EVALUATION AND PRODUCTION OF FORTIFIED BLENDS OF MAIZE-BAMBARA GROUNDNUT MALT AND MAIZE-COWPEA MALT COMPLEMENTARY FOODS FOR IMPROVED IRON AND ZINC CONTENTS

BY

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CHAPTER ONE
1.0 INTRODUCTION

1.1 Background

Complementary foods are any nutrient containing food or liquid other than breast-milk given to young children along with breast-milk (Gibbs, 2010) within the 6-23 month window. The foods, therefore, must contain sufficient amounts of the essential nutrients to complement breast milk. Infants who do not receive enough complementary foods are stunted, malnourished or both as a result of deficiencies in protein, energy and micronutrients such as calcium, iron, zinc, vitamin A and iodine (Brown, 1991).

Infancy is a time of rapid physical growth as well as physiological, immunological, and mental development. During the first year of life, nutritional requirements are at their highest in the entire life cycle. Deficiency in energy or any of the essential nutrients can have dire consequences, some of which are long-lasting. Micronutrient deficiencies especially of iron and zinc are common during infancy (Adu-Afarwuah et al., 2008; Vinodini, 2003).

Iron, zinc, and calcium are problem micronutrients in proprietary and traditional complementary foods (CFs) for infants because their concentrations fall below the calculated requirements for breast-fed infants. Complementary diets of infants and young children in Asia often contain inadequate levels of the problem micronutrients and sometimes, high levels of phytate which inhibit the absorption of these micronutrients. Consumption of fortified complementary diets is therefore highly encouraged (Gibbs, 2010) because the nutrient composition of complementary foods in developing countries (Faber, 2004) are inadequate, especially for iron, zinc and calcium. Even in the United States, iron and zinc are problem nutrients in the first year of life despite widespread availability of fortified foods. The situation for calcium, vitamin A, thiamin, folate and vitamin C depends on which desired levels are deemed most appropriate (Nestel et al., 2003).

The recommended dietary allowance for some micronutrients per 100g of complementary food for infants aged 6 to 12 month old are: 500mg calcium, 27.5mg iron, 12.5 mg zinc and 500µgRE vitamin A (Lutter and Dewey, 2003). With an average
composition and intake of breast-milk, complementary foods should provide approximately 12% of the vitamin A, 75 to 100% of zinc and iron (Gibson and Hotz, 2000).

Plant-based complementary foods fall below the RDA of micronutrients especially iron and zinc for 6-12 months infants. Attempts to improve calcium, iron, zinc and vitamin A in maize-bambara groundnut and maize-cowpea complementary foods by food-to-food fortification showed that the best results were obtained by wet-mix fortification but the values for iron and zinc (0.15 and 0.02 g/kg) were below the calculated requirements (Uvere et al., 2010; Onyekwere, 2007; Attaugwu, 2007). The present study therefore sought to improve iron and zinc contents by modifying the wet-mix fortification procedure.

1.2 Problem Statement

1. Micronutrient malnutrition is responsible for a wide range of non-specific physiological impairments, leading to reduced resistance to infections, metabolic disorders, and delayed or impaired physical and psychomotor development. In 2000, the World Health Report identified iodine, iron, vitamin A and zinc deficiencies as being among the world’s most serious health factors (Allen et al., 2006).

2. The WHO/UNICEF review of complementary feeding in developing countries recognized that iron and zinc requirements will be difficult to meet from nonfortified complementary foods (Bhutta and Dewraj, 2000).

3. Micronutrient deficiencies in infancy and early childhood abound in many developing countries (Phu et al., 2010). In this region, the prevalence of anemia reaches and in some countries exceeds 50% in one-year old children (Anon, 2002). Children who have iron-deficiency anemia in infancy are at risk for long-lasting developmental disadvantage as compared with their peers with better iron status (Lozoff et al., 1998). Iron deficiency is the most common form of nutritional deficiency.

4. Zinc deficiency in children is widespread in different regions of the world (Rosado, 2003) and results in significant delays in linear growth and weight gain, loss of a proper sense of taste and smell (ODS, 2009; Higdon et al., 2010).
5. Multiple micronutrient deficiencies including iron, zinc, and vitamin A are associated with a decreased growth rate in children and increased susceptibility to and/or severity of infections and impaired immunity (Phu et al., 2010).

1.3 Justification

This study attempts to improve iron and zinc contents of fermented maize-cowpea malt and maize-bambara groundnut malt complementary food blends by food-to-food fortification. When this research is completed,

(i) information useful for nutrition education and further research would be available,

(ii) complementary foods that are adequate for meeting the Ca, Fe, Zn and Vit. A needs of infants aged 6 to 12 months would be available.

1.4 Aims and Objectives

The goals of this research include:

(i) to produce maize-bambara groundnut malt (MBm)b and maize-cowpea malt (MCm)b complementary foods for infants,

(ii) to fortify the foods by wet mixing with a blend of processed cattle bone, roselle calyces and palm oil,

(iii) to evaluate the fortified complementary food for sensory attributes and iron and zinc contents.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Micronutrients and Infant Foods

The critical micronutrients in infant food include calcium, iron and zinc (Adu-Afarwuah et al., 2008; Gibbs, 2010; WHO, 2007). Calcium helps in proper bone formation. Vitamin A helps to boost resistance to infections and for proper vision. Iron is associated with psycho-motor development and proper functioning of the oxygen-carrying capacity of the blood. Zinc supports normal growth and development during childhood and is required for proper sense of taste and smell (ODS, 2009). Iron and zinc are critical micronutrients in infant foods (Vinodini, 2003) but their requirements will be difficult to meet from nonfortified complementary foods (Bhutta and Dewraj, 2000).

Micronutrient malnutrition in children

Among infants and pre-schoolers, the more prevalent forms of nutrient deficiencies are those of iron, vitamin A, iodine, protein-energy, riboflavin, calcium and vitamin D (Vinodini, 2003.) The overt effects of micronutrient malnutrition, such as blindness, anaemia and goiter have been known for many centuries. Iron deficiency, one of the most prevalent problems of micronutrient malnutrition, occurs in both developing and industrialized countries (Yeung, 1998). Thirty percent (30%) of the world’s population is affected by vitamin A, iron or iodine deficiency. About 700 million persons suffer from clinical forms of these deficiencies and another two billion from sub-clinical forms. Deficiencies of micronutrients such as zinc, calcium, folic acid and other vitamins are widely prevalent in the developing world. Globally, it is estimated that there are nearly 20 million children who are severely malnourished, most of them living in South Asia and in Sub-Saharan Africa (WHO, 2007, Ciliberto et al., 2005).

Infants feeding characteristics and nutrient requirements

In addition to the physical limitations of small gastric capacity, complementary feeding behaviours influencing food intake include: the number of meals and amount of food offered to the infant, and the encouragement and engagement in feeding between caregiver and child. At the individual level, the child’s appetite will also be a major determinant of the quantity of complementary food consumed.
In response to the low nutrient adequacy of complementary diets in low-income countries, the World Health Organization (WHO) have recommended the consumption of fortified complementary foods (WHO, 2007). Manufactured fortified complementary foods have the potential to provide more nutrient-dense foods to the complementary diets of infants (Gibbs, 2010)

Infants are particularly susceptible to nutrient deficiency, for a number of reasons:

- Their food choices are limited. Babies have a strong sense of taste and do not need the flavour enhancers. By the age of 6 months, cereals based foods could be introduced. Honey may carry botulism spores and is not recommended for infants under the age of 12 months. The digestive system of children and adults can destroy these harmful spores but a baby cannot. Beets and spinach have high concentrations of naturally-occurring nitrates that can reduce the ability of the baby's haemoglobin to transport oxygen. These foods should be used in moderation or not at all until the baby reaches the first birthday. Raw eggs and raw milk are not appropriate for babies. These foods may be sources of infections that can be dangerous to infants. Other foods which babies should not take include desserts, carbonated beverages, caffeine-containing beverages and candy. They provide calories with few nutrients. If they take the place of nutritious foods and beverages, they can be harmful. Soft drink mixes sweetened with sugar or NutraSweet(TM) are not good for babies. NutraSweet(TM) is considered safe in moderate amounts for children and adults, but safety for babies is not yet fully established. Besides, babies need calories for growth and development (Schafer and Fradgley, 1995).

- The amount of food they consume is relatively low (due to the small capacity of their stomach), while the demand for nutrients is high (due to their body’s fast development).

The primary cause of under-nutrition during infancy is a lack of suitable nutrient-dense complementary foods containing micronutrients that are bioavailable during the weaning period. In the light of an infant’s limited gastric capacity and high nutrient requirements, it is now widely acknowledged that malnutrition is not merely an issue of
insufficient intake of food, but also a consequence of the poor nutritional quality of the complementary diet (Gibson and Hotz, 2000, Brown, 1991). Often complementary foods are prepared as thin watery gruels that only provide minimal amounts of energy and micronutrients per meal to the infant.

One of the ways to deliver more vitamins and minerals to infants is through fortification of foods. Cereal flours (wheat and maize) are currently the most common vehicles for iron and zinc fortification to reach the general population. Implementation of complementary food fortification or preventive supplementation is important to meet their daily iron requirements (Compaore et al., 2011).

*Iron Deficiency*

Iron deficiency develops gradually and usually begins with a negative iron balance, when iron intakes do not meet the daily need for dietary iron. This negative balance initially depletes the storage form of iron (ferritin) while the blood haemoglobin level, a marker of iron status, remains normal. Iron deficiency anaemia is an advanced stage of iron depletion. It occurs when storage sites of iron are deficient and blood levels of iron cannot meet daily needs. Blood haemoglobin levels are below normal with iron deficiency anaemia. The RDA for iron varies considerably based on age, gender and source of dietary iron (ODS, 2007).

Iron deficiency is one of the most prevalent forms of malnutrition in the world. Approximately 2,000 trillion people worldwide suffer from anaemia due to iron deficiency. The highest incidence occurs in developing countries. Iron-deficiency anaemia reduces oxygen carrying capacity and interferes with aerobic functions. Iron deficiency has also been shown to increase the risk of childhood lead poisoning (Yeung, 1998). There is evidence that the absorption of iron and other divalent metals such as lead and cadmium is more efficient in iron-deficient persons. Thus, preventing and controlling iron deficiency during infancy and early childhood has important public health implications. Vitamin A helps to mobilize iron from its storage sites, so a deficiency of vitamin A limits the body’s ability to use stored iron. This results in an “apparent” iron deficiency because hemoglobin levels are low even though the bones can maintain normal amounts of stored iron. This problem is seen in developing countries where vitamin A deficiency often occurs.
Zinc Deficiency

Zinc deficiency is usually due to insufficient dietary intake, but can be associated with malabsorption, acrodermatitis enteropathica, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy and other chronic illnesses. The clinical signs of marginal zinc deficiency are depressed immunity, impaired taste and smell, onset of night blindness, impairment of memory and decreased spermatogenesis. Zinc supplementation in children is associated with substantial reduction in the duration and severity of diarrhoea and pneumonia in developing countries. There are some suggestions that zinc supplementation might improve the neuropsychological performance of children with marginal zinc deficiency. The association with diet is the main reason for the much higher incidence of zinc deficiency in rural areas of Mexico (Rosado, 2003).

Zinc nutritional status is difficult to measure adequately using laboratory tests due to its distribution throughout the body as a component of various proteins and nucleic acids. Plasma or serum zinc levels are the most commonly used indices for evaluating zinc deficiency, but these levels do not necessarily reflect cellular zinc status due to tight homeostatic control mechanisms (ODS, 2009).

Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes and it plays a role in immune function, protein synthesis, wound healing, DNA synthesis, cell division, and proper sense of taste and smell. Zinc also supports normal growth and development during childhood. A daily intake of zinc (12.5g/100g) is required to maintain a steady state because the body has no specialized zinc storage system (ODS, 2009).

2.2 Complementary foods

Complementary foods are any nutrient containing food or liquid other than breast-milk given to young children along with breast-milk (Gibbs, 2010) within the 6-23 month window. During the time they are consumed, complementary foods make up a large proportion of the infant’s diet and contribute a significant amount of the nutrients that are necessary for growth and development. The foods, therefore, must contain sufficient amounts of the essential nutrients to complement breast-milk. Infants who do not receive enough complementary foods may be stunted or malnourished or both.
Cereals are the first complementary foods to be introduced to infants, sometime between 4 and 6 months of age. They are followed by vegetables, fruits, fruit juices, and meat products. By following this progression, a balanced diet containing all the essential nutrients can be achieved when the infant reaches eight to nine months. This dietary regimen obviates the need for nutrient supplements (Yeung, 1998).

Complementary food problems

The complementary diets of infants and young children in low-income countries often contain inadequate levels of the problem micronutrients (calcium, iron and zinc) and the absorption of these micronutrients is often inhibited by the high levels of phytate in the traditional diets (Gibbs, 2010).

The other problem with complementary food is the potential liability of food contamination due to unclean water or poor personal hygiene (Vinodini, 2003). The food should be free from objectionable matter. It must not contain any substance originating from microorganisms or any poisonous or deleterious substances, including antinutritional factors, heavy metals or pesticides in amounts that may represent a hazard to health. The product should comply with the recommended international code of hygienic practice for foods for infants and children (Gibbs, 2010).

Complementary food for infant feeding are produced at both the factory and home or traditional levels. The main issues in child nutrition is the prevention of linear growth retardation and anemia and high mortality rate in infants aged 0-3 years (Lutter and Dewey, 2003). Complementary foods are associated with problems such as nutrient inadequacy, microbiological purity and safety and non accessibility of food due to high cost.

1. Nutritional adequacy of complementary foods

For complementary food to be termed adequate, it has to provide 6-11g protein per 100g of complementary food eaten by infants (6-23 months), supply sufficient energy- 202 kcal for infants 6-8 months, 307 kcal for infants 9-11 months and 548 kcal for infants in the 12-23 month bracket (Dewey and Brown, 2003) and enough micronutrients such as 225 µg iodine, 500 mg calcium, 27.5 mg iron, 12.5 mg zinc and 500 µgRE vitamin A to meet a growing child’s nutritional needs (Lutter and Dewey, 2003).
Traditional complementary foods do not meet these requirements because, they are made from foods of plant origin which:

a. are deficient in micronutrients such as calcium, iron, zinc and vitamin A. This generally leads to growth-associated problems such as rickets, anaemia, linear growth retardation, low weight gain and increased susceptibility to disease and infection:

b. have high phytic acid and fibre contents which impair the bioavailability of iron and zinc in the body as phytic acid forms complexes with them; and

c. have high viscosity or bulk as porridges. They are mainly starchy porridges with low energy and nutrient densities; unreasonably large amounts would need to be consumed by infants to meet their requirements. Considering the small stomach capacity of the children by six months, a baby will consume 180–240 ml at each of four or five feedings in twenty-four hours. On average, a baby should take in about 75 ml of formula a day for every pound (453 grams) of body weight, but probably will regulate intake from day to day to meet individual specific needs (AAP, 2012). Children will need to be fed more frequently than desirable to meet both their energy and micronutrient requirements.

2. Microbiological purity

At the transition between exclusive breast-feeding and a mixed diet, there is an increase in diarrhoeal morbidity called weaning diarrhoea. Some reports suggest that complementary foods may be an important vehicle for such diseases. It is generally unknown what proportion of the infectious diseases in infants is food-borne, and what comes from water, feeding bottles, and unhygienic environments. While the importance of clean water and hygiene should not be underplayed, in Nigeria it has been established that installation of drinking water and sanitation systems have remarkably little effect on the morbidity from diarrhoea in infants (Hutley et al., 1990). All these point to microbiological impurity and instability of complementary foods as an important factor.

3. Food availability and cost

Owing to increased urbanization and subsequent increase in the number of working class mothers, the need for commercially produced, easy-to-prepare complementary foods has become a critical necessity. But the high cost of commercially
produced complementary foods may make it inaccessible to low-income families. High cost of complementary foods especially when animal product(s) are included makes industrially processed complementary foods out of reach to low-income earners and poor families (Nout and Ngoddy, 1997).

2.3 Dietary Allowances for Iron and Zinc

Recommendations for iron and zinc are provided in the Dietary Reference Intakes (DRIs) developed by the Institute of Medicine of the National Academy of Sciences, United States of America. Dietary Reference Intakes is the general term for a set of reference values used for planning and assessing nutrient intake for healthy people.

Three important types of reference values included in the DRIs are
1. Recommended Dietary Allowances (RDA)
2. Adequate Intakes (AI)
3. Tolerable Upper Intake levels (UL)
   - The RDA recommends the average daily intake that is sufficient to meet the nutrient requirements of nearly all healthy (97-98%) individuals in each age and gender group.
   - An AI is set when there is insufficient scientific data available to establish a RDA. AIs meet or exceed the amount needed to maintain a nutritional state of adequacy in members of a specific age and gender group.
   - Tolerable Upper Intake level (UL): this is the maximum daily intake unlikely to cause adverse health effects.

Recommended Dietary Allowance (RDA) for iron and zinc

The Recommended Dietary Allowances for iron and zinc in complementary food are 27.5 mg iron and 12.5 mg zinc per 100g (Lutter and Dewey, 2003). Since a sensitive indicator of zinc nutritional status is not readily available, the RDA for zinc is based on a number of different indicators of zinc nutritional status and represents the daily intake likely to prevent deficiency in nearly all individuals in a specific age and gender group (Higdon et al., 2010).

2.4 Food Fortification

The addition of minerals to foods began in 1833 when Boussingault J.B., a French chemist, recommended the addition of iodine to salt as a public health
measure to prevent goitre. Food fortification is generally carried out to restore nutrients that are lost during storage, cooking or processing of foods. This process is known as nutrient restoration of foods and is applied to many foods including cereals, for which many countries have regulatory laws (Rosado, 2003). Food fortification may also be a strategy for increasing the intake of some nutrients that are known to be deficient in specific population groups, and thus fortification contributes to reducing the incidence of nutrient deficiencies, especially of vitamins and minerals.

When the objective of food fortification is to increase the intake of specific nutrients that are known to be deficient in a population, the selection of an adequate vehicle is crucial for program success. The following characteristics should be met when choosing a vehicle for such purposes (Vinodini, 2003).

(1) The food vehicle should be ingested by the target population in sufficient quantities and with a small variability in the amount ingested.

(2) The fortified food should be stable and the physiochemical properties such as appearance, texture and flavour should not change when the nutrient is added.

(3) The added nutrient should be relatively bioavailable and well tolerated.

(4) Fortification should be carried out with available ingredients and technology and preferably at low cost so that it does not significantly increase its price.

Food fortification is a more cost effective and sustainable solution to micronutrient deficiency. It plays a major role in improving the diet and meeting the micronutrient needs of the population. This must be viewed as part of an integrated food-based strategy which includes dietary diversification, homestead production and improved food processing and storage. In industrialized countries, where processed foods are widely consumed and the industry is streamlined, food fortification has played a major role in improving the diet and several nutritional deficiencies have been eliminated.

Fortification offers a unique opportunity for the industry to simultaneously expand its market and profitability while playing a key role in improving health and nutritional status of the population (Vinodini, 2003). All added mineral salts and vitamins should be on the Advisory list of mineral salts and vitamin compounds for use in foods.
for infants and children (Gibbs, 2010). Experience from industrialized countries shows that one of the best ways to ensure that infants consume all the essential nutrients in adequate amounts is to provide culturally acceptable foods that are affordable and fortified with the nutrients that are commonly missing in traditional diets.

*Iron fortification*

The contribution of iron from fortified complementary foods has great potential because it provides the major source of iron at a critical time in infant growth and brain development (Ricardo et al., 2002, Yeung, 1998). There are two major technical constraints when cereals are selected as vehicles: high levels of phytic acid and the extreme sensitivity of unsaturated fat to oxidation during storage in the presence of the highly reactive forms of iron, ferrous sulfate or fumarate (Ricardo et al., 2002). One option for increasing absorption is to hydrolyze the phytic acid in cereals, but nearly all of it needs to be removed. Activating natural phytases from legumes and some cereals (rye, buckwheat and wheat) helps to hydrolyze phytic acid. When iron is added to cereal foods as water–soluble, highly bioavailable compounds such as ferrous sulfate, the soluble iron rapidly catalyses fat oxidation resulting in organoleptically unacceptable rancid products. Furthermore, water–soluble iron compounds can cause unacceptable colour reactions during storage and food preparation. Thus, food manufacturers are often obliged to use water-insoluble iron compounds to fortify foods; elemental iron powders and ferric pyrophosphate are widely used to fortify cereal flours and infant cereals (Davidsson et al., 2009).

Elemental iron powders are not very reactive, but this form of iron has extremely poor bioavailability and should not be used in complementary foods. A new form of elemental iron (atomized iron) appears promising and is already being used. The presence of inhibitors and enhancers should be critically assessed to ensure bioavailability. Ethylenediaminetetraacetic acid (EDTA) and ascorbate act as enhancers and have additive effects. Bioavailability studies are crucial in the selection of fortificant for specific complementary foods but do not ensure effectiveness of the fortified food product (Ricardo et al., 2002). Choice of iron fortificant should be based on compatibility and bioavailability within the specific food matrix.
Electrolytic iron has better absorption and is widely used in commercial infant cereal-based foods. The choice of food matrix and iron source should be based on the optimal combination considering that biological impact is dependent on both. The process of selecting the best food vehicle and iron source may appear simple but is actually a complex process that requires evaluation at every step (Ricardo et al., 2002). Although, the conditions for successful iron fortification programmes are at hand, specific problems still exist:

1. Arbitrary criteria have often been adopted to select iron compounds;
2. Fortification programs lack quality assurance systems, and countries have not implemented monitoring and surveillance systems;
3. Legislation has not been adjusted in accordance with needed changes to mandate fortification with specific sources, to prevent contraband and to ensure monitoring and quality control.

**Zinc fortification**

Zinc is part of every tissue in the body and is an important component in literally hundreds of body functions. It plays an integral role in muscle growth, injury healing and immunity building. Zinc bioavailability can be enhanced with the addition of the enzyme phytase to inactivate phytases (Shelke and Feder, 2006).

The Food and Drug Agency (FDA) lists zinc chloride, zinc gluconate, zinc oxide, zinc stearate and zinc sulfate gluconates as GRAS for foods. Acidic forms of glucose, are better absorbed than sulfates and oxides. Amino acid chelates – a stable chemical complex of the mineral with amino acids – are produced by different processes and therefore have different degree of absorption properties. Independent research indicates amino acid chelates are better absorbed than their inorganic counterparts (Evans, 2010). Similarly superior absorption properties are reported for zinc sulfate and zinc oxide. Zinc picolinate chelate reportedly is absorbed better than zinc gluconate and zinc citrate. Zinc and iron with lactate and citrate ligands are gaining popularity for their enhanced functional bioavailability.

Zinc is involved in the body’s development of protein essential for the body to function properly. Apart from being responsible for how insulin is stored and released, zinc is also directly involved in the way it works. Zinc is essential for wound healing and
for many other basic metabolic processes, forming an integral part of a number of enzymes as well as playing an integral role in many other essential functions within the body (Evans, 2010).

According to a fact sheet issued by the Department of Human Nutrition, Ohio State University, any food that is naturally rich in protein is an excellent source of zinc in the diet (Evans, 2010). Exclusively breast-fed infant of mothers with adequate zinc nutriture can satisfy their zinc requirements for the first 4-5 months of life. Therefore, complementary foods with high content of absorbable zinc are required to satisfy the growing needs. In many developing countries, cereals or tubers are used as a basis for such additional foods (Reddy, 2005).

Zinc deficiencies are common in developing countries where there is little access to animal proteins and nuts, which are the best sources of this mineral (USDA/ARS, 2002). Zinc fortification of cereal flour is a safe and effective, low cost method to increase zinc intake, total absorbed zinc and zinc status (Brown et al., 2009).

2.5 Nutrient Bioavailability

Since minerals are added to foods to increase nutritive value, the major factor to be considered in choosing the appropriate compound to be added to a particular food is bioavailability. However, using an exceedingly bioavailable compound does not make sense if the food is rendered unpalatable or unacceptable as a result of physicochemical changes. Minerals are chemically reactive compounds and their bioavailability will be greatly affected by interactions with food components when added or during processing and storage. These properties include solubility, charge density, reduction potential and pH, complex formation and the effect of processing. Successful fortification of a food depends on the fortificant acting in a relatively benign manner in the food (Rosado, 2003). The added minerals should be water-soluble and should not form insoluble components when mixed together (WHO, 2007).

Absorption of iron in food is influenced by multiple factors. One important factor being the form of the iron. Heme iron, found in animal sources, is highly available for absorption. Non-heme iron on the other hand, found in vegetable sources (lentils, beans etc) is less available (Tsang, 2002). Heme iron is absorbed better than nonheme iron, but most dietary iron is nonheme iron (ODS, 2007). The zinc in whole grain products and
plant proteins is less bioavailable due to their relatively high content of phytic acid, a compound that inhibits zinc absorption. The enzymatic action of yeast reduces the level of phytic acid in foods. Therefore, leavened whole grain breads have more bioavailable zinc than unleavened whole grain breads (Higdon et al., 2010).

*Anti-nutritional factors in plants and its reduction*

Anti-nutritional factors are deleterious substances which interfere with digestive processes and reduce food intake and/or efficient utilization of the nutrients. These anti-nutritional factors reduce efficient utilization of the nutrients thereby negatively affecting its bioavailability. Their levels vary in foods of plant origin with species, cultivars and post-harvest treatments such as drying. Examples of such substances include:

- Protease inhibitors: found in all legumes
- Tannins: These are polyphenolic substances
- Phytates: A hexaphosphate derivative of inositol
- Oxalates: A dibasic acid, widely distributed among legumes and vegetables

Anti-nutritional factors are reduced in foods by processing using methods such as soaking/steeping, malting, fermentation and heat treatment (Obizoba and Egbuna, 1992).

Fortification of staple/complementary foods and condiments can be successfully implemented with proper attention to the selection of fortificants that are bioavailable and with due consideration to the balance of inhibitors (phytate, tannins and oxalates) and enhancers (ethylenediaminetetraacetic acid (EDTA) and ascorbate) (Ricardo et al., 2002). Phytates bind zinc and inhibit its absorption (Rosado, 2003).

*Iron Bioavailability*

The amount of iron available for absorption (bioavailability) in the gut is dependent on its solubility in gastric juice, which in turn is dependent on the chemical and physical characteristics of the compounds (size, shape and surface area of particles) as well as on gastric acid secretion in the individual, the presence of dietary absorption enhancers or inhibitors in the meal, and the iron status of the individual (Nestel and Nalubola, 2002). A critical problem in some food fortification programs is the lack of bioavailability of iron compounds (Ricardo et al., 2002). The most absorbable iron compounds are the most reactive with the food matrix. This limits the amount of iron added to foods and determines in practice their potential biological impact. Absorption of
iron from food is influenced by multiple factors including the form of the iron. Heme iron, found in animal sources, is highly available for absorption. Non-heme iron on the other hand, found in vegetables is less available (Tsang, 2002) and is the source of most dietary iron. Absorption of nonheme iron is decreased by tannins found in tea, calcium, polyphenols, and phytates found in legumes and whole grains.

Vitamin A helps to mobilize iron from storage sites, so a deficiency of vitamin A limits the body’s ability to use stored iron (ODS, 2007). There is evidence that the absorption of iron and other divalent metals such as lead and cadmium is more efficient in iron-deficient persons (Yeung, 1998).

Zinc bioavailability

The bioavailability of zinc from grains and plant foods is lower than that from animal foods, although, many grain and plant-based foods are still good sources of zinc. Phytates – which are present in whole-grain breads, cereal, legumes, and other foods bind zinc and inhibit its absorption. Research have not determined whether differences exist among forms of zinc in absorption, bioavailability, or tolerability. Zinc gluconate is one compound used for the delivery of zinc as a dietary supplement. Even though zinc oxide is more widely used; it however has very low solubility. The use of zinc oxide has some advantages over the use of other zinc compounds because it is more stable and does not significantly change the food to which it is added (Rosado, 2003).

Interactions between iron and zinc and other nutrients

Interactions between iron and zinc and other nutrients affect its bioavailability. Minerals with similar physical and chemical properties and electronic structure act antagonistically to each other in biological systems.

Another concern is that many zinc-fortified foods also contain added iron; however, the effect of supplemental iron on zinc absorption does not appear to be significant when supplements are consumed with food. Studies testing the effect of iron on zinc absorption showed that iron decreased zinc absorption when added together in water, but only one study showed a negative effect of iron on zinc absorption when added to foods. Fortification of foods with iron does not significantly affect zinc absorption. However, large amounts of supplemental iron (greater than 25 mg) might decrease zinc absorption. Taking iron supplements between meals helps decrease its effect on zinc
absorption (ODS, 2009). Supplemental iron (38-65 mg/day of elemental iron but not dietary levels of iron) may decrease zinc absorption. This interaction is of concern in the management of iron supplementation during pregnancy and lactation and has led some experts to recommend zinc supplementation for pregnant and lactating women taking more than 60 mg/day of elemental iron (Higdon et al., 2010).

There is also evidence that calcium from supplements and dairy food may inhibit iron absorption, but it has been very difficult to distinguish between the effects of calcium on iron absorption versus other inhibitory factors such as phytate (ODS, 2007).

There is some concern that the addition of zinc to foods could increase zinc intake to a level that could affect copper absorption (Rosado, 2003). It has been reported that intake of more than 50 mg of zinc (diet and supplements) can lead to improper copper metabolism, altered iron function, reduction of high density cholesterol, and reduced immune function. High intakes of zinc can induce synthesis of the copper-binding metallothionein in the mucosal cell; this protein sequesters copper, making it unavailable for transfer and thus decreases copper absorption (Rosado, 2003). Excessive absorption of zinc suppresses copper and iron absorption.

2.6 Plant Sources of Iron and Zinc

Plant sources of iron include soybeans, lentils, lima beans, black-eyed peas (cowpea), spinach, Hibiscus sabdariffa L. Plant sources of zinc include cashew, almonds, peanuts, beans, chickpeas, Hibiscus sabdariffa L.

Hibiscus sabdariffa L

Hibiscus sabdariffa L (Family Malvaceae) commonly known as roselle, is widely grown in Africa, South East Asia and some tropical countries of America. Roselle is an annual, erect, bushy, herbaceous sub shrub, with smooth or nearly smooth, cylindrical, typically red stems. The most exploited part of a roselle plant is its calyces (Ismail et al., 2008). Roselle is an important source of vitamins, minerals and bioactive compounds, such as organic acids, phytosterols, and polyphenols, some of them with antioxidant properties (Sayago-Ayerdi et al., 2007). Differences in the composition of the roselle flower could arise from genetic variety and type of soil. The mineral content is dependent on soil type and plant growth environment. Table 1 shows analysis of the fresh calyces of Hibiscus sabdariffa in Guatemala.
Table 1: Food value of edible portion of *Hibiscus sabdariffa* fresh calyces (Guatemala)

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<tr>
<th>Constituents</th>
<th>Per 100g (edible portion)</th>
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<tr>
<td>Moisture</td>
<td>9.2g</td>
</tr>
<tr>
<td>Protein</td>
<td>1.145g</td>
</tr>
<tr>
<td>Fat</td>
<td>2.61g</td>
</tr>
<tr>
<td>Fiber</td>
<td>12.0g</td>
</tr>
<tr>
<td>Ash</td>
<td>6.90g</td>
</tr>
<tr>
<td>Calcium</td>
<td>1,263mg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>273.2mg</td>
</tr>
<tr>
<td>Iron</td>
<td>8.98mg</td>
</tr>
<tr>
<td>Carotene</td>
<td>0.029mg</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.117mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.277mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>3.765mg</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>6.7mg</td>
</tr>
</tbody>
</table>

*Source: Morton (1987).*

The leaves of *Hibiscus sabdariffa* were found to contain useful amounts of micronutrients such as iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) (Sayago-Ayerdi *et al.*, 2007). Cu, Fe and Zn content in the leaves of the Roselle are higher at the vegetative stage while Mn content is lower. The vegetative stage corresponding to 25 days after sowing is the recommended optimal harvest time of Roselle for maximum nutrient content (Atta *et al.*, 2010).

*Chemical nature*

Pharmacological activity has been identified in the flowers, petals, and seeds. The health effects are numerous: cardioprotective action; reduction of urinary concentrations of creatinine, uric acid, citrate, tartrate, calcium, sodium, potassium, phosphate; antihypertensive action; effectiveness against low-density lipoprotein oxidation and hyperlipidemia. The phenolic content in the plant consists mainly of anthocyanins like delphinidin-3-glucoside, sambubioside, and cyanidin-3-sambubioside; other flavonoids like gossypetin, hibiscetin, and their respective glycosides; protocatechuic acid, eugenol, and sterols like β-sitosterol and ergosterol (Sayago-Ayerdi *et al.*, 2007). The antioxidative effect of roselle seed extract increases when combined with other oxidant compounds compared to when used alone (Ismail *et al.*, 2008).
Other constituents

Other constituents such as lysine, arginine, leucine, phenylalanine and glutamic acid are particularly rich in the seeds. The red anthocyanin pigments in the calyces are used as food colouring agents (Ali et al., 2005).
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Healthy mature seeds of bambara groundnut (*Voandzeia subterranea* Thouars), cowpea (*Vigna unguiculata*), cattle bone, proprietary formula (Nutrend, a commercially available cereal-legume based infant formula), roselle calyces (*Hibiscus sabdariffa*), red palm oil, yellow maize (*Zea mays*), and *achi* (*Brachystegia eurycoma*), were purchased from Ogige market, Nsukka, Nigeria.

3.2 Methods

The processing methods was based on the methods of Uvere *et al.* (2010) with some modification consisting essentially of the elimination of the fermentations of individual food fortificants prior to formulation of the fortificant mix.

3.2.1 Production of maize-bambara and maize-cowpea food blends

The maize, bambara groundnut and cowpea seeds were cleaned by winnowing and handsorting.

*Production of degermed maize flour*

The maize grains (700g) were tempered in excess water for 15 minutes, degermed using a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK), dried at 32±0.26°C, winnowed and the grits milled into flour (Figure 1) using a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK). The flour was packed in polyethylene bags and stored in the refrigerator at 4°C.

*Production of bambara groundnut and cowpea flours*

The production of malted and unmalted bambara groundnut and cowpea flours is shown in Figure 1. One lot (500g) of bambara groundnut seeds was steeped in excess tap water at 28±0.56°C for 8 hours, wet-dehulled by abrasion between the palms and dried at 50°C in a hot air Gallenkamp oven (Model IH-150, Gallenkamp, England). The dried grains were milled into flour using a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK), packed in polyethylene bags and stored in the refrigerator at 4°C.

Five hundred grams (500g) of cleaned cowpea seeds were tempered in excess tap water at 28±2°C for 10 minutes, wet dehulled by abrasion between the palms, dried in a
Gallenkamp oven at 50°C before milling into flour (Figure 1) using the Bentall attrition mill. The flour was packed in a plastic container and stored in a refrigerator at 4°C.

Lots of bambara groundnut seeds (200g each) were weighed into porous malting bags (25 cm x 45 cm) for malting at 28±0.56°C by the methods of Uvere et al. (2010). The seeds were steeped in tap water for 8 hours, air rested for 4 hours and re-steeped in clean tap water for 8 hours. The out-of-steep seeds were spread in malting bags and allowed to germinate in a dark room for 72 hours during which they were turned once every 24 hour. The samples were moistened on alternate days by dipping the malting bags containing the germinating grains in water for 30 seconds (Figure 1). The green

**Figure 1: Production of maize, bambara groundnut malt, cowpea malt and flours.**
Malts were dried in a convection Gallenkamp oven (Model IH-150, Gallenkamp, England) at 50°C for 12 hours after which the seeds were cleaned of sprouts and hulls by abrasion between the palms and winnowed. The malts were milled into flour using a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK) and stored in polyethylene bags in a refrigerator at 4°C.

Lots of sorted cowpea seeds (500g) were soaked for 4 hours followed by 2 hour air rest and another 4 hour water steep before sprouting in the dark for 72 hours in the malting bags. The malts were dried in a Gallenkamp oven (Model IH-150 Gallenkamp, England) and cleaned of sprouts. The dried malts were milled into flour (Figure 1) with a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK) packed in a plastic container and stored in a refrigerator at 4°C.

Malting loss was calculated as the loss in weight of bambara groundnut and cowpea after steeping, germination and drying. The root length of the germinating bambara groundnut malt and cowpea malt were determined on a daily basis using a metre rule (Bhutta, 2006).

Diastatic activity

The diastatic activities of the bambara groundnut malt and cowpea malts were determined by the method of Hulse et al. (1980). The slurry of each of the flours (5g) was made with distilled water (1:5w/v). The temperature of the slurry was gradually raised from 35°C to 70°C over one hour by heating in a regulated Gallenkamp water bath. The digest was filtered using Whatman filter paper No 1 (11 microns). The concentration of sugar in the filtrate was determined using a hand held refractometer (Berlington and Stanley Ltd. London). The percent total sugar was expressed as diastatic activity in degrees Lintner (°L).

Production of fermented composite flour blends.

Composite flours from maize and malts of bambara groundnut and cowpea were formulated in a 70:30 ratio (Table 2) (Cameron and Hofvander, 1983).
Table 2: Ratio of maize to bambara groundnut malt/cowpea malt and fortificants

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flour blend</strong></td>
<td>11.02</td>
</tr>
<tr>
<td>Maize flour</td>
<td>2.33</td>
</tr>
<tr>
<td>Bambara groundnut /cowpea malt</td>
<td>1</td>
</tr>
<tr>
<td><strong>Fortificants</strong></td>
<td>1</td>
</tr>
<tr>
<td>Roselle Calyces ash</td>
<td>1</td>
</tr>
<tr>
<td>Cattle bone ash</td>
<td>5.51</td>
</tr>
<tr>
<td>Emulsified red palm oil (red palm oil, distilled water and B. eurycoma (1:1:2))</td>
<td>1.81</td>
</tr>
</tbody>
</table>

The maize-bambara groundnut malt or maize-cowpea malt blends were fermented for 72 hours by backslopping and the fermentation was monitored by pH determination using a hand-held pH meter (Hanna Instruments Woonsocket, R102895, Bedfordshire, UK). The ratio of flour blend to water during fermentation by backslopping was 60:40 (Table 3).

Table 3: Quantity of fortificant and water during fermentation

<table>
<thead>
<tr>
<th>DAY</th>
<th>Flour blend : Water</th>
<th>Fortificant : Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(60%)</td>
<td>(40%)</td>
</tr>
<tr>
<td>DAY 1</td>
<td>0.9168</td>
<td>0.6112</td>
</tr>
<tr>
<td>DAY 3</td>
<td>61.5952</td>
<td>54.3968</td>
</tr>
<tr>
<td>Total</td>
<td>91.68</td>
<td>61.12</td>
</tr>
</tbody>
</table>

3.2.2 Processing of foods used as fortificants

Processing of cattle bones into meal

Cattle bones used as source of calcium was cracked open using Bench Vice Ma (Model HI-Duty Vice Paramo, England), washed with hot water at 90°C to remove the marrow and oil, then dried in a Gallenkamp oven (Model IH-150, Gallenkamp, England) at 50°C for 12 hour. The dried bones (100g) were then ashed at 600°C for 5 hour (Figure 2).
**Processing of roselle calyces into flour**

Roselle calyces used as source of iron and zinc were hand-sorted to remove dirt and extraneous materials. One hundred grams (100g) was dried at 50°C milled into flour and ashed at 450°C (Figure 2).

**Processing of red palm oil**

Red palm oil used as source of vitamin A was processed by forming a 24 hour stable emulsion with *Brachystegia eurycoma*. *B. eurycoma* seeds were roasted at 150°C for 30 minutes, soaked in excess water for 3 hour, dehulled by abrasion and milled into powder using a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK). The powder was used to form a 24 hour stable emulsion of red palm oil, water and *B. eurycoma* (1:1:2, v/v/w).

**Formulation of fortificant mix**

The quantity of each processed food fortificant in the mix (1:1.81:5.51 for roselle, emulsified red palm oil and cattle bone, respectively, (Table 2) was based on the level of the required nutrient as analysed and the RDA for the nutrient for 6 to 12 month old infants. Thereafter, the fortificant mix was fermented by backslopping for 72 hour and monitored by pH determination using a hand-held pH meter (Hanna Instruments Woonsocket, R102895, Bedfordshire, UK.). The ratio of fortificant to water during the three days of fermentation by backslopping (Table 3) was 60:40.
3.2.3 **Formulation of fortified maize-bambara groundnut and maize-cowpea malt complementary food blends**

The fermented maize-bambara groundnut and maize-cowpea malt complementary foods were wet-mixed with the fermented fortificant mix (Figure 3) in a ratio of 11.02 : 1 (Table 2) and dried in a convection Gallenkamp oven (Model IH-150, Gallenkamp, England) at 50°C followed by milling into flour in a Bentall attrition mill (Model 200 L090, E. H. Bentall, UK), packaging in polyethylene bags, sealed and stored at 4°C.
Figure 3: Modified Post-fermentation procedure for production of maize-bambara groundnut malt or maize-cowpea malt complementary foods.
3.2.4 Chemical Analyses of the Fortified Complementary Food blends

3.2.4.1 Proximate Analysis

Moisture content
The moisture content of the samples was determined by the method described in AOAC (2010). Two gram (2g) of sample was weighed into an aluminum dish with cover and placed in an oven previously regulated to 135±2°C and dried for 2 hours. The cover was placed on dishes and transferred to a desiccator to cool and then re-weighed. The percentage moisture content of the samples was calculated using the formula:

\[ \text{Weight difference} \times \frac{100}{\text{Original weight of the sample}} \]

Where weight difference = original weight of sample – final weight of sample

Crude protein content.

The crude protein content was determined using the micro-Kjeldahl method as described by AOAC (2010). Half a gram (0.5g) of malt flour was weighed into 30ml Kjeldahl flask. Fifteen milliliter (15ml) of concentrated Sulphuric acid (H\textsubscript{2}SO\textsubscript{4}) was added followed by 1g of kjeldahl catalyst mixture. The mixtures were heated cautiously on a digestion rack in a fume cupboard until a greenish colored solution was obtained. After the digest had cleared (about 30 minutes), it was heated for 30 minutes and allowed to cool. Some 10ml of distilled water was added to avoid caking and the contents later transferred to a Kjeldahl distillation apparatus. Ten milliliter (10ml) of 40% NaOH solution was added to the digested sample and the mixture distilled into 50ml receiver flask containing 5ml boric acid indicator solution. Hydrochloric acid (0.1N) was used in titration against the mixture containing boric acid until a purple colour was obtained. The average of three titre determinations was used to calculate the crude protein content.

\[
\% N = \frac{14 \times (V_1 - V_2) \times \text{Normality of NaOH} \times \text{Dilution factor (50)}}{1000 \times \text{weight of sample}} \times \frac{100}{1}
\]

\[ \% \text{ crude protein} = \% N \times 6.25 \]
\( V_1 = \text{Titre value} \)

\( V_2 = \text{Blank titre value} \)

**Fat content**

The fat content of the samples was determined using the Soxhlet extraction method described in AOAC (2010). Two gram (2g) of the sample was weighed into a thimble. Flat-bottomed fat extraction cups were weighed and placed on the platform of the Tecator fat extraction unit. The thimbles were attached to the soxhlet extractor and the samples extracted with petroleum ether (b.p. = 40–60°C) for 1 hour. The solvent-free fat in the cups was dried in an air oven for 30 minutes at 80°C; the cup with its contents was cooled in a desiccator and re-weighed.

\[
\% \text{ fat} = \frac{(\text{weight of extract + cup}) - \text{weight of cup}}{\text{Original weight of sample}} \times 100
\]

**Ash content**

The ash content of the samples was determined using the method described in AOAC (2010). Two grams (2g) of each sample was weighed into a porcelain crucible and ignited in a temperature-controlled furnace at 600°C for 2 hours. The crucible with its contents was removed, cooled in a desiccator and weighed. The percentage weight of the ash was calculated as shown below:

\[
\% \text{ Ash} = \frac{(\text{weight of the sample + crucible}) - \text{weight of crucible}}{\text{Original weight of sample}}
\]

**Carbohydrate content**

The carbohydrate in each sample was obtained by difference, that is, by subtracting the amount of moisture, protein, fat, and ash from 100% (AOAC, 2010).

3.2.4.2 **Micronutrient Analysis**

**Determination of calcium, iron and zinc**

The spectrophotometric method of Christian (2004) was used. One gram (1g) of the dried sample was weighed into a digestion flask and 20ml of acid mixture (650ml conc Nitric acid (HNO₃), 80ml Perchloric acid (PCA) and 20ml conc Sulphuric acid (H₂SO₄) were added. The flask was heated until a clear digest was obtained. The digest was diluted with distilled water to 100ml.

Standard solutions were prepared as follows:
**Calcium:** Oven dried calcium carbonate (2.497g) was dissolved and diluted to 100ml with de-ionized water to give a solution containing 100mg/ml Ca$^{2+}$ ions. From this stock solution, calcium standard solutions with the following concentrations (0.0, 3.0, 6.0 and 9.0 mg/ml) were prepared.

**Iron:** A stock solution containing 100mg/ml of Fe$^{3+}$ was prepared from 1g of pure iron wire. The wire was dissolved in 100ml conc. HNO$_3$, boiled on a water bath and diluted to 100ml with distilled water. Standard solution of concentrations 0.0, 0.5, 1.0, 2.0 and 4.0ppm were prepared from this stock solution.

**Zinc:** A stock solution containing 100mg/ml of zinc was prepared by dissolving one gram (1g) of zinc ribbon in 10ml of conc. hydrochloric acid (HCl). The solution was evaporated almost to dryness and the salt redissolved in 100ml of de-ionized water. Standard solutions of concentrations 0.0, 0.5, 1.0 and 1.5ppm were prepared from this stock solution.

A calibration curve was prepared for each element using the standard solution. The appropriate lamp and wavelength for each element was used to read the concentration of the element in each sample digest in an Atomic Absorption Spectrophotometer, Model 6800 (Shimadzu, Japan) located at NARICT (National Research Institute for Chemical Technology) Zaria, Kaduna. The wavelength (nm) and slit number (nm) were 422, 248.3, 213.9 and 0.7, 0.2, 0.7 for Ca, Fe and Zn respectively.

**Determination of vitamin A**

A stock solution was prepared by dissolving 25 mg Retinol in 85 ml Isopropanol (99%). Standard solutions of concentrations 0.0, 5.0, 10.0, 15.0 and 20.0 were prepared from this stock solution. The standard solution was used to prepare a calibration curve.

Twenty millilitres (20 ml) of Isopropyl alcohol was added to 5g of the sample in a test tube and allowed to stand for 45 minutes at room temperature (28±0.56°C). The mixture was gently swirled and filtered using Whatman filter paper No. 1 (11 microns). The filtrate was measured at 325 nm for vitamin A using UV-Visible Spectrophotometer, Model 2550 (Shimadzu, Japan) located at NARICT (National Research Institute for Chemical Technology) Zaria, Kaduna (Biesalski et al., 1986).
3.2.4.3 Determination of antinutrients:

**Tannin**

The tannin content was estimated spectrophotometrically by the Folin-Denis method (Makkar *et al.*, 1993). The method is based on oxidation of the molecules containing a phenolic hydroxyl group. The tannin and tannin-like compounds reduce phosphomolybdic acid in alkaline solution to produce a highly coloured blue solution; the intensity of which is proportional to the amount of tannin and can be estimated against standard tannic acid solution at wavelength of 725nm.

*Sample preparation and extraction of tannins:*

The sample was dried at 55±1°C and ground to pass through a sieve of 1mm diameter. Tannin extraction was done using 400mg ground sample in conical flask with 40ml diethyl ether containing 1% acetic acid (v/v) and mixed to remove pigments. The supernatant was carefully decanted after 5 minutes and then 20ml of 70% aqueous acetone was added. The flask was sealed with cotton plug covered with aluminum foil and kept in an electrical shaker (Clarkson MX 001014 Helix 150 BLR, USA) for 2 hour for extraction. Then it was filtered through Whatman filter paper No. 1 (11 microns) and the extract was kept in a refrigerator at 4°C until analysis.

Standard calibration curve was prepared from the stock solution of tannic acid (0.5mg/ml) using 0, 10, 20, 30, 40 and 50µg respectively. Then 0.5ml Folin reagent and 2.5ml of 20% sodium carbonate was added and the contents mixed properly; and after 40 minutes, the absorbance reading at 725nm was read in a BIOCHROM 4049 UV spectrophotometer (BIOCHROM LTD. UK.) located at NARICT (National Research Institute for Chemical Technology) Zaria, Kaduna.

Fifty microlitres of tannin extract for each sample was taken in a test tube and the volume made up to 1.0ml with distilled water. Then, 0.5ml Folin Ciocalteu reagent was added and mixed properly followed by 2.5ml of 20% sodium carbonate solution. These were added, mixed and kept for 40 mins at room temperature (28±0.56°C). The optical density was read at 725nm in a BIOCHROM 4049 UV spectrophotometer and the concentration was estimated from the standard curve.
**Calculation:**

\[
\text{\% tannin} = \frac{A_n \times D_f}{A_s \times W} \times 100
\]

Where: \( A_n \) = absorbance of test sample; \( A_s \) = absorbance of standard tannic acid
\( C \) = concentration of standard tannic acid (mg/ml)
\( D_f \) = dilution factor = \( \frac{V_{ex}}{V_a} \);
\( W \) = weight of test sample (mg)
\( V_{ex} \) = total volume of extract;
\( V_a \) = volume of extract analyzed

**Phytic acid**

The phytic acid was determined using the procedure described by Lucas and Markakas (1975). Two grams (2.0g) of the sample was weighed into 250ml conical flask to which 100ml of 2% hydrochloric acid was added and allowed to stand for 3 hours before filtering. Fifty millilitres of each filtrate was placed in 250ml beaker and 107ml of distilled water added in each case to give proper acidity.

Ten millilitres of 0.3% Ammonium thiocyanate solution was added into each solution as indicator and titrated with standard iron chloride solution, which contained 1.95mg iron per ml. The end point was slightly brownish-yellow persisting for 5 minutes.

The percentage phytic acid was calculated using the formula:

**Calculation:**

\[
\text{% phytic acid} = Y \times 1.19 \times 100
\]

Where: \( Y \) = titre value x 1.95mg.

**Oxalate**

Oxalate was determined using the method of Oke (1969). Two grams of the sample was digested with 10ml of 6M HCl for one hour and made up to 250ml in a volumetric flask after which it was filtered using Whatman filter paper No.1 (11 microns).

The pH of each filtrate was adjusted with conc. NH\(_4\)OH solution until the colour of solution changed from salmon pink to faint yellow. Thereafter, the solution was heated on a water bath to 90°C and allowed to stand for 8 hour and then the suspension is now centrifuged at 2500rpm, after which the supernatant was decanted and precipitate completely dissolved in 10ml of hot 20% (v/v) H\(_2\)SO\(_4\).

The total filtrate resulting from the dissolution in H\(_2\)SO\(_4\) was made up to 300ml. An aliquot of 125ml of the filtrate was heated until near boiling point and then titrated
against 0.05M of standardized KMnO₄ solution to a faint pink colour which persisted for about 30 seconds after which the burette reading was taken. The oxalate content was evaluated from the titre value.

The overall redox reaction is:

\[
\text{MnO}_4^- + C_2O_4^{2-} + 8H^+ \rightarrow Mn^{2+} + 4H_2O + 2CO_2
\]

**Calculation**

The oxalate content was calculated using the formula:

\[
\frac{T \times Vme \times Df}{M_E \times Mf} \times 100
\]

where T is the titre of KMnO₄ (ml), Vme is the volume-mass equivalent (ie. 1cm³ of 0.05M KMnO₄ solution is equivalent to 0.00225g anhydrous oxalic acid), Df is the dilution factor (VT/A = 2.4 where VT is the volume of titrate (300ml) and A is the aliquot used (125ml), Mₑ is the molar equivalent of KMnO₄ in oxalate (KMnO₄ redox reaction) and Mf is the mass of sample used.

**Molar ratio of phytate to iron, zinc and calcium**

The phytate to iron, zinc and calcium molar ratios were calculated by the following equation (Gibbs, 2010).

\[
\text{Phytate: mineral molar ratio} = \frac{(\text{phytate (mg)/phytate molecular weight})}{(\text{mineral (mg)/mineral atomic weight})}
\]

where phytate molecular weight = 660

- Calcium atomic weight = 40.08
- Iron atomic weight = 56
- Zinc atomic weight = 65.4

**3.2.5 Sensory Evaluation**

Maize–bambara groundnut malt and maize-cowpea malt complementary food were evaluated for sensory acceptance using a fifteen (15) man trained panel. The evaluation was by assessing the degree of preference of the complementary foods from the proprietary formula (control) in terms of appearance, texture, flavor, taste and overall acceptability using a 9 point Hedonic scale where 9 represented like extremely and 1 represented dislike extremely (Iwe, 2002).
3.2.6 Statistical Analysis

Data analysis was carried out using one-way analysis of variance (ANOVA) in a completely randomized design (CRD); mean separation was by Duncan’s New Multiple Range Test (Steel and Torrie, 1980). Significance was accepted at p<0.05.
CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Malting of bambara groundnut and cowpea

Mobilisation of nutrients for growth was highest between 24 hours and 48 hours (between day 1 and day 2) for Bambara groundnut while for cowpea was between 0 and 24 hours (between day 0 and day 1) (Figure 4). The average root length of bambara groundnut and cowpea for the three days of malting were 0.35cm, 1.13cm, 1.66cm and 2.05cm, 3.05cm, 3.96cm, respectively; bambara groundnut and cowpea had the highest values for root length on the third day (1.66 cm for bambara groundnut and 3.95 cm for cowpea) while the first day had the lowest (0.35 cm for bambara groundnut and 2.05 cm for cowpea).

Fig. 4: Root length of germinating cowpea and bambara groundnut malts

The increase suggests improved modification of endosperm resulting from enhanced hydrolytic enzyme secretion and activity (Palmer and Bathgate, 1976) and was
significantly (p<0.05) higher than other malting days. This could account for the high diastatic activity of 45.8°L for bambara groundnut malt and 45.3°L for cowpea malt (Table 4) leading to increased respiration of the embryo and outgrowth of the root and resulting in the high malting loss of 20% for bambara groundnut malt and 17.11% for cowpea malt (Table 4). Bambara groundnut has sugar content of 64.5mg/g (Apata, 2008) while cowpea has 16.9mg/g (Islam et al., 2012) and contribute to the high respiration rate and hence malting loss. In addition, leaching of nutrients during steeping and degree of grain modification during germination (Uvere et al., 2010) may also contribute to the malting loss.

Table 4: Malting characteristics of bambara groundnut and cowpea seeds

<table>
<thead>
<tr>
<th>Sample</th>
<th>Malting yield (%)</th>
<th>Malting loss (%)</th>
<th>Diastatic Activity (°L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B&lt;sub&gt;m&lt;/sub&gt;</td>
<td>80.00±0.01</td>
<td>20.00±0.01</td>
<td>45.3±0.20</td>
</tr>
<tr>
<td>C&lt;sub&gt;m&lt;/sub&gt;</td>
<td>82.89±0.01</td>
<td>17.11±0.01</td>
<td>45.8±0.28</td>
</tr>
</tbody>
</table>

B<sub>m</sub> = bambara groundnut malt, C<sub>m</sub> = cowpea malt

The diastatic activity of bambara groundnut malt (45.3°L) was lower than the 45.8°L of cowpea. This may be attributed to the higher carbohydrate content (86%) in bambara groundnut (Eltayeb et al., 2011) since enzyme activity is related to the substrate concentration.

4.2 pH changes during fermentation by backslipping

The pH of the fermenting slurries decreased during the 72 hour of fermentation (Table 5). The pH drop after the three days of fermentation were 1.76, 0.92, 0.15, 0.31 and 0.15 for MB<sub>m</sub>, MC<sub>m</sub>, Fortificant, Fortificant+MB<sub>m</sub> and Fortificant+MC<sub>m</sub>, respectively. Compared to MC<sub>m</sub>, the difference in pH drop in MB<sub>m</sub> is 0.84 and might be attributed to a higher carbohydrate (sugar) and lower protein content in MB<sub>m</sub>. Bambara groundnut has a carbohydrate content of 86% and a protein content of 17.70% (Eltayeb, 2011) while cowpea has a carbohydrate content of 63.60% and a protein content of 24.80% (Davis et al., 1991).

Addition of fermented fortificant reduced the pH drop in MB<sub>m</sub> to 0.31 and in MC<sub>m</sub> to 0.15, giving a difference in pH drop of 0.16. This implies that the addition of the fortificant mix increased the buffering capacity of MB<sub>m</sub> more than MC<sub>m</sub>. Considering the
composition of the fortificant mix, the alkalinity and buffering capacity may be due to palm oil and the protein content of 9.98% *B. eurycoma* (Uzomah and Odusanya, 2011).

### Table 5: pH of maize-bambara groundnut and maize-cowpea malt flour blends during fermentation

<table>
<thead>
<tr>
<th>Day</th>
<th>MB&lt;sub&gt;m&lt;/sub&gt;</th>
<th>MC&lt;sub&gt;m&lt;/sub&gt;</th>
<th>Fortificant</th>
<th>Fortificant + MB&lt;sub&gt;m&lt;/sub&gt;</th>
<th>Fortificant + MC&lt;sub&gt;m&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>6.93&lt;sup&gt;a&lt;/sup&gt; ± 0.06</td>
<td>6.20&lt;sup&gt;a&lt;/sup&gt; ± 0.10</td>
<td>11.23&lt;sup&gt;a&lt;/sup&gt; ± 0.12</td>
<td>9.43&lt;sup&gt;a&lt;/sup&gt; ± 0.15</td>
<td>9.53&lt;sup&gt;a&lt;/sup&gt; ± 0.06</td>
</tr>
<tr>
<td>Day 2</td>
<td>5.33&lt;sup&gt;b&lt;/sup&gt; ± 0.06</td>
<td>5.50&lt;sup&gt;b&lt;/sup&gt; ± 0.10</td>
<td>11.15&lt;sup&gt;a&lt;/sup&gt; ± 0.09</td>
<td>9.21&lt;sup&gt;b&lt;/sup&gt; ± 0.02</td>
<td>9.40&lt;sup&gt;ab&lt;/sup&gt; ± 0.10</td>
</tr>
<tr>
<td>Day 3</td>
<td>5.17&lt;sup&gt;c&lt;/sup&gt; ± 0.06</td>
<td>5.28&lt;sup&gt;c&lt;/sup&gt; ± 0.03</td>
<td>11.08&lt;sup&gt;a&lt;/sup&gt; ± 0.03</td>
<td>9.02&lt;sup&gt;c&lt;/sup&gt; ± 0.03</td>
<td>9.38&lt;sup&gt;b&lt;/sup&gt; ± 0.03</td>
</tr>
</tbody>
</table>

Results are the means of three replications. Values carrying different superscripts in the same column are significantly different (p<0.05). MB<sub>m</sub> = maize-bambara groundnut malt flour blend, MC<sub>m</sub> = maize-cowpea malt flour blend.

### 4.3 Proximate composition of fortified maize-bambara groundnut malt and maize-cowpea malt complementary food blends:

The proximate composition of the fortified maize-bambara groundnut malt and maize-cowpea malt complementary food blends (Table 6) show that the moisture content of the samples was low [(3.35 and 2.95 for (MB<sub>m</sub>)<sub>b</sub> and (MC<sub>m</sub>)<sub>b</sub> respectively)] and could be due to hydrolysis of macromolecules such as starch and protein during malting and fermentation resulting in loss of water holding capacity. However, the moisture content of the proprietary formula was lower (2.50%) than that for (MB<sub>m</sub>)<sub>b</sub> and (MC<sub>m</sub>)<sub>b</sub>.

The crude protein content of the (MB<sub>m</sub>)<sub>b</sub> was 20.80mg and for (MC<sub>m</sub>)<sub>b</sub>, 22.50mg. These values are higher than the 15.00% value for the proprietary formula. The higher crude protein content could be attributed to microbial biomass and additional contributions from the crude protein content (9.98%) of *B.Eurycoma* (Uzomah and Odusanya, 2011), the 17.70% of bambara groundnut (Eltayeb, 2011) and 24.80% of cowpea (Davis *et al*., 1991).

(MB<sub>m</sub>)<sub>b</sub> contained a higher amount of fat than (MC<sub>m</sub>)<sub>b</sub>, (14.05 vs 11.65 mg) probably because bambara groundnut contains more fat than cowpea. The fat content of the blends was higher than that of the proprietary formula (9.00 mg) which could be due to contributions from red palm oil and could improve vitamin A absorption from the food. Bambara groundnut contains about 6.58% of fat (Eltayeb *et al*., 2011) while cowpea contains between 0.60 and 1.90% (Davis *et al*., 1991, Henshaw, 2008).
Table 6: Proximate composition of complementary food blends (mg/100g) and RDA

<table>
<thead>
<tr>
<th>Proximate content</th>
<th>(MB&lt;sub&gt;m&lt;/sub&gt;&lt;sub&gt;b&lt;/sub&gt;)</th>
<th>(MC&lt;sub&gt;m&lt;/sub&gt;&lt;sub&gt;b&lt;/sub&gt;)</th>
<th>N</th>
<th>6-11mo (40g)&lt;sup&gt;2&lt;/sup&gt;</th>
<th>6-23 mo (50g)&lt;sup&gt;2&lt;/sup&gt;</th>
<th>12-23mo (60g)&lt;sup&gt;2&lt;/sup&gt;</th>
<th>6-23mo (100g)&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>3.35</td>
<td>2.95</td>
<td>2.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Crude protein</td>
<td>20.80</td>
<td>22.50</td>
<td>15.00</td>
<td>3- 4.5</td>
<td>3- 5.5</td>
<td>4- 6.5</td>
<td>6-11</td>
</tr>
<tr>
<td>Fat</td>
<td>14.05</td>
<td>11.65</td>
<td>9.00</td>
<td>4.20</td>
<td>6.30</td>
<td>8.20</td>
<td>12.70</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>1.30</td>
<td>1.40</td>
<td>7.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>55.73</td>
<td>56.72</td>
<td>64.20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ash</td>
<td>4.77</td>
<td>4.78</td>
<td>2.30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Results are the means of three replications, (MB<sub>m</sub><sub>b</sub>) = maize-bambara groundnut malt complementary food, fermented and fortified, (MC<sub>m</sub><sub>b</sub>) = maize-cowpea malt complementary food, fermented and fortified, N = proprietary formula.

Based on 24% of energy as fat for infants ages 6-11 months, 28% of energy as fat for children ages 12-23 months, and 26% of energy as fat for infants ages 6-23 months (Lutter and Dewey 2003)

The ash content of the fermented and fortified maize-bambara groundnut malt and maize-cowpea malt were 4.77 mg/100g and 4.78 mg/100g, respectively, and were higher (p<0.05) than that for the proprietary formula (2.30 mg/100g). This increase might be attributed to the effect of fortification and loss of organic matter during fermentation (Obizoba and Atti, 1991).

The low fibre contents of 1.3 mg for (MB<sub>m</sub><sub>b</sub>) and 1.4 mg for (MC<sub>m</sub><sub>b</sub>) may be due to the use of dehulled raw materials in the formulation. The fibre content of the proprietary formula, (7.0), was considerably higher than 1.3mg for (MB<sub>m</sub><sub>b</sub>) and 1.4mg (MC<sub>m</sub><sub>b</sub>).

The carbohydrate content of 55.73 mg/100g and 56.72 mg/100g for (MB<sub>m</sub><sub>b</sub>) and (MC<sub>m</sub><sub>b</sub>), respectively were lower compared to the 64.2 mg/100g for the proprietary formula.

*Calcium, iron, zinc and vitamin A contents*

The calcium, iron, zinc and vitamin A contents of the ingredients and fortified blends are shown in Table 7. The calcium content of the fortified maize-bambara
groundnut malt, (MB<sub>m</sub>)<sub>b</sub> and maize-cowpea malt, (MC<sub>m</sub>)<sub>b</sub> complementary foods was considerably low [(MB<sub>m</sub>)<sub>b</sub> = 0.0535 mg/g; (MC<sub>m</sub>)<sub>b</sub> = 0.0544 mg/g] compared with the RDA of 2.50 – 5.00 mg/g. These low values for calcium may be due to calcium utilization by fermenting microorganisms (Adewusi et al., 1999). This derives from the fact that the calcium content of malted bambara groundnut (0.0621 mg/g), malted cowpea (0.0554 mg/g) and maize (0.059 mg/g) are higher than the calcium content of (MB<sub>m</sub>)<sub>b</sub> and (MC<sub>m</sub>)<sub>b</sub>.

The iron content of the maize-cowpea malt (MC<sub>m</sub>)<sub>b</sub> complementary food was significantly (p<0.05) higher than that of maize-bambara groundnut malt (MB<sub>m</sub>)<sub>b</sub> complementary food (7.08 mg/g vs 5.30 mg/g). Both (MC<sub>m</sub>)<sub>b</sub> and (MB<sub>m</sub>)<sub>b</sub> were significantly (p<0.05) higher than that in the proprietary formula (0.100 mg/g), and the recommended dietary allowance for 6-23 month infants (0.125 mg/g). The high iron content derives from the effect of concentration during malting of the legumes, contributions from processed roselle, maize and B. eurycoma used to emulsify palm oil. The higher iron content of (MC<sub>m</sub>)<sub>b</sub> may therefore be due to the higher iron content of cowpea.
Table 7: Calcium, iron, zinc (mg/g) and vitamin A (µgRE/g) content of complementary food blends and raw materials used in production.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Ca</th>
<th>Fe</th>
<th>Zn</th>
<th>Vitamin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_b</td>
<td>0.0062±</td>
<td>4.82±</td>
<td>0.28±</td>
<td>1.38±</td>
</tr>
<tr>
<td>B_u</td>
<td>0.065±</td>
<td>1.29±</td>
<td>0.42±</td>
<td>1.64±</td>
</tr>
<tr>
<td>B_m</td>
<td>0.062±</td>
<td>1.83±</td>
<td>3.84±</td>
<td>1.68±</td>
</tr>
<tr>
<td>C_u</td>
<td>0.065±</td>
<td>1.95±</td>
<td>0.83±</td>
<td>0.87±</td>
</tr>
<tr>
<td>C_m</td>
<td>0.055±</td>
<td>2.32±</td>
<td>0.69±</td>
<td>3.42±</td>
</tr>
<tr>
<td>R</td>
<td>0.064±</td>
<td>2.88±</td>
<td>0.75±</td>
<td>1.98±</td>
</tr>
<tr>
<td>M</td>
<td>0.059±</td>
<td>1.47±</td>
<td>0.50±</td>
<td>3.85±</td>
</tr>
<tr>
<td>B_eu</td>
<td>0.054±</td>
<td>0.17±</td>
<td>0.05±</td>
<td>1.72±</td>
</tr>
<tr>
<td>P</td>
<td>0.411±</td>
<td>0.77±</td>
<td>0.43±</td>
<td>19.58±</td>
</tr>
<tr>
<td>P_e</td>
<td>0.069±</td>
<td>2.21±</td>
<td>0.49±</td>
<td>6.61±</td>
</tr>
<tr>
<td>(MB_m)</td>
<td>0.054±</td>
<td>5.30±</td>
<td>1.58±</td>
<td>3.93±</td>
</tr>
<tr>
<td>(MC_m)</td>
<td>0.054±</td>
<td>7.08±</td>
<td>1.37±</td>
<td>3.36±</td>
</tr>
<tr>
<td>N</td>
<td>3.900</td>
<td>0.100</td>
<td>0.060</td>
<td>4.500</td>
</tr>
<tr>
<td>RDA+</td>
<td>2.5 – 5.0</td>
<td>0.275</td>
<td>0.1 – 0.125</td>
<td>5.0</td>
</tr>
<tr>
<td>WHO+</td>
<td>5.25</td>
<td>0.11</td>
<td>0.028</td>
<td>3.5</td>
</tr>
<tr>
<td>IOM+</td>
<td>2.7</td>
<td>0.11</td>
<td>0.03</td>
<td>5.0</td>
</tr>
<tr>
<td>FAO/WHO+</td>
<td>4.0</td>
<td>0.093</td>
<td>0.041</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Results are the means of three replications. Values carrying different superscripts in the same column are significantly different (p<0.05). C_b = cattle bone meal, B_u = unmalted bambara groundnut, B_m = malted bambara groundnut, C_u = malted cowpea, Cu = unmalted cowpea, R = roselle, M = maize, B_eu = Brachystegia eurycoma, P = palm oil, P_e = emulsified palm oil, (MB_m) = maize-bambara groundnut malt complementary food, fermented and fortified, (MC_m) = maize-cowpea malt complementary food, fermented and fortified, N = proprietary formula, RDA = Recommended Dietary Allowance, *(Lutter and Dewey 2003), WHO = World Health Organization, IOM = Institute of Medicine, FAO = Food and Agriculture Organisation

In the case of zinc, however, (MB_m) had a significantly higher content of 1.58 mg/g than (MC_m) with 1.37 mg/g. Both values were higher than RDA (0.125 mg/g) and the value for the proprietary formula (0.060 mg/g). The increased zinc contents derives
from the same reasons adduced for iron content. But (MB<sub>m</sub>)<sub>b</sub> had a higher zinc content than (MC<sub>m</sub>)<sub>b</sub> because of the higher zinc content of bambara groundnut malt (3.84 mg/g). The iron and zinc contents of (MB<sub>m</sub>)<sub>b</sub> [5.30 mg/g; 1.58 mg/g] and (MC<sub>m</sub>)<sub>b</sub> [7.08 mg/g; 1.37 mg/g] were significantly (p<0.05) higher than the 0.134 mg/g and 0.015 mg/g reported by Uvere <i>et al.</i> (2010).

The vitamin A content of 3.93 µgRE/g in (MB<sub>m</sub>)<sub>b</sub> was significantly higher than the 3.36 µgRE/g of (MC<sub>m</sub>)<sub>b</sub> in spite of the significantly higher vitamin A content in cowpea malt. This suggests that (MC<sub>m</sub>)<sub>b</sub> may have components that sequester vitamin A. The values were however less than RDA (5.00 µgRE/g) and the proprietary formula (4.50 µgRE/g). Palm oil contained the highest amount of vitamin A (19.58 µgRE/g) but the emulsified palm oil contained a lower amount of 6.61 µgRE/g. This suggests that emulsification with <i>B. eurycoma</i> reduces the quantity of vitamin A. Malting increased the vitamin A content of bambara groundnut from 1.64 to 1.68 µgRE/g and cowpea from 0.87 to 3.42 µgRE/g.

**Tannin, Oxalate and Phytate Contents**

The oxalate, phytate and tannin contents of (MB<sub>m</sub>)<sub>b</sub>, were 0.315, 0.002, and 0.076 mg/100g and for (MC<sub>m</sub>)<sub>b</sub> 0.0068, 0.38, 0.002 and mg/100g, respectively (Table 8). These low values possibly derived from the low content of these antinutrients in the unprocessed raw materials, dehulling of bambara groundnuts and cowpea, degerming of maize, malting of bambara groundnut/cowpea and fermentation (Uvere <i>et al.</i>, 2010; Obizoba and Atti, 1991; Enwere 1998).
Table 8: Tannin, oxalate and phytate composition of maize-bambara groundnut and maize-cowpea malt food blends

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tannin</th>
<th>Oxalate</th>
<th>Phytate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(MB&lt;sub&gt;m&lt;/sub&gt;)b</td>
<td>0.315&lt;sup&gt;c&lt;/sup&gt; ± 0.005</td>
<td>0.002&lt;sup&gt;c&lt;/sup&gt; ± 0.000</td>
<td>0.076&lt;sup&gt;a&lt;/sup&gt; ± 0.001</td>
</tr>
<tr>
<td>(MC&lt;sub&gt;m&lt;/sub&gt;)b</td>
<td>0.380&lt;sup&gt;d&lt;/sup&gt; ± 0.001</td>
<td>0.002&lt;sup&gt;c&lt;/sup&gt; ± 0.000</td>
<td>0.0068&lt;sup&gt;b&lt;/sup&gt; ± 0.001</td>
</tr>
<tr>
<td>B&lt;sub&gt;u&lt;/sub&gt;</td>
<td>0.478&lt;sup&gt;a&lt;/sup&gt; ± 0.001</td>
<td>0.003&lt;sup&gt;b&lt;/sup&gt; ± 0.001</td>
<td>0.007&lt;sup&gt;c&lt;/sup&gt; ± 0.001</td>
</tr>
<tr>
<td>B&lt;sub&gt;m&lt;/sub&gt;</td>
<td>0.456&lt;sup&gt;c&lt;/sup&gt; ± 0.000</td>
<td>0.001&lt;sup&gt;d&lt;/sup&gt; ± 0.000</td>
<td>0.007&lt;sup&gt;c&lt;/sup&gt; ± 0.000</td>
</tr>
<tr>
<td>C&lt;sub&gt;u&lt;/sub&gt;</td>
<td>0.463&lt;sup&gt;b&lt;/sup&gt; ± 0.001</td>
<td>0.004&lt;sup&gt;a&lt;/sup&gt; ± 0.000</td>
<td>0.005&lt;sup&gt;c&lt;/sup&gt; ± 0.001</td>
</tr>
<tr>
<td>C&lt;sub&gt;m&lt;/sub&gt;</td>
<td>0.308&lt;sup&gt;f&lt;/sup&gt; ± 0.000</td>
<td>0.001&lt;sup&gt;d&lt;/sup&gt; ± 0.001</td>
<td>0.006&lt;sup&gt;d&lt;/sup&gt; ± 0.000</td>
</tr>
<tr>
<td>SL&lt;sup&gt;+&lt;/sup&gt;</td>
<td>150 – 200</td>
<td>400 – 500</td>
<td>301</td>
</tr>
</tbody>
</table>

Results are the means of three replications. Values carrying different superscripts in the same column are significantly different (p<0.05). (MB<sub>m</sub>)b = maize-bambara groundnut malt complementary food, fermented and fortified (MC<sub>m</sub>)b = maize-cowpea malt complementary food, fermented and fortified, B<sub>u</sub> = unmalted bambara groundnut, C<sub>u</sub> = unmalted cowpea, MB = malted bambara groundnut, MC = malted cowpea, SL = safe level. *Phytate: Heaney et al. (1991), Oxalate: Oke O.L. (1969), Tannin: Schiavone et al. (2007)

The tannin content of (MC<sub>m</sub>)b, 0.380 mg/100g was higher than that of (MB<sub>m</sub>)b, 0.315 mg/100g in spite of the higher tannin content of B<sub>m</sub> (0.456) compared to C<sub>m</sub> (0.308) suggesting that fermentation may have more reductive effect on cowpea malt tannins than on bambara groundnut malt or that it could be due to effects relating to the fortificant mix. The low tannin content of the fortified and fermented maize-bambara groundnut malt and fermented maize-cowpea malt complementary food could be attributed to the activity of malt enzymes. Similar results were reported by Obizoba and Egbuna (1992).

The decrease in oxalate content of the maize-bambara groundnut malt and the maize-cowpea malt complementary foods could be as a result of steeping and fermentation as reported by Aworh (1993) and Quinteros et al. (2003). Malting reduced oxalate contents of bambara groundnut and cowpea by 0.002 and 0.003 units, respectively, suggesting that the oxalate contents of (MB<sub>m</sub>)b, 0.002 mg/100g and (MC<sub>m</sub>)b, 0.002 mg/100g may be due to contributions from the fortificant mix or due to concentration as a result of fermentation of (MB<sub>m</sub>)b and (MC<sub>m</sub>)b.

Malting had no effect on the phytate in bambara groundnut but increased that of cowpea. The phytate in (MB<sub>m</sub>)b, 0.076 mg/100g was significantly (p<0.05) higher than (MC<sub>m</sub>)b, 0.0068 mg/100g and were significantly less than the safe levels of 300. The
phytate content of (MC<sub>m</sub>)<sub>b</sub> was significantly (p<0.05) lower than that of (MB<sub>m</sub>)<sub>b</sub> while the tannin contents were in a reverse relationship.

**Phytate:mineral molar ratios.**

The maize-bambara groundnut malt and maize-cowpea malt complementary foods had phytate:mineral molar ratios (Table 9) which are by far lower than the maximum suggested desirable levels and suggests that the relative mineral bioavailability of (MB<sub>m</sub>)<sub>b</sub> and (MC<sub>m</sub>)<sub>b</sub> would be high (Gibson *et al*., 2010; Gibson and Hotz, 2000).

(MB<sub>m</sub>)<sub>b</sub> had phytate:mineral molar ratios of 0.000012, 0.000048, 0.00000047 for Ca, Fe and Zn. (MC<sub>m</sub>)<sub>b</sub> had 0.00000042, 0.0000081, 0.000049 for the respective minerals in the samples. (MB<sub>m</sub>)<sub>b</sub> had higher ratios for Fe and Ca than (MC<sub>m</sub>)<sub>b</sub>, while (MC<sub>m</sub>)<sub>b</sub> had higher ratio for zinc than (MB<sub>m</sub>)<sub>b</sub>.

**Table 9: Phytate:mineral molar ratio of maize-bambara groundnut and maize-cowpea malt complementary foods**

<table>
<thead>
<tr>
<th>Minerals (mg/g)</th>
<th>Phytate:Mineral molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>Phytate</td>
</tr>
<tr>
<td>(MB&lt;sub&gt;m&lt;/sub&gt;)&lt;sub&gt;b&lt;/sub&gt;</td>
<td>0.00076</td>
</tr>
<tr>
<td>(MC&lt;sub&gt;m&lt;/sub&gt;)&lt;sub&gt;b&lt;/sub&gt;</td>
<td>0.00068</td>
</tr>
<tr>
<td>SDL&lt;sup&gt;+&lt;/sup&gt;</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

(MB<sub>m</sub>)<sub>b</sub> = maize-bambara groundnut malt complementary food, fermented and fortified. (MC<sub>m</sub>)<sub>b</sub> = maize-cowpea malt complementary food, fermented and fortified. *Gibson et al* (2010), SDL: suggested desirable levels; molar mass of phytate = 660, calcium = 40.08, iron = 56, zinc = 65.4

**Sensory evaluation**

The flour from (MB<sub>m</sub>)<sub>b</sub> was more preferred (Table 10) than (MC<sub>m</sub>)<sub>b</sub> on the basis of the texture and flavour (Table 10) but were not statistically different. However, (MB<sub>m</sub>)<sub>b</sub> was inferior to the proprietary formula (N) which appeared better in appearance, texture, flavour and taste.
Appearance

The proprietary formula was scored highest (8.53 ± 0.64) and was significantly (P<0.05) different from (MB_m)_b blend (7.07 ± 0.8) and (MC_m)_b blend (7.13 ± 0.02). (MB_m)_b and (MC_m)_b blends did not differ between each other (P > 0.05).

Texture

The proprietary formula was scored highest in texture (8.00 ± 0.93). However, this was not found to be significantly (P > 0.05) different from the scores of (MB_m)_b blend (7.60 ± 1.40) and (MC_m)_b blend (7.53 ± 1.36).

Flavour

The flavor of the proprietary formula was significantly (P < 0.05) more acceptable (8.67 ± 0.62) compared to the flavor of the (MB_m)_b blend (4.53 ± 1.36) and (MC_m)_b blend (4.13 ± 0.99). Also, the flavor of (MB_m)_b did not significantly (P > 0.05) differ from that of (MC_m)_b blend.

Taste

The taste of the proprietary formula was highly accepted (8.13 ± 1.13) compared to the taste of the (MB_m)_b blend (4.27 ± 1.39) and (MC_m)_b blend (4.40 ± 1.55), but the taste of the (MB_m)_b blend did not differ significantly (P>0.05) from that of (MC_m)_b blend.

Overall acceptability

The proprietary formula was highly accepted (8.53 ± 0.52) and was significantly (P < 0.05) different from the acceptability of (MB_m)_b blend which scored (5.27 ± 1.33) and the (MC_m)_b blend which scored (5.20 ± 1.26). The acceptability of (MC_m)_b blend did not differ from that of (MB_m)_b blend.
Table 10: Sensory scores for maize-bambara groundnut and maize-cowpea malt flour samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Appearance</th>
<th>Texture</th>
<th>Flavour</th>
<th>Taste</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8.53a ± 0.64</td>
<td>8.00a ± 0.93</td>
<td>8.67a ± 0.62</td>
<td>8.13a ±1.13</td>
<td>8.53a ± 0.52</td>
</tr>
<tr>
<td>(MB\textsubscript{m})\textsubscript{b}</td>
<td>7.07b ± 0.80</td>
<td>7.60a ± 1.40</td>
<td>4.53b ± 1.36</td>
<td>4.27b ± 1.39</td>
<td>5.27b ± 1.33</td>
</tr>
<tr>
<td>(MC\textsubscript{m})\textsubscript{b}</td>
<td>7.13b ± 0.02</td>
<td>7.53a ± 1.36</td>
<td>4.13b ± 0.99</td>
<td>4.40b ± 1.55</td>
<td>5.20b ± 1.26</td>
</tr>
</tbody>
</table>

Results are the means of three replications. Values carrying different superscripts in the same column are significantly different (p<0.05). (MB\textsubscript{m})\textsubscript{b} = maize-bambara groundnut malt complementary food, fermented and fortified (MC\textsubscript{m})\textsubscript{b} = maize-cowpea malt complementary food, fermented and fortified, N = proprietary formula.

When the flours were prepared into gruels as used for infant feeding, (MB\textsubscript{m})\textsubscript{b} had the overall best rating (Table 11) as a result of a significantly (p<0.05) better rating for its colour, texture, flavor and taste. (MC\textsubscript{m})\textsubscript{b} had the least acceptance.

Table 11: Sensory scores for maize-bambara groundnut and maize-cowpea malt gruels

<table>
<thead>
<tr>
<th>Samples</th>
<th>Appearance</th>
<th>Texture</th>
<th>Flavour</th>
<th>Taste</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>(MB\textsubscript{m})\textsubscript{b}</td>
<td>7.73a ± 1.53</td>
<td>8.27a ± 0.96</td>
<td>6.73a ± 2.37</td>
<td>6.67a ± 2.23</td>
<td>7.07b ± 1.94</td>
</tr>
<tr>
<td>N</td>
<td>6.47ab ± 1.96</td>
<td>7.33ab ± 1.63</td>
<td>5.53ab ± 2.85</td>
<td>5.07ab ±7.94</td>
<td>6.13ab ± 2.64</td>
</tr>
<tr>
<td>(MC\textsubscript{m})\textsubscript{b}</td>
<td>6.00b± 1.89</td>
<td>7.13b ± 1.41</td>
<td>3.93b ±1.53</td>
<td>3.73b ± 1.62</td>
<td>4.67b ± 1.35</td>
</tr>
</tbody>
</table>

Results are the means of three replications. Values carrying different superscripts in the same column are significantly different (P<0.05). (MB\textsubscript{m})\textsubscript{b} = maize-bambara groundnut malt complementary food, fermented and fortified (MC\textsubscript{m})\textsubscript{b} = maize-cowpea malt complementary food, fermented and fortified, N = proprietary formula.
CHAPTER FIVE; CONCLUSION AND RECOMMENDATION

Conclusion

Fortified maize-bambara groundnut malt and maize-cowpea malt complementary foods for infants could be produced using a combination of household technologies such as malting, fermentation, fortification and drying. The use of processed cattle bone, roselle calycies and palm oil as fortificants for calcium, iron, zinc and vitamin A, respectively, improved the Fe and Zn contents but did not improve the Ca and vit. A contents.

The values of Ca, Fe, Zn and vit. A values respectively for \((MB_m)_b\) is 0.054 mg/g, 5.30 mg/g, 1.58 mg/g and 3.93 µgRE/g while for \((MC_m)_b\) is 0.054 mg/g, 7.08 mg/g, 1.37 mg/g and 3.36 µgRE/g. These values compared with RDA values 5.00 mg/g, 0.28 mg/g, 0.125 mg/g and 5.00 µgRE/g showed that only Fe and Zn contents were improved. The processing methods were therefore effective/efficacious.

Recommendations

Animal studies to investigate the toxicity level, if any, and confirm the bioavailability of the micronutrients. Studies to investigate the reason for the high pH of the blends and fortificant mix.
REFERENCES


