humans do not have the required enzymes. There are two subcategories of fibers, that is, soluble and insoluble, which are present in whole grains, fruits and vegetables. The nutritional benefits of fibers are numerous, for instance, soluble fiber binds to bile acids in the small intestine, making them less likely to enter the body; this in turn lowers cholesterol levels in

the blood (Anderson *et al.*, 2009). Soluble fiber also attenuates the absorption of sugar, reduces sugar response after eating, normalizes blood lipid levels and, once fermented in the colon, produces short-chain fatty acids as byproducts with wide-ranging physiological activities. Insoluble fiber is associated with reduced diabetes risk (Weickert and Pfeiffer, 2008).

***Protein*:** Proteins are the primary structural and functional polymers in living systems. They have a broad range of activities, including catalysis of metabolic reactions and transport of vitamins, minerals, oxygen, fuels and structure of tissues; while others function in nerve transmission, muscle contraction and cell motility, blood clotting, immunologic defenses, hormones and regulatory molecules. Proteins are synthesized as a sequence of amino acids linked together in a linear polyamide (polypeptide) structure, but they assume complex three-dimensional shapes in performing their function.

Proteins are the basis of many animal body structures (e.g. muscles, skin, and hair). Each molecule is composed of amino acids, which are characterized by inclusion of nitrogen and sometimes sulphur. The body requires amino acids to produce new proteins and to replace damaged proteins (Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies, 2008). For all animals, some amino acids are essential (an animal cannot produce them internally) and some are non-essential (the animal can produce them from other nitrogen-containing compounds) (Voet and Voet, 2004). A diet that contains adequate amounts of amino acids is particularly important for growth and development of children (Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies, 2008).

The quality of protein depends on the level at which it provides the nutritional amounts of essential amino acids needed for overall body health, maintenance and growth (FAO/WHO/UNU, 2007). Animal proteins, such as eggs, cheese, milk, meat and fish are considered high quality, or complete proteins, because they provide sufficient amounts of the

essential amino acids. Plant proteins, such as grain, corn, nuts, vegetables and fruits, are lower quality, or incomplete proteins, because many plant proteins lack one or more of the essential amino acids, or because they lack a proper balance of amino acids. Incomplete proteins can be combined to provide all the essential amino acids, though combinations of incomplete proteins must be consumed at the same time, or within a short period, to obtain the maximum nutritive value from the amino acids (FAO/WHO, 1998; Ikujenlola and Fashakin, 2005). Such combination diets generally yield a high-quality protein meal, providing sufficient amounts and proper balance of the essential amino acids needed by the body to function.

There are about three hundred known amino acids present in animals only 23 are important for the human diet (Atanasova, 2008). These amino acids are divided into groups according to their necessity in the human diet. Those that may be present in the diet but can be omitted without threatening life are called dispensable or nonessential amino acids (NEAA), while those that are required to maintain life are called indispensable or essential amino acids (EAA). Essential amino acids are those that cannot be synthesized de novo by the organism, and therefore must be supplied in food (Atanasova, 2008). Eight amino acids generally regarded as essential for humans are isoleucine, leucine, lysine, threonine, tryptophan, methionine, valine, and phenylalanine (Atanasova, 2008); however, Cysteine (or sulphur-containing amino acids), tyrosine (or aromatic amino acids), histidine and arginine are additionally required by infants and young children (Imura and Okada, 1998; FAO/WHO/UNU, 2007). Some essential amino acids have been observed to be deficient in different plant food products; for example, lysine is deficient in wheat, tryptophan in maize, and methionine in legumes (Atanasova, 2008). Conditionally essential, that is, arginine, cysteine, glycine, glutamine, histidine, proline, serine and tyrosine, meaning the amino acids are not normally required in the diet, and are synthesized from the metabolism of other amino acids (Reeds, 2000; Fürst *et al*., 2004). Nonessential amino

acids are mutually interchangeable as sources of nonspecific nitrogen, there is experimental evidence suggesting that some amino acids (histidine and arginine) commonly classified as nonessential should be present in the diet of rapidly growing animals to promote maximal growth and nitrogen retention and, therefore, maximal utilization of amino acids (Imura and Okada, 1998; Tuan *et al*., 1999; FAO/WHO/UNU, 2007).

Use of essential amino acids: Foods that lack essential amino acids are poor sources of protein. Complete essential amino acids are necessary for a high degree of net protein utilization, which is the mass ratio of amino acids converted to proteins to amino acids supplied. Food from animal sources provides all the essential amino acids (Centers for Disease Control and Prevention, 2008). Several studies have reported that near-complete proteins are also found in plant sources, such as cereals and legumes (Achi, 2005; Wakil and Onilude, 2009). By consuming a wide variety of plant foods, a full set of essential amino acids will be supplied and the human body can convert the amino acids into proteins.

The net protein utilization is profoundly affected by the limiting amino acid content (the essential amino acid found in the smallest quantity in the foodstuff), and somewhat affected by salvage of essential amino acids in the body. It is therefore a good idea to mix foodstuffs that have different deficient of essential amino acid distributions; and thereby improved on the overall status of essential amino acids composition of the mixes. The limiting amino acids of some selected food crops are as follows, that is, wheat is deficient in lysine, rice in lysine and legumes in tryptophan, while maize deficient in lysine and tryptophan (Adepeju *et al*., 2014) and pulses are deficient in methionine or cystein (Minarro *et al.,* 2012)

Table 2.1: Recommended Daily Allowances (RDA) of essential amino acids for children (2-5 years) and adult

Amino acid	Adults (mg/kg body weight)	Children (mg/kg body weight)
Arginine	-	-
Histidine	-	19
Isoleucine	20	28
Leucine	39	66
Lysine	30	58
Methionine + Cystein	15	25
Phenylalanine + Tyrosine	25	63
Threonine	15	34
Tryptophan	4	11
Valine	26	35

Source: *FAO/WHO/UNU, 2007*

Minerals: Dietary minerals are the chemical elements required by living organisms, other than the four elements carbon, hydrogen, nitrogen, and oxygen that are present in common organic molecules. Minerals can be supplied from foods in which they occur naturally or at least as complex compounds, or sometimes even from natural inorganic sources (such as calcium carbonate from ground oyster shells). Minerals are classified as macro minerals (i.e., elements with recommended dietary allowance (RDA) greater than 200 mg/day, such as calcium, phosphorus, potassium, sodium, chloride, sulfur and magnesium and trace minerals i.e., elements with recommended dietary allowance less than 200 mg/day, such as copper, cobalt, chromium, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc. Mineral nutrients are metabolized for growth, development, and vitality of living organisms (Adame, 2002).

***Vitamins*:** Vitamins constitute a group of organic compounds, which are essential in small quantities for the normal metabolism of other nutrients and maintenance of physiological well-being. Vitamins are found in varying quantities in different foods. Absence or relative deficiency of vitamins in the diet can lead to a characteristic deficiency state and disease (Lakhan and Vieira, 2008; Boy *et al.*, 2009). These deficiencies can be avoided by consuming a wide variety of foods in adequate amounts.

The vitamins are classified according to their solubility in water and fat solvents. The fat-soluble vitamins include vitamin A (retinol), vitamin D (calciferol), vitamin E (tocopherol), and vitamin K (menadione). The water-soluble vitamins include vitamin B_1 (thiamin), vitamin B_2 (riboflavin), vitamin B_3 (niacin), biotin, vitamin B_6 (pyridoxine), pantothenic acid, folate, vitamin B_{12} (cobalamin) and vitamin C (ascorbic acid). Fat-soluble vitamins are stored in appreciable amounts in body tissues and, do not have to be supplied daily in the diet, while

water-soluble vitamins are not stored largely and therefore need to be included in the diet every day.

***Water*:** About 70% of the non-fat mass of the human body is made of water. To function properly, the body requires between one and seven liters of water per day to avoid dehydration; the precise amount depends on the level of activity, temperature, humidity, and other factors.

2.3. Phytochemicals/Anti-nutritional factors in Human Diets

Phytochemicals are found in edible plants, especially colorful fruits and vegetables. Study has reported on the health benefits of phytochemicals in man (Agaie, 2004). Plant phytochemicals exhibit diverse pharmacological and biochemical actions when ingested by animals and man (Amadi *et al*., 2006; Soetan, 2008). One of the principal classes of phytochemicals is polyphenol antioxidants, chemicals, which are known to provide certain health benefits to the cardiovascular system and immune system. These chemicals are known to down-regulate the formation of reactive oxygen species, key chemicals in cardiovascular disease. Recent study has reported that zeaxanthin, a yellow-pigmented carotenoid present in many yellow and orange fruits and vegetables prevents the age-related mucular degeneration (AMD) (Seddon *et al*., 1994).

Anti-nutritional factors are those substances generated in natural food stuffs by the normal metabolism of species and by different mechanisms (e.g. inactivation of some nutrients, diminution of the digestive process, or metabolic utilization of feed) which exert effects contrary to optimum nutrition (Kumar, 1992; Akande *et al*., 2010). Anti-nutritional factors interfere with the digestion, absorption, or some other aspect of metabolism of a nutrients or nutrients contained in those or other foods (Soetan and Oyewole, 2009; Akande *et al*., 2010). Some of these antinutritional factors, such as enzyme inhibitors, phytates, lectins, polyphenols and allergic factors are quite common in food materials, while others may be specific to just a few

plant species, such as cyanogens in cassava, gossypol in cotton seed and favism factors in broad beans and other legumes. These compounds generally make up a small fraction of the weight of foods, and their analysis problematic. Most of the toxic and anti-nutrient effects of these compounds in plants could be removed by several processing methods such as soaking, germination, boiling, autoclaving, fermentation, genetic manipulation and other processing methods (Soetan, 2008; Soetan and Oyewole, 2009).

2.3.1 Common types of anti-nutritional factors in food materials

***Protease inhibitors*:** All seeds, especially the legumes, contain enzyme inhibitors. Of these, the most harmful are the protease inhibitors that block the activity of pancreatic proteolytic enzymes, such as trypsin and chymotrypsin, resulting in pancreatic hyperplasia hypersecretion in animals (Liener, 1994). Therefore, it is generally important to remove these protease inhibitors from foods prior to consumption (Vidal-Valverde *et al*., 1994). Processing techniques such as heat, roasting and wet autoclaving denatured protease inhibitors (Soetan and Oyewole, 2009). Boiling, however, may be insufficient to deactivate these substances fully, so dry heat (toasting and roasting) is the preferred methods for home processing.

***Phytates*:** Phosphorus is stored in plant seeds linked to inositol to form different compounds of inositol phosphste, known as phytates. Phytates can make up as much as 1-5% of the dry weight of some grains. Depending on the number of phosphorus molecules they contain, phytates may bind strongly to minerals and trace elements in the diet, rendering them unavailable for absorption (Lopez *et al*., 2002). Phytates also form stable bonds with protein and inhibit the activity of some enzymes (Akande *et al*., 2010). In contrast to its anti-nutritive effects, the potential benefits of phytic acid, such as a delayed postprandial glucose absorption (Yoon *et al*., 1983), a decrease in plasma cholesterol and triglycerides (Katayama, 1995), chelating with heavy metals such as cadmium (Rimbach and Pallauf, 1997) and lead (Rimbach *et al.*, 1996)and

thereby reducing the toxicity of heavy metals. Rcently sudy revealed that phytate may have beneficial roles as an antioxidant and anticarcinogen (Shamsuddin, 1995; Jeanb and Thompsonm, 2002).

The molar ratios of phytate and minerals are one of the methods used to determine the bioavailability of minerals in the human body (Morris and Ellis, 1989). The molar ratio is determined by dividing the weight of phytate and minerals with its atomic weight (phytate: 660g/mol; Fe: 56g/mol; Zn: 65g/mol; Ca: 40 g/mol). Studies on the phytate and mineral molar ratios established the following critical values: phytate: calcium > 0.24 (Morris and Ellis, 1985), phytate:iron > 1 (Hallberg *et al*., 1989), phytate:zinc >15 (Turnlund *et al*.,1984; Sandberg *et al*., 1987; Morris and Ellis, 1989), phytate:calcium/zinc > 200 mmol/ 1000 kcal (4.2 MJ) (Davies *et al*., 1985; Bindra *et al*., 1986). The calculated [Ca][Phytate]/[Zn] molar ratio is considered a better index for predicting zinc bioavailability compared with the phytate:zinc ratio because of the calcium to phytate interaction (Akindahunsi and Oboh 1999). The calculated phytate and mineral ratios below the critical levels are indication of bioavailability of dietary minerals (Ma *et al.*, 2007).

Phytate is present at high levels in unrefined cereals, legumes, nuts, and most of the phosphorus in these foods is present mostly as phytate. Phytate contains negatively charged phosphate ligands which complex with positively charged ions such as Zn^{2+}, Ca^{2+}, Mg^{2+} and Fe^{2+}. Absorption of these metals in the small intestine can be inhibited due to their chelation by phytate (Graf and Eaton, 1984; Sandstead, 1991). Synergistic effects of phytate and fiber on iron and zinc bioavailability in children compared with adults is well-established (Kelsay, 1987; Williams and Bollella, 1995). In adults, up to 32 g per day of dietary fiber and 2 g per day of phytic acid may exert no adverse effects on iron and zinc bioavailability (Kelsay, 1987). Among

children, up to 25 g per day of dietary fiber and 1 g per day of phytic acid is unlikely to have a deleterious effect on iron and zinc bioavailability (Williams and Bollella, 1995).

***Lectins*:** Lectins, or phytohemagglutinins, are found in plant such as legumes and some oil seeds. Lectins are proteins, which agglutinate red blood cells. Lectins are high molecular weight proteins that have a high affinity for sugar molecules and are characterized by their ability to combine with carbohydrate membrane receptors. Lectins have the ability to bind directly to the intestinal muscosa (Almeida *et al*., 1991; Santiago *et al*., 1993), interacting with the enterocytes and interfering with the absorption and transportation of nutrients (particularly carbohydrates) during digestion (Santiago *et al*., 1993) and causing epithelial lesions within the intestine (Oliveira *et al*., 1989).

***Tannins and other polyphenols*:** Polyphenols are a diverse group of compunds that may be more or less polymerized or condensed. The condensed polyphenols, like tannins, consist of polymerized proanthocyanidins that are neither hydrolysed nor absorbable. They are abundant in some cereals, such as sorghum and legume seeds. Tannins interfere with protein digestibility and lysine availability, but may also have antioxidative beneficial effects. Tannins are not denatured by heat treatment, so avoidance requires elimination of those parts of the plants that have the highest tannin contents. Soaking and dehulling had been reported to remove tannin and polyphenol compound in legumes (Kaur and Kapoor, 1990; Bishnoi *et al.* 1994). Since polyphenolic compounds are present in the periphery of the grain, their passing out into the soaking medium through seed coat is possible (Kaur and Kapoor, 1990; Bishnoi *et al.* 1994).

***Alpha-galactosides*:** Alpha-galactosides are found in legume seeds. The galactose, oligosaccarides, contains glycosidic bonds, which cannot be hydrolysed by digestive enzymes in the human intestine (ASP and N-GL. 1995; Porzucek *et al*., 2002). Intake of oligosaccharide (i.e., raffinose and stachyose) passes into large intestine where it is undergo anaerobic

fermentation by bacteria, especially *Clostridium* spp. (Nowak, 1992), causing gas, distension, flatulence and possibly diarrhea (Castillo *et al*., 1990). Alph-galactosides can be eliminated partially by solubilization or enzymatic hydrolysis during germination (Mubarak, 2005) or fermentation (Granito *et al*., 2003; Adewumi and Odunfa, 2009).

2.4 Infant nutrition: Breastfeeding and Complementary Feeding Practices

Infancy is a period of rapid physical growth as well as physiological, immunological and mental development when nutritional requirements like calories and proteins are at their highest (Ossai, 2013). Scientific studies have shown that most brain development, for which protein, cholesterol, zinc and some essential fatty acids are required; occur in the first two years after birth (Guthrie, 1989). Most tooth development, requiring calcium, phosphorus and vitamins A, D and C occurs in the first one to five years of life (Uphoff, 1993). Deficiency of these and other nutrients can therefore have dire consequences, some of which can be long lasting or even lead to death (Moterjemi, 2000; Oluwafemi and Ibeh, 2011). WHO/UNICEF (1997) reported that millions of children, particularly in developing countries, are physically and mentally disabled due to macronutrient and micronutrient deficiencies. These shortfalls have been attributed to shortened period of exclusive breastfeeding, low dietary intake and poor bioavailability of nutrients in complementary foods (Lutter and Rivera , 2003)

Nutritious dietary intake during this critical period, that is, infant therefore becomes very vital and priority must be accorded to its improvement. Study has recommended that, after 6 months, nutritious and safe foods (also known as complementary foods) be gradually introduced and continued up to two years of age, thereafter the normal family diet be introduced (Yeung, 1998). The use of complementary foods does not only prime the infant's system to an adult diet, but also serves as a source of additional nutrients required for optimal growth and development (Fernandez *et al*, 2002). Proper weaning practices coupled with lengthened breastfeeding period

of at least two years can eliminate or at least reduce the high incidence of infant mortality and morbidity experienced in developing countries (Walker, 1990). It is believed that gradual introduction of complementary foods after six (6) months of age to the infant diet would have no adverse or shock effect on the digestive system of the child (Okungbowa, 1986). A number of studies have shown that children are particularly vulnerable to infections, malnutrition, cognitive retardation and deaths during the transition from breast milk to complementary foods (Drewett *et al.*, 1993 and Dewey and Brown, 2003; Moterjemi, 2000; Oluwafemi and Ibeh, 2011)

The nutritional adequacy is essential to the prevention of infant morbidity and mortality (ACC/SCN, 2000). The linear growth retardation acquired early on in infancy cannot be easily reversed after the second year of life (Martorell *et al*., 1994). Therefore, providing infants with adequate nutrition in the early years of life is a major determinant of healthy growth and development throughout childhood and of good health in adulthood (Sule, 2014). High prevalence of protein-energy and micronutrient malnutrition in Nigeria has been attributed to the poor complementation of breastmilk with cereal gruels(a complementary food) that are of low energy density and high cost of commercial complementary foods (Nemer *et al.*, 2001; Müller *et al.*, 2003; Black *et al.*, 2003; FAO 2004; NPC/ICFM, 2009, Eka *et al.*, 2010). Nutritional investigations have shown that the commercial complementary foods are available and nutritious; however, most of the infant formulae are priced beyond the reach of the majority of the population in the rural areas (Traoré, 2005; Bruyeron *et al*., 2010; Muhimbula *et al*., 2011; Ijarotimi *et al*., 2012).

2.4.1 Nutrient Requirements of Infants

A proper complementary food should consists the following features: high energy content, low viscosity (i.e. of an acceptable thickness or consistency), balanced protein (containing all essential amino acids) content, required vitamins and minerals (Iron, folic acid,

calcium) content, no (safe level) anti-nutritional components and pleasant taste (palatable) (Ugwu, 2009).

***Energy*:** For a newborn infant exclusively breast-fed, breast-milk provides the entire required calorie (95–145 kcal/kg (150 ml)) for the first 6 months. By 6 months, energy needs increases for a very good nurtured infant by 32 kcal, and a restless infant by 60 kcal (Dewey and Brown, 2003). It is recommended that energy density of complementary food should be 4 kcal per gram on dry weight basis (Huffman and Martin, 1994). Infants and young children have a limited gastric capacity and an energy requirement per unit body weight about three times as high as for the adults. If a diet has a very low energy density children may not be able to eat adequate amounts because of the bulkiness of the diet. The energy density is most important for children with wasting, as they have an increased energy need for catch-up growth (Daelmans and Saadeh, 2003). Low energy density complementary foods caused by high bulk gruels have long been implicated in protein-energy-malnutrition.

***Proteins*:** Proteins are major sources of essential amino-acids and source of energy during times of energy deprivation, although fat and carbohydrate are utilized preferentially by the body (Eschleman, 1984). Protein deficiency is always accompanied by inadequate energy intake and the two together leads to protein–energy malnutrition, one of the commonest forms of malnutrition worldwide. Severe protein–energy malnutrition results in the clinical syndromes of marasmus, kwashiorkor or marasmic-kwashiorkor. To maintain cellular integrity and function and to ensure health and growth, an adequate supply of dietary protein is vital. Cereals, legumes and/or oil seed flours, alone or preferably mixed, can constitute an appropriate source of proteins. Provided they are prepared in such a way that in the finished product the proteins in the mixture should fulfill the protein digestibility corrected amino acid score (PDCAAS) and should

not be less than 0.70 and the energy from protein content should not be less than 10% of the total energy from the product (Huffman and Martin, 1994).

***Fat*:** Dietary fat in complementary foods should provide approximately 30 to 45% of the total energy required (Bier *et al*., 1999; Frongillo, 2000); and this would guarantee adequate intake of essential fatty acids, good energy intake and uptake of fat soluble vitamins A, D, E and K (PAHO/WHO, 2003). Fat not only provides energy in the diet, it also has an important role for promoting good health in humans (Eschleman, 1984). Fat accounts for approximately 50% of the energy in breast-milk and is the main source of energy for infants less than 6 months old. With the introduction of complementary food, fat is gradually replaced by carbohydrate as the chief energy source, and together they meet the energy needs of the growing child.

***Carbohydrates*:** Starch is likely to be a major constituent of many complimentary foods for older infants and young children (Ugwu, 2009). To ensure that its energy value is realized, this starch should be provided in a readily digestible form (Ugwu, 2009). The early years of life are a period of very rapid growth. Appropriate nutrition is essential during this period: children who do not receive sufficient energy and nutrients will not sustain their expected growth and development (Stephen *et al*., 2012). Digestible dietary carbohydrates, primarily lactose, are one of the main sources of dietary energy in this crucial period (Stephen *et al*., 2012). Additionally, other key sources are starch and sugars children (Ugwu, 2009). In infants, the minimum carbohydrate intake should be close to that provided by human milk, that is, 40% of total energy, and lactose should be the main digestible carbohydrate (WHO, 2003). After the second semester and until 2 years of age, children should gradually increase the intake of digestible carbohydrates, for example, starch up to 55%, although lactose should remain the major carbohydrate. Children of later ages should have a range of digestible carbohydrates similar to that recommended for adults (WHO, 2003).

***Vitamins and minerals*:** Vitamins and mineral are micronutrients, which are needed in minute quantities for the normal functioning of the body. Micronutrients are normal chemical components of foods in their active forms or as precursors of the active forms, which are needed for metabolic reactions in the body (Daelmans and Saadeh, 2003). These micronutrients include vitamins A and B12, iron, folic acid, iodine, and zinc. Prolonged inadequate intake of foods rich in these micronutrients result in their deficiencies (Uchendu, 2011). Micronutrient deficiencies are becoming more widespread especially in those countries in the developing part of the globe (Uchendu, 2011). Micronutrients have become more prevalent in developing countries including Nigeria following economic stress and food insecurities faced by populations in these countries. The consequences of these are retardation in physical growth, cognitive development academic performance (REAP, 2010).

Many local foods, fruits, and plants have been reported to be good sources of micronutrients. They are available in abundance and very cheap. Consumption of varieties of local foodstuffs will help the children have adequate nutrient stores especially during their season when the fruits, vegetables and foodstuffs are usually wasted due to poor storage facilities (Uchendu, 2011).

Adequate intakes of micronutrients such as iron, zinc and calcium are important for ensuring optimal health, growth, and development of infants and young children (Huffman and Martin, 1994). In developing countries, dietary intakes of iron, zinc and calcium are low, especially among young children living in rural areas and probably contribute to high prevalence of iron deficiency anemia and mild zinc deficiency among this age group. Cereals and legumes are important food sources of iron, zinc, and calcium for young rural infants and children. Notwithstanding, phytic acid; a dietary factor found primarily in unrefined cereals, grains, legumes, and oil seeds are a potent inhibitor of iron, zinc, and calcium absorption. Hence, to

ensure absorption of these minerals from a meal, it is important to consider the molar ratios of phytate: mineral of each plant based food in it. The desirable phytate: mineral molar ratios, for mineral absorption, are less than one for phytate: iron and less than 0.17 for phytate: calcium (Krebs and Westcott, 2002).

***Dietary fiber*:** Dietary fiber is the functional food that can affect one or more targeted function in the body in positive manner. Higher fiber content of weaning food may inhibit mineral absorption and reduce the digestibility of protein in foods (Amuna *et al*., 2000). There is much evidence that dietary fiber (DF) may contribute to present future health benefits in young children. For example, dietary fiber has a major influence on the bacterial colonization of the gastrointestinal tract and its maturation, in promoting laxation, and in establishing healthy eating patterns while eating foods high in dietary fiber is recognized as important, controversy over recommendations for infant dietary fiber intake exists (Bultosa, 2007). The Institute of Medicine in the United States has recommended a specific value of 19 g/d for children between 1 and 3 years of age. This value, based on total dietary fiber intakes of 3.3 g/1000 kJ (14 g/1000 kcal), is based on a reduced risk of coronary heart disease in adults aged 19 years and older (National Research Council, 1996).

2.4.2 Infant breastfeeding practices

Exclusive breastfeeding (EBF) is defined as the process whereby infant received only breast milk from his/her mother or a wet nurse, or expressed breast milk and no other liquids, or solids, with the exception of drops or syrups consisting of vitamins, minerals supplements, or medicines (WHO, 1991). Exclusive breastfeeding is adequate in quality as well as quantity in terms of energy, protein, water etc. for an infant's need under six months of age (WHO, 2002). Breastfeeding is the natural and normal means of feeding and caring for infants (Adepeju *et al.*, 2014), and it is one of the defining characteristic of being a mammal. Breast milk contains all the

nutrients that babies need to stay healthy and grow (Ikujenlola and Adurotoye, 2014). Despite these advantages, it is evident that only 39% of all infants aged 0 to 5 months in the developing world are exclusively breastfed while over 60% of those aged 6 to 9 months continues to be breastfed while receiving complementary foods (UNICEF, 2009b).

Nutritional and medical investigations have shown that formula-fed infants face higher risks of infectious morbidity in the first year of life compared with breastfed infants (Hamosh *et al*., 2001; Li *et al*., 2007). These differences in health outcomes may be explained, in part, by specific and innate immune factors present in human milk. For instance, an infant who is breastfed has a reduced risk of developing diarrhea, protect infants against acute respiratory illnesses (ARI), enhances brain development, protects babies from illnesses that can cause malnutrition, hearing problems and learning difficulties (American College of Obstetrics and Gynecology, 2007).

2.4.3 Factors influencing infant breastfeeding practices

Demographic and socioeconomic factors affect infant feeding practices. Investigation has established a strong, positive correlation between maternal age and education level and breastfeeding initiation and duration (Ford and Labbok, 1990). Studies have shown that full-time employment and school enrollment are associated with decreased breastfeeding duration due to environmental barriers at both work and school (Spisak and Gross, 1991; Littman *et al*., 1994). Married women breastfeed their infants exclusively more often than single women (Arora *et al*., 2000). Arora *et al*. (2000) observed that the attitudes of married women concerning breastfeeding were more positive than that of single mothers. Evidence has shown that negative attitudes and lack of knowledge on the part of healthcare providers can also affect successful infant feeding practices (Black *et al*., 1990).

2.4.4 Infant complementary feeding practices

Infancy is a time of rapid physical growth as well as physiological, immunological and mental development (Ogbonnaya *et al*., 2012). Adequate nutrition during infancy and early childhood is fundamental to the development of each child's full human potential. Rapid growth of infants during the first year of life and specifically the first six postnatal months requires an adequate supply of nutrients to cope with the rapid development of body muscles and other tissues (Domellof *et al*., 2002). Deficiency of energy or any of the essential nutrients can have dire consequences on the growth and development of children. Epidemiological studies have consistently shown that infancy period is the peak age for growth faltering, deficiencies of certain micronutrients and common childhood illnesses such as diarrhea, which augment the problem of malnutrition in children (Neumann and Harrison, 1994; Ibrahim *et al*., 1998; Shrimpton *et al.*, 2001). After the age of two years, earlier stunting is often irreversible (Martorell *et al.*, 1994). Poor complementary feeding practices, coupled with high rates of infectious diseases, have been reported as the principal causes of malnutrition during the first two-year of postnatal period.

Complementary feeding is the process of introducing other foods and liquids into the child's diet when breast milk alone is no longer sufficient to meet nutritional requirements and therefore other foods, that is, complementary foods, in solid or liquid forms are needed, along with breast milk (WHO/UNICEF, 2000). The target age range for complementary feeding is generally from six to twenty-four months of age although breastfeeding may continue well beyond the second year (WHO/UNICEF, 2000).

Complementary feeding period is the time when malnutrition starts in many infants, contributing significantly to the high prevalence of malnutrition in children less than 5 years of age worldwide (Daelmans and Saadeh, 2003; Imdad *et al*., 2011; Bassey *et al*., 2013). Poor

feeding practices as well as lack of suitable complementary foods are responsible for under nutrition with poverty exacerbating the whole issue. Early introduction of complementary foods increases morbidity, mortality, growth and cognitive retardation in children (Sheth and Dwivedi, 2006; Dewey and Brown, 2003 You *et al*., 2010).

2.4.5. Complementary foods

Complementary foods are the first foods whether manufactured or locally prepared, suitable as a complement to breast milk or to infant formula, when becomes insufficient to satisfy the nutritional requirement of the infant (WHO/OMS, 2000). Although the causes of malnutrition are many and diverse, inadequate intake of foods and essential nutrients has been reported to be a major contributory factor (Kikafunda *et al*., 2003). Complementary foods are needed to fill the gap in energy, iron and other essential nutrients, between what is provided by exclusive breastfeeding and the total nutritional requirements of the infant. Complementary foods are needed in order to improve the nutritional status of the child at various stages in the life cycle and to combat malnutrition as the child transit from exclusive breastfeeding to family foods. Complementary foods can be categorized as home foods, fortified foods, or industrially manufactured foods. However, home foods, which are less expensive, readily available and culturally acceptable often, do not have the nutrient density needed by infant and young children to satisfy their daily requirement (Uavy, 2003).

The composition of local complementary foods varies from place to place and from country to country. In Nigeria, the common family diets and staple foods on which infants are weaned include dishes from cereals, starchy tubers, legumes and vegetables (Ifediora, *et al*., 2006). Some of the diets are single, while some are mixed diets. In Nigeria for instance, the main traditional complementary food is ‘Ogi’ -a fermented maize porridge (Enwere, 1998; Ikujenlola, 2008; Ibe, 2008). The availability, minimal cost, and ease of preparation make ‘pap’ the

preferred complementary food for the majority of Nigerian infants (Ogbonnaya *et al*., 2012). Other traditional complementary foods are based on local staple foods such as rice, millet, sorghum, groundnuts, soybeans and beans singly or in combination (Nkama *et al*, 1995). Traditional complementary foods have been reported to be low in essential nutrients (Ukegbu and Anyika, 2012) and grossly inadequate when compared to nutritional needs of infants (Ladeji, 2000; Fernandez *et al*, 2002; Kikafunda *et al*. 2006). When introduced too early or too frequently, they displace breast milk (Villapando, 2000; WHO, 2002) as the main sources of nutrition in infants. Traditional infant foods made of cereals or tubers are known for their high bulkiness and concentrations of fiber and inhibitors, which reduce their nutritional benefits (Michaelsen and Friis, 1998; Urga and Narasimha, 1998; Hurrell, 2003; Mbithi *et al*., 2002). Bulkiness, often associated with gelatinization of the starch during boiling is a major problem in cereal-based complementary foods (Bennet *et al*., 1999). To obtain a reasonable viscosity, it is therefore necessary to add large amounts of water during preparation. Hence, the energy density of such porridges is typically below the daily requirement (Michaelsen and Friis, 1998).

In Nigeria, a number of cereal–legume combinations have been formulated. It is well established that cereal-based complementary foods are generally low in protein and limiting in some essential amino acids, particularly lysine and tryptophan (Eshun *et al*., 2011; Minarro *et al.*, 2012). Therefore, supplementation of cereals with locally available legumes, which is rich in lysine, although, often limiting in sulphur amino acids, increases the protein content of cereal-legume blends and their protein quality through mutual complementation of their individual amino acids (Achi, 2005; Ibe 2008; Nwamarah and Amadi 2009; Wakil and Onilude, 2009).

Evidences have shown that single plant-based complementary foods are not beneficial to the growth and development of the children; and that two or more plant-based food combinations was found to be superior in nutrient content (Müller *et al*., 2003; Black *et al*., 2003; FAO 2004;

NPC/ICFM, 2009, Eka *et al*., 2010). Investigators emphasized the use of local foods formulated in the home and guided by the principles of (i) high nutritional value to supplement breastfeeding, (ii) acceptability, (iii) low price, and (iv) use of local food items (Dewey and Brown, 2003; Pelto *et al*., 2003).

2.4.6 Time of introduction of complementary foods and physiological development and maturation

Timely introduction of appropriate complementary foods promotes good nutritional status and growth in infants and young children (Michaelsen *et al*., 2000). Too early or too late introduction of complementary foods is inadequate feeding practice as it carries many risks which contributes to persistent child malnutrition **(**Drewett *et al.*, 1993 and Dewey and Brown, 2003; Muhimbula and Issa-Zacharia, 2010). Unfortunately, both too early introduction of complementary foods (< 6 month) and delayed introduction have been reported in many developing countries (Onyango, 2003; Hussein, 2005). This is because too early introduction of complementary foods is reported to stress the immature gut, kidneys and immune system, increased allergies and morbidity due to diarrhea (Mila, 1986; Kelly *et al*., 1989; Fergusson *et al*., 1990; Wilson *et al*., 1998). In addition, early introduction of complementary foods pre-disposes infants to reduced protective benefits of the breast milk, risks of microbial contamination, because of poor hygiene practices and growth retardation (Trowbridge, 2002). In addition, the micronutrients in complementary foods are not absorbed as well as those in breast milk (Trowbridge, 2002) thus increasing the chances of slow growth pattern among infants. Delayed introduction of complementary food predisposes the child to increased risks of growth faltering, decreased immune protection, malnutrition (PAHO/WHO 2003) and feeding problems (Northston *et al*., 2001; Food and Nutrition Service, 2008). Delayed introduction of complementary foods to infants is also associated with local acceptance of the complementary

foods, because the infants become accustomed to only sucking from the breast or feeding bottle over a longer time. The ability of infant to consume “solid” food requires certain factors:

Neuromuscular coordination: Maturation of the neuromuscular system influences the timing of the introduction of “solid” foods and the ability of infants to consume them. Many of the feeding reflexes exhibited during the different stages of development either facilitate or interfere with the introduction of different types of food. Before 4 months, infants do not have the neuromuscular coordination to form a food bolus, transfer it to the oropharynx and swallow it (Sheppard and Mysak, 1984). Head control and back support are immature and make it difficult for infants to maintain a position for successful ingestion and swallowing of semisolid foods (Sheppard and Mysak, 1984; Stevenson and Allaire, 1991). Infants start to bring objects to their mouth at about 5 months of age, and development of the “munching reflex” at this time permits consumption of some solid foods, regardless of whether or not teeth have appeared. By 8 months, most infants have sufficient tongue flexibility to swallow thicker boluses of food (Sheppard and Mysak, 1984).

Digestion and absorption: The secretion of gastric, intestinal and pancreatic digestive enzymes is not developed to adult levels in young infants . For instance, during early infancy the secretion of bile salts is only marginally adequate to permit micelle formation, and the efficiency of fat absorption is lower than in childhood and adulthood. By about 4 months, gastric acid is more effective and gastric pepsin is produced to digest protein fully. The pancreatic amylase is more effective to digest starches only at the end of the first year (De Vizia *et al*., 1975).

Renal function: Renal solute load refers to the sum of solutes that must be excreted by the kidneys. It mainly comprises nonmetabolizable dietary components, primarily the electrolytes sodium, chloride, potassium and phosphorus, which have been ingested in excess of body needs, and metabolic end-products, of which the nitrogenous compounds resulting from the digestion

and metabolism of protein are the most important (Fomon, 1993). The newborn baby has limited renal capacity to deal with a high solute load and at the same time conserve fluids. By around 4 months, renal function has matured considerably and infants can conserve water better and deal with higher solute concentrations. Thus, recommendations on complementary feeding do not ordinarily need to be modified to take account of the stage of renal development.

Defense system: The development and maintenance of an effective mucosal barrier in the intestine is an essential defence mechanism. In the neonate, the mucosal barrier is immature, making it vulnerable to injury by enteropathic microorganisms and sensitive to some antigenic food proteins (Fomon, 1993). Human milk contains a wide range of factors, absent from commercial infant formulas that stimulate the development of active defense mechanisms and help to prepare the gastrointestinal tract for the ingestion of transitional foods. The relatively poor defenses of the young infant's digestive tract, together with reduced gastric acidity, contribute to the risk of injury to the mucosa by foreign food and microbiological proteins, which can cause direct toxic or immunologically mediated damage. Some foods contain proteins that are potentially antigenic, such as soya protein, gluten (present in some cereals), cow's milk, egg and fish proteins, which have been associated with an enteropathy. It is therefore prudent to avoid introducing these foods before 6 months of age, particularly where there is a family history of food allergy.

2.4.7 Principle of complementary feeding of infants

According to the World Health Organization, complementary food should be timely, adequate in protein content, high energy value per unit of food volume, soft texture, low fiber content, adequate vitamins and minerals, absence of anti-nutritional factors, and given in sufficient quantity (WHO, 2002). Several strategies have been employed to improve complementary food practices (Dewey and Adu-Afarwuah, 2008). These include nutritional

counseling to mothers designed to promote healthy feeding practices; provision of complementary foods offering extra energy (with or without micronutrient fortification); and increasing energy density of complementary foods through simple technology (Caulfield *et al*., 1999; Dewey and Adu-Afarwuah, 2008).

Infants need to be exposed to a new food eight to ten times until they accept it well (Sullivan and Birch, 1994). The small amount of complementary foods initially offered should be gradually increased with age. The amount and frequency of foods should be based on infants acceptance, which varies according to individual needs, the amount of breastmilk ingested and the content of complementary foods (Dewey and Brown, 2003; PAHO/WHO, 2003). The infant should be encouraged to eat until he/she feels satiated (PAHO/WHO, 2003). WHO recommends two to three meals a day with complementary foods for breastfed infants between 6 and 8 months of life and three to four meals a day for those between 9 and 24 months, with additional nutritious snacks once or twice a day at 12 months. If energy content or the amount of complementary foods per meal is small, or if the infant has been completely weaned or children are not growing well, a higher frequency of meals may be necessary (PAHO/WHO, 2003; Dewey and Brown, 2003).

The main factors influencing the extent to which an infant can meet his or her energy and nutrient requirements are the consistency and energy density (energy per unit volume) of the complementary food and the frequency of feeding (Onyango, 2003; Muhimbula *et al.*, 2011). Starch often provides the principal source of energy, but when heated with water starch granules gelatinize to produce a bulky, thick (viscous) Porridge (Grando and Gormez, 2005). These physical properties make the porridge difficult for infants to ingest and digest. Furthermore, the low energy and nutrient density means that large volumes of food have to be consumed to meet the infant's requirements. This is not usually possible, owing to the infant's limited gastric

capacity and to the limited number of meals offered per day. Dilution of thick porridges to make them easier to swallow will further reduce their energy density. Traditional complementary foods tend to be of low energy density, protein and high fiber content, and although their liquid consistency makes them easy to consume, the volumes needed to meet infant energy and nutrient requirements often exceed the maximum volume the infant can ingest (Grando and Gormez, 2005; Temesgen, 2013).

Complementary foods should be rich in energy, protein and micronutrients, and have a consistency that allows easy consumption. In some parts of the developing world, this problem has been addressed through the addition of amylase-rich flour to thick porridges, which reduces their viscosity without reducing their energy and nutrient contents (Weaver, 1994). Amylase rich flour is produced by the germination of cereal grain, which activates amylase enzymes that then break down starch into sugars (maltose, maltodextrins and glucose). As starch is broken down, it loses its ability to absorb water and swell, and therefore porridge made with germinated flour rich in amylase has a high energy density while retaining a semi-liquid consistency, but increased osmolarity. These flours are time-consuming and tedious to prepare, but can be made in large quantities and added in small amounts to liquefy porridges as required (Walker and Pavitt, 2007).

Evidence has shown that contaminated complementary foods are the major route of transmission of diarrhea among infants (Black *et al*., 1989). Epidemiologically study established that higher incidence of diarrhea among children coincides with the intake of complementary foods (Martinez *et al.*, 1992). Proper maternal hygiene practices regarding the management, preparation, administration and storage of complementary foods may reduce microorganism contamination of complementary foods (Feachem *et al.*, 1983). The adoption of proper hygiene practices for complementary foods can be affected by lack of clean water, soap and utensils, but

may be considerably enhanced by health education intervention programmes (Monte *et al*., 1997; PAHO/WHO, 2003).

2.4.8 Production of Complementary Foods in Nigeria

The quality and quantity of nutrients present in the food consumed by people in Nigeria and other developing countries is very low compared to the actual daily requirements for a normal growth and health maintenance (FAO, 2004). Poor resource family in Nigeria and other developing countries cannot afford animal products, which are rich sources of protein because they are either too expensive or simply unavailable (Olujobi, 2012). This situation has made many people to depend mainly on carbohydrate diets, comprising cereal grains or starchy roots and tuber crops with low protein, thus leading to high level of malnutrition, particularly among children (Olujobi, 2012). Maize products are the cheapest and readily available fermented foods for infants and young adults in most tropical countries (Torre *et al,* 1991), and maize products are important energy food rich in carbohydrates and traces of vitamins. The traditional preparation of maize Ogi involves soaking of maize in water for 1 to 3 days followed by wet milling and sieving to remove bran, hulls and germs (Otitoju, 2009; Akinleye *et al*., 2014). The pomace is retained on the sieve and later discarded as animal feed while the filtrate is fermented (for 2-3 days) to yield Ogi, which is sour, white starchy sediment (Akinleye *et al*., 2014).

Ogi generally have been implicated for kwashiorkor among infant (Akanbi *et al*., 2003) and this has lead to many researcher attempt to fortify it to improve its nutritional value with plant protein sources (Akanbi *et al*., 2003). In addressing this nutritional problem in Nigeria, quite a number of researchers have made use of locally available foods to formulate infant foods. For instance, Adewuyi *et al*. (2014) incorporated red palm oil into ogi to improve its vitamin A status; Yusufu *et al*. (2013) formulated complementary food from sorghum, African yam bean (*Sphenostylis stenocarpa*) and mango mesocarp flour. Similarly, Osundahunsi and Aworh (2003)

incorporated mellon seed flour into ogi, Akingbala *et al*. (2005) supplemented ogi with okro seed flour, Sanni *et al*. (2001), Egounlety *et al.* (2002) and Nnam (2000) improve nutritional quality of ogi with the supplementation of cowpea and soybean respectively

2.4.9 Nutritional Status of Traditional Complementary Foods

Appropriate nutrition includes feeding children with a variety of foods to ensure that nutrient requirements are met. Complementary feeding, that is, acts of introducing foods other than breast milk to infant, usually begins at 6 months (WHO/OMS, 2000; Oyarekua, 2011**)**. Investigations have shown that the age at which complementary foods are introduced to infant is the stage that the nutritional requirements of many infants are not met, thus leading to the onset of protein-energy malnutrition among under 5 years of age children worldwide (Daelmans and Saadeh, 2003; Anigo *et al*., 2009). Anigo *et al*. (2009) reported high prevalence of malnutrition among children in the areas where introduction of plant-based complementary foods at earlier age than the 6^{th} month recommended by World Health Organisation. Studies have shown that a single plant-based complementary food is insufficient to meet the needs of micronutrients and protein; and those different types of economical protein rich plant mixtures can be used to formulate qualitative infant diets (Ibe 2008; Nwamarah and Amadi, 2009).

The traditional complementary foods in Nigerian and other part of developing countries are cereal based (Nnam, 2002; Ikujenlola, 2008, Oyarekua, 2011); and are characterized by low protein (e.g., lysine and tryptophan) (Oyarekua, 2011), low energy density and bulky (meaning, food of high viscosity but low energy density) (Kikafunda *et al*., 2006; Ukegbu and Anyika, 2012). The factors limiting energy intake of an infant weaned on such low energy foods are the volume the child can consume at a time and the frequency of feeding (Walker, 1990). The traditional complementary food, such as ogi (corn gruel) and other family diets (yam, gari, amala etc.) constituted the major complementary foods in Nigeria and other developing countries

(Mosha *et al*., 2000). These complementary foods often fails to meet the nutritional needs of the infant due to poor nutritive values (Fernandez *et al*, 2002); hence, they have been implicated in the aetiology of protein–energy malnutrition in the community where they are solely used as the complementary food (Kikafunda *et al*., 2006).

In Nigeria, complementary foods are usually consist mainly of un-supplemented cereal porridges made from maize, sorghum or millet (Ikujenlola, 2008) and family diets, like fruits and roots/tubers such as cocoyam, yam or cassava (Mosha *et al*. 2000; Eka *et al*., 2010). Nutritional studies have reported that young children fed on these traditional complementary diets are not receiving essential nutrients; hence, leading to high prevalence of malnutrition-related deaths that occurs among children less than five years of age (FMOH, 2005; Power *et al*., 2005; Galobardes *et al*., 2006; Lawlor *et al*. 2006). The immediate causes of malnutrition among under five years old children are inappropriate breastfeeding, complementary feeding practices and low nutritional quality of complementary foods contamination (UNICEF, 2001; Anigo *et al*., 2007, 2009).

2.5 Childhood Malnutrition and its Consequences

Malnutrition in the form of undernutrition is the most significant risk factor for the burden of disease in developing countries (Nemer *et al*., 2001), causing approximately 300,000 deaths per year accountable for more than half of the deaths occurring in children in the developing countries (Müller *et al*., 2003; Black *et al*., 2003). Malnutrition causes a great deal of human suffering physically and emotionally (Ijarotimi, 2013) It is estimated that malnutrition alone accounts for more than half of children's deaths annually (WHO, 2011). In developing countries, approximately 183 million children are underweight-for-age, 67 million are underweight-for-height (wasted), and 226 million are low height-for-age (stunted) (WHO, 2011). Evidence has shown that malnutrition is associated with more than half of all children's deaths

worldwide (Pelletier *et al*., 2003) and that children who suffered malnutrition are less physically and intellectually productive at adulthood (REAP Health, Nutrition, Care and Educational Performance, 2010). It has been documented that malnutrition in young children causes disturbances in the morphological and functional development of the central nervous system, thus affecting the cognitive and emotional development of the child (Northstone *et al*., 2010). Both protein-energy and micronutrient malnutrition have profound negative consequences for children' health and survival.

2.5.1 Prevalence of Child Malnutrition in Nigeria

Childhood malnutrition is a major public health problem in Nigeria (FMOH, 2007); and is one of the principal underlying causes of death for many children in in many parts of developing countries worldwide (Muller *et al*., 2005; Faruque *et al*., 2008). Prevalence of child malnutrition in Nigeria is increasing due to early or late introduction of complementary foods, low nutritional quality of complementary foods and high levels of microbial contami877nation (UNICEF, 2001; Anigo *et al*., 2007, 2009; Nwosu *et al*., 2014). Recently, Nigeria ranks 14th in global assessment of under-five mortality rate and approximately one million children die annually in Nigeria before the 5th year of age (Bryce *et al*., 2005). Nigeria is among the 20 countries in the world that account for 80% of undernourished children. The causes of this public health problem in Nigeria are complex and multidisciplinary. However, poor quality and quantity of foods given to children play a major role (UNICEF, 2007). Poor feeding practices and high cost of fortified nutritious proprietary complementary foods are the most important direct factors responsible for high prevalence of malnutrition and illness amongst children in Nigeria (Nnam, 2002; Solomon, 2005).

Epidemiological studies have shown that poverty, inaccessibility to animal-based foods, lack of nutrition education, poor child breastfeeding and complementary feeding practices and

cultural beliefs and practices, are the major factors responsible for protein-energy malnutrition among infants and young children in the poor socio-economic groups of developing countries (Bwibo and Neumann, 2003; Hamidu *et al*, 2003). Hence, the scientists have worked extensively using locally available food materials to formulate complementary foods in order to solve problem of protein-energy malnutrion in Nigeria (Ijarotimi and Olopade, 2009; Eka *et al*., 2010; Emmanuel- Ikpeme *et al*., 2012; Makinde and Ladipo, 2012; Ogbonnaya *et al*., 2012; Ukegbu and Anyika 2012; Okorie *et al*., 2013; Oyarekua, 2013; Madukwe *et al*., 2013). Despite all these efforts, prevalence of malnutrition among weaning-aged children in Nigeria is still persist, due to poverty and poor complementation of local food materials in the production of qualitative complementary foods by the rural nursing mothers; hence they solely depend on available low-cost food mixtures to wean their infants (Eka *et al.*, 2010).

2.6 Cereals and its Application in Food Production

The term cereal is a derivative from Latin word 'cerealis' meaning ‘grain’, which is botanically, a type of fruit called a caryopsis, composed of the endosperm, germ, and bran. The cereals are annual common grass members of the grass family (a monocot family Poaceae, also known as Gramineae), which usually have long, thin stalks, such as wheat, rice, maize, sorghum, millet, barley and rye, whose starchy grains are used as food (Muhammad *et al*., 2013). Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crops; they are therefore staple food crops (Muhammad *et al.*, 2013). All cereal grains have high-energy values, mainly from the starch fraction, but, also from the fat and protein portions. Apart from moisture content and inediblesubstances such as cellulose, cereal grains containcarbohydrates- mainly starches (comprising 65 to 75% of their total weight), as well as proteins (6 to 12%) and fat (1 to 5%) along with traces of minerals and vitamins (Muhammad *et al*., 2013).

2.6.1 Nutritional contents of whole grain cereals

The grains consist of three major parts, which are bran (the outer layer of the grain, which contains fiber omega-3 fatty acids, vitamins and dietary minerals; endosperm (the main part of the grain which is mainly starch), and germ (the smallest part of the grain which contains the (vitamin E, folate, thiamine, phosphorus, magnesium). The wholegrain cereals are a rich source of many essential vitamins, minerals and phyto-chemicals, which have health benefits (Sarwar, 2008, 2009; Sarwar et al., 2004). Cereals form a major portion of human diet and are an important source of starch and other dietary carbohydrates (dietary fibre), which play an important role in the energy requirement and nutrient intake of human (Verma and Patel, 2013). Cereals provide the bulk (45%) of food calories of humanity (Oyarekua, 2011). Generally cereal grains contain large proportion of carbohydrates (70 - 80%), as starch, protein (15%) and lipids in small proportions (<5%) (Abdoulaye *et al*., 2011). However, the nutritional quality of cereals and the sensorial properties of their products are sometimes inferior or poor in comparison with animal-based food products. The reasons behind this are the lower protein content, the deficiency of certain essential amino acid**s** (lysine), the low starch availability, the presence of anti-nutrients **(**phytic acid**,** tannins and polyphenols) and the coarse nature of the grains. Cereals are a major source of micronutrients, the bioavailability of minerals such as iron, zinc, calcium, magnesium, is low because they are present as insoluble complex with food components such as phytic acid (Abdoulaye *et al*., 2011).

Cereal is an excellent source of B-complex vitamins, including folate, a good source of many minerals such as iron, magnesium, copper, phosphorus; zinc, and a good source of antioxidants and phytochemicals that can help to lower blood cholesterol levels (Sarwar, 2008, 2009; Sarwar et al., 2004). These phytochemicals, that is, lignans, which can lower the risk of coronary heart disease, and regress or slow cancers in animals; phytic acid, which reduces the

glycaemic index of food, and it is important for people with diabetes, and saponins, which lower blood cholesterol, and phenolic compounds, which have antioxidant effects (Sarwar, 2008, 2009; Sarwar *et al*., 2004).

Cereal grains have been the principal component of human diet and have played a major role in shaping human civilization for thousands of years. Around the world; rice, wheat, and maize, and to a lesser extent, sorghum and millet are important staples and critical to daily survival of billions of people. More than 50% of world's daily caloric intake is derived directly from cereal grains consumption. Most of the grains used for human food are milled to remove the bran (pericarp) and germ, primarily to meet sensory expectations of consumers (Awika, 2011). The milling process strips the grains of important nutrients including dietary fiber, phenolics, vitamins and minerals, which are beneficial to health (Awika, 2011).

2.6.2 Application of cereal in complementary foods production

The complementary effect of amino acids from one protein on the nutritive value of another is an economical and practical way to improve the protein quality as well as the quantity of the combined complementary protein. Among vegetable crops, legumes contained the highest amount of proteins, which can be used to supplement the nutrient content of cereals (Ene-Obong, 2008). Some essential amino acids have been indentified to be deficient in different plant food products, for example, lysine is deficient in cereals and legume is deficient in sulfur-containing amino acids, but rich in lysine, while cereal grains have an adequate amount of sulfur containing amino acids, but relatively low in lysine (Atanasova, 2008). It is therefore logical that the proteins of legumes should complement the protein of cereal grain (Enwere, 1998; Fernandez *et al*., 2002; Solomon, 2005; Ene-Obong, 2008). Based on this, several studies have shown several applications of legume proteins, which have a potential role as substrates for the development of complementary foods due to the high nutritional value (Ijarotimi and Famurewa, 2006; Anigo *et*

al., 2010; Muhimbula *et al*., 2011; Ijarotimi and Keshinro, 2012 (a&b); Oyarekua, 2013; Madukwe *et al*., 2013). Legumes and cereals have complementary nutritional effects and their consumption together fulfils the need of balanced protein. To improve the nutritional quality and organoleptic acceptability of leguminous seeds, processing techniques have been employed to reduce or destroy the anti nutrients present in them (Esenwah and Ikenebomeh, 2008).

In Nigeria and other developing countries, several researchers have formulated complementary foods from the combinations of cereals and legumes, and their findings showed that single plant-based food products are deficient in essential nutrients compared with when two or more plant-based food materials are used (Oyarekua, 2010; Anigo *et al*. 2010; Makinde and Ladipo, 2012; Ijarotimi and Keshinro, 2012; Emmanuel-Ikpeme *et al*., 2012; Bassey *et al*., 2013; Malomo *et al*., 2013; Nabuuma *et al*., 2013 ; Amegovu *et al*., 2013; Malunga *et al*., 2014; Sule, 2014; Ibironke,, 2014; Ikujenlola and Adurotoye, 2014; Opara *et al*., 2014; Amegovu *et al*., 2014).

2.6.3 Popcorn (a type of maize)

Maize, a cereal, represents the staple food for most part of the population of Africa, Nigeria inclusive. However, maize is relatively poor cereal when it comes to the quality of protein, because it has limiting amounts of two essential amino acids, lysine and tryptophan (Azevedo *et al*., 1997). In Nigeria, maize (*Zea mays* L.) is used both for human consumption and livestock feed. The maize is eaten either at the green stage, as boiled or roasted ears, or dried and prepared into a jelly-like “pap” or “eko” (Alika *et al*., 1988).

Popcorn a type of maize originated from a wild grass. Popcorn scientific name is *Zea mays* everta, and it is the only type of corn that pop. Popcorn (*Zea mays* everta), which is grown solely for human consumption and is now becoming popular in Nigeria (Iken and Amusa, 2010). The production and utilization of popcorn enhances the availability of the field corn for livestock

and for other industrial uses. Popcorn has approximately the same nutrient composition and feeding value as other maize corn (Iken and Amusa, 2010). The use of popcorn as snacks at amusement parks, motion- picture theatres, or around family televisions and while traveling, has greatly increased the demand for popcorn, thus making it a profitable outlet for commercial production (Iken and Amusa, 2010). Recently, Ijarotimi *et al*. (2012) reported on the utilization of popcorn in complementary food formulation. Popcorn provides a full complement of nutritional benefits, including dietary fibre, protein and B-vitamin (Iken, 1991).

The nutritional composition of popcorn showed that the kernel contained crude fat, crude protein, reducing sugars and starch, in which 27.0–28.5% of the starch is amylase (Donkeun *et al*., 2000). The major fatty acids in the popcorn hybrids were linoleic and oleic acids (Ijarotimi and Keshinro, 2011). Energy value (380 kcal/100g) and other essential nutrients of the kernel are higher when compared with other cereals like sweet corn and sorghum (Donkeun *et al*., 2000).

Table 2.2: Nutrient composition of popcorn kernel

Nutrient	Units	Value per 100g
Proximate composition		
Moisture	g	3.32
Energy	kcal	387
Protein	g	12.94
Fat	g	4.54
Carbohydrate	g	77.90
Fiber	g	14.5
Minerals		
Calcium	mg	7
Iron, Fe	mg	3.19
Magnesium, Mg	mg	144
Phosphorus, P	mg	358
Potassium, K	mg	329
Sodium, Na	mg	8
Zinc, Zn	mg	3.08
Vitamins		
Vitamin C	mg	0.0
Thiamin	mg	0.104
Riboflavin	mg	0.083
Niacin	mg	2.308
Vitamin B-6	mg	0.157
Folate	mcg	31
Vitamin B-12	µg	0.00
Vitamin A	Mcg	10
Vitamin A, IU	IU	196
Vitamin E (alpha-tocopherol)	mg	0.29
Vitamin D (D2 + D3)	µg	0.0
Vitamin D	IU	0
Vitamin K (phylloquinone)	µg	1.2

* Source: USDA Nutrient Data Laboratory (2014)

Plate 1: Picture of popcorn kernel

2.7 Legume and its Application in Food Production

From time immemorial, Legumes continue to be the world most important sources of staple food (John, 2005). Legumes belong to the family *Leguminosae*. Legumes can be classified into two classes: oilseeds, such as soybeans and peanuts, which are grown for both their protein and oil content; and grain legumes, including common beans, lentils, lima beans, cowpeas, fava beans, chickpeas (garbanzos) and common peas, which are grown primarily as a protein source. In the tropics, legumes are the next important food crop after cereals (Uzoechina, 2009). Legumes are sources of low-cost dietary vegetable proteins and minerals when compared with animal products such as meat, fish and egg (Apata and Ologhobo, 1997; Ghadge *et al.*, 2008). Indigenous legumes are an important source of affordable alternative protein to poor resource people in many developing countries where consumption of animal protein is limited because of economic, social, cultural or religious factors (Phillips and McWatters, 1991; Ahmed *et al*., 2006; Doss *et al*., 2011), especially in Nigeria where legumes are predominantly consumed. Legume seeds have an average of twice as much protein as cereals and the nutritive value of the proteins are usually high (Vijayakumari *et al*., 1997).

2.7.1 Nutritional contents and health benefits of legumes

Legumes are rich in protein and their chemical composition varies depending on variety, species and region (Liu, 1997). Legumes are unique foods because of their rich nutrient content, including starch, vegetable protein, dietary fibre, oligosaccharides, phytochemicals (especially the isoflavones in soy) and minerals. The carbohydrate and dietary fibre contents of legumes contribute to their low glycaemic indices, which benefit diabetic individuals (Jenkins *et al*., 1991) and reduce the risks of developing diabetes mellitus (Sameron et al., 1997).

Legumes contain anti-nutritional factors such as lectins, saponin, haemagglutin, protease inhibitor, oxalate, goitrogen, phytates, trypsin inhibitor and tannin (Apata and Ologhobo, 1997).

These compounds reduce protein digestibility and availability. Some anti-nutritional factors in legumes have been reported to have health benefits (Amarowicz and Pegg, 2008). Tannin, a polyphenolic compound is reported to possess antioxidative activity (Amarowicz and Pegg, 2008). Raw legumes have higher content of anti-nutritional factors but can be eliminated or reduced by processing. Legumes are also good sources of carbohydrates, minerals, dietary fibres and water soluble vitamins which are important in human health. Dietary fibre consists of indigestible polymers, which are made up of cellulose, hemicellulose, pectin and lignin. They provide bulk in natural food and are resistant to hydrolysis by enzymes in the alimentary tract (Fennema, 1996). Dietary fibre is important in aiding absorption of water from the digestive track. It also has health benefits such as lowering of blood pressure and serum cholesterol, protection against cardiovascular diseases, diabetes, obesity and colon cancer (Ubom, 2007). Legumes have complex sugars such as raffinose and starchyose which are responsible for flatulence. Legumes are important both in human and animal nutrition especially in tropical Africa where they are more consumed (Fasoyiro *et al*., 2012). Legumes are processed into various semi- finished and finished products (Ojimelukwe, 2009).

The protein content of legumes is twice or triples that of cereals depending on the type of the legume. The protein of legumes though adequate in essential amino acid lysine is however deficient in sulphur containing amino acids methionine and cystine (Bressani and Elias, 1974). Legumes, however, form good supplements for cereals, which are lacking in essential amino acid lysine. Improved nutritional quality can therefore be achieved by combining legumes with cereals. The nutrient compositions of selected legumes are presented in Table 2.3.

Table 2.3: Nutrient composition of selected legumes (g/100g)

Legumes	Protein	Fat	Carbohydrate	Fibre	Ash
Soybean	37-41	18-21	30-40	4-6	4-5
Cowpea	22-26	1-2	60-65	4-5	3-4
Groundnut	20-33	42-48	22-25	3-4	2-3
Hyacinth bean	24-28	1-2	65-70	7-9	4-5
Common bean	20-27	1-3	60-65	4-5	4-5
Pigeon pea	15-29	1-2	60-66	5-10	3-4
Lima bean	19-25	1-2	70-75	4-6	3-5
Winged bean	30-40	15-20	35-45	6-7	3-5
Bambara groundnut	6-18	6-8	50-57	3-6	3-4

Source: Borget, 1992.

2.7.2 African locust bean (Parkia biglobosa)

The African locust bean *(Parkia biglobosa*) is a spreading tree of medium size, with compound leaves and numerous leaflets. The tree is a perennial deciduous *leguminous* tree with pods ranging from pink brown to dark brown (Leaky, 1999). The pods are reported to contain up to 30 seeds embedded in a yellow pericarp. The seed is widely distributed in the natural grassland of the northern states and in derived savannah zones of the Western States of Nigeria. Among the leguminous plants used by man in Nigeria and other African countries, it is the Africaln locust bean (*P. biglobosa*). The seeds are well known for their used in the production of local food and medicinal agent (Hassan and Umar, 2005). The most popular form of consumption of African locust beans is in its traditional fermented tasty food condiment called dawadawa (Hausa) or Iru (Yoruba), which is used as a flavour intensifier for soups and stews and adds protein to a protein-poor diet (Lockeett *et al*., 2000; Achi, 2005). African locust bean is a leguminous plant with an outstanding protein quality and its protein and amino acid compositions have been reported by several researchers (Lockeett *et al*., 2000; Hassan and Umar, 2005; Elemo *et al.*, 2011; Ijarotimi and Keshinro, 2012).

To improve the nutritional quality and organoleptic acceptability of leguminous seeds, processing techniques, such as, soaking, boiling, alkaline or acidic solutions, sprouting, autoclaving, roasting, dehulling, microwave treatment, steam blanching and fermentation have been reported by several investigators to reduce or destroy the anti nutrients present in the seeds (Esenwah and Ikenebomeh, 2008).

Table 2.4: Nutrient composition of African locust bean

Nutrient	Value (%)
Proximate	
Moisture content	6.20
Crude protein	31.07
Crude fat	23.22
Crude fiber	13.22
Total ash	7.20
Vitamin Composition (µg/100g)	
Vitamin B1	0.10
Vitamin B2	1.02
Vitamin C	5.00
Vitamin D	11.16
Vitamin E	18.13
Beta carotene	11.37
Minerals composition (mg/kg)	
Calcium	2398.33
Magnessium	45.90
Iron	29.85
Manganese	35.30
Potassium	110.15
Sodium	2.90
Zinc	0.38
Phosphorous	17.0

Source: Olujobi, 2012.

Plate 2: Picture of African locust beans

2.7.3 **Bambara Groundnuts** (*Vigna subterranea L. Verdc*)

Bambara groundnuts (*Vigna subterranea* L. Verdc), and belongs to the plantea of the family of fabaceae and sub family of Faboidea. The common names of Bambara groundnut are Nyimo Beans in Zimbabwe and Jugo Beans in South Africa. The beans are underutilized legume widely cultivated in sub-Saharan Africa. Bambara groundnuts originated from Bambara, near Timbuktu in Central Mali, West Africa, hence its name Bambara groundnut (Kay, 1979). The seed is widely distributed and grown in Asia, parts of Northern Australia, and South and Central America (Baudoin and Mergeai, 2001).

Bambara groundnut is economically important because it is an inexpensive source of high quality protein. It is highly valued among the Eastern and Northern states of Nigeria (Oloyede *et al*., 2010). Bambara nut is a novel legume of African origin grown mainly by subsistence female farmers intercropped with major commodities such as maize, millet, sorghum, cassava, yam, peanut and cowpea (Omoikhoje, 2008). Bambara groundnut is the third most important legume after groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) in Africa and usually eaten in various forms as source of protein (Abdulsalami and Sheriff, 2010). It is one of the most adaptable of all plants and tolerates harsh conditions better than most crops. It yields on poor soils in areas of low rainfall and does not yield well in times of heavy rainfall. The cultivars had distinct colour differences ranging from cream through brown, maroon to black, with variations in the seed sizes and seed coat thicknesses (Nti, 2009).

The seeds can be consumed at different stages of maturities (Omoikhoje, 2008). The young fresh seeds are consumed as snack or produce as moimoi or okpa (bean porridge) in some parts of Nigeria. It is evident that the seed can be used in bread making (Brough *et al*., 1993), milk production (Poulter and Caygill, 2006) and supplemented with fermented maize (Ogi) (Mbata *et al*., 2009). The nut is an important source of food security, because of the vital nutrient

like essential fatty acids, amino acids (lysine and methionine) and micronutrients (Ijarotimi and Esho, 2009; Abdulsalami and Sheriff, 2010). Despite the nutrient composition, bambara nut are still among the lesser known and under-utilized crop in Nigeria.

The beans can be eaten fresh after harvest or dried and stored for future use. Bambara groundnut is regarded as a balanced food, because the nut contains sufficient quantities of amino acids (lysine and methionine) (Onimawo *et al*., 1998; Adu-Dapaah and Sangwan, 2004; Mune-Mune *et al*., 2011), carbohydrates and essential fatty acids (linoleic, palmitic and linolenic acids) (Minka and Bruneteau, 2000), Calories (414 kcal/100g). The seed also contain appreciable amount of minerals like iron (Brough and Azam-Ali, 1992; Abdulsalami and Sheriff, 2010; Mahala and Mohammed 2010). The unique properties and composition of Bambara groundnut make it serve as a balanced food, which contains almost all the vatal nutrients that promotes good health for people living in Africa (National Reserch Council, 2006).

Table 2.5: Nutrient composition of Bambara groundnut

Nutrient	Value
Proximate compostion	
Moisture content	9.82
Ash	3.10
Crude protein	22.46
Crude fat	5.80
Crude fibre	4.50
Carbohydrate	55.00
Minerals composition	
Potassium	1702
Sodium	135.30
Magnesium	347.15
Phosphotous	738.04
Iron	18.51
Calcium	256.56
Manganese	10.46

Source: Oyeleke et al., 2012

Plate 3: Picture of Bambara groundnut seeds

2.8 Traditional Methods of Foods Processing

Processing is the set of methods and techniques used to transform raw ingredients into food or to transform food into other forms for consumption by humans or animals either in the home or by the food processing industry. Food processing dates back to the prehistoric ages when crude processing incorporated slaughtering, fermenting, sun drying, preserving with salt, and various types of cooking (such as roasting, smoking, steaming, and oven baking). Processing methods, such as soaking, sprouting, fermentation and cooking have been reported to improve the nutritional and functional properties of plant seeds (Jirapa *et al*., 2001; Yagoub and Abdalla, 2007). The effect of food processing methods on nutrient content depend on the sensitivity of the nutrient to the various conditions prevailing during the process, such as heat, oxygen, pH and light. The nutrient retention may vary with a combination of conditions, such as the characteristics of the food being processed, and the concentration of the nutrient in the food.

Foods are processed for a variety of reasons: to render them edible if they are not; to permit storage; to alter texture and flavor; and to destroy microorganisms, undesirable enzymes, and other toxins (Friedman, 1991; Friedman, 2004; Friedman, 2008). Although processing of foods can improve nutrition, food microbiology, quality and safety, these processing alternatives can occasionally lead to the formation of toxic compounds. These consequences of food processing result from molecular interactions among nutrients and with other food ingredients, both natural and added (Friedman and Levin, 2008). An improved understanding of the molecular changes during food processing and the resulting nutritional and safety consequences is needed to optimize beneficial effects such as food quality, bioavailability, and food safety and to minimize the formation and facilitate the inactivation of deleterious compounds.

2.8.1 Dehulling and its nutritional benefits: Dehulling removes the fibrous outer layers of grains. The dehulling reduces the levels of anti-nutrient in cereals and legumes (Rehman and Salariya, 2005). These reductions in anti-nutrients will improve the bioavailability of minerals. Thus, dehulling is a viable means of improving the nutritional quality of millet, sorghum, and other cereals that have anti-nutritive factors in the seed coat (Grala *et al*., 1998; Dietrych and Oleszek, 1999; Duhan *et al*., 2000; Zeb *et al*., 2002; Abiodun and Adepeju, 2011).

***2.8.2 Soaking and its nutritional benefits*:** Soaking is widely applied at both household and industrial scale. It is the most important operation in the germination or fermentation process of cereals. During cereals soaking for food making, a natural fermentative acidification takes place and this is regarded as important for food quality. Soaking cereal or legume improves the bioavailability of amino acids (especially lysine, niacin, and thiamine) in edible grains (Mubarak, 2005). The level of phytates was reduced by 16.9% after whole maize grains were soaked (Mubarak, 2005; Karkle and Beleia, 2010). In West Africa, soaking of maize grains for many hours reduced the pH of the grains to around 5.0 and could account for the reported reduction in phytate (Mbithi-Mwikya *et al*., 2000; Perlas and Gibson, 2002). Soaking also removes other anti-nutritive factors, such as saponins, trypsin inhibitors, and polyphenols (el-adawy *et al*., 2000; Siddhuraju *et al*., 2001). However, tannin-free whole-grain sorghum soaked for 24 hours had 50% less phytates (Hotz *et al*., 2001). The observation of reduced levels of phytates and increased bioavailability of minerals like iron and zinc by 2-23% provides scientific support for the traditional practice of soaking maize before milling and fermentation in the preparation of complementary foods (Lestienne *et al*., 2005). A second soaking of the milled flour in the preparation of Nigerian fermented maize dough could further reduce the levels of phytates, but this could have implications for food safety, since the antimicrobial substance resulting from fermentation of maize is water-soluble and could be lost in the decanted water.

2.8.3 Thermal processing and its nutritional benefits: Thermal processing may improve the bioavailability of micronutrients such as thiamin and iodine by destroying certain anti-nutritional factors (e.g., goitrogens, thiaminases). There is some evidence that boiling and blanching of seeds or green leaves (Erdman and Pneros-Schneier, 1994; Yeum and Russell, 2002; Yadav and Sehgal, 2002) induce moderate losses (i.e., 5–15%) of phytic acid. Thermal processing can also enhance the bioavailability of thiamin, vitamin B-6, niacin, folate, and carotenoids by releasing them from entrapment in the plant matrix (Yeum and Russell, 2002). Roasting is another forms of thermal processing that can improve the protein digestibility, but at higher temperature protein is denatured (Fasasi, 2009) and produced undesirable flavors and darkened colors due to heat-enhanced chemical reactions. The goal of roasting is to improve sensory qualities and achieve inactivation of destructive enzymes, which improves the storage and nutritional quality of the product. Shinde *et al*. (1991) reported that during roasting total phenols and tannins decreased. Malik *et al*. (2002) observed the reduction in mineral contents during roasting; due to heating at high temperature**.** Heat can kill or inactivate potentially harmful organisms including bacteria and viruses.

2.8.4 Fermentation and its nutritional benefits: Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases, and lipases, hydrolyze the polysaccharides, proteins and lipids to form nontoxic products and produce flavors, aromas, and textures that are pleasant and attractive to the consumer (Steinkraus, 1995).

The natural fermented foods prepared from most common types of cereals are common in many parts of the world, particularly developing countries. In most of these products, the fermentation is natural and involves mixed cultures of yeasts, bacteria and fungi. Some

microorganisms may participate in parallel, while others act in a sequential manner with a changing dominant flora during the course of the fermentation. The common fermenting bacteria are species of *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Micrococcus* and *Bacillus*. The fungi in general A*spergillus*, *Paecilomyces*, *Cladosporium*, *Fusarium*, *Penicillium* and *Trichothecium* are the most frequently found in certain products. The common fermenting yeasts are species of *Saccharomyces*, which results in alcoholic fermentation.

Nutritionally, fermented foods have been noted for their superior nutritional value and digestibility compared to the unfermented counterpart (Ochanda *et al*., 2010). Fermentation improves the quantity as well as quality of the food proteins as expressed by biological value, water soluble vitamins and organoleptic properties foods are generally increased, while the anti-nutritional factors like phytic acid and polyphenols decline during fermentation (Ochanda *et al*., 2010). A variety of products is fermented worldwide, including complementary foods. Fermentation causes changes in food quality indices including texture, flavor (i.e., presence of diacetyl acetic acid and butyric acid that make fermented products appetizing), appearance, nutrition and safety. For instance, Mugula *et al*. (2003) observed a decrease in the concentration of starch and soluble sugar**s** in the production of Togwa (a Tanzanian fermented food), due to hydrolysis of starch and processing of fermentable sugars to produce organic acids, like lactic acid**,** acetic acid, pyruvic acid, formic acid and the citric acid.

The increase in protein content of fermented food products reflects the decrease of starch solids and other constituents, which the mold might have consumed for growth. The decrease of carbohydrates in wheat after fermentation indicated that the mold had used the carbohydrates as an energy source (Wang, 1986). Fermentation process provides a means through which the protein content of high starch substrates can be increased for the benefit of consumers needing higher protein intakes (Azoulay *et al*., 1980). Inyang and Zakari (2008) reported that

fermentation increased fat content of pearl millet and the lipids are hydrolysed to the fatty acids. Odunfa and Adeyele (1985) showed that fermentation reduced the concentration of raffinose and stachyose, sugars causing flatulence. Study has reported on the effects of fermentation on the increased and bioavailability of micronutrients like copper, zinc, magnesium, calcium, phosphorus and iron (Sripriya *et al*., 1997) during the fermentation of millet. It is evident that the ethanol produced by yeasts, organic acid**s** produced by bacteria and anaerobic conditions during fermentation inhibit the development of pathogenic microorganisms (Helland *et al*., 2004). The antimicrobial effect associated with the lactic fermentation is due to the action of a variety of metabolites (organic acid**s,** the diacetyl, Co_2, antibiotics and bacteriocins) synthesized during the fermentation process.

2.8.5 Germination and its nutritional benefits: Germination enhances the nutritive value of cereals and legumes through the formation of enzymes that eliminate or reduce the antinutritional and indigestible factors in legumes (Abdelrahaman *et al*. 2007). The germinated legume grain is rehydrated during germination process and a number of complex chemical reactions are activated. These include breakdown of stored starch to dextrins sucrose, maltose, glucose and fructose, because of the utilisation of some sugars during the growth metabolic activity (Sripriya *et al*., 1997; Nnam, 2000; Mamudu *et al*., 2005; Fasasi, 2009). The porridge from sprouted cereal or legume have some nutritional advantages like high energy density, increased in protein and micronutrients (Kayode *et al*., 2005).

Germination increased protein and ash content (Onwuka *et al*. 2009), while antinutrient factors, fat content and energy values of germinated food samples decreased (Abdelrahaman *et al*. 2007; Ocheme and Chinma, 2008). The increased in protein content in germinated seed could be attributed to synthesis of some amino acid**s** in excess of the requirement during germination process (Marero *et al*., 1988). Germination increases the vitamin content, resulting in improved

bioavailability of niacin, riboflavin, and vitamin C, protein quality and digestibility are enhanced (Svihus *et al*., 1997; Gibson *et al.*, 1998; Ariahu *et al.*, 1999; Egli, 2001; Helland *et al*., 2002). The production of alpha amylase, an enzyme that converts insoluble starch to soluble sugars, resulting in a thinning effect, is the most important nutritional effect of germination. Amylases convert starch, especially sorghum and millet, to dextrins and maltose, thus reducing the viscosity of thick cereal porridges without dilution with water while simultaneously enhancing their energy and nutrient densities (Gibson *et al*., 1998).

The products of starch hydrolysis during germination do not gelatinize after cooking, so porridges from germinated grain can have a markedly reduced viscosity; hence, as much as three times more starch-digested flour can be used to prepare porridge of the same consistency as normal non-starch-digested porridge (Gibson *et al*., 1998). This allows a higher nutrient density in porridge paste of a given viscosity. This is useful in overcoming the problems that children have in consuming bulky, low-nutrient dense porridges (Gibson *et al*., 1998).

2.9 Functional Properties of Food and Nutritional Applications

The functional properties of foods refer to their solubility, hydration, gel formation, coagulation, foaming, emulsifying properties, viscosity, and so on. Viscosity is commonly used as an objective measure of the consistence (i.e., the degree of density, firmness, viscosity, or resistance to movement or separation of constituent particles) of starch-based gruels. The viscosity of a material is its resistance to flow under mechanical stress, or, in quantitative terms, the ratio of the shear stress to the shear rate. Viscosity is the main factors influencing the extent to which an infant can meet his or her energy and nutrient requirements.

Functionality of a food is the property of a food ingredient, apart from its nutritional value, that has a great impact on its utilization (Mahajan and Dua, 2002). Obatolu and Cole (2000) reported that in the processing of most complementary foods emphasis is usually on the

nutritional quality and quantity of the ingredients rather than their functional properties. Functional properties of complementary diets (gelation, bulk density, swelling index, emulsifying capacity, water binding capacity) are very important for the appropriateness of the diet to the growing child (Mahajan and Dua, 2002; Omueti *et al.*, 2009). The consistency and energy density (energy per unit volume) of the complementary food and the frequency of feeding is also important in determining the extent to which an infant can meet his energy and nutrient requirements (Omueti *et al*., 2009).

In developing countries, most complementary diets are starch and cereal based. Starch often provides the principal source of energy, but when heated with water, starch granules gelatinize to produce a bulky, thick (viscous) porridge (Omueti *et al*., 2009). These complementary foods tend to be of low energy density and protein content, although their liquid consistency makes them easy to consume, the volumes needed to meet infant energy and nutrient requirements often exceed the maximum volume the infant can ingest (Omueti *et al*., 2009). These physical properties make the porridge difficult for infants to ingest and digest. Furthermore, the low energy and nutrient density means that large volumes of food have to be consumed to meet the infant's requirements. This is not usually possible, owing to the infant's limited gastric capacity and to the limited number of meals offered per day. According to WHO (2003), a good quality complementary diet must have high nutrient density, low bulk density, viscosity and appropriate texture thus, complementary foods should be rich in energy, protein and micronutrients, and have a consistency that allows easy consumption.

In some parts of the developing world, this problem has been addressed through the addition of amylase-rich flour to thick porridges, which reduces their viscosity without reducing their energy and nutrient contents (Weaver, 1994). Amylase rich flour is produced by the germination of cereal grain, which activates amylase enzymes that break down starch into sugars

(maltose, maltodextrins and glucose). As starch is broken down, it loses its ability to absorb water and swell, and therefore porridge made with germinated flour rich in amylase has a high energy density while retaining a semi-liquid consistency (Walker and Pavitt, 2007), but increased osmolarity.

The lower value of least gelation energy facilitates the gelating ability of a food component (Obatolu and Cole, 2000). The ability of complementary foods to form a gel at a higher concentration implies that the diets have poor gelating ability, hence will not form a thick gel, this is a good functional property for a complementary diet. The implication of a thick gel to a complementary diet is that the volumes needed to meet infant energy and nutrient requirements often exceed the maximum volume the infant can ingest; and it can affect the gastric system of the child since they have limited gastric capacity to metabolize thick or viscous foods (Omueti *et al*., 2009). The importance of high least gelation energy to the complementary diet is however that the diet will have reduced viscosity, plasticity and elasticity hence the diet will have a low dietary bulk, which is highly favourable for a good complementary diet (Obatolu and Cole, 2000).

According to Iwe and Onalope (2001), water absorption capacity (WAC) is the ability of a product to associate with water under a condition where water is limiting, while water-bidding capacity (WBC) connotes water that is retained by the protein after processing. The significance of a lower water absorption capacity and water bidding capacity of the complementary diets is that it will have lower water absorption and binding capacity, which is desirable for making thinner gruels with high caloric density per unit volume (Elkahalifa *et al*., 2005). The high water absorption capacity and water bidding capacity of food products is attributed to their protein content, this is because proteins are hydrophilic in nature and will make the food materials to absorb and bind more water (Badries and Mellowes 1992; Otegbayo *et al*., 2000).

The advantage of low bulk density in complementary food is that the gruel or porridge made from food sample will have a lower dietary bulk. This is important in complementary foods, because high bulk limits the caloric and nutrient intake per feed per child, and infants are sometimes unable to consume enough to satisfy their energy and nutrient requirements (Desikachar, 1980). Apart from dietary bulk of the gruel or porridge, the bulk density is also important in the packaging requirement and material handling of the complementary diet (Karuna *et al*., 1996). The loose pack density (LPD) is related to the bulk density, the higher the LPD the higher the bulk density. This is because the LPD indicates the free space between the foods when packed. A large free space is undesirable in packaging of foods because it constitute a large oxygen reservoir whereas a low LPD and lower bulk density result in greater oxygen transmission in the packed food.

Swelling power refers to the expansion accompanying spontaneous uptake of solvent while solubility index (SI) is the amount of water-soluble solids per unit weight of the sample (Eke-Ejiofor and Owuno, 2012). According to Kinsella (1976), swelling causes changes in hydrodynamic properties of the food thus impacting characteristics such as body, thickening and increase in viscosity to foods. This implies that complementary foods with high content of carbohydrate will have high swelling power than less carbohydrate based complementary foods. According to WHO (2003), appropriate complementary diet is one which produce a gruel or porridge that is neither too thick (when it is too thick, it will be difficult for the infant to ingest and digest because of limited gastric capacity) for the infant to consume nor so thin that energy and nutrient density are reduced.

The protein solubility is an index of protein functionality such as denaturation and its potential applications. The high solubility of complementary food shows that the protein component of the diets are still functional or probably underwent little denaturation.

2.10 Microbiological Status of Complementary Foods and Health Consequences

Introduction of complementary foods in resource-poor settings can result in diets that are nutritionally inadequate and microbiologically unsafe, which can lead to multiple nutrient deficiencies (Hotz *et al.,* 2001; Kimmons *et al*., 2005). Foodborne microbial agents can cause diarrhoeal diseases and ill health in infants (Mosha *et al*., 2000; Black *et al*., 2003).

2.10.1 Complementary foods and sources of microbial contaminations

Infants and young children are very susceptible to food borne diseases and if they consume contaminated foods, they are likely to contract infections or intoxications leading to illness and often death. Numerous studies in developing countries have shown that weaning foods prepared under unhygienic conditions are heavily contaminated with pathogenic agents and are a major risk factor in the transmission of diseases, especially diarrhea. It is generally recognized that contamination of complementary foods may occur because of poor hygiene of food handlers, household equipments and the environment where the preparation of food takes place (Sheth *et al*., 2000). Other possible sources of contamination of complementary foods are raw food materials and water. Water used for the preparation of food itself is a source of pathogenic agents in many parts of developing countries (Kung'u *et al*., 2009). Improper storage and handling of cooked food is equally responsible for foodborne illnesses, as during storage especially at ambient temperature (28 to 35°C) there is the risks of multiplication of pathogenic organisms.

Various pathogens have been identified as the major causes of diarrhoeal diseases and most of these pathogens have been isolated from complementary foods commonly consumed in developing countries. These include bacteria such as *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Vibrio cholera, Campylobacter jejuni* (Black *et al*., 1989; Gomes, 1991), *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium perfringens*. Infections due to pathogenic *E. coli* are the

commonest illnesses in developing countries and produce up to 25% of all diarrhoeal episodes (Motarjem *et al*., 1993) and *E. coli* transmission has been substantially associated with complementary foods.

2.10.2 Health consequences of microbial food contamination

Contamination of food, including drinking water, with microbial agents is one of the major causes of diarrhoeal diseases and ill health in infants and young children (WHO, 1989; Motarjemi *et al*., 2000; Kung'u *et al*., 2009). Certain pathogens are opportunistic and affect mainly infants and young children. Some have particularly severe, others only mild, health consequences. Examples are *Aeromonas hydrophila* and other motile aeromonads, enterohaemorrhagic *Escherichia coli, Cryptosporidium parvum* and *Listeria monocytogenes*.

Worldwide, diarrhoea is the second leading cause of death in children (after neonatal disorders) (Black *et al*., 2003) and is a leading cause of growth faltering and malnutrition (Motarjemi *et al*., 1993). A great proportion (~70%) of diarrhoeal episodes occurs due to foodborne pathogens transmitted by unhygienic preparation of foods in households (Motarjemi *et al*., 1993). Socio-cultural constraints, such as social infrastructure, ignorance, incorrect beliefs and practices, taboos, poverty, insufficient food, lack of safe water and sanitation, and shortage of fuel and time may aggravate the situation (Motarjemi *et al*., 1993).

The serious implication of foodborne infections is the reduction in food intake owing to anorexia, which leads to poor nutritional status (Muhimbula and Issa-Zacharia, 2010). Poor food intake, aggravated by the loss of nutrients from diarrhea and mal-absorption over an extended period, may lead to nutritional deficiencies with serious consequences for growth, cognitive development and immune function in infants and children (Motarjemi *et al*., 1993). Thus, an infant whose resistance is suppressed becomes vulnerable to other diseases and is subsequently caught in a vicious circle of malnutrition and infection.

2.11 Evaluation of Protein Quality of Formulated Food Products

Protein quality is defined, as the ability of a food to meet the protein nutritional needs of an organism or individual species. In any given organism this is indicated by how well protein is digested, absorbed, and utilized for other life sustaining processes (Tuan *et al.*, 1999; Michaelsen *et al*., 2009). The essential amino acid that is found in the least quantity is defined as the limiting essential amino acid. The limiting essential amino acid ultimately determines the nutritional value of the protein for humans (Tuan *et al*., 1999). The quality of a protein is determined by the quantity and balance of each amino acid provided the availability of these amino acids after digestion, and the individual's requirements (Tuan *et al*., 1999).

The quality of food is assessed in terms of nutritional value, nutrient bioavailability, functional, organoleptic properties and ease of preparation. The bioavailability of nutrients is includes a protein's digestibility, absorbability, and metabolic utilizability (Heaney, 2001).
Several dietary factors may affect the nutrient bioavailability of plant foods when they are consumed, including: (1) the chemical form of the nutrient in the food and the nature of the food matrix; (2) interactions occurring between nutrients and other organic components within the plant food; (3) pretreatment of the food during processing and/or preparation (4) nutrient absorption and/or utilization in man (Sandstro¨m, 2001)

The *in vitro* and *in vivo* protein digestibilities are methods for predicting bioavailability of food products. The *in vitro* protein digestibility is a method for predicting bioavailability of food products using enzyme combination to imitate human and animal digestive systems (FAO/WHO, 1989). However, the *in vivo* method, which involves using young growing rats, is quite reliable; but time consuming and expensive (FAO/WHO, 1989). Efforts have been made to correlate *in vivo* methods to *in vitro* methods in order to develop reliable methods for protein efficiency ratio (PER) measurement. Protein efficiency ratio (PER) was the most widely used

index for evaluating protein quality. It is defined as body weight gain divided by the amount of test protein consumed by a young growing rat. Protein that has a higher PER value than 2.7 is considered an excellent quality protein (FAO/WHO, 1989). An important disadvantage of the PER is the differences in growth patterns between rats and humans and the different amino acid requirements (Schaafsma, 2005). Rats have dissimilar protein requirement than humans. This is due to all furry animals requiring more sulfur amino-acids- (methionine, cysteine) to make sulfur-rich hair proteins (Keratins). A rat has a whole-body coat of hair, hence requiring more of methionine/cysteine than in humans.

Table 2.6: Protein efficiency ratio (PER) for selected food samples

Food	**Protein Efficiency Ratio (PER)**
Almonds	0.4
Barley	1.7
Beans, navy (dry)	1.51
Beans, black	1.61
Bread, white	1
Casein	2.5
Chick peas, cooked	2.32
Corn, whole	1.4
Dried whey	2.6
Egg white	3
Egg, whole	3.1
Fish	2.7
Kidney beans	1.55
Lentils, whole green	1.3
Milk	2.5
Oats, rolled	1.8
Pea flour	1.2
Peas, split yellow	1.42
Peanuts	1.7
Pinto beans	1.64
Poultry	2.7
Rice	1.5
Soybeans, heated	2.3
Soy protein	2
Sunflower seed	1.2
Wheat, whole	0.8
White flour	0.7

Source: Health Canada, 1996

2.11.1 Protein Digestibility (PD)

The proportion of food protein absorbed is called “Protein Digestibility” (PD). This rating scale also elevates the vegetable amino acid profile of soybean to its animal-based counterparts. It should be noted that combinations of plant proteins appear to exceed or equal the amino acid profile of animal-based proteins. The standard values for protein digestibility method of protein quality are shown in Table 2.3.

2.11.2 Biological Value (BV)

The biological value (BV) is an accurate indicator of biological activity of protein, measuring the actual amount of protein deposited per gram of protein absorbed. Biological value is defined as the proportion of protein retained in the human body for growth and body maintenance (Ignjatović-micić *et al*., 2008). The biological value measure of protein quality expresses the rate of efficiency with which protein is used for growth. High biological value proteins are better for nitrogen retention, immunity. IGF-I stimulation, and are superior for reducing lean tissue loss from various wasting states than proteins with a low biological value score. Generally, high biological value proteins are more anti-catabolic than low biological value proteins. The standard values of protein biological value are shown in Table 2.4.

Table 2.7: Standard Value for protein digestibility for single and combination of proteins

Food samples	**Protein digestibility**
Wheat + Soy Protein	0.87
Milk	1.0
Fish Flour + Millet + Peanut Flour	0.87
Egg	0.97
Rice, Polished	0.84
Corn + Beans	0.82
Corn-Soy blend	0.92
Wheat, Whole	0.79
Indian Rice Diet + Milk	0.92
Soybeans	0.78
Corn, beans, Milk	0.90
Maize	0.76
Wheat, Refined	0.89
Soy protein, Isolated	0.88

Sources: FAO/WHO, 1989

Table 2.8: Biological values (BV) of selected foods from animal and plant sources

Food	**Biological value (%)**
Egg	93.7
Milk	84.5
Fish	76.0
Soybeans	72.8
Beans, dry	58.0
Rice, Polished	64.0
Wheat, whole	64.0
Beef	74.3
Corn	60.0

Source: FAO/WHO, 1989

2.12 Digestible indispensable amino acid score (DIAAS)

The digestible indispensable amino acid score (DIAAS) is a more recent method to evaluate protein quality and has been introduced, because of the weakness of other indexes such as protein efficiency ratio (PER) and protein digestibility corrected amino acid score (PDCAAS). DIAAS has been adopted by FAO/WHO as the method of choice for evaluating protein quality in human nutrition (FAO, 2013). The highest DIAAS value that any food can achieve is by definition 100%, which means that 100% or more of indispensable amino acids can be fully digested and utilized whenever consumed. A score above 100% should by definition be truncated to 100%, because any amino acids in excess of what is required for building and repairing tissues are catabolized.

2.13 Heamatological Properties of Animal Fed with the Formulated Diets

Blood is a complex fluid containing a large variety of dissolved suspended inorganic and organic substances (Stewart, 1991) or specialized circulating tissues and cells suspended in the intercellular fluid substance (Dellman and Brown, 1976). Blood circulates in the arteries, veins and capillaries of man and animals (Kronfield and Mediway, 1975). Its primary function is to transport oxygen from respiratory organs to body cells (Duke, 1975), distributing nutrients and enzymes to cells and carrying away waste products, thereby maintaining homeostasis of the internal environment (Bentrick, 1974). The various functions of blood are made possible by the individual and collective actions of its constituents – the biochemical and haematological components.

Generally, the quantity and quality of food and the level of anti-nutritional factors present in the food influence both the biochemical and haematological blood components (Akinmutimi, 2004). Biochemical components are sensitive to elements or factors present in the food (Akinmutimi, 2004), including elements of toxicity, and they are used to monitor protein quality

of foods. Haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood (Oyawoye and Ogunkunle, 1998). Reduction in the concentration of pack cell volume (PCV) in the blood usually suggests the presence of a toxic factor (e.g. haemaglutinin), which has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998). The haematopoietic system is an important index of physiological and pathological status in animals and man, since it is the one, which becomes exposed to a high concentration of toxic agents first. Frandson (1986) reported that the number of neutrophils in the blood increases rapidly when acute infection is present, hence a blood count showing increase is useful in diagnosis of infections. Eosinophils, which normally are scarce, increase in numbers in certain chronic diseases, such as infection with parasites and in allergic reactions.

2.12.1 Haemoglobin concentration (Hb)

Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of the blood in vertebrates and other animals. In mammals the haemoglobin makes up about 97% of the red cell's dry content, and around 35% of the total content (including water) (Eshaghian *et al*., 2006). Haemoglobin transports oxygen from the lungs or gills to the rest of the body, such as to the muscles, where it releases its load of oxygen. Haemoglobin also has a variety of other gas-transport and effect-modulation duties, which vary from species to species, and may be quite diverse in invertebrates. The name haemoglobin is the concentration of heame and globin, reflecting the fact that each subunit of haemoglobin is a globular protein with an embedded haem group; each haeme group contains an iron atom, and this is responsible for the binding of oxygen. The most common type of haemoglobin in mammals contains four such subunits, each with one haem group (Campbell, 1999). In humans, each haem group is able to bind one oxygen molecule, and thus, one hemoglobin molecule can bind four oxygen molecules.

The chemical empirical formula of the most common human haemoglobin is C738H1166N812O203S2Fe, but haemoglobin vary widely across species, and even (through common mutations) slightly among subgroups of human **(**Hardison, 1996). Decrease in haemoglobin, with or without an absolute decrease in red blood cells, leads to symptoms of anaemia (Hardison, 1996). Anaemia has many different causes, although iron deficiency and its resultant iron deficiency anaemia are the most common causes in the world. The absence of iron decreases haem synthesis and red blood cells production.The normal levels range from 11.0 - 14.0 g/dl for infants, 12.0-15.0 g/dl for women and 13.0-18.0 g/dl in adult men (Hercberg, *et al*., 1991). Low haemoglobin values may indicate: anemia, erythropoietin deficiency, haemolysis, haemorrhage, lead poisoning, malnutrition, nutritional deficiencies of Iron, folate, Vitamin B12 and Vitamin B6 and over hydration. Abu *et al.* (1988) reported that low level haemoglobin (Hb) of treatment diets could imply that dietary proteins were not of high quality. Diets containing poor protein would usually result in poor transportation of oxygen from the respiratory organs to the peripheral tissues (Roberts *et al*., 2000). High haemoglobin may include congenital heart disease, pulmonary fibrosis, polycythemia vera and increased red blood cell formation associated with excess erythropoietin.

2.12.2 White Blood Cell Count (WBC)

White blood cells or leukocytes are cells of the immune system, which defend the body against both infectious disease and foreign materials. High white blood cells count is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system. There are different types of leukocytes namely: neutrophil, small lymphocyte, large lymphocyte, monocyte, eosinophil and basophils are produced from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system. The number of leukocytes in the blood is often an

indicator of disease. There are normally between 4×10^9/l and 10×10^9/l white blood cells in a liter of blood, making up approximately 1% of blood in healthy human beings (Skala *et al*., 1981; Sarchielli and Chandra, 1991). Low white blood cell count may indicate: viral, bacterial, parasitic infections, drugs (e.g. cytotoxic) and reaction to chemicals, hypersplenism, a plastic anaemia, foliate and Vitamin B12 deficiency (megaloblastic anaemia). High white blood cell counts may indicate the following: acute infections, inflammation and tissue necrosis of burn fractures arthritis etc., metabolic disorders e.g. eclampsia, uremia, diabetic coma and acidosis, poisoning e.g. chemicals, acute haemorrhage, malnutrition (Skala *et al*., 1981).

2.12.3. Serum Albumin

Serum albumin is the most abundant plasma protein in humans and other mammals. Albumin is essential for maintaining the osmotic pressure needed for proper distribution of body fluids between intravascular compartment and body tissues. Bovine serum albumin (BSA) is commonly used in molecular biology laboratories. Albumin is negatively charged and the main functions of albumin are to maintains osmotic pressures, transport thyroid hormones, transport other hormones particularly fat-soluble one, transport fatty acids, transports unconjugated bilirubin, transports many drugs and competitively binds calcium ion (Ca^{2+}) and buffer's pH (Jakus *et al*., 1999). Serum albumin levels have been used extensively to assess the nutritional status of individuals with and without malnutrition and chronic renal failure (CRF) (Mohamadi-Nejad *et al*., 2002). Serum albumin levels may fall modestly with a sustained decrease in dietary protein, and energy intake and may rise with increased protein or energy intake (Kanska and Boratynski, 2002). Conversely, serum albumin levels may fall acutely with inflammation or acute or chronic stress and increase following resolution or recovery (Kanska and Boratynski, 2002).

Normal serum albumin levels range from 38-51 g/l (Corti *et al*., 1994). Low albumin levels may be caused by a poor diet (malnutrition, severe burnt, kidney disease, liver disease, uncontrolled diabetes hyperthyroidism and heart failure).

2.12.4 Total Protein

Total Protein is a rough measure of serum protein. Total serum protein has been reported as an indication of the protein retained in the animal body (Akinola and Abiola, 1991; Esonu *et al*., 2001). Protein measurement can reflect nutritional state, kidney disease, liver disease and many other conditions (Akinola and Abiola, 1991; Esonu *et al*., 2001). If total protein is abnormal, further tests must be performed to identify which protein fraction, and then which specific protein, is abnormal. Proteins are important constituents of all cells and tissues. There are many different kinds of proteins in the body with many different functions. Enzymes, some hormones, haemoglobin (oxygen transport), low-density lipoproteins (LDL), cholesterol transport fabric oxygen (blood clothing) collagen, (structure of bone and cartilage) and immunoglobulin's (antibodies) are some examples. Proteins are separated into two groups: albumin and globulins, that is, total protein equals albumin plus globulins. Total blood protein and creatinine contents have been shown to depend on the quantity and quality of dietary protein (Iyayi, 1998; Awosanya *et al*., 1999; Esonu *et al*., 2001). Muscle wasting has been shown to be the source of excess creatinine in the blood of animals and is normally due to creatinine phosphate catabolism during this process.

Table 2.9: Normal physiological ranges of haematological and biochemical components for rabbits

Parameters	**Range**
Erythrocytic data	
Erythrocytes (RBC) (10^{12}/L)	6-9
Haemoglobin (g/dL)	11-17
Haemotocrit	0.38-0.5
Mean cell volume (MCV)	50-62
Mean cell haemoglobin (MCH)(pg)	17-22
Mean cell haemoglobin concentration (MCHC) (g/dl)	31-36
Recticulocytes (%)	1-4
Recticulocytes (10^9/L)	50-350
Leukocytes data	
Total leukocytes (WBC) (10^9/L)	4-17
Lymphocytes (10^9/L)	3-15
Neutrophils (10^9/L)	0-4
Monocytes (10^9/L)	0-2
Eosonophils (10^9/L)	0-0.5
BAsophils (10^9/L)	Rare
Lymphocytes (%)	65-70
Neutrophils (%)	5-30
Monocytes (%)	1-6
Eosonophils (%)	0-3
Basophils (%)	Rare
Platelet data	
Platelet count	800-1400

Source: Evans, 2008

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Materials

Food materials: The popcorn kernels (*Zea mays averta*) and Bambara groundnut seeds (*Voandzeia subterranean* L) were obtained from Federal College of Agriculture Research Farm in Akure; while African locust beans (*Parkia biglobosa*), Cerelac (NOA supermarket) and 'Ogi' (vendor) were purchased from Erekesan market in Akure Township of Ondo State.

Experimental Animals: The Albino Wistar strain weanling rats (male and female) of aged three weeks were purchased from the Central Animal House, College of Medicine, University of Ibadan, Ibadan, for the animal experimentation aspects of the study.

3.2 Production of Complementary Food Samples

The samples, that is popcorn, African locust beans and Bambara groundnut were subjected to biological processing methods, that is, fermentation and germination methods. Fermentation and germination processing methods were employed in this study, because evidences have shown that these processing methods increase the digestibility, bioavailability of vitamins, minerals, amino acids, proteins and phytochemicals, and also decrease the anti-nutrients and starch component of food samples (Egli, 2001; Helland *et al*., 2002; Enujiugha *et al*., 2003; Oladele and Oshodi, 2008).

3.2.1 Production of germinated and fermented popcorn flour

***Germinated popcorn flour*:** The popcorn kernels were weighed (1 kilogram), sorted, winnowed, soaked for 4 hours and drained using sieves. The drained popcorn kernel were spread on moistened jute sack and allowed to germinate. The germinated kernels after 3 days were washed, drained and oven dried (Galenkamp, size 3, hotbox, London, UK) at 60^{o}C for 20 hours. The

dried grains were milled using attrition mill (Cyclotec 1093 sample mill, Tecator, Sweden), sieved using a 60 mm wire mesh sieve (British Standard) and packed in plastic container sealed with aluminum foil and stored at room temperature prior to analyses (Fig. 3.1).

***Fermented popcorn flour*:** Fermentation was performed using the microorganisms naturally present on the grain surface. Popcorn kernels were weighed (1 kilogram), cleaned, dried and maintained in water at normal room temperature for three days. The soaked grains were washed, drained and wet milled with attrition mill (Cyclotec 1093 sample mill, Tecator, Sweden). The wet milled slurry was sieved using muslin cloth and fermented for three days, decanted and drained. The drain paste was oven dried in hot air oven (Galenkamp, size 3, hot box, London, UK) at 60^{o}C for 20 hours, re-milled using attrition mill (Cyclotec 1093 sample mill, Tecator, Sweden), sieved using a 60 mm wire mesh sieve (British Standard) and packed in plastic container sealed with aluminum foil and stored at room temperature prior to analyses (Fig. 3.1).

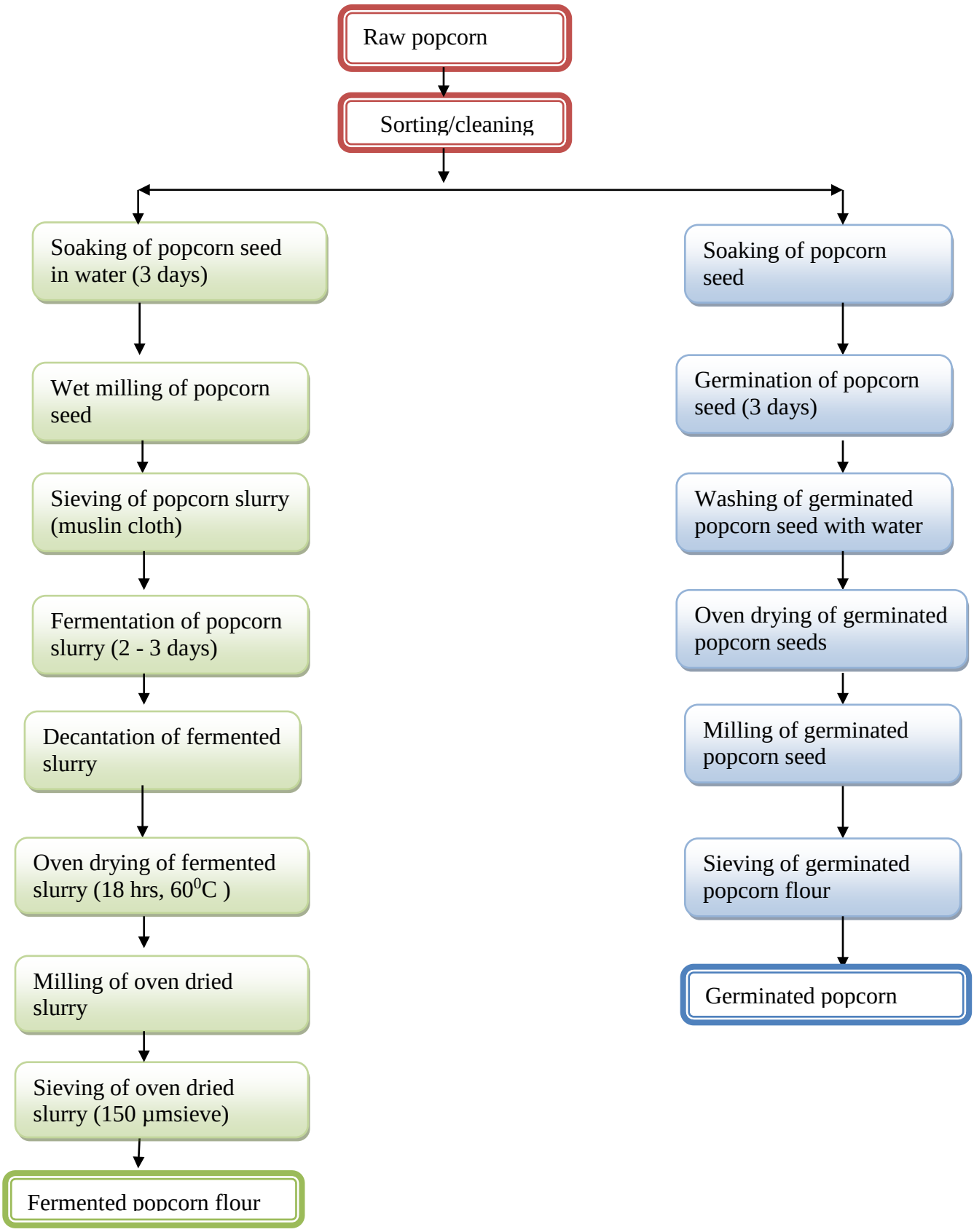

Fig.3.1: Flowchart of production of popcorn grains using fermentation and germination

3.2.2 Production of Germinated and Fermented African locust Bean Flour

***Fermented African locust bean flour*:** Fermentation was performed using the microorganisms naturally present on the beans surface. The African locust beans were weighed (1 kilogram), soaked in warm water for four days and dehulled. The dehulled seeds were cooked for thirty minutes, fermented for forty-eight hours, oven dried (Galenkamp, size 3, hot box, London, UK) at 60^0C for 20 hours, milled using an attrition mill, sieved through 60 mm wire mesh and packed in plastic container and stored at room temperature prior to analysis (Fig. 3.2).

***Germinated African locust bean flour*:** The African locust bean seeds were weighed (1 kilogram), soaked for 12 hours, drained and spread on wet jute bag and cover with another jute bag. The seeds were watered every morning and evening until the seeds germinated. The germinated seeds were dehulled manually, oven dried (Galenkamp, size 3, hot box, London, UK) at 60^0C for 18 hours, milled using a Philips laboratory blender (HR2811 model), sieved using 60mm wire mesh screen (British standard screen) and stored at room temperature prior to analysis (Fig. 3.2.).

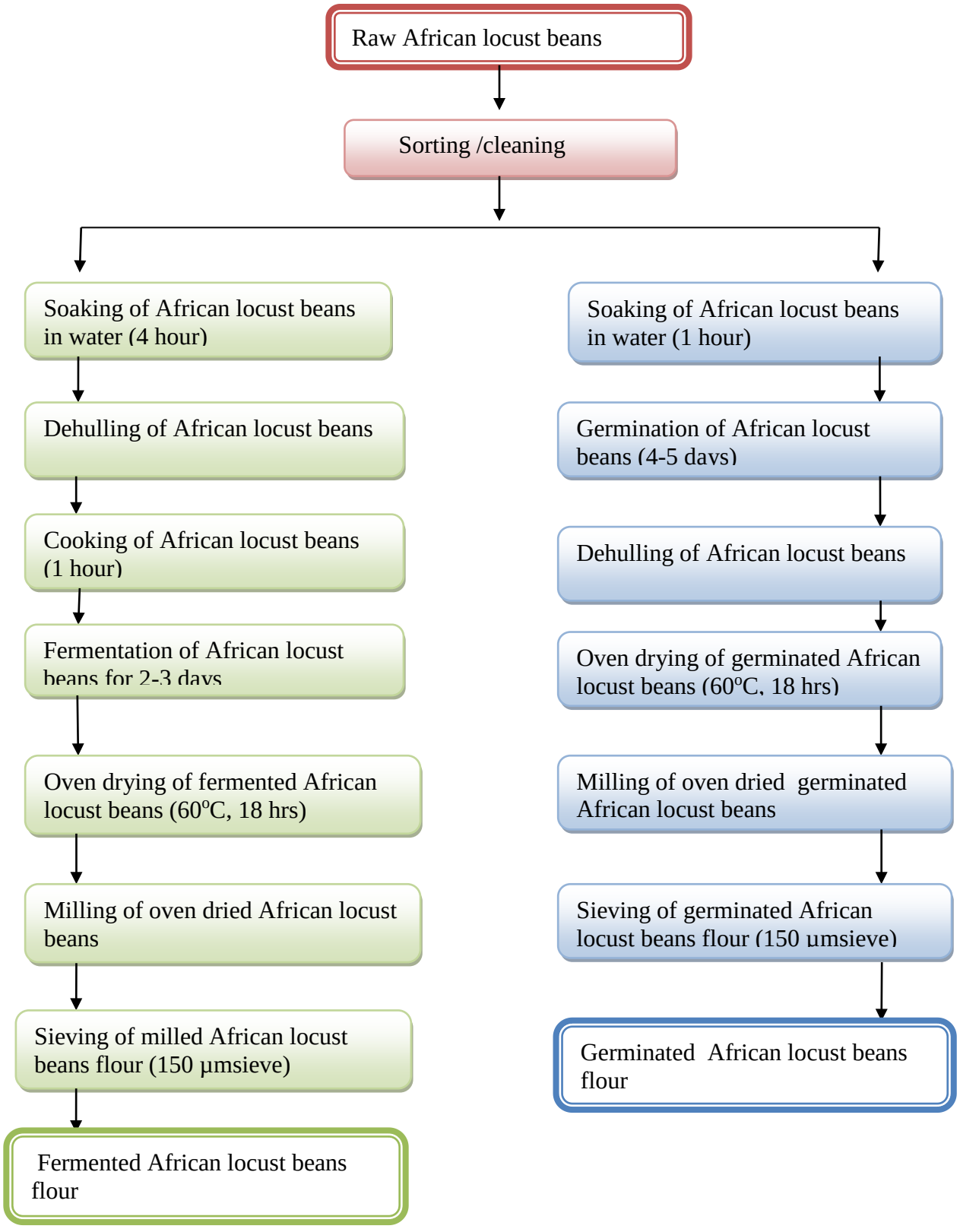

Figure 3.2: The flowchart illustrating the production of fermented and germinated African locust beans

3.2.3 Production of Germinated and Fermented Bambara Groundnut Seeds Flour

***Fermented Bambara groundnut flour*:** Fermentation was performed using the microorganisms naturally present on the seed surface. The Bambara groundnut seeds were weighed (1 kilogram), soaked in warm water for 24 hours and dehulled. The dehulled beans were precooked and fermented for three days, oven dried (Galenkamp, size 3, hot box, London, UK) at 60^0C for 20 hours, milled using a Philips laboratory blender (HR2811 model), sieved using 60 mm wire mesh screen (British standard screen) and packed in plastic container and stored at room temperature prior to analysis (Fig. 3.3).

***Germinated Bambara groundnut seed flour*:** The Bambara groundnut seeds were weighed (1 kilogram), soaked for 12 hours, drained and spread on wet jute bag and cover with another jute bag. The seeds were watered every morning and evening until they were germinated. The germinated seeds were dehulled manually, oven dried (Galenkamp, size 3, hot box, London, UK) at 60^0C for 18 hours, milled using a Philips laboratory blender (HR2811 model), sieved using 60 mm wire mesh screen (British standard screen) and stored at room temperature prior to analysis (Fig. 3.3).

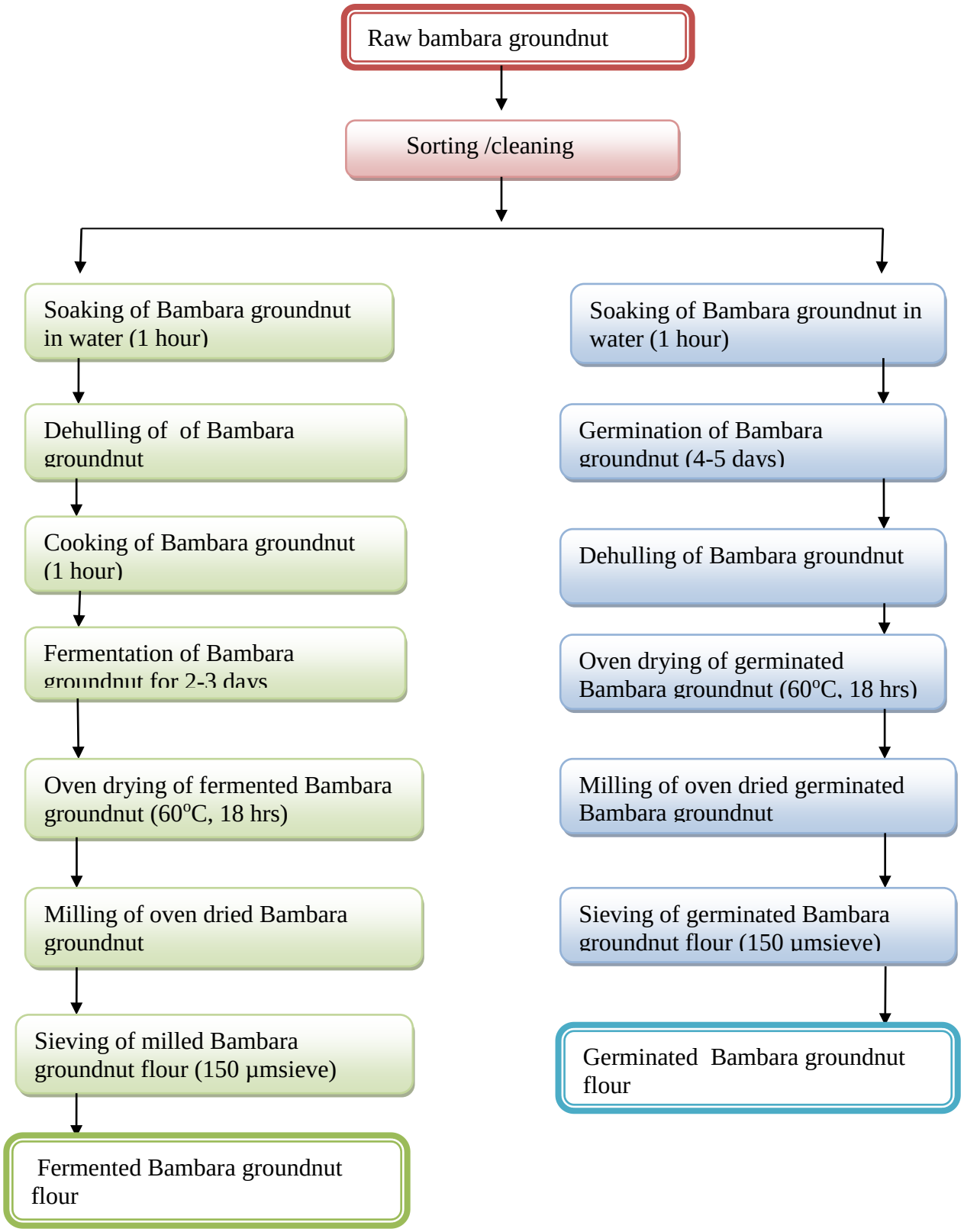

Figure 3.3: The flowchart showing production of fermented and germinated Bambara groundnut seeds.

3.3 Determination of Flour Sample Proportions in the Formulated Complementary Foods

Food formulations were obtained by blending different components of food samples in the appropriate ratios according to their nutrient contributions in order to achieve a proper food mix that meets the energy and protein needs of a 6 months old baby (Muhimbula *et al*., 2011). The ratios of the corresponding flour samples (Table 3.1) were blended in a ratio obtained using NutriSurvey for Linear Programming Software developed by J. Erhardt and A. Briend (2007) to optimize nutrient density of the novel formulations (Darmon *et al*., 2002). The NutriSurvey Linear Programming Software was used to target 18% protein, 9% fat (Amankwah *et al.,* 2009) and minimum energy value of 380 kcal/100g dry matter requirement specifications in the weaning blend formulation for the age group of 6 to 18 months.

NutriSurvey for Linear Programming Software gives the possibility of minimizing any linear function of a set of variables, when complying with a multiple set of constraints. The importance of the equation in determining the cheapest amount of food materials to be combined to meet the nutritional requirement of the target individuals (<5 years) has been reported by several researchers. For instances, linear programming (LP) is a design method that can be used in the development of novel ready to use therapeutically food (RUTF) and complementary foods (Amegovu *et al.,* 2013). Linear programming analysis is an operational research approach that is used to model complex multifactorial problems, including diet-related ones (Ferguson *et al*., 2006). Since 1959, linear programming has been applied to human nutrition (Smith, 1959). More recently, with the aid of specific software, linear programming was used for complementary feeding programs (Briend *et al*., 2003) or in the design of diets for specific populations (Darmon *et al*., 2002a; Darmon *et al*., 2002b). Linear programming is also used to design personal diets, define institutional nutritional practices, support decision-making in nutrition education or agricultural programs, define food fortification activities, and undertake analysis of economic

constraints on human diets (Darmon *et al*., 2002a; Darmon *et al.*, 2002b). Linear programming can be used for assessing the economic value of fortified food supplements and in predicting limiting nutrients in specific diets (Briend *et al*., 2003). Lnear programming is a suitable decision tool for designing novel food-based formulations. The method helps by identifying the cheapest possible combination of food ingredients that meet a set of nutritional requirements (Anderson *et al*., 1983; Darmon *et al*., 2002a), avoiding a "trial and error" approach (Westrich *et al*., 1998; Briend *et al*., 2003).

Table 3.1: Proportions of popcorn, African locust bean and bambara groundnut flour in the formulated diets

Sample	Popcorn (%)	African locust bean (%)	Bambara groundnut (%)
Germinated Popcorn+ Locust bean (GPA)	70	30	-
Germinated Popcorn+ Bambara nut (GPB	70	-	30
Germinated Popcorn+ Bambara+Locust bean (GPAB)	70	15	15
Fermented Popcorn+ Locust bean (FPA)	70	30	-
Fermented Popcorn+ Bambara nut (FPB)	70	-	30
Fermented Popcorn+ Bambara+Locust bean (FPAB)	70	15	15

3.4 Control Food Samples

Nestle *Cerelac* (a commercial complementary food) and Ogi (a corn gel and traditional complementary food) were used as the control food samples.

3.4.1 Cerelac (a commercial formula)

Cerelac was purposely selected as the control food sample for this study, because of its nutritional quality. Cerelac is a maize-based infant formula, which is enriched with milk, traces of soybean and fortified with micronutrient. Quite a number of studies have validated the use of commercial formulas, such as *Cerelac* and Nutrend, as control food samples during bioassay (Solomon, 2005; Ijarotimi *et al*., 2009; Asemi *et al*., 2010; Sodipo and Fashakin, 2011; Udensi *et al*., 2012).

3.4.2 Ogi, a local complementary food

Ogi- acid-fermented cereal gruel is a staple food of several communities in Nigeria (Omemu, 2011). It is traditionally made from maize, sorghum or millet in West Africa (Omemu, 2011). It is a popular breakfast cereal meal and infant weaning food in Nigeria (Torre *et al*., 1991; Otitoju, 2009; Omemu, 2011). According to Fashakin (1989), the low-income earners find ogi as a better alternative in infant food. Ogi production involves two stages, that is, steeping and souring (Teniola and Odunfa 2002). It is prepared by steeping clean grains in water at room temperature (25+2oC) for 48-72 h. The steep water is decanted and the fermented grain is washed with clean water and then wet-milled. The bran is removed by wet sieving and the sievate is allowed to settle for another 24-48 h, a process referred to as souring during which time fermentation also proceeds and the solid starchy matter, *ogi*, sediments (Akingbala *et al.*, 1981). The wet *ogi* usually has a smooth texture, a sour flavor resembling that of yoghurt and a characteristic aroma that differentiate it from starch and flour. The color of *ogi* depends on the type of cereal used: cream for maize, light brown for sorghum and greenish to grey for millet

(Banigo and Muller, 1972). The wet o*gi* can be boiled at 8-10% total solids into a porridge or pap, which serves as weaning food for infants, breakfast for children and convenient meal for the convalescence (Onyekwere *et. al.,* 1989).

Fermentation of *ogi* is by microorganisms from the environment and quality control is absent in the traditional method of preparation (Onyekwere *et. al.*, 1989; Halm *et. al.,* 1993). Many nutrient losses occur during processing of cereals for *ogi* production (Omemu, 2011); hence, several attempts have been made to improve the nutritional status of *ogi* by fortifying it with protein rich substrates (Omemu, 2011). A lot of nutrient losses occur during processing of cereals for *ogi* production; hence, several attempts have been made to improve the nutritional status of *ogi* by fortifying it with protein rich substrates. However, nutritional improvements of these fermented cereal gruels with proteineous foods lowered their pasting viscosities and sometimes affected their sensory attributes adversely. These factors are likely to influence consumer acceptability (Osungbaro, 2009).

3.5 Preparation of diets for the experimental wistar rats

Iso-nitrogenous of the formulated complementary foods and control samples (Ogi and *Cerelac*) were calculated with reference to 10% of protein content (Sodipo and Fashakin, 2011).

Iso-nitrogenous calculation

Iso-nitrogenous (IN) diets were obtained by diluting the analysed protein content of the formulated complementary foods to 10% protein level with basal diet (Olapade and Aworh, 2012).

Equations

$$\text{IN} = \frac{original\ protein\ conten\ of\ test\ food\ samples\ (\%)}{100} x\ X = \frac{10}{100} x\ Y$$

Where:
X = weight of sample required for the new feed mixture
Y = proposed total weight of the basal diet to be added to the feed (e.g., 100%)

For germinated popcorn-African locust bean blend (GPA) (protein =23.85 %)

$$\text{IN} = \frac{23.85\ (\%)}{100}\, x\, X = \frac{10}{100}\, x\ 100$$

0.2385X = 10
X = 10/0.2385
X = 41.9% (weight of GPA food sample
Y = 100 – 41.9 %
Y = 58.1% (weight of the basal diet required in the new mixture to make the protein level of 10%)

The iso-nitrogenous formulated food samples (that is, GPA, GPB, GPAB, FPA, FPB and FPAB) and control food samples (that is, *Cerelac* and Ogi) were pelleted (Appendix I). The pelletted food samples were used to feed the animals during bioassay analysis.

3.6. Determination of the proximate composition of flour and formulated complementary food samples

Nutrient composition of fermented (popcorn, African locust beans and Bambara groundnut) flour, germinated (popcorn, African locust beans and Bambara groundnut) flour and formulated food samples (i.e., popcorn-African locust bean, popcorn-Bambara groundnut and popcorn-African locust bean-Bambara ground flour blends) were determined. The analyses were determined in triplicates as follows:

3.6.1 Proximate analyses of the food samples

The nutrient composition of the food samples was determined using the standard procedures of Association of Official Analytical Chemists (AOAC, 2005).

3.6.1.1 Moisture content determination

The standard method of AOAC (2005) was used to determine the moisture contents of the formulated complementary foods. Clean petri dishes with lids were labeled and dried in an oven at 100^{o}C for 30 minutes, cooled in a dessicator containing reignited CaO as desiccant, and weighed to a constant weight (W_1) using Mettler balance scale. Five grammes (5 g) of each sample were weighed into respective petri – dishes. The dishes and food samples were weighed again before drying (W_2). The petri – dishes and food samples were transferred into the oven (Galenkamp, size 3, hot box, London, UK) maintained at 105^{0}C for 3 hours. The dishes and content were removed and quickly transferred into a dessicator containing CaO as dessicant to cool and re-weighed. The samples were returned into the oven and re – dried for further one hour, cooled and weighed. The procedure was repeated until a constant weight was attained (W_3). Triplicate analyses were determined on each sample.

Calculation

The moisture content of each sample was calculated as the difference in weights before and after drying to constant weights. Values were expressed as percentage moisture.

$$\text{Moisture content}(\%) = \frac{W2 - W3}{W2 - W1} x100$$

3.6.1.2 Crude fibre determination

Crude fibre is the organic residue, which remains after the food sample has been treated under standardized conditions with petroleum spirit, boiling dilute H_2SO_4, boiling dilute NaOH solution, dilute HCl, alcohol and ether (AOAC, 2005). About 0.5g of the sample was weighed into 1 litre conical flask (W_1), followed by the addition of 200ml of boiling 25% H_2SO_4. The

solution was boiled gently for 30 minutes. Using cooling fingers to maintain a constant volume. The solution was then filtered through muslin cloth, stretched over 9 cm butchner funnel and rinsed with hot distilled water. The residue was scrapped back into the flask with spatula and 200ml of boiling 1.25% NaOH was added and the solution was allowed to boil gently for 30 minutes with boiling fingers used to maintain a constant volume. This was again washed thoroughly with hot distilled water and it was rinsed once with 10% HCl and twice with industrial methylated spirit. The residue was rinsed finally three times with petroleum ether (40 – 60^0C boiling range) and was allowed to drain, dried and scrapped into a crucible. The residue is dried overnight at 105^0C in the oven (Galenkamp, size 3, hotbox, London, UK), cooled in a dessicator and then weighed (W_2). Ashed at 550^0C for 90 minutes in a muffle furnace and then cooled in a dessicator and reweighed (W_3).

Calculation

$$\% \text{ Crude Fibre} = \frac{W_2 - W_3}{W_1} \times 100$$

3.6.1.3 Determination of Crude Protein

The crude protein was determined according to micro kjedahl method.

Digestion: 0.2g of the sample was weighed into microkjeldahl digestion flask and selenium catalysts were added. 5ml of concentrated H_2SO_4 was added. The carbon present was oxidized to carbon(iv)oxide, nitrogen to ammonium sulphate $(NH_4)_2SO_4$, sulphur and phosphorus to oxides, which were given off as gases. The mixtures were heated on an electro thermal heater until clear solution was obtained. Chemical reactions involved during the digestion process are as follows:

$$\text{- N - } + \text{Conc. } H_2SO_4 \xrightarrow{\text{Reduction}} (NH_4)_2\,SO$$

$$\text{- C - } \xrightarrow{O_2} CO_2$$

$$\text{- S - } \xrightarrow{O_2} SO_2$$

Distillation: This involves steam distillation of the diluted sample with 40% NaOH. 5ml of 25 boric acid solution was pipette into a 100ml conical flask to which 3 drops of mixed indicator were added. The conical flask was placed in such a way that delivery tube touched the boric acid level inside the flask while tubes were being cooled with cold water. The 40% NaOH solution was used to liberate ammonia out of the whole digest during the distillation. Distillation was carried out until the solution turned cloudy thus, indicating that the solution has become alkaline. During distillation, all other outlet was tightly closed to avoid loss of ammonia. The liberated ammonia was trapped in boric acid solution and the pink coloured solution of boric acid in the receiver flask turned light green indicating the presence of ammonia acid complex ion or reaction of ammonia with boric acid. Distillation was continued until about 50ml of distillate was collected into the receiver flask. Chemical reactions involved are as follows:

$$(NH_4)_2\,SO_4 + 40\%\ NaOH \rightarrow 2NH_3 + Na_2\,SO_4 + H_2O$$

$$NH_3 + H_3BO_3 \xrightarrow[\text{complex}]{} NH_3 - H_3BO_3$$

Titration: The solution obtained was titrated with 0.1M HCl until the initial pink colour was observed at end point. The chemical reaction involved is as follows:

$$NH_3 + H_3BO_3 + HCl \Rightarrow NH_4Cl + H_3BO_3$$

Calculation

Percentage nitrogen in the food samples were calculated as follows:

$$\%\text{Nitrogen} = \frac{(a-b)x\ 0.01x14.005xCx100}{D\ x\ Ex\ 1000}$$

Where:

a = average titre value for samples
b = titre value for blank
C = volume to which the digest was made up to.
D = aliquot taken for distillation
E = weight of dried samples taken for digestion.

% Crude Protein = %Nitrogen x 6.25 (AOAC, 2005).

3.6.1.4 Determination of crude fat

The fat content was determined using the soxhlet apparatus. A considerable quantity of the sample was put into a pre – weighted filter paper, weighed, dried in an oven and tied with thread. The filter paper containing the food sample was placed in soxhlet extractor. Normal hexane of boiling point range 60 – 68% was used as solvent for extraction; a 500ml round bottom flask was fixed to the soxhlet extractor with a reflex condenser and placed in an electromantle heater. Extraction began as the solvent refluxed several times and was continued for 4hours after which the condenser was detached. The filter paper containing the defatted sample were removed and dried to a constant weight in an oven at 50^{o}c. The difference in the sample before extraction and after extraction was recorded in order to obtain the fat extracted.

Calculation

$$\% \text{ Fat Content} = \frac{\text{Weight of fat extracted}}{\text{Initial weight of sample}} x \frac{100}{1}$$

3.6.1.5 Ash content determination

The ash content was determined by the method of AOAC (2005). The crucible for ashing were dried in hot air oven and allowed to cool in a desicator. The cooled crucible was weighed and about 1g of food sample was put in the crucible and weighed. The crucible and its content were then transferred into a muffle furnace (Model M-525) at temperature of 550^{0}C for 18 hours. The ashing continued until no black speck or white grey ash was obtained. The crucible was taken out and immediately transferred into a dessicator to cool and later weighed.

Calculation

The difference in weight or loss in weight of the crucible and samples before ashing gave the organic matter content of each diet sample, while the difference between the weight of the

crucibles alone and crucible plus ash, gave the weight of ash of each sample. Values for ash were calculated and expressed in percentages.

$$\% \text{ Ash} = \frac{\text{Weight of Ash (g)}}{\text{Weight of Sample (g)}} x100$$

3.6.1.6 Carbohydrate determination

The carbohydrate content was determined by subtracting the total moisture content, crude protein, crude fiber, ash, and fat from the total dry weight (100 g) of the food sample differences.

% Carbohydrate = 100 – (Moisture Content + Protein + Fat + Ash + Crude Fibre)

3.7 Determination of Energy Values of formulated food samples

Energy value (calorific value) was determined using an indirect calculation method. The three groups of nutrients, which provide the body with energy, are carbohydrates, fats and proteins. One gram of carbohydrate (C) was assumed to give 4.0 kcal. energy; one gram of fat (F) 9.0 kcal energy and one gram of protein (P) 4.0 kcal. The energy values for one gram of the three groups of nutrients, which provides the body with energy, were calculated by using specific values of Atwater factors for protein, fat, and total carbohydrate (Iombor *et al*., 2009).

Calculation

Energy value = (P* 4.0) + (F* 9.0) + (C* 4.0) in Kcal/100g of the sample

Where;
P = Protein content (%).
F = Fat content (%).
C = Available total carbohydrate (%).

3.8 Determination of Mineral Composition of the Flour and Formulated Food Samples

The minerals content of the food samples were analyzed using the method of Association of Analytical Chemist (AOAC) (2005). About 1g of food sample was poured into a crucible and placed inside a muffle furnace at 550^0C for 5 hours to ash; and after ashing the sample was

transferred to dessicator to cool. The ash was dissolved in 10% HCl and filtered, then, it was made up to the mark in 100ml standard flask with de-ionized water. The solution was aspirated into atomic absorption spectrometer Buck 200 scientific to obtain the mineral content. The mineral analyses include calcium, magnesium, potassium, copper, zinc, iron, lead, aluminum, sodium and phosphorus. For the calcium, magnesium, iron, copper and zinc the atomic absorption spectrophotometer (SF9 mole) was used to determine these elements.

The standard solutions were prepared separately for each of the elements and values determined from atomic absorption spectrophotometer (AAS). The values measure were plotted against the strength of the solution. The value of the various digests were measured from the atom i.e. absorption spectrophotometer and the strength traced on the respective standard curve to give the corresponding values, which would give the original value of the element present in the digest.

Sodium and potassium determination: Flame photometer was used to determine the concentrations of the elements in the food samples. The standard solutions were prepared separately using sodium chloride and potassium chloride for sodium and potassium determinations respectively. The standard solutions were measured from the flame photometer and the value obtained was plotted against the strength of various solutions. The digests were determined from the flame photometer. The values were plotted in the respective standard value to read the original values of the elements.

Phosphorus determination: The phosphorus was determined using Vanodo-molybdate method. To series of 100mL volumetric flasks 0.0, 2.5, 5.0, 7.5, 11.0, 15.0, 20.0, 20.0, 40.0, 50.0 mL of the standard phosphate solution was made acidic by addition of 2 ml nitric acid (2:1). After which 25 mL of the Vanodo-molybdate reagent was added. The solution was diluted to the

mark, mixed thoroughly and allowed to stand for 10 min. The optical density was measured at 47mu.

3.9 Determination of Amino Acids Profile of the flour and Formulated Food Samples

The amino acid contents of the flour, formulated foods and control food samples (*Cerelac* and Ogi) were determined using the method described by Spackman *et al*. (1958). The known (2.0 g) sample was oven dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into Technicon sequential Multi- sample Amino Acid Analyzer (TSM-1).

Defatting of amples: Two grams of the dried sample was weighed into extraction thimble and the fat extracted with chloroform/methanol mixture (1:1v/v) using soxhlet extraction apparatus as described by AOAC (2005) the extraction lasted for 15 hours.

Hydrolysis of the samples: 40mg of each defatted sample was separately weighed into glass ampoules. 7mL of 6N HCl, was added and oxygen expelled by passing nitrogen into the ampoules (This was to avoid possible oxidation of some amino acids during hydrolysis). The glass ampoules were then sealed with flame and put into an oven preset at 105^{o}C and left for 22 hours to hydrolyze. The ampoules were allowed to cool and the tips broken. Contents were then filtered. The filtrates were evaporated to dryness at 40^{o}C under vacuum in a rotary evaporator. Residues were dissolved in 5 ml acetate buffer (pH=2.0) and stored in plastic sample bottles at –4^{o}C until required.

Loading of the hydrolysate into the TSM-1 analyzer: Ten microliter of each hydrolysate was dispensed into the cartridge of the analyser. The amino acid analysis was done by ion-exchange chromagraphy (Spackman *et al*., 1958) using a Technicon Sequential Multisample Amino Acid Analyser (Technicon Instruments Corporation, New York, USA). The analyzer then separated and analyzed free acidic, neutral and basic amines, which lasted for 76 hours. Norleucine was

employed as the internal standard. Ten micro-liters (10µL) of the standard solution mixture of amino acids was also loaded into the analyzer. Values of both the standard and samples were recorded and printed out as chromatogram peaks by the chart recorder.

Calculation from the peaks: The net height of each peak produced on the chromatogram (each representing an amino acid) was measured. The half-height of each peak was located and the width of the peak at half-height accurately measured. Approximate area of each peak was then obtained by multiplying the height with the width of half height. All measurements were in millimeters (mm). The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated as:

$$NE = \frac{\text{Area of norleucine peak}}{\text{Area of each amino acid in the standard mixture.}}$$

A constant (Sstd) for each amino acid in the standard mixture was then calculated.

Sstd = NEstd x mol.weighed of amino acid x µmole AAstd.

The amount of each amino acid (in g/100g protein) in each diet sample was calculated as follows:

$$\text{Concentration of amino acid (g/100g protein)} = NH \times \frac{NH}{2} \times Sstd \times C$$

Where

$$C = \frac{\text{Dilution} \times 160}{\text{Sample wt} \times \%N \times 10 \times \text{volume loaded} \times NH \times W(nleu)}$$

NH = net height
W = width at half height
nleu = norleucine

Determination of tryptophan in the food samples

Tryptophan was estimated by the ninhydrin method of Pintér-Szakács and Molnán-Perl (1990). One gram of sample was introduced into a 25 ml polypylene test tube with caps and then 10 ml of 0.075 mol/L NaOH was added and mixed until there were no lumps. The dispersion

was shaken for 30 min and centrifuged at 5000 r/min for 10 min and the supernant was transferred to a clean test tube. To 0.5 mL of supernant, 5 ml of ninhydrin reagent (1.0 gram of ninhydrin in 100 mL mixture of 37% HCl and 96% HCOOH at a ratio of 2:3) was added and then solution was incubated at 35 °C for 2 h, and then cooled to room temperature after which the volume was made up to 10 mL with diethyl ether, thoroughly mixed with a Vortex mixer, filtrated and the clear filtrate was read at 380 nm. A standard tryptophan curve was prepared using 0~100 μg tryptophan. From the standard graph, the concentration of tryptophan was calculated and expressed as g/100 g protein.

3.10. Determination of Fatty acid Composition of the Flour and Formulated Food Samples

Extraction of lipid for fatty acid analyses: About 0.25 gram of each sample was weighed into the extraction thimble. 200 mL of petroleum ether (40-60 ^{o}C boiling range) was measured and then added to the dried 250 mL capacity flask. The covered porous thimble with the sample was placed in the condenser of the Soxhlet extractor arrangement that has been assembled (AOAC, 2005). The lipid was extracted for 5 h. The extraction flask with the oil was oven dried at 105^{o}C for 1 hr. The flask containing the dried oil was cooled in the desiccators and the weight of the cooled flask with the dried oil was measured.

***Preparation of methyl esters and analyses*:** 50 mg of the extracted oil was saponified for 5 min at 95^{o}C with 3.4 mL of 0.5 M KOH in dry methanol. The mixture was neutralized by 0.7 M HCl. 3 ml of 14 % boron triflouride in methanol was added (AOAC, 2005). The mixture was heated for 5 min at 90^{o}C to achieve complete methylation process. The fatty methyl esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1 ml for analysis and 1μ l was injected into the injection pot of the gas chromatograph (GC). The fatty acid methyl esters were analysed using an HP 5890 powered with HP gas chromatograph (HP 5890 powered with HP ChemStation rev. A09.01) [1206] software [GMI, Inc, Minnesota, USA])

fitted with a flame ionisation detector. Nitrogen was the carrier gas with a flow rate of 20-60 ml/min. The oven programme was: initial temperature at 60^{o}C, first ramping at 10^{o}C/min for 20 min, maintained for 4 min, second ramping at 15^{o}C/min for 4 min and maintained for 10 min. The injection temperature was 250^{o}C whilst the detector temperature was 320^{o}C. A capillary column (30m x 0.25 mm) packed with a polar compound (HP INNOWAX) with a diameter (0.25 μm) was used to separate the esters. Split injection type was used having a split ratio of 20:1. The peaks were identified by comparison with standard fatty acid methyl esters.

Calculations: Other fatty acid parameters calculated were total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), PUFA/SFA, n-6/n-3, LA/ALA and MUFA/SFA

3.11 Determinations of Anti-nutrient Composition of Food Samples

3.11.1 Phytate determination

Phytate was determined using the method of AOAC (2005). Phytate was extracted using dilute HCl and the extract was mixed with Na_2EDTA – NaoH solution, and placed in an ion-exchange column. The extracted phytate was diluted with 0.7ml NaCl solution and wet-digested with H_2SO_4/HNO_3 mixture to release phosphate, which was measured colorimetrically after reacting with ammonium molybdate solution. The amount of phytate in original sample was obtained as hexaphosphate equivalent.

Procedure: Two grams of each diet sample was separately weighed into labeled 150 mL Erlenmeyer flasks. Exactly 40 ml of 2.4% HCl was added to each sample, covered and shaken vigorously on a MSE orbital shaker for 3 hours at room temperature. Meanwhile, a column was prepared by adding 3 ml of distilled H_2O to the slurry of 0.5g-anion exchanger resin AG 1-x 4 chlorides obtained from BIO-Rad laboratories. This was allowed to settle and then washed with 15ml of 0.7M NaCl solutions, followed by 15 mL of distilled water. Samples were removed from

the shakers and filtered through Whatman filter paper No. 42. One millilitre of each filtrate was separately mixed with 1.0ml Na_2 EDTA – NaOH reagent in a 25ml volumetric flask. Mixtures were diluted to the mark with distilled water, mixed and transferred quantitatively to the prepared column. The first eluent from the column was discarded. The column was washed with 15ml 0.7M NaCl and fraction collected into a digestion flask. Concentrated H_2SO_4 (0.5 mL) and 3.0 mL concentrated HNO_3 were added to the flask. Before the next sample was added to the column, 15ml of distilled water was passed through it. Mixtures in flasks were digested on micro-Kjeldahl rack at 50^oC until active boiling ceased and thick yellow vapour was given out. Heating continued for another 10 minutes before burner was turned off. Flasks were allowed to cool to room temperature. Exactly 10 mL-distilled water was added to flask and swirled to dissolve the digests. All solutions were separately transferred quantitatively to labeled 50ml volumetric flasks. 2.0 mL molybdate solution was added to each sample, mixed thoroughly, then followed by 1.0ml sulfonic acid reagent and mixed again. The solutions were then diluted to volume with more distilled water. Mixtures were allowed to stand for 15 minutes and absorbance read at 640nm. A blank solution was prepared by mixing 1ml 2.4% HCl with 1.0ml Na_2EDTA – NaOH reagent, and diluted to 25ml with distilled water before pouring into column and treated as samples

Preparation of standard curve

A standard curve was prepared by pipetting 1.0, 3.0, 5.0, 7.0ml phosphate standard solution containing 80, 240, 400 and 560µg phosphorus respectively, into labeled 50 mL volumetric flasks. 20 mL distilled water was added to each flask, mixed thoroughly, followed by 2.0 mL Molybdate solution with continuous mixing. 1.0ml sulfonic acid solution was added and mixed well, diluted to volume with distilled water and mixed again. Absorbance of the solutions

was read at 640nm. A standard curve was generated. Triplicate determinations were carried out on all the samples and standards.

Calculation

Phytate concentration in the diet samples was extrapolated from the generated standard curve, and expressed as mg/100g sample.

3.11.2 Determination of tannin in the food samples

Tannin contents were determined by the modified vanillin-HCl methods (Burns, 1971). A 2 g sample was extracted with 50 ml 99.9% methanol for 20 minutes at room temperature with constant agitation. After centrifugation for 10 min. at 653 rpm, 5 ml of vanillin-HCl (2% vanilli and 1% HCl) reagent were added to 1 ml aliquots, and the colour developed after 20 min. at room temperature was read at 500nm. Correction for interference light natural pigments in the sample was achieved by subjecting the extract to the conditions of the reaction, but without vanillin reagent. A standard curve was prepared using catechin (Sigma Chemical, St. Louis, MO) after correcting for blank, and tannin concentration was expressed in g/100g.

3.11.3 Determination of oxalate in the food samples

Total oxalate in the food samples was assayed using the method of AOAC (2005). Oxalate was precipitated as insoluble calcium oxalate, which is collected by centrifuging. The precipitate is dissolved in an excess of hot dilute H_2SO_4 and the oxalate titrated (in hot) with standardized $KMnO_4$. Two grammes of the diet samples were separately weighed into labeled 250 ml beaker, and 150 ml distilled water and 55 ml 6M HCl was added. Two drops of alcohol were added and mixture boiled for 15 minutes, cooled, and transferred quantitatively into 500 ml volumetric flask, diluted to volume with distilled water and mixed again. Mixture was allowed to stand overnight, mixed thoroughly, and filtered through No. 42 Whatman filter paper. Exactly

25ml of the filtrates were separately pipetted into labeled 50 ml flasks and then 5 ml tungstophosphoric acid added, mixed and allowed to stand for 5 hours. Mixtures were filtered through Whatman filter paper, and 20 mL of filtrates were pipetted again into centrifuge tubes followed by ammonium hydroxide solution drop wise until a pH of 4.5 was achieved using indicator paper. 5ml acetate buffer (pH 4.5) was then added to maintain a constant pH. Mixtures were allowed to stand again overnight at room temperature, after which they were centrifuged for 15 minutes at 1700 rpm to compact the precipitate. Supernatants were carefully decanted and calcium oxalate precipitates washed three times with centrifugation and decantation using cold washing liquid (12.5 mL HoAc, diluted to 250ml with distilled water). Precipitates were re-dissolved in 5ml dilute H_2SO_4 (1:9v/v). The dilute H_2SO_4 also served as the blank solution. All mixtures were then heated in a boiling water bath for 15 minutes and the hot solutions titrated with 0.01N $KMnO_4$ until a persistent pink colour was obtained. Triplicate titrations were carried out on each sample.

Calculation

The volume of $KMnO_4$ used to titrate the hot solution of each sample was used to calculate the oxalate content of each sample as follows:

$$\text{Mg oxalate/100g sample} = \frac{\text{ml of 0.01N KMnO4 x 1350}}{\text{Weight of sample taken}}$$

Where 1350 = 0.45 (mg oxalic acid equivalent to 1ml 0.01N KMnO4) x [(30/20) x (50/25) dilution factors] x 100 (to convert to 100g sample)

3.11.4 Determination of Trypsin Inhibition Activity (TIA) in the food samples

The trypsin inhibition activity was assayed in terms of the extent to which an extract of the defatted flour inhibited the action of Bovine Trypsin (EC 3.4.21.4) on the substrate benzoyl-DL-arginine-p-nitrianilide (BAPNA) hydrochloric (Kakade *et. al.,* 1969). The samples (1g each)

was extracted continuously at ambient temperature for 3 hours with 50mL, 10 mM NaOH using a mechanical shaker (GallenKamp orbital shaker Surrey, UK). The pH of the resulting slurry was adjusted to 9.4 -9.6 with 1M NaOH. After extraction, the suspension was shaken and diluted with distilled water such that 1 cm^3 of the extract produced trypsin inhibition of 40-60% at 37^oC. The respective dilutions will be noted. Consequently, TIA was calculated in terms of mg pure trypsin (Sigma type lll, lot 20H0868)

$$TIA = \frac{2.632DA}{S} \text{ mg pure trypsin inhibited g}^{-1} \text{ sample}$$

Where D is the dilution factor, A is the change in absorbance at 410mm due to trypsin inhibition per cm^3 diluted sample extract and S is the weight of the sample.

3.11.5. Determination of chocking property in germinated and fermented popcorn

Thirty albino rats of the Wistar strain (males and females), weaned at 21 days, were obtained from the disease-free stock of the central animal house of College of Medicine, University of Ibadan. The rats were reared on a balanced commercial stock diet (Pfizer Livestock Feed Ltd, Ikeja, Nigeria) until they were 30 days old. The rats were grouped into six containing 5 rats per group as follows:

Group 1: GPA- Germinated popcorn-African locust bean diet

Group 2: GPB- Germinated popcorn-Bambara groundnut diet

Group 3: GPAB- Germinated popcorn-African locust bean-Bambara groundnut diet

Group 4: FPA- Fermented popcorn-African locust bean diet

Group 5: FPB- Fermented popcorn-Bambara groundnut diet

Group 6: FPAB- Fermented popcorn-African locust bean-Bambara groundnut diet

The animals were housed in perforated Perspex cages, fed with the formulated food samples for 5 weeks. The percentage and survival probability (S_t) were calculated for the groups of animals as described by Kaplan and Meier (1958).

$$St = \frac{\text{Number of subjects living at the start - Number of subjects died}}{\text{Number of subjects living at the start}}$$

3.12. Relationship between phytate and selected minerals molar ratios as indices of their bioavailability in human body

There are many techniques used to determine the bioavailability of minerals in the human body. One of the methods is by measuring the molar ratios of phyate/minerals in the food and diet (Morris and Ellis, 1989). The mole of phytate and minerals was determined by dividing the weight of phytate and minerals with its atomic weight (phytate: 660g/mol; Fe: 56g/mol; Zn: 65g/mol; Ca: 40 g/mol). The molar ratio between phytate and mineral was obtained after dividing the mole of phytate with the mole of minerals. The calculated phytate/mineral molar ratios were compared with the critical values, that is, phytate: calcium > 0.24 (Morris and Ellis, 1985), phytate: iron > 1 (Hallberg *et al*., 1989), phytate : zinc >15 (Turnlund *et al*.,1984; Sandberg *et al*., 1987; Morris and Ellis, 1989), phytate : calcium/zinc > 200 (Davies *et al*.,1985; Bindra *et al*., 1986; Hemalatha *et al*., 2007).

3.13. Determination of Functional Property of the Formulated Food Samples

3.13.1 Determination of water absorption capacity (WAC)

Water absorption capacity of the food sample was determined using the method of Sathe *et al*. (1998a) as modified by Adebowale *et al.,* (2005). 10 ml of distilled and deionized water was added to 1.0 g of the sample in a beaker. The suspension was stirred using magnetic stirrer for 5 minutes. The suspension obtained was centrifuge at 3500 rpm for 30 minutes and the supernatant was measured into a 10 ml graduated cylinder. Water absorbed was calculated as the

difference between the initial volume of water added to the sample and the volume of the supernatant.

3.13.2 Determination of least gelation property

The least gelation property of the food sample was determined using the method described by Sathe *et al*. (1998a). Sample suspensions of 2 – 16% were prepared in distilled water. 10 ml of each of the prepared dispersions was transferred into a test tube and heated in a boiling water bath for 1hour, cooled rapidly in a cold water bath, and was allowed to cool further at 4^0C for 2 hours. The least gelation concentration was determined when the sample from the inverted test tube did not slip or fall.

3.13.3 Determination of bulk density

The bulk density (Packed Bulk density and Loose Bulk density) was determined using the procedure of Akpapunam and Markakes, (1981) as modified by Narayana and Narasinga Roa (1984). A known quantity of the flour mixes was put into a known weighed 5ml measuring cylinder (W_1). For packed bulk density (PBD), the measuring cylinder was gently tapped to eliminate air spaces between the flour mixes in the measuring cylinder and the volume was noted to be the volume of the sample used. The new mass of the sample and the measuring cylinder were recorded as (W_2). The Bulk density (BD) was expressed as:

$$\text{BD} = \frac{W2 - W1}{Volume\ of\ sample\ used} x\ 100$$

For loosed bulk density (LBD) space was eliminated by tapping.

3.13.4 Determination of swelling capacity (SC)

The swelling capacity was determined by the method described by Leach *et al*. (1959) as modified by Abraham (1993). Samples (5g) were weighed into dry test tube 50ml centrifuge tube. About 30 ml distilled water was added and mixed gently. The slurry was heated in water bath at desire temperatures- 40^0C, 50^0C, 60^0C, 70^0C, 80^0C, 90^0C for 30mins in a thermostat water bath. During heating, the slurry was stirred gently to prevent clumping of the starch. On completion of the 30 mins, the tube containing the paste was cooled and centrifuged at 2,200 rpm for 15 min. The supernatant was decanted immediately after centrifugation. The tubes were dried at 50 ^{0}C for 30mins, cooled and then weighed (W_2). Centrifuge tubes containing sample alone were weighed (W_2). Centrifuge tubes containing sample alone were weighed prior to adding distilled water (W_1). Swelling capacity was calculated as follows:

$$\text{SC} = \frac{W2 - W1}{Weight\ of\ sample} x\ 100$$

3.14 Determinations of Microbiological Status of Flour Samples

The microbial analysis was carried out on the food samples as described by Olutiola *et al.,* (1991). The analyses were carried out in triplicates. The mean colony obtained from the countable triplicate plates, were expressed as colony forming unit per gram (cfu/g).

Sterilization of Materials Used: All glasswares were washed with detergent and rinsed with clean tap water, air dried and then oven sterilized at 150^0C for 2 hours. Laboratory benches and inoculating chamber were swabbed with cotton wool moistened with 70% ethanol before and after investigation.

Preparation and sterilization of culture media: Nutrient Agar (NA) Eosine methylene blue (EMB), MacConkey Agar (MCA), Deoxocholate Agar (DCA), potatoes malt extract agar and

dextrose agar were used as culture media for the investigation. The media were prepared by dissolving 28g of the dehydrated nutrient agar (NA) powder in a liter of distilled water in a conical flask. While 50g of malt extract agar powder was dispensed in 1litre of distilled water. The agar were allowed to soak for 10 minutes, gently swirled to mix, cotton plugged, covered with aluminum foil and then sterilized at 121^0C for 15 minutes.

Sample preparation: One gram of each samples were aseptically transferred into a sterile beaker and macerated with sterile glass rod for about 5 minutes. The sample was thoroughly mixed to form a homogenous solution. 20 ml of sterile distilled water was added while the maceration continues for another 5 minutes until the sample formed a paste. 1ml aliquot of the samples was pipetted into sterile 20 cm^3 test tube and serially diluted in another six sets of test tube each containing 9 ml of sterile distilled water to dilution ratio 10^{-6}. 1ml portion of the diluents from the forth (10^{-4}) and second (10^{-2}) dilution factors were aseptically separately pipetted into different sterile Petri dishes and cool (45^0C) sterile malt agar media was added, swirled gently for even distribution of the inoculums, allow to set (solidify) and incubated at 37 ± ^{0}C for 24 hours.

Isolation and enumeration of microorganisms: Mould and yeast count were carried out using potato dextrose agar (PDA) (DIFCO, Detroit, MI). Twenty-five grams (25g) of each sample was blended and homogenated by shaking the mixture. One milliliter (1ml) was pipetted into 9ml of buffered peptone water (BPW) and aspirated 10 times with pipette. One milliliter (1ml) of each dilution was plated in corresponding duplicate marked plates containing Potato Dextrose Agar (PDA) and allowed to solidify. The plates were incubated at 25^0C for 5 days. Colonies were enumerated and counted (cfu/g). Mould isolates were purified on PDA, subcultured and identified by the method described by Nelson *et al.* (1983).

Escherichia coli count was done with Violet Red Blue Agar (VRB). Twenty-five grammes (25g) of each sample were mixed by shaking with 225ml of buffered peptone water (BPW). One milliliter (1ml) of each solution was pipette into 9 ml BPW. One milliliter (1ml) of each homogenate (dilution) was plated in corresponding duplicate marked plates containing Violet Red Blue (VRB) agar and allowed to solidify. Incubation was carried out at 44.5^0C for 48h and the counts done.

Coliform count was carried out with Lauryl Sulphate Broth. Twenty-five grams (25g) of each blended sample was mixed with 225 ml BPW and homogenated. One milliliter (1ml) of solution was pipetted into 9ml BPW in a tube. Each of the Lauryl Sulphate Broth (LSB) in tubes was inoculated with 1ml of each dilution using sterile pipettes. Incubation was carried out for each of the LSB tubes at 37 ^{o}C for 48 h. Confirmatory test for the coliform bacteria was done by transferring a loop from each of the LSB tubes into a separate tube of Brilliant Green Bile Agar (BGBA) broth. The tubes were incubated at 37 ^{0}C for 48 h. Gas formation confirmed the presence of coliform bacteria.

3.15 Determination of Nutritional Quality: *in vivo* protein digestibility

Procurement of Animals- Seventy (70) healthy albino rats (males and females) of 4 weeks old maturity randomly distributed into ten (10) groups containing seven animals per group with reference to sex and weight as follows:

Animal grouping

Group 1: GPA- Germinated popcorn-African locust bean diet

Group 2: GPB- Germinated popcorn-Bambara groundnut diet

Group 3: GPAB- Germinated popcorn-African locust bean-Bambara groundnut diet

Group 4: FPA- Fermented popcorn-African locust bean diet

Group 5: FPB- Fermented popcorn-Bambara groundnut diet

Group 6: FPAB- Fermented popcorn-African locust bean-Bambara groundnut diet

Group 7: Control (Ogi, a traditional complementary food)

Group 8: Control (Cerelac, a commercial complementary food)

Group 9: Basal diet (This comprised fermented corn flour, vegetable oil, minerals and vitamins premix)

Group 10: Baseline group.

Formulated complementary foods and *Cerelac* (a control sample) were prepared by incorporating basal diet to achieve an iso-nitrogenous diet at 10% protein level. The rats were housed in individual metabolic cages. Weighed diets and water were given *ad libitum* for 28 days and unconsumed diets were collected and weighed daily. The length and weight of the animals were measured daily, while their faeces and urine were collected and pooled for each group. The faeces and urine were analysed for nitrogen using AOAC (2005) method. At the end of the test period, the animals were sacrificed with chloroform, the kidney, liver and heart were quickly excised and weighed. The values of weight gained by the animals and feacal and urinary nitrogen determination were used to evaluate the protein qualities of the formulated diets using the following mathematical equations described by FAO/WHO (1989) and AOAC (2000).

Biological value (BV):

$$\text{BV} = \frac{Ni - (Nf - Nef) - (Nu - Neu)}{Ni - (Nf - Nu)} x\ 100$$

Where

Ni = Nitrogen intake, Nf = Fecal nitrogen, Nef = endogenous fecal nitrogen, Nu = urinary nitrogen, Neu = endogenous urinary nitrogen

Food efficiency (FE):

$$\text{FE} = \frac{Weight\ gained}{Food\ intake}$$

Net protein utilization (NPU): NPU (= BV x TD)

$$\text{NPU} = \frac{Ni - (Nf - Nef) - (nu - Neu)}{Ni} x\ 100$$

Nitrogen retention (NR) (dietary nitrogen retained in the body):

$$\text{NR} = Ni - (Ni - Nef) - (Nu - Neu)$$

Protein efficiency ratio (PER):

$$\text{PER} = \frac{Weight\ gained}{Protein\ intake}$$

True protein digestibility (TPD):

$$\text{TPD} = \frac{Ni - (Nf - Nef)}{Ni} x\ 100$$

Protein rating (PR)

$$PR = PER\ x\ Dailly\ protein\ intake(g)$$

3.16. Metabolizable Energy (ME) and Digestible Energy (DE)

The metabolizable energy (ME) and Digestible energy (DE) were calculated according to the method described by Goranzon *et al*. (1983)

ME = Energy intake – (energy in feaces + energy in urine)

$$\text{DE} = \frac{Gross\ energy\ intake - Gross\ energy\ content\ of\ feaces}{Gross\ energy\ intake} x\ 100$$

3.17 Calculation of Digestibility Indispensable amino Acid Score (DIAAS)

The digestible (dietary) indispensable amino acid score (DIAAS) was calculated using the method described by FAO (2013). The digestible (dietary) indispensable amino acid score (DIAAS) can be obtained from the digestible indispensable amino acid (DIAA) content in 1 g protein of tested food sample(s) and recommended amino acid scoring patterns for humans (6 months to 3 years) ratio FAO (2013). These values can be calculated using the following equations

$$\text{DIASS}(\%) = 100\ x\ lowest\ value[\frac{mg\ of\ digestible\ EAA\ in\ 1g\ of\ dietary\ protein)}{mg\ of\ the\ same\ EAA\ in\ 1g\ of\ reference\ protein}$$

Where *EAA = essential amino acids*

Or

$$\text{DIASS}(\%) = 100\ x\ lowest\ value\ (digestible\ EAA\ reference\ ratio\ for\ a\ given\ amino\ acid\ scoring\ pattern$$

Where DIAA is calculated as

Digestible IAA content = mg of IAA in 1 g protein of food multiplied by the true ileal digestibility coefficient for the same dietary indispensable amino acid (the digestibility coefficient is the percentage value divided by 100, e.g. digestibility = 90%, coefficient = 90/100 = 0.90).

3.18 Determination of Growth Patterns of the Wistar Rats fed with the Formulated Complementary Foods and Control Food Samples

Anthropometric measurements: The anthropometric measurements of the experimental rats were determined.

Weight measurement: The weights of the animals were determined in a weighing balance (Salter scale, SL20348, UK). During the measurement, the animal was centered in the weighing

tray and the weight recorded in grams. The weighing balance was checked and frequently adjusted to zero weight before each measurement was taken to minimize error.

***Length measurement*:** The animal was placed on fixed hardboard with a length measuring device (Rotring meter rule, Germany) that was marked in centimeters segments, with the zero ends at the edge of the head of the animal on the hard board. The animal was stretched by holding the back whiles pressing it firmly against the board to allow the animal to stretch appropriately. The body lengths of the rats were measured in centimeters starting from the nose to the anus (Novelli *et al*., 2007; Altunkaynak and Özbek, 2009).

Nutritional status classifications: Nutritional indices like body mass index (BMI), weight-for-age, weight-for-height and length-for-age were used to classify the animals into different categories of nutritional status using animals in control group, that is, those animals that were fed with the commercial formula and ogi as the reference standards. The following anthropometrical indices were used to determine the animal growth patterns: height-for-age portrays performance in terms of linear growth, and essentially measures long-term growth faltering (stunting); weight-for-height reflects body proportion, or the harmony of growth, and is particularly sensitive to acute growth disturbances (wasting); weight-for-age represents a convenient synthesis of both linear growth and body proportion (underweight) and Body mass index (BMI) = body weight (g)/length^2 (cm^2) (Novelli *et al*., 2007; Altunkaynak and Özbek, 2009)

3.19 Determination of Haematological Properties of Wistar Rats Fed with the Formulated Complementary Foods and Control Diets

Blood for haematological analysis was drawn by cardiac puncture after the animal was sacrificed using chloroform. Blood samples were collected using syringe and preserved in tubes containing ethylenediaminetetraacetate (EDTA) solution. The haematological analyses were determined as described by Lamb (1981).

3.19.1 Determination of haemoglobin of Albino rats using colourimetric method

A 0.2ml of whole blood was pipette with RAININ Pipet (plus L60926, U.S.A) into a test tube containing 5ml Drabkin's reagent. The pipette was rinsed 3 to 4 times with the content in the test tube. The content in the test tube was mixed thoroughly by swirling and allowed to stand for 15 minutes at room temperature (25°C). The absorbance of the content was measured using an auto analyzer (heamalyzer junior, U.K) against the reagent blank at a wavelength of 540 nm. The value of the haemoglobin level was determined based on the calculation method shown below.

$$HB = \frac{\text{Absorbance of standard X Standard concentration (g/dl)}}{\text{Absorbance of sample}}$$

3.19.2 Determination of packed cell volume (PCV)

The packed cell volume was determined by the microhaematocrit method (Hercberg *et al*., 1991). Two capillary tubes (length 75 mm, internal diameter 1.16 mm, wall thickness 0.20 mm) were filled with blood to between 5.5 and 6 mm of the total length. The dry ends of the tubes were sealed and centrifuged for 5 minutes at 12,000 x g. The volume occupied by the red blood cells was measured by a microhaematocrit reader and expressed as a percentage of the total blood in the tubes. Blood samples (0.02 ml) were mixed with anticoagulant (sequestering) and diluted in 4 ml formal citrate solution. The diluted blood was placed in a counting chamber, and red blood cells were counted under a dry objective lens.

3.19.3 Determination of White blood count (WBC) of Albino Rats using Neubauer ruled Chamber

A 0.38ml of white blood count (WBC) diluting fluid was measured and dispensed into small containers of twenty micro liters (20µl) of well mixed EDTA and anti coagulated venous

blood was added and mixed. The counting chamber was assembled making sure that the central grid areas and the special haemolytometer cover glass are completely clean and dry. The cover glass is slide in a position over the grid areas and pressed down on each side until the rainbow color (Newton's ring) was seen. Prior to moistening of the chamber surface of each grid areas the cover glass was strongly adhered to the chamber. A capillary, Pasteur pipette was held at angle of about 45^{o} was used to remix the diluted blood. One of the grids chamber was filled with the sample by making sure that it did not over fill the area. The chamber was left undisturbed for about 2 minutes to allow the white blood cells to settle. The underside of the chamber was dried and placed on the microscope stage. The white blood cells were counted in the four large corner squares of the chamber. The number of white cells per liter of blood was determined using the following sample calculation: The total number of cells counted divided by 20 and expressed as 10^{9}/l of white cells.

Calculations

The haematological indices of mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were calculated using the heamoglobin concentration (Hb) and hematocrit (Hct) according to the following formulae (Dacie and Lewis, 2001; Ochang *et al*., 2007):

Equations

$$MCHC\ (g\ l^{-1}) = [Hb\ (g\ dl^{-1}) \times 10]/Hct \times 100$$

$$MCH\ (pg) = [Hb\ (g\ dl^{-1}) \times 10]/\ RBC\ (10^{6}\ \mu l^{-1})$$

$$MCV\ (fl) = Hct/RBC\ (10^{6}\ \mu l^{-1})$$

3.20 Sensory Evaluation of Formulated Foods and Control Food Samples

The formulated food samples were prepared into light gruels, using about 20 g and 60 ml of water. The reconstituted formulated food samples and the control food samples (i.e., *Cerelac*, a commercial weaning food and Ogi, a traditional weaning food) were coded and presented to 30 untrained panelists (Nursing mothers) that were familiar with the existing complementary foods (i.e., *Cerelac* and Ogi). The panel members were assigned individually to well illuminate laboratory booths and the gruels prepared were served at 40 ^{0}C in white and transparent glass cups coded with random three digits. The panelists were instructed to rank the gruels on the basis of appearance (color), taste, odour, texture (mouth feel) and overall acceptability using a nine point Hedonic scale as described by Ihekoronye and Ngoddy (1985) and Ruston *et al*., (1996) (where, 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5= neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely).

The participation of nursing mothers in complementary food formulation and acceptability testing encourages them to gain nutrition knowledge and positive attitudes towards dietary improvements (Pelto *et al*., 2003).

3.21 Cost Production of the formulated Food Samples

The estimated costs of food materials used for the formulation, that is, popcorn kernel, Bambara groundnut and African locust bean, were based on the current market prices. The control food sample, that is, *Cerelac* was bought for N850 per a tin (450g).

3.22 Selection Criteria for Determining Optimal Complementary Food

Ranking of formulated complementary food samples to determine the optimal nutritional profile was determined as described by Griffith *et al*. (1998) with slight modification. However, the scale has not been validated. The ranking system was determined using some nutritional criteria, such as protein content, energy value, mineral molar ratios, bioavailability of minerals, total essential amino acids, fatty acids, sensory attributes, nutritional status, protein quality and heamatological properties were considered. Based on the relative importance and interrelationship of these criteria, ranking was reported on an equal weight basis. The weighting of those criteria as to relative importance produced identical conclusive results. The six blends were ranked from 1 to 6 (i.e., worst to best) to objectively determine the choice complementary blend. The blend yielding the highest score was considered to possess the most suitable nutritional characteristics.

3.23 Statistical Analysis

Data were collected in triplicate. The statistics software of Microsoft Excel (2007) and Statistical Package for Social Sciences (SPSS) version 16.0 for Windows were used to analyse the results. The mean and standard error of the mean (SEM) of the results were calculated. The comparison of the mean differences in proximate, mineral, amino acids, fatty acids, antinutritional factors, sensory attributes and functional properties between food groups was determined using one-way analysis of variance (ANOVA). Significant difference was determined at $p<0.05$. Duncan's Multiple Range Test (DMRT) was used to separate the means.

CHAPTER FOUR

RESULTS

4.1 Nutrient Composition of Processed Flour Samples of the Formulations - Popcorn, Bambara Groundnut and African Locust Bean Flour

4.1.1 Nutrient composition of popcorn flour

The proximate compositions of popcorn flour are presented in Table 4.1. The protein content of fermented popcorn (FPC) flour was 14.37±0.52 g/100g, while that of germinated popcorn flour (GPC) was 14.24±0.69 g/100g. Comparatively, protein contents of both germinated and fermented popcorn flour samples were significantly higher ($p<0.05$) than that of raw popcorn flour (RPC) (12.13±0.56 g/100g) sample. Energy value of the popcorn flour samples ranged from 322.53±8.91 kcal in FPC to 424.38±7.75 kcal in RPC. Statistically, the protein contents in FPC was significantly higher ($p<0.05$)when compared with the protein content in GPC and RPC respectively.

The mineral composition of raw, germinated and fermented popcorn flour samples are presented in Table 4.2. The mineral compositions ranged from 1.37±0.13mg/100g (Mn) to 143.55±9.13mg/100g (P) in raw popcorn flour, 1.74±0.21 mg/100g (Mn) to 156.93±13.21 mg/100g (P) in germinated popcorn flour and 1.35±0.04 mg/100g (Cu) to 142.51±14.62 mg/100g (P) in fermented popcorn flour sample. Germinated popcorn flour had higher phosphorous, potassium, calcium, magnesium, iron and copper contentthan raw and fermented popcorn flour samples.

Table 4.1: Proximate compositions (g/100g dry weight matter) of raw, germinated and fermented popcorn flour

Nutrient/Sample	Raw Popcorn Flour	Germinated Popcorn Flour	Fermented Popcorn Flour	*Recommended values (g/100g)
Moisture	6.68[a] ±0.88	4.84[c] ±0.78	5.43[b] ±0.47	<5
Protein	12.13[b] ±0.56	14.24[a] ±0.69	14.37[a] ±0.52	>15
Fat	6.86[a] ±1.59	6.39[a] ±1.14	5.85[a] ±1.63	10-25
Ash	1.49[a] ±0.08	1.39[a] ±0.11	0.87[b] ±0.07	<3
Fiber	1.12[a] ±0.16	1.12[a] ±0.17	0.81[a] ±0.21	<5
Carbohydrate	78.31[a] ±1.89	76.85[a] ±0.89	78.09[a] ±1.25	64
Energy (Kcal)	424.38[a] ±7.75	421.93[a] ±5.58	322.53[b] ±8.91	400-425

Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$).
*FAO/WHO (1991).

Table 4.2: Mineral compositions (mg/100g) of raw, germinated and fermented popcorn flour

Nutrient/Sample	Raw popcorn flour	Germinated popcorn flour	Fermented popcorn flour	*Recommended value
Phosphorous	143.550[a] ±9.13	156.93[a] ±13.21	142.51[a] ±14.62	456
Potassium	101.985[c] ±6.62	141.24[a] ±3.12	122.59[ab] ±17.68	516
Sodium	113.26[b] ±0.07	137.15[a] ±0.65	140.71[a] ±0.45	296
Calcium	116.21[c] ±0.06	174.60[a] ±0.03	134.80[b] ±0.07	500
Magnesium	28.100[c] ±0.017	31.97[b] ±0.16	31.44[b] ±0,96	76
Iron	2.16[b] ±0.01	4.96[a] ±0.08	4.12[b] ±0.04	16
Zinc	2.00[b] ±0.08	2.28[a] ±0.25	2.84[a] ±0.32	3.2
Copper	1.97[b] ±0.03	2.36[a] ±0.02	1.35[c] ±0.04	160
Manganese	1.37[b] ±0.13	1.74[a] ±0.21	1.96[a] ±0.11	32
Nickel	-	-	-	-
Rubidium	-	-	-	-
Molybdenum	-	-	-	-
Cadmium	-	-	-	-
Bromine	-	-	-	-
Strontium	-	-	-	-
Astatine	-	-	-	-
Lead	-	-	-	-
Aluminium	-	-	-	-
Iodine	-	-	-	-

(-) Not detected; Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$); *FAO/WHO (1991).

Table 4.3 shows the amino acid profile of raw, germinated and fermented popcorn flour. The ranged values of amino acid profile of raw, germinated and fermented popcorn flour sample were 0.82±0.03 to 9.08±0.03 mg/100g of protein, 0.26±0.02 to 7.59±0.01 mg/100g of protein and 0.41±0.01 to 12.09±0.04mg/100g of protein respectively. Total non-essential amino acid compositions of the samples ranged from 20.90 mg/100g in fermented popcorn flour to 23.71 mg/100g in raw popcorn flour. For the conditionally essential amino acids, the values ranged from 6.64 mg/100g in fermented popcorn to 14.78 mg/100g in raw popcorn flour sample; while total essential amino acids (TEAAs) plus histidine ranged from 13.52 mg/100g in fermented popcorn to 26.55 mg/100g in the raw popcorn.

Fatty acid compositions of raw, germinated and fermented popcorn flour are presented in Table 4.4. Total saturated fatty acid composition of germinated popcorn (62.18 mg/100g) was significantly higher ($p<0.05$) than that of fermented popcorn (13.19 mg/100g) and raw popcorn (4.50 mg/100g) flour samples respectively. For polyunsaturated fatty acid (PUFA) compositions, fermented popcorn flour sample (68.74 mg/100g) was significantly higher ($p<0.05$) than raw popcorn flour (28.30 mg/100g) and germinated popcorn (2.47 mg/100g) samples respectively. Total monounsaturated fatty acid compositions ranged from 17.94 mg/100g in fermented popcorn to 67.20 mg/100g in raw popcorn flour samples.

Table 4.3: Amino acid compositions (mg/100g protein) of raw, germinated and fermented popcorn flour

Amino acids	Raw Popcorn flour	Fermented Popcorn flour	Germinated Popcorn flour
Non essential amino acids			
Alanine	3.40^{c}	5.85^{a}	4.27^{b}
	±0.02	±0.05	±0.05
Aspartic acid	8.21^{a}	7.21^{b}	4.34^{c}
	±0.01	±0.02	±0.03
Serine	3.025^{a}	0.26^{b}	0.57^{c}
	±0.03	±0.02	±0.04
Glutamic acid	9.08^{b}	7.59^{c}	12.09^{a}
	±0.03	±0.01	±0.04
Total	23.71	20.90	21.27
Conditionally essential amino acids			
Proline	2.14^{a}	0.55^{c}	1.06^{b}
	±0.01	±0.01	±0.02
Glycine	4.18^{a}	0.36^{c}	2.03^{b}
	±0.03	±0.01	±0.07
Arginine	4.16^{a}	4.06^{a}	3.69^{b}
	±0.01	±0.02	±0.05
Cysteine	1.05^{a}	0.54^{b}	0.41^{c}
	±0.02	±0.05	±0.01
Tyrosine	3.25^{a}	1.14^{c}	1.33^{b}
	±0.05	±0.01	±0.04
Total	14.78	6.64	8.52
Essential amino acids + Histidine			
Lysine	5.04^{a}	2.18^{c}	2.71^{b}
	±0.06	±-.02	±0.02
Threonine	2.58^{a}	1.23^{c}	2.03^{b}
	±0.03	±0.01	±0.02
Valine	4.09^{a}	1.38^{b}	1.60^{c}
	±0.03	±0.01	±0.03
Methionine	2.175^{a}	0.56^{c}	0.81^{b}
	±0.05	±0.02	±0.02
Isoleucine	3.25^{a}	2.27^{b}	0.92^{c}
	±0.02	±0.01	±0.10
Leucine	5.19^{a}	3.78^{b}	3.28^{c}
	±0.02	±0.04	±0.04
Phenylalanine	4.23^{a}	2.13^{b}	2.28^{b}
	±0.01	±0.02	±0.08
Histidine	2.10^{a}	0.55^{c}	1.02^{b}
	±0.10	±0.05	±0.01
Tryptophan	0.82^{b}	1.12^{a}	1.10^{a}
	±0.03	±0.02	±0.13
Total	26.55	13.52	13.61

Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$).

Table 4.4: Fatty acids composition (mg/100g protein) of raw, germinated and fermented popcorn flour

Fatty acids	Raw popcorn	Germinated Popcorn	Fermented Popcorn
Saturated Fatty acids (SFA)			
Myristic acid	0.01[a]	0.00[b]	0.01[a]
Capric acid	Trace	Trace	-
Behenic acid	Trace	Trace	Trace
Lignoceric acid	Trace	Trace	Trace
Lauric acid	Trace	Trace	Trace
Palmitic acid	2.33[c]	50.42[a]	9.62[b]
Stearic acid	1.99[b]	2.48[a]	3.54[a]
Arachidic acid	0.17[b]	9.01[a]	0.15[b]
Total	4.50 [c]	62.18 [a]	13.19 [b]
Poly unsaturated fatty acids (PUFA)			
Linolenic acid	8.92[a]	0.01[b]	0.01[b]
Linoleic	19.38[b]	2.48[c]	68.73[a]
Arachidonic acids	-	-	-
Docohexanoic acid	-	-	-
Total	28.30 [b]	82.46 [c]	68.74[a]
Mono unsaturated fatty acid (MUFA)			
Palmitoleic acid	0.15[b]	0.85[a]	1.11[a]
Oleic acid	67.06[a]	34.49[b]	16.83[c]
Total	67.21[a]	35.34[4b]	17.94 [c]
P:S	6.29	0.04	5.21

Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$).

4.1.2 Nutrient Compositions of African Locust Bean Flour

The proximate compositions of raw, germinated and fermented African locust beans flour are presented in Tables 4.5. The protein content of raw, germinated and fermented African locust bean (ALB) flour were 33.64±0.41 g/100g, 41.49±1.84 g/100g and 35.36±0.23 g/100g respectively. Statistically, protein content of germinated ALB flour was significantly higher ($p<0.05$) than that of fermented and raw ALB samples respectively. The energy values of fermented ALB flour was 457.20±2.15 kcal., and was significantly higher when compared with the value of germinated ALB flour (446.71± 7.06 kcal.) and that of raw ALB flour sample (442.79±2.32 kcal) respectively.

Mineral compositions of ALB samples ranged from 1.29±0.02 mg/100g for Mn to 223.17±0.05 mg/100g for K in raw ALB flour, 1.72±0.03 mg/100g for Mn to 375.60±0.15 mg/100g for K in germinated ALB flour sample; while that of fermented ALB flour was between 1.25±0.01 mg/100g for Cu and 144.01±0.05 mg/100g for Ca. Statistically, the concentration of potassium, sodium, calcium, iron and zinc in germinated ALB flour sample were found to be significantly higher ($p<0.05$) than those of the raw and fermented ALB flour samples.

Table 4.5: Proximate compositions (g/100g Dry matter) of raw, germinated and fermented African locust beans flour

Nutrient/Sample	Raw African locust bean flour	Germinated African locust bean flour	Fermented African locust bean flour	*Recommended values (g/100g)
Moisture	5.67 [b]	3.89 [b]	8.16 [a]	<5
	±0,.42	±0.13	±1.25	
Protein	33.64[b]	41.49[a]	35.36[b]	>15
	±0.41	±1.84	±0.23	
Fat	18.21[a]	18.64[a]	18.63[a]	10-25
	±0.38	±0.83	±0.19	
Ash	3.99[a]	4.34[a]	2.34[b]	<3
	±0.49	±0.38	±0.21	
Fiber	8.08[a]	7.28[ab]	6.65[b]	<5
	±0.36	±0.35	±0.50	
Carbohydrate	36.08[a]	28.24[b]	37.01[a]	64
	±0.24	±1.92	±0.38	
Energy (Kcal)	442.79[b]	446.71[ab]	457.20[a]	400-425
	±2.32	±7.06	±2.15	

Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$).
*FAO/WHO (1991).

Table 4.6: Mineral compositions [mg/100g] of raw, germinated, fermented African locust bean flour

Nutrient/Sample	Raw African locust bean flour	Germinated African locust bean flour	Fermented African locust bean flour	*Recommended value
Phosphorous	108.50^{a} ±0.20	90.85^{b} ±0.45	75.75^{c} ±0.15	456
Potassium	223.17^{b} ±0.05	375.60^{a} ±0.15	130.14^{c} ±0.15	516
Sodium	143.60^{b} ±2.50	161.70^{a} ±0.04	132.70^{c} ±0.15	296
Calcium	122.07^{c} ±0.14	173.50^{a} ±0.04	144.01^{b} ±0.05	500
Magnesium	4.86^{a} ±0.01	4.16^{b} ±0.05	3.60^{c} ±0.10	76
Iron	2.20^{c} 0.10	6.20^{a} ±0.10	2.63^{b} ±0.02	16
Zinc	2.55^{b} ±0.15	3.03^{a} ±0.01	1.27^{c} ±0.01	3.2
Copper	4.15^{a} ±0.15	2.26^{b} ±0.01	1.25^{c} ±0.01	160
Manganese	1.29^{c} ±0.02	1.72^{b} ±0.03	2.20^{a} ±0.10	32
Nickel	-	-	-	-
Rubidium	-	-	-	-
Molybdenum	-	-	-	-
Cadmium	-	-	-	-
Bromine	-	-	-	-
Strontium	-	-	-	-
Astatine	-	-	-	-
Lead	-	-	-	-
Aluminium	-	-	-	-
Iodine	-	-	-	-

[-] Not detected; Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$)**;** *FAO/WHO (1991).

Table 4.7 shows the amino acid profile for the raw, germinated and fermented African locust bean flour samples. Total non-essential amino acids for the raw, fermented and germinated ALB samples were 46.22 mg/100g, 48.87 mg/100g and 44.98 mg/100g respectively. Aspartic acid and serine were found to be the highest and least concentration for non-essential amino acid respectively. The values for total conditionally essential amino acids were 16.11 mg/100g, 18.97 mg/100g and 15.34 mg/100g for the raw, fermented and germinated ALB flour sample respectively. For total essential amino acids plus histidine, the values were 27.51 mg/100g, 29.96 mg/100g and 26.13 mg/100g of nitrogen in the raw, fermented and germinated ALB flour sample respectively. The lysine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan and histidine content of fermented ALB were observed to be significantly higher ($p<0.05$) than in germinated ALB flour samples and raw ALB flour samples.

Fatty acid compositions of ALB flour samples are presented in Table 4.8. Total saturated fatty acid composition of the African locust bean samples were 46.90 mg/100g in the raw flour, 45.30 mg/100g in germinated flour and 49.44 mg/100g in fermented flour sample. For the total polyunsatured fatty acid compositions, the values were 33.69 mg/100g in the raw ALB flour, 31.78 mg/100g in germinated ALB flour and 29.10 mg/100g in fermented ALB flour sample. The linoleic acid value of germinated ALB flour (31.58 mg/100g) was observed to be higher than that of fermented ALB flour (28.70 mg/100g), but lower than in the raw ALB flour (33.69 mg/100g) sample.

Table 4.7: Amino acid compositions [mg/100g protein] of raw, germinated and fermented African locust bean flour

Amino acids	Raw African locust bean flour	Fermented African locust bean flour	Germinated African locust bean flour
Non essential amino acids			
Alanine	4.72[b]	5.02[a]	4.32[c]
	±0.01	±0.01	±0.02
Aspartic acid	22.82[b]	23.15[a]	21.79[c]
	±0.01	±0.02	±0.01
Serine	3.86[b]	4.20[a]	3.54[c]
	±0.05	±0.01	±0.01
Glutamic acid	14.83[b]	16.50[a]	15.15[b]
	±0.02	±0.01	±0.15
Total	46.22	48.87	44.98
Conditionally essential amino acids			
Proline	4.29[a]	4.29[a]	3.88[b]
	±0.02	±0.02	±0.01
Glycine	3.42[c]	5.30[a]	3.42[b]
	±0.01	±0.01	±0.01
Arginine	4.15[b]	4.84[a]	3.96[c]
	±0.01	±0.01	±0.01
Cysteine	1.70[a]	1.76[a]	1.85[a]
	±0.02	±0.01	±0.25
Tyrosine	2.55[a]	2.52[a]	2.24[b]
	±0.01	±0.02	±0.01
Total	16.11	18.97	15.34
Essential amino acids			
Lysine	5.34[b]	5.92[a]	5.04[c]
	±0.01	±0.02	±0.01
Threonine	2.55[b]	3.17[a]	2.27[c]
	±0.01	±0.01	±0.01
Valine	4.18[a]	4.24[a]	3.79[b]
	±0.01	±0.01	±0.02
Methionine	0.88[a]	0.90[a]	0.81[b]
	±0.01	±0.01	±0.01
Isoleucine	3.32[b]	3.50[a]	3.29[b]
	±0.01	±0.01	±0.01
Leucine	5.74[b]	5.81[a]	5.52[c]
	±0.01	±0.01	±0.02
Phenylalanine	3.80[b]	4.12[a]	3.70[c]
	±0.02	±0.01	±0.05
Histidine	1.73$_b$	2.32[a]	1.74[b]
	±0.02	±0.01	±0.03
Tryptophan	0.52[c]	0.84[a]	0.75[b]
	±0.01	±0.01	±0.02
Total	27.51	29.96	26.13

Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$).

Table 4.8: Fatty acid compositions (mg/100g protein) of raw, germinated and fermented African locust bean

Fatty acids	Raw African locust bean flour	Germinated African locust bean flour	Fermented African locust bean flour
Saturated Fatty acids (SFA)			
Myristic acid	Trace	Trace	Trace
Lauric acid (C12:0)	Trace	Trace	Trace
Lignoceric acid (C24:0)	0.73^{b}	2.02^{a}	0.01^{c}
Palmitic acid (C16:0)	17.26^{a}	15.48^{b}	12.88^{c}
Stearic acid (C18:0)	18.09^{b}	18.07^{c}	19.72^{a}
Arachidic acid (C20:0)	3.82^{b}	3.31^{c}	5.17^{a}
Behenic acid (C22:0)	7.74^{c}	8.44^{b}	11.68^{a}
Total	47.64	47.32	49.46
Poly unsaturated fatty acids (PUFA)			
Linoleic acid (C18:2)	33.69^{a}	31.58^{b}	28.70^{c}
Linolenic acid (C18:3)	Trace	Trace	0.40
Arachidonic acids (C20:4)	-	-	-
Docohexanoic acid (C22:2)	-	-	-
Total	33.69	31.58	29.10
Mono unsaturated fatty acid (MUFA)			
Palmitoleic acid (C16:1)	0.01^{b}	0.01^{b}	0.14^{a}
Oleic acid (C18:1)	18.59^{c}	20.83^{a}	20.15^{b}
Total	18.60	20.84	20.29
P:S	0.718	0.701	0.589

Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$).
(-) Not detected

4.1.3 Nutrient Compositions of Bambara Groundnut Flour

Proximate compositions of the raw, germinated and fermented Bambara groundnut (BG) flours are shown in Table 4.9. The protein content of Bambara groundnut flours ranged from 20.92±1.30 g/100g in raw BG to 32.58±3.79 g/100g in germinated BG flour sample. Statistically, the protein content of germinated BG flour sample was significantly higher ($p<0.05$) when compared with fermented BG and that of raw BG flour sample respectively. Energy values of germinated BG (422.52 ±1.72 kcal) and fermented BG (419.69±1.22 kcal.) flour were significantly higher ($p<0.05$) than raw BG flour sample (414.76±1.31 kcal). However, there was no significant difference between the energy values of germinated and fermented BG flour samples ($p>0.05$).

The mineral compositions of raw, germinated and fermented Bambara groundnut flours are presented in Table 4.10. Mineral composition of the food flour ranged from 1.03±0.02 mg/100g for copper to 426.02±0.71 mg/100g for potassium in raw BG flour. For germinated BG flour, the values ranged between 1.69±0.02 mg/100g for manganese and 347.97 mg/100g for potassium; while mineral contents of fermented BG flour sample ranged from 1.45±0.02 mg/100g for copper to 166.9±0.11 mg/100g for potassium. Statistically, the mineral compositions of germinated and fermented BG flour were significantly lower ($p<0.05$) for K, Na, Ca, Mg and Zn than that of the raw BG flour samples.

Table 4.9: Proximate compositions (g/100g dry weight matter) of raw, germinated and fermented Bambara groundnut flour

Nutrient/Sample	Raw Bambara groundnut flour	Germinated Bambara groundnut flour	Fermented Bambara groundnut flour	*Recommended values (g/100g)
Moisture	1.99[b]	5.02[a]	5.05[a]	<5
	±0.95	±0.59	±0.66	
Protein	20.92[b]	32.58[a]	27.94[ab]	>15
	±1.30	±3.79	±2.12	
Fat	6.19[b]	9.99[a]	8.94[ab]	10-25
	±0.03	±1.03	±0.82	
Ash	2.18[a]	3.82[a]	2.75[a]	<3
	±0.09	±0.57	±0.50	
Fiber	1.87[b]	3.03[ab]	3.50[a]	<5
	±0.02	±0.30	±0.47	
Carbohydrate	68.83[a]	50.58[b]	56.88[ab]	64
	±2.41	±5.70	±3.93	
Energy (Kcal.)	414.76[b]	422.52[a]	419.69[a]	400-425
	±1.31	±1.72	±1.22	

Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$).
*FAO/WHO (1991).

Table 4.10: Mineral compositions (mg/100g) of raw, germinated, fermented Bambara groundnut flour

Nutrient/Sample	Raw Bambara groundnut flour	Germinated Bambara groundnut flour	Fermented Bambara groundnut flour	*Recommended value
Phosphorous	87.48^{b} ±0,33	77.93^{c} ±0.21	90.47^{a} ±0.12	456
Potassium	426.02^{a} ±0.71	347.97^{b} ±0.23	166.90^{c} ±0.11	516
Sodium	191.17^{a} ±0.48	126.27^{c} ±1.48	141.67^{b} ±0.12	296
Calcium	163.80^{a} ±0.32	118.40^{c} ±0.05	126.67^{b} ±0.20	500
Magnesium	5.13^{a} ±0.09	2.73^{b} ±0.09	2.68^{b} ±0.01	76
Iron	4.73^{a} ±0.12	4.86^{a} ±0.01	3.73^{b} ±0.11	16
Zinc	5.33^{a} ±0.14	2.97^{b} ±0.01	2.67^{c} ±0.03	3.2
Copper	1.03^{c} ±0.02	2.17^{a} ±0.11	1.45^{b} ±0.02	160
Manganese	1.79^{b} ±0.02	1.69^{b} ±0.02	2.60^{a} ±0.06	32
Nickel	-	-	-	-
Rubidium	-	-	-	-
Molybdenum	-	-	-	-
Cadmium	-	-	-	-
Bromine	-	-	-	-
Strontium	-	-	-	-
Astatine	-	-	-	-
Lead	-	-	-	-
Alumininum	-	-	-	-
Iodine	-	-	-	-

(-) Not detected; Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$)**;** *FAO/WHO (1991).

Table 4.11 shows the amino acid profile of raw, germinated and fermented Bambara groundnut flour samples. The values of total non-essential amino acid composition of the samples were 29.98 mg/100g in raw BG flour, 28.14 mg/100g in fermented BG flour and 27.58 mg/100g in germinated BG flour sample. Glutamic acid was the highest among the amino acids for the raw, fermented and germinated BG samples, while alanine and serine were the least in the raw, germinated and fermented BG flour sample respectively. The values of total conditionally amino acid of the samples ranged from 13.59 mg/100g in the raw BG flour to 15.27 mg/100g in germinated BG flour sample. Arginine and cystein were found to be the highest and the least concentration for the conditionally essential amino acid group respectively. Total essential amino acid plus histidine compositions of the flour samples ranged from 27.17 mg/100g in fermented BG to 30.16 mg/100g in raw BG flour sample. Leucine and tryptophan were observed to be the highest and lowest concentration for essential amino acids respectively.

Fatty acid compositions of the raw, germinated and fermented Bambara groundnut flours are presented in Table 4.12. The values of saturated fatty acid compositions of the samples were as follows: 66.16 mg/100g, 55.04 mg/100g and 51.91 mg/100g in the raw, germinated and fermented BG flour samples respectively. For polyunsaturated fatty acid, the values ranged from 6.05 mg/100g in raw BG flour to 55.04 mg/100g in germinated; while that of mono unsaturated fatty acid were 28.26 mg/100g, 15.85 mg/100g and 22.62 mg/100g in raw, germinated and fermented BG flour respectively.

Table 4.11: Amino acid compositions (g/100g crude protein) of raw germinated and fermented bambara groundnut flour samples

Amino acids	Raw Bambara groundnut flour	Fermented Bambara groundnut flour	Germinated Bambara groundnut flour
Non essential amino acids			
Alanine	2.50[a]	2.72[a]	1.95[a]
	±0.20	0.68	±0.15
Aspartic acid	9.59[b]	10.23[a]	9.29[b]
	±0.02	±0.11	±0.07
Serine	3.27[a]	1.87[b]	3.17[a]
	±0.06	±0.09	±0.15
Glutamic acid	14.62[a]	13.32[b]	13.17[b]
	±0.11	±0.25	±0.18
Total	29.98	28.14	27.58
Conditionally essential amino acids			
Proline	1.97[a]	2.30[a]	2.35[a]
	±0.04	±0.01	±0.15
Glycine	3.05[b]	4.00[a]	3.93[a]
	±0.05	±0.03	±0.01
Arginine	4.79[a]	4.50[a]	4.99[a]
	±0.08	±0.66	±1.03
Cysteine	0.93[a]	1.17[a]	1.12[a]
	±0.08	±0.13	±0.2
Tyrosine	2.85[a]	2.85[a]	2.88[a]
	±0.05	±0.15	±0.32
Total	13.59	14.82	15.27
Essential amino acids			
Lysine	5.12[a]	5.08[a]	4.77[a]
	±0.11	±0.28	±0.42
Threonine	2.57[a]	2.36[a]	2.44[a]
	±0.02	±0.31	±0.19
Valine	3.10[ab]	2.50[b]	3.48[a]
	±0.20	±0.10	±0.22
Methionine	1.16[a]	1.27[a]	1.34[a]
	±0.02	±0.03	±0.30
Isoleucine	3.97[a]	4.11[a]	3.49[b]
	±0.17	±0.06	±0.06
Leucine	7.37[a]	5.69[a]	5.89[a]
	±0.06	±0.61	±1.24
Phenylalanine	3.76[a]	3.92[a]	3.33[a]
	±0.03	±0.38	±0.15
Histidine	2.47[a]	1.75[a]	2.54[a]
	±0.07	±0.35	±0.06
Tryptophan	0.64[b]	1.02[a]	0.73[b]
	±0.07	±0.02	±0.08
Total	30.16	27.17	28.01

Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$).

Table 4.12: Fatty acid compositions (g/100g protein) of raw, germinated and fermented Bambara groundnut

Fatty acids	Raw Bambara groundnut flour	Germinated Bambara groundnut flour	Fermented Bambara groundnut flour
Saturated Fatty acids (SFA)			
Myristic acid	-	-	-
Caprylic	-	-	-
Capric	-	-	-
Margaric	-	-	-
Erucic acid	-	-	-
Lauric acid (C12:0)	-	-	-
Lignoceric acid (C24:0)	-	1.94^{a} ±0.01	0.99^{b} ±0.03
Palmitic acid (C16:0)	37.14^{a} ±0.47	13.53^{c} ±0.51	21.04^{b} ±0.46
Stearic acid (C18:0)	21.29^{b} ±0.01	25.43^{a} ±0.07	21.38^{b} ±0.14
Arachidic acid (C20:0)	7.73^{a} ±0.01	5.49^{b} ±0.04	3.03^{c} ±0.01
Behenic acid (C22:0)	0.004^{c} ±0.001	10.59^{a} ±0.49	6.18^{b} ±0.01
Total	66.16	55.04	51.63
Poly unsaturated fatty acids (PUFA)			
Linoleic acid (C18:2)	$6..05^{b}$ ±0.01	26.10^{a} ±0.04	24.76^{a} ±0.52
Linolenic acid (C18:3)	0.002 ±0.001	-	-
Arachidonic acids (C20:4)	-	-	-
Docohexanoic acid (C22:2)	-	-	-
Total	6.05	26.10	24.76
Mono unsaturated fatty acid (MUFA)			
Palmitoleic acid (C16:1)	0.91^{a} ±0.01	0.77^{a} ±0.50	-
Oleic acid (C18:1)	27.35^{a} ±0.02	15.08^{c} ±0.06	22.62^{b} ±0.231
Total	28.26	15.85	22.62
P:S	0.09	0.47	0.48

Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$).

4.2. Proximate Compositions and Energy Values of Germinated and Fermented Complementary Foods from Popcorn, African Locust Bean and Bambara groundnut

The proximate compositions of complementary foods formulated from popcorn, African locust bean and Bambara groundnut combinations are presented in Table 4.13. The moisture contents of germinated and fermented complementary foods ranged from 5.73±0.01 in GPB to 10.19±1.76 in GPA and 10.07±0.03 in FPAB to 10.70±0.73 in FPA respectively. The moisture contents of germinated complementary foods (GCF) were significantly lower than those of fermented complementary foods (FCF), but were higher when compared with control samples, that is, *Ogi and Cerelac* ($p<0.05$). Comparatively, the moisture contents of the formulated complementary foods were higher than the FAO/WHO recommended values (<5%) for infant foods (Fig. 4.1). Protein contents of GCF ranged from 23.85 ±1.54 g/100g in GPA to 28.89 ±1.02 g/100g in GPAB, while fermented complementary foods ranged from 20.49±1.08 in FPA to 26.87±1.07g/100g in FPAB. The protein contents of formulated diets in this study were higher than '*Ogi*' (a traditional complementary food), *Cerelac* (a commercial complementary food) and FAO/WHO recommended values for infant foods (<15%) respectively ($p<0.05$). Fat contents of GCF and FCF ranged from 9.89±0.01g/100g in GPAB to 12.14±0.03g/100g in GPB and 12.31±0.11g/100g in FPB to 15.59±0.15g/100g in FPAB respectively. There were significant difference between the fat contents of the formulated complementary foods and control samples ($p<0.05$). Energy values of germinated complementary foods ranged from 434.47±2.04 kcal in GPAB to 444.11±2.47 kcal in GPA, while those of FCF ranged from 441.41 ±3.05 kcal in FPA to 464.94±1.22 kcal in FPAB. Comparatively, the nutrient compositions values of both germinated and fermented complementary foods were similar, and were within the ranged values of FAO/WHO recommendation for infant food. Figures 4.2 and 4.3 compared the protein contents and energy values of formulated complementary foods with FAO/WHO

recommended dietary allowance (RDA) for infant. The protein contents and energy values of the formulations were observed to be higher than the recommended daily allowance for infant (14 g/day) (Fig. 4.2). Similarly, energy values of the formulations were higher than that of FAO/WHO recommended value for children (344 kcal/day) (Fig. 4.3).

Table 4.13: Proximate compositions (g/100g dry weight matter) of complementary foods from popcorn, African locust bean and bambara groundnut blends

Nutrient	GPA	GPB	GPAB	FPA	FPB	FPAB	Ogi	Cerelac
Moisture	10.05^{a}	5.73^{cd}	6.30 ^{bc}	10.70^{a}	10.27^{a}	10.07^{a}	7.80^{b}	4.03^{d}
	±0.76	±0.01	±0.01	±0.73	±0.01	±0.03	±0.57	±0.50
Protein	23.85^{c}	28.42^{ab}	28.84^{a}	20.49^{d}	20.87^{d}	26.87^{b}	6.52^{f}	16.67^{e}
	±1.54	±1.12	±1.02	±1.08	±1.02	±1.07	±0.31	±0.01
Fat	11.74^{d}	12.14^{c}	9.89^{e}	12.76^{b}	12.31^{c}	15.59^{a}	5.17^{g}	9.38^{f}
	±0.28	±0.03	±0.01	±0.20	±0.11	±0.15	±0.11	±0.02
Ash	1.91^{bcd}	2.26b^{c}	2.53^{b}	3.87^{a}	2.96^{b}	1.85b^{cd}	1.09^{cd}	2.4^{b}
	±0.08	±0.10	±0.12	±0.25	0.10	±0.01	±0.01	±0.01
Fiber	1.74^{c}	1.79^{c}	1.22^{d}	1.72^{c}	2.05^{c}	2.41^{b}	0.85^{e}	5.21^{a}
	±0.19	±0.01	±0.02	±0.27	±0.21	±0.18	±0.01	±0.01
Carbohydrate	60.75^{cd}	55.39^{fg}	57.50^{ef}	58.96^{de}	61.78^{c}	54.27^{g}	86.38^{a}	66.35^{b}
	±3.76	±2.04	±3.01	±1.43	±1.01	±2.11	±0.21	±0.01
Energy	444.11^{b}	444.45^{b}	434.47^{c}	441.41^{b}	441.48^{b}	464.94^{a}	418.08^{c}	431.58^{d}
(Kcal.)	±2.47	±1.07	±2.04	±3.05	±3.05	±1.22	±0.47	±0.01

Means (±SEM) with different alphabetical superscripts in the same row are significantly different ($P<0.05$).

Key
GPA (Germinated popcorn-African locust bean blend),
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend)

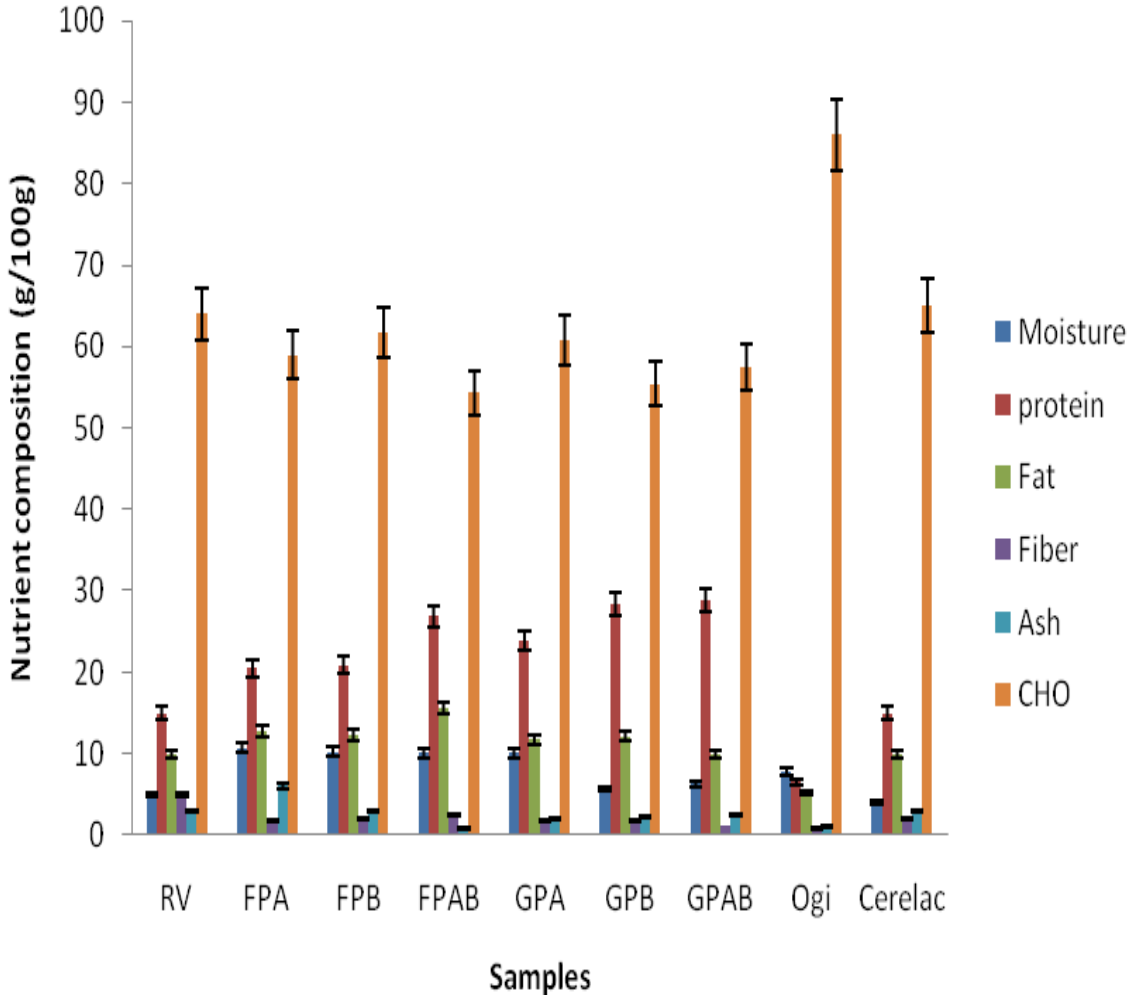

Fig. 4.1: Comparison between nutrient contents in 100g of formulated food samples with WHO recommended values (RV) for infant foods.

Key
GPA (Germinated popcorn-African locust bean blend),
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend)
RV (FAO/WHO, 1991, recommended values for infant foods)

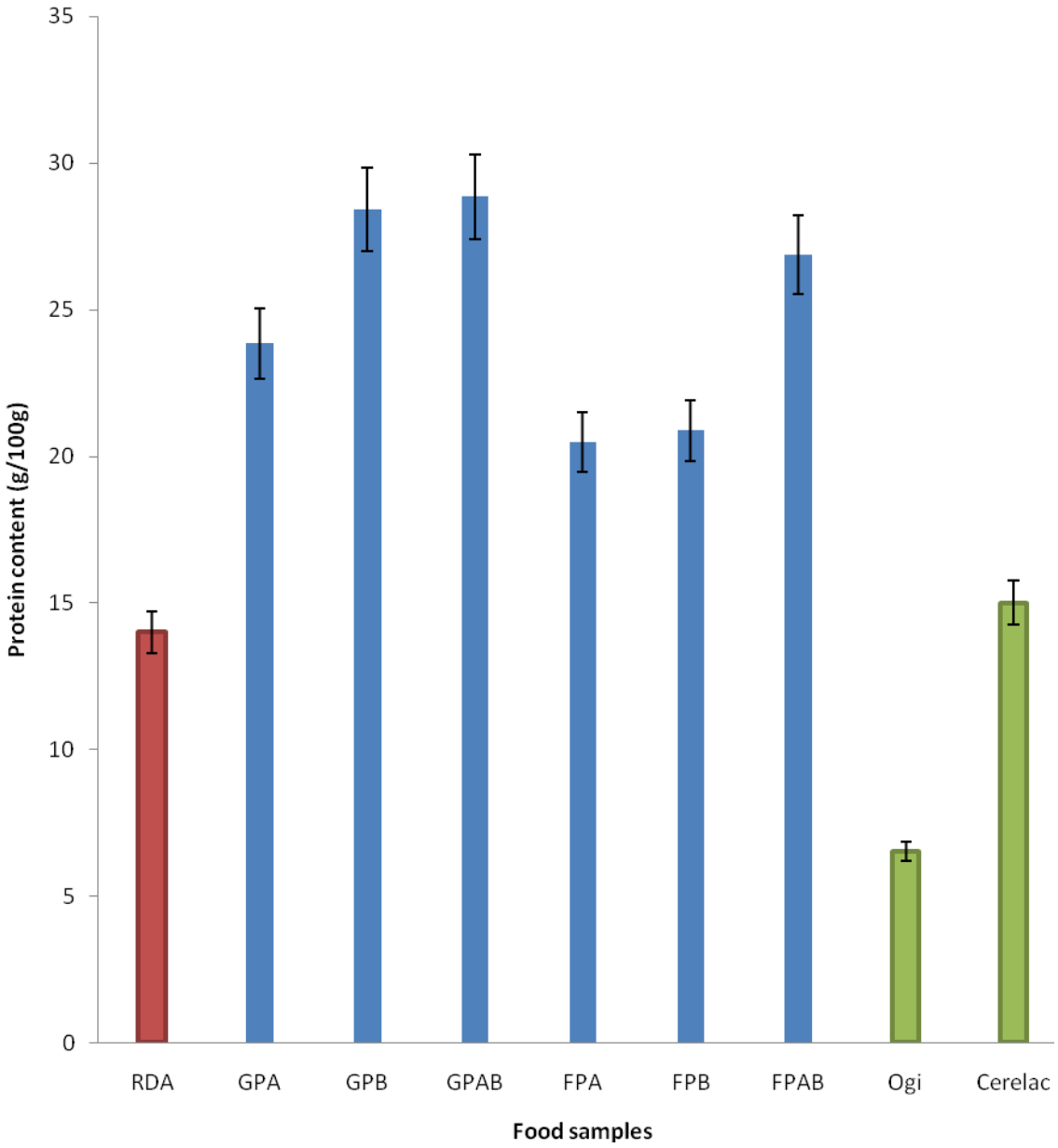

Fig. 4.2: Comparison between protein contents in 100g of formulated food samples with control food sample and recommended daily requirements of children.

Key
GPA (Germinated popcorn-African locust bean blend),
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).
RDA (recommended daily allowance)(FAO/WHO, 1991)

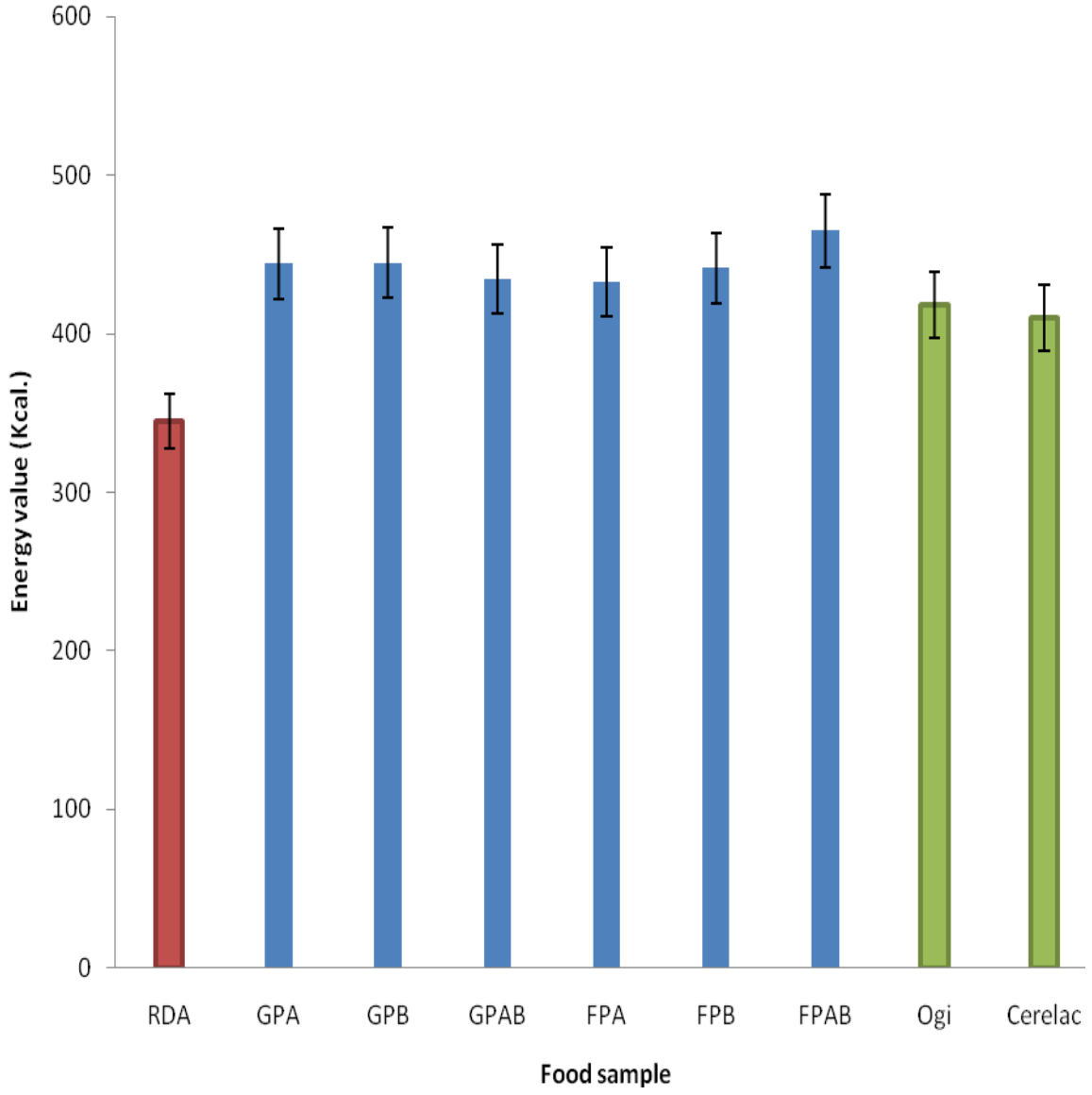

Fig. 4.3: Comparison of energy values (kcal.) in 100g of formulated food samples with control food sample and recommended daily requirement for children ($p<0.05$)

Key
GPA (Germinated popcorn-African locust bean blend),
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).
RDA (recommended daily allowance)(FAO/WHO, 1991)

4.3 Mineral Compositions of Germinated and Fermented Complementary foods from Popcorn, African Locust Bean and Bambara groundnut

Mineral compositions of the formulated complementary food samples are presented in Table 4.14. The mineral compositions of germinated and fermented complementary food samples ranged from 1.66±0.21 mg/100g to 564.30±0.20 mg/100g and 1.31±0.02 mg/100 to 173.75±0.21 mg/100g respectively. Potassium had the highest concentration, while copper had the least concentration in both the germinated and fermented complementary food samples. Statistically, the concentrations of phosphorous, potassium, calcium, iron, zinc, copper and manganese in the formulated food samples were significantly higher than in '*Ogi*', but were lower when compared with the *Cerelac* sample ($p<0.05$). The molar ratios of Na/K and Ca/K in the germinated complementary food diets were lower than that of fermented food samples; however, the values were higher when compared with the values in control samples (Ogi and *Cerelac*). The Ca/P molar ratios in germinated complementary foods were higher when compared with the fermented complementary foods and control food samples. Comparactively, the Na/K, Ca/P and Ca/K molar ratios of the formulations met the recommended values of FAO/WHO.

Table 4.14: Mineral compositions (mg/100g) of germinated popcorn, African locust bean and bambara groundnut flour blends

Nutrient/Sample	GPA	GPB	GPAB	FPA	FPB	FPAB	Ogi	Cerelac	*Ref
Phosphorous	86.70^{d} ±0,20	77.75^{g} ±0.15	88.78^{c} ±0.17	78.95^{f} ±0.35	88.90 ^{c} ±0.21	96.90^{b} ±0.32	85.95^{e} ±0.02	400.00^{a} ±0.01	456
Potassium	496.55^{c} ±0.15	564.30^{b} ±0.20	386.80^{d} ±0.11	173.75^{e} ±0.21	157.45^{f} ±0.25	132.75^{g} ±0.15	102.39^{h} ±1.01	635.00^{a} ±0.00	516
Sodium	152.90^{a} ±0.30	136.35^{e} ±0.25	141.75^{d} ±0.15	148.75^{b} ±0.15	129.30^{f} ±0.20	127.90^{g} ±0.31	14.56^{h} ±0.04	145.00^{c} ±0.00	296
Calcium	174.65^{c} ±0.15	212.75^{b} ±0.21	170.10^{d} ±0.20	136.91^{f} ±0.20	139.75^{e} ±0.15	136.68^{f} ±0.10	68.66^{g} ±0.35	600.00^{e} ±0.01	500
Magnesium	3.90^{d} ±0.21	3.20^{e} ±0.10	4.29^{c} ±1.10	2.86^{f} ±0.02	3.17^{e} ±0.03	4.98^{b} ±0.02	34.91^{a} ±0.01	-	76
Iron	5.75^{b} ±0.15	4.76^{c} ±0.01	5.88 ^{b} ±0.02	4.14^{d} ±0.01	4.25^{d} ±0.03	3.79^{e} ±0.05	0.26^{f} ±0.01	7.50^{a} ±0.01	16
Zinc	3.55^{b} ±1.10	2.76^{b} ±0.03	2.68^{b} ±0.11	2.89^{b} ±0.05	3.16^{b} ±0.02	3.83^{ab} ±0.04	0.80^{c} ±0.00	5.00^{a} ±0.00	3.2
Copper	2.26^{a} ±0.15	1.66^{b} ±0.21	2.35^{a} ±1.50	1.31^{c} ±0.02	1.35^{c} ±0.15	1.38^{c} ±0.01	1.29^{c} ±0.04	-	0.89
Manganese	1.69^{c} ±0.03	2.20^{b} ±0.10	1.68^{c} ±0.18	2.09^{b} ±0.02	2.15^{b} ±0.16	2.65^{a} ±0.05	1.93^{c} ±0.11	-	2.2
Na /K	0.31	0.24	0.37	0.86	0.82	0.96	0.14	0.23	1.4-3.4
Ca/P	2.01	2.74	1.92	1.73	1.57	1.41	0.80	1.50	1.6-3.6
Ca/K	0.35	0.38	0.44	0.79	0.89	1.03	0.67	0.94	2.2-6.2
Zn/Cu	1.57	1.66	1.14	2.21	2.34	2.78	0.62	-	2.0-4.0
Fe/Cu	2.54	2.87	2.50	3.16	3.15	2.75	0.20	-	0.2 – 1.6

(-) Not detected; Means (±SEM) with different superscripts in a row are significantly different at P<0.05. *FAO/WHO, 1991.

Key
GPA (Germinated popcorn-African locust bean blend),
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

Figure 4.4 shows the percentage of recommended daily allowance (RDA) met by the selected essential minerals (Ca, P, Zn and Fe) in the formulated complementary food samples. The formulated diets were observed to provide over half of the recommended daily allowance of zinc and iron of the infant requirements.

4.4 Amino acid profiles of Germinated and Fermented Complementary foods from Popcorn, African Locust Bean and Bambara groundnut

Amino acid compositions of the formulated food and control food samples are presented in Table 4.15. The total amino acids profile of the germinated and fermented formulations ranged from 50.83±0.31 g/100g of protein in FPAB to 75±0.85 g/100g of protein in GPA. Statistically, the total amino acid profiles in the germinated complementary foods were significantly higher than in the fermented complementary foods and '*Ogi*' (67.0±0.09 g/100g of protein) respectively, however, the values were lower than in *Cerelac* (88.81±0.11 g/100g of protein) ($p<0.05$). For the total essential amino acids, the values were higher in germinated formulations (27.73- 31.09 g/100g of protein) than fermented complementary food samples (19.0-27.91 g/100g of protein), these values were however significantly higher ($p<0.05$) when compared with that of '*Ogi*' (a local complementary food sample) (18.32 mg/100g), but lower than in *Cerelac* (a commercial food sample) (31.73 mg/100g).

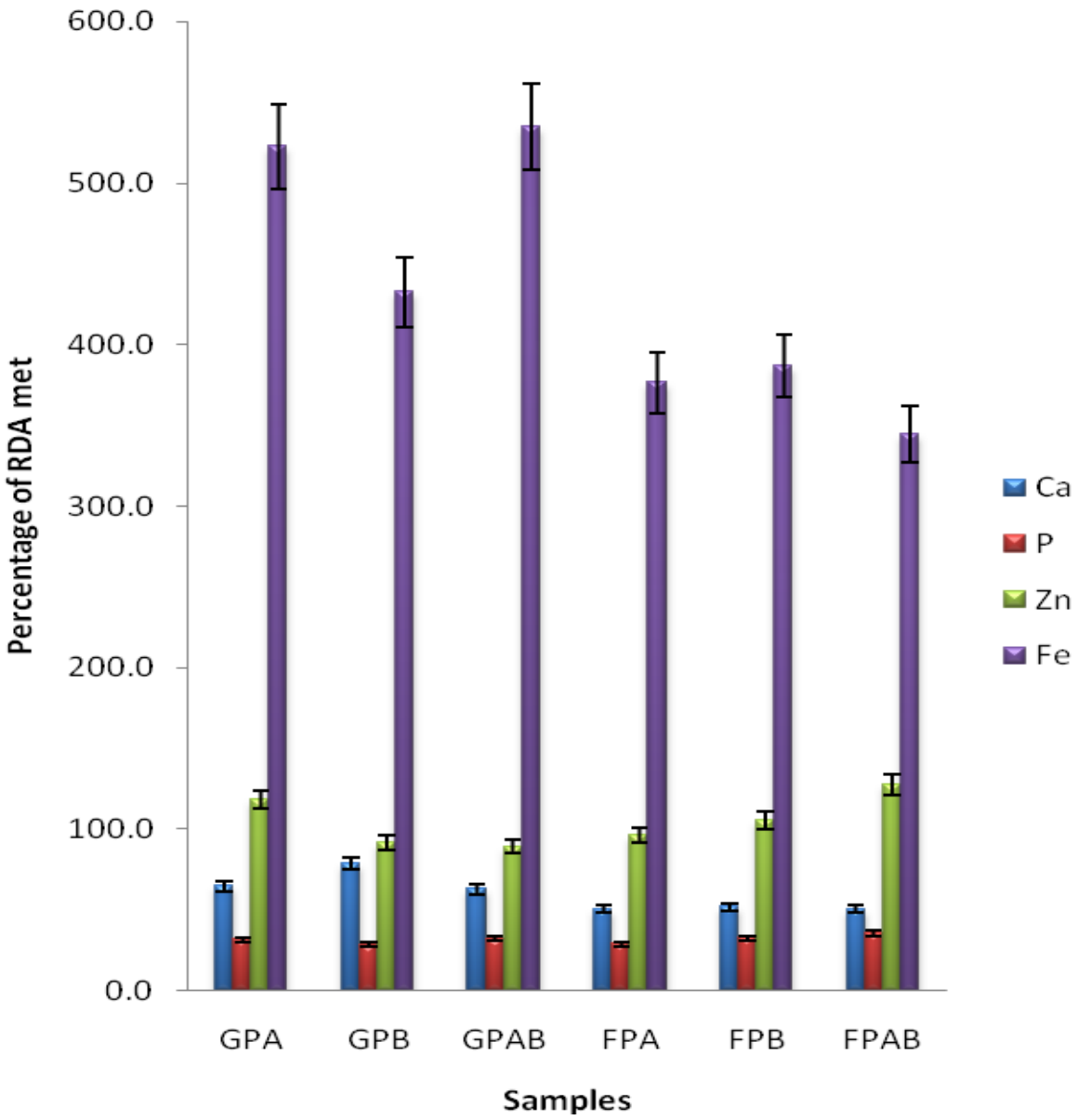

Figure 4.4: Percentage of Recommended Daily Allowance (RDA) met by selected minerals of formulated complementary food samples.

Key
GPA (Germinated popcorn-African locust bean blend),
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend)
RDA (recommended daily allowance) (FAO/WHO, 1991)

Table 4.15: Amino acid compositions (g/100g crude protein) of formulated food samples from germinated popcorn, African locust bean and Bambara groundnut flour blends

Parameters	GPA	GPB	GPAB	FPA	FPB	FPAB	Ogi	Cerelac
Non essential amino acids (TNEAA)								
Alanine	3.48^{b}	2.33^{d}	2.65^{c}	2.09^{e}	2.49^{cd}	2.47^{cd}	3.61^{b}	4.44^{a}
	±0.02	±0.09	±0.10	±0.01	±0.02	±0.11	±0.15	±0.01
Aspartic acid	8.37^{b}	3.17^{d}	3.40^{d}	6.30^{c}	2.51^{e}	1.30^{f}	6.12^{c}	9.26^{a}
	±0.02	±0.07	±0.30	±0.01	±0.02	±0.03	±0.04	±0.01
Serine	5.08^{a}	2.91^{e}	2.69^{f}	3.36^{d}	2.22^{g}	1.92^{h}	4.78^{b}	4.32^{c}
	±0.04	±0.06	±0.02	±0.01	±0.09	±0.06	±0.11	±0.01
Glutamic acid	12.65^{de}	13.14^{c}	13.01^{cd}	9.17^{g}	12.13^{e}	12.30^{ef}	17.63^{b}	19.53^{a}
	±0.02	±0.02	±0.31	±0.10	±0.17	±0.20	±0.13	±0.05
∑NEAA	29.58	21.55	21.75	20.92	10.35	17.99	32.14	37.55
Conditionally essential amino acids (TCEA)								
Proline	3.62^{a}	1.74^{d}	1.75^{d}	2.03^{d}	1.84^{d}	1.34^{e}	2.49^{c}	3.26^{b}
	±0.15	±0.05	±0.15	±0.01	±0.15	±0.19	±0.15	±0.01
Glycine	4.00^{b}	1.67^{d}	1.58^{e}	3.10^{c}	1.46^{e}	1.11^{f}	4.14^{b}	5.88^{a}
	±0.03	±0.10	±0.09	±0.01	0.03	±0.03	±0.05	±0.10
Arginine	6.65^{b}	4.16^{e}	3.29^{g}	4.27^{e}	3.97^{f}	4.42^{d}	4.83^{c}	9.14^{a}
	±0.01	±0.05	±0.08	±0.02	±0.03	±0.05	±0.01	±0.01
Cysteine	1.65^{ab}	1.39^{ab}	1.71^{ab}	0.68^{c}	1.15^{bc}	1.29^{b}	1.94^{a}	1.15^{bc}
	±0.19	±0.02	±0.04	±0.02	±0.01	±0.05	±0.01	±0.01
Tyrosine	3.25^{ab}	3.60^{a}	3.55^{a}	2.10^{c}	3.26^{ab}	2.66^{bc}	4.02^{a}	2.36^{c}
	±0.28	±0.20	±0.10	±0.01	±0.62	±0.09	±0.01	±0.03
∑CEAA	19.17	12.56	11.88	12.18	11.68	10.82	17.42	21.79
Essential amino acids (TEAA) + Histidine								
Lysine	3.39^{f}	4.62^{c}	4.36^{d}	2.38^{g}	5.28^{a}	4.92^{b}	1.71^{h}	4.12^{e}
	±0.15	±0.05	±0.12	±0.01	±0.10	±0.04	±0.01	±0.01
Threonine	3.06^{c}	3.50^{b}	2.84^{cd}	1.84^{e}	2.69^{d}	2.64^{d}	1.09^{f}	3.80^{a}
	±0.06	±0.20	±0.06	±0.01	±0.04	±0.07	±0.01	±0.11
Valine	3.79^{b}	4.71^{a}	3.95^{b}	2.69^{c}	2.72^{c}	2.60^{c}	2.69^{c}	4.79^{a}
	±0.01	±0.07	±0.03	±0.02	±0.46	±0.14	±.02	±0.01
Methionine	1.10^{bc}	1.48^{abc}	1.95^{a}	0.84^{c}	1.29^{abc}	1.47^{abc}	1.13^{bc}	1.63^{ab}
	±0.01	±0.11	±0.52	±0.01	±0.04	±0.10	±0.11	±0.01
Isoleucine	3.04^{c}	3.24^{c}	3.08^{c}	1.83^{d}	4.41^{a}	4.28^{a}	3.56^{b}	4.23^{a}
	±0.06	±0.09	±0.12	±0.01	±0.06	±0.10	±0.01	±0.02
Leucine	5.70^{a}	4.14^{d}	3.73^{e}	4.14^{d}	4.62^{c}	4.79^{c}	3.75^{e}	5.25^{b}
	±0.06	±0.24	±0.09	±0.02	±0.11	±0.10	±0.02	±0.01
Phenylalanine	4.08^{b}	4.60^{a}	4.75^{a}	2.40^{d}	3.71^{c}	3.84^{bc}	2.04^{e}	4.61^{a}
	±0.13	±0.03	±0.16	±0.10	±0.05	±0.10	±0.15	±0.01
Histidine	2.37^{b}	3.63^{a}	3.56^{a}	1.65^{de}	1.74^{d}	1.51^{e}	1.56^{de}	2.04^{c}
	±0.02	±0.01	±0.15	±0.05	±0.03	±0.02	±0.02	±0.01
Tryptophan	1.20^{abc}	1.17^{abc}	0.89^{b}	1.23^{abc}	1.45^{a}	1.21^{abc}	0.85^{c}	1.26^{ab}
	±0.10	±0.14	±0.02	±0.04	±0.25	±0.03	±0.11	±0.03
∑EAA	27.73	31.09	29.11	19.00	27.91	27.26	18.32	31.73
TSAA(Meth+cystein)	2.75	2.87	3.66	1.52	2.44	2.76	3.07	2.78
TArAA(Phenyl+Tyro)	7.33	8.2	8.3	4.5	6.97	6.5	6.06	6.97
∑AA	75.27^{b}	64.01^{cd}	61.82^{d}	54.83^{e}	57.44^{e}	50.83^{f}	67.0^{c}	88.81a
	±0.85	±1.03	±2.13	±1.35	±1.14	±0.31	±0.09	±0.11
(∑EAA/∑AA)%	36.84	48.57	47.09	34.65	48.59	53.63	27.34	35.73

Means (±SEM) with different alphabetical superscripts in the same row are significantly different (P<0.05).

Figure 4.5 shows the percentage of Recommended Daily Allowance (RDA) met per 100 grammes of essential amino acids in the formulated food samples. It was observed that all the formulated complementary foods, except FPA, were suitable of providing over three-quarters of the infant RDA for essential amino acid, and these were quite higher when compared with that of ogi, but comparable to that of commercial formula (*Cerelac*). The amount of formulated complementary food samples required for the weaning-aged children to meet the recommended daily allowance for essential amino acids were calculated (Fig. 4.6). It was observed that the amount of complementary food samples that were required to provide the needed total essential amino acids for the growing infant were lower in the formulated diets than '*Ogi*', however, the amount were higher than that of *Cerelac*.

Figure 4.7 shows the Digestible Indispensable Amino Acid Score (DIAAS) of formulated complementary foods and control food samples (Ogi and *Cerelac*). The calculated DIAAS of FPAB sample had the highest value, while FPA had the lowest value. In comparison to the control food samples, it was observed that the calculated DIAAS of the formulated food samples were higher than that in Ogi, but lower than in Cerelac.

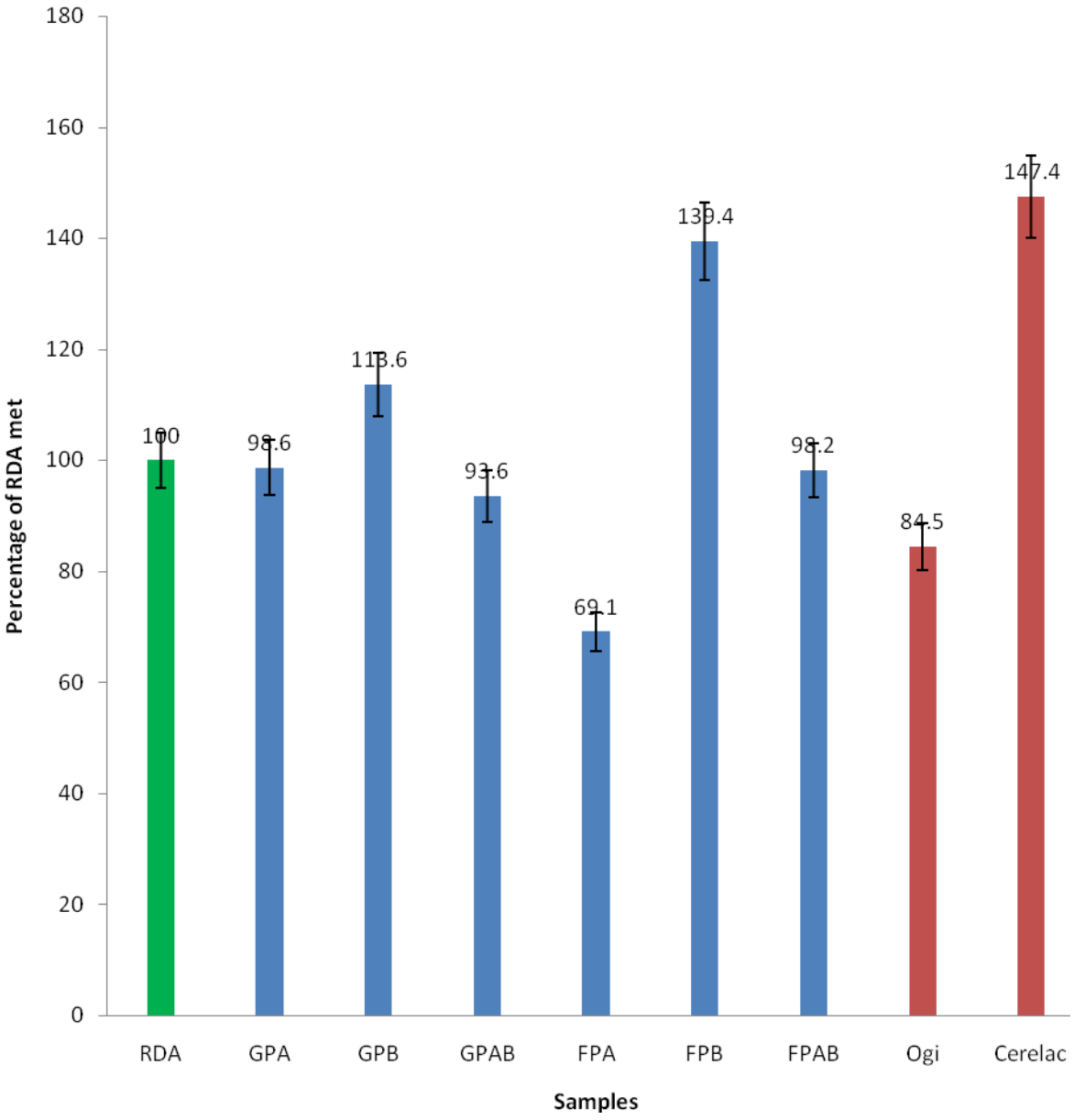

Fig. 4.5: Comparison of recommended daily allowance (RDA) percentage met of total essential amino acid + histidine + Arginine) per 100g of formulated complementary foods, ogi and Cerelac with reference to infant requirements (<1year).

Key
GPA (Germinated popcorn-African locust bean blend),
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend)
RDA (Recommended Daily Allowance)(FAO/WHO, 1991)

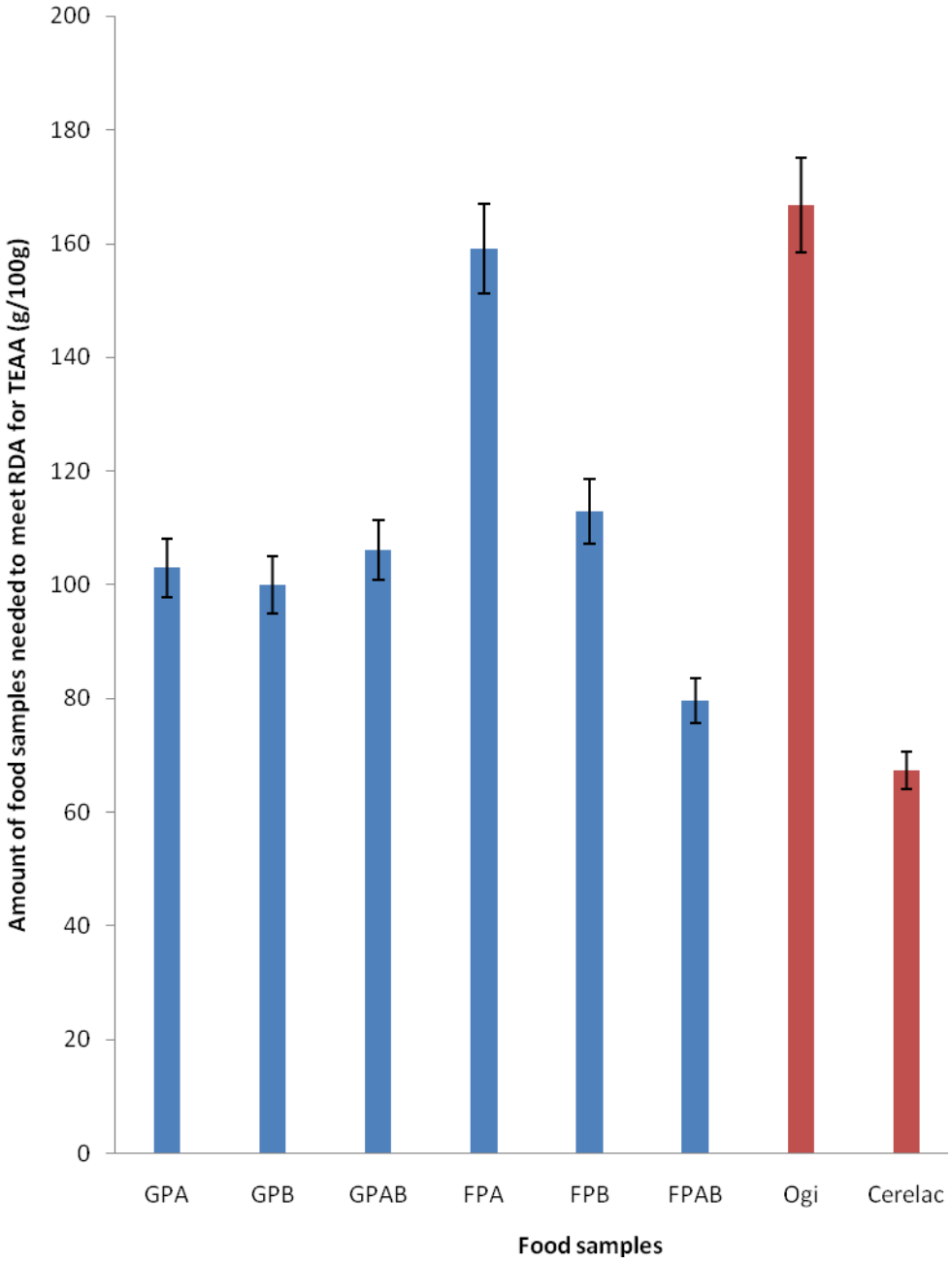

Figure 4.6: Relative amount of formulated food samples give total essential amino acids (TEAAs) for infant ($p<0.05$).

Key
GPA (Germinated popcorn-African locust bean blend),
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend)

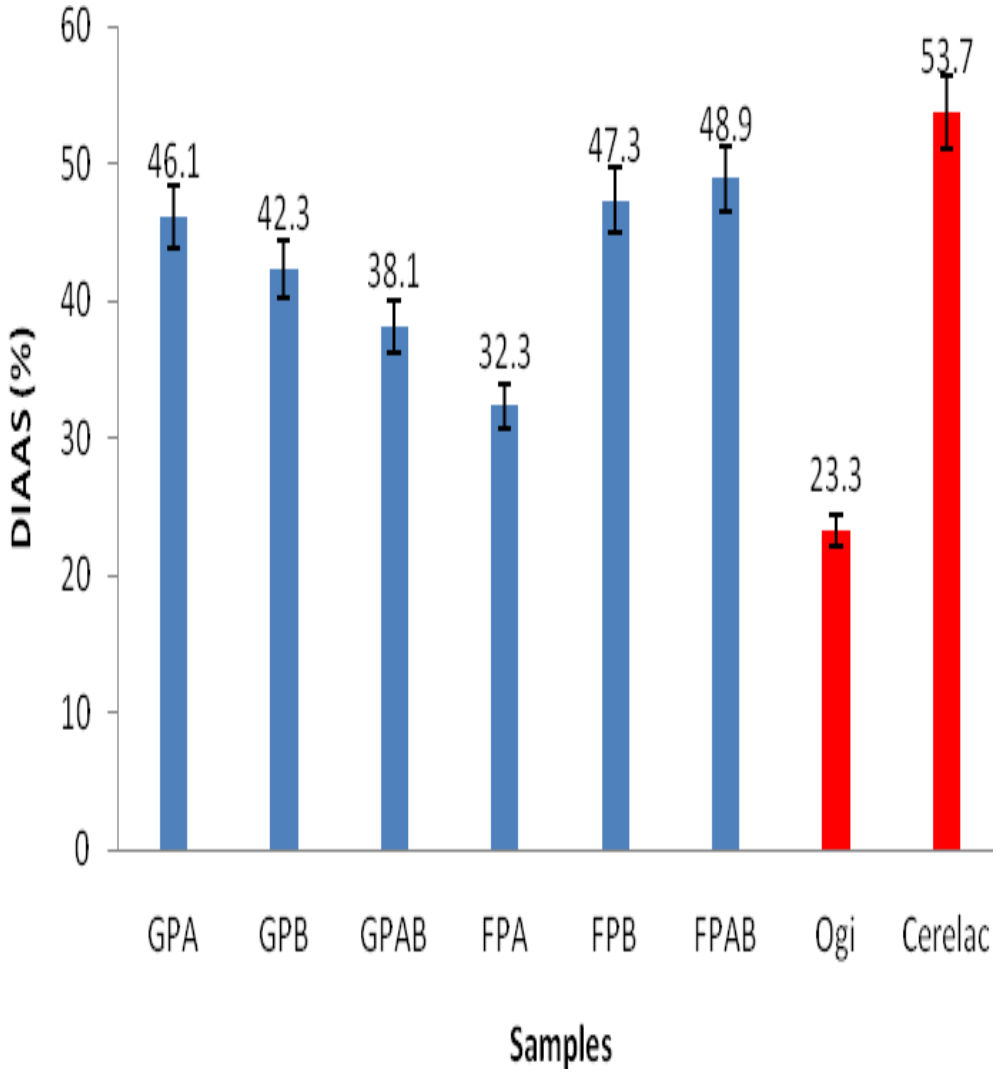

Fig. 4.7: Digestible indispensable amino acid score (DIAAS) of formulated complementary foods and control samples (Ogi and Cerelac)

Key
GPA (Germinated popcorn-African locust bean blend),
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend)

4.5 Fatty acid compositions of Germinated and Fermented Complementary foods from Popcorn, African Locust Bean and Bambara groundnut

Table 4.16 shows the fatty acid compositions of germinated and fermented popcorn, African locust bean and bambara groundnut flour blends. The results showed that total saturated fatty acid composition (SFA) of the germinated food samples varied from 40.02 mg/100g in GPA to 51.10 mg/100g in GPB, while that of fermented food samples ranged from 29.66 g/100g in FPA to 45.91 g/100g in FPB. For total polyunsaturated fatty acid (PUFA), the values ranged from 29.51g/100g to 33.45 mg/100g in GPB and GPAB respectively, while that of fermented food samples ranged from 33.76 mg/100g to 44.29 mg/100 in FPB and FPA respectively. The monounsaturated fatty acids of germinated complementary food samples ranged from 19.16 mg/100g to 28.46 mg/100g in GPB and GPA respectively, while that of fermented food samples ranged from 19.35 mg/100g to 25.91 g/100g in FPB and FPA respectively. Statistically, the polyunsaturated and monounsaturated fatty acid values of the formulated food samples were comparable to that of control food samples. Polyunsaturated fatty acid and saturated fatty acid molar ratios (P/S) of germinated and fermented complementary foods ranged from 0.62 in GPA to 1.26 in GPAB, and the values were significantly ($p<0.05$) lower than control samples (Ogi, 4.96; *Cerelac*, 4.33) (Fig. 4.8). Table 4.17 shows the fatty acids composition of germinated and fermented complementary foods compared with FAO/WHO recommendations. It was observed that the total unsaturated fatty acid compositions of germinated complementary foods ranged from 48.67 to 59.98 mg/100g and were lower than fermented complementary foods (53.11 – 70.2 mg/100g), but the values were within FAO/WHO (1991) recommendations (44.8-52.4 mg/day).

Table 4.16: Fatty acid compositions (mg/100g) of germinated popcorn, African locust bean and bambara groundnut flour blends

Parameters	GPA	GPB	GPAB	FPA	FPB	FPAB	Ogi	*Cerelac*
Caprylic	-	-	-	-	-	-	-	-
Capric	-	-	-	-	-	-	-	-
Lauric acid (C12:0)	-	-	-	-	-	-	-	-
Margaric	-	-	-	-	-	-	-	-
Arachidonic acid	-	-	-	-	-	-	-	-
Erucic acid	-	-	-	-	-	-	-	-
Myristic acid (C14:0)	0.01^{a} ±0.00	-	-	0.01^{a} ±0.00	-	-	-	-
Palmitic acid (C16:0)	16.69^{b} ±0.03	16.32^{c} ±0.01	9.66^{g} ±0.02	14.63^{d} ±0.01	17.51^{a} ±0.04	10.94^{f} ±0.02	12.33^{e} ±0.01	0.27^{h} ±0.02
Palmitoleic acid (C16:1)	0.91^{b} ±0.02	0.27^{c} ±0.02	0.20^{d} ±0.01	2.13^{a} ±0.05	-	0.13^{e} ±0.03	-	-
Stearic acid (C18:0)	19.29^{b} ±0.01	21.25^{a} ±0.01	19.35^{b} ±0.01	15.03^{e} ±0.05	17.23^{d} ±0.10	21.18^{a} ±0.05	4.44^{f} ±0.02	18.50^{c} ±0.02
Oleic acid (C18:1)	27.55^{b} ±0.02	18.89^{e} ±0.02	21.67^{e} ±0.01	23.75^{d} ±0.15	19.35^{e} ±0.02	19.69^{f} ±0.03	35.42^{a} ±0.01	25.11^{c} ±0.02
Linoleic acid (C18:2)	31.5^{g} ±0.01	29.15^{h} ±0.01	33.12^{f} ±0.01	44.29^{c} ±0.02	33.76^{e} ±0.03	34.22^{d} ±0.02	47.80^{b} ±0.02	52.28^{a} ±0.01
Linolenic acid (C18:3)	0.03^{f} ±0.005	0.36^{c} ±0.01	0.33^{d} ±0.01	0.05^{e} ±0.001	-	0.58^{b} ±0.01	-	3.84^{a} ±0.01
Arachidic acid (C20:0)	3.73^{a} ±0.01	3.45^{b} ±0.03	3.12^{c} ±0.02	-	3.00^{d} ±0.01	2.75^{e} ±0.01	-	-
Behenic acid (C22:0)	0.002^{e} ±0.001	10.09^{b} ±0.03	11.67^{a} ±0.02	-	8.1671^{d} ±0.01	9.66^{c} ±0.03	-	-
Lignoceric acid (C24:0)	-	0.94^{b} ±0.02	0.88^{c} ±0.02	-	0.99^{a} ±0.01	0.84^{d} ±0.02	-	-
∑SFA	40.02	51.1	43.8	29.66	45.91	44.54	16.78	18.77
∑PUFA	31.52	29.51	33.45	44.29	33.76	34.8	47.80	56.12
∑MUFA	28.46	19.16	21.87	25.91	19.35	19.82	35.42	25.11

Means (±SEM) with different alphabetical superscripts in the same column are significantly different ($P<0.05$).

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend)
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

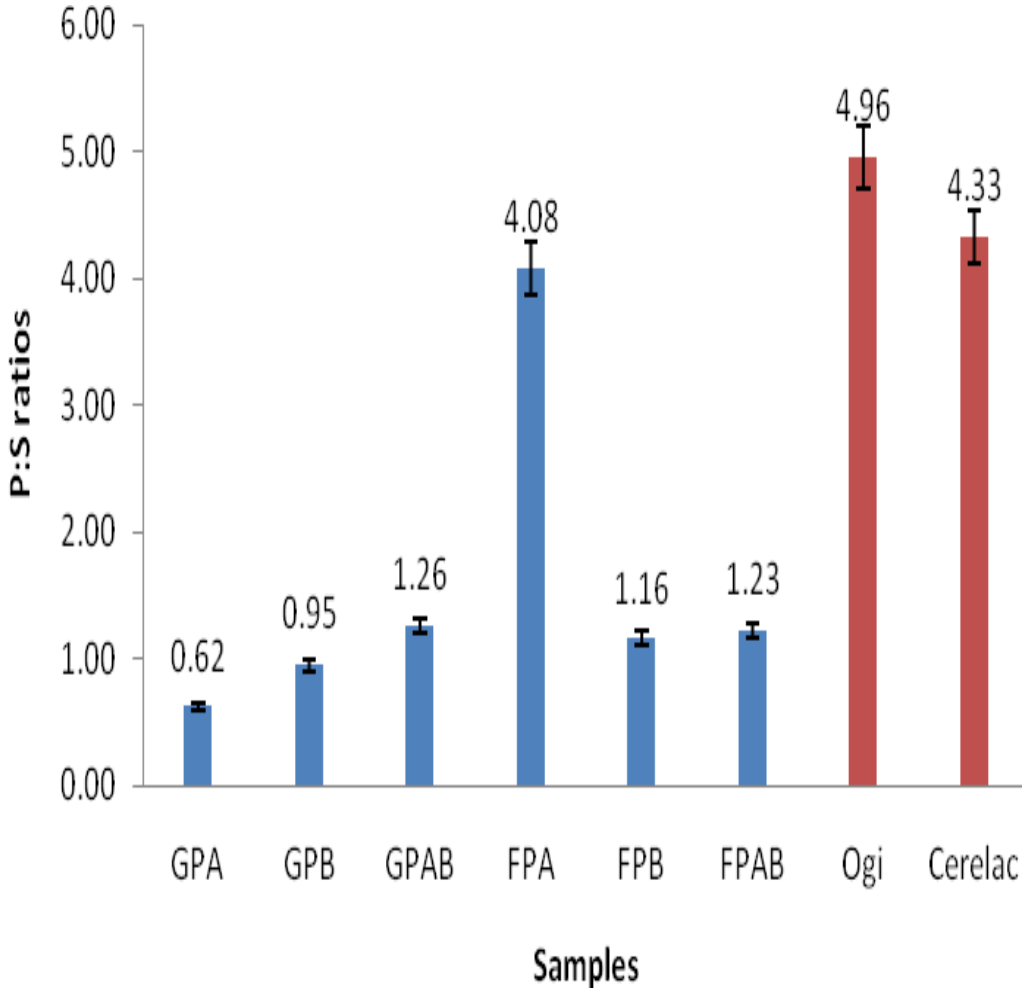

Fig. 4.8 Comparison of polyunsaturated fatty acid/saturated fatty acid ratio (P/S) of formulated complementary food and control samples

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend)
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

Table 4.17: Fatty acid compositions (g/100g) of the formulated complementary foods compared with FAO/WHO recommendations

Parameters	GPA	GPB	GPAB	FPA	FPB	FPAB	*Recommended
Linoleic acid 18:2n-6	31.49	29.15	33.12	54.29	33.76	34.22	11-12
α- Linolenic acid 18:3n-3	0.03	0.36	0.33	0.05	0.00	0.58	0.8-0.9
Arachidonic acid 20:4n-6	-	-	-	-	-	-	0.5-0.6
Total unsaturated FA	59.98	48.67	55.32	70.2	53.11	54.62	44.8-52.4
Total saturated FA	40.02	51.10	43.80	29.66	45.90	44.54	45.2-53.5
Unsaturated/saturated fatty acid ratio	0.24	0.58	0.76	2.76	0.74	0.78	0.84:1-1.16:1

*Source: Koletzko *et al.*, 1992; FAO/WHO, 1991.

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

4.6 Phytochemicals Concentration in Germinated and Fermented Complementary Foods from Popcorn, African Locust Bean and Bambara groundnut

The antinutritional factors in the formulated food samples are presented in Table 4.18. Oxalate concentration in germinated food samples ranged from 0.015±0.04 mg/g in GPAB to 1.925±1.02 mg/g in GPA, while fermented food samples ranged from 0.153±0.01 mg/g in FPB to 2.141±0.02 mg/g in FPA sample. Statistically, the oxalate concentration in germinated food samples, particularly GPB and GPAB, were lower when compared with the fermented food samples ($p<0.05$). Tannin concentration in germinated food samples ranged from 0.014±0.0 mg/g in GPB to 0.026±0.01 mg/ in GPA, while tannin concentration in fermented food samples ranged from 0.034±0.01 mg/g in FPA to 0.507±0.09 mg/g in GPAB sample. In comparison, there were no significant difference between the tannin contents in the formulated complementary food samples and those of control food samples. Phytate concentration in germinated complementary foods ranged from 15.31±0.17 mg/g in GPB to 32.90±2.12 mg/g in GPAB, while fermented food samples ranged from 15.21±0.25 mg/g in FPAB to 19.25±2.12 mg/g in FPA. It was observed that the phytate content in germinated food samples, particularly in GPA and GPAB, were higher than fermented food samples. Comparatively, the phytate in formulated complementary food samples were significantly higher ($p<0.05$) than in Ogi and *Cerelac*. Trypsin inhibition activities in the germinated food samples ranged from 0.018±0.01 mg/g to 0.057±0.02 mg/g, while fermented food samples ranged from 0.049±0.01 mg/g in FPB to 0.118±0.02 mg/g, and were significantly lower than Ogi (0.278 ±0.04mg/g) and *Cerelac* (0.294 ±0.01 mg/g) respectively. Chocking properties of popcorn-based complementary food sample was determined using animal model (Table 4.19). The percentage survival of the albino rats fed with the germinated and fermented popcorn-based complementary foods was 96.4%. This observation indicated that the consumption of popcorn-based complementary foods would not cause any mortality in the animal fed with any of these formulations.

Table 4.18: Phytochemical compositions (mg/g) of popcorn, African locust bean and bambara groundnut blend

Parameters	Oxalate	Tannin	Phytate	Trypsin
GPA	1.925^{ab}	0.026^{b}	21.41^{b}	0.057^{bc}
	±1.02	±0.01	±1.82	± 0.02
GPB	0.018^{c}	0.014^{b}	15.31^{e}	0.018^{c}
	±0.04	±0.09	±0.17	±0.01
GPAB	0.015^{c}	0.015^{b}	32.90^{a}	0.018^{c}
	±0.20	±0.09	±2.47	±0.04
FPA	2.141^{a}	0.034^{b}	19.25^{c}	0.118^{b}
	±0.02	±0.01	±2.12	±0.02
FPB	0.153^{c}	0.118^{ab}	18.01^{d}	0.049^{bc}
	±0.00	±0.00	±2.01	±0.01
FPAB	0.330^{bc}	0.507^{a}	15.21^{e}	0.088^{bc}
	±0.01	±0.01	±0.25	±0.01
Ogi	0.18^{c}	0.016^{b}	16.41^{d}	0.278^{a}
	±0.01	±0.01	±0.13	±0.04
Cerelac	0.04^{c}	0.011^{b}	3.71^{f}	0.294^{a}
	±0.00	±0.00	±0.04	±0.01
*Lethal dose	2.5g/kg	30mg/kg	50-60g/kg	2.5g/kg

Means (±SEM) with different alphabetical superscripts in the same column are significantly different ($P<0.05$). *Source: Inuwa *et al*. (2011)

Key
GPA (Germinated popcorn-African locust bean blend),
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend)
RDA (Recommended Daily Allowance)(FAO/WHO, 1991

Table 4.19: Percentage of survived albino rats fed with the formulated complementary foods (an index of choking properties of popcorn)

Time Period	Animals At Risk	Became Unavailable (Censored)	Died	Survived	*Survival Probability Estimate (S_t)	Percentage of Survival
Week 1	30	1	0	30	1	100
Week 2	29	1	0	29	1	100
Week 3	28	1	1	28	0.964286	96.4
Week 4	26	1	0	26	0.964286	96.4
Week 5	25	1	0	25	0.964286	96.4

*Source: Kaplan and Meier (1958)

4.7 Relationship between Phytate and Bioavailability of Calcium, Iron and Zinc in Germinated and Fermented Complementary foods from Popcorn, African Locust Bean and Bambara groundnut

Table 4.20 shows the relationship between phytate and bioavailability of selected minerals like zinc, iron and calcium of the formulated food samples. Germinated food samples, phytate:zinc molar ratio ranged between 0.546 mol/kg in GPB and 1.21 mol/kg. in GPAB, and phytate:calcium molar ratio values ranged between 0.004 mol/kg in GPB and 0.012 mol/kg in GPAB, (calcium)(phytate):zinc was between 2.59 mol/kg in GPA and 5.14 mol/kg in GPAB, while that of phytate:iron was between 0.27 mol/kg in GPB and 0.48 mol/kg in GPAB; while that of fermented food samples, phytate:zinc molar ratio ranged between 0.391 mol/kg in FPAB and 0.656 mol/kg in FPA. Phytate:calcium molar ratio, the values ranged from 0.007 mol/kg in FPAB to 0.008 mol/kg in FPB, and (calcium)(phytate):zinc was between 1.336 mol/kg in FPAB and 2.245 mol/kg in FPA, while that of phytate:iron was between 0.341 mol/kg in FPAB and 0.395 mol/kg in FPA. In comparison, these molar ratios (phytate/zinc, phytate/calcium, (calcium) (phytate)/zinc and phytate/iron) of germinated and fermented food samples were within the same range; and that the values were lower than the recommended critical values, that is, phytate/zinc >15.0, phytate/calcium >0.24, (calcium)(phytate)/zinc >200 and phytate/iron >1.0.

Table 4.20: Relationship between phytate and minerals as index of bioavailability of selected minerals (zinc, iron and calcium) (mol/kg) in complementary foods from popcorn, African locust bean and bambara groundnut blends

Parameters	Phytate:Zinc	Phytate:Calcium	(Ca)(Phytate):zinc	Phytate:Iron
GPA	0.594	0.007	2.593	0.316
GPB	0.546	0.004	2.904	0.273
GPAB	1.209	0.012	5.141	0.475
FPA	0.656	0.009	2.245	0.395
FPB	0.564	0.008	1.971	0.361
FPAB	0.391	0.007	1.336	0.341
Ogi	5.352	20.189	0.014	3.4655
Cerelac	0.042	0.073	0.000	1.096
*Critical values	>15.0	>0.24	>200	>1.0

***Sources:** phytate: calcium > 0.24 (Morris and Ellis, 1985), phytate : iron > 1 (Hallberg *et al*., 1989), phytate : zinc >15 (Turnlund *et al*.,1984; Sandberg *et al*., 1987; Morris and Ellis, 1989), phytate : calcium/zinc > 200 (Davies *et al*., 1985; Bindra *et al*., 1986; Gibson 2006)

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

4.8 Functional Properties of Germinated and Fermented Complementary Foods from Popcorn, African Locust Bean and Bambara groundnut

Table 4.21 shows physical properties of formulated complementary foods compared with control food samples (i.e., Ogi and *Cerelac*). Bulk density of germinated food samples ranged from 0.71±0.11 g/cm^3 in GPAB to 0.82±0.03 g/cm^3 in GPA sample, while fermented food samples ranged from 0.79±0.03 g/cm^3 in FPA to 0.88±0.12 g/cm^3 in FPAB sample. Comparatively, the bulk density of germinated and fermented food samples were within the same ranged values, however, they were higher than Ogi, 0.66±0.01 g/cm^3 and *Cerelac*, 0.56 ±0.01 g/cm^3). Water absorption capacity (WAC) of the germinated food samples was higher in GPA (2.19 ± 0.19 ml/g), while GPB (1.96 ± 0.02 ml/g) had the least value. For fermented food samples, FPAB (2.34 ± 0.12 ml/g) had the highest value, while FPA (0.58±0.02 ml/g) had the least value. Water absorption capacities of the formulated diets were within the same-ranged values, but their values were higher than 'Ogi', but similar to that of *Cerelac*. Swelling capacity (SC) of GPA was the least in germinated food samples, while GPAB had the highest. For the fermented samples, FPB (4.97±0.04) had the highest swelling capacity, while FPA (0.53±0.17) had the lowest value. Comparatively, swelling capacity of the formulated food samples, that is, GPB, GPAB, FPB and FPAB, were higher than in 'Ogi' and *Cerelac*. The least gellation property of germinated food samples ranged from 11.5 ±0.05 in GPB to 16.00 ±2.01 in GPA, while fermented food samples ranged from 10.5 ±0.7 to 12.50 ±0.50 in FPAB and FPA samples respectively. The least gellation values of the formulated food samples were similar, and they were higher than 'Ogi'; but lower than that of *Cerelac*.

Table 4.21: Functional properties of formulated complementary foods compared with control food samples (i.e., Ogi and *Cerelac*).

Parameters	Bulk Density (g/cm^3)	Water absorption capacity (ml/g)	Swelling Capacity(%)	Least Gellation (%)
GPA	0.82^{a}	2.19^{a}	0.67^{c}	16.00^{a}
	±0.03	±0.19	±0.03	±2.01
GPB	0.78^{ab}	1.96^{a}	4.22^{ab}	11.5^{cde}
	±0.01	±0.02	±0.01	±0.05
GPAB	0.71^{bc}	2.04^{a}	2.45^{bc}	14.50^{ab}
	±0.11	±0.08	±0.75	±1.50
FPA	0.79^{ab}	0.58^{b}	0.53^{c}	11.0^{de}
	±0.03	±0.02	±0.17	±1.00
FPB	0.80^{ab}	2.31^{a}	4.97^{a}	12.50^{bcd}
	±0.01	±0.20	±0.04	±0.50
FPAB	0.88^{a}	2.34^{a}	3.30^{ab}	10.5^{de}
	±0.12	±0.12	±0.80	±0.7
Ogi	0.66^{c}	1.82^{a}	0.90^{c}	9.00^{e}
	±0.01	±0.02	±0.03	±1.11
Cerelac	0.56^{d}	2.31^{a}	2.43^{bc}	14.00^{abc}
	±0.03	±0.21	±0.03	±1.21

Means (±SEM) with different alphabetical superscripts in the same column are significantly different ($P<0.05$).

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
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FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

4.9 Microbial Status of Food Samples of Germinated and Fermented Complementary Foods from Popcorn, African Locust Bean and Bambara groundnut

The microbial loads of germinated and fermented popcorn, Bambara groundnut and African locust bean flour sample is presented in Table 4.22. The total viable count of the germinated food samples ranged from 1.95 x 10^2 cfu/g in germinated bambara groundnut flour sample to 5.50 x 10^2 cfu/g in germinated popcorn flour sample, while that of fermented food samples ranged from 1.15 x 10^3 cfu/g in fermented popcorn flour samples to 2.27 x 10^4 cfu/g in locust bean flour sample. For the mould/yeast count, the values ranged from 4.05 x 10^1 cfu/g – 1.55 x 10^2 cfu/g in germinated food samples, while fermented food samples ranged from 5.0 x 10^1 to 4.25 x 10^2 cfu/g in fermented flour samples. However, Escherichia coli and Coliform were not detected in any of the flour samples.

Table 4.22 Mean microbial count of popcorn, African locust bean and Bambara groundnut flour (cfu g^{-1})

Parameters	***Escherichia coli*** (cfu/g)	**Total viable count** (cfu/g)	**Mould/Yeast** (cfu/g)	**Coliform** (cfu/g)
Germinated Popcorn	-	$5.50x10^{2d}$	$4.05x10^{1d}$	-
Germinated locust bean	-	$3.15x10^{2e}$	$4.21x10^{1d}$	-
Germinated Bambara groundnut	-	$1.95x10^{2f}$	$1.55x10^{2c}$	-
Fermented Popcorn	-	$1.15x10^{3b}$	$3.05x10^{2b}$	-
Fermented locust bean	-	$2.27x10^{4a}$	$5.01x10^{1d}$	-
Fermented bambara groundnut	-	$1.52x10^{3c}$	$4.25x10^{2a}$	-
*Acceptable values	<10	$<10^5$	$<10^3$	$<10^3$

Means (±SEM) with different alphabetical superscripts in the same colum are significantly different ($P<0.05$). *FAO/WHO (1991)

4.10 Sensory Attributes of Germinated and Fermented Complementary Foods from Popcorn, African Locust Bean and Bambara groundnut

The sensory scores for the evaluation of gels from the flour mixes are shown in Table 4.23. The aroma of fermented food samples were significantly scored higher by the panelist than germinated food samples ($p<0.05$), but both the germinated and fermented food samples were scored lower when compared with Ogi and *Cerelac*. For colour, there were no significant difference ($p>0.05$) between the germinated and fermented complementary food samples; however, panelists scored formulated food samples lower in colour than in the control sample. For taste and texture parameters, There were no significant difference between the germinated and fermented food samples ($p<0.05$); but the formulated food samples were significantly scored lower than control foods ($p<0.05$). For the overall mean of sensory parameters (Fig. 22), FPAB sample had highest scored when compared with other formulated food samples, however, it was scored lower than Ogi and *Cerelac* ($p>0.05$) respectively.

Table 4.23: Sensory attributes of formulated complementary food samples, ogi (a traditional complementary food) and *Cerelac* (a commercial formula)

Parameters	Aroma	Colour	Taste	Texture	Overall acceptability
GPB	5.3^{c}	6.3^{c}	5.2^{bc}	5.8^{c}	6.5^{bc}
	±0.3	±0.4	±0.4	±0.4	±0.4
GPA	5.0^{c}	6.3^{c}	5.8^{bc}	6.1^{c}	6.3^{bc}
	±0.2	±0.2	±0.2	±0.3	±0.3
GPAB	5.2^{c}	5.8^{c}	5.2^{bc}	5.9^{c}	6.1^{bc}
	±0.4	±0.3	±0.4	±0.3	±0.5
FPB	6.1^{bc}	6.1^{c}	5.3^{bc}	5.6^{c}	5.9^{bc}
	±0.3	±0.3	±0.3	±0.4	±0.4
FPA	5.6^{c}	6.5^{bc}	4.8^{c}	6.2^{c}	5.4^{c}
	±0.3	±0.3	±0.4	±0.3	±0.4
FPAB	6.1^{bc}	5.5^{c}	5.9^{b}	6.5^{bc}	6.9^{b}
	±0.2	±0.2	±0.4	±0.3	±0.2
Ogi	6.7^{b}	7.4^{ab}	7.6^{a}	7.3^{b}	7.1^{b}
	±0.4	±0.2	±0.3	±0.3	±0.4
Cerelac	8.3^{a}	7.7^{a}	8.3^{a}	8.6^{a}	8.5^{a}
	±0.3	±0.3	±0.3	±0.2	±0.3
Range	5.0-8.3	5.5-7.7	4.8-7.6	5.6-8.6	5.4-8.5

Means (±SEM) with different alphabetical superscripts in the same column are significantly different ($P<0.05$).

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

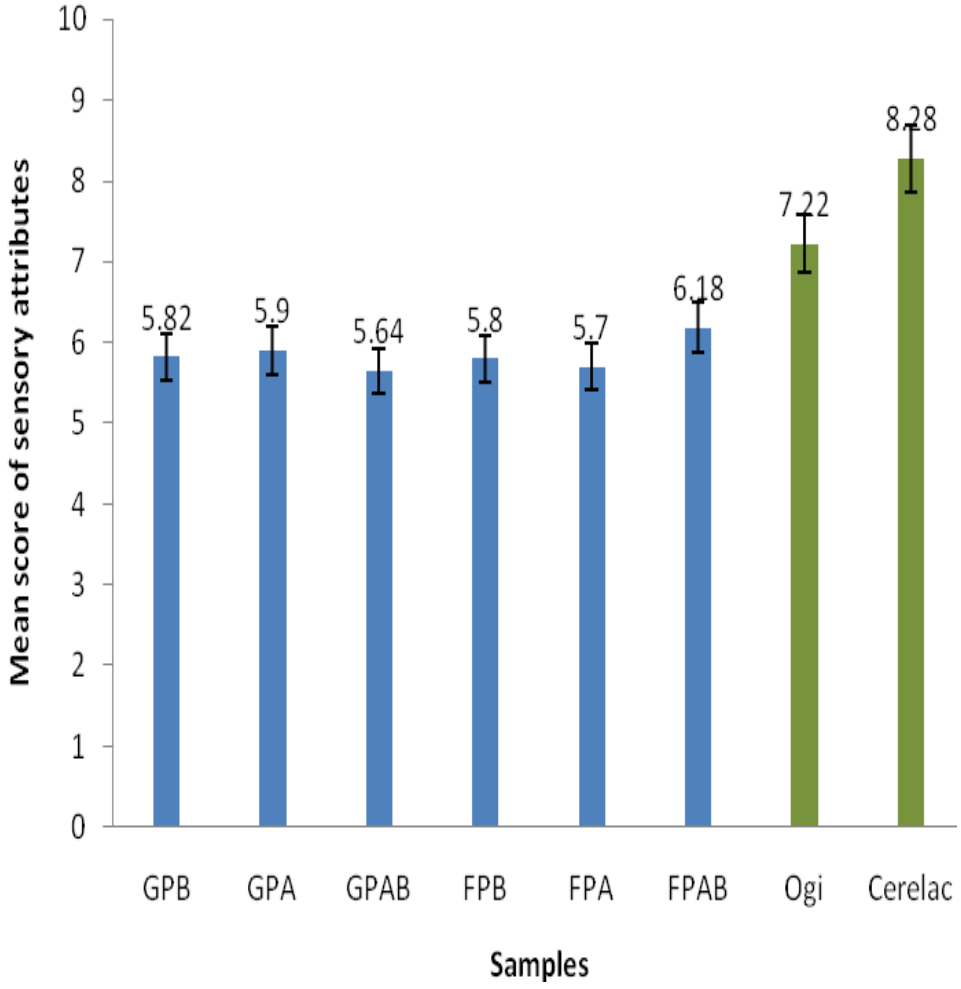

Fig. 4.9: Mean scores of sensory parameters of formulated complementary food samples and control complementary foods ($p<0.05$)

Key
GPA (Germinated popcorn-African locust bean blend),
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend)

4.11. Protein and Energy Digestibility of Germinated and Fermented Complementary Foods from Popcorn, African Locust Bean and Bambara groundnut using *Invivo*

Protein quality and metabolizable energy values of the formulated diets and control food samples are shown in Table 4.24. The biological values (BV) of germinated complementary food samples ranged from 51.4 % for GPA to 59.3% for GPAB, while fermented food samples ranged from 41.2% for FPA to 60.2% for FPAB sample. True protein digestibility of germinated food samples ranged from 50.9% for GPA to 59.2% for GPAB, while that of fermented food samples ranged from 41.1% for FPA to 60.0% for FPAB sample. Protein efficiency ratio, GPAB had the least value (1.99) and GPA had the highest value (2.33) for germinated complementary food samples, while FPB had the lowest (2.33) and FPAB had the highest value (3.41) for fermented food samples. Comparatively, the biological values (BV), true protein digestibility (TPD) and protein efficiency ratios (PER) of formulated complementary food samples were higher than Ogi (BV=10.0%, TPD=9.5%, PER=1.88), however, these values were comparable to the *Cerelac* values (BV= 70.4%, TPD=70.4%, PER=4.29).

Metabolizable energy (ME) of germinated complementary foods ranged from 146.9 kcal in GPB to 167.3 kcal in GPA, while fermented food samples ranged from 145.3 kcal in FPA to 186.5 kcal in FPAB; and the values were higher than Ogi, but lower when compared with the value of *Cerelac*. The digestible energy (DE) of GPB had the lowest value (78.0 kcal) and GPAB had the highest value (79.2 kcal) in germinated food samples, while FPA had the least (76.2 kcal) and FPAB (80.5 kcal) had the highest in fermented food samples. The metabolozable and digestible energy values of formulated complementary foods were comparatively higher than Ogi, but lower than *Cerelac.*

Table 4.24: Protein quality and metabolizable energy evaluation of formulated food samples and control

Parameters	**GPA**	**GPB**	**GPAB**	**FPA**	**FPB**	**FPAB**	**Ogi**	**Cerelac**	Mean	SD	CV%
FE	0.56	0.62	0.58	0.57	0.49	0.65	0.32	0.68	0.56	0.11	20.190
NR	2.06	1.17	1.37	0.65	0.63	1.49	0.00	1.24	1.08	0.63	58.765
BV (%)	51.37	55.41	59.26	41.15	44.24	60.20	10.03	70.43	49.01	18.28	37.301
NPU (%)	51.37	55.42	59.26	41.16	44.25	60.20	10.06	70.43	49.02	18.27	37.276
TD (%)	50.88	55.47	59.21	41.05	43.74	60.03	9.48	69.89	48.72	18.36	37.687
PER	2.33	2.18	1.99	2.80	2.33	3.41	1.88	4.29	2.65	0.82	31.050
PR	26.96	27.50	26.78	26.48	23.66	32.84	12.74	48.80	28.22	10.09	35.751
ME (kcal.)	167.32	146.91	155.20	145.29	167.63	186.48	114.1	256.37	167.41	41.72	24.921
DE (kcal.)	78.95	78.04	79.17	76.17	80.38	80.48	70.58	84.08	78.48	3.92	4.996

Key
FE (Food efficiency),
NR (Nitrogen retention),
BV (Biological value),
NPU (Net protein utilization),
TPD (True protein digestibility),
PER (Protein efficiency ratio),
PR (Protein rating,
ME (Metabolozable energy,)
DE (Digestible energy),
GPA (Germinated popcorn-African locust bean blend)
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-African locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-African locust-bambara groundnut blend),
SD (Standard deviation)
CV% (Coefficient of variation)

Weight gained by the organs of Albino rats fed with the formulated complementary food samples and control samples are shown in Figure 4.10. The weight gained by the kidney, liver and heart of the rats fed with FPAB sample were higher than those fed with other formulated complementary food samples. Statistically, the weight gained by the organs of animals fed with the experimental complementary food samples were significanly higher than those animals fed with ogi, but lower than those rats fed with Cerelac ($p<0.05$).

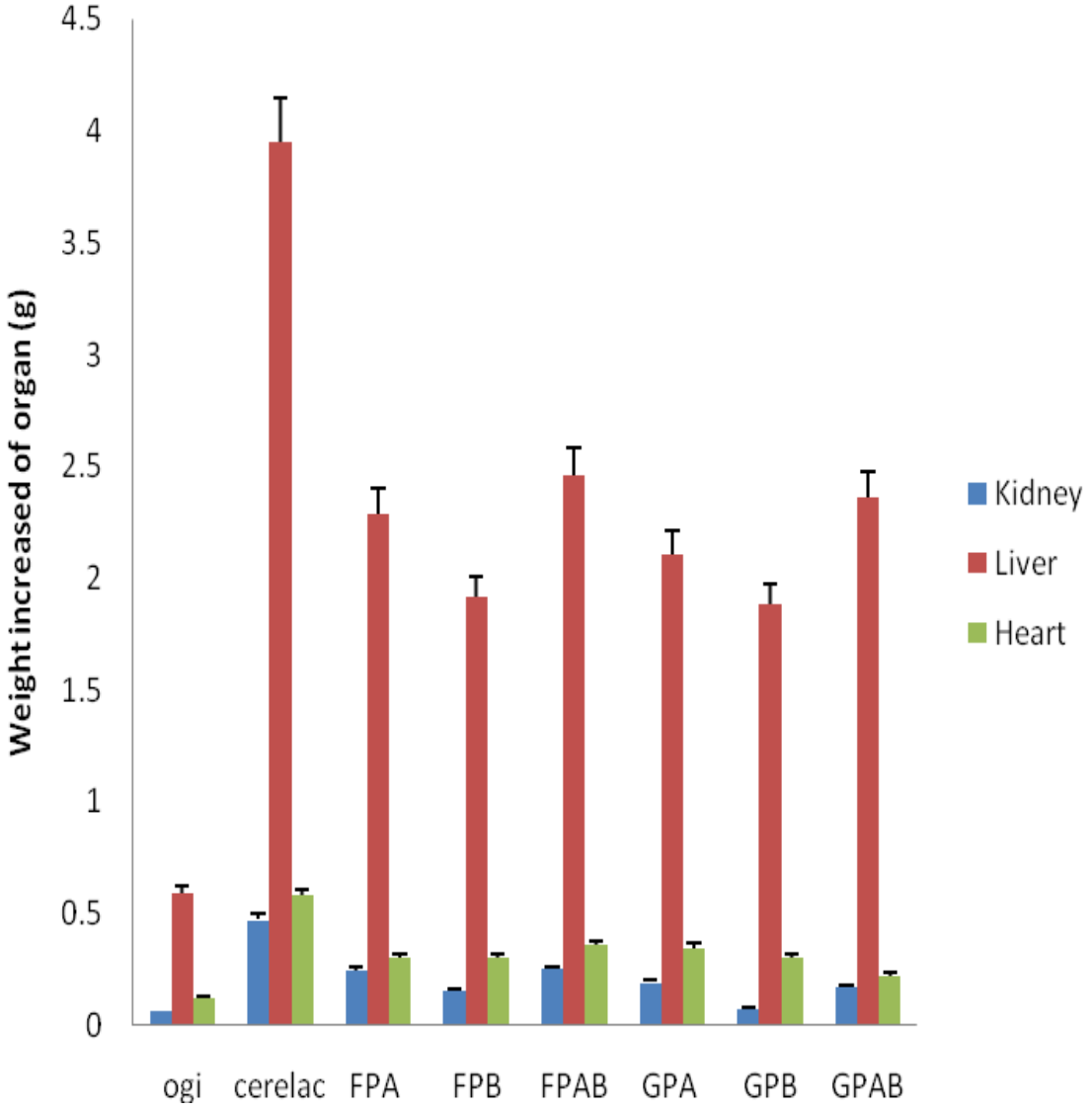

Figure 4.10: Weight gained (g) by the organs (i.e., kidney, liver and heart) of animals fed with formulated and control food samples

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

4.12 Heamatological Properties of Wistar Rats Fed with the Germinated and Fermented Complementary foods from Popcorn, African Locust Bean and Bambara groundnut

Heamatological properties of albino rats fed with the germinated and fermented complementary foods and control samples are presented in Table 4.25. Pack cell volume (PCV) of albino rats fed with the germinated food samples ranged from 43% for GPB to 49% for GPAB, while fermented complementary foods ranged from 43% for FPAB to 48% for FPB. Comparatively, the PCV values of rats fed with either germinated or fermented complementary foods were higher than those rats fed with ogi, but less than those rats in *Cerelac* group. Red blood cells (RBC) of rats fed with GPAB ($8.31x10^3$ mm^3) sample had highest value, while those fed with FPAB (7.27 $x10^3$ mm^3) sample had the least value. Similarly, it was also observed that the red blood cells of rats fed with either germinated or fermented food samples were higher than those fed with 'Ogi', but lesser than those rats in *Cerelac* group. The heamoglobin (Hb) concentration of animals fed with the germinated foods ranged from 14.30 g/100mL in GPB to 15.65 g/100mL in GPAB blends, while fermented complementary foods ranged from 14.30 g/100mL in FPAB sample to 16.10 g/100mL in FPB blend. The heamoglobin concentration of rats fed with the formulated complementary foods were significantly higher than those in 'Ogi' group ($p<0.05$), but comparable to rats in Cerelac group. Erythrocytes sedimentation rate (ESR), monocytes, eosinophils, basophils and mean cell heamoglobin concentration (MCHC), there were no significant difference between the values of animals fed with the formulated diets and control food samples (ogi or Cerelac) ($p>0.05$); however for other parameters, such as lymphocytes, neutrophils and mean cell heamoglobin (MCH) and mean cell volume (MCV), there were significant difference ($p<0.05$) between the rats fed with the formulated diets and control samples.

Table 4.25: Heamatological properties of albino rats fed with germinated and fermented complementary food and control food samples

Parameters	**GPA**	**GPB**	**GPAB**	**FPA**	**FPB**	**FPAB**	**Ogi**	**Cerelac**	***Range**
ESR (mm^3)	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	-
PCV (%)	47.00^{a}	43.00^{b}	49.00^{a}	47.0^{a}	48.0^{a}	43.0^{b}	41.00^{c}	44.00^{b}	30-50
RBC ($x10^6mm^3$)	7.89^{c}	6.90^{f}	8.31^{a}	7.97^{bc}	8.05^{b}	7.27^{e}	6.66^{g}	7.51^{d}	4 - 8
WBC ($x10^3mm^3$)	9.03^{c}	9.15^{c}	10.65^{b}	11.38^{a}	10.3^{b}	8.5^{cd}	10.28^{b}	7.95^{d}	5-12
Hb (g/100ml)	15.65^{c}	14.30^{e}	16.35^{a}	15.65^{c}	16.10^{b}	14.30^{e}	13.65^{f}	14.65^{d}	8-17.5
Lymphocytes(%)	66.50^{a}	53.00^{d}	62.00^{b}	61.50^{b}	57.50^{c}	55.00^{cd}	62.00^{b}	68.50^{a}	25-50
Neutrophils (%)	24.50^{d}	36.00^{a}	28.50^{c}	29.00^{c}	32.50^{b}	36.50^{a}	30.00^{bc}	22.50^{d}	36-55
Monocytes (%)	5.50^{b}	7.50^{a}	6.50^{ab}	6.50^{ab}	7.00^{ab}	5.50^{b}	5.50^{b}	7.00^{ab}	-
Eosinophils (%)	2.50^{a}	2.50^{a}	2.50^{a}	2.00^{a}	2.50^{a}	2.00^{a}	2.50^{a}	2.00^{a}	0-5
Basophils (%)	1.00^{a}	1.00^{a}	0.50^{ab}	1.00^{a}	1.00^{a}	1.00^{a}	0.00^{b}	1.00^{a}	-
MCHC (%)	33.29^{a}	33.26^{a}	33.37^{a}	33.29^{a}	33.54^{a}	33.26^{a}	33.17^{a}	33.29^{a}	-
MCH (picogram)	27.95^{c}	25.54^{e}	29.20^{a}	27.95^{c}	28.75^{b}	25.54^{e}	24.29^{f}	26.16^{d}	-
MCV(cubicmicrons)	94.50^{b}	86.50^{c}	98.50^{a}	95.00^{b}	96.50^{ab}	87.00^{c}	83.00^{d}	88.50^{c}	-

Means (±SEM) with different superscripts in a row are significantly different at $P<0.05$
**Sources: Hillyer (1994) and Jenkins (1993)*

***Key**:*
ESR (Erythrocytes sedimentation rate), PCV (Packed cell volume), RBC (Red blood cells), WBC (white blood cells), Hb (heamoglobin), MCHC (mean cell heamoglobin concentration), MCH (Mean cell heamoglobin), MCV (mean cell volume), GPA (Germinated popcorn-african locust bean blend; GPB (Germinated popcorn-bambara groundnut blend; GPAB (germinated popcorn-african locust-bambara groundnut blend), FPA (Fermented popcorn-african locust bean blend), FPB (Fermented popcorn-bambara groundnut blend). FPAB (Fermented popcorn-african locust-bambara groundnut blend).

4.13 Nutritional status of Wistar Rats Fed with the Germinated and Fermented Complementary foods from Popcorn, African Locust Bean and Bambara groundnut

The growth patterns of albino rats fed with the formulated complementary foods and control food samples are presented in Figures 4.11 - 4.14. The Nutritional status of the experimental rats showed that the rats in FPAB group were classified higher than their counterparts in other groups, while rats in GPB group were classified lower when compared with other rats from the remaining groups using weight-for-age (WFA) (underweight, a measure of combination of chronic and acute malnutrition.), length-for-age (LFA) (stunting, a measure of past nutritional status) and weight–for–length (WFL) (wasting, an indicator of short-term fluctuation in nutritional status). Comparatively, the nutritional status of experimental rats using WFA, LFA and WFL nutritional indices showed that the rats fed with the formulated complementary foods grow better than those rats in Ogi group, but lower in growth rate when compared with those rats in *Cerelac* group. For body mass index (BMI) (a measure of underwight/obese), The experimental rats BMI ranged from 0.13 g/cm^3 for rats in GPB group to 0.143 g/cm^3 in FPAB group, and were higher than the ratss in *Ogi* group (0.118 g/cm^3), but comparable to those rats in *Cerelac* group (0.146 g/cm^3).

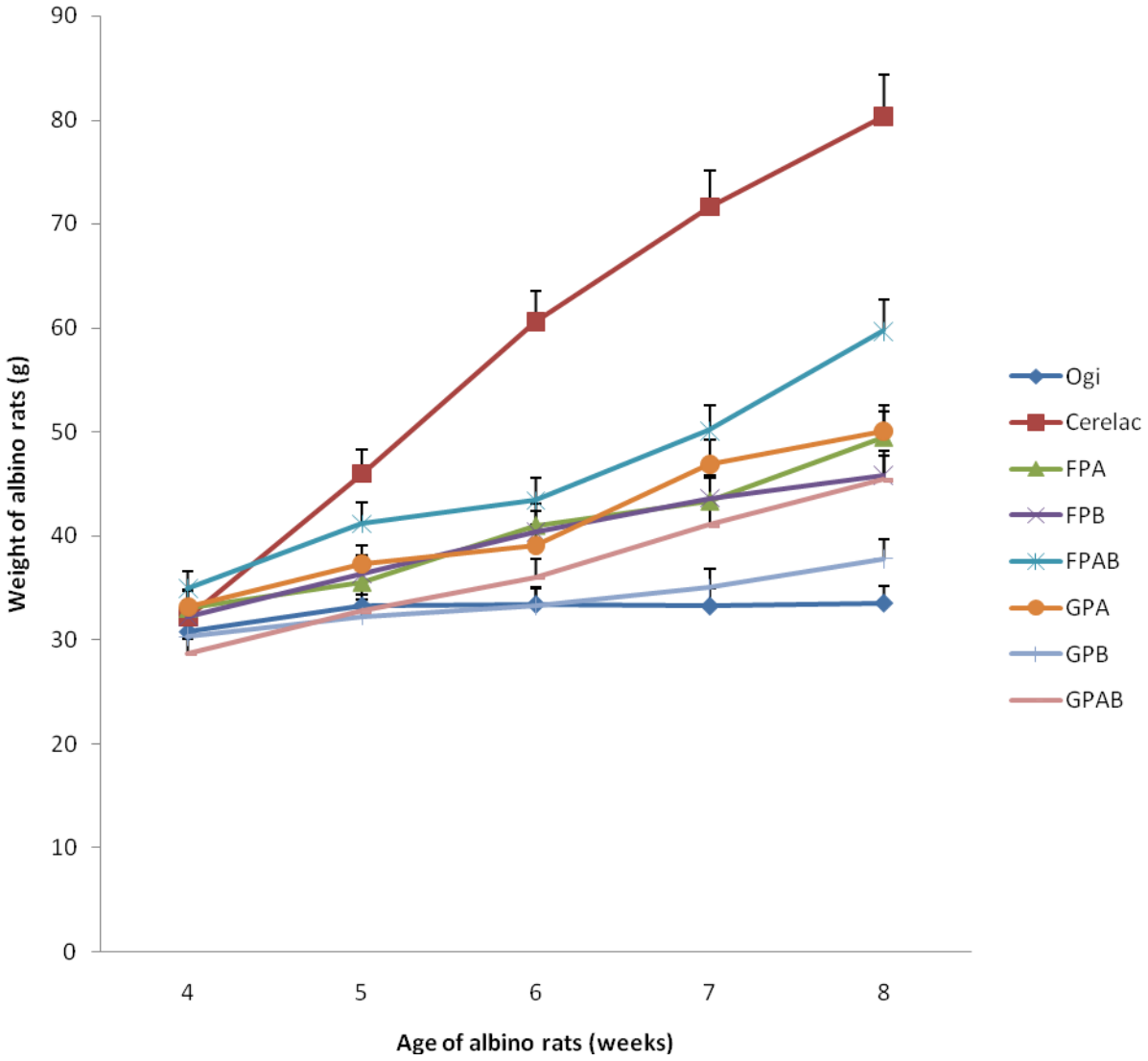

Fig. 4.11: Comparison of weight-for-age of albino rats fed with the germinated and fermented formulated diets and control food samples ($p<0.05$)

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

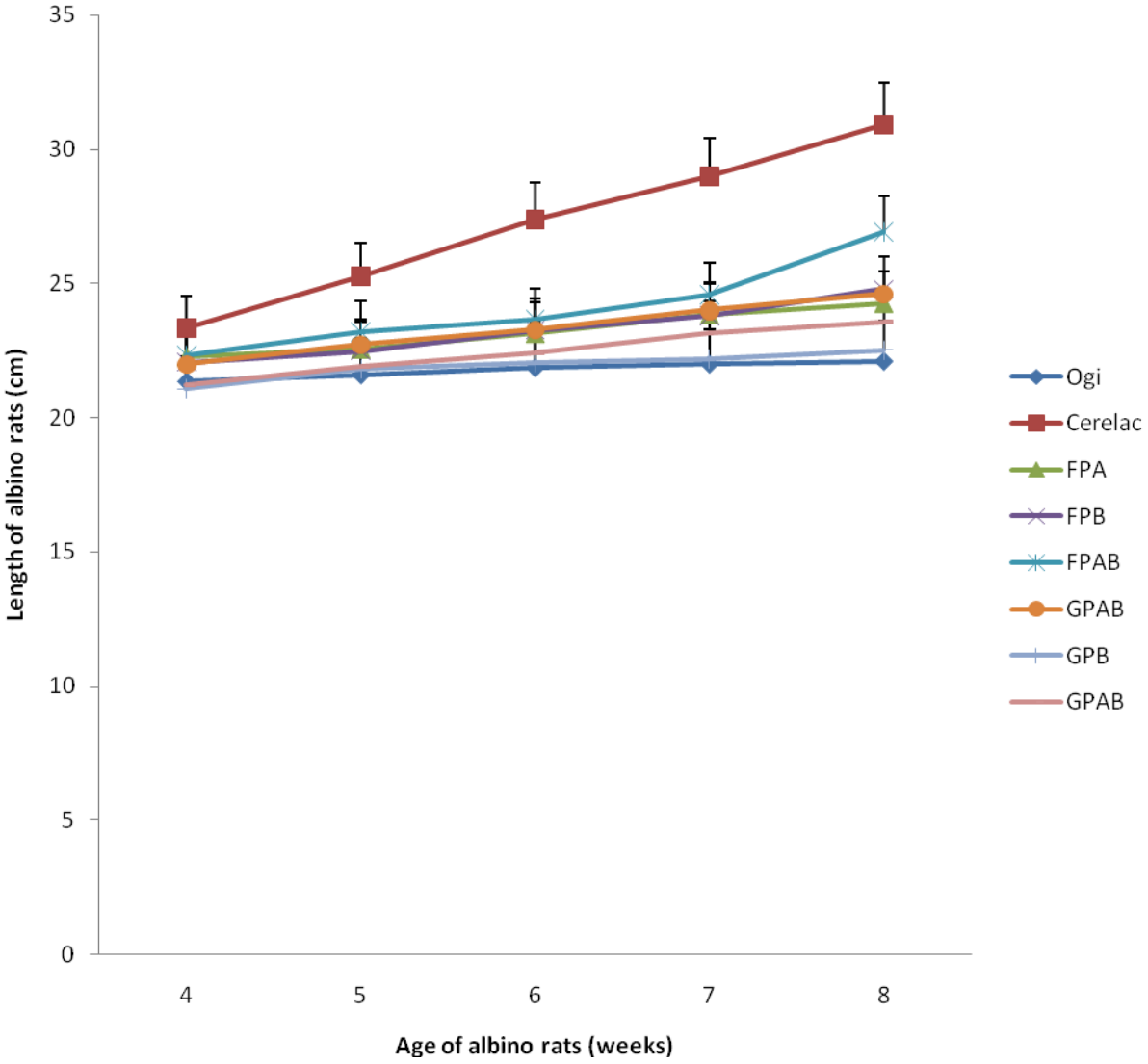

Fig. 4.12: Comparison of Length-for-age of albino rats fed with the germinated and fermented formulated diets and control food samples ($p<0.05$)

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

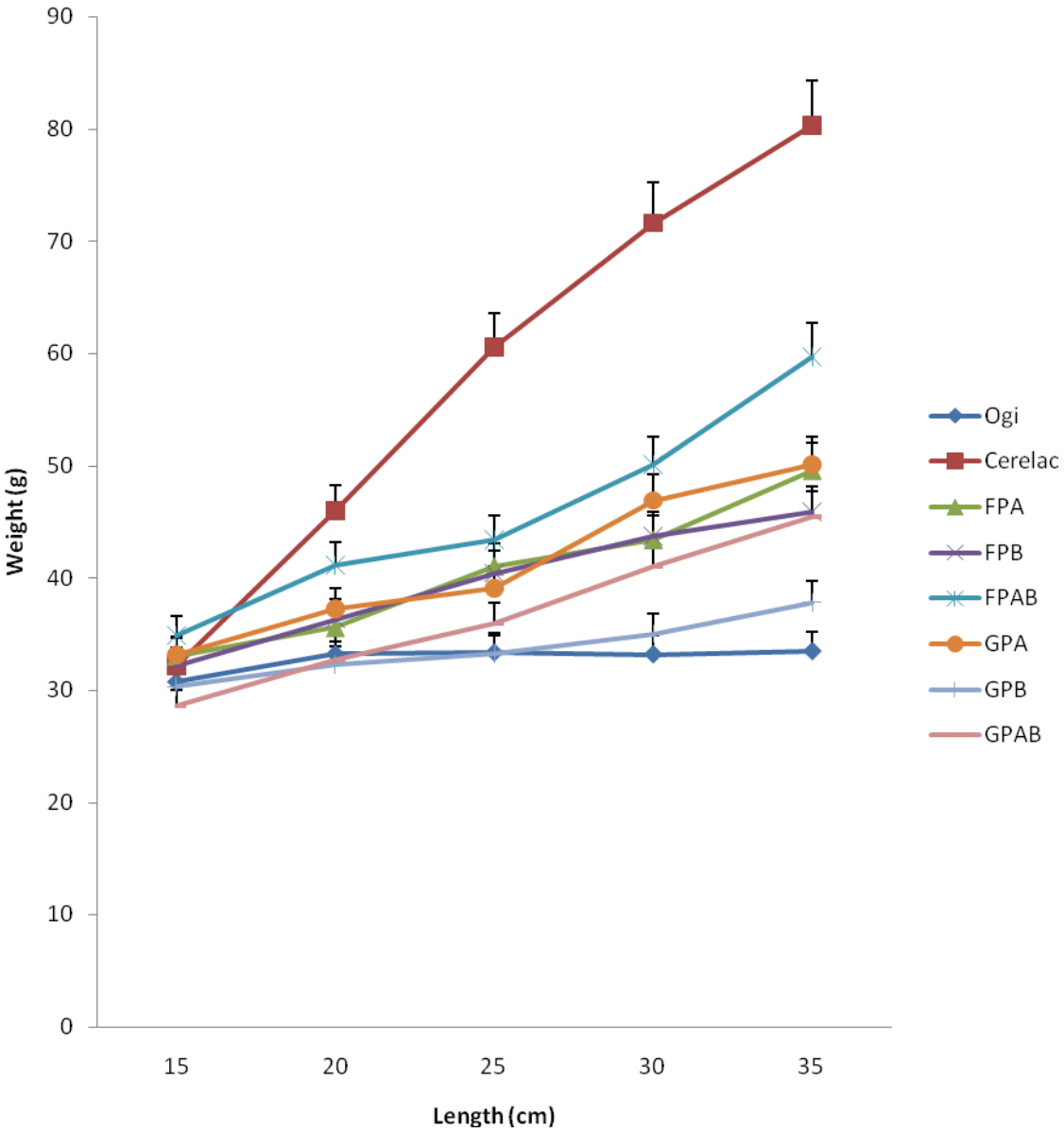

Fig. 4.13: Comparison of weight-for-age of albino rats fed with the germinated and fermented formulated diets and control food samples ($p<0.05$)

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

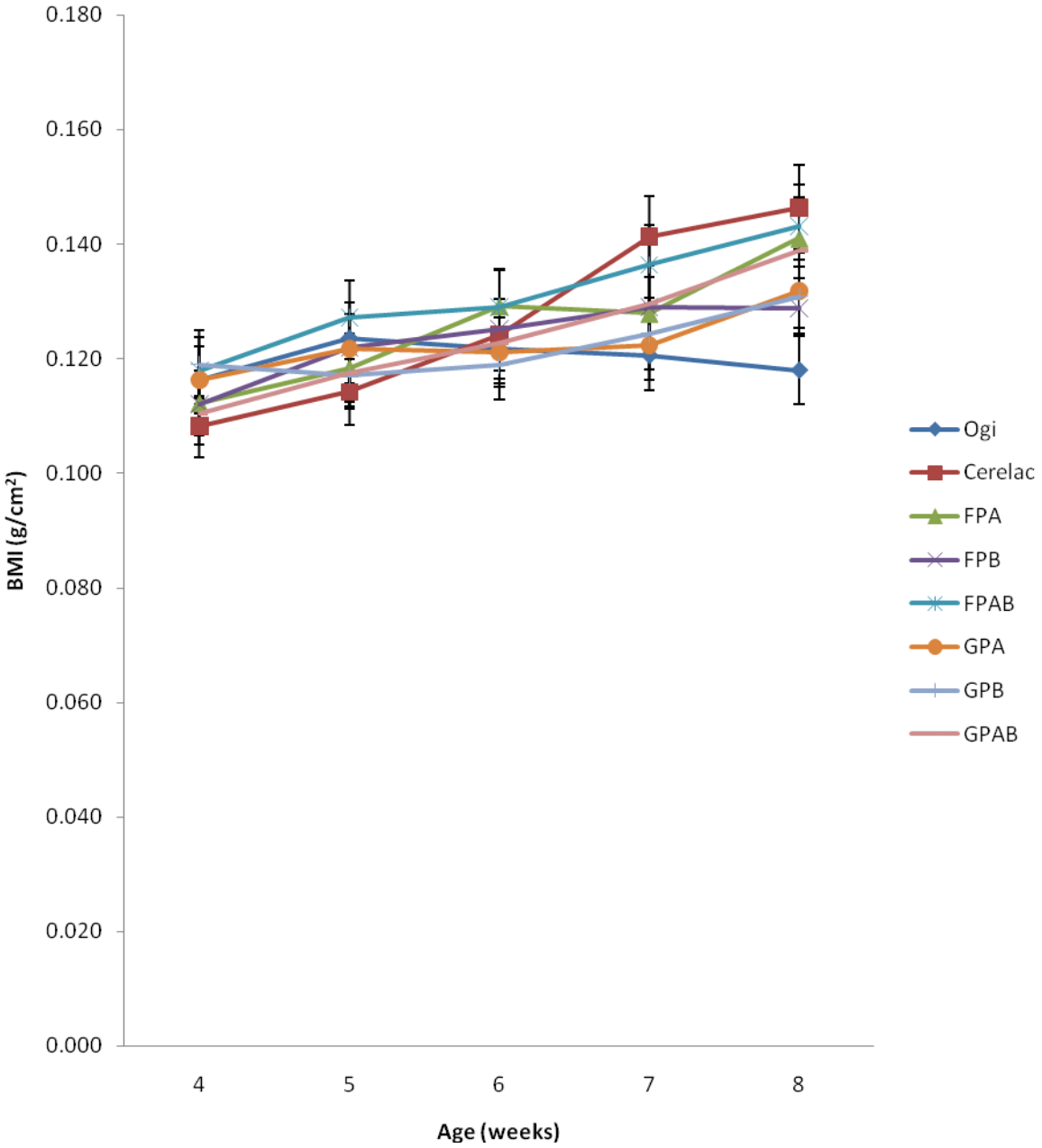

Fig 4.14: Body mass index (BMI) (g/cm^2) of Abino rats fed with the germinated and fermented formulated diets and control food samples ($p<0.05$)

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

4.14: Estimated Cost of the Formulated Food Samples

Figure 4.15 shows the estimated cost of approximately 100g of each formulated food samples and control complementary food samples based on the current prices of foodstuff used in the market, and the cost of a 450g tin of control diet (Nestle Cerelac). The estimated costs of the formulated complementary foods ranged from N52.10 to 53.81, and were significantly ($p<0.05$) lower when compared with the cost of *Cerelac* (N188.89). This observation agreed with the report of Solomon (2005), who established that the costs of locally produced complementary foods ranged from N50.00 to N100.00, which were lower than the cost of Cerelac (a commercial complementary food).

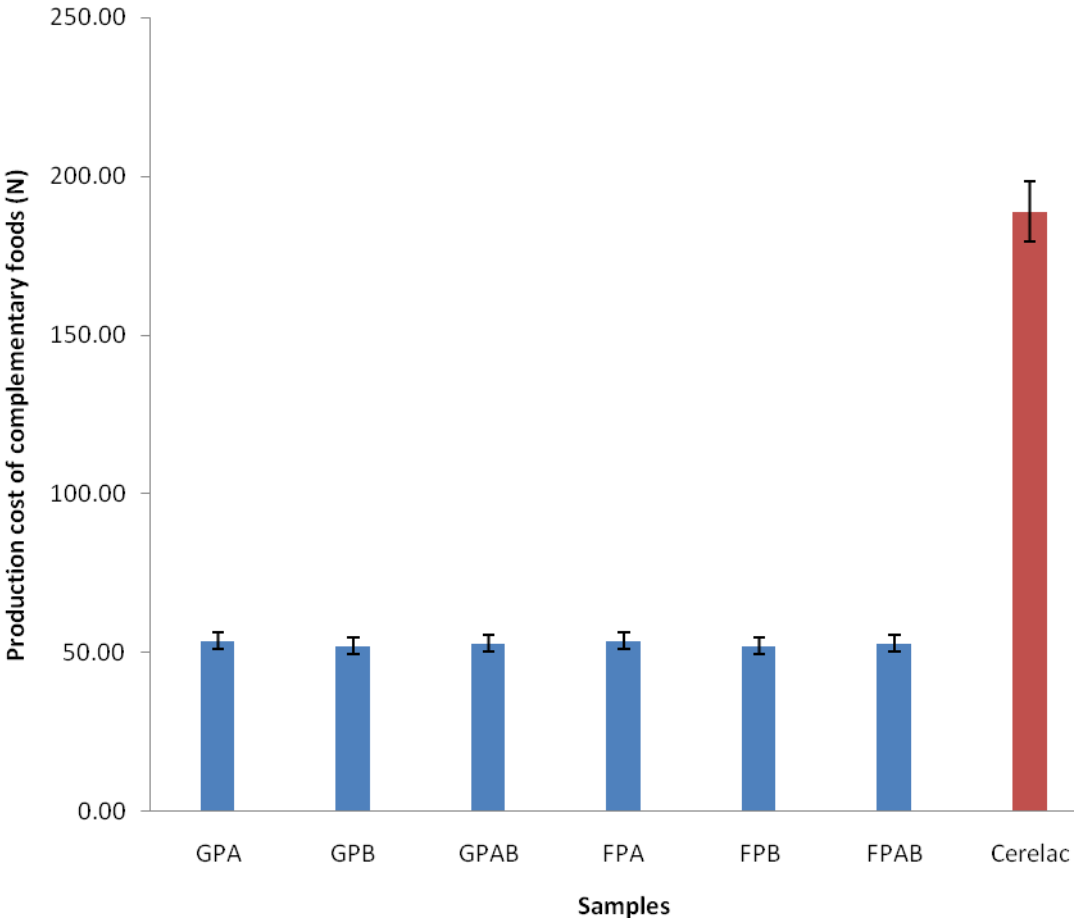

Fig. 4.15: Estimated cost of the formulated food samples per 100g

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

4.15 Selection Criteria for Determining Optimal Complementary Food

Ranking of formulated complementary foods to determine optimal nutritional profile is presented in Fig. 4.15. The ranking of the formulated food samples to determine the best nutritional profile of the samples showed that fermented popcorn-African locust bean- Bambara groundnut blend (FPAB) was ranked best compared with the remaining formulated food samples, while FPA was ranked the least of all the formulations.

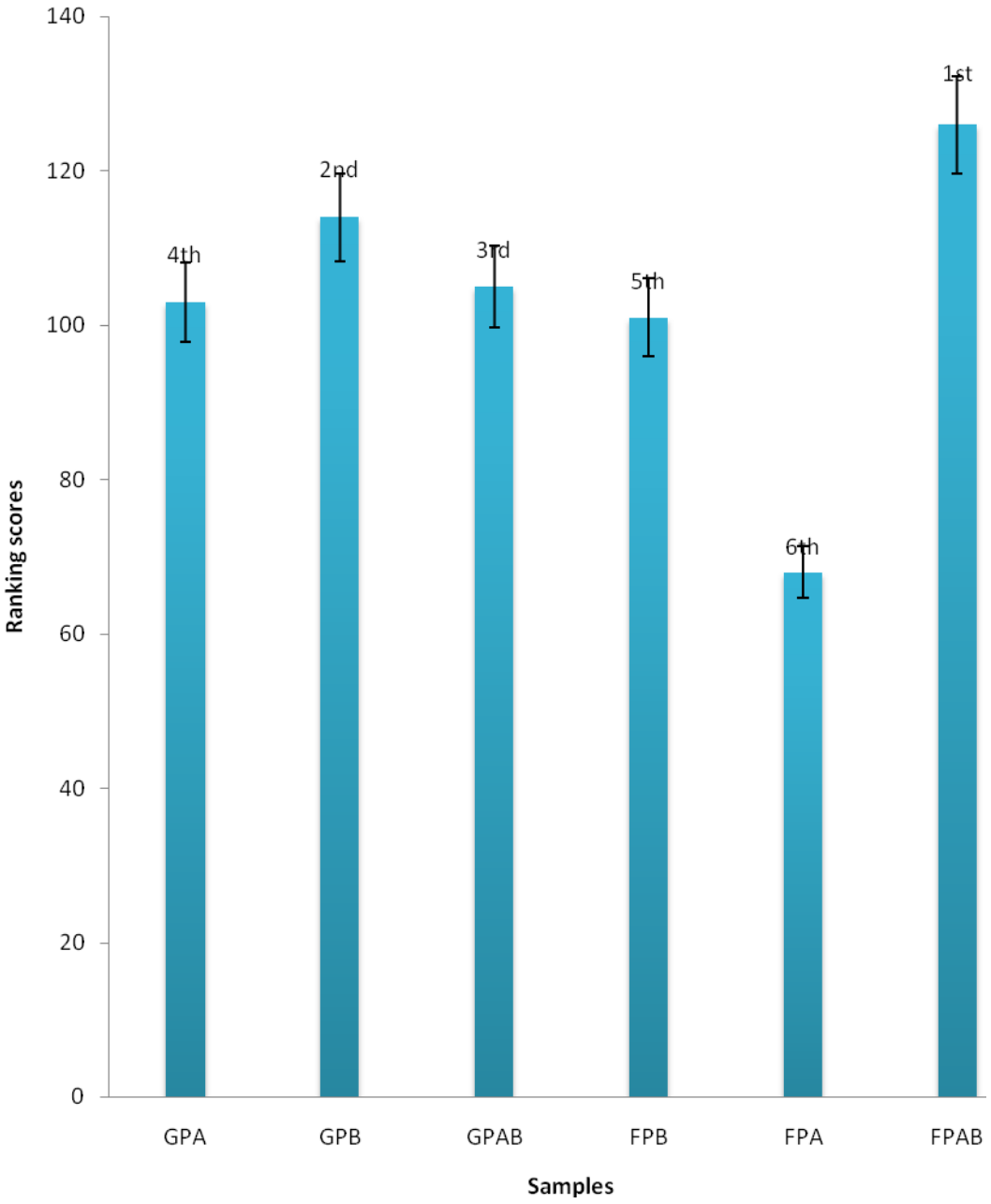

Fig. 4.16. Ranking of formulated complementary foods to determine optimal nutritional profile

Key: *GPA (Germinated popcorn-African locust bean blend),*
GPB (Germinated popcorn-bambara groundnut blend),
GPAB (germinated popcorn-African locust-bambara groundnut blend),
FPA (Fermented popcorn-african locust bean blend),
FPB (Fermented popcorn-bambara groundnut blend),
FPAB (Fermented popcorn-african locust-bambara groundnut blend).

CHAPTER FIVE

DISCUSSION, CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

5.1.1 Proximate Composition of Formulated Complementary Food Samples

The moisture content (MC) in germinated complementary foods were lower than fermented complementary food samples, however, the moisture content in both germinated and fermented complementary food samples were higher than Ogi (a local complementary food), *Cerelac* (a commercial complementary food) and FAO/WHO (1991) recommended value for infant and young children complementary foods. In comparison with other finding, it was observed that the moisture contents in the present study were similar to the value reported for the complementary food formulated from rice, soybean and egg powder (Bangoura and Zhou, 2007). Scientific investigations have reported that low moisture content in food samples is a desirable phenomenon, since this would reduce microbial activities and thereby increased the storage periods of the food products (Noorlidah and Nawawi, 2000; Olitino *et al*., 2007; Alozie *et al*., 2009). In contrary, high moisture content in foods encourage microbial growth; hence, facilitate food spoilage and reduction in nutritional qualities of the food products (Udensi *et al*., 2012; Oyarekua, 2013). In view of the high moisture content that were observed in the present formulated complementary foods, it is therefore advisable not to store the products for a long time in order to avoid spoilage. However, in Nigeria, most of traditional complementary foods are characterized with high moisture content that encourage microbial growth (Temple *et al*., 1996). Mothers often prepare large quantities of infant foods and keep in containers, to avoid frequent processing, and to have spare time and energy for other domestic activities. In view of this, it is important therefore, that food commodities, which intended to be used in the

preparation of dry weaning foods, should be properly dried, and only small quantities are to be prepared at a time to avoid prolonged storage and microbial attack.

The fiber content in both germinated and fermented complementary food samples were higher than 'Ogi', but lower than that of *Cerelac* and FAO/WHO (1991) recommended values for infant complementary foods. This observation agreed with the report of Stransky *et al*. (1992), who recommended low level of fiber content in infant and young children complementary foods; and that low fiber content in complementary food reduces bulkiness of the food and encourages high digestibility and absorption of essential nutrients like proteins and minerals, particularly in children. Fiber is a non-digestible carbohydrate originating from plants, and it is characterized as either dietary fiber, found naturally in fruits, vegetables and legumes; or functional fiber, which has been extracted from those natural sources and added into various foods, beverages or supplements. Research shows that fiber aid to improve heart health, reducing risk of chronic diseases, promoting a healthy gastrointestinal tract and improving bowel regularity, and that children are not consuming enough in their diets (Mosfegh *et al*., 2005; Anderson *et al*., 2009).

The protein contents in germinated and fermented complementary food samples were significantly higher than Ogi (a local complementary food) and *Cerelac* ($p<0.05$); and were also higher when compared with popcorn flour. This observation could be attributed to the supplementation of popcorn flour with two different legumes like Bambara groundnut and African locust bean flour, which are high in protein content (Ijarotimi and Esho, 2009; Ijarotimi and Keshinro, 2012). Investigations have shown that the protein content of two or more plant-based food materials, particularly cereal-legume combinations, are usually better than those produced from a single cereal or other plant based food materials (Solomon, 2005; Achi, 2005; Wakil and Onilude, 2009; Ibironke, 2014). Similarly, the present study also established that the

protein content of germinated complementary food samples were higher when compared with that of fermented complementary foods. This finding could be attributed to the fact that during germination the growing plant produces produces additional protein needed for its growth and development by utilizing other nutrients (carbohydrate and fat) for making protein (Enujiugha *et al*., 2003; Ocheme and Chinma, 2008; Fasasi, 2009; Oyarekua, 2014). Nutritionally, the protein contents in germinated and fermented food samples were higher than that in 'Ogi' (a traditional complementary food) and *Cerelac* (a commercial complementary food). Comparatively, it was observed that the protein content of the present experimental complementary food samples were either similar or higher than the report of other findings (Onabanjo *et al*., 2009; Bassey *et al*., 2013; Adepeju *et al.*, 2014; Amegovu *et al*., 2014; Ikujenlola and Adurotoye, 2014; Sule, 2014). Studies have shown that sprouting and fermentation processing techniques usually improved the nutritional (i.e., protein and vitamin content) and functional properties of food products (Jirapa *et al*., 2001; Yagoub and Abdalla, 2007; Assohoun *et al*., 2013; Oyerukua, 2014). Nutritionally, the high protein contents that were observed in the formulated complementary food samples in the present study would be beneficial for the growth and development of infants. Essential nutrients, like proteins, are needed for biochemical activities, building and repair of new tissues in the organs of the body (Oyarekua, 2010).

The energy values of formulated complementary food samples were higher than that in 'Ogi', but comparable to *Cerelac* and FAO/WHO (1991, 1998) recommended ranged values for infant complementary foods. The high-energy values of the formulated complementary food samples in this present study indicate that infants may need to consume less quantity of the complementary foods to meet their daily energy requirements of 200, 300 and 550 kcal for ages 6 to 8, 9 to 11 and 12 to 23 months respectively when compared with 'Ogi' (Dewey and Brown, 2003). This would provide more beneficial to the infant considering the capacity of the stomach

size. It is evident that most complementary foods used in Nigeria are locally produced from cereals, and are characterized with low energy density (Adepeju *et al*., 2014).

The common maize used in traditional complementary foods production in Nigeria and other developing countries; has two significant nutritional problems like all cereals. Firstly, it is low in protein (9-10%) and secondly, it does not provide the essential amino acids (lysine and tryptophan) in sufficient quantities to meet the nutritional needs of infants (Adepeju *et al*., 2014). Hence, it has been implicated as the major factor in the etiology of nutritional related diseases such as protein-energy malnutrition among underprivileged children who were solely weaned on Ogi (Kikafunda *et al*. 2006; Mohamed and Huiming, 2007; Temesgen, 2013). Epidemiological study has revealed that protein-energy malnutrition constitutes a serious nutritional and health problem for children between 6 to 18 months of age in Nigeria and other developing countries, due to poor child feeding practices, low quality protein commonly associated with plant-based single diets and nutrient losses during processing (Temple *et al*., 1996; Daelmans and Saadeh, 2003). This nutrition problem is responsible for growth retardation, increase in morbidity and mortality rate among children falling within the low-income families who cannot afford the high cost of fortified nutritious proprietary complementary foods (Traoré, 2005; Bruyeron *et al*., 2010; Muhimbula *et al*., 2011).

5.1.2 Amino acid Composition of Formulated Complementary Foods and Control Samples

The total amino acids profile (TAA) and total essential amino acids (TEAA) in germinated food samples were higher than fermented food samples. This observation agreed with the findings of other researchers, who reported that the protein contents of germinated food products were higher when compared with the food products that were processed with other processing techniques (Onwuka *et al*. 2009; Temesgen, 2013). Germination is a food processing

method by which the quality of a cereal and legumes can be improved for both digestibility and physiological functions (Temesgen, 2013). Germination is practical, cost-effective, and sustainable process for production of weaning foods with minimum paste viscosity, high energy and nutrient density (Temesgen, 2013). Total essential amino acid contents of both germinated and fermented food samples were higher when compared with 'Ogi', but comparable to *Cerelac*. This finding indicated that the formulated complementary foods could provide adequately the essential amino acids required by infants for the growth and development. It was equally observed in this present study that the essential amino acids profile of popcorn-based complementary foods supplemented with African locust bean and bambara groundnut were higher than in popcorn flour alone. This observation agreed with other findings, who reported that a single plant-based food, such as Ogi (corn gruel), is usually low in essential amino acids (Badamosi *et al.*, 1995); but when combined with legume or other protein-rich food materials, the nutritional quality of the combinations usually improved and better than unfortified single plant-based food product (Omueti *et al*., 2009). It is well known that a child, who solely depends on Ogi is usually prone to nutritional problems like stunted growth and poor cognitive development (Tizazu *et al*., 2010). Nutritionally, the percentage ratio of TEAA to TAA in the formulated complementary foods were well above the values considered to be adequate for ideal protein for infants (39%), children (26%) and adults (11%) (FAO/WHO/UNU, 1985**)**, hence, the essential amino acids in the formulated diets may adequately support growth and development in infant and young children. Study has shown that in addition to the essential amino acid, histidine and arginine are equally important to children, because the metabolic pathways that synthesize these amino acids are not fully developed (Lourenço and Camilo, 2002), and these were adequately provided in the formulated complementary foods of this study. It is well known that histidine is important in the repair of tissue, maintenance of the myelin sheaths that protect

nerve cells and production of both red and white blood cells, while arginine is used for the production of nitric oxide (Tejero *et al*., 2008).

5.1.3 Fatty Acid Composition of Formulated Complementary Foods and Control Samples

Essential fatty acid compositions in germinated complementary food samples were lower than in fermented food samples. This observation could be attributed to the utilization of essential fatty acid during biochemical processes of the sprouted seeds; hence, there was reduction in the fatty acid composition of germinated food products compared with the food products from other processing techniques. In comparison, the fat contents in the formulated diets were comparable with that of the *Cerelac* a proprietary formula. This could be attributed to the inclusion of oil-dense African locust bean and Bambara ground nut in the formulations. This observation agreed with the FAO/WHO (1991) recommendation that advocated for the inclusion of vegetable oils into infants and young children complementary foods in order to increase the energy density, and also, to facilitate the absorption of fat-soluble vitamins. Similarly, other researcher utilised oil seeds like soybean in the production of infants and young children complementary foods, and reported that the food products contain appreciable amount of linoleic acid and α-linolenic acid that could provide adequate amounts of essential fatty acids for the growing children (Bond *et al*., 2005). However, the formulated complementary foods in this study were devoid of some essential fatty acids, like arachidonic and docosahexanoic acids, but contain appreciable amounts of linoleic acid and linolenic acid. This observation also agreed with the study of Fernandez *et al.* (2002), who reported that some Nigerian weaning foods were devoid of arachidonic and docosahexanoic acids, but high in linoleic and linolenic acids. Evidence showed that essential fatty acids (EFA) (α-linolenic acid and linoleic acid) are precursors to docosahexaenoic acid and arachidonic acid. The docosahexaenoic acid and arachidonic acid are critical for brain growth and development, particularly of the neural

development in the first 6 months of life (Innis, 1992), and that low intakes of these fatty acids may lead to impaired cognitive, visual, and motor skill development (Innis, 1992; Martinez, 1992). The ratio of polyunsaturated fatty acid to saturated fatty acid in fermented formulated complementary foods was higher than in germinated food samples. This observation showed that the fermented food samples contain appreciable amount of polyunsaturated fatty acid compared to saturated fatty acid. Nutritional studies have reported that an increased in dietary P/S ratio to 1.0 or higher is recommended as part of measure to lower serum cholesterol, and that such high dietary ratio of polyunsaturated to saturated fatty acid provides health benefits like preventing cardiovascular diseases (Singman *et al*., 1980). It is well known that saturated fatty acids and trans fatty acids cause negative effects on human health, but polyunsaturated fatty acids (PUFA) have a positive effect on human health as regards coronary heart disease (Blanch and Grashom, 1995; Bhatnagar and Durrington, 2003; Erkkila *et al*., 2003; Meyer *et al*., 2003). The consumption of unsaturated oils in diet is therefore recommended both to decrease high cholesterol intake and to increase the ratio of polyunsaturated to saturated fatty acids to prevent the development of atherosclerosis (Singman *et al*., 1980; Cutler, 1991). Polyunsaturated fatty acids (PUFA) are prone to free-radical reactions leading to lipid peroxidation, which is known to play a significant role in the development of cancer, aging, diabetes mellitus, atherosclerosis etc ((Esterbauer *et al*., 1993; Halliwell, 1994; Mayes, 1995)

5.1.4 Mineral Content of Formulated Complementary Foods and Control Samples

Essential minerals like calcium, phosphorous, iron and zinc were higher in germinated food samples than in fermented food samples. This observation agreed with other researchers, who reported that sprouting or germination increased bioavailability of minerals and other essential nutrients in food products (Camacho *et al*., 1992; Egli, 2002; Helland *et al*., 2002; Egli *et al*., 2004). This study showed that the mineral compositions of the formulated diets were lower

than the FAO/WHO (1991) recommended values for infant's complementary foods and in the *Cerelac* (a proprietary formula), however, the values were higher than in Ogi, a traditional complementary food. This observation could be attributed to high content of anti-nutritional factors and poor bioavailability of minerals in plant-based foods (Badamosi *et al*., 1995; Temple *et al*., 1996), as well as loses of minerals during processing (Nigerian Nutrition Network, 2000). There is a need therefore to fortify the formulated complementary foods with micronutrients in order to improve on the mineral status of the formulations. It is evident that commercial formulas are usually fortified with micronutrient during production in order to compensate for loses, and this was actually responsible for the high mineral content of the *Cerelac* when compared with the present study formulations. These however may make the product unaffordable to many low-income families (Traoré, 2005; Bruyeron *et al*., 2010; Muhimbula *et al*., 2011).

In developing countries, studies have shown that most of the complementary foods for infant are usually low in vital minerals like iron and zinc, due to processing methods and antinutritional factors in plant-based complementary foods (Na¨vert *et al*., 1985; Hallberg *et al*., 1989; Temesgen, 2013). Iron deficiency is the most common causes of anemia although other nutrition and non-nutrition related cause could be involved in the origin of anemia. Hallberg *et al*. (1989) reported that anemia is most prevalent in children between 6 and 24 months of age and the major causes are inadequate dietary intake of bio-available iron, malaria and parasitic infections. Infants need iron for the production of red blood cells and growth. Deficiency of iron can be severe resulting in anemia, which in turns a risk factor for abnormal cognitive, social development as well and neuro-psychomotor development. Iron values in this study were lower than the reference daily requirement of 12mg/100g adopted by FAO/WHO (1991) on formulated complementary foods for older infants and young children. These formulated complementary

foods therefore need to be fortified with iron supplement in order to improve on the iron status of the diets and to meet the daily requirements of target-aged group.

Calcium and phosphorous are needed for bone and teeth formation, and deficiency of these minerals can lead to rickets in infant and children. Calcium values in this study were higher in germinated samples than fermented samples, and were both lower than *Cerelac*. Food is considered "good" if Ca/P ratio is above one and "poor" if the ratio is less than 0.5, while Ca/P ratio above two helps to increase the absorption of calcium on the small intestine (Nieman *et al*., 1992). The results of Ca/P ratios in the formulated diets in this present study were not only good but also gave an indication that they would help to increase the absorption of calcium in the small intestine and promote bone and teeth formation in children.

Sodium (Na) and potassium (K) are required to maintain osmotic balance of body fluid and the pH of the body regulate muscle and nerve irritability, control glucose absorption and enhance normal retention of protein during growth (NRC, 1989). Sodium to potassium ratios in this report were lower than the recommended value (<1.0), hence the formulated complementary foods may not pose any threat to the heart and blood pressure of the consumers, particularly the children. A Na/K ratio less than one is recommended in the diets of people who are prone to high blood pressure and similarly for children with immature heart (Langford 1983; Cappuccio and McGregor, 1991).

5.1.5 Anti-nutrient Concentration of Formulated Complementary Foods and Minerals Bioavailability

5.1.5.1 Anti-nutritional factors

The anti-nutrient concentrations in both germinated and fermented complementary foods were low, and this could be attributed to the effects of germination and fermentation processing techniques adopted during the samples preparations. For instance, the oxalate, tannin, phytate

and trypsin inhibitor compositions of fermented food samples were lower than in germinated diets, and both were within the tolerable level. This finding agreed with the studies of Peace *et al*. (1992) and Anigo *et al*. (2010), who reported reduction in trypsin inhibitor, tannin and phytate concentration of fermented food samples over malted cereals, soybeans and groundnut. In comparison, anti-nutrient concentrations in the formulated complementary foods in terms of oxalate, phytate, tannin and trypsin inhibition activities were higher than the values of *Cerelac*, but lower than the recommended range values of lethal dose for oxalate (2.5 g/kg), tannin (30mg/kg), phytate (50-60 g/kg) and trypsin inhibition activities (2.5 g/kg) (Inuwa *et al*., 2011).

Quite a number of researchers had reported that various domestic processing methods like soaking, dehulling, sprouting, cooking and fermentation usually bring about reductions in anti-nutritional composition of food products (Badau *et al*., 2005; Gilani *et al*., 2005; Anju *et al*., 2008; Ijarotimi and Keshinro, 2011; Ijarotimi and Keshinro, 2012; Temesgen, 2013). The presence of anti-nutritional factors in food products affects their nutritional qualities (Lopez *et al*., 2002; Weaver and Kanna, 2002). Nutritional quality is the ability of food to provide usable forms of nutrients, protein, carbohydrate, vitamins and minerals to support growth and promote good health (Temesgen, 2013). Phytic acid is a powerful chelating agent and it is usually chelates with divalent or trivalent cations (calcium, magnesium, zinc and iron) and macro-elements and form a complex with protein, protease and amylases of the intestinal tract, thereby reduces their bioavailability (Lopez *et al*., 2002; Weaver and Kanna, 2002; Temesgen, 2013). Polyphenols decrease the digestibility of carbohydrates the availability of vitamins and minerals (Rao and Deosthale, 1982, Saharan, *et al*., 2001) and interact with protein to make them insoluble (Singh, 1984; Gilani *et al*., 2005). Trypsin inhibitor disrupts digestive process of protein and may lead to other undesirable physiological reactions (Gilani *et al*., 2005). Recent findings have also reported on the nutritional and health benefits of some phytochemicals in

plant-based foods, for instance, anti-nutritional compounds like phenolic compounds and saponins, play important health benefits associated with control of anti-nutritional cardiovascular disorders (blood cholesterol reaction) and anti oxidant activities (Temesgen, 2013).

5.1.5.2 Relationship between Phytate and Bioavailabilities of Selected Minerals

Phytate, which is also known as inositol hexakisphosphate, is a phosphorus-containing compound that binds with minerals and inhibits mineral absorption. The presence of phytate in foods has been associated with reduced mineral absorption due to the structure of phyate, which has high density of negatively charged phosphate groups, which form very stable complexes with mineral ions causing non-availability for intestinal absorption (Walter *et al*., 2002). Phytates are generally found in food high in fibre especially in cereals and legumes (Lori *et al*., 2001). There are many techniques used to determine the bioavailability of minerals in the human body. One of the methods is by measuring the molar ratio of phyate/minerals in the food (Morris and Ellis, 1989). The proportion of samples with ratios above the suggested critical values has been calculated: phytate: calcium > 0.24 (Morris and Ellis, 1985), phytate: iron > 1 (Hallberg *et al*., 1989), phytate: zinc >15 (Turnlund *et al*., 1984; Sandberg *et al*., 1987; Morris and Ellis, 1989), phytate: calcium/zinc> 200 (Davies *et al*., 1985; Bindra *et al*., 1986; Hemalatha *et al*., 2007).

The calculated Phytate:Zinc, Phytate:Calcium, (Ca)(Phytate):Zinc and Phytate:Iron molar ratios (indices for predicting minerals bioavailability) of the formulated complementary foods were lower when compared with the critical values reported by other investigators (Morris and Ellis, 1985; Davies *et al*., 1985; Bindra *et al*., 1986; Gibson *et al*., 1991; Gibson 2006). The importance of a food as a source of dietary zinc, calcium and iron depends upon the total contents of these minerals and the level of phytic acids in the diet that affect the bioavailability of these minerals. Phytic acid may reduce the bioavailability of dietary zinc and calcium by forming insoluble mineral chelate at physiological pH. The inhibitory effect of phytate on

minerals (zinc, calcium and iron) absorption has been quantified by the molar ratios of phytate to minerals in the diet. Ratios greater than the critical values have been associated with biochemical and/or clinical evidence of minerals deficiencies (Akindahunsi and Oboh 1999).

Nutritionally, zinc, iron and calcium are essential trace elements in human nutrition, particularly for the children (Kono and Yoshida 1989). Children are more vulnerable to sub-optimal zinc, iron and calcium status with adverse effects on their growth rate and cognitive development (Hambidge *et al*., 1985), presumably because of their high zinc, iron and calcium requirements for growth and development (Kono and Yoshida 1989). Mineral deficiencies, especially of iron, calcium, and zinc, have a negative effect on human health (Sandsteed, 2000). Minerals play a vital role in the maintenance of human health. Iron for instance, is an important component of blood and enzymes involved in electron transfer. Its deficiency results in fatigue, headache and sore tongue in addition to anemia. Calcium is needed for bone and teeth formation, while zinc is essential for protein and nucleic acid synthesis, carbohydrate metabolism, successful pregnancy, delivery and normal development (Wintrobe and Lee, 1974).

5.1.6 Functional Properties of Formulated Complementary Food Samples

The functional properties, that is, bulk density, water absorption capacity, swelling capacity and least gellation of the formulated food samples were compared with Ogi and *Cerelac* samples. It was observed that the fermented complementary food samples, particularly popcorn- African locust bean- Bambara groundnut flour mixes (FPAB), had higher values for bulk density (BD), water absorption capacity (WAC) and swelling capacity (SC) than other formulated complementary foods and control food samples. The higher value of water absorption capacity (WAC) of the formulated foods compared with Ogi and *Cerelac* could be attributed to the protein content of the diet. Proteins are hydrophilic in nature and make the food to absorb and bind with more water (Badries and Mellowes 1992; Otegbayo *et al*., 2000). Water absorption

capacity depends on the hydrophobicity of proteins and the polar amino-acids that brings about the interactions between water and proteins (Kuntz, 1977). When the lipid content is high in the flour, the water absorption decreases because lipids block the polar sites of the proteins attenuating the absorption of water (Sathe and Salunkhe, 1981). The differences in the water absorption capacities of the formulated complementary foods could be explained by their respective protein contents with hydrophilic properties, which bind more water than lipids (Mbaeyi, 2005). In addition, the low water absorption capacity observed in some of the formulations could be attributed to high content of fat in the foods that usually combined with protein or carbohydrate to form a complex substance with reduced ability to absorb water (Mbaeyi, 2005). Water absorption capacity is an index of the maximum amount of water that a food product would absorb and retain (Marero *et al*., 1988; Mosha and Lorri, 1987). With respect to water absorption capacity, Giami and Bekeham (1992) reported that the microbial activities of food products with low water absorption capacity would be reduced. Hence, the shelf life of such food product would be extended.

Fermented popcorn-African locust bean-Bambara groundnut flour blends (FPAB) had the highest bulk density (BD) while germinated popcorn-African locust bean-Bambara groundnut flour mixes (GPAB) had the least value. The bulk density value is of important in packaging (Sharma *et al*., 2012; Adebayo *et al*., 2013). The lower loose bulk density implies that less quantity of the food samples would be packaged in constant volume thereby ensuring an economical packaging. However, the packed bulk densities would ensure more quantities of the food samples being packaged, but less economical. Also the bulk density is related to the loose pack density (LPD), the higher LPD indicates the free space between the foods when packed. A large free space is undesirable in packaging of foods because it constitute a large oxygen reservoir within the packed food. Nutritionally, loose bulk density promotes easy digestibility of

food products, particularly among children with immature digestive system (Gopaldas and John, 1991; Osundahunsi and Aworh, 2002).

Swelling capacity refers to the expansion accompanying spontaneous uptake of solvent. Among the complementary diets, FPB had the highest swelling power followed by GPB with the lowest being FPA. According to Kinsella (1976), swelling causes changes in hydrodynamic properties of the food thus imparting characteristics such as body, thickening and increased in viscosity to foods. This implies that among these complementary diets, FPB with the highest swelling power will produce a thick viscous gruel compared to other formulated diets. This is could be due to higher carbohydrate content in the sample than in the other diets. According to WHO (2003), appropriate complementary diet is one which produce a gruel or porridge that is neither too thick (when it is too thick, it will be difficult for the infant to ingest and digest because of limited gastric capacity) for the infant to consume nor so thin that the energy and nutrient density are reduced.

Germinated popcorn-African locust bean flour mixes (GPA) had the highest least gellation (LGE) while fermented popcorn-African locust bean-Bambara groundnut flour mixes (FPAB) had the least value. The lower the least gellation (LGE), the better the gelating ability of a food component. The ability of food amples to form a gel at a higher concentration implies that the food samples have poor gelating ability, hence will not form a thick gel, this is a good functional property for a complementary food. This means that the diet will have a low dietary bulk. The implication of a thick gel to a complementary diet is that it can affect the gastric system of the child since they have limited gastric capacity. The importance of high LGE to the complementary food is that it will reduced viscosity, plasticity and elasticity; hence, the food sample will have a low dietary bulk, which is highly favourable for a good complementary food. The result in this present study was similar to the finding of Obatolu and Cole (2000) on the

soybean flour blends. Gelation is aggregation of denatured molecules (Kinsella, 1976), during gelation protein gels are composed of three-dimensional network of intertwined, partially associated, polypeptides in which water is entrapped. Since gelation depends not only on the quantity and quality of the protein alone, but also on the non-protein component of the blend, the presence of fat that introduces a hydrophobic component to the diet can also increase the LGE thereby contributing to the poor gelating ability of the complementary diet.

Functionality of a food is the property of a food ingredient, apart from its nutritional value, that has a great impact on its utilization (Mahajan and Dua, 2002). Obatolu and Cole (2000) reported that in the processing of most complementary foods emphasis is usually on the nutritional quality and quantity of the ingredients rather than on their functional properties. Functional properties of complementary diets (gelation, bulk density, swelling index, emulsifying capacity, water-binding capacity) are very important for the appropriateness of the diet to the growing child. The consistency energy density (energy per unit volume) of the complementary food and the frequency of feeding is also important in determining the extent to which an infant can meet his or her other energy and nutrient requirements.

In developing countries, most complementary foods are starch and cereal based. Starch often provides the principal source of energy, but when heated with water, starch granules gelatinize to produce a bulky, thick (viscous) porridge. Such complementary foods tend to be of low energy density and protein content, although their liquid consistency makes them easy to consume, the volumes needed to meet infant energy and nutrient requirements often exceed the maximum volume the infant can ingest. These physical properties make the porridge difficult for infants to ingest and digest. Furthermore, the low energy and nutrient density means that large volumes of food have to be consumed to meet the infant's requirements. This is not usually possible, owing to the infant's limited gastric capacity and to the limited number of meals

offered per day. According to WHO (2003), a good quality complementary food must have high nutrient density, low bulk density, viscosity and appropriate texture thus, complementary foods should be rich in energy, protein and micronutrients, and have a consistency that allows easy consumption.

5.1.7 **Microbiological Status of Formulated Complementary Food Samples**

The microbial load of fermented food samples in terms of bacterial counts were lower when compared with germinated food samples. This observation could be attributed to the production of lactic acid that was produced during the fermentation processing, and thereby not conducive for further proliferation of the microorganism in the fermented samples. The mould/yeast counts were significantly higher in fermented African locust bean, but lower in germinated Bambara groundnut ($p<0.05$). E. Coli and Coliform were not detected in both germinated and fermented food samples. However, the microbial loads of both germinated and fermented food samples were comparatively lower than that of FAO/WHO (1991) recommended values. Studies have shown that processing techniques, particularly fermentation, inhibit the growth of microorganisms in food products; and besides, they improve on the appearance, taste of some foods and reduce the energy required for preparing food and enhanced nutritive value (Afoakwa, *et al.,* 2007; Oyarekua, 2011). Fermentation is traditional processing of food subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification of food (Temesgen, 2013). It is a very interesting process used in plant foods to increase the nutritional quality and remove undesirable compounds. Fermentation involving lactic acid bacteria offers potential for widespread applications, particularly with respect to the preservation of cereals, legumes and root crops and the provision of safe, low-cost weaning foods for developing countries (Temesgen, 2013).

In contrary to these nutritional benefits of food processing, several epidemiological investigations have established that there is strong association between complementary foods and development of diarrhoeal diseases in infants; due to microbial contamination (WHO, 1998; Hop *et al*., 2000; Saha *et al*., 2008). One of the serious nutritional implications of microbial infections is the effect on nutritional status. Microbial infections can lead to a reduction in food intake owing to anorexia. Reduction in food intake leads to weight loss, lowered immunity, mucosal damage, invasion by pathogens, and impaired growth and development in children (Katona and Katona-Apte, 2008). A sick child's nutrition is further aggravated by diarrhea, mal-absorption, loss of appetite, diversion of nutrients for the immune response, and urinary nitrogen loss, all of which lead to nutrient losses and further damage to defence mechanisms (Katona and Katona-Apte, 2008). There is a strong relationship between malnutrition and infection and infant mortality, because poor nutrition can leave children underweight, weakened, vulnerable to infections and subsequently caught in a vicious circle of malnutrition and infection (Katona and Katona-Apte, 2008).

5.1.8 Sensory Attributes of Formulated Complementary Food Samples

The aroma, colour, taste, texture and overall acceptability parameters of the formulated food samples were rated lower by the panelists compared with the Ogi (a traditional complementary food) and *Cerelac* (a commercial formula). However, there was no significant difference between the overall acceptability of the experimental food samples and that of Ogi ($p>0.05$); while *Cerelac* was significantly rated higher in terms of overall acceptability over the formulated food samples ($p<0.05$). The disparity observed in this present study between the formulated complementary food samples and control food samples (*Cerelac* and 'Ogi') in terms of taste, aroma and overall acceptability could be due to the familiarity of the panelists with the 'Ogi' and *Cerelac* over the new formulated products. The disparity between the germinated and

fermented formulated products could be due to different in processing techniques. For instance, it has been scientifically reported that processing methods such as germination technique negatively affect the organoleptic properties of food products (Nnanna *et al.*, 1990; Bau *et al*., 2000; Uwaegbute *et al*., 2000). To improve the on the sensory attributes of the present study formulations, there is a needs to add sweetners and flavourant.

5.1.9 Protein Digestibility, Heamatological Properties and Nutritional Status of Rats fed with the Formulated Complementary Foods Samples

5.1.9.1 Protein qualities of formulated complementary foods

The biological value (BV), true protein digestibility (TD), protein efficiency ratio (PER) and protein rating (PR) of germinated food samples were higher than in fermented food samples, except in FPAB sample. However, both the germinated and fermented complementary food samples were higher than in 'Ogi', but they were lower than in *Cerelac*. The metabolizable energy (ME) and digestibility energy (DE) in germinated food samples were lower than in fermented food samples, and both samples were higher than in control samples, except in *Cerelac*. Comparatively, the BV and PER of the formulated food samples did not met the FAO/WHO (1989) recommended values of 70% and 2.7 respectively. These indicated that the protein contents in the formulated complementary foods may not adequately support growth and development in infant without complementing the diets with other source of protein based foods like breastmilk.

The Digestible Indispensable Amino Acid Scores (DIAAS), a nutritional index to predict the quality and suitability of protein in man, of the formulated diets were observed to be higher than in Ogi (a traditional complementary food), but lower than that of *Cerelac* (a commercial formula). This finding could be attributed to the fact that *Cerelac* was formulated from cereal and milk. It is well known that animal-based foods, such as milk, is usually high in biological

value and essential amino acids compared with plant-based food products. Nutritionally, of all the formulated diets FPAB had the highest DIAAS value that was comparable to that of *Cerelac*; therefore, FPAB blend could be used as complementary food for infant and young children. Digestible Indispensable Amino Acid Score has been recommended to be the most suitable method for routine evaluation of protein quality and highly digestible food products for human nutrition (FAO, 2013).

Scientific study has indicated that combination of cereals with two or more inexpensive plant protein sources like that of legume can be highly beneficial, since the biological values of the products are improved (Amankwah *et al*., 2009). Quantitatively, the amount of FPAB needed to meet essential amino acids requirement of infant was lower than that of ogi and other formulated complementary foods; but higher when compared with that of Cerelac.

5.1.9.2 Heamatological properties of rats fed with the formulated complementary foods

The hematological variables, that is, red blood cell (RBC), pack cell volume (PCV) and heamoglobin (Hb) concentrations in the blood of rats fed both germinated and fermented complementary food samples were within the same ranged values, but were higher than in 'Ogi' and comparable with the values in the blood of rats fed *Cerelac*. The white blood cells (WBC) in rats fed both germinated and fermented food samples were within the same ranged values, and these values were lower than in control food samples, except for the 'Ogi'. Comparatively, The PCV, RBC and Hb concentrations of the formulated complementary foods were within the range of values reported by Jenkins (1993) and Hillyer (1994). These observations indicated the adequacy of the formulated diets in blood formation and promotion of good health status. Scientific study has proven that diets containing poor protein would usually result in poor production of heamoglobin and poor transportation of oxygen from the respiratory organs to the peripheral tissues (Roberts *et al*., 2000). Hence, low haemoglobin (Hb) concentration is an

indication that the dietary proteins were not of high quality. White blood cells (WBC) of the animals fed on formulated complementary foods were within the recommended values *(*Hillyer, 1994; Jenkins, 1993). This indicated that the complementary food samples were free of toxic and microorganisms. It is well known that high white blood cells counts indicated an acute infections and toxicity of diets consumed (Nwankpa *et al*., 2014). Blood is one of the specialized body fluid responsible for the transportation of nutrients, oxygen, hormones and other metabolites to the body's cell and metabolic waste products away from those cells to sites of elimination (Nwankpa *et al*., 2014). It is known to be the most important body fluid that regulates various vital functions of the body (Nwankpa *et al*., 2014). Mammalian circulation of blood transports such specific chemical substances as nutrients, gases, minerals, metabolic products and hormones between different tissues and organs (Baynes and Dominiczak, 2005).

5.1.9.3 Anthropometric Measurements and Nutritional Classifications of Wistar Rats Fed on Formulated complementary Food Samples

The nutritional status (weight-for-age, weight-for-length, height-for–age and body mass index (BMI) nutritional indices) of animals fed on FPAB sample had a better growth rate when compared with those animals fed on other formulated complementary foods. However, in comparing with the control food samples, it was observed that the growth patterns of animals fed on the formulated complementary foods were significantly higher than those animals fed on 'Ogi' (a traditional complementary foods), but lower than those animals fed on *Cerelac* ($p<0.05$). The body mass index (BMI) of the experimental rats in this present study were comparatively lower than the report of Novelli *et al*. (2007). For the development of kidney, liver and heart, the study established that FPAB food sample enhanced the development of these organs in the rats fed with this diet better than other formulated complementary foods and 'Ogi', but lower than in those rats fed on *Cerelac*. Considering these findings, it could be deduced that these formulated

food samples, particularly FPAB, are suitable as infant complementary foods, especially for those infants belonging to low-income families who cannot afford animal protein-based complementary foods or those children who are solely weaned on traditional complementary foods like 'Ogi', which are low in protein content.

Anthropometric measurements can be used to monitor changes in growth of children, and that it is an important determinant of a nation's health. The measurements of height, weight and nutrient intake are the reliable means to evaluate the nutritional status of children (Parimalavalli and Sangeetha, 2011). Recently, several findings have reported on the increased in protein-energy malnutrition from many parts of developing countries due to low nutritional quality of traditional complementary foods and high cost of commercial complementary foods (Mosha *et al*., 2000; Hurrel, 2003; Mbithi-Mwikya *et al*., 2002; Ikujenlola and Adurotoye, 2014; Adepeju *et al.*, 2014). The high cost of commercial formula has compelled many nursing mothers to depend on low nutrient-dense and bulky traditional complementary foods produced from cereal (Malunga *et al*., 2014). World Food Programme (WFP) defines malnutrition as "a state in which the physical function of an individual is impaired and can no longer maintain adequate bodily performance process such as growth, physical work, and resisting or recovering from disease" (WFP, 2000). Epidemiological findings have established that a malnourished child experiences retardation in growth and cognitive development (Hamadani *et al*., 2001; Berkman *et al*., 2002) and stands a greater risk of morbidity and death when compared with their normal counterparts (WFP, 2000; Umeta *et al*., 2000; Rivera *et al*., 2003; Fishman, 2003; Kim *et al*., 2007).

It is well known that poverty remains the major contributor to poor nutrition and health among children in many parts of developing countries, including Nigeria (Claeson and Waldman, 2000). Traditional complementary foods are generally deficient in vital micronutrients as well as macronutrients (Eka *et al.*, 2010). Lack of education especially among nursing

mothers further disadvantages the vulnerable children in delivery of healthy practices like breastfeeding and consumption of nutritious foods (Amegovu *et al*., 2014). In order to combat protein-energy malnutrition (PEM) effectively in developing countries, a low-cost complementary food that is high in protein and energy-dense is a desirable substitute for expensive imported complementary foods and low qualities local complementary foods (Agbede and Aletor, 1997; Ijarotimi and Olopade, 2009; Ijarotimi and Keshinro, 2013).

5.1.10 Estimated Cost of Formulated Complementary Foods and Control Complementary Foods

The estimated cost of producing the formulated complementary foods in this present study was quite low when compared with the Cerelac (a commercial complementary food). This indicates that with proper selection of local foodstuffs, it is possible to prepare nutritious complementary foods that would be acceptable, readily available, affordable and nutritionally adequate. Therefore, the present formulations can be affordable and accessible to most underpriviledged mothers who cannot afford the commercial complementary foods. Quite a number of nutritional survey have been reported in many countries including Nigeria that commercial complementary foods are energy-dense and rich in high quality protein to meet the nutritional requirements of infants in both developed and developing countries. However, the products as marketed are too expensive for the target groups in developing countries (Ijarotimi *et al*., 2012; Yusufu *et al*., 2013; Adepoju and Etukumoh, 2014).

5.1.11 Selection of Optimal formulated Complementary Food Samples

The ranking of the formulated complementary food samples to determine the best nutritional profile of the samples showed that fermented popcorn-African locust bean- Bambara groundnut blend (FPAB) was ranked best compared with the remaining formulated complementary food samples, while FPA was ranked the least of all the formulations. This

observation showed that fermentation improved the nutritional qualities of food products. However, the fermented popcorn African locust bean (FPA) blend was ranked least, and this may be attributed to the composition of the blended flour sample or effects of fermentation, which affects its flavor.

5.2 Conclusion

The study formulated and evaluated nutritional qualities of complementary foods using home-based methods and locally available food materials like popcorn, African locust bean and Bambara groundnut. The findings showed that the protein contents, energy values, biological values, protein efficiency ratios, heamatological indices like pack cell volume, red blood cells and heamoglobin concentration, and growth patterns of both germinated and fermented complementary foods were better than in 'Ogi', a local complementary food, but comparable with the Nestle *Cerelac*, a commercial complementary food. The fermented popcorn, African locust bean and Bambara groundnut (FPAB) flour combinations however was more efficacious in terms of nutritional quality and supporting growth in animals than in other formulated complementary foods. Therefore, FPAB sample is recommended as infant complementary food, and it could be used as a substitute for Ogi, a local complementary food, low in protein and energy density, which has been implicated as one of the factors responsible for protein-energy malnutrion among 6-59 months old children in Nigeria.

5.3 Recommendations

In view of the findings in this present study, the following recommendations are therefore made:

1. processing techniques other than germination and fermentation should be used to further improve on nutritional quality of the formulated diets.
2. fortification and or supplementation of the formulated food samples with micronutrient that are marginal should be explored.
3. protein quality evaluation of the formulated blends should be conducted in infants to further substantiate the nutritional potentials of the product to support growth and development.
4. shelf-life studies of the formulation should be undertaken.
5. findings from this study should be disseminated at scientific and community levels.

5.4 Contribution to Knowledge

At the end of the research, the study provided additional information on the utilization and nutrient compositions of popcorn, African locust bean and Bambara groundnut seeds. Also, it provided qualitative information concerning the nutritional qualities of novel complementary foods formulated from the combinations of these locally available food samples. The production and ultilisation of these formulated complementary foods as complementary food as infant diets would further improve child health and nutritional wellbeing, and thereby reduce morbidity and mortality rates among weaning aged children in Nigeria and other developing countries. The findings of this present study would be useful in nutrition and health sectors, to establish or update local food composition tables and dietary guidelines for achieving the millennium development goals (MDGs) in child feeding. The findings would also be useful for the agricultural and food industries policy makers in Ondo State and Nigeria as a whole.

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Appendix I: Preparation of formulated complementary food samples for the rat feeding

Plate. 4: Pelleting of formulated complementary food samples for rat feeding

Appendix II: Sensory evaluation form

UNIVERSITY OF IBADAN, IBADAN, NIGERIA
DEPARTMENT OF HUMAN NUTRITION
COLLEGE OF MEDICINE

SENSORY EVALUATION FORM

PANELIST'S NAME:…….....

INSTRUCTIONS: You have been presented with seven samples, which are coded as shown below. Please evaluate these samples for sensory/organoleptic using a 9 points hedonic scale, and indicate the level that best described your perception of each sample on the scales given below. Please rate as applicable.

OBSERVATION	**SCORE**
Extremely like	9
Very much like	8
Moderately like	7
Slightly like	6
Neither like nor dislike	5
Slightly dislike	4
Moderately dislike	3
Very much dislike	2
Extremely dislike	1

SAMPLE CODE	**222**	**333**	**444**	**555**	**666**	**777**	**888**
COLOUR							
TASTE							
AROMA							
TEXTURE							
OVERALL ACCEPTABILITY							

Please comments briefly on your observations on the samples……
……

Signature…… **Date……**

Appendix III: A nursing mother during sensory evaluation

Plate 5: Picture of a panelist during sensory evaluation