Development and evaluation of complementary foods based on traditional foodstuffs in Western Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

To my charming wife Nancy,

To my son Eddie Lloyd and others to come,

To men and women who cherish the knowledge of science.

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ABBREVIATIONS

AOCS	American Association of Oil Chemists
AOAC	Association of Official Analytical Chemists
CFU	Colony Forming Units
CHW	Community Health Worker
CSB	Corn Soy Blend
CSB+	Corn Soy Blend plus
CSB++	Corn Soy Blend plus plus
FAO	Food and Agriculture Organisation
GC	Gas Chromatography
HPLC	High Pressure Liquid Chromatography
JKUAT	Jomo Kenyatta University of Agriculture and Technology
RDA	Recommended Daily Allowance
UNICEF	United Nations Children’s Fund
UNU	United Nations University
UV-VIS	Ultra-Violet Visible Spectrophotometer
WFP	World Food Programme
WHO	World Health Organisation

DEFINITION OF TERMS

Complementary feeding	Starts when breast-milk alone is no longer sufficient to meet the nutritional requirements and other foods are needed, along with breast milk
Complementary food	Solids and/or liquids fed together with the breast milk to meet the nutritional requirements
Food	A substance developed using foodstuff
Foodstuffs	Ingredients used to process complementary foods
Indigenous foods	Foods known to be grown in a particular area that can't be grown elsewhere
Traditional foods	Includes wild indigenous plants and animals collected from land and aquatic environments, semi-domesticated indigenous plants and animals, locally available staple foods processed using indigenous techniques

LIST OF PUBLICATIONS

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ABSTRACT

In a bid to address the challenge of child malnutrition occasioned by inadequate complementary feeding, this study aimed at utilizing traditional foodstuffs to develop nutrient dense complementary foods in Western Kenya. The components studied included evaluation of traditional foods with potential for complementary feeding and evaluation of nutrient and anti-nutrient factors of selected traditional foods. Formulation of complementary foods (CF1, CF2, CF3) composed of non-germinated amaranth grain (*Amaranthus cruentus*), maize (*Zea mays*), termites (*Macrotermes subhylanus*) and *dagaa* fish (*Rastreneobola argentea*). Four other complementary foods (CF4, CF5, CF6 and CFC) composed of germinated amaranth, maize, termite, *dagaa* fish were developed. Nutrient content, functional properties and consumer acceptability of the four foods was evaluated.

In order to select the traditional foodstuffs appropriate for processing complementary foods, the study identified 26 leafy vegetables, 5 grains and pulses, 3 tubers, 1 seed, 6 insects, and 1 fish. Amaranth grain, different termite species, and *dagaa* fish had 18.6, 33.5-39.3, and 58.2 g/100g protein content respectively. The termites had 53.33 - 115.97 mg/100g iron content while finger millet had 102.98 mg/100g of iron. Finger millet had the highest phytic acid content (2287.02 mg/100g) even though it had the lowest phytic acid/iron ratio (1.88) compared to amaranth (7.23) and maize (2.86). The level of unsaturated fatty acids in termites was found to be 50.5 – 66.2%, amaranth grain 69.1%, finger millet 50.9% and maize (77.8%).

Complementary foods CF1, CF2 and CF3 contained high phytic acid content (914.5 - 1234.0 mg/100g) and low bioavailability (<5%) of non-heme iron. Phytates reduction on the amaranth grain was significant after 24 hours of germination followed by significant reduction of molar ratios ($p<0.05$). Complementary foods CF4, CF5, CF6 and CFC contained 396.0 – 442.0 kcal/100g energy, 13.7 – 19.0 g/100g protein, 10.40 – 27.41 mg/100g iron, 4.19 – 5.96 mg/100g zinc. CF5 and CFC were preferred more by children than CSB+ and none of the foods showed signs of microbial or aflatoxin contamination.

The findings of this study provide evidence that Western Kenya has traditional foodstuffs which are nutrient dense. Traditional grains studied have anti-nutrients and reducing them is necessary before processing to complementary foods. Germinating amaranth grain reduced phytic acid further improving mineral bioavailability. Termite and *dagaa* fish can be utilized in processing nutrient dense and acceptable complementary foods. It's therefore recommended that both traditional animal and plant foods be exploited. Appropriate pre-processing steps such as germination should be incorporated before processing traditional grains. Facilitation to commercialize and patent the process and products should be done to enable full exploitation.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background Information

Childhood malnutrition remains a common problem in much of the developing world (UNICEF, 2009; Mosha and Bennink, 2005). Nutrition and health in the first year of life affect growth and development of children and most growth faltering occurs during this time (Shrimpton et al., 2001; Semproli and Gualdi-Russo, 2007). Multiple micronutrient deficiencies – particularly of iron, zinc and vitamin A are common in Kenya as in other resource poor settings (UNICEF, 2009; Gibson, 1994). According to the Kenya Demographic and Health Survey (KNBS, 2010a), 35% of children less than 5 years of age are stunted children, 7% wasted and 16% underweight. The report further indicates that child malnutrition rates are higher in rural than urban areas. In Western Kenya 34.2% of children <5 years of age are stunted, 2.3% are wasted while 11.8% are underweight (KNBS, 2010a). Causes of malnutrition are the combination of diseases and dietary inadequacies interacting in a mutually reinforcing manner.

Inadequate food intake is one key cause of malnutrition. Usually, in resource poor settings, the availability of nutritionally sound complementary foods is a major limitation in ensuring optimum feeding of infants and young children after 6 months of age when breastfeeding alone becomes inadequate (Dewey, 2005). In addition, complementary foods often fail to meet the nutritional requirements of infants (WHO, 2008) because they are mostly plant foods with low energy and nutrient density and contain anti-nutrient factors such as phytic acid, tannins and phenolics that limit the absorption of essential micronutrients (Towo et al., 2006; Gibson et al., 1998a). Mineral deficiencies are likely to occur if these foods are processed in a way that does not ensure enhanced bioavailability of the micronutrients (Towo et al., 2006).

Protein-energy malnutrition and micronutrient deficiencies begin during weaning and/or immediately thereafter as most foods used for weaning do not provide adequate amounts of energy, protein and micronutrients (Mosha and Bennink, 2005). In Africa, traditional weaning foods are based on starchy cereals such as maize (*Zea mays*), sorghum (*Sorghum bicolor* L), finger millet (*Eleucine coracona*) and rice (*Oryza sativa*) or non-cereals such as cassava (*Manihot esculenta*), round potato (*Solanum tuberosum*), sweet potato (*Ipomea batatas*), yams (*Dioscorea spp*) and plantains (*Musa paradisiaca sapientum*). These foods have been widely associated with nutrient deficiencies among pre-school age children (Walker, 1990).

Developing nutrient dense, low-cost weaning foods from the locally available food ingredients has been strongly recommended as a viable and sustainable approach to address the problem of malnutrition in developing countries (WHO/UNICEF, 1998; Dijkhuizen, 2000; WHO, 2002; Dewey and Brown, 2003; Lutter, 2000; Mensah and Tomkins 2003; Lutter 2003; Lutter and Dewey, 2003). Such foods would provide reliable options for many families and can thus contribute immensely in ameliorating protein-energy and micronutrient malnutrition among older infants and young children (WHO/UNICEF, 1998; Dewey and Brown, 2003). These nutrient dense, low cost foods must be stable during storage so as to allow ample time for transportation, storage, and marketing while still maintaining their nutritional and sensory wholesomeness (King and Burgess, 1993). The foods should also be free from pathogenic microorganisms and free from any substances originating from microorganisms in amounts which may represent a hazard to health. They should also not contain any other poisonous or deleterious substances in amounts which may represent a hazard to health of the infants or young children (Codex, 1991).

The potential of utilizing traditional foodstuffs and traditional food processing techniques in developing nutrient dense complementary foods is a viable one. Amaranth grain has in the recent past been promoted as a health food for lactating mothers and infants in Western Kenya after years of neglect (Muyonga et al., 2008). Consumption of termites and *dagaa* fish is a common practice in western Kenya and exploiting the resource in improving infant and child feeding is both viable and acceptable. The two types of food are rich in minerals and essential fatty acids. Germination is one of the oldest techniques of food processing. In western Kenya, beverages made from germinated grains are common and so it’s an indigenous household level food technology and this makes it sustainable. It’s touted to improve taste and texture. Germination often leads to an improvement in the nutritional value of foods by reducing nutrient inhibitors such as phytic acid (Gison et al., 1998).

1.2 Problem statement

A major contributor of the widespread childhood malnutrition in Western Kenya is inadequate complementary feeding both in quantity and quality due to over reliance on cereals for complementary feeding. Recent demographic and health survey shows that 72% of infants are weaned using cereal based complementary foods. These foods have high bulk density and high anti-nutrient content and therefore do not offer adequate nutrient density and bioavailable minerals. The complementary foods are also lacking in animal source foodstuffs partly due to high costs, limitations on processing techniques and insufficient knowledge on nutritional and health benefits. With civilisation, indigenous processing methods which are affordable and likely to enhance nutrient density and bioavailability have been neglected. A strategy to combine plant and animal foodstuffs in development of complementary foods will result in adequate nutrient density and high mineral bioavailability.

1.3 Justification

WHO recommends use of traditional foodstuffs in complementary feeding. Western Kenya has diverse traditional plant and animal foodstuffs most of which are nutrient rich. Animal foodstuffs have high mineral content; the minerals are more bioavailable and have been found to improve bioavailability of minerals in plant foodstuffs. Appropriate indigenous processing techniques will reduce anti-nutrients thus further improve mineral bioavailability. The foodstuffs are culturally acceptable and affordable in Western Kenya and uptake of the developed foods will be high.

1.4 Objectives

1.4.1 Main Objective

To develop and evaluate the nutrient density, mineral bioavailability and acceptability of complementary foods developed using traditional foodstuffs.

1.4.2 Specific Objectives

1.4.2.1 To evaluate the traditional foods consumed and the processing techniques utilized to prepare them in Western Kenya

1.4.2.2 To determine the nutrient and anti-nutrient content of the selected traditional foods

1.4.2.3 To develop a process for development of complementary foods from the selected traditional foodstuffs and determine their mineral bioavailability

1.4.2.4 To utilize germinated amaranth grain in development of complementary foods and determine the microbial safety, functional properties, storage stability and consumer acceptability of foods

CHAPTER TWO

LITERATURE REVIEW

2.1 Traditional foods in Western Kenya

Traditional foods include wild indigenous plants and animals collected from land and aquatic environments, semi-domesticated indigenous plants and animals. It also includes locally available staple foods processed using indigenous techniques. They are culturally acceptable and may have no or low commercial value (Ogoye-Ndegwa and Aagaard-Hansen, 2003). According to the same authors, Kenya has a diverse traditional food system with important dietary contributions. Persistent use of a large number of these traditional foods plays an important role in the continued adaptation of the communities of Western Kenya (Orech et al., 2007a).

Plants provide most of the foods consumed by peasant farming communities in tropical countries. Communities in Western Kenya, depend on many plant products for subsistence and for generation of cash income. Sizeable areas are dedicated to the cultivation of dietary staples and crops, many of which are not traditional (Johns and Kokwaro, 1991). Traditional foods adapted to local conditions contribute to the diet in periods of seasonal scarcity (Ogoye-Ndegwa and Aagaard-Hansen, 2003; Orech et al., 2007a), thus contributing to an important traditional buffer against periodic famines that are becoming increasingly prevalent in other areas of the tropics.

The *Luo* and *Luhya* communities of western Kenya are particularly unique in their culture of harvesting and consumption of different species of insects from the wild during the rainy seasons (Christensen et al., 2006; Ayieko and Oriaro 2008; Ayieko et al., 2010a; Kinyuru et al., 2010a). Currently, the staple foods are restricted to maize and to a lesser extent, millet and sorghum (Geissler et al. 2002) which also happens to be the foods utilized as complementary foods among infants and young children.

2.2 Nutritional potential of traditional foods

Traditional plant and animal foods consumed in the western region of Kenya may constitute the cheapest sources of macronutrients and micronutrients, especially providing minerals, carbohydrates, protein and polyunsaturated fatty acids (Johns and Kokwaro 1991; Orech et al., 2007b; Ayieko 2007; Kinyuru et al. 2010a) that are essential for optimal infant growth and development. Edible insects are culturally accepted in western Kenya, are nutritious with significant level of iron, zinc, protein and are moderate sources of omega 3 fatty acids (Kinyuru et al., 2010a; Ayieko et al., 2012). Some plant food species have also been associated with treatment of different ailments and this is sometimes related to their nutritional contribution (Geissler et al., 2002). However, limited data are available on the nutrient and anti-nutrient composition of many traditional foods thereby hampering their utilization (Hongo, 2003). Varied content of nutrient inhibitors have been reported in traditional food plants such as millet and sorghum (Tizazu et al., 2011; Sharma and Kapoor, 1996). These hamper availability of minerals such as iron, zinc and calcium resulting to mineral deficiency.

According to the WHO (2001), mineral deficiency, among other deficiencies, is widespread in developing countries. Anti-nutrients such as phytic acid may inhibit absorption of some minerals, even at low concentrations (Hortz and Gibson, 2007). Dietary phytates, tannins and polyphenols inhibit non-heme iron absorption (Miller, 1996). Iron, zinc and phytic acid form insoluble complexes and the negative effect of such complexes on absorption can be predicted by phytate - to - mineral molar ratios (Oberleas and Harland, 1981). High amounts of calcium exacerbate the inhibitory effect of phytate on zinc absorption by forming a calcium–zinc–phytate complex in the intestine that is even less soluble than phytate complexes formed by either ion alone (Gibson et al., 1998a). Anti-nutrients are not a serious challenge in traditional animal foods such as edible insects. Omotoso (2006) reported insignificant amounts of phytic acid in *Ciria forda* larva. Enujiugha and Ayodele-Oni (2003) reported that tannin was not detected in *C. forda* larva.

2.3 Complementary feeding in the developing countries

According to the Kenya Demographic Health Survey of 2008 – 2009, 72% of infants are weaned using foods made from cereal grains (KNBS, 2010a). Sometimes the grains are substituted with foods made from roots and tubers while legumes are introduced at a much later age. This is the case in most developing countries where complementary foods are made of a mixture of flour from cereals mostly rich in carbohydrates. The foods are sometimes fortified with some proteins, with a little oil and sugar added (Walker, 1990). Foods rich in protein such as powdered milk, fish, peanut butter or soya are out of reach of the poorest people in some of these countries. Locally available foodstuffs e.g. edible insects can successfully replace the expensive sources of protein, for they are rich in digestible proteins of good nutritional quality (Kinyuru et al., 2010a).

Most of the foods used during the complementary feeding stage are frequently characterized by low nutrient density and high bulk, which can adversely affect infants' health (Owino et al., 2007). Infants are usually either weaned directly onto the family diet early in life or continue to be exclusively breastfed, both resulting in suboptimal nutrient intake with adverse impact on optimum growth. The high cost of commercial complementary foods adds to the difficulty of providing good nutrition to infants (Asma et al., 2006).

In resource poor settings such as western Kenya, preparation of a complementary food should be based on foods available locally. The food habitually eaten by the family (family meal) can also be used as the basis to make a fluid complementary food by adding water and enriching it during the cooking (Lung'aho and Glahn, 2009).

2.4 Current trends in development of complementary foods

Complementary foods in developing countries have for a long time been cereal based. Recently in Africa, supplementation of cereals with locally available legumes as a protein source has been exploited such as soya bean, groundnut (Martin, 2010; Anigo et al., 2010) cowpeas (Oyarekua, 2010; Muhimbula et al., 2011) pigeon peas (Asma et al., 2006) common bean and bambara nuts (Muhimbula et al., 2011; Owino et al., 2007) among others. In addition, World Food Programme (WFP) has promoted corn-soy-blend *plus* (CSB+) and corn-soy-blend *plus plus* (CSB++), food supplements utilized in management of moderate acute malnutrition (WFP, 2010).

An observational study showed that school going children in Kenya consuming diets high in animal source foods grew better (Grillenberger et al., 2006). However, studies on complementary foods development with animal foods as ingredients are fewer or have not been widely reported. Existing work have been based on the use of fish (Mosha and Bennink, 2005; Haug et al., 2010). Bwibo and Neumann (2003) observed underutilization of animal foods in complementary foods and recommended the need to increase animal source foods among Kenyan children. Foods made from some animal food sources are better sources of minerals such as calcium, iron and zinc, vitamins and lipids. There has been an awakening on utilization of edible insects to fight food and nutrition security in Kenya (Ayieko et al., 2010b) and therefore their utilization in complementary feeding may be of interest.

2.5 Inhibition of mineral bioavailability by phytates

Plant-based complementary foods often contain high levels of phytate, a potent inhibitor of iron, zinc, and calcium bioavailability. One of the common methods of predicting mineral bioavailability is phytate:mineral molar ratio. Gibson et al., (2010) reported that 62% of indigenous and 37% of processed complementary foods in low income countries have phytate:mineral molar ratios that exceed suggested desirable levels for mineral bioavailability (i.e., phytate:iron <1, phytate:zinc <15, phytate:calcium <0.17). Mineral bioavailability from these products is typically low due to the presence of the phytic acid (Cook et al., 1997; Egli et al., 2002; Davidsson, 2003). Several recent *in vivo* isotope studies in adults (Mendoza et al., 1998; Egli et al., 2004; Hambige et al 2004) and infants (Davidsson et al., 2004) have reported improvements in absorption of iron, zinc, and calcium in cereal-based foods prepared with a reduced phytate content.

Dietary phytates inhibit iron absorption whereas ascorbic acid and meats enhance it (Miller, 1996). Phytic acid is a major inhibitor of zinc absorption, especially when the content of animal protein is low. Zinc and phytic acid form insoluble complexes and the negative effect of such complexes on zinc absorption can be predicted by phytate-to–zinc molar ratios (Oberleas and Harland, 1981). High amounts of calcium exacerbate the inhibitory effect of phytate on zinc absorption by forming a calcium–zinc–phytate complex in the intestine that is even less soluble than phytate complexes formed by either ion alone (Davidsson *et al*. 1994; Cook, 1997; Egli *et al*. 2002; Davidsson, 2003).

2.6 Indigenous strategies for reducing phytic acid in plant foods

2.6.1 Germination

Germination has been an indigenous technique practiced in western Kenya. Though the locals attribute the practice to solely improved taste of beverages made for germinated grain, the practice also improves their nutritional value of the foods. Germination increases endogenous phytase activity in cereals through *de novo* synthesis, activation of intrinsic phytase, or both. Tropical cereals such as maize and sorghum have a lower endogenous phytase activity than do rye, wheat, triticale, buckwheat, and barley (Egli et al., 2002). Specifically, germination has a reducing effect of phytic acid on amaranth grain (Colmenares and Bressani, 1990). Egli et al.,

(2002) reported a 1.5-fold increase in phytase activity in unspecified amaranth grain species germinated for 48 hours. Hence, a mixture of cereal flours prepared from germinated and ungerminated cereals will promote some phytate hydrolysis when prepared as porridge for infant and young child feeding (Colmenares and Bressani, 1990; Marero et al., 1991; Sharma and Kapoor, 1996). The rate of phytate hydrolysis varies with the species and variety as well as the stage of germination, pH, moisture content, temperature (optimal range 45–57^{o}C), solubility of phytate, and the presence of certain inhibitors (Egli et al., 2002). Lower inositol phosphates have less binding capacity and have little influence on mineral bioavailability in humans. This degradation of phytic acid has been reported to increase absorption of both iron (Hurrell *et al*. 1992; Davidsson et al., 1994; Sandberg et al., 1999) and zinc (Egli et al., 2004).

Certain tannins and other polyphenols in legumes (e.g., *Vicia faba*) and red sorghum may also be reduced during germination as a result of the formation of polyphenol complexes with proteins and the gradual degradation of oligosaccharides (Camacho et al., 1992). The α-Amylase activity is also increased during germination of cereals, especially sorghum and millet. This enzyme hydrolyzes amylase and amylopectin to dextrins and maltose, thus reducing the viscosity of thick cereal porridges without dilution with water. This simultaneously enhances the energy and nutrient densities of the porridge (Gibson et al, 1998b).

2.6.2 Soaking

Soaking has been an indigenous technique practiced in western Kenya especially for cereal grains before cooking. It said to soften the grain and therefore cooks faster. The practice has other advantages. Soaking cereal in water can result in passive diffusion of water-soluble phytates, which can then be removed by decanting the water (Hortz and Gibson, 2007). The extent of the phytate reduction depends on the species, pH, and length and conditions of soaking. Some polyphenols and oxalates that inhibit iron and calcium absorption, respectively, may also be lost by soaking (Erdman and Pneros-Schneier, 1994).

2.6.3 Fermentation

Fermentation is an age old practice in Kenya and the world over. Fermentation induces phytate hydrolysis via the action of microbial phytase enzymes, which hydrolyze phytate to lower inositol phosphates which do not inhibit nonheme iron absorption (Sandberg et al., 1999; Hurrel, 2004). Microbial phytases originate either from the microflora on the surface of cereals and legumes or from a starter culture inoculate (Sandberg and Svanberg, 1991). The extent of the reduction in higher inositol phosphate levels during fermentation varies; sometimes 90% or more of phytate can be removed by fermentation of maize, soy beans, sorghum, cassava, cocoyam, cowpeas, and lima beans. In cereals with a high tannin content (e.g., bulrush millet and red sorghum), phytase activity is inhibited, making fermentation a less-effective phytate-reducing method for these cereal varieties (Sandberg and Svanberg, 1991).

2.6.4 Mechanical processing

Household pounding of cereal grains has been practiced in Kenya for a long time. It is used to remove the bran and/or germ from cereals, which in turn may also reduce their phytate content when it is localized in the outer aleurone layer (e.g., rice, sorghum, and wheat) or in the germ (i.e., maize) (Nout and Ngoddy, 1997). Hence, bioavailability of minerals may be enhanced, although the content of minerals and some vitamins of these pounded cereals is simultaneously reduced. Household mechanical processing may only be possible for large seeds such as maize but may be cumbersome for small ones like amaranth grains or millet thus making it less viable in some settings.

2.7 Addition of animal foods to improve mineral bioavailability

The bioavailability of iron is generally higher from animal foods than from plant foods (Lartey et al., 1999; Allen, 2003). Addition of even a small amount of animal-source foods is one of the strategy to improve the content and bioavailability of micronutrients in plant-based diets in resource-poor settings.

Fish has been used in development of complementary foods leading to enhanced iron, zinc and calcium content and bioavailability (Mosha et al., 2005; Haug et al., 2010). Inclusion of red meat has been found to significantly increase non-heme iron bioavailability (Engelm-ann et al., 1998; Davidsson, 2003; Sorensen et al., 2007a) in plant based foods. There have been negative associations between the avoidance of animal source foods and mineral deficiency in young children from developed countries (Black, 2002; Black et al., 2002; Dagnelie et al., 1994). According to

Kinyuru et al. (2009) and Ayieko et al., (2010b), it is possible to develop commercial products from edible insects in Kenya. Wheat based products developed with incorporation of edible insects have been observed to have high nutrient content and to be highly acceptable among consumers.

2.8 Consumer acceptability of complementary foods

Many factors affect acceptance of a complementary foods both by mothers and infants including culture and food properties like taste, color and consistency (Codex 1991). Traditional foods are locally accepted and therefore pose no challenge to consumers. If a food is to be cooked to gruel, consistency has been found to affect acceptability (Owino et al., 2007). Some studies have found that complementary food formulations with addition of sugar were found to be more tasty and appealing than those without sugar by mothers and infants (Martin et al., 2010; Muhimbula et al., 2011).

CHAPTER THREE

EVALUATION OF TRADITIONAL FOODS SUITABLE FOR DEVELOPMENT OF COMPLEMENTARY IN WESTERN KENYA

3.1 INTRODUCTION

Traditional foods are culturally acceptable, are an integral part of local food habits, have no or low commercial value and are either collected for consumption or traded locally. They may contribute to a nutritionally balanced diet by supplying essential vitamins and minerals (FAO 1988). Moreover, traditional foods adapted to local conditions contribute to the diet in periods of seasonal scarcity (Ogoye-Ndegwa and Aagaard-Hansen, 2003; Orech et al., 2007a), thus contributing to an important traditional buffer against periodic famines that are becoming increasingly prevalent in other areas of the tropics.

Traditional plant and animal foods may play a very crucial role of providing macronutrients and micronutrients and may more importantly be utilized to alleviate childhood malnutrition (Roos et al., 2007). The retention of knowledge by the local people in western Kenya on the use of traditional foods attests to the continuing importance of these resources for subsistence and as part of the cultural heritage of the *Luo*. This study was aimed at identifying and documenting traditional foods with potential in formulation of improved complementary foods in Western Kenya based on their nutritional value, perceived nutritional benefits and availability.

3.2 METHODOLOGY

3.2.1 Study area and population

The study was conducted in the rural areas of Nyanza province, Kisumu West District namely: Kanyoto, Kapuonja “A”, Karateng West, Marera, Ong’io, Meronda, and Karateng’ East villages of Maseno Division and Seme, Reru and Kaura villages of Kombewa Division. Kisumu West District lies between a latitude of -0.25 (0° -15' 0 S) and a longitude of 34.92 (34° 55' 0 E), with a land area of 919 km^2, with an approximate population of 1 million according to the 2009 population and housing census (KNBS, 2010b). Majority of the inhabitants in the District are people with Luo ethnic background whose main economic activities are fishing and subsistence farming; maize, sorghum, and fresh vegetables. With an approximate altitude of 1500 meters above sea level, Kisumu West District is part of the extension of the humid tropical zone of central Africa into western Kenya.

3.2.2 Study design

The study was a cross-sectional study with random sampling used in order to allow the generalization of conclusions. The methodological protocol closely followed a multidisciplinary approach combining botanical inventorying; collection of voucher plant specimens and taxonomic assessment; semi-structured and informal interviews (Martin, 1995; Grenier, 1998) which came up with a list of Luo (local) names of the different foods mentioned. Secondary data on the nutritional content of the foods was obtained from food composition tables.

3.2.3 Field survey

This field survey targeted 50 women of child bearing age identified using a snow balling technique beginning with a Community Health Worker (CHW). The study was conducted in the rural areas of Kisumu West District namely: Kanyoto, Kapuonja "A", Karateng West, Marera, Ong'io, Meronda, and Karateng' East villages of Maseno Division and Seme, Reru and Kaura villages of Kombewa Division. The purpose of the study was explained to the selected mothers and oral consent to participate in the study was sought. Only consenting mothers were included in the study. Home and farm plots of some interviewees were randomly selected and surveyed for food species (Appendix 1). Traditional food species were collected from vegetation types such as scrubs, thickets, grasslands as well as from kitchen gardens, farmlands, built-up areas, hedges and wastelands.

3.2.4 Key informant interviews

Six (6) key informants were purposively selected with the help of interviewees, elders and the local administrators (Appendix 2) each representing a village within the study area. The interviewer read out names of foods and sought explanation why those foods were eaten. The survey tool included a list of all plant and animal foods originating from the field survey described above. The objective was to find reasons why the earlier listed foods were consumed and determine whether the consumption of these foodstuffs was linked to nutritional and/or health benefits. Common phrases such as, 'increase of blood levels', 'addition of energy', 'enhancement of breast milk by lactating mothers' from the informants were noted. Availability of the foods and cooking methods were probed.

3.2.5 Secondary data

Literature on iron and zinc content of the foodstuffs was obtained from the Kenya and Tanzania food composition databases (Sehmi, 1993; Lukmanji et al., 2008).

3.2.6 Data analysis

Vernacular names of the foods were annotated with scientific names. Foods were separated according to food groups namely; fruits and vegetables, cereal grains, pulses, seeds, tubers/roots and animal foods. Foods with perceived nutritional and health benefits were tabulated.

3.3 RESULTS AND DISCUSSION

3.3.1 Traditional food groups and the edible parts

Among the plant source traditional foods, 26 fruits and vegetables were identified (Table 3.1). Leaves were the most consumed amongst the traditional vegetables while a few fruits were consumed together with their seeds. One (1) vegetable, wandering jew (*Commelina sinensis)* is consumed as a whole plant. Amaranth (*Amaranthus hybridus* L.) was found to be consumed as a leafy vegetable. *Amaranthus cruentus* L. was a tall plant sometimes growing up to 1.5 M high. Some of the fruits such as forest star (*Mussaenda arcuata),* bush plum *(Carissa spinarum)* and guava *(Psidium guavajum*) are consumed together with the seeds.

Most of the vegetables and fruits presented in table 3.1 grow abundantly and are easy to procure in large quantities from various habitats of study area, during short and long rainy seasons. Some of the species such as amaranth *(Amaranthus hybridus),* 'atipa' *(Asystasia mysorensis),* black jack *(Bidens pilosa),* wandering jew *(Commelina sinensis),* Jute mallow *(Corchorus trilocularis),* wild lettuce *(Launaea cornuta)* and African nightshade (*Solanum scabrum)* are invasive weeds in cultivated fields, and thus are sometimes uprooted and fed to domesticated animals. They are however abundant all year round and hence are important food resource during periods of drought or poor harvests. The vegetables, amaranth and spider plant are self sustaining and once planted, persist in the field through self-seeding for many years.

Table 3.1: Vegetables, fruits and their edible parts according to the interviewees

Common name	*Dholuo* name	Scientific name	Edible parts
Forest star	‘Anyuka’	*Mussaenda arcuata*	Fruit with seed
Pepper	‘Apilo’	*Capsicum frutescens L.*	Leaves & fruits
Climbing spinach	‘Nderma’	*Basella alba* L.	Leaves
Black jack	‘Onyiego’	*Bidens pilosa* L.	Leaves
Common bean	‘Oganda’	*Phaseolus vulgaris* L.	Leaves
Amaranth	‘Ododo’	*Amaranthus hybridus* L.	Leaves
Amaranth	‘Ododo’	*Amaranthus cruentus* L.	Leaves
Oxalis	‘Awayo’	*Oxalis latifolia* Kunth	Leaves
Sesame	‘Onyulo’	*Sesamum calycimum* Welw. *var.*	Leaves & seeds
Bush plum	‘Ochuoga’	*Carissa spinarum L.*	Fruit with seed
-	‘Atipa’	*Asystasia mysorensis* T. Anderson	Leaves & fruits
Spider plant	‘Akeyo/Dek’	*Cleome gynandra* (L.) Briq.	Leaves
Cassava	‘Omwogo’	*Manihot esculentum*	Leaves
Cucumber	‘Budho’	*Cucumis africanus* L.	Leaves
Jute mallow	‘Apoth’	*Corchurus olitorius* L.	Leaves
Pumpkin	‘Susa budho’	*Curcurbita maxima* Duchesne ex. Lam.	Leaves & fruits
Egyptian riverhemp	‘Osao’	*Sesbania sesban* L.	Leaves
Wild Mustard, Ethiopian cabbage	‘Kandhira’	*Brassica juncea* (L.) Czern.	Leaves
Slender leaf rattlebox	‘Mitoo’	*Crotalaria ochroleuca* G. Don.	Leaves
African nightshade	‘Osuga’	*Solanum scabrum* L.	Leaves
Cowpeas	‘Bo’	*Vigna unguiculata* (L.) Walp.	Leaves
Guava	‘Mapera’	*Psidium guajavum* L.	Fruit with seed
Sweet potato	‘Rabuon nyaluo’	*Ipomoea batatas* (L.) Lam.	Leaves
Wandering jew	‘Odielo’	*Commelina sinensis L.*	Whole plant
Simsim	‘Nyim’	*Sesamum indicum* L.	Leaves
Wild lettuce	‘Achak’	*Launaea cornuta* (Hochst) C. Jeffrey	Leaves

- Common names lacking; n =50

Sorghum *(Sorghum bicolor),* finger millet *(Eleusine coracana),* maize *(Zea mays)* and amaranth *(Amaranthus cruentus)* were mentioned as the cereal grains traditionally utilized in the area (Table 3.2).

Table 3.2: Cereal grains, pulses, seeds, tuber/roots and their edible parts according to the interviewees

Common name	***Dholuo* name**	**Scientific name**	**Part consumed**
Sorghum	'Bel'	*Sorghum bicolor* (L.) Moench	Grains
Cassava	'Omwogo'	*Manihot esculentum* Crantz.	Tubers
Finger millet	'Kal'	*Eleusine coracana* Gaertn.	Grains
Sweet potato	'Rabuon'	*Ipomoea batatas* (L.) Lam.	Tubers
Yam	'Rabuon nduma'	*Dioscorea alata* L.	Tubers
White maize	'Oduma'	*Zea mays* L.	Grains
Simsim	'Nyim'	*Sesamum indicum* L.	Seeds
Cow peas	'Ng'or'	*Vigna unguiculata*	Pulses
Amaranthus	'Ododo'	*Amaranthus cruentus* L.	Grains

n =50

None of the cereal grains reported is harvested from the wild. They are all cultivated. Cowpea (Vigna unguiculata) was mentioned as pulse while sweet potato (*Ipomea batatas)* and cassava (*Manihot esculentum)* were mentioned as tubers with special attachment to the community. Cassava was mentioned *as* an important food especially during dry spells due to its ability to withstand harsh environmental conditions. Simsim (*Sesamum indicum*) is consumed as a seed and it is considered to be very nutritious.

Among the animal source foods, 6 edible insects and 1 fish (Table 3.3) were identified. Of the edible insects, four (4) species were edible termites; one (1) was the long-horned grasshopper (*Ruspolia differens*) and the winged black ants (*Calebara vidua*). The edible insects are collected from the wild. The termites were reported to be harvested during the rainy seasons. Some of the species were reported to make termite moulds and thus harvesting is done near the moulds while others simply emerge from the ground during the rains.

Table 3.3: Traditional animal source foodstuffs and their edible parts according to the interviewees

Common name	***Dholuo* name**	**Scientific name**	**Edible parts**
Winged termite	*Sisi*	*Pseudacanthotermes militaris* (Hagen)	Whole;de-winged
Winged termite	*Riwo*	*Macrotermes bellicosus* (Smeathman)	Whole; de-winged
Winged termite	*Agoro*	*Macrotermes subhylanus* (Rambur)	Whole; de-winged
Winged termite	*Oyala*	*Pseudacanthotermes spiniger (*Sjostedt*)*	Whole; de-winged
Black ant	*Onyoso*	*Carebara vidua* (Smith)	Abdomen; de-winged; whole
Long-horned grasshopper	*Senesene*	*Ruspolia differens* (Serville)	Whole; de-winged
Dagaa fish	*Omena*	*Rastrineobola argentea* (Pellegrin)	Whole

n =50

Some interviewees could not differentiate among termite species with some referring to them as *ng'wen,* a general Luo name for winged termites. However, the common termite species were *agoro* (*Macrotermes subhylanus)* and *riwo* (*Macrotermes bellicosus)* while *sisi* (*Pseudocanthotermes militaris)* and *oyala* (*Pseudocanthotermes spiniger)* were considered scarce. The locals considered *M. subhyalinus* as the most delicious followed by *P. militaris*. Although the *M. bellicosus* are generally larger than those of *P. militaris,* the former was reported to cause running stomach. All the insects are consumed whole with some people de-winging them except *C. vidua* whose fatty abdomen is the preferred delicacy. However, some respondents mentioned that it is also consumed whole or de-winged. It was revealed that *C. vidua* is currently facing extinction with only a few interviewees confirming to have consumed the insect.

Dagaa fish (*Rastrineobola argentea*) commonly known as *Omena*, is harvested from Lake Victoria in large numbers. *R. argentea* was reported to be a very popular food around the study area since it borders the lake. Harvesting of *dagaa* employs a lot of people along the lake involved in fishing, preservation and selling. The fish is consumed whole with no gutting reported by the interviewees.

In general, members of the Luo community are well versed in the native names of traditional plants used for both food and medicine (Johns and Kokwaro, 1991; Orech et al., 2007b). Traditional food plants are particularly important in the driest regions, which are most vulnerable to drought (Orech et al., 2007a). According to Johns and

Kokwaro (1991), the vegetable species namely *amaranth*, *Basella alba, Cleome gynandra, Cucurbita maxima, Solanum scabrum,* and *Vigna unguiculata* are of critical importance both in the days of food shortage and days of abundance. Unfortunately, the vegetable species have continued to become rare. For example, spider plant is presently rare in the region, but if found only grows fertile loamy and clay soil around abandoned homes, home gardens, farmlands or cattle pastures.

Currently, there are efforts to domesticate some of the plants to avoid extinction and at the same time help fight food insecurity and malnutrition. This has been boosted by numerous studies conducted on them. Traditional crops in this area have received a considerable evaluation related to their nutritional, pharmacological, and toxicological properties (Abukutsa-Onyango, 2003; Ogoye-Ndegwa and Aagaard-Hansen, 2003; Orech et al., 2007b). Most of them are a major source of micro-nutrients and are, at the same time, toxicologically safe.

Similarly, traditional animal foods have received their fair share of interest in the scientific world. Edible insects have been evaluated for their distribution and abundance in Lake Victoria region of Western Kenya (Nyeko and Olubayo, 2005; Ayieko et al., 2010b) in addition to their consumption and associated cultural values (Ayieko and Oriaro, 2008). A modern trap to harvest winged termites has been developed and tested (Ayieko et al., 2011).

3.3.2 Perceptions of nutritional, health benefits and availability of selected traditional foods

Perceptions on health and nutritional benefits of the traditional foods are summarised in Table 3.4. The most common benefits included provision of energy as well as ‘increasing’ blood in the human body. Other health benefits included prevention of stomach pains, bloating and constipation. Ododo (*Amaranth sp.*) was said to increase breast milk in lactating women. The winged termite (*Macrotermes subhylanus*) and *dagaa* fish (*Rastrineobola argentea*) were said to have a body building function, enhance good health and ‘increase’ blood. ‘Increasing’ blood may be associated with high iron content.

Availability of most of the foods mentioned ranged from moderate to high. However, availability for jute mallow and oxalis were rated to be low and therefore amassing enough amounts for product development would be a challenge. The winged termites were reported to be widely available during different seasons of the year with *dagaa* fish being available in abundance. A key informant lamented the decline in availability of the termites and associated decline of this food resource with increased farming, especially accompanied by use of insecticides.

Table 3.4: Perceived nutritional and health benefits, availability of selected foodstuffs according to key informants and their iron and zinc contents

Common name	Scientific name	Perceived nutritional & health benefits	Availability of the food	Iron[1] (mg/100g)	Zinc[2] (mg/100g)
Finger millet	*Eleusine coracana* Gaertn.	Source of energy	High	20.0	1.2
Sorghum	*Sorghum bicolor* L.	Source of energy	High	13.0	0.8
Amaranth grain	*Amaranthus cruentus* L.	Increases breast milk in mothers; Increases energy & blood; Reduces constipation	High	21.0	-
Simsim	*Sesamum indicum* L.	Body building & source of energy	High	9.5	7.8
White maize	*Zea mays* L.	Source of energy	High	4.5	1.8
Jute mallow	*Corchurus olitorius* L.	Increases blood and energy	Low	25.0	-
Oxalis	*Oxalis latifolia* Kunth	Enhances appetite; Source of energy	Low	28.0	-
Cassava leaves	*Manihot esculenta* Crantz	Source of energy	Moderate	7.7	0.4
Spider plant	*Cleome gynandra* (L.) Briq.	Prevents stomach pains and bloating; Prevents difficult deliveries in pregnant women; Increases blood and energy	High	30.0	-
Wandering jew	*Commelina sinensis* L.	Source of energy & increases blood; Reduces constipation	Moderate	28.0	-
-	*Asystasia mysorensis* T. Anderson	Prevents stomach pains and bloating; Reduces joints pains & increases blood	Moderate	6.0	-
Winged termite	*Macrotermes subhylanus* (Rambur)	Body building; Increases blood	Moderate	21.0	2.5
Dagaa fish	*Rastrineobola argentea* (Pellegrin)	Body building ; Promotes good health	High	7.0	5.2

[1]Source: National food composition tables and the planning of satisfactory diets in Kenya (Sehmi 1993); [2]Source: Tanzania food composition tables (Lukmwanji *et al*., 2008); - Common names or values missing

Review of secondary data (Table 3.4) indicated that among the grains, amaranth had the highest iron content (21.0 mg/100g) while among the leafy vegetables, spider plant had the highest (30.0 mg/100g). Winged termite had the highest iron content (21.0 mg/100g) among the animal source foods. White maize had significant iron (4.7 mg/100g) and high energy content (Sehmi, 1993). Termites have high iron (Sehmi, 1993) and zinc (Lukmanji et al., 2008) content. Termites emerged to be widely consumed within the study area especially during the rainy season. *Dagaa* fish had iron content of 7.0 mg/100g (Sehmi, 1993).

Recently, a lot of interest has also been directed at evaluating the nutritional potential of some insects consumed among the Luo of Western Kenya. Some insects have been found to be a rich source of minerals, fat soluble vitamins and even highly digestible proteins (Christensen et al., 2006; Ayieko, 2007; Kinyuru et al., 2010a; Kinyuru *et al.*, 2010b). Research has shown that consumption of edible larva of Westwood (*Cirina forda*) in Nigeria does not pre-dispose neurotoxicity or hepatotoxicity to study animals (Akinnawo et al., 2002). This can be further complemented by the fact that no toxicity disaster associated with consumption of insects has been reported by the consuming communities in Kenya.

3.3.3 Food preparation methods

Different food preparation methods were recorded (Table 3.5). Traditionally, food was prepared by the mother alone who is acquainted with good cooking skills and maintains high hygienic standards. The interviewees argued that the father has no skills and knowledge on good preparation of food.

Table 3.5: Traditional foodstuffs preparation methods according to the interviewees

Food group	Preparation method
Vegetables	Boiling
Cereals	Milling, boiling, fermentation, germination
Roots/tubers	Pounding, fermenting
Legumes	Pounding; boiling
Seeds	Pounding; boiling
Insect	Sun-drying; frying
Fish	Sun drying; Boiling

n = 50

The study found that most vegetables were consumed after boiling. According to Orech et al., (2005), some traditional vegetables contain agents that can cause possible acute or chronic toxicities when consumed in large quantities or over a long period of time. However, Luo women know such species and prepare them using traditional cooking methods to make consumption of the vegetables safe.

Most cereals were traditionally prepared into porridges while seeds and legumes were pounded, then cooked by boiling. The selection of flour for porridge preparation depends on available food types, largely influenced by the agroecological zone. Roots such as cassava was pounded and the flour used for making gruels. Sometimes the flour was pounded and further fermented.

Traditionally, preparation of termites for consumption involves mostly frying in their own oil or sun-drying fresh harvests. They are dried for consumption as snacks or sauces of various types. Consumption of live termites with or without salt, and preparing a sauce of fresh (not dried) alates were also reported to be common. The *dagaa* (*R. argentea*) were processed and preserved by the traditional sun drying method and boiled for consumption. Consumption of *dagaa* could be greatly enhanced if they are processed using modern and improved preservation and processing methods (Bille & Shemkai, 2006; Dampha et al., 1995).

3.4 CONCLUSION

Finger millet, sorghum, amaranth grain and maize were perceived to be a source of energy. Amaranth grain was perceived to increase breast milk in lactating mothers in addition to being a source of iron. Termites and *dagaa* fish were perceived to be important in body building and a source of iron and zinc. Availability of the animal foodstuffs within the study area suggests that they maybe be incorporated in cereal based foods to develop high energy, iron and zinc content complementary foods. Fermentation and germination were mentioned as techniques for processing cereals and may therefore be exploited in development of complementary foods.

CHAPTER FOUR

NUTRIENT AND ANTI-NUTRIENT CONTENT OF SELECTED TRADITIONAL FOODS CONSUMED IN WESTERN KENYA

4.1 INTRODUCTION

Amaranth grain, maize, finger millet, winged termites, ant and *dagaa* fish were selected for nutrient and anti-nutrient analysis. These were potential candidates in developing a cereal based complementary food incorporating animal source foods widely available and affordable within the region. The foods selected are habitually eaten by the family as signified by their wide availability within the study area.

Most of the complementary foods in Western Kenya are basically made of a mixture of flour from cereals mostly rich in carbohydrates. Finger millet and maize have traditionally been utilized in complementary feeding. Amaranth grain is also becoming an important cereal grain in the region (Muyonga et al., 2008). Nutrient analysis of amaranth grain will therefore give a clearer picture on its nutritional significance. Insects and d*agaa* fish are not commonly processed into complementary foods and utilizing them for such will bring in the much needed and under utilized nutrient dense animal foodstuffs.

Proximate composition; iron, zinc and calcium; fatty acid composition; phytic acid, tannins and total free polyphenols in plant foods were analysed while minerals to phytic acid molar ratios were calculated. The aim was to assess the nutrient and anti-nutrient content of the selected foods and hence inform appropriate processing techniques in the subsequent phase of complementary food development.

4.2 MATERIAL AND METHODS

4.2.1 Sampling design

Traditional foodstuffs were obtained from the local markets within Western Kenya following the field survey (Chapter 3). However, sample collection spread beyond Kisumu West District to the wider Western Kenya in order to get a more representative picture of the foods in Western Kenya. Six samples of foodstuff weighing 0.25kg – 2kg each were sampled randomly from the local markets. Sun-dried maize and finger millet were purchased in the local markets of Kombewa and Bondo areas while sun-dried amaranth grains were purchased from farmers in Teso District. Sun-dried d*agaa* fish samples were purchased from retailers in Kibuye market of Kisumu city. Sun-dried insects were purchased from local harvesters and distributors in Maseno Market in Kisumu West District, Luanda Market in Emuhaya District and Kakamega Market in Kakamega District. Amaranth, finger millet and maize samples were packed in kraft paper bags and sealed to avoid loss and transported to the laboratory. Insect and *dagaa* fish samples were packed in 300 gauge zip-lock polythene bags, packed in cool boxes lined with frozen ice packs and transported to the food analysis laboratory at Jomo Kenyatta University of Agriculture and Technology.

4.2.2 Sample analysis

A 200 g portion of each sample was milled to a fine powder within 24 hours of reception at the laboratory. They were then stored at -20^{o}C until analysis. Edible portions of each foodstuff were analyzed in duplicate. Proximate composition (moisture, protein, fat, ash, dietary fibre, available carbohydrates); iron, zinc and calcium content; fatty acid composition; phytic acid, tannins and total free polyphenols in plant foods were analysed. Whole grains were analysed while the insects were de-winged before analysis.

4.2.3 Determination of proximate composition

Moisture content was assayed by the drying method, crude fat by Soxhlet extraction method and crude protein by semi-micro-Kjeldhal method (AOAC, 1996). Nitrogen values obtained were converted to crude protein using a factor of 6.25. Crude ash was determined by incinerating in a muffle furnace at 550 ^{o}C. Dietary fibre was determined by enzymatic gravimetric method - Prosky (AOAC 1995). Available carbohydrate value was calculated as the difference between 100 and the sum of the percentages of water, protein, lipids, ash and dietary fibre.

4.2.4 Determination of iron, zinc and calcium content

The quantification of iron, zinc and calcium was done by atomic absorption spectrometry (AAS) (Shimadzu AA-6200, Tokyo, Japan) according to AOAC methods (AOAC, 1996). Quantification was done using external standards (Sigma-Aldrich Chemie, Steinheim, Germany).

4.2.5 Determination of phytic acid

Determination of phytic acid was done by HPLC analysis method of phytic acid according to Camire and Clydesdale, (1982) with modifications as follows, 50 mg of sample was weighed into a 125 ml Erlenmeyer flask and 10 ml of 3% H_2SO_4 added. The flasks were placed on a shaker at a moderate speed for 30 minutes at room temperature and filtered using a fast filter paper (Shaker Model KS 250 basic, Germany). The filtrate was transferred to a boiling water bath (BWB) for 5 minutes and 3 ml of $FeCl_3$ solution (6mg ferric iron per ml in 3% H_2SO_4) added. A second BWB heating was done for 45 minutes to complete precipitation of the ferric phytate complex. Centrifugation followed at 2500 rpm for 10 min and the supernatant discarded. The precipitate was washed with 30ml distilled water, centrifuged and the supernatant discarded. Three (3) ml of 1.5 N NaOH was added to the residues and the volume brought to 30ml with distilled water. Heating was done for 30 minutes in a BWB to precipitate the ferric hydroxide. Cooled samples were centrifuged and the supernatant transferred into a 50 ml volumetric flask. The precipitate was rinsed with 10 ml distilled water, centrifuged and the supernatant added to the contents of the volumetric flask. A sample of 20 µl of the supernatant was injected into a HPLC fitted with a 50377 RP-18 (5 µm) column at an oven temperature of 30 ºC and a refractive index detector. The mobile phase was 0.005 N sodium acetate in distilled water and flowing at a flow rate of 0.5 µl/ minute. Quantification was done using external standards (Merk IV, multi element standard solutions).

4.2.6 Determination of phytate/mineral molar ratio

The mole of phytate and minerals was obtained and molar ratio was calculated by dividing the mole of phytate with the mole of minerals (Norhaizan and Nor, 2009).

4.2.7 Determination of tannin

Tannin content was determined colorimetrically (Singleton and Rossi, 1965) with modifications as follows, 20g of samples were homogenized with 40 ml water and filtered. The filtrate was centrifuged at 4 ^{o}C, 12000 rpm for 2 minutes. Then 0.1ml aliquot of the solution was mixed with 5.0ml of 0.2 N Foli-Ciocalteau reagent and 4.0ml off saturated sodium carbonate. After 2 hours, the absorbance was read at 765 nm in a spectrophotometer (UV mini 1240, Kyoto, Shimadzu, Japan). The concentration was quantified from a standard curve prepared using gallic acid.

4.2.8 Determination of total free polyphenols

Total free polyphenol content was determined colorimetrically (Singleton and Rossi, 1965) with modifications as follows, 20 g sample was homogenized with 40 ml of water and filtered. The filtrate was centrifuged at 4 ^{o}C, 12000 rpm for 2 minutes. A 0.1 ml aliquot of the solution was mixed with 5.0ml of 0.2 N Foli-Ciocalteau reagent and 4.0ml off saturated sodium carbonate. After 2 hours, the absorbance was read at 765 nm in a spectrophotometer (UV mini 1240, Kyoto, Shimadzu, Japan). The concentration was quantified from a standard curve prepared using tannic acid.

4.2.9 Determination of fatty acid composition

The fatty acid profile was determined by gas chromatography. The extraction of the lipids was done by Folch extraction method (Folch et al., 1957). Prior to methylation, the extracted lipid was redissolved to a concentration of 10 mg/mL in chloroform: methanol (2:1, v/v) solution. The samples were methylated according to Bligh and Dyer, (1959). Then 0.2 µl was injected into the Gas Chromatography (GC) capillary column (Supelcowax, internal diameter 30m x 0.53mm) maintained at an injection/detection temperature of 220 ^{o}C under a flame ionization detector. Identification of the fatty acid methyl esters was by comparison of retention times with standards and was expressed as percentages of total methyl esters. Fatty acid standards were obtained from Sigma Chemical Co. The polyunsaturated fatty acids/saturated fatty acids ratio (PU/SA) and n-6: n-3 fatty acids ratios were calculated according to Koletzko et al., (2005).

4.2.10 Data analysis

All results are given as mean ± SD; n refers to the number of observations.

4.3 RESULTS AND DISCUSSION

4.3.1 Proximate composition

Table 4.1 presents the proximate composition of the selected traditional foods. Amaranth grain had the highest protein (18.5 g/100g) and fat content (14.4 g/100g) among the analysed cereal grains in this study. This was higher than amaranth varieties grown in Uganda, 12.0 - 13.0 g/100g protein content (Muyonga et al., 2008). Consumption of 100g of amaranth grain would provide 200% of RDA protein for 6 month old infants (WHO, 1998). The carbohydrates in amaranth grain consist primarily of starch made up of both glutinous and non-glutinous fractions (Muyonga et al. 2008; Teutonico and Knorr, 1985). Due to the unique size and composition of amaranth starch, the starch may exhibit distinctive characteristics which could be of benefit to the development of complementary foods (Singhal and Kulkarni, 1990a,b). Finger millet and maize were found to compare with samples grown elsewhere within the country (Sehmi, 1993; Muyonga et al., 2008). Of interest however, was the high carbohydrate level in both amaranth and finger millet meaning they can be exploited as energy source foods for infant.

The fat content of the termites (44.8 – 47.3 g/100g) was lower than the values reported by Sehmi, (1993) for an unspecified dried termite (53.4 g/100g). The values were also slightly higher than those of *Nausitermes spp*. reported by Oyarzun et al. (1996) at 40.23%. Table 4.1 also shows a fairly high level of fat content in black ant (*Calebara vidua*), probably one of the reasons why the insect is a favourite of many in the community. The abdomen, which the villagers prefer is composed of approximately 50% fat (Ayieko et al. 2012).

Table 4.1: Proximate composition of selected traditional foodstuffs (g/100g)

Foodstuff	Protein	Fat	Total ash	Dietary fibre	Available Carbohydrate
Amaranth grain	18.5 ± 0.4	14.4 ± 0.6	2.5 ± 0.0	7.1 ± 0.0	63.8 ± 1.5
Finger millet	8.4 ± 0.8	1.4 ± 0.0	7.2 ± 0.1	8.0 ± 1.1	74.8 ± 1.2
White maize	8.2 ± 0.2	4.8 ± 0.8	1.1 ± 0.0	5.9 ± 1.1	79.8 ± 1.0
Dagaa fish	58.1 ± 0.6	26.6 ± 0.9	11.1 ± 0.0	3.0 ± 1.0	11.0 ± 1.3
Agoro termite	39.3 ± 0.1	44.8 ± 2.8	7.5 ± 0.0	6.3 ± 1.1	1.8 ± 0.7
Sisi termite	33.5 ± 0.8	46.5 ± 2.1	4.5 ± 0.0	6.5 ± 0.0	8.7 ± 1.8
Oriwo termite	39.7 ± 0.6	47.0 ± 1.0	4.6 ± 0.0	6.2 ± 2.0	2.3 ± 0.9
Oyala termite	37.5 ± 0.1	47.3 ± 0.1	7.2 ± 0.3	7.2 ± 0.4	0.7 ± 0.0
Onyoso ant	40.8 ± 1.1	47.5 ± 2.7	1.5 ± 0.1	5.91 ± 1.9	0.2 ± 0.0

Values are mean ± SD on dry weight basis; n= 6

The protein content of the studied termites (33.5 – 39.7 g/100g) was within the range reported for an unspecified dried termite (35.7 g/100g) in NFCT (Sehmi, 1993). Consumption of 100g of termites would provide 3 - 4 times more protein than RDA for 6 month old infants (WHO, 1998). The protein content exhibited by the insects analysed in this study was significantly higher than that of red meats reported by Williams (2007) and therefore insects may offer an alternative source of protein to counteract the protein malnutrition in Kenya. Protein profiles of insects indicate high protein quality, beneficial for human nutrition (Ramos-Elorduy et al. 1997; Verkerk et al. 2007, Kinyuru et al. 2010b), especially in an otherwise plant-dominated diet, typical in Kenya.

High fibre content was also found in the plant foods (5.9 – 8.0 g/100g) and this may pose a challenge in utilizing the grains in complementary foods. The dietary fibre in insects (5.9 – 7.2 g/100g) is largely composed of chitin whose effect on the nutrients in a complementary food is not known.

4.3.2 Calcium, iron and zinc

Calcium, iron and zinc content were the minerals of interest in the selected traditional foods as shown in Table 4.2.

Table 4.2: Mineral composition of selected traditional foodstuffs (mg/100g)

Foodstuff	Calcium	Iron	Zinc
Amaranth grain	12.2 ± 0.1	15.0 ± 0.0	3.4 ± 0.0
Finger millet	86.2 ± 3.2	102.9 ± 0.6	1.7 ± 0.6
White maize	43.6 ± 3.0	9.4 ± 0.0	0.9 ± 0.1
Dagaa fish	3505.4 ± 6.2	25.7 ± 0.2	12.0 ± 0.9
Agoro termite	58.7 ± 1.2	53.3 ± 1.4	8.1 ± 2.8
Sisi termite	48.3 ± 7.0	60.2 ± 1.1	12.8 ± 0.9
Riwo termite	63.6 ± 6.5	115.9 ± 3.4	10.7 ± 1.9
Oyala termite	42.8 ± 1.7	64.7 ± 2.6	7.1 ± 1.8
Black ant	22.3 ± 1.6	10.6 ± 1.0	5.6 ± 0.7

Values are mean ± SD on dry weight basis, n= 6

Amaranth had the highest zinc content while finger millet had the highest iron and calcium content among the cereal grains. Muyonga et al., (2008) reported 17.0 mg/100g iron and 3.7 mg/100 g zinc content in amaranth harvested in Eastern Uganda. Amaranth grains contain twice the level of calcium found in milk, five times the level of iron in wheat (Becker et al., 1981). The content of iron in finger millet was higher than the values reported by Sehmi (1993). This could however be explained by regional variations. Black ant had the least mineral content among the insects and fish analysed, however, others had substantially high calcium, iron and zinc. Consumption of 100 g of amaranth grain would provide 1.6 times more iron than the RDA for 6 month old infants (WHO, 1998) consuming 100 g of the termites will provide 5 – 7 times more iron than RDA.

The most notable finding was the high calcium content in *dagaa* fish, as well as zinc and iron in the different species of termites. Consumption of 100 g of *dagaa* fish would provide 8 times more calcium than the RDA for 6 month old infants (WHO, 1998). Among the termites, *Riwo* termite had the highest calcium and iron content even though *sisi* termite had the highest zinc content. The high levels of calcium, iron and zinc of the insects and *dagaa* fish collected for this study are in line with previous studies in Africa (Christensen et al., 2006; Onigbinde and Adamolekun, 1998; Kinyuru et al. 2010a; Kabahenda et al. 2011). The bioavailability of the minerals from the fish, black ant and the termites is likely to also be higher than from the plant foods (Christensen et al., 2006) since animals contain heme iron which is more bioavailable.

4.3.3 Anti-nutrient content

Phytic acid measured as myoinositol hexa-inositol phosphate (IP6), tannins and total free polyphenols composition of the traditional foods are reported in Table 4.3.

Table 4.3: Anti-nutrient content of selected traditional plant foodstuffs (mg/100g)

Foodstuff	Phytic acid	Tannins	Total free polyphenols	Phytate /Iron ratio	Phytate /Zinc ratio	Phytate / Calcium ratio
Amaranth grain	1285.4 ± 206.3	7.1 ± 1.4	30.3 ± 1.4	7.2 ± 1.1	36.3 ± 5.4	6.3 ± 1.0
Finger millet	2287.0 ± 66.2	21.7 ± 0.3	143.0 ± 2.2	1.8 ± 0.0	77.6 ± 3.3	0.9 ± 0.1
White maize	318.5 ± 11.2	12.2 ± 0.2	69.0 ± 2.9	2.8 ± 1.0	2.8 ± 1.0	34.7 ± 1.2
Critical limits*				1	15	0.24

Values are mean ± SD on dry weight basis, n= 6

*Source: Norhaizan and Nor (2009)

Finger millet had the highest phytic acid (2287.0 mg/100g), tannin (21.7 mg/100g) and total free polyphenols (143.0 mg/100g). Maize had the lowest phytic acid content (318.5 mg/100g) indicating its appropriateness in complementary food development.

Amaranth grain had high phytic acid (1285.4 mg/100g) though lower than in finger millet. Other researchers have reported a wide variation of phytic acid content in amaranth grain. Becker et al., (1981) reported 0.2 - 0.2 g/100g in *Amaranthus caudatus*, while 1.3 g/100g was reported in unspecified amaranth grain specie (Egli et al., 2002) and 0.3 g/100g in *Amaranthus cruentus* (Colmenares and Bressani, 1990). Kanensi et al., (2011) reported 0.01 g/100g phytic acid in *Amaranthus cruentus* from Kenya. This means phytic acid content in amaranth should be ascertained for the specific grain species in focus before utilization.

High phytic acid in both cereals pose a major challenge in formulation of food rich in bioavailable calcium, iron and zinc (Garcia-Estepa et al. 1999; Norhaizan and Nor, 2009). Phytic acid binds the minerals and results to insoluble salts with poor bioavailability of minerals in plant foods (Rhou and Erdman, 1995).

Therefore, a process to reduce the phytic acid content is essential in lowering the anti-nutrients during the processing of a complementary food using either amaranth or finger millet grains.

Phytic acid/ mineral molar ratios were calculated and the findings presented as shown in Table 4.3. All the samples analysed were beyond the critical limits for phytate/ iron ratio and phytate/ calcium ratio while phytate/ zinc ratios in amaranth and finger millet were beyond the critical limits (Norhaizan and Nor, 2009). This means that bioavailability of the minerals is low in these foods if consumed in their current form. The ratio is an indicator of bioavailability of the minerals with respect to the set critical limits for each mineral.

The bioavailability of added and intrinsic non-heme iron is largely determined by the solubility of the iron in the upper gastrointestinal tract (Sorensen et al, 2007a). Dietary phytates inhibit non-heme iron absorption (Miller, 1996). Zinc and phytic acid form insoluble complexes and the negative effect of such complexes on zinc absorption can be predicted by phytate-to–zinc molar ratios when the dietary zinc intake is close to the requirement (Oberleas and Harland, 1981). High amounts of calcium exacerbate the inhibitory effect of phytate on zinc absorption by forming a calcium–zinc–phytate complex in the intestine that is even less soluble than phytate complexes formed by either ion alone (Egli et al., 2004).

4.3.5 Fatty acid composition

Fatty acid composition in the oil of the selected traditional foodstuffs is shown in Table 4.4. The oil obtained from amaranth grain was predominantly composed of unsaturated fatty acids (69.1 %), with 2.5 % being linolenic acid while finger millet had 3.8 % linolenic acid. The level of saturation in *dagaa* fish oil was higher than expected with palmitic acid (58.6 %) being the dominant fatty acid. This can be explained by the uncontrolled sun-drying process undertaken by the fishers after harvesting. The fish is dried on racks along the lake shores and drying may take a week (Owaga et al., 2010). Lipid oxidation will result to loss of the unsaturated fatty acids.

Arachidonic acid was only detected in *dagaa* fish (0.1 %) and black ant (1.3%). The fatty acid was not detected in the termites analysed. Studies in Nigeria have reported small amounts of arachidonic acid in insects (Oyarzun et al, 1996; Ekpo and Onigbinde, 2007).

The ratio of n-6: n-3 in foods is of interest to the fat quality following their reported significance in complementary foods is well recognised, especially for the healthy development of infants and young children (Lauritzen et al., 2001). The recommendation for n-6: n-3 is between 5 and 15:1 in infant foods (Koletzko et al., 2005). Sufficient intake of n-3 fatty acids is the most critical to meet in plant dominated diets (Michaelsen et al. 2011) common in developing countries due to low animal foodstuffs especially fish. The plant foods analysed had an n-6:n-3 ratio of 5.3 – 94.8:1 (Table 4.4).

Table 4.4: Fatty acid composition of oil from selected traditional foodstuffs

Fatty acid (%)	Foodstuff								
	Amaranth grain	Finger millet	White maize	*Dagaa* fish	*Agoro* termite	*Sisi* Termite	*Riwo* termite	*Oyala* termite	Black ant
Caprylic acid (C10:1)	nd	nd	1.3 ± 0.5	nd	nd	Nd	0.4 ± 0.2	0.3 ± 0.0	nd
Capric acid (C10:0)	nd	nd	1.2 ± 0.4	nd	nd	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	nd
Lauric acid (C12:0)	nd	nd	nd	nd	nd	nd	0.1 ± 0.1	0.2 ± 0.0	2.2 ± 1.0
Myristic acid (C14:0)	nd	18.7 ± 0.0	nd	8.6 ± 2.1	1.0 ± 0.0	nd	1.1 ± 0.2	0.7 ± 0.0	1.8 ± 0.0
Palmitic acid (C16:0)	30.0 ± 1.0	27.6 ± 1.8	19.3 ± 4.5	58.6 ± 0.2	26.1 ±3.5	26.0 ± 2.3	38.3 ± 3.3	28.0 ± 0.1	28.7 ± 3.2
Palmitoleic acid (C16:1)	nd	nd	nd	3.0 ± 1.1	3.9 ± 0.4	5.8 ± 0.3	0.6 ± 0.0	3.6 ± 0.0	2.9 ± 1.1
Stearic acid (C18:0)	0.8 ± 0.1	2.7 ± 0.3	2.0 ± 0.4	8.4 ± 2.1	6.0 ± 0.0	5.9 ± 0.4	9.5 ± 0.5	6.1 ± 0.1	5.9 ± 0.0
Oleic acid (C18:1)	27.8 ± 2.0	26.4 ± 1.3	33.9 ± 2.3	15.4 ± 3.3	46.0 ±3.5	50.2 ± 1.6	41.7 ± 2.6	49.2 ± 0.0	46.7 ± 1.3
Linoleic acid (C18:2)	38.8 ± 1.4	20.6 ± 0.7	41.6± 2.1	2.6 ± 0.6	10.1 ±0.6	11.5 ± 0.4	5.0 ± 0.1	10.4 ± 0.1	10.2 ± 1.7
Linolenic acid (C18:3)	2.5 ± 0.5	3.8 ± 0.0	0.4 ± 0.0	2.9 ± 0.6	1.3 ± 0.1	0.2 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	nd
Arachidonic (C20:2)	nd	nd	nd	0.1 ± 0.0	nd	nd	nd	nd	1.3 ± 0.0
Total saturated [b]	30.8 ± 2.3	49.0 ± 0.4	22.6 ± 1.1	75.7 ± 4.1	32.9 ± 0.8	32.1 ± 0.5	49.4 ± 0.2	35.8 ± 0.0	38.7 ± 2.1
Total unsaturated [c]	69.1 ± 3.7	50.9 ± 0.4	77.3 ± 1.1	24.2 ± 1.1	67.0 ± 0.9	67.8 ± 0.5	50.5 ± 0.2	64.5 ± 0.0	61.2 ± 3.5
Monounsaturated [d]	27.8 ± 0.6	26.4 ± 0.4	35.2 ± 0.9	18.5 ± 2.2	9.9 ± 0.2	56.1 ± 0.6	44.6 ± 0.2	53.2 ± 0.0	49.7 ± 1.2
Polyunsaturated [e]	41.3 ± 8.3	24.5 ± 0.4	42.1 ± 1.3	5.7 ± 0.2	56.2 ± 2.1	11.7 ± 0.3	5.9 ± 0.1	11.2 ± 0.1	11.5 ± 0.0
P/S	1.3 ± 0.3	0.5 ± 0.0	1.8 ± 0.1	0.0 ± 0.1	0.4 ± 0.2	0.3 ± 0.0	0.1 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
n6:n3	15.4:1	5.3:1	94.8:1	0.9:1	7.5:1	57.7:1	5.8:1	13.4:1	-

[b]Sum total percentage of C10:0, 12:0, 14:0, 16:0, 18:0; [c]Sum total percentage of C16:1, 18:1, 18:2, 18:3; [d]Sum total percentage of 16:1, 18:1; [e]Sum total percentage of C18:2, 18:3, 20:2; nd- Not detected

Values are mean ± SD on dry weight basis, n= 6

Among the insects, n-6:n-3 ratio ranged between 5.8:1 and 57.7:1 in the termite oil analysed. Termite *P. militaris* had a marginally high n-6:n-3 ratio (57.7:1) while *sisi* (*M. bellicosus)* had the lowest (5.8:1). In addition to the amaranth and maize foods, termites and *dagaa* can offer high quality fat for the human diet among the communities practicing entomophagy. The recommendation for a n-6:n-3 ratio is between 5 and 15:1 in infant formula (Koletzko et al., 2005) and therefore the anlaysed foodstuffs maybe an important component of complementary feeding as well as being a moderate source of n-3 PUFA. The fatty acids of the termites are similar to those of poultry and fish in their degree of unsaturation, but higher in the polyunsaturates (DeFoliart, 1991). DeFoliart, (1991) and Kinyuru et al. (2010a), among other authors have reported that insect oils have lower degree of saturation than that of beef and pork (Williams, 2007).

The presence of essential fatty acids such as linoleic and linolenic acid points to the nutritional value of the traditional foods' oil analyzed in this study. Nutritionally, a high level of saturated fatty acids in foods might be undesirable because of the linkage between saturated fatty acids and atherosclerotic disorders (Reiser, 1973). P/S ratio of below 0.2 has been associated with high cholesterol level with high risk of coronary heart disorders (Mannan, 2002). All the foods analysed had P/S ratios above 0.2 except *riwo* (*M. belicosus*) termite and *dagaa* fish. The acceptable P/S ratios of amaranth as well as some of the termites suggest that the insects can be associated with health benefits to the human diet. Similar P/S ratios have been reported for other edible insects (Kinyuru et al., 2010b).

4.4 CONCLUSION

Amaranth grain had the highest protein content (18.55 g/100 g) among plant foodstuffs while *dagaa* fish had the highest protein content (58.1 g/100 g) among animal foodstuffs. Iron content ranged between 9.4 – 102.9 g/100 g among the cereals and 10.6 – 64.7 g/100 g in among insects and fish. The animal foodstuffs had higher level of zinc (5.6 -12.8 g/100 g) compared to the cereal grains (0.93 – 3.48 g/100 g). High anti-nutrient content in amaranth and finger millet were found. Finger millet had the highest phytic acid content (2287.0 g/100 g) compared to amaranth grain and maize. The foods analysed met the RDA for protein, fat, iron, zinc, and calcium and may be used in development of nutrient dense complementary foods.

CHAPTER FIVE

PROCESS DEVELOPMENT AND IRON BIOAVAILABILITY IN COMPLEMENTARY FOODS PROCESSED WITH AMARANTH GRAIN, TERMITE AND *DAGAA* FISH

5.1 INTRODUCTION

Traditional foods are rich in nutrients and maybe utilized in development of nutrient-dense complementary foods. Of interest in this study is the high mineral content in the foods. Some of the plant foods, such as amaranth grain, have lower phytic acid levels than other foods evaluated. This means that amaranth may be processed into complementary foods with high iron bioavailability compared to phytates rich finger millet.

Termite and *dagaa* fish, being an animal food is a source of more bioavailable heme iron. Inclusion of edible termites and *dagaa* fish during processing of complementary foods is therefore a strategy to improve non-heme iron bioavailability abundant in plant foodstuffs. These animal foodstuffs are also a major source of iron and zinc among other minerals. It is hoped that being animal foods, they may enhance non-heme iron bioavailability (Sorensen et al., 2007a). Therefore varying amounts of amaranth grain with termite and *dagaa* fish may achieve high iron content, low phytic acid content and high iron bioavailability.

Extrusion cooking has significant nutrient retention owing to high temperature and short time required in addition to retaining natural color, flavour and reducing microbial load of the food (Fellows, 2000; Bhandari et al., 2001). Singh et al., (2007) reported that extrusion cooking has marginal effect on phytic acid and therefore its effect on phytic reduction was not expressly evaluated in this study.

Pathogenic bacteria are a major health hazards in animal source foods such as fish (Owaga et al., 2009). The challenge is complicated by the increased manual handling during harvesting and drying of the *dagaa* fish and termite. Good harvesting practices and heat pre-treatment of the foodstuffs is necessary to reduce the contamination. Blanching is utilized in order to reduce microbial contamination. The study was therefore aimed at optimizing a process to develop a safe, nutrient dense complementary food with high *in-vitro* iron bioavailability.

5.2 MATERIAL AND METHODS

5.2.1 Description of food optimization methodology

Amaranth grain (*Amaranthus cruentus*), white maize (*Zea mays*), *dagaa* fish (*Rastreneobola argentea*) and winged termite (*Macrotermes subhylanus*) formed the basis of the formulations of complementary foods (Appendix 3). The content of each ingredient was determined by manual iteration to optimise iron and zinc contents balanced with a moderate energy and protein content using MS Excel (2007 version). Combinations of these ingredients were incorporated into the original draft set of complementary foods to produce alternative sets of complementary foods by varying food amounts. Three foods, CF1, CF2 and CF3 were formulated (Table 5.1).

Table 5.1: Formulations for CF1, CF2, and CF3 complementary foods (%)

Product	Amaranth grain	Maize	*Dagaa* fish	Termite	Soy bean oil	Sugar
CF1	73.0	15.0	3.0	8.0	0.6	0.4
CF2	71.0	15.0	3.0	10.0	0.6	0.4
CF3	66.0	15.0	3.0	15.0	0.6	0.4

Energy content of selected ingredients were obtained from Kenya and Tanzania food composition tables (Sehmi, 1993; Lukmanji et al., 2008) while other nutrients values were results of analysis in this research study (see chapter 3). The foods were formulated to meet the RDA for 6 month olds as follows; energy 400 Kcal, Protein 9.1 g, iron 9.3 g, zinc 4.6 g and calcium 400g (Codex 1991; WHO, 1998; FAO/WHO, 2002; FAO/WHO, 2004) [Appendix 4].

5.2.2 Preparation of ingredients and processing of complementary foods

Amaranth grains were sorted to remove extraneous materials; *dagaa* fish and termites were sorted to remove pebbles and other extraneous materials, washed in clean water and blanched for 1 minute. A single layer of the blanched fish and termite was then spread on aluminium trays and oven dried for 1 hour at 120^{o}C with continuous turning.

The mixture was processed by extrusion cooking and ground into flour that could be reconstituted into porridge for child feeding as recommended by Codex (1991). Extrusion was carried out at All-grain co. LTD, Kenya, with a barrel length/diameter ratio of 25 using a locally fabricated extruder. The extrusion conditions were as follows: Feed moisture content 12-14.4%, moisture injection 9%, feed rate 12.5 kg/h, screw-speed 300 rev/min, barrel temperatures 70^{o}C (zone 1), 100^{o}C (zone 2), 127^{o}C. The process flow for production of the complementary foods was as shown (Figures 5.1).

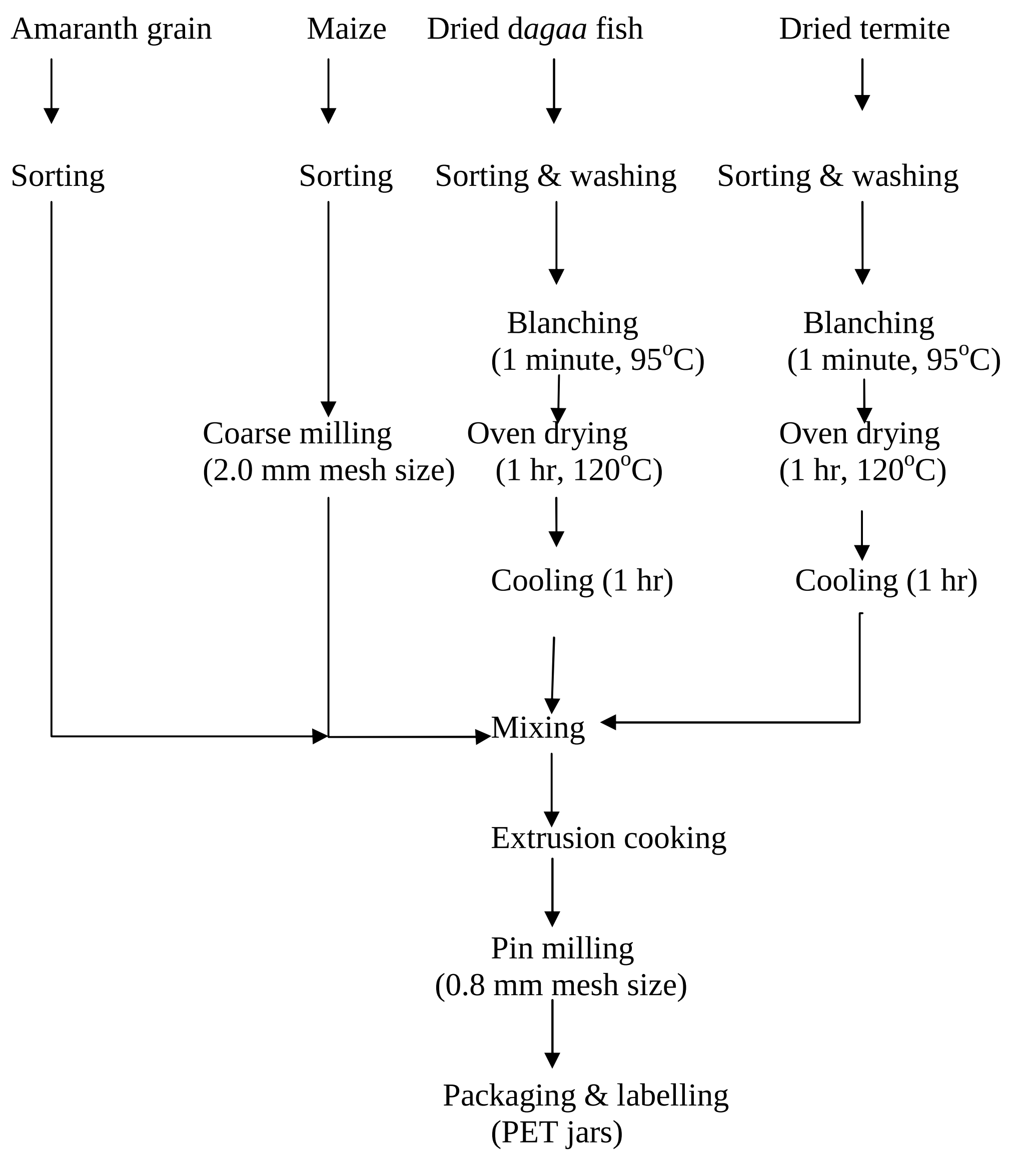

Figure 5.1: Flow diagram for processing of complementary foods

5.2.3 Moisture, protein and mineral content

Moisture, protein and mineral contents were analysed as outlined in Chapter 4 sections 4.2.3 and 4.2.4.

5.2.4 In-*vitro* non-heme iron bioavailability in complementary foods

This was determined according to a method described by Sorensen et al., (2007a) with slight modifications. *In-vitro* iron availability was measured as Fe (II) bioavailability obtained by a method combining *in-vitro* protein digestion and dialysis (IVPD-dialysis). IVPD imitates the conditions in the duodenum and the proximal jejunum of the human gastrointestinal tract.

The reagents used were: Sodium taurocholate (500 mg/mL), porcine pepsin (885 units/ mg protein; 5 mg/ mL in 50 mM acetate buffer pH 4.5), porcine pancreatin (at least 3xUSP specification; 7.5 mg/ mL in 1 mM HCl), trichloroacetic acid (TCA), PIPES buffer (piperazine - N, N'-bis [2-ethane-sulphonic acid] disodium salt; 75 mM, pH 6.1), HEPES buffer (N-2-hy droxylethylpiperazine- N'-2-ethanesulphonic acid, sodium salt; 0.3 M, pH 9.9), tris-glycine buffer (trishydroxymethylaminomethane (Trizma Base) (10.4 g/L), glycine (6.9 g/L), and EDTA (Tritriplex III) (1.2 g/ L) in water and adjusted to pH 8), SDS solution (7.25 g/ L), and Ellmans reagent (5,5' dithio-bis-(2-nitrobenzoic acid; 0.35 g/L) were diluted in the tris-glycine buffer . All solutions and ferrozine chromogen solution (3-(2-pyridyl)-5,6 bis(4-phenyl sulfonic acid)1,2,4-triazine, disodium salt; 5 mg/ mL in water) were prepared from reagents purchased from Sigma - Aldrich (St. Louis, MO). The used dialysis tubing with a molecular weight cut-off of 6000–8000 Da was from Spectrapore®, Millipore.

Slurry of the food was prepared by homogenizing with water. The homogenate was prepared to contain 50 mg protein/ mL of slurry. To 7.5 mL of the homogenate, 10 mL 0.1 M HCl was added resulting in pH 2.5. The remaining 13.5 mL of homogenate were incubated with pepsin (1.40 mL) for 1 h in a 37^{o}C water bath. Subsequently, aliquots (6.6 mL) were drawn and mixed with 3.4 mL water resulting in the pepsin digests. Finally, the residual assay mixtures were mixed with $NaHCO_3$ (2 mL, 600 mM), NaOH (100 mL, 1 M), pancreatin solution (2.15 mL), and sodium taurocholate (500 mL) resulting in pH 7, and further incubated for 1 hour at 37^{o}C, resulting the pepsin/pancreatin digests. Control and blank samples were without samples. Dialysis was performed by placing a dialysis bag containing 1 mL of PIPES buffer in 5 mL of the different digests and continuing dialysis until equilibrium was obtained for 4 hours at 37^{o}C with stirring.

Fe (II) bioavailability and iron-reducing capacity were measured immediately after dialysis by determining iron in the retentate and dialysate of pepsin-digested, and pepsin/pancreatin-digested samples from IVPD dialysis using a micro-scale method based on reaction with chromogen ferrozine. The assay was adapted to microtiter plates. Fe(II) content was analysed by adding 100 m L non-reducing protein precipitate (TCA, 1 g; HCl, 37%, 1 mL , adjusted to 10 mL with water) to aliquots of dialysate and retentate, respectively (200 m L). The detection range of the microassay was between 4 mM and 0.5 mM Fe. The aliquots to which were added either non- reducing or reducing protein precipitate were left over night at room temperature followed by centrifugation (2575 g; 1 0 min). The supernatants and $FeCl_3$ dilutions (100 mL) in double determinations were placed in microtiter wells (96-well ELISA plates), HEPES (0.3 M; 200 mL) and ferrozine solution (5 mg/mL; 25 mL) were added and the absorbance was measured at 570 nm (ELISA reader EL340 Microplate) immediately after ferrozine addition for the quantification of Fe (II) and after 1 h for determination of total (Fe (II) + Fe (III)) iron content. This colorimetric assay has been optimised for the application in microtiter plates with the measurement of absorbance at 570 nm for sensitive estimation of iron content and oxidative status (Sorensen et al, 2007b). Bioavailable Fe (II) and reducing capacity were calculated. Reducing capacity of Fe (II) is a measure of the soluble but not bioavailable iron.

% Bioavailability of Fe (II) = [(Fe(II) in dialysate (mM) x 1total volume (mL))/*total (Fe(II) + Fe (III)) (µmol) x 100.

% Reducing capacity of Fe (II) = (Fe (II) in dialysate (mM) x dialysate volume (mL)] + [Fe (II) in retentate (mM) x retentate volume (mL))/ *total (Fe (II) + Fe (III)) (µmol)] x 100

*Dialysate and retentate

5.2.5 Phytic acid content

This was done by HPLC analysis method of phytic acid according to Camire and Clydesdale, (1982) with modifications as outlined in Chapter 4 section 4.2.5.

5.2.6 Predicting mineral availability by phytate/mineral molar ratio

This was done was done by calculating the phytate/mineral molar ratio as outlined in Chapter 4.2.6.

5.2.7 Data analysis

All results are given as mean ± SD; n refers to the number of observations. The data was analysed by Student’s t-test for paired or unpaired variates and $p < 0.05$ was considered significant using SAS (2004) software.

5.3 RESULTS AND DISCUSSION

5.3.1 Moisture, protein, iron and phytic acid content

Moisture, protein, iron and phytic acid content of the developed foods is shown (Table 5.2). The moisture content was within safe moisture content of below 12% to ensure a stable product in pre-cooked complementary foods (WFP, 2010).

Table 5.2: Moisture, protein, iron, phytic acid and molar ratio in the developed foods

Food	Moisture (g/100g)	Protein (g/100g)	Iron (mg/100g)	Phytic acid (mg/100g)	PA/Fe* molar ratio
CF1	8.0 ± 0.3^b	17.8 ± 1.2^b	16.2 ± 0.0^b	1234.0 ± 22.4^a	3.6 ± 0.3^a
CF2	8.0 ± 0.1^b	17.5 ± 1.4^b	16.5 ± 0.0^b	1110.6 ± 31.6^b	3.2 ± 0.0^b
CF3	8.8 ± 0.6^b	18.9 ± 0.8^a	17.0 ± 1.0^a	914.5 ± 15.6^c	2.5 ± 0.0^c

*Critical limit = 1
Mean ± standard deviation; *n*=3
Values on the same column with different superscripts are significantly different ($p<0.05$)

5.3.2 *In-vitro* non-heme iron bioavailability and reducing capacity

Bioavailability of iron is shown in Figure 5.2. It was observed that amount of bioavailable iron after pepsin digestion was low in the foods (<5.0%) though it increased with increase in termite content i.e. CF1 (0.7%), CF2 (1.2%), CF3 (3.3%). There was a significant increase between CF2 and CF3 ($p<0.05$). Bioavailable iron was higher on pepsin+ pancreatin digestion than in pepsin digestion in CF1 and CF2. CF2 had significantly higher bioavailable iron ($p<0.05$) than CF1 upon pepsin+ pancreatin digestion.

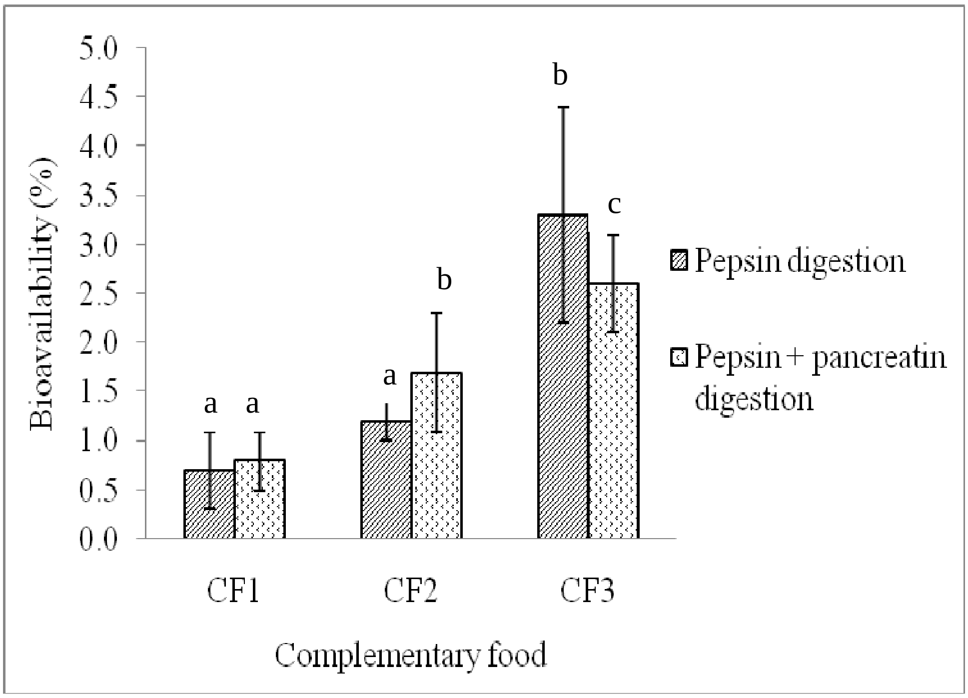

Figure 5.2: *In-vitro* non-heme iron bioavailability of the complementary foods

Values on the same variable with different letters are significantly different ($p<0.05$)

It's postulated that majority of iron absorption *in vivo* occurs in duodenum and the proximal part of jejunum. Gunshin et al. 2001 and Sorensen et al., 2007a found that iron bioavailability should be higher on pepsin digestion than on pepsin+ pancreatin digestion. However, in this study only CF3 showed such a tendency.

The findings of this study indicate that presence of termite in a food matrix increases Fe (II) bioavailability though the results are not significant at some of the levels of termite used in this study. This means that though the termite may enhance, there is possibility of an inhibitor in the food matrix that is prohibiting the gains. It is therefore be important to significantly reduce the phytic acid before addition of iron enhancers such as animal source foods. Increasing the termite content in the formulation may be a strategy to further improve bioavailability of the iron but that is likely to affect the processability, storage and consumer acceptability properties due to the high fat and protein content of termite. The animal source foods in the formulation not only enhanced bioavailability of non-heme iron, but also contributed heme iron resulting in high amount of readily bioavailable iron in the foods.

The capacity to reduce Fe (III) to Fe (II) in the samples was higher in the pepsin+ pancreatin digested than pepsin digested samples in all the foods (Figure 5.3). Reducing capacity was not significantly different between CF1 and CF3 upon pepsin. High reducing capacity shows that a significant amount of Fe (II) is present in the digested samples in a non-bioavailable but yet soluble form, which could be polymeric soluble aggregates or Fe (II) complexes with phytates. Means of making this iron bioavailable should therefore be explored during processing of a complementary food with these ingredients so as to raise the level of available iron. A process that breaks complexes formed between iron and inhibiting food components such as phytates may also be employed.

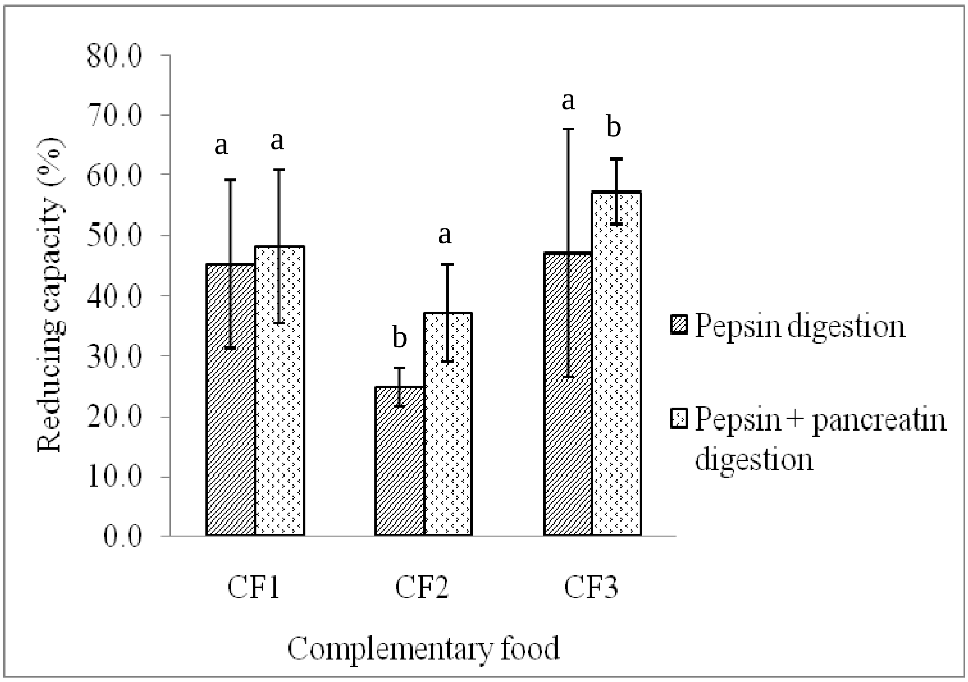

Figure 5.3: Reduction capacity of the complementary foods

Values on the same variable with different letters are significantly different ($p<0.05$)

4.4 CONCLUSION

A process to optimize inclusion of termites and *dagaa* fish was developed and pre-cooked products obtained. Phytate/iron molar ratio was beyond the critical limits for all the foods. The foods contained up to 3.3% bioavailable iron after pepsin digestion and up to 2.5% bioavailable iron after pepsin+ pancreatin digestion. Incorporation of termites and *dagga* fish in processed complementary foods containing of phytic acid does not translate into large mineral bioavailability and therefore techniques to further reduce the inhibitor are necessary. An indigenous process such as germination maybe evaluated in order to reduce phytic acid before processing the grains into complementary foods.

CHAPTER SIX

UTILIZATION OF GERMINATED AMARANTH GRAIN IN DEVELOPMENT OF NUTRIENT DENSE AND ACCEPTABLE COMPLEMENTARY FOODS

6.1 INTRODUCTION

Formulated complementary foods should have a high nutritional value to complement breastfeeding, be acceptable and affordable (WHO, 2003). Today, an international interest exists to promote affordable, nutritionally dense diets during complementary feeding prepared with traditional foods (Briend and Darmon, 2000; Owino et al., 2007).

Germination is an indigenous process that been found to reduce phytates in millet, sorghum and other cereal grains (Egli et al., 2002). It reduces the phytate/mineral molar ratio translating to high mineral bioavailability. This indigenous technique has been exploited in food processing to produce high value products with nutritional and functional benefits.

Complementary foods were developed with germinated amaranth grain, incorporating termite and *dagaa* fish. The objective of this study was therefore to determine the nutritional quality, storage stability and consumer acceptability of the complementary foods.

6. 2 MATERIAL AND METHODS

6.2.1 Germination of amaranth grain and food processing

Amaranth grains were sorted to remove extraneous materials and washed in clean water. They were covered with water and steeped for 5 hours at room temperature after which the water was drained. A 1 inch depth of grain was spread on perforated aluminium trays and germinated for 72 hours at room temperature (22 - 25^{o}C). The sprouts were sun dried for 24 hours with temperature range of 25^{o}C to 45^{o}C. To further achieve moisture content below 10%, circulating air oven drying was done at 55^{o}C. Preparation of d*agaa* fish and termite were prepared as previously outlined in Chapter 5 section 5.2.2. Four foods namely CF4, CF5, CF6 and CFC were formulated as shown in Table 6.1 using the methodology outlined in Chapter 5 Section 5.2.1. The foods were formulated to meet the RDA for 6 month olds as follows; energy 400 Kcal, Protein 9.1 g, iron 9.3 g, zinc 4.6 g and calcium 400g (Codex 1991; WHO, 1998; FAO/WHO, 2002; FAO/WHO, 2004) [Appendix 5]. Extrusion cooking properties are also outlined in Chapter 5 Section 5.2.2.

Table 6.1: Formulations for CF4, CF5,CF6 and CFC complementary foods (%)

Product	Germinated amaranth grain	Maize	*Dagaa* Fish	Edible termites	Soy bean oil	Sugar
CF4	73.0	15.0	3.0	8.0	0.6	0.4
CF5	71.0	15.0	3.0	10.0	0.6	0.4
CF6	66.0	15.0	3.0	15.0	0.6	0.4
CFC	84.0	15.0	0.0	0.0	0.0	0.0

6.2.2 Nutritional analysis of the products

Protein, fat, zinc, iron and calcium contents were analysed as outlined in Chapter 4 Sections 4.2.3 and 4.2.4. Energy content was measured by bomb calorimetry using Gallenkamp Ballistic Bomb calorimeter (AOAC, 1996).

6.2.3 *In-vitro* protein digestibility of the complementary foods

Digestibility of protein in the developed foods was determined by the method outlined by Mertz et al, (1984). Initial protein content of the samples was determined using semi-micro-kjeldahl nitrogen determination method. The second stage involved pepsin digestion, where 0.2g of the sample was weighed into centrifuge tubes. Then 20ml buffered pepsin was added and mixed. A blank was prepared in the same way but without a sample. The tubes were placed in a water bath at 37ºC for 2 hours with gentle shaking after every 20 minutes. The tubes were then placed in an ice bath for 30 minutes to attain a temperature of 4ºC followed by centrifugation at 6000 rpm for 15 minutes. The supernatant was discarded and 10ml of buffer solution added, then shaking and centrifugation was done again. The supernatant was discarded and the residue filtered using a filter paper. The filter paper was rolled and inserted into a Kjeldahl flask and dried for 15 minutes in the oven. Ten millilitres of concentrated sulphuric acid, 1g potassium sulphate and 1ml of 10% copper sulphate solution were added to the Kjeldahl flask containing the dried filter paper and sample. Then digestion, distillation and titration were done according to the micro-Kjeldahl nitrogen determination.

Protein digestibility (%) = (A - B)/A

Where A = % protein content in the sample before pepsin digestion

B = % protein in the sample after pepsin digestion

6.2.4 Particle size distribution analysis of the complementary foods

The particle size of flour samples were determined with modifications according to Oladunmoye et al., (2010) using a set of eight Endicott test sieves (Endicott Ltd., London, UK) ranging from 1000 microns to 250 microns sieve sizes arranged in decreasing order of pore size. About 100 g of each sample was sieved for 15 minutes on an Endecott's sieve shaker (Endicott Ltd.). The flour retained on each sieve and in the receiver pan was weighed and expressed as the percentage of total flour. Appropriate calculations were made; cumulative graphs and histograms were drawn to obtain the average particle size and the most common particle size of each flour sample.

6.2.5 Determination of water absorption capacity of complementary foods

The water absorption capacity was determined according to the method described by Akubor (2005) with modifications. One-gram sample was mixed with 10 ml distilled water (specific gravity, 0.904 kg/m^3) and allowed to stand at ambient temperature (25^oC) for 30 min, and then centrifuged at 3000 rpm for 30 min using centrifuge model 800D (Hettich, Universal 11, Herford, Germany). Water absorption capacity was expressed as percent water bound per 100g flour.

6.2.6 Determination of bulk density of the complementary foods

In determining the bulk density the method described by Oladunmoye et al., (2010) was used. A dish of known volume was washed, dried and weighed with its lid. Each flour sample was filled into the dish, tapped thrice and then weighed. Bulk density was calculated from the volume and weight of the flour sample.

6.2.7 Determination of dispersability of the complementary foods

Dispersability was measured using a method described by Asma et al., (2006). It was done by placing 10 g of the sample flour in a 100-mL stoppered measuring cylinder and then increasing the volume to 100 ml. The mixture was vigorously shaken and allowed to stand for 3 hours. The volume of the settled particles was subtracted from the total volume and the difference was expressed as percentage dispersability.

6.2.8 Determination of viscosity of the complementary foods

Viscosity was measured using a method described by Ikujenlola and Fashakin (2005) with modifications. Gruels of the formulated complementary foods were prepared by mixing 40 g of each food in 250 ml water and boiled for 10 minutes (Codex, 1991). The gruels were then held in a water bath at 40^0C for temperature standardization. The viscosity values in centipoises, (cP) was measured at 40^0C using a viscometer (Model: BL, Shimadzu, Japan) and spindle number 52 at a shear rate of six revolutions per minute.

6.2.9 Determination of storage stability of the complementary foods

Microbiological and total aflatoxin evaluation was done by the Kenya Bureau of Standards according to KEBS, (2008). The micro-organisms and test methods used were, *Salmonella spp* (TES/MIC/TM/08), *E-coli* (TES/MIC/TM/17), total viable count (TES/MIC/TM/10), yeast and molds (TES/MIC/TM/11), total aflatoxins (TES/06/TM/30). Peroxide value was determined according to AOCS (1996) method. These tests were done at 0 month, 3 months and 6 months after were products packed in PET bottles and stored at room temperature (22-25^{o}C).

6.2.10 Cost analysis of the complementary foods

The cost per kilogram of extrusion-cooked complementary foods was analysed as described by Owino et al. (2007). Machine and man hour costs were based on estimates provided by All Grain Co. Ltd. A profit margin of 15% was assumed based on figures used by All Grain Co. Ltd, Kenya.

6.2.11 Consumer acceptability of the complementary foods

6.2.11.1 Sensory evaluation by a semi-trained panel

Sensory evaluation by a semi-trained pane was done to select two foods that were to be presented for consumer evaluation by mothers and infants. Product weighing 40g was thoroughly mixed in 250ml of cold water to a fine consistency with no lumps. The mixture was then allowed to boil for 5 minutes. All the samples were then allowed to cool to 45^{o}C and maintained in a water bath at that temperature. Sensory evaluation of the foods in relation to aroma, taste, appearance, texture and overall consumer preference were carried out by 17 pre-trained panelists recruited from staff and graduate students of JKUAT using a questionnaire. Samples of about 30 ml of the cooked product were placed in plastic cups coded with three-digit random numbers. A 5-point hedonic scale (1 - dislike extremely to 5 - like extremely) with equivalent intervals between the categories was used (Meilgaard et al. 2007). In addition, each panelist was required to indicate the product they preferred most (Appendix 6).

6.2.11.2 Consumer acceptance by mother and infants

The evaluation was based at Makunga District Hospital and involved both mothers and their infants (6-24 months old). Two foods selected after sensory evaluation by semi trained panelists were compared with CSB+ processed according to WFP (2010) specifications. Fifty seven child /mother pairs from Isongo location of Mumias District of western Kenya were recruited into the study. The inclusion criteria was; 6-24 months of age as authenticated by birth records; good health (obtained by self-reporting from the mothers) and well nourished as determined by mid-upper-arm-circumference (MUAC> 12.5). The infants were admitted to the study together with their mother/caregiver. The purpose of the study was explained to the mothers and written consent obtained (Appendix 7).

The porridges were prepared by the study team prior to testing as outlined in section 6.2.12.1 above. Each mother tested all the three porridges as detailed by Meilgaard *et al*. (2007). Color, taste, texture and smell of the porridges were assessed based on a 5-point hedonic scale (from “5” – like very much, to “1” - dislike very much). Infants were randomised to test one food per day (CFC [n=15], CF5 [n=17], CSB+ [n=15]) for a total of three days with a wash out day in between. The child's reaction (non-verbal cues) during feeding was observed by a research assistant at different instances; when child first sees the foods, on initial food offer and on subsequent offers until refusal, satisfaction or finishing the food. The non-verbal cues were interpreted by the mother as indicators of acceptance and scored on a 5-point scale (Appendix 7).

6.2.12 Data analysis

All results are given as mean ± SD; n refers to the number of observations. Nutrient values, nutrient density and functional characteristics were analysed by one way ANOVA. Means were separated by Tukey's studentized test and $p < 0.05$ were considered significant using SAS (2004) software.

6.3 RESULTS AND DISCUSSION

6.3.1 Phytic acid reduction in germinated amaranth grain

The effect of germination of *Amaranthus cruentus* grains on phytic acid reduction is shown in Figure 6.1.

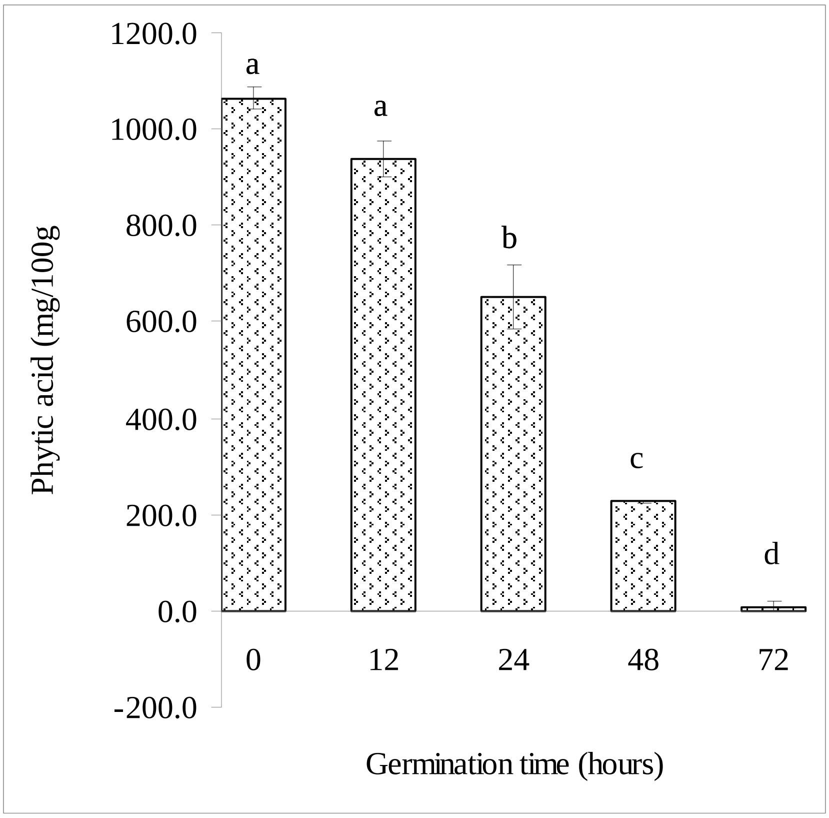

Figure 6.1: Phytic acid reduction on germination of amaranth grain
Values with different letters are significantly different ($p<0.05$)

Phytic acid reduced as the germination time increased from 1064.7 mg/100g at 0 hours after soaking to 8.6 mg/100g after 72 hours of germination. This translates to 11% reduction after 12 hours of germination, 38% reduction after 24 h, 78% reduction after 48 h and 99% phytic acid reduction after 72 h of germination. The reduction was not significant after 12 h of germination ($p>0.05$) though it was significant ($p<0.05$) in subsequent germination times.

The decrease in phytic acid at 72 h germination may influence iron bioavailability, as phytic acid is a potent bioavailability inhibitor even at low levels (Hurrell et al., 1992). Longer germination period showed better phytic acid reduction. However, long germination periods may result in significant losses in dry matter and larger sprouts and rootlets which may reduce energy density as well as hamper processing and consumer acceptability (Wijngaard et al., 2005; Kanensi et al., 2011).

6.3.2 Phytic acid/mineral ratio in germinated amaranth grain

Phytic acid measured as myoinositol hexa-inositol phosphate (IP6) and minerals molar ratio in germinated *Amaranthus cruentus* grain are reported in Table 6.2.

Table 6.2: Zinc, iron, calcium and phytate/ mineral molar ratios of germinated amaranth grains

Time (h)	Zinc (mg/100g)	Phytate/ Zn molar ratio	Iron (mg/100g)	Phytate/ Fe molar ratio	Calcium (mg/100g)	Phytate/ Ca molar ratio
0	3.3 ± 0.3^a	31.9 ± 3.8^b	14.7 ± 1.0^a	7.1 ± 0.6^a	56.8 ± 3.5^b	1.8 ± 0.1^a
12	2.9 ± 0.0^a	38.3 ± 2.0^a	14.9 ± 1.9^a	6.2 ± 1.0^a	83.1 ± 3.8^a	1.1 ± 0.0^b
24	2.8 ± 1.6^a	26.3 ± 2.9^c	14.7 ± 0.8^a	4.3 ± 0.6^b	60.8 ± 0.9^b	1.0 ± 0.0^b
48	3.3 ± 0.7^a	6.9 ± 1.6^d	15.2 ± 0.7^a	1.4 ± 0.0^c	35.5 ± 3.0^c	0.6 ± 0.0^c
72	2.6 ± 0.0^a	0.3 ± 0.3^e	15.0 ± 0.1^a	0.1 ± 0.0^d	32.9 ± 0.1^c	0.1 ± 0.0^d
*Limits		15.0		1.0		0.24

* Source: Norhaizan and Nor (2009);
Mean ± standard deviation (n=3);
Values on the same column with different superscripts are significantly different ($p<0.05$); *n*=3

The phytate/iron molar ratio reduced as the germination time increased reducing to below the critical limits after 72 h of germination. Phytate/zinc molar ratio had reduced below the critical limit by 48 h of germination while 72 h of germination had reduced phytate/ calcium molar ratio to 0.03, way below the critical limit. A similar trend was reported for the phytate/ calcium molar ratio with 72 h of germination reducing the ratio to below critical limits.

The results of this study were similar to those of Egli et al., (2002) who found that phytic acid content decreased up to 81% of the initial values in a wide range of cereal grains including amaranth. Reduction of phytic acid will translate to higher predicted bioavailability of iron, zinc and calcium due to improved phytate/ mineral molar ratio. When the phytate/iron molar ratio is >1.0, it predicts very low iron bioavailability (Norhaizan and Nor, 2009). Phytate/ zinc molar ratio >15.0 predicts relatively low zinc bioavailability, phytate/ zinc molar ratio between 5.0 and 15.0 predicts medium zinc bioavailability and phytate/ zinc molar ratio ≤5 predicts relatively good zinc bioavailability. The phytate/ calcium molar ratio >0.24 is a predictor of impaired calcium bioavailability (Norhaizan and Nor, 2009; Guasheng *et al*. 2005; Umeta et al., 2005; Mendoza et al., 2001).

The high phytic acid in *Amaranthus cruentus* poses a major challenge in formulation of food rich in iron, zinc and calcium due to reduction of mineral bioavailability. Germinating the grain to 72 h may reduce the phytates thereby improve phytate/ mineral molar ratio and hence improved bioavailability.

6.3.3 Nutrient density in developed complementary foods

Table 6.3 presents the protein, carbohydrate, energy and mineral composition of complementary foods on dry weight basis. CF6 had the highest energy (442.40 kcal) and fat content (10.42 g/100g) while CFC had the least energy (396.0 kcal/100g) and fat (7.05 g/100g) even though it had the highest carbohydrate content (59.8 g/100g). The lack of the fat rich termites and *dagaa* fish may be a probable explanation for the low energy.

CF6 had the highest iron (27.41 mg/100g) while CF4 had the highest zinc (5.96 mg/100g) and calcium (75.86 mg/100g) though calcium levels were not significantly different between CF4, CF5 and CF6. All the foods met the minimum recommended compositional requirement for energy, carbohydrates, protein and iron. Carbohydrates are an essential source of energy for infants especially taking into account the glucose needs of the human brain (Koletzko et al. 2005).

CF4 and CF5 met the minimum zinc requirement while CF6 and CFC were slightly less. All the foods were low in calcium content in reference to the minimum requirement for complementary foods for 6 – 24 month infants and young children. All the foods met the recommended nutrient density for carbohydrate, protein, iron and zinc.

Table 6.3: Nutrient values and nutrient density of the developed complementary foods (CF's)

Nutrient and nutrient density	CF4	CF5	CF6	CFC	Recommended level in CF's		Values in CSB+
					Nutrient density	Nutrient content	
Moisture (g/100g)	4.65 ± 1.34[c]	4.88 ± 0.99[b]	4.73 ± 1.33[c]	8.87 ± 1.20[a]	Na	Na	10.0
Energy (kcal/100g)	429.42 ± 1.24[c]	436.02 ± 0.06[b]	442.40 ± 2.72[a]	396.00 ± 1.92[d]		400[1]	380.0
Carbohydrate (g/100g)	57.20 ± 3.99[b]	54.70 ± 0.78[c]	51.60 ± 2.66[d]	59.80 ± 1.64[a]		45[5]	-
Carbohydrate (g/100kcal)	13.33 ± 3.22[b]	12.54 ± 0.61[c]	11.65 ± 0.98[d]	15.10 ± 0.85[a]	9 -14[2]		-
Protein (g/100g)	17.86 ± 0.99[b]	17.55 ± 1.33[c]	19.00 ± 1.81[a]	13.72 ± 1.00[d]		9.1[5]	14.0
Protein (g/100kcal)	4.16 ± 0.80[b]	4.02 ± 1.04[c]	4.29± 0.67[a]	3.46 ± 0.52[d]	2.25 - 3[2]		3.68
Fats (g/100g)	9.27 ± 1.31[a]	9.75 ± 0.36[a]	10.42 ± 0.82[a]	7.07 ± 0.51[b]		10[1]	6.0
Fats (g/100kcal)	2.16 ± 1.06[a]	2.24 ± 0.28[a]	2.36 ± 0.30[a]	1.79 ± 0.27[b]	4.4 - 6[2]		1.58
Iron (mg/100g)	20.71 ± 0.88[c]	23.94 ± 1.00[b]	27.41 ± 0.54[a]	10.40 ± 1.99[d]		9.3[3,7]	6.25
Iron (mg/100kcal)	4.82 ± 1.41[c]	5.49 ± 0.78[b]	6.20 ± 0.20[a]	2.63 ± 1.04[d]	0.45 - 2[2]		1.64
Zinc (mg/100g)	5.96 ± 0.21[a]	4.87 ± 0.82[a]	4.22 ± 0.06[b]	4.19 ± 0.87[b]		4.6[4,6]	5.0
Zinc (mg/100kcal)	1.39 ± 0.17[a]	1.12± 0.64[a]	0.95 ± 0.02[b]	1.06 ± 0.45[b]	0.5 - 1.5[2]		1.32
Calcium (mg/100g)	75.86 ± 4.46[a]	72.14 ± 0.81[b]	72.58 ± 2.67[b]	18.73 ± 1.84[c]		400[7]	130.0
Calcium(mg/100kcal)	17.68 ± 0.28a	16.55 ± 0.87a	16.41 ± 0.98a	4.73± 0.96b	50 - 140[2]		105.23

Values on the same row with different superscripts are significantly different ($p<0.05$)   n=6;   nd: not detected;
na: not available; [1]Codex (1991); [2]Koletzko et al. 2005; [3] Assuming 10% bioavailability; [4] Assuming moderate bioavailability; [5]WHO (1998); [6]FAO/WHO (2002); [7]FAO/WHO (2004)

The developed complementary foods had nutritional attributes that compared closely with nutritional specification set for CSB+ (WFP, 2010). All the CF's had higher energy, fat and iron content than CSB+. CF4, CF5 and CF5 had higher protein content that CSB+ while CF4 had higher zinc content. Unlike CSB+ which has micronutrient fortification, the developed complementary foods utilized traditional foods with no exogenous micronutrient fortification. Though it was difficult to achieve a food that met the requirements for calcium, the study is a pointer that micronutrient fortification is not the only option in attaining an iron and zinc dense complementary food. With proper handling and processing, traditional foods can be utilized to achieve even better results.

The RDA used in this comparison was for 6 – 11 months old infants. In general, the younger infants consume the lowest amounts of complementary food per day. Depending on the nutrient in question, their specific nutrient needs may be as great as or greater per body weight than those of children aged 12–23 months because of their rapid rate of growth and development (Lutter and Dewey, 2003). For example, a single formulation targeted for infants 6 - 11 months of age will result in excessive intakes of iron if consumed by children 12 - 23 months of age since it's up to six times the RDA for iron, whereas a formulation targeted for children 12 - 23 months olds will provide insufficient levels of iron for infants 6 - 11 month olds. An option for resolving this dilemma therefore include developing a high nutrient-density product but specifying a maximum ration per day.

6.3.4 Particle size distribution of complementary foods

Particle size distribution is an important physical property of powders and dusts the distribution for the developed foods is shown in figure 6.2. In all the foods, 100% of the particles passed through a 1000 microns sieve. Similarly, 100% of CF4 and CFC particles passed through a 600 microns sieve while 95% of both CF5 and CF6 passed through a similar sieve. None of the foods had particle sizes <250 microns.

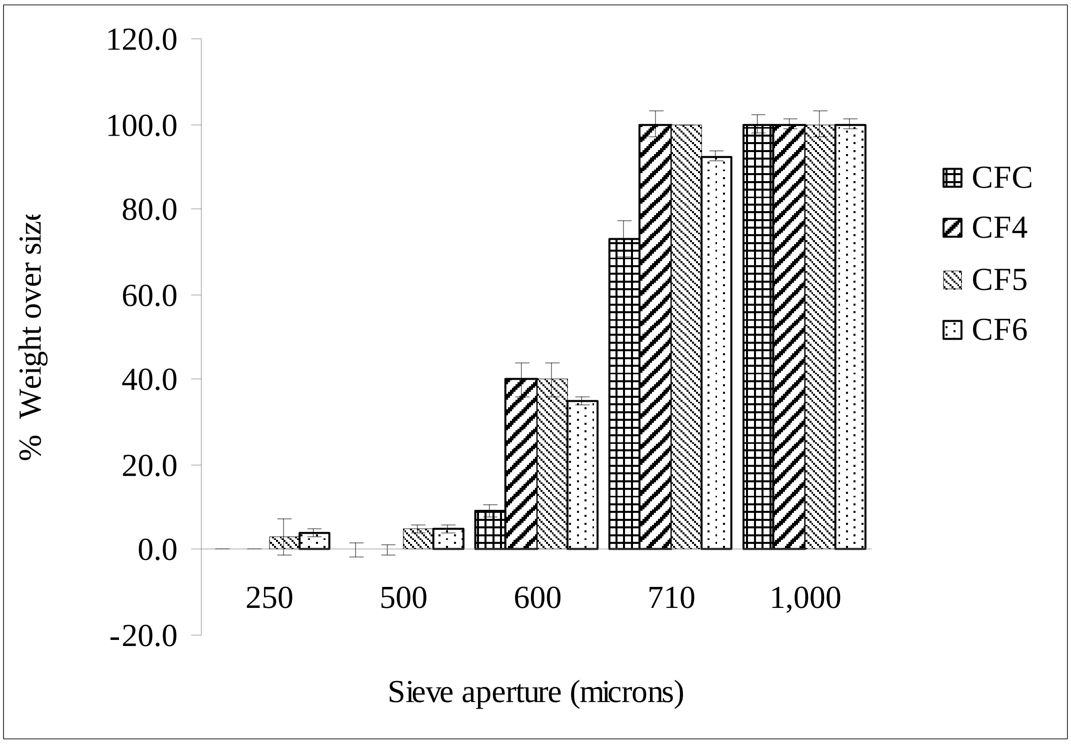

Figure 6.2: Particle size distribution of complementary foods

Particle size distribution is a function of processability of the complementary food; a high oil product may interfere with the processing characteristics in CF5 and CF6 thus produce slightly larger particle sizes. According to WFP (2010), the fortified food, CSB+, should have a uniform fine texture where 95% of the particles must pass through a 600 microns sieve and 100% must pass through a 1000 microns sieve.

Therefore, all the food flours were within the expected range for complementary foods.

6.3.5 Functional properties and in-vitro protein digestibility

The functional properties of the developed complementary foods are shown in Table 6.4. The water absorption capacity (WAC) of the complementary foods ranged from 18.4 to 19.8 mL/100g though CF4 and CF5 were not significantly different (p>0.05), with high dispersability (52.7% - 63.5%). The high dispersability of the foods indicates their good reconstitutability. It means that the food shall be free from lumping or balling when mixed with water of ambient temperature.

Table 6.4: Functional properties and protein digestibility of complementary foods

Food	WAC (mL/100g)	Bulk density (g/cm^3)	Dispersability (%)	Consistency (cP)	Protein digestibility (%)
CF4	19.5 ± 0.0^a	0.4 ± 0.0^c	62.5 ± 0.7^b	1740.0 ± 2.2^b	87.6 ± 1.0^b
CF5	19.8 ± 0.1^a	0.4 ± 0.0^c	62.2 ± 1.0^b	1460.0 ± 0.0^c	88.7 ± 1.2^a
CF6	18.4 ± 1.7^b	0.4 ± 0.0^b	63.5 ± 0.7^a	1230.0 ± 2.4^d	79.3 ± 0.7^d
CFC	19.2 ± 0.8^a	0.5 ± 0.0^a	52.7 ± 0.3^c	2510.0 ± 2.4^a	85.2 ± 0.6^c

WAC - water absorption capacity
Values on the same column with different superscripts are significantly different (p<0.05) n=6

The bulk density of the foods was low (0.4 – 0.5 g/cm^3) with CF4 and CF5 not being significantly different (p>0.05). A low bulk density is desirable for a complementary food since it translates to a higher nutrient content in a specific volume of food (Imtiaz, et al. 2011). Germinating amaranth grain and incorporating termite and fish reduced bulk density making them desirable ingredients in the formulation. The consistency of the gruel prepared from the developed complementary foods were within the range reported for different complementary feeding gruels (Ikujenlola and Fashakin, 2005; Owino et al., 2007; Tizazu et al., 2011). Low consistency increase the nutrient density of the gruels, which is highly beneficial to the infants as infants can easily consume more (Tizazu et al., 2011). Significant reductions in viscosity allow incorporation of higher solids concentrations during food preparation thereby increasing final nutrient density of the product. Grain germination offers significant potential for increasing energy density of weaning foods used in many developing areas of the world.

The foods had *in-vitro* protein digestibility ranging between 79.3 – 88.7%, with CF6 having the lowest. *In-vitro* protein digestibility of formulated plant based complementary foods was comparable to those commonly used complementary foods in other parts of Africa (Anigo et al., 2010). *In-vitro* protein digestibility is part of the parameters that determine protein quality. According to Kinyuru et al., (2010b), edible termites have a high *in-vitro* protein digestibility and therefore should not pose a challenge on the digestibility of the complementary foods. Reduction of phytates by germinating the cereal grains may also have contributed to the high protein digestibility as phytates bind proteins reducing digestibility (Solomon et al. 2008). Extrusion cooking may also increase protein digestibility by solubilizing insoluble fibers to some degree (Michaelsen et al., 2009).

6.3.6 Storage stability of the complementary foods

As shown in Table 6.5, all the foods met the set standards for for processed cereal-based foods for infants and young children (4th Edition) specification for microbial and aflatoxin quality up to 6 months of storage.

Table 6.5: Microbial quality, aflatoxin content and peroxide value of complementary foods

Food	Time (months)	Total viable count (cfu/g)	Yeasts and molds (cfu/g)	E-coli (cfu/g)	Salmonella (cfu/ 30g)	Aflatoxin (ppm)	Peroxide value (meq/kg)
CF4	0	1.7×10^3	4.7×10^2	nd	nd	nd	0.0
	3	2.0×10^3	5.0×10^2	nd	nd	nd	0.0
	6	3.0×10^3	5.5×10^2	nd	nd	nd	0.2
CF5	0	2.5×10^3	4.7×10^2	nd	nd	nd	0.0
	3	5.3×10^3	4.7×10^2	nd	nd	nd	0.0
	6	7.3×10^3	5.3×10^2	nd	nd	nd	0.3
CF6	0	3.0×10^3	5.2×10^2	nd	nd	nd	0.0
	3	3.5×10^3	5.5×10^2	nd	nd	nd	0.0
	6	4.2×10^3	6.1×10^2	nd	nd	nd	0.6
CFC	0	<10	<10	nd	nd	nd	0.0
	3	1.8×10^2	2.1×10^1	nd	nd	nd	0.0
	6	2.2×10^3	2.2×10^2	nd	nd	nd	0.0
Limits		[2]100,000	[2]100,000	[2]Nil	[2]Nil	[2]≤10	[1]≤10

[1]Codex, 1991
[2] KS 1808 Kenya standards composite flour specification
nd – not detected

Peroxide value was below detectable values and therefore below the maximum limits set by Codex (1991) for fortified complementary foods. The foods were therefore safe for use in the Kenyan market as well as shelf stable. Peroxide value is a measure of lipid oxidation, while total viable count, *E-coli* and *salmonella spp* may indicate processing and product handling hygiene.

According to Codex (1991), similar products shall be free from pathogenic microorganisms; shall not contain any substances originating from microorganisms in amounts which may represent a hazard to health and shall not contain any other poisonous or deleterious substances in amounts which may represent a hazard to health. The developed complementary foods therefore met these requirements.

6.3.7 Cost analysis of complementary foods

Table 6.6 shows cost analysis per kilogram of extruded complementary food. The cost of ingredients was the highest accounting for about 70% in CF4, CF5, CF6 and this increased with an increase in edible termite content. CFC had the lowest cost (USD 1.89/ kg) due to the fact that it did not have the termites. The cost of the foods was lower than an average commercially processed complementary food which retails at about USD 7.0/ kg in Kenya. One way to reduce the cost is by encouraging more termite harvesters to harvest thereby increasing supply and consequently lowering the price.

Table 6.6: Cost analysis of the complementary foods (USD/kg)

Production input	CF4	CF5	CF6	CFC
Amaranth grain (1.0)	0.77	0.75	0.70	0.89
Maize (0.5)	0.09	0.09	0.09	0.09
Termite (11.7)	0.94	1.18	1.76	0.00
Dagaa fish (3.5)	0.11	0.11	0.11	0.11
Soybean oil (3.5)	0.02	0.02	0.02	0.02
Sugar (1.3)	0.01	0.01	0.01	0.01
Processing (0.4)*	0.24	0.24	0.24	0.24
Packaging and labeling (0.4)	0.41	0.41	0.41	0.41
Total basic cost†	2.58	2.79	3.33	1.65
Indirect costs				
(15% of basic cost)	0.39	0.42	0.50	0.25
Total costs	2.97	3.21	3.83	1.90
Profit (15% of total costs)	0.45	0.48	0.57	0.29
Grand total	3.42	3.69	4.40	2.19

1 USD = Kshs 85.00;
*Machine and man hours during germination, mixing, extrusion and milling
†Cost of ingredients, processing, packaging and labeling
Values in brackets are unit costs for each input

6.3.8 Sensory evaluation by a semi-trained panel

Sensory evaluation was performed on the products CF4, CF5, CF6 and CFC and the scores obtained are shown in Table 6.7. Appearance, aroma, taste and texture are important parameters when evaluating sensory attribute of food as they affect acceptability of the product.. CF4, CF5 and CFC had an appearance score of 4.0 which was significantly higher ($p < 0.05$) than the value for CF6 (3.0). The attractive brownish appearance of the products was maintained though it gradually changed to a darker product as the insect concentration increased. The 15% termite content maybe responsible for the low score in appearance of CF6 since it imparts a darker color compared to other foods.

Table 6.7: Sensory evaluation of the foods by a semi-trained panel

Food	Attributes				
	Appearance	Aroma	Taste	Texture	Overall Acceptability
CF4	4.0 ± 0.8^a	3.0 ± 0.9^b	3.0 ± 1.0^b	4.0 ± 1.0^a	3.0 ± 0.9^b
CF5	4.0 ± 1.0^a	4.0 ± 1.0^a	4.0 ± 1.4^a	4.0 ± 1.1^a	4.0 ± 0.8^a
CF6	3.0 ± 1.0^b	3.0 ± 0.8^b	3.0 ± 1.2^b	3.0 ± 1.0^b	3.0 ± 0.9^b
CFC	4.0 ± 0.9^a	4.0 ± 0.9^a	3.0 ± 1.2^b	3.0 ± 1.1^b	3.0 ± 1.1^b

Values on the same column with different superscripts are significantly different ($p<0.05$); n=17

CF5 was rated significantly higher ($p<0.05$) than CF4, CF6 and CFC which were given a taste score of 3.0. The foods had a smell score of 3.0 to 4.0. CF5 had significantly higher scores ($p < 0.05$) in terms of aroma than the rest of the foods. CF4 and CF5 had significantly higher ($p < 0.05$) score for texture than CF6 and CFC. CF5 was the highest overall acceptability score (4.0) amongst all the foods while CF4, CF6 and CFC had a significantly lower score (3.0). The mean score of all the foods was not less than 3.0 indicating that based on the test parameters these foods were liked by the panelists.

Out of the 17 semi-trained panellists, 53% preferred CF5 over the other foods (Figure 6.3). CF6 which contains the highest amount of termites (15%) was least preferred together with CFC, the food that did not have termites or *dagaa* fish. The inclusion of animal source foods into the formulation may improve the consumer preference as evidenced by the increase in percentage preference from CFC to CF5. However, a further increase in termite content reduced the consumer preference as shown by product CF6.

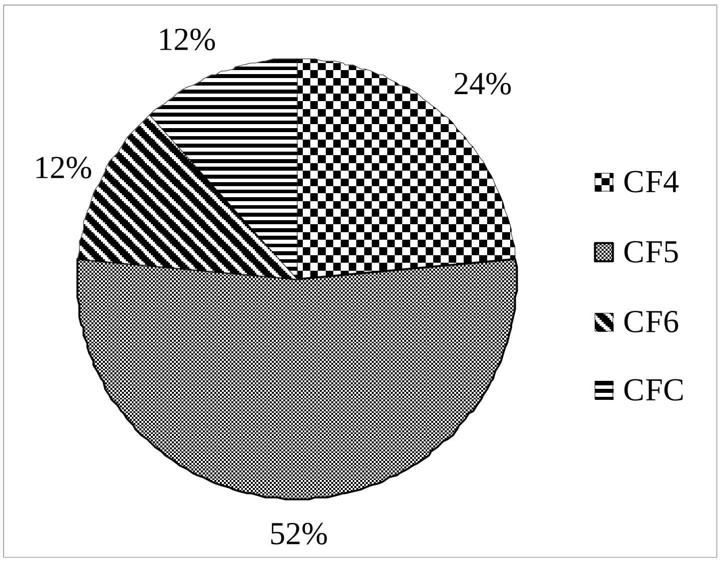

Figure 6.3: Proportion of preference for complementary foods

6.3.9 Maternal food acceptability

Results of maternal evaluation of the foods are reported in Table 6.8. The scores for CFC and CSB+ were above 3.0 indicating that the two foods were liked most. However the mothers slightly disliked the taste and smell of CF5 even though they liked the texture.

Table 6.8: Consumer acceptance of the complementary foods by mothers

Food	Attributes			
	Color	Aroma	Taste	Texture
CF5	3.0 ± 0.2[b]	2.0 ± 0.1[b]	2.0 ± 0.2[b]	4.0 ± 0.2[a]
CFC	4.0 ± 0.1[a]	4.0 ± 0.1[a]	4.0 ± 0.1[a]	4.0 ± 0.1[a]
CSB+	4.0 ± 0.2a	4.0 ± 0.1[a]	4.0 ± 0.2[a]	3.0 ± 0.2[b]

Mean ± SD; n=57; Values on the same column with different superscripts are significantly different ($p \leq 0.05$)

The mothers indicated that they preferred their product of choice due to the inherent properties of color, smell, taste and texture and none indicated that their preferences was based on extrinsic non-study related factors. The observation that both CFC and CF5 porridges were generally liked agrees with previous observations that mothers often find complementary foods processed from local foodstuffs attractive (Mensa and Tomkins, 2003; Owino et al., 2007).

6.3.10 Infant food acceptability

The interpretation of the infant non-verbal cues indicated that all the foods were liked along the three feeding regimes (Table 6.9).

Table 6.9: Consumer acceptance of the complementary foods by infants

Complementary Food	Sees food first	Initial offer	Subsequent offers
CF5	4.0 ± 0.2[a]	4.0 ± 0.2[a]	3.0 ± 0.2[b]
CFC	4.0 ± 0.1[a]	4.0 ± 0.1[a]	4.0 ± 0.1[a]
CSB+	3.0 ± 0.2[b]	3.0 ± 0.2[b]	3.0 ± 0.2[b]

Mean ± SD; n=57; Values on the same column with different superscripts are significantly different ($p \leq 0.05$)

The infants preferred CFC more in comparison to other foods at all the three levels of feeding. There was no significant difference ($p>0.05$) between CF5 and CFC except upon subsequent feeding offers. CSB+ was the least preferred by the infants. Infants tended to dislike CSB+ more than their mother did. It's also probable that taste and smell did not affect their preference for CF5 like in their mothers. Infant age has been found to affect food preferences with infants not showing dislike for some foods that mothers reportedly dislike (Parker et al., 1998). However, it is necessary to ensure that there is maternal preference of complementary food if a food is to be widely accepted. The approach of using non-verbal cues by infants has been used before by researchers and it is the practice of choice in the baby-food industry (Kevin, 1995; Neumann *et al*., 2002).

6.4 CONCLUSION

Germination was found to reduce phytic acid levels to insignificant levels after 72 hours and the grain was utilized in processing of complementary foods. CF4, CF5 and CF6 provided 100% of energy requirements while CFC provided 99% of the RDA for energy. All the foods met the RDA for iron and zinc providing more than the minimum density of the two minerals. CF5 was the most preferred food by semi-trained panelists while the mothers consistently scored CFC higher than CF5 and CSB+. The preference for CFC was higher than CF5 by the children in all the food offers. They also disliked CSB+ more than the other foods. All the foods were shelf stable for up to 6 months.

CHAPTER SEVEN

CONCLUSION AND RECOMMENDATIONS

7.1 CONCLUSION

The study showed the diversity of traditional foods and their perceived nutritional benefits in the study area. Finger millet, sorghum, amaranth grain and maize were perceived to be a source of energy. Amaranth grain was perceived to increase breast milk in lactating mothers in addition to being a source of iron. Termites and *dagaa* fish were perceived to be important in body building and a source of iron and zinc. Availability of the animal foodstuffs within the study area suggested that they maybe be incorporated in cereal based foods to develop high energy, iron and zinc content complementary foods. The grains have previously been utilized in the region while *dagaa* fish and termites were to be a much needed animal source food in complementary feeding. Amaranth grain, finger millet, maize, *dagaa* fish and termites were selected for further analysis.

Nutrient analysis found that amaranth grain had the highest protein content (18.55 g/100g) among plant foodstuffs while *dagaa* fish had the highest protein content (58.1 g/100g) among animal foodstuffs. Iron content ranged between 9.4 – 102.9 g/100g among the cereals and 10.6 – 64.7 g/100g in among insects and fish. The animal foodstuffs had higher level of zinc (5.6 -12.8 g/100g) compared to the cereal grains (0.93 – 3.48 g/100g). High anti-nutrient content in amaranth and finger millet were found. Finger millet had the highest phytic acid content (2287.0 mg/100g) compared to amaranth grain (1285.4 mg/100g) and maize (318.5 mg/100g). The foods analysed may be used in development of nutrient dense complementary foods.

A process to optimize inclusion of termites and *dagaa* fish was developed and pre-cooked products obtained. The process incorporated blanching and oven drying of termites and *dagaa* fish as unit operations. Extrusion cooking was used to achieve pre-cooked complementary foods. Phytate/ iron molar ratio was beyond the critical limits for all the developed foods. The foods contained up to 3.3% bioavailable iron after pepsin digestion and up to 2.5% bioavailable iron after pepsin+pancreatin digestion. An indigenous process such as germination maybe evaluated in order to reduce phytic acid before processing the grains into complementary foods.

Germinating amaranth grain up to 72 hours after soaking was found to reduce phytic acid to insignificant amounts. The germinated grain was utilized in processing of nutrient dense, acceptable complementary foods. CF4, CF5 and CF6 provided 100% of energy RDA while CFC provided 99% of the RDA. All the foods met the RDA for iron and zinc providing more than the minimum density of the two minerals. CF5 was the most preferred food by semi-trained panelists while the mothers consistently scored CFC higher than CF5 and CSB+. The preference for CFC was higher than CF5 by the children in all the food offers. They also disliked CSB+ more than the other foods. All the foods were shelf stable for up to 6 months.

7.2 RECOMMENDATIONS

From the study, it is recommended that;

i. Both animal and plant traditional foods should be exploited in development of nutrient dense complementary foods

ii. Appropriate pre-processing steps such as germination should be incorporated as means to reduce anti-nutrients in traditional grains before processing to complementary foods

iii. Facilitation to commercialize and patent the process and products required to enable full exploitation.

7.3 SUGGESTION FOR FURTHER RESEARCH

i. Methods to further improve acceptability qualities of the foods should be evaluated

ii. The foods should be subjected to a controlled clinical trial to determine their efficacy in maintenance of good nutrition

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APPENDICES

Appendix 1: A questionnaire for Luo community traditional food names given by individual informants

To be filled by interviewer.

Name: ___________________________Age: ______ Profession: _____ _____

No.	Traditional food item	Local /Luo name	English/Common name	Characteristics- frequency of use, cooking method
1				
2				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				
13.				
14.				
15.				
17.				
18.				
19.				
20.				
21.				
-				
-				
100.				

Appendix 2: A questionnaire for reasons for consumption of particular foods by the Luo community

Background:
Previously, a list of traditional foods consumed among the Luo community of Kisumu West District has been generated, but we are unable to identify which of these foodstuffs was consumed for improvement of health and specifically iron status (add blood as is locally known). The idea is to engage elderly people in respective communities to obtain proxy indication as to which of the foodstuffs were used for that purpose.

Overall objective:
To find reasons why these people consumed these foods and we want to see if there were any nutritional reasons, and if possible whether the consumption of these foodstuffs were linked to 'enhancement of blood levels'

Methodology:
In this regard the interviewer will introduce the topic in general stating that he wants to be explained for why those foods were eaten, who ate them and why. In the course of the discussion the interviewer will drive the discusser to touch on nutritional reasons.

The interviewer will read out the foodstuffs in the order in which they appear on the list. It is expected that interviews will be with at least 3 discussants, and at least one should be a woman.

Traditional plant and animal foods consumed by the Luo community

No	Local (*Luo*) name of foodstuff	Which part is consumed? Plants-whole, leaves, fruits, roots, grains, seeds Animals-whole, dewinged)	Who in the family is the food meant for?	Why was the food consumed?	Any nutritional reason? (*To be inferred from discussion by interviewer)*	Any iron-related reason? (*To be inferred from discussion by interviewer)*
1.	*Anyuka*					
2.	*Apilo*					
3.	*Nderma*					
4.	*Onyiego*					
5.	*Oganda*					
6.	*Ododo*					
7.	*Ododo*					

8.	*Awayo*					
9.	*Onyulo*					
10.	*Ochuoga*					
11.	*Atipa*					
12.	*Akeyo/Dek*					
13.	*Omwogo*					
14.	*Budho*					
15.	*Apoth*					
17.	*Susa budho*					
18.	*Osao*					
20.	*Kandhira*					
21.	*Mitoo*					
22.	*Osuga*					
23.	*Bo*					
25.	*Mapera*					
26.	*Rabuon nyaluo*					
27.	*Bel*					
28.	*Omwogo*					
29.	*Kal*					
30.	*Rabuon*					
31.	*Rabuon nduma*					
32.	*Oduma*					
33.	*Nyim*					
34.	*Ng'or*					
35.	*Ododo*					
36.	*Sisi*					
37.	*Riwo*					
38.	*Agoro*					

39.	*Oyala*					
40.	*Onyoso*					
41.	*Tsenesene*					
42.	*Omena*					

Appendix 3: Images of traditional foodstuffs utilized in development of the complementary foods

Winged termites

Amaranth plant (head loaded with grains)


Maize grain


Dagaa fish

Appendix 4: Development of formulations for CF1, CF2 and CF3

Formulation for CF1

Ingredients (%)		73.00	15.00	3.00	8.00	0.60	4.00	100.00	
Nutrient	Units	Value per 100g	Value per 100g	Value per 100g	Value per 100g	Value per 100g	Value per 100g	Value per 100g	RDA
		Amaranth grain	Maize	Omena (Dagaa)	Termite	Oil (soy bean)	Sugar	Product	
Water	g	9.9	11.9	9.2	6.5	0.0	0.3	9.8	1100.0
Energy	kcal	319.0	1476.0	143.0	656.0	884.0	387.0	517.8	400.0
Protein	g	18.5	8.2	58.1	39.3	0.0		19.6	9.1
Fat	g	14.3	4.8	26.6	44.8	100.0		16.2	10.0
Carbohydrate	g	63.8	79.8	11.0	1.8		99.9	59.4	
Dietary fiber	g	7.1	0.0	9.7	3.4			5.7	
Calcium	mg	12.2	43.6	2790.0	58.7			103.8	400.0
Phoshorus	mg	322.7	231.0	277.0	182.3			293.1	80.0
Magensium	mg	219.4	92.1	86.0	42.6			180.0	80.0
Potassium	mg	324.3	287.0	461.0	259.6			314.4	400.0
Sodium	mg	7.9	35.0	55.0	123.6			22.6	320.0
Iron	mg	15.0	9.4	25.7	53.3			17.4	9.3.0
Zinc	mg	3.4	0.9	12.0	8.1			3.6	4.6.0
Copper	mg	1.7			1.7			1.3	0.3
Manganese	mg	2.5			3.2			2.0	0.2

Formulation for CF2

Ingredients (%)		71.00	15.00	3.00	10.0	0.6	0.4	100.00	
Nutrient	Units	Value per 100g	Value per 100g	Value per 100g	Value per 100g	Value per 100g	Value per 100g	Value per 100g	RDA
		Amaranth grain	Maize	Omena (Dagaa)	Termite	Oil (soy bean)	Sugar	Product	
Water	g	9.9	11.91.0	9.2	6.5		0.3	9.6	1100.0
Energy	kcal	31	1476.0	143.0	656.0	884.0	387.0	521.4	400.0
Protein	g	18.5	8.2	58.1	39.3			19.9	9.1
Fat	g	14.4	4.8	26.6	44.8	100		16.7	10.0
Carbohydrate	g	63.8	79.8	11.0	1.8		99.9	57.5	
Dietary fiber	g	3.1		9.7	3.4			2.8	
Calcium	mg	12.2	43.6	2790.0	58.7			104.6	400.0
Phoshorus	mg	322.7	231.0	277.0	182.3			287.1	80.0
Magensium	mg	219.4	92.1	86.0	42.6			174.2	80.0
Potassium	mg	324.3	287.0	461.0	259.6			309.9	400.0
Sodium	mg	7.9	35.0	55.0	123.6			24.8	320.0
Iron	mg	15.0	9.4	25.7	53.3			18.0	9.3.0
Zinc	mg	3.4	0.9	12.0	8.1			3.7	4.6.0
Copper	mg	1.7			1.7			1.3	0.3
Manganese	mg	2.5			3.2			2.0	0.2

Formulation for CF3

Ingredients (%)		66.00	15.00	3.00	15.00	0.60	0.40	100.00	
Nutrient	**Units**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**RDA**
		Amaranth grain	**Maize**	**Omena (Dagaa)**	**Termite**	**Oil (soy bean)**	**Sugar**	**Product**	
Water	g	9.9	11.9	9.2	6.5		0.3	9.5	1100.0
Energy	kcal	319.0	1476.0	143.0	656.0	884.0	387.0	541.4	400.0
Protein	g	18.5	8.2	58.1	39.3			21.1	9.1
Fat	g	14.4	4.8	26.6	44.8	100.0		18.3	10.0
Carbohydrate	g	63.8	79.8	11.0	1.8		99.9	55.1	
Dietary fiber	g	3.1		9.7	3.4			2.8	
Calcium	Mg	12.2	43.6	2790.0	58.7			107.1	400.0
Phoshorus	Mg	322.7	231.0	277.0	182.3			283.3	80.0
Magensium	Mg	219.4	92.1	86.0	42.6			167.6	80.0
Potassium	Mg	324.3	287.0	461.0	259.6			309.9	400.0
Sodium	Mg	7.9	35.0	55.0	123.6			30.7	320.0
Iron	Mg	15.0	9.4	25.7	53.3			20.1	9.3.0
Zinc	Mg	3.4	0.9	12.0	8.1			4.0	4.6.0
Copper	Mg	1.7			1.7			1.3	0.3
Manganese	Mg	2.5			3.2			2.1	0.2

Appendix 4: Development of formulations for CF4, CF5, CF6 and CFC

Formulation for CF4

Foodstuffs (%)		73.00	15.00	3.00	8	0.6	0.4	100.00	
Nutrient	**Units**	**Value Per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**RDA**
		Germinated Amaranth	**Maize**	**Omena (Dagaa)**	**Termite**	**Oil (soy bean)**	**Sugar**	**Product**	
Water	g	9.9	11.9	9.2	6.5		0.3	9.8	1100.0
Energy	kcal	319.0	1476.0	143.0	656.0	884.0	387.0	517.8	400.0
Protein	g	18.5	8.2	58.1	39.3			19.6	9.1
Fat	g	14.4	4.8	26.6	44.8	100.0		16.2	10.0
Carbohydrate	g	63.8	79.8	11.0	1.8		99.9	59.4	
Dietary fiber	g	7.1		9.7	3.4			5.7	
Calcium	mg	12.2	43.6	2790.0	58.7			103.8	400.0
Phoshorus	mg	322.7	231.0	277.0	182.3			293.1	80.0
Magensium	mg	219.4	92.1	86.0	42.6			180.0	80.0
Potassium	mg	324.3	287.0	461.0	259.6			314.4	400.0
Sodium	mg	7.9	35.0	55.0	123.6			22.6	320.0
Iron	mg	15.0	9.4	25.7	53.3			17.4	9.3.0
Zinc	mg	3.4	0.9	12.0	8.1			3.6	4.6.0
Copper	mg	1.7			1.7			1.3	0.3
Manganese	mg	2.5			3.2			2.0	0.2

Formulation for CF5

Foodstuffs (%)		71.00	15.00	3.00	10.00	0.60	0.40		
Nutrient	**Units**	**Value Per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**RDA**
		Germinated Amaranth	**Maize**	**Omena (Dagaa)**	**Termite**	**Oil (soy bean)**	**Sugar**	**Product**	
Water	g	9.9	11.9	9.2	6.5		0.3	9.6	1100.0
Energy	kcal	319.0	1476.0	143.0	656.0	884.0	387.0	521.4	400.0
Protein	g	18.5	8.2	58.1	39.3			19.9	9.1
Fat	g	14.4	4.8	26.6	44.8	100.0		16.7	10.0
Carbohydrate	g	63.8	79.8	11.0	1.8		99.9	57.5	
Dietary fiber	g	3.1		9.7	3.4			2.8	
Calcium	Mg	12.2	43.6	2790.0	58.7			104.6	400.0
Phoshorus	Mg	322.7	231.0	277.0	182.3			287.1	80.0
Magensium	Mg	219.4	92.1	86.0	42.6			174.2	80.0
Potassium	Mg	324.3	287.0	461.0	259.6			309.9	400.0
Sodium	Mg	7.9	35.0	55.0	123.6			24.8	320.0
Iron	Mg	15.0	9.4	25.7	53.3			18.0	9.3.0
Zinc	Mg	3.4	0.9	12.0	8.1			3.7	4.6.0
Copper	Mg	1.7			1.7			1.3	0.3
Manganese	Mg	2.5			3.2			2.0	0.2

Formulation for CF6

Foodstuffs (%)		66.00	15.00	3.00	15.00	0.60	0.40		
Nutrient	**Units**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**RDI**
		Germinated Amaranth	**Maize**	**Omena (Dagaa)**	**Termite**	**Oil (soy bean)**	**Sugar**	**Product**	
Water	g	9.9	11.9	9.2	6.5	0.0	0.3	9.6	1100.0
Energy	kcal	319.0	1476.0	143.0	656.0	884.0	387.0	541.5	400.0
Protein	g	18.6	8.2	58.2	39.3	0.0	0.0	21.1	9.1
Fat	g	14.4	4.8	26.6	44.8	100.0	0.0	18.4	
Carbohydrate	g	63.8	79.9	11.0	1.9	0.0	100.0	55.1	
Dietary fiber	g	3.1	0.0	9.7	3.4	0.0	0.0	2.9	
Calcium	mg	12.2	43.7	2790.0	58.7			107.1	400.0
Phoshorus	mg	322.8	231.0	277.0	182.3			283.3	80.0
Magensium	mg	219.5	92.1	86.0	42.6			167.6	80.0
Potassium	mg	324.4	287.0	461.0	259.6			309.9	400.0
Sodium	mg	8.0	35.0	55.0	123.6			30.7	320.0
Iron	mg	15.1	9.5	25.8	53.3			20.1	9.3
Zinc	mg	3.5	0.9	12.0	8.1			4.0	4.6
Copper	mg	1.7			1.7			1.4	0.3
Manganese	mg	2.5			3.3			2.1	0.2

Formulation for CFC

Foodstuffs (%)		84.00	15.00	0.00	0.00	0.60	0.40		
Nutrient	**Units**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**RDI**
		Germinated Amaranth	**Maize**	**Omena (Dagaa)**	**Termite**	**Oil (soy bean)**	**Sugar**	**Product**	
Water	g	9.9	11.9	9.2	6.5	0.0	0.3	10.1	1100.0
Energy	kcal	319.0	1476.0	143.0	656.0	884.0	387.0	496.2	400.0
Protein	g	18.6	8.2	58.2	39.3	0.0	0.0	16.8	9.1
Fat	g	14.4	4.8	26.6	44.8	100.0	0.0	13.4	
Carbohydrate	g	63.8	79.9	11.0	1.9	0.0	100.0	66.0	
Dietary fiber	g	3.1	0.0	9.7	3.4	0.0	0.0	2.6	
Calcium	mg	12.2	43.7	2790.0	58.7			16.8	400.0
Phoshorus	mg	322.8	231.0	277.0	182.3			305.8	80.0
Magensium	mg	219.5	92.1	86.0	42.6			198.2	80.0
Potassium	mg	324.4	287.0	461.0	259.6			315.5	400.0
Sodium	mg	8.0	35.0	55.0	123.6			12.0	320.0
Iron	mg	15.1	9.5	25.8	53.3			14.1	9.3
Zinc	mg	3.5	0.9	12.0	8.1			3.1	4.6
Copper	mg	1.7			1.7			1.4	0.3
Manganese	mg	2.5			3.3			2.1	0.2

Appendix 6: Sensory evaluation questionnaire for the developed complementary foods

Hedonic Test

Name:________________________________Gender (M/F):_____________ Date________________

You are provided with four (4) coded samples of complementary porridge.

A. Please rate the samples (1-5) according to the scale provided below by filling in the table against each sample and attribute with 1, for Disliking extremely and 5, for liking extremely. Rinse your mouth with clean water after tasting each food.

1. Dislike extremely
2. Dislike slightly
3. Neither like nor dislike
4. Like slightly
5. Like extremely

Sample Code	Appearance	Aroma	Taste	Texture	Overall consumer preference
281					
262					
211					
284					

Any other comments:____

B. Which product do you prefer most? ___________________

Appendix 7: Questionnaire for testing maternal and infant acceptability of the complementary foods

CHILD ID:- __/__/__/ DAY ___ DATE:- __ __/__ __/2011
FOOD ID:- __/__/__/

<u>1.0 General instructions</u>

1. Select children who are between the ages of 6 months to 1year (Use vaccination cards, or health card to ascertain age).
2. Explain to each carer and that this is a new food and we want to know what they think of it so that we can make it even better.
3. Emphasize that the food is nutritious and that it has been tested in certified laboratory.
4. Ensure friendly environment for each child to taste the food, without other children prompting a response.
5. After explaining the study, ask the parent/guardian to sign the informed consent form.
6. Measure and record child's weight, height/length as per demographic table below only on the FIRST day.
7. Each mother evaluates each of the prepared porridges.
8. Each child will be served porridge individually in graduated cups.
9. Amount of porridge served and amount left after feeding must be recorded.
10. During feeding observe child's behavior and record (ie 1) child signaling for more, 2) child turning head away at second offer, 3) child spitting food, 4) child smiling, 5) child crying, etc).

<u>2.0 Informed consent</u>

I, being the parent/ guardian of --- (name of the child), have been given the explanation about this study. I have understood what has been explained to me and my questions have been answered satisfactorily. I understand that I can change my mind any time and it will not affect the management of my child.

Please fill the boxes as required:

☐ (*Please tick*) **I have agreed to allow my child to participate**

Parent/guardian's signature------------------ Date------------------ (dd/mm/yyyy)

I certify that I have followed all the study procedures in the S.O.P for obtaining consent.

Investigator's signature ------------------- Date -------------------- (dd/mm/yyyy)

*The Parent/Guardian's thumb print as named above if he /she cannot write*___________________

3.0 Demographic information

<table>
<tr><td colspan="5">DEMOGRAPHIC INFORMATION AND ANTHROPOMETRY</td></tr>
<tr><td>Age</td><td colspan="3">Months</td><td>Residence</td></tr>
<tr><td>Sex</td><td colspan="3">Male ☐ Female ☐</td><td>Length ___ ____ . ___ cm</td></tr>
<tr><td>Birth weight</td><td colspan="3">___ ____ . ____Kg</td><td></td></tr>
<tr><td>Current weight</td><td colspan="3">___ ____ . ____Kg</td><td>MUAC ____
____ .____cm</td></tr>
<tr><td rowspan="5">Others</td><td colspan="3">No of siblings</td><td>Birth order _____out of</td></tr>
<tr><td colspan="3">No of children <5yrs in the</td><td></td></tr>
<tr><td colspan="3">Total No of people living in the household</td><td></td></tr>
<tr><td>Is the child</td><td>Y</td><td>N</td><td>Is child weaned? Y</td></tr>
<tr><td colspan="4">If, weaned, Is the child receiving any kind of purchased/donated food?</td></tr>
</table>

4.0 Sensory evaluation by mothers/caregivers

Name:______________________________Gender (M/F):____________ Date__
____

Instructions:

A. You are provided with three (3) coded samples of complementary porridge. Please taste and rate the samples (1-5) according to the scale provided below by filling in the table against each sample and attribute with 1, for Disliking extremely and 5, for liking extremely. Rinse your mouth with clean water after tasting each food.

1. Dislike extremely
2. Dislike slightly
3. Neither like nor dislike
4. Like slightly
5. Like extremely

Sample Code	Appearance	Aroma	Taste	Texture
841				
869				
871				

Any other comments:____
____
____

B. Which product do you prefer most? ______________

C. Why did you prefer the flour?_____________________________________
____
____

5.0 Infant feeding observation

Provide porridge in graduated cup to the parent / guardian together with a plastic spoon. Ask the mother to feed the CHILD with porridge and take notes.

1. Watch the child's facial expressions as they first see the food. Record the child's expressions according to the scale (1-5) provided below:-
    1. Dislike extremely
    2. Dislike slightly
    3. Neither like nor dislike
    4. Like slightly
    5. Like extremely
2. Ask the mother to feed the baby. Observe and record expression as the child receives food first. Use scale (1-5) as below:-
    1. Dislike extremely
    2. Dislike slightly
    3. Neither like nor dislike
    4. Like slightly
    5. Like extremely

3. Observe and record expression as the child receives food in subsequent offers. Use scale (1-5) below:-
    1. Dislike extremely
    2. Dislike slightly
    3. Neither like nor dislike
    4. Like slightly
    5. Like extremely

4. Observe and record child's behaviour during subsequent offers of porridge
    1. Child signalling for more aggressively
    2. Child accepts porridge passively
    3. Child turns head away,
    4. Child spitting food,
    5. Child smiling while eating,
    6. Child crying, refusing to eat
    7. Child crying, but eats
    8. Child vomits
    9. Any other observations on the child's reaction to the food.
    ----

5. Write down any other comment made by the mother / carer or the child that are not captured above.

Appendix 8: Publications