FORTIFICATION OF MAIZE FLOUR WITH GRAIN AMARANTH FOR IMPROVED NUTRITION

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DECLARATION

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

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DEDICATION

This thesis is dedicated to my father, Peterson Kamotho, mother, Alice Wanjiru and sister, Ivy Wangari, for their love, encouragement and continuous support throughout my study.

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ABBREVIATION AND ACRONYMS

AACC	Association Association of Cereal Chemists
AAS	Atomic Absorption Spectroscopy
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ARLMP	Arid Land Resource Management Project
ASAL	Arid and Semi-Arid Land
DALYs	Disability Adjusted Life Years
FANTA	Food and Nutrition Technical Assistance
FAO	Food and Agriculture Organisation
GOK	Government of Kenya
HHI	Hidden Hunger Index
HIV	Human Immuno-Deficiency Virus
JKUAT	Jomo Kenyatta University of Agriculture and Technology.
KALRO	Kenya Agricultural and Livestock Research Organisation
KDHS	Kenya Demographic and Health Survey
KES	Kenya Shillings
LDL	Low Density Lipoproteins
PDA	Potato Dextrose Agar
TPC	Total Plate Count
VRBA	Violet Red Bile Agar
WFP	World Food Program
WHO	World Health Organization

ABSTRACT

Malnutrition, a widespread problem especially in developing countries, affecting all age groups particularly children and reproductive age women. Among the causes that have led to perennial malnutrition levels in Kenya, is the over reliance on starchy staples like maize, sorghum, rice and millet. These staples are characterized by low nutrient densities that are further lowered during processing. In a bid to combat the problem, the government targeted increased production of the micronutrient (minerals, vitamins) fortified maize flour. This is mostly based on chemical fortificants, a short term strategy aimed at increasing micronutrient intake. Therefore, there is need to think about long term and sustainable strategies such as food to food fortification. This study aimed at providing a better and more sustainable approach by fortifying maize based foods with grain amaranth. Maize was procured from National Cereals and Produce Board and processed to refined flour and whole meal flour while amaranth grain that had been toasted at 100ºC for 5 minutes and milled into flour was obtained from Annicos Limited. The refined maize flour and whole maize meal flour were then mixed with varying amounts of grain amaranth flour (0-40%). The proximate composition and mineral content of the raw materials (control samples) and the blends were determined using standard methods while protein digestibility was determined after enzyme digestion. Nutritional composition of amaranth grain flour differed significantly (p=0.001) from refined and whole meal maize flour. Amaranth grain flour was found to be superior in proteins (15.82%), lipids (7.61%), ash (2.54%) and fibre (4.39%) as compared to refined maize flour; proteins (6.29%), lipids (1.92%), ash (0.55%) and fibre (0.76%), and whole meal maize flour; proteins (9.81%), lipids (3.89%), ash (1.14%) and fibre (2.68%). Adding grain amaranth flour to refined and whole meal maize flour at the different ratios increased the nutrient density significantly (p=0.001), particularly protein, iron, calcium and zinc. However, it decreased the digestibility of protein significantly (p=0.001). The results indicate that although adding 40% grain amaranth flour to refined and whole meal maize flour gave the highest nutritional profiles, the most acceptable blends had 20% grain amaranth addition. Therefore, food to food fortification is an approach that can be adapted to meet the nutrition requirements of our society.

Key words: Nutrition, complementary, blending, amaranth flour, maize flour

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Malnutrition is defined as "a state of nutrition in which a deficiency, or excess, of energy, protein and micronutrients causes measurable adverse effects on tissue/body form (body shape, size and composition) and function, and clinical outcome" (Stratton *et al*., 2003). Protein-energy malnutrition and micronutrient malnutrition are the common forms of malnutrition that result in deficiencies with adverse effects, ranging from mild to severe malnutrition. Policies and programs aimed towards reducing malnutrition include food based strategies such as food fortification, dietary diversification and nutrition education and non-food based strategies such as supplementation (WHO and FAO, 2006). In the past, food based approaches have become attractive options towards increasing micronutrient uptake due to increased sustainability. In particular, food fortification is the process in which micronutrients such as minerals and vitamins are added to food materials. This is increasingly gaining importance as channeling interventions through food vehicles that are important to a population reduces deficiencies and enhances effectiveness of the programs (WHO and FAO, 2006).

Maize flour is among the main vehicle in fortification programs, for it is the staple food in many African countries, thus increased possibilities of nutritional interventions. In Kenya, "the food, drugs and chemical substances act of 2015 requires all packaged wheat flour, maize meal, salt and cooking fats and oils to be fortified with basic nutrients" (GOK, 2015). As a result, maize flour is fortified with vitamins A, B_1, B_2, B_6, niacin and folate; iron and zinc. However, concerns on bioavailability, lipid oxidation and sensory quality resulting from fortification have previously been raised (Hurrell *et al*., 1991). Despite fortification being a great tool towards mitigating malnutrition and more so to the vulnerable groups, the use of chemical fortificants is a short term measure that targets reducing the level of micronutrient malnutrition. Therefore, there is need to think about long term and sustainable strategies. In this research, a food to food fortification approach is used in which grain amaranth is used to fortify maize flour.

Amaranth grain has a high nutritional value (Kariuki *et al.*, 2013), thereby providing most of the nutrients required by the body. Additionally, it contains exceptionally complete protein for plant sources. In particular, it has a relatively high proportion of lysine, an essential amino acid, compared to other foods, leading to its effective utilization as a protein source. Besides protein, amaranth grain is a good source of fats, carbohydrates, sugars, vitamins such as A, C, E and folic acid as well as minerals such as calcium, iron, magnesium, phosphorous, copper and manganese. It contains fairly high levels of poly-unsaturated fats (Kariuki *et al.,* 2013; Mburu *et al*., 2011)

The grain is also high in dietary fibre, a leading factor in prevention and treatment of chronic diseases (Silva-Sanchez *et al*., 2008) lowers blood cholesterol levels (Saunders and Becker, 1983), lowers the risk of coronary heart disease and high blood pressure, enhances weight control and reduces the risk of intestinal cancer by improving digestive functions (Martirosyan, *et al*., 2007). Therefore, it is anticipated that blending maize flour with grain amaranth will result in a more sustainable food fortification strategy since grain amaranth is locally available and the nutritional outcomes are likely to be better.

1.2 Problem Statement

Malnutrition is a widespread problem in Kenya manifested mainly through under-nutrition (protein-energy malnutrition) and micronutrient deficiencies. It is associated with devastating consequences including weakening the immune systems and worsening illness. On a national level, 26% of children under 5 are stunted, 11% are underweight and 4% are wasted (KNBS, 2015). In fact, in terms of proportions, Nairobi is leading since a study conducted in two urban slums in Nairobi indicated that close to 40% of the children were stunted (Abuya *et al.,* 2012). In addition, children are the most vulnerable group with others being individuals that are pregnant, lactating mothers, the elderly and refugees (FAO, 2005).

Maize is the main staple food in Kenya, with others being sorghum, rice and millet. The protein quality in these cereals is inadequate, especially in essential amino acids. Milling of maize involves removal of bran. This further reduces the nutrient density of the end product since fibre, some vitamins and minerals are lost in the process. Some of the micronutrients affected are zinc, calcium and iron (FAO, 1997).

In rural populations, cases of repeated consumption of the staple food on all the 3 daily meals in a household over prolonged periods are common. Although they provide the body with the required energy, they are largely deficient in some of the nutrients, therefore the need to diversify our meals. It is therefore important to stress the need to educate families to exploit locally produced foods to produce nutritionally adequate products (WHO and FAO, 2006).

Existing efforts for food fortification largely depend on adding specific minerals. However, the amounts added may either be too little or above the required limit set by Kenya Bureau of Standards. This might lead to other unknown health problems since fortification of maize flour is still at its infancy. Moreover, compared to blended foods, chemical fortificants may pose a threat to the health of Kenyans if not added in the right amounts.

1.3 Justification

The use of chemical fortificants is a short term measure which targets reducing the level of malnutrition while creating better livelihoods. On the other hand, food to food fortification and diet diversification are better and more sustainable approaches towards meeting the nutrition requirements. These approaches are food based and targets increasing the nutritional benefits to the consumer. In this research, grain amaranth has been selected as the fortificant of choice because it does not only address the micronutrient deficiency but also the protein deficiency (Mugalavi *et al*., 2013). Furthermore, it is governmental policy to fortify all packaged maize meal (GOK, 2015).

Amaranth grain contains exceptionally complete protein for plant sources. In particular, it has a relatively high proportion of lysine, an essential amino acid, compared to maize, leading to its effective utilization as a protein source (FAO, 1997; Kariuki *et al*., 2013). It is also a good source of fats, carbohydrates, fibre, sugars, vitamins such as A, C, E and folic acid and micronutrients such as iron, calcium and zinc (Kariuki *et al*., 2013).

The grain also thrives in poor soils and arid conditions (Kauffman and Weber, 1990). This means a lot of arid and semi-arid areas can be converted to amaranth producing zones without compromising maize production. It is also a fast growing crop with the ability to perform under marginal conditions (Muriuki *et al.,* 2014). The crop requires 40-50% less moisture as compared to other cereals e.g. maize and survives better than most crops under dry and hot conditions. Harvesting of the grain occurs between 60-90 days, while that of the leaves as from 4 weeks. In addition, the grain stores very well once it is well dried and is not easily infested by pests (Muyonga *et al*., 2008). In Kenya, production of grain amaranth has been demonstrated and a number of high yielding and nutritious grain amaranth varieties already identified (Kariuki *et. al.,* 2013)

1.4 Objectives

1.4.1 Main Objective

To improve the nutritional quality of maize based diets by blending maize flour using grain amaranth flour

1.4.2 Specific objectives

i. To carry out a feasibility study on the purchase of grain amaranth and its products in supermarkets and companies
ii. To determine the nutritional value of refined maize flour, whole meal maize flour and their respective amaranth flour blends
iii. To evaluate the protein digestibility of the processed and optimized blends
iv. To determine the shelf life and sensory acceptability of the developed products

1.5 Hypothesis (Ho)

i. Grain amaranth is not purchased in supermarkets and companies
ii. Raw grain amaranth does not have a high nutritional content as compared to maize flour
iii. Blending of maize with amaranth flours improves protein digestibility
iv. Blending of the flours does not affect the sensory characteristics and shelf life of the developed products

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

2.1.1 Overview of Food Security

Food security is defined as "condition where all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (World Food Summit, 1996). Globally, it is estimated that two billion people are affected by chronic deficiencies of micronutrients, collectively known as hidden hunger (WHO, 2002; WHO, 2004; WHO, 2008 and WHO 2009). The most widespread micronutrient deficiencies in the world are of iron, zinc, vitamin A, iodine, and folate. Developing countries are the most affected by food insecurity with factors such as high prices of food commodities and policy barriers elevating the food crisis (Weaver, 1994). This is evident for multiple micronutrient deficiencies often occur together in the same population (Allen *et al*., 2009). Young children, women, and refugees especially those living in low-income countries are among the most vulnerable. These deficiencies account for approximately 7% of the global disease burden annually (Ezzati *et al*., 2004).

It has been reported that deficiencies of vitamin A and zinc were responsible for 0.6 million and 0.4 million child deaths respectively, and a combined 9% of global childhood Disability Adjusted Life Years (DALYs) (Black *et al*., 2008). Iron deficiency was associated with 115,000 maternal deaths (Ezzati *et al*., 2004). These deficiencies mainly lead to cognitive impairment, poor physical growth, increased morbidity from infectious diseases in infants and young children, and decreased work productivity in adulthood (Elmadfa *et al*., 2003). Generally, the effects of prolonged deficiency tend to be irreversible (Mclaren and Frigg, 2001). In Africa, Kenya ranked second to Niger with the highest Hidden Hunger Index (HHI) score for preschool-age children (Muthayya *et al*., 2013). The HHI was estimated based on national estimates of the prevalence of stunting, anemia due to iron deficiency, and low serum retinol concentration. On a national level, 26% of children under five are stunted, 11% are underweight and 4% are wasted (KNBS, 2015). In ASAL regions, under nutrition rates have been reported to be high with 80-98% of the children being iron deficient (ARLMP, 2008; GoK, 2008).

2.1.2 Strategies to address micronutrient deficiencies

Adoption of a comprehensive approach is key in reducing malnutrition and hidden hunger. Therefore, this indicates the importance of promoting activities that increase accessibility, sufficiency and utilization of adequate quantity and quality of foods. Policies and programs aimed towards reducing malnutrition include food based strategies such as food fortification, dietary diversification and nutrition education and non-food based strategies such as supplementation (WHO and FAO, 2006). The application of a strategy is highly dependent on the conditions and needs of a targeted population.

Currently, food based approaches such as food fortification and diet diversification are becoming more attractive options towards increasing micronutrient uptake due to increased sustainability. Food fortification is the process in which micronutrients such as minerals and vitamins are added to food materials. This is increasingly gaining importance as channeling interventions through food vehicles that are important to a population reduces deficiencies. Food fortification can be achieved through the use of micronutrient powders as well as food to food fortification (Mulokozi *et al*., 2004; Horton *et al*., 2008).

Diet diversification involves increasing the range of foods consumed. It can be achieved through foods of plant or animal origin Increasing dietary diversity is advantageous as it does not only improve the micronutrient intake but also the overall nutritional status. However, limitations of acceptance and change in behavioral patterns in the population remain a challenge. Previously, studies by FANTA and FAO (2007) showed that dietary diversification is a good indicator of nutrition security and is associated with lower morbidity. Nutrition education through creating awareness of these foods from production to consumption is important in the implementation of diet diversification. This is because inclusion of a wide variety of foods in the diet facilitates adequate nutrient intake (Hotz and Gibson, 2001).

Micronutrient supplementation is achieved through the use of mainly tablets, capsules, oil solutions or powders or syrups (WHO and FAO, 2006). Supplementation programmes are widely applicable especially in developing countries providing micronutrients to pregnant women, children under 5 years and postnatal depressed women. Supplements for fat solubles vitamins are consumed 2-3 times a year while those of water solubles are more frequent. For an effective supplementation program, purchases, distribution systems and consumer compliance are key (WHO and FAO, 2006).

The use of chemical fortificants is a short term measure which targets reducing the level of malnutrition. This strategy has been in existence and found not sustainable for it is financially expensive and therefore not accesible to all populations in the country (GOK, 2015). However, excessive intake of synthetic form of micronutrients reportedely causes toxicity of micronutrients (Alais and Linden, 1991). Although the use of supplements is often the fastest at increasing micronutrient intake, food based strategies are viewed to be more sustainable in the long run (FAO, 1997; Hotz and Gibson, 2001; Oniang‘o, 2001; Yadav and Sehgal, 1995).

2.2 Maize

2.2.1 Origin of maize

Maize (Z ea mays ssp. mays) belongs to the tribe Maydae, family Poaceae and was originated in Mexico and Central America. It possesses somatic chromosome number of 20, a genome size of 2.3 gigabase and more than 32,000 genes (Schnable *et al*., 2009). Maize grows well in various agroecologies and is unparalleled to any other crop due to its ability to adapt in diverse environments. It has emerged as a crop of global importance owing to its multiple end uses as a human food and livestock feed and serves as an important component for varied industrial products. Besides, maize serves as a model organism for biological research worldwide.

2.2.2 Maize as a staple food

Maize ranks as the second most widely produced cereal crop in the world after wheat (Johnson, 2000). More than 90% of the white maize is produced in the developing countries. Main producers include South Africa, Zimbabwe, Kenya, Malawi, Tanzania and Zambia. Maize meal flour is the dominant staple food in Eastern and Southern Africa (Jayne *et al*., 1996) where white maize varieties are used more for human consumption than yellow varieties (FAO, 1997). White maize represents approximately 90 percent of total cereals production. It plays a major role in the diet primarily in sub-saharan Africa and parts of Central America. In Africa, maize is mainly used for human consumption with other uses being livestock feed production and brewing. Data for between 1995 and 1997 indicates that Eastern and Southern Africa used an average of 72%, Western and Central Africa used 66% and North Africa used 45% of the total maize national requirements for human consumption (Aquino *et al*., 2000).

Maize flour is among the main vehicle in fortification programs for it is the staple food in many African countries thus increased possibilities of nutritional interventions. It provides the body with significant amounts of nutrients, particularly calories. In Kenya, maize meals are being fortified with vitamins A, B_1, B_2, B_6, niacin and folate; iron and zinc mainly based on the use of micronutrient powders (GOK, 2015).

2.2.3 Maize grain

Maize remains an important cereal, especially in Kenya where white maize is highly consumed. The major parts of the maize kernel are the endosperm which contains mostly of starch and the germ which contains most of the oil. Maize is processed by four main methods: wet milling, the dry grind process for ethanol production, dry milling and alkaline processing. Alkaline processed and dry milled maize goes directly for human consumption (Watson & Ramstad, 1987; Shukla & Cheryan, 2001).

There are two different systems used to dry-mill the maize grain: Non-degerming and Degerming (Johnson, 2000). The non-degerming system involves grinding of the maize grain into meal with very minimal levels of separation (Johnson, 2000). This process results in whole meal which contains the bran, germ and endosperm similar to the proportions found in the whole kernel. Refined meals are produced through the degerming system by removing part of the germ and bran, resulting in a lower extraction rate than whole meals. Super-refined meal is highly-refined and with much lower extractions rates than refined meals (Jayne *et al.*, 1996). Maize grains contain about 7.9% protein as shown in Table 1 which is almost the same as other cereal grains. Most of this protein is in the form of zein, a poor-quality protein containing only small amounts of lysine and tryptophan.

Table 1: Nutrient content of maize

Nutrient	Refined meal		Whole meal	Nutrient	Refined meal		Whole meal
	Extraction rates				Extraction rates		
	65%	85%	96-99%		65%	85%	96-99%
Protein	7.9	9.3	10.0	Calories (Kcal)	334	341	343
Fat	1.2	2.4	3.8	Calcium (mg)	6.0	7.0	12.0
Carbohydrates	78.4	75.1	73.4	Iron (mg)	1.1	2.0	2.5
Fibre	0.6	1.1	1.9	Thiamin (mg)	0.14	0.30	0.35
Ash	0.5	0.7	1.3	Riboflavin (mg)	0.05	0.08	0.13
				Niacin (mg)	1.0	1.8	2.0

Source: West *et al.*, 1987

Milling reduces the nutritive value of maize just as it does that of other cereals. This means that the increased popularity and use of highly milled maize meal as opposed to traditionally ground or lightly milled maize results in deficiency of B vitamins. However, vitamin B constituents lost in milling, as shown in Table 2, may be replaced in maize meal, as in other cereal flours, by fortification (FAO, 1997). Maize is not a common allergenic food, although in some case-studies, allergic reactions were reported (Hefle, 1996). These reported allergic effects for maize include skin, gastrointestinal and respiratory complaints.

In Kenya, urban sifted maize meal consumption is highly consumed due to its taste and cooking attributes as compared to whole meal (Jayne and Argwings-Kodhek, 1997). The success of higher consumption of the sifted maize results from wide range of advertisements by large scale milling industries. Currently, more households are purchasing sifted maize meal due to convenience as compared to whole meal (De Groote and Kimenju, 2012). Moreover, more of those who consumed whole meal chose it because it was cheaper and nutritious. This indicates that many urban households were already aware of whole meal's superior nutritional quality at the time of this survey (Mukumbu and Jayne, 1995).

Table 2: Effects of milling on vitamin B content of maize

Level of processing of maize	Thiamine (mg/100g)	Riboflavin (mg/100g)	Niacin (mg/100g)
Whole grain	0.35	0.13	2.0
Lightly milled	0.30	0.13	1.5
Highly milled (65 percent extraction)	0.05	0.03	0.6

Source: FAO 1997, Human nutrition in the developing world

2.2.4 Importance of maize meal in Africa

At the household level, maize meal is consumed as dough or thick porridges. This is quite common in Southern Africa (Van der Merwe *et al*., 2001); in Zambia (Laleye and Wesley, 2001), in Zimbabwe, South Africa, East Africa including Kenya, Tanzania, Uganda, Burundi, Rwanda, and Democratic Republic of the Congo, West Africa particularly in Nigeria (Tembo, 2007) and light porridges at breakfast (Sammon, 1999). Indeed, maize meal porridge is used as complementary food for infants in many African countries (Lartey *et al*., 1999; Huffman *et al*., 2000; Mamabolo *et al*., 2004; Faber *et al*., 2005). For example Eastern and Southern Africa, fermented starch-based porridges are particularly popular; uji, togwa, kenkey, mahewu, ogi and enjera (spongy bread) (Steinkraus, 1996).

Industrial food applications of maize meal include its use in production of non-alcoholic fermented products such as commercial production of ‘mageu’ (Holzapfel, 1989). Maize meal is also used in the commercial production of African alcoholic products at industrial level. Factory brewers almost invariably use maize grits (or maize meal) as starchy adjuncts (Haggblade and Holzapfel, 1989).

On the other hand, extrusion products are beginning to have a huge market in Africa, especially for children’s ready-to-eat snacks. Maize flour is one of the major ingredients in extruded products (Martinez-Bustos *et al*., 1998; Onwulata *et al*., 2001a, 2001b; Wen *et al*., 1990; Zhang and Hoseney, 1998). Maize flour is also used extensively in animal feeds as an energy source in Africa. In livestock feeding, yellow maize is preferred because it gives poultry meat, animal fat and egg yolk the yellow colour appreciated by consumers in many countries.

2.3 Amaranth

2.3.1 Origin of amaranth

Amaranthus is an annual herb that belongs to the family Amaranthaceae (Stephens, 2009). There are more than 60 amaranth species and between 4000 – 6000 varieties (Yarger, 2008). Amaranth is native to Central and South America and considered among the oldest cultivated plant species. It dates back to Mayan, Incan and Aztec civilisations having been cultivated for more than 8,000 years (Yarger, 2008). The grain amaranth was a staple food to the Aztecs and was included into their religious ceremonies until in the 1516 when the spaniards conquistadors prohibited its production (O’Brien and Price, 1983). In the past 30 years, amaranth has gained more attention and it has been shown to hold many unique nutritional qualities (Kariuki *et al*., 2013; Njoki *et al.*, 2014). Amaranth species thrives mostly in tropical and temperate regions. The crop is cultivated in the world as cereals, leaf vegetables, and ornamental plants, while others grow naturally as weeds (Railey, 1993).

In the recent past, production and consumption of grain amaranth varieties was limited to only a few areas in the Nyanza region (Yongo, 2009). Crop production is now widespread in the country; grown for both subsistence and cash purposes.The crop mainly grows naturally in open fields especially in the rural areas. Average yields in farmers' fields have ranged from 0.25 to 1 tonne/hectare. This is highly dependent on the weather patterns and cultural practices (Grubben and van Sloten, 1981; O' Brien and Price, 1983). Generally, amaranth leaves were more commonly consumed than the grains until recently (Alemu, 2005). There are two main grain amaranth varieties grown, the short and tall varieties. The short varieties are better suited for low rainfall areas while the tall varieties are for high rainfall regions. Some of the vegetable species grown are *Amaranthus cruentus*, *Amaranthus blitum*, *Amaranthus dubius* and *Amaranthus tricolor* while the grain species are *Amaranthus hypochondriacus*, *Amaranthus cruentus* and *Amaranthus caudatus*. The colour of the grains range from white, yellow, black or pink (Railey, 1993). Most of the ethnic groups in Kenya have a name for the vegetable amaranth. Kikuyu's call it *Terere*, Waswahili's *Mchicha*, Luhya's *Omboga*, Luo's *Ododo*, Pokot's *Sikukuu*or *Chepkuratian*, Turkana *Lookwa* or *Epespes* and Teso *Ekwala* (Alemu, 2005).

2.3.2 Production of grain amaranth

a) Ecological requirements

Grain Amaranth grows in a wide range of soil conditions. It grows in soil pH ranging from 4.5 – 8.0. It can also grow in loam soils and silty loams with good water holding capacity. The optimal growth temperature ranges from 22 - 30ºC with 15 - 17ºC being the least temperature for seed germination and biomass accumulation. Also, the growth of is optimal under humid conditions and do well under rainfed as well as irrigation. The grain is characterised with large flower heads laeding to high evapotranspiration rates thus the need to water for improved production (Mwangi, 2003). Adequate soil moisture is key during germination and also in the first weeks of growth. Temperatures above 35ºC cause a decline in seed yield due to decreased seed germination (Modi, 2006). In the tropics, it grows at altitudes of 1000 m to 3500 m. Although the optimal conditions, it is extremely adaptable to adverse growing conditions. This is seen by its ability to tolerate drought and soils with low fertility. Grain Amaranth is drought tolerant for it has deep roots which extend down up to 2 metres in search of water. During high temperatures, its C_4 photosynthetic pathway is highly efficient in utilization of light and nutrients. In extreeme cases the plant has the ability to go dormant (O'Brien and Price, 1983) and recovers easily when moisture is available (Myers, 1996). Grain amaranth water requirements are lower as compared to other cereals such as wheat and maize (Mwangi, 2003). It does well under conditions ideal for maize (O'Brien and Price, 1983) thus possibilities of intercropping.

b) Grain preparation

Grain amaranth generally takes about 7-8 weeks to mature. However, this is highly dependent on the variety type whereby short varieties mature in 45 – 60 days while tall varieties take 70- 120 days. Mature seeds hang very loosely on husks when they are ready for harvest. Also, no liquid oozes out from the seeds when crushed (Myers, 1996). Harvesting of the mature seeds is followed by winnowing whereby the seeds are cleaned and foreign materials removed. The grain should be dried to a moisture content not exceeding 13% to limit growth of moulds and enhance the keeping quality. The grains can be stored in clean plastic paper bags in a cool room where rodents are completely avoided and with adequate ventilation. Sisal bags, which are widely used for storage of grains, are also quite suitable. Most stored grains are usually susceptible to infestation; however, due to their small size, infestation by weevils and grain borers is greatly reduced. With correct storage conditions, post-harvest losses are minimal and the grain can be stored up to seven years (Weber, 1987; Myers and Putnam, 1988).

c) Processing of grain amaranth

The amaranth grain is processed under conditions that do not damage its protein and its essential amino acids availability, like moist heat cooking and extrusion, presents good protein quality, similar to casein (Mendonza and Bressani, 1987). The digestibility and the protein efficiency ratio are improved if the grain is heat processed (Kauffman and Weber, 1990). Heat removes lectins and improves the protein efficiency ratio of the amaranth flour (Singhal and Kulkani, 1988).

There are a number of viable methods for processing, including popping, toasting, heat-rolled flakes, extrusion, and wet cooking as gruel. Excessive thermal processing reduces the quality of amaranth grain (Bressani and Elias, 1984). Processing methods involving heat reduce the anti-nutrirent content especially moist heat (Njoki et al., 2014).

Processing is important with respect to the protein quality of amaranth grain. According to Pederson *et al.*, (1987), processing increased the protein quality of amaranth grain. However, if the processing is carried out under more extreme conditions of time and temperature, it destroys the quality of the product by reducing available lysine content (Pedersen *et al.*, 1987). Of interest is the extrusion process, which for *A. cruentus* and *A. caudatus* yielded cooked flour equal in protein quality to casein (Mendoza and Bressani, 1987).

2.3.3 Utilization of amaranth

a) Amaranth grain

Amaranth grain flour is obtained through milling by the use of a mechanized mill. Other milling methods such as the use of a pestle and mortar or grinding using a stone can also be used. Generally, at the household level, milling is done on small scale so as to avoid spoilage of the flour. The main disadvantage of milling is grain loss during the process. This is because the amaranth grains are quite small that sometimes they come out of the milling machine. To curb this, amaranth is milled with other grains such as maize, millet and sorghum among others to produce composite flour. The flour can be used to cook porridge and to make tortillas, pinole or toasted meal, confectionery, pastries, biscuits, atoles, small savoury pancakes, desserts and bread (Muyonga *et al*., 2008; Yarger, 2008). Soups and stews can also be made from whole grain; *alegria*, a confection made from popped amaranth in Mexico; *atolea*, a fermented Mexican drink made from roasted amaranth flour; *chichi*, which is a form of beer made from amaranth in Peru; *sattoo*, a gruel consumed in Nepal, and *chapatti* made in different parts of Asia (Kauffman and Weber, 1990).

Grains can be popped at temperatures of about 220ºC for 10 – 15 seconds resulting in increase of volume of upto 1,050 %. Popped amaranth can be used as breakfast cereals with milk as well as in confections bound with sorghum, molasses or honey (Muyonga *et al*., 2008). The seeds can also be sprouted before milling. Sprouting increases digestibility and bioavailability of nutrients (Colmenares De Ruiz and Bressani, 1990). Roasting of the grains can be done in an oven at around 120°C for 5 - 10 minutes before milling. The toasted seeds are brownish and give a nutty flavour as well when milled. The grains can also be fermented or malted for beer production.

b) Amaranth leaf

Amaranth leaf is highly nutritious and rich in micronutrients such as zinc, folic acid, vitamin E, B vitamins, ascorbic acid, selenium, β - carotene, calcium and iron (Funke, 2011; Mwangi, 2003; Yadav and Sehgal, 1995; Yarger, 2008; Muriuki *et al*., 2014.). The leaves, often freshly picked, are widely used as vegetables for human consumption. Mostly, they are normally cooked like spinach and other greens (Muyonga *et al*., 2008; Yarger, 2008). Amaranth leaves do not differ significantly with other green vegetables in terms of appearance, texture and flavour except for nutritive value (Mwangi, 2003). In fact, cooking and discarding the water removes potentially harmful oxalates and nitrates (Yarger, 2008). On the other hand, fresh amaranth leaves can be dried and stored using appropriate technology to ensure availability throughout the year (Masarirambi *et al*., 2010). The root of mature amaranth can also be used in making of soups, to prepare curries and also as a stir-frying vegetable.

c) Animal feed

Amaranth can also be used in feeding of animals. The foliage is used in stock breeding as a green fodder, silage component and for obtaining protein-vitamin flour and concentrates (Ofitserov, 2001). Research has shown that the use of cooked or autoclaved amaranth grain as chicken feed gives good production results (Yarger, 2008). For instance, in China, forage is fed to hogs, rather than harvesting the grain (Myers, 1996; National Academy of Sciences, 2006).

2.3.4 Amaranth grain market outlets in Kenya

Amaranth grain markets in Kenya could be divided into four main categories; household consumer, institutional consumer, industrial consumer and animal feeds markets (Bahilgwa, 2006). At the household level, the exact quantity of amaranth grain consumed in Kenya is not well known. Generally, the market is very small as compared with the other market outlets (institutional consumer market, industrial consumer market, and livestock feeds market). There are also a few middlemen who buy the grain from the farmers and market it to the larger companies. Apart from these few big brokers, there is not much wholesaling of amaranth grain in the country because the sub-sector is still small at the moment.

With respect to amaranth grain products of human food processors (e.g. Annicos limited, Kirinyaga flour mills etc.) and human food and livestock processors, there is a relatively high intensity of wholesaling. Human food processors make wholesales to institutional consumers such as world food program (WFP) for refugee feeding. Similarly, the human food and livestock feed processors make wholesales to human food retailers such as the supermarkets and also to livestock feed.

Certified amaranth grain seeds of different varieties can be acquired in most regions of the country. Various research institutions such as KALRO are carrying out more adaptive research on amaranth grain such as production of high quality seeds. KALRO also provides improved amaranth seed varieties.

2.3.5 Nutritional value of grain amaranth

The nutritional composition of different varieties of the grain has been extensively studied (Petr *et al*., 2003; Kariuki *et al*., 2013). Variations in the nutritional composition can be attributed to the species grown, the geographical location, harvesting season and fertilizer application among others. The protein content ranges from 12-19%, which is higher than most grains except in soybean that contains about 37% (Akpagu *et al.,* 2015). Its protein quality is characterized by the high levels of the essential amino acid lysine, methionine and cysteine which are in low levels in commonly consumed cereal grains. The average protein content of commonly consumed grains such as wheat, maize and rice ranges from 6 – 14% (Goldberg, 2003).

The lipid content of grain amaranth ranges from 5.4 - 10% which is almost 3 times higher when compared to other cereal grains (Kariuki *et al*., 2013). Studies indicate that the grain contains a high amount of unsaturated fatty acids with linoleic acid (35-55%) and oleic acid (18-38%) dominating. The saturated/unsaturated fatty acid ratio ranges from 0.29 to 0.43. Linoleic acid is an essential fatty acid which is important in human nutrition especially in children for proper growth and development. Stearic acid (3-4%) and palmitic acid (20-23%) were found to be the dominating saturated fatty acids (Berganza *et al*, 2003; Escudero *et al.*, 2004; Kariuki *et al*., 2013).

The grain is also high in micronutrients such as especially vitamin A, iron and zinc (Caulfield *et al*., 2006). The carbohydrates in grain amaranth consist primarily of starch made up of both glutinous and non-glutinous fractions. Starch granules of the amaranth grain are much smaller (1 to 3 µm) compared to other cereal grains such as maize (5 to 25µm). Due to the size, the grain exhibits greater water-binding capacity, higher swelling power, lower gelatinization temperature and high resistance to amylases and could therefore benefit the food industry. For instance, studies have been done on use of grain amaranth starch in food preparation such as custards have been done (Singhal and Kulkarni, 1990a; Singhal and Kulkarni, 1990b). The grain does not contain gluten. Allergens are also not observed in the grain thus making it an alternative source for non-allergenic food products (Thanapornpoonpong, 2004).

Table 3: Nutrition profile of amaranth grain

Nutrients	Amount (%)	Nutrients	Amounts (IU/mg/g)
Proteins	19	Vitamin A	6100
Fibre	8	Vitamin B1	1.29
Minerals	3.1	Vitamin B2	2.1
Carbohydrates	65	Vitamin B3	8.4
Unsaturated oils	8	Vitamin C	4.63
Moisture	9	Folic	49.0
		Vitamin E	1.03

Source: Pedersen, *et al.*, 1990

2.3.6 Nutrition and health benefits of grain amaranth consumption

Consumption of grain amaranth has a range of nutritional and health benefits. Some of them include general improvement in well-being to prevention and improvement of specific ailments and symptoms, recovery of severely malnourished children and an increase in the body mass index of people formerly wasted by HIV/AIDS (SRLP, 2005; Tagwira *et al*., 2006). Tagwira *et al*., (2006) documented perceived benefits of consuming grain amaranth among communities in Zimbabwe. The communities claimed that eating grain amaranth made them feel healthier and they noticed improvements in the health of their children. Specific health improvements noted included improvement in appetite, fast healing of mouth sores and herpes zoster, and weight gain for people living with HIV and AIDS. Amaranth consumption was also associated with higher milk production among breast feeding mothers. The improvements in general well-being and health reported by people who included grain amaranth in their diets are generally explainable by its high nutritional value (Tagwira *et al*., 2006).

Amaranth oil lowers total serum triglycerides and levels of low-density lipoproteins (LDL) in animals (Esculedo *et al*., 2006). Consequently, similar effects have been reported in humans (Martirosyan *et al*., 2007). High levels of serum LDL are associated with coronary heart disease. The serum LDL lowering effect of amaranth has been attributed by tocotrienols (unsaturated forms of vitamin E) and squalene in amaranth oil. This is because these compounds affect cholesterol biosynthesis in humans (Martirosyan *et al*., 2007).

Studies indicate the oil to have anti-tumor and antioxidative activity (Kim *et al*., 2006a), pointing to potential anti-cancer effects. In addition, supplementation of patients with coronary heart disease with amaranth oil has been shown to contribute to a decrease or disappearance of headaches, weakness, increased fatigue, shortness of breath during a physical activity, edema of the legs towards the evening hours and feeling of intermission of heart function in most patients (Martirosyan *et al*., 2007).

Consumption of grain amaranth has potential benefits to diabetics. Indeed, studies suggest that supplementation of diets with amaranth grain and amaranth oil improves glucose and lipid metabolism in diabetic rats (Kim *et al*., 2006b). Moreover, it also contains dietary fibers important in prevention of coronary heart disease and cancer of the colon. Consumption of the grain has been known to enhance human growth and development, improve general health and strengthen body immunity (Alemu, 2005; Legacy, 2003; Spetter and Thompson, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Summary of activities

Figure 1 below indicates the activities that were carried out.

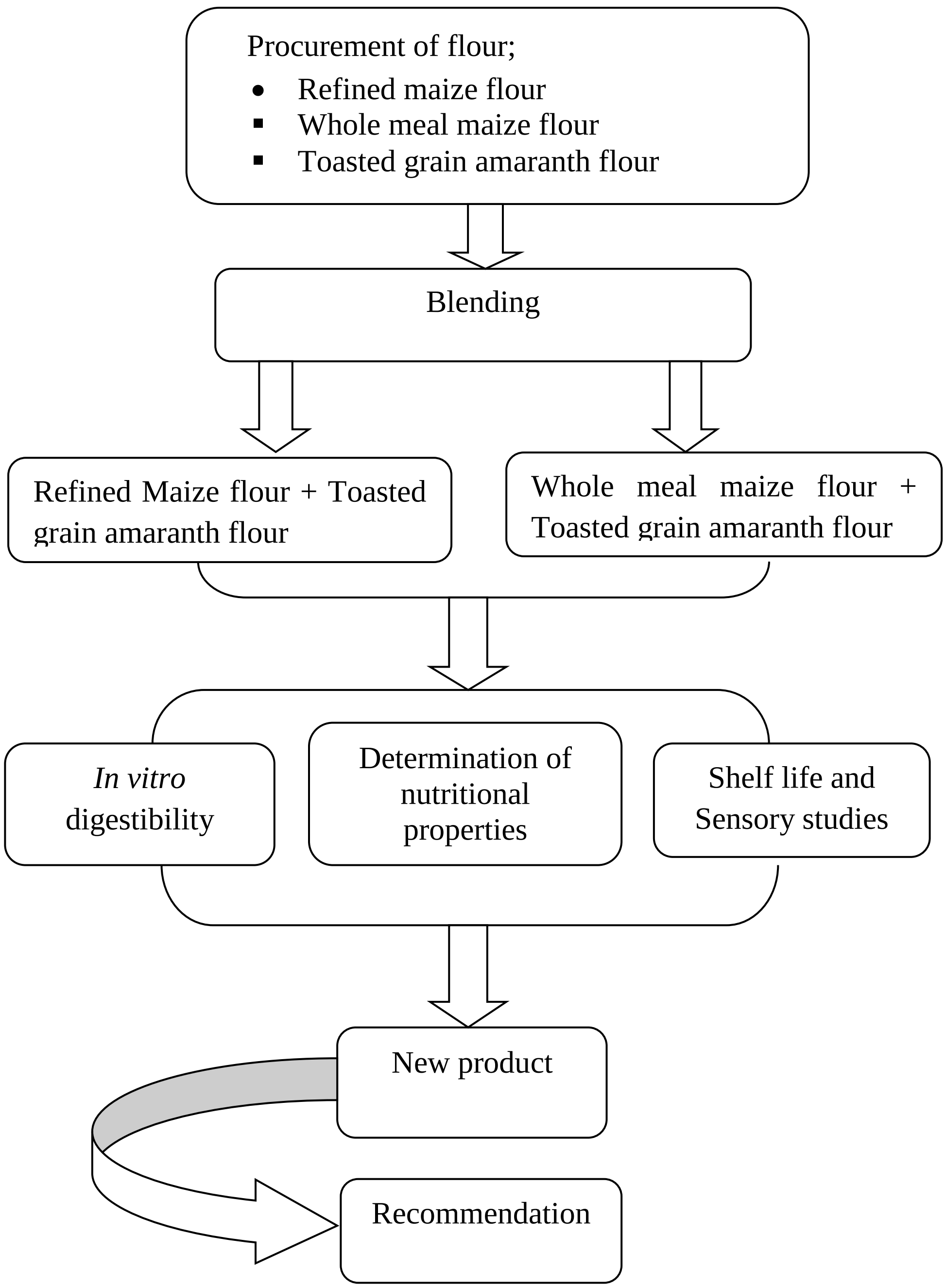


Figure 1: Flowchart of research activities

3.2 Data collection

3.2.1 Feasibility Study

A feasibility study was carried out in order to determine the popularity and the general public acceptability of grain amaranth. This was done by the use of semi-structured questionnaires (refer to appendixes 2 and 3) in supermarkets and industries respectively. The study was conducted in Nairobi region whereby three supermarket chains (*"Tuskys", "Uchumi" and "Nakumatt"*) were purposively selected based on popularity. This was then followed by a random selection of three supermarkets from each chain where the questionnaire was administered. "*Tuskys"* branches selected were *"Imara", "greenspan"* and *"adams". "Uchumi"* branches selected were *"capital centre", "Koinange"* and *"sarit centre"*. Lastly, *"Nakumatt"* branches selected were *"mega", "prestige"* and *"downtown"*. A total of 5 companies dealing with grain amaranth flour *("Kirinyaga millers", "Annico Limited", "Promote Amaranth", "Ngong Amaranth foods" and "Nature's Pure Health")* were also purposively selected and questionnaires administered.

3.3 Determination of optimal blends of maize and grain amaranth

Maize grains were procured from National Cereals and Produce Board in Nairobi and milled to whole meal flour and refined flour. Milling was done in Juja at *"Kwest Millers".* Amaranth grain which had been toasted at 100ºC for 5 minutes and milled into flour was obtained from *"Annico Limited"*. Complementary diets were then prepared by blending the flours. Both the whole meal flour and the refined flour were mixed with inclusions of 0, 20, 30 and 40% of the grain amaranth flour as shown in Table 4 and 5.

Table 4: Formulation of refined maize and amaranth flour blends

Flour type	RMF	RA1	RA2	RA3	GAF
Refined Maize Flour	100	80	70	60	0
Grain Amaranth Flour	0	20	30	40	100
Total Weight (g)	100	100	100	100	100

RMF- Refined Maize flour; GAF- Grain Amaranth flour; RA1- 80:20 (RMF: GAF); RA2-70:30 (RMF: GAF); RA3- 60:40 (RMF: GAF).

Table 5: Formulation of whole meal maize and amaranth flour blends

Flour type	WMF	WA1	WA2	WA3
Whole Meal Maize Flour	100	80	70	60
Grain Amaranth Flour	0	20	30	40
Total Weight (g)	100	100	100	100

WMF- Whole Meal Maize flour; GAF- Grain Amaranth flour; WA1- 80:20 (WMF: GAF); WA2-70:30 (WMF: GAF); WA3- 60:40 (WMF: GAF).

Chemical analyses were carried for raw materials (refined maize flour, whole meal maize flour and grain amaranth) and the blended samples each in triplicate.

3.4 Proximate analysis

3.4.1 Determination of moisture content

Moisture was determined according to Association of Official Analytical Chemists (AOAC) methods specification 950 46, method 925.10-32.10.03 (AOAC, 1995). Five grams of sample was accurately weighed into a moisture dish and transferred in a hot-air oven previously heated to around 105^{o}C and then dried for one hour. The final weight of the sample was then taken after drying and cooling in a dessicator. The residue was taken as the total solids and loss in weight as the moisture content of the sample. Calculation was done using the formula:

% Moisture = $\frac{W1-W2}{W1} \times 100$

W1 - Weight of sample before drying

W2 - Weight of sample after drying

3.4.2 Determination of protein content

Protein was determined using the semi-micro kjeldahl method, specification 950.46, method 20.87-37.1.22 (AOAC, 1995).

Sample weights of about 2 g were weighed into a digestion flask together with a combined catalyst of 5 g K_2SO_4 and 0.5 g of $CuSO_4$ and 15 ml of concentrated H_2SO_4. The mixture was heated in a fume hood until the digest color turned blue. This signified end of the digestion process. The digest was cooled, transferred to 100 ml volumetric flask and topped up to the mark with deionized water. A blank digestion with the catalysts was also made. Approximately 10 ml of diluted digest was transferred into the distilling flask and washed with about 2 ml of distilled water. Approximately 15 ml of 40% NaOH was then added and this washed with 2 ml of distilled water. Distillation was done to a volume of about 60 ml distillate. The distillate was titrated using 0.02N HCl to an orange color of the mixed indicator, which signified the end point. Calculation was done using the formula:

%N= $(\{(V1 - V2) \times N \times F \times 100\}/(V \times 100))/S$

V1 - Titer for sample in ml

V2 - Titer for blank in ml

N - Normality of standard HCl solution (0.02)

F - Factor of the standard HCl solution

V - Volume of diluted digest taken for distillation (10 ml)

S - Weight of sample taken (1 g)

% Crude protein = %N × PF

Protein factor (PF) = 6.25

3.4.3 Determination of crude fat content

Crude fat was done using the soxhlet method 920.85-32.1.13, (AOAC, 1995). This gave an intermittent extraction of oil with excess of fresh condensed organic solvent used. Approximately 5 g of sample was weighed into extraction thimbles and initial weight of extraction flasks taken. Fat extraction was done using petroleum spirit in Soxhlet apparatus for 8 hours. The extraction solvent was rotary evaporated and the fat extracted dried in a hot air oven for 15 minutes before the final weight of flasks with extracted oil taken. Calculation was done using the formula:

% Crude fat= $(W1/W2) \times 100$

W1 - Weight of fat extracted

W2 - Weight of the sample

3.4.4 Determination of crude ash

Ash content was determined by incinerating in a muffle furnace according to method 923.03-32.1.05, (AOAC, 1995). Sample weights of about 5 g were weighed in pre-conditioned crucibles. First, the sample was charred by a flame to eliminate carbons before being incinerated at 550^0C in a muffle furnace, to the point of white ash. The residues were cooled in a dessicator and the weights taken. Calculation was done using the formula:

% Crude ash = $(W1/W2) \times 100$

W1 - Weight of ash

W2 - Weight of the sample

3.4.5 Determination of crude fiber

Crude fiber was determined according to (AOAC, 1995, Method 920.86-32.1.15). Approximately 2 g of sample was weighed into a 500 ml conical flask. About 200 ml of boiling 1.25% H_2SO_4 was added and boiled for 30 minutes under reflux condenser. Filtration was done under slight vacuum with Pyrex glass filter (crucible type) and the residue washed to completely remove the acid with boiling water. Approximately 200 ml of boiling 1.25% NaOH was added to the washed residue and boiling done under reflux for another 30 minutes. Filtration was done using the same glass filter previously used with the acid. The residue was rinsed with boiling water followed by 1% HCl and again washed with boiling water to rinse the acid from the residue. The residue was washed twice with alcohol and thrice with ether. It was then dried in a Hot-air oven at 105ºC in a porcelain dish to a constant weight. Incineration was then done in a muffle furnace at 550ºC for 3 hrs and the dish cooled in a dessicator. The final weight taken and calculation was done using the formula:

% Crude fiber=$(\mathrm{W1} - \mathrm{W2})/\mathrm{W} \times 100$

W1 - Weight of acid and alkali digested sample

W2 - Weight of incinerated sample after acid and alkali digestion

W - Weight of sample taken.

3.4.6 Determination of total carbohydrate

Carbohydrate was estimated by difference based on Müller and Tobin (1980) method.

Total carbohydrates % $=100 - (Moisture\ content + crude\ protein + crude\ fiber + ash + crude\ lipid)$

3.5 Determination of mineral content

Mineral analysis was determined according to AOAC (1995) method. The ash that was previously determined (refer to 3.4.4) was cooled. This was then followed by addition of 15 ml of 6N HCl to samples in crucibles before transferring to 100 ml volumetric flasks. Distilled water was used to top up to the mark (100 ml). A standard solution of calcium, iron and zinc at different concentrations was prepared and was subjected to atomic absorption spectrophotometer Standards of (refer to appendix 1). Atomic Absorption Spectroscopy (AAS) was used for all the minerals (Model A-6200, Shimadzu, Corp., Kyoto, Japan).

3.6 Determination of *in vitro* protein digestibility

The pepsin digestion method was used based on that of Hamaker *et al*., (1987). Accurately weighed samples (200 mg) were digested with P7000100G pepsin, activity 863 units/mg proteins for 3 hours at 37°C and products of digestion was pipetted off using a Pasteur pipette. The residues were washed with distilled water and clear supernatant pipetted off. The residues were then dried in an oven at 100°C overnight. The residual protein was determined by the Dumas combustion method (American Association of Cereal Chemists (AACC) International, 2000).

Protein digestibility (%)= $\{(\text{Total protein} - \text{Residual protein}) \div \text{Total protein}\} \times 100$

3.7 Shelf life determination based on microbial growth

3.7.1 Total plate count determination

Using the aerobic plate count technique on plate count agar, serial diluents in ratios of 1:10 using peptone water was done. Further stepwise serial dilutions were done appropriately in series tubes. Twenty milliliters of plate count agar was poured into each of the test tubes at 45ºC and incubated at 35ºC for 48 hours. Duplicate plates of at least one of three dilutions between 30-300 colony ranges were considered to compute the average count per gram and reported as CFU/ml (AOAC 966.23, 2000).

3.7.2 Total yeast count determination

Using the potato dextrose agar (PDA), serial diluents in ratios of 1:10 using peptone water were done. Further stepwise serial dilutions were done appropriately in series tubes. Twenty milliliters of the PDA agar was poured into each of the test tubes and incubated at 25ºC for 48 hours. Duplicate plates of at least one of three dilutions between 20-200 colony ranges were considered to compute the average count per gram and reported as CFU/ml.

3.7.3 Escherichia coli determination

Using the violet red bile agar (VRBA), serial diluents in ratios of 1:10 with peptone water were done. Further stepwise serial dilutions were done appropriately in series tubes. Twenty milliliters of the VRBA was poured into each of the test tubes and incubated at 37ºC for 24 hours. Duplicate plates of at least one of three dilutions between 20-200 colony ranges were considered to compute the average count per gram and reported as CFU/ml.

3.8 Sensory analysis

Thirty semi-trained panelists consisting of staff members and graduate students from Jomo Kenyatta University of Agriculture and Technology evaluated '*ugali*' made from the different blends. The samples were prepared by use of clean drinking water boiled for 10 minutes and the flour blends added and mixed to make '*ugali'*. Characteristics evaluated were: a) Appearance, b) Color, c) Mouth feel, d) Texture, e) Taste and f) Overall acceptability (refer to appendix 4). A 9 point Hedonic scale (Rangana, 1994) was used to measure the consumer acceptability of the products. The relative importance of each factor was compared numerically on a scale of 1 to 9; 1 = dislike extremely, 9 = like extremely). Each panelist gave a score. The average score of each sample was then calculated.

3.9 Data analysis

Data on feasibility study was analysed using Microsoft excel. For nutrient content analysis, three independent replications were conducted and the data obtained subjected to Analysis of Variance (ANOVA) using Genstat 14th edition, 2012 (VSN international, UK). Mean comparisons for treatments were made using Duncan's Multiple Range Tests. Significant difference was accepted at $P \leq 0.05$ (Steel and Torrie, 1980).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Feasibilty study

4.1.1 Supermarkets

Supermarkets were selected as they are one of the fastest growing retail shops in Kenya. In urban Kenya, they have risen from a tiny niche a half decade ago to a fifth of food retail, targeting all income groups. In addition, they are expected to provide a wide range of quality and variety as a means to appeal to consumers (Terblance and Boshoff, 2004). Therefore, the study was carried out in three main supermarkets in Nairobi. The results showed that a wide variety of grain amaranth flour and its products were sold. The composite flour pack sizes were 500g, 1Kg and 2Kg. Packaging materials for processed amaranth grain products was polythene bags and paper boards. Flour packed in polythene bags was hardly branded while those in paper boards were branded with the Kenyan standardization mark. The prices of grain amaranth flour products ranged from as low as KES 55/kg to as much as KES 115/kg. These prices on the final products were dependent on the pack size and the flour constituents. In addition, the products were also either fermented or unfermented. Those meant for complementary feeding were unfermented whilst those targeting family consumption were mainly fermented. The composite flours were meant for "*uji*"- (a thick gruel porridge) preparation and also for *"ugali"* preparation. This study indicated that in the month of March and April 2014, *"Nakumatt"* supermarket sold the most quantity (54%) of amaranth grain products while *"Uchumi"* supermarket sold the least (8%) as shown in Figure 2.

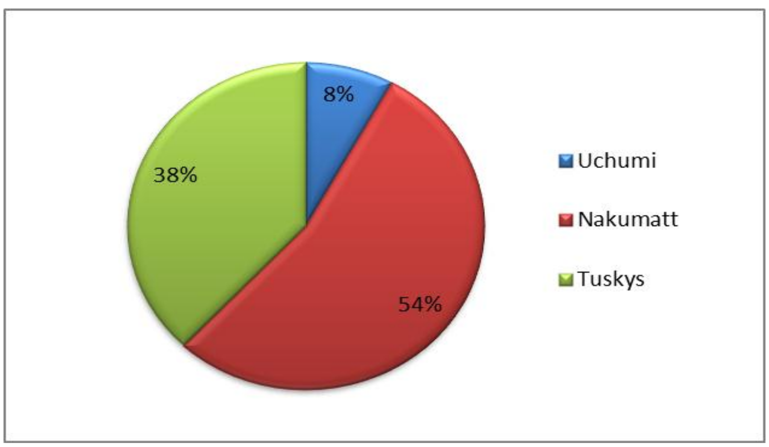


Fig 2: Market share of amaranth grain products sold in March and April 2014

According to information on the labels of grain amaranth products, grain amaranth flour was mixed with flours such as finger millet, cassava, soya, groundnuts, beans and green grams. In fact, some companies also had fortified pure amaranth grain flour with vitamins and minerals. Despite these mixtures and additions, grain amaranth flour was hardly mixed with maize flour.

4.1.2 Companies

An industrial survey on grain amaranth and its products is shown in Table 6. From this study, raw grain amaranth is locally obtained from Western and Nyanza regions in Kenya. The grain is either sent directly to the processors or gets into the hands of middlemen and finally to the processors. Industries purchased a kilo of grain amaranth from as low as KES 70 to KES 130. The industry that had the highest consumption of grain amaranth per month was*" Kirinyaga millers"* (2 tonnes per month while *"Nature's Pure Health"* and *"Promote Amaranth"* which used 0.5 tonnes per month had the lowest consumption. Pre-processing procedures included cleaning and drying in all the industries. Cleaning and drying is important for removal of contaminants and prevention of mold growth. The main process subjected to the grain was milling with others being toasting, popping, puffing and fermentation. The temperature-time combination during thermal processing was mainly 120ºC for 5 seconds. There were high variations in the blending ratios of amaranth flour with other flours. These variations depended largely on the company as well as consumer needs.

Table 6: Industrial survey of grain amaranth

NAME OF INDUSTRY	SOURCE OF RAW MATERIALS	AMARANTH PRICE/KG	GRAIN USED/MONTH	PRE-PROCESSING	GRAIN PROCESSING	TIME - TEMP	BLENDING RATIOS
Kirinyaga millers	Kakamega,Bungoma, Budalangi (Western)	70 – 130	2 tonnes	Cleaning, drying	Milling	None	Dependent on consumer needs
Annico Enterprises	Budalangi, Suba, Bondo, Lugali, Busia	85	1 tonne	Cleaning, drying	Milling Toasting Puffing	120°C-5 sec	Maize: amaranth 3:1 Amaranth: rice 7:3
Promote Amaranth	Bondo, Suba	70	0.5 tonne	Cleaning, drying	Milling Popping Toasting	120°C-5 sec	Maize+wheat: Amaranth 3:1
Ngong Amaranth Foods	Kakamega, Suba, Bondo	80-100	1 tonne	Cleaning, drying	Milling Popping Fermentation	120°C-5 sec	Amaranth: other flours 1:2
Nature's Pure health	Budalangi, Suba, Bondo, Lugali, Busia	80-100	0.5 tonne	Cleaning, drying	Milling Toasting	120°C-5 sec	Amaranth: millet 1:3

4.2 Proximate analysis of the blends

The nutritional composition of the raw materials and the formulated blends are shown in Tables 7 and 8. Table 7 indicates the nutritional quality of refined maize flour blends while Table 8 indicates the nutritional quality of whole meal flour blends. Each of the nutrition parameters will be discussed independently for the raw materials, whole and refined maize flour blends.

Table 7: Proximate composition of raw materials and refined maize flour blends (g/100g)

Samples	Moisture	Crude fat	Crude Protein	Crude Ash	Crude Fibre	CHO
RMF	12.73[d]±0.09	1.92[a]± 0.27	6.29[a]± 0.17	0.55[a]± 0.05	0.76[a]± 0.10	77.75[c] ±0.26
GAF	8.34[a]±0.02	7.61[d]± 0.21	15.82[e] ±0.22	2.54[d] ±0.02	4.50[d]± 0.13	61.19[a] ±0.57
RA1	12.57[d]± 0.02	3.75[b]± 0.19	6.62[b] ±0.22	0.83[b]± 0.01	1.78[b] ±0.19	77.44[d] ±0.51
RA2	12.15[c]± 0.03	4.11[bc]± 0.08	7.92[c]± 0.24	1.04[c] ±0.01	2.15[b] ±0.26	72.63[c]± 0.24
RA3	11.11[b]± 0.11	4.63[c]± 0.13	8.80[d] ±0.12	1.10[c]± 0.03	2.99[c] ±0.18	71.38[b]± 0.22
LSD 5%	0.371	0.585	0.627	0.121	0.662	1.179
Grand Mean	11.38	4.41	9.09	1.21	2.43	71.48
P value	0.001	0.001	0.001	0.001	0.001	0.001

Means within the same column with different alphabetic superscripts were significantly different at (p=0.001). Values are presented as means ± standard error, n=3. RMF- Refined Maize flour; GAF- Grain Amaranth flour; RA1- 80:20 (RMF: GAF); RA2-70:30 (RMF: GAF); RA3- 60:40 (RMF: GAF); CHO – Carbohydrates, LSD – Least Significant Difference

4.2.1 Moisture content

There were significant differences (p=0.001) in the moisture content of the refined maize flour blends and whole meal flour blends. The moisture content of GAF was found to be 8.34%, which was lower than RMF, WMF and all the blends. This could be as a result of longer conditioning periods prior to storage of the grain. The moisture content of RMF and WMF was found to be 13.05% and 12.67% respectively, which was within the recommended limit of 15.5% (WFP, 2012). The flour blends had moisture content ranging from 10.75 -

12.57%. The low moisture contents observed in this study could be an indicator of longer shelf life of the products. Generally, grain of higher moisture content is highly susceptible to deterioration. Furthermore, moisture content is highly dependent on the duration of the drying process thus an index of storage stability of the flour (Brewbaker, 2003).

Table 8: Proximate composition of raw materials and whole meal flour blends (g/100g)

Samples	Moisture	Crude fat	Crude Protein	Crude Ash	Crude Fibre	CHO
WMF	12.67^{d}±0.10	3.89^{a}± 0.28	9.81^{a}± 0.26	1.14^{a}± 0.01	2.68^{b}± 0.13	67.99^{c} ±0.61
GAF	8.34^{a}±0.03	7.61^{d}± 0.21	15.82^{e} ±0.22	2.54^{e} ±0.02	4.50^{a}± 0.27	61.19^{a} ±0.57
WA1	11.64^{b}± 0.10	5.55^{b}± 0.27	10.43^{b} ±0.17	1.43^{b}± 0.01	3.14^{a} ±0.15	67.80^{c} ±0.22
WA2	11.47^{c}± 0.03	6.08^{bc}± 0.08	11.83^{c}± 0.12	1.55^{c} ±0.10	3.40^{a} ±0.41	65.66^{b}± 0.46
WA3	10.75^{c}± 0.06	6.49^{c}± 0.11	12.65^{d} ±0.19	1.67^{d}± 0.01	3.42^{a} ±0.50	65.05^{b}± 0.37
LSD 5%	0.4621	0.5566	0.6146	0.0695	1.024	1.353
Grand Mean	10.98	5.93	12.11	1.67	3.43	65.90
P value	0.001	0.001	0.001	0.001	0.001	0.001

Means within the same column with different alphabetic superscripts were significantly different at (p=0.001), Values are presented as means ± standard error, n=3**.** WMF- Whole Meal Maize flour; GAF- Grain Amaranth flour; WA1- 80:20 (WMF: GAF); WA2-70:30 (WMF: GAF); WA3- 60:40 (WMF: GAF); CHO – Carbohydrates, LSD – Least Significant Difference

4.2.2 Fat content

GAF had significantly (p=0.001) higher lipid content (7.61%) than RMF (1.92%) and WMF (3.89%). Crude fat of GAF fell within the reported range of 5.60 to 10.9% (Mlakar *et al* 2009 and Kariuki *et al*., 2013). Ijabadeniyi and Adebolu (2005) determined the fat content of three maize grain varieties grown in Nigeria which ranged from 4.77 - 5.00%. Abiose *et al*., (2014) reported values of 4.50% of whole meal flour which is higher than the reported value in this study. Values of 1.4% of maize flour that had been degermed and dehulled have been reported (Adeyeye *et al*., 2014) which is slightly lower than the reported value in this study. Onyango (2014) reported commercial sifted maize meal brands purchased from a supermarket in Nairobi ranging from 0.95 - 3.25%. The amount of crude fat is highly dependent on the refinement degree of maize flour thus reduces with eliminating much of the germ portion. Most of the oil is found mainly in the germ. Significant differences (p=0.001) in lipid content were observed among the blends. The lipid content of the refined maize flour blends and the whole meal flour blends ranged from 3.75 - 4.63% and 5.55 - 6.49% respectively.

The higher the proportion of amaranth flour in the blend the higher was the fat content. This trend is similar to maize fortified with *Moringa olifera* seed flour whereby an increase of up to 4.40% (Aluko *et al.*, 2013) was observed and 8.34% for maize fortified with defatted pumpkin flour (Ikujeniola *et al.*, 2013).

4.2.3 Protein content

Similarly, GAF had significantly (p=0.001) higher protein content (15.82%) than RMF (6.29%) and WMF (9.81%). The protein content of GAF was similar to that reported values ranging from 13.1-21% (Kariuki *et al.*, 2013). Onyango (2014) reported commercial sifted maize meal brands purchased from a Nairobi ranging from 7.90 - 11.61% which is higher than the reported value in this study. Abiose *et al.*, (2014) reported values of 9.80% of whole meal flour which is in agreement with the results of this study. Ijabadeniyi *et al.,* (2005) determined the fat content of three maize grain varieties grown in Nigeria which ranged from 4.77 - 5.00%. Significant differences (p=0.001) in protein content were observed among the blends. Addition of grain amaranth significantly improved the protein content of the blends. The protein content of the refined maize flour blends and the whole meal flour blends ranged from 6.62 - 8.80% and 10.43 - 12.65% respectively. This trend is similar to maize fortified with *Moringa olifera* seed flour whereby an increase of up to 4.82% (Aluko *et al*., 2013) was observed and 15.86% for maize fortified with defatted pumpkin flour (Ikujeniola *et al*., 2013). Therefore the use of grain amaranth flour in blending increases the biological value (Bressani *et al*., 1990).

4.2.4 Fibre content

GAF had significantly (p=0.001) higher fibre content (4.50%) than RMF (0.76%) and WMF (2.68%). The fibre content of GAF was similar to reported values ranging from 3.1 – 5.0% (Kariuki *et al.,* 2013). Onyango (2014) reported commercial sifted maize meal brands purchased from a Nairobi ranging from 1.34 – 2.20% which is higher than the reported value in this study. Ullah *et al*., (2010) reported a range of 0.89 – 2.32% of different maize varieties grown in Pakistan. Abiose *et al*., (2014) reported values of 2.60% of whole meal flour which is slightly lower than the results of this study. Ajabadenyi *et al*., (2005), reported fibre content in the range of 2.07 – 2.97% for maize variety grains in Nigeria. Significant differences (p=0.001) in fibre content were observed among the blends. Furthermore, grain amaranth significantly improved the fibre content of maize flur blends. The fibre content of the refined maize flour blends and the whole meal flour blends ranged from 1.78 – 2.99% and 1.43 – 1.67% respectively. This trend is similar to maize fortified with *Moringa olifera* seed flour whereby an increase of up to 1.55% (Aluko *et al*., 2013) was observed and 1.90% for maize fortified with defatted pumpkin flour (Ikujeniola *et al*., 2013). Crude fiber is highly a characteristic of the kernel seed coat (87% of the seed coat), but is also found in smaller amounts in the endosperm and germ walls. The fiber content of processed, dehulled kernels is drastically lower than that of whole-grain options, because fiber is concentrated in the pericarp.

4.2.5 Ash content

GAF had significantly (p=0.001) higher ash content (2.54%) than RMF (0.55%) and WMF (1.14%). Crude ash of GAF is within the range of 2.5 - 4.4% reported by Mlakar *et al.*, (2009). Crude ash of RMF is within the range of 0.46 - 0.87% reported by Calvin (2014) of commercial sifted maize meal brands purchased from a supermarket in Nairobi. Crude ash of WMF is also within range of 1.0 - 2.0% reported by Aisha and El – Tinay (2004). The higher the ash content the greater the proportion of non-endosperm material. Significant differences (p=0.001) were observed among the blends. The ash content of the refined maize flour blends and the whole meal flour blends ranged from 0.83 - 1.10% and 1.43 - 1.67% respectively. All the samples were within the acceptable ranges of up to 3 % (WFP, 2012).

4.2.6 Carbohydrates

RMF had significantly (p=0.001) higher carbohydrate content (77.75%) than WMF (67.99%) and GAF (61.19%). GAF was within the range that has been reported of 55 - 69% (Kariuki *et al*., 2013). Significant differences ($p \leq 0.05$) were observed in the blends. The carbohydrate content of the refined maize flour blends and the whole meal flour blends ranged from 71.38 - 77.44% and 65.05 - 67.80% respectively. Maize is generally known to be high in carbohydrate and as such a good source of calories (Nuss and Tanumihardjo, 2011). The high carbohydrate content observed in this study indicates that the samples are energy dense foods which thus can be incorporated in addressing energy malnutrition.

4.3 Mineral composition

The mineral composition of the raw materials and the formulated blends are shown in Table 9 and 10. Generally, increase of amaranth flour significantly (p=0.001) increased the calcium, iron and zinc content of the blends. From the studies, sample WA3 had the highest amounts of the minerals analyzed followed by RA3. This indicates that grain amaranth can successfully be used to improve the micronutrient density of refined maize flour and whole meal flour. Differences in mineral composition of the samples can be attributed to genetic factors and environmental factors such as soil composition, irrigation frequency and type of fertilizer used. Concentration levels of the minerals in the corn are lower than the levels in wheat (Kulp & Ponte, 2000). The mineral constituents lost in milling may be replaced in maize meal, as in other cereal flours, by fortification with grain amaranth flour as indicated by the results.

Table 9: Mineral composition of raw materials and refined maize flour blends (mg/100 g)

Samples	Calcium	Iron	Zinc
RMF	2.46^{a}±0.15	0.72^{a}± 0.08	0.67^{a}± 0.02
GAF	290.13^{e}±1.30	9.58^{e}± 0.14	4.38^{e} ±0.03
RA1	57.1^{b}± 0.53	2.21^{b}± 0.21	1.41^{b} ±0.10
RA2	88.8^{c}± 1.22	3.06^{c}± 0.25	1.77^{c}± 0.07
RA3	114.8^{d}± 1.13	4.05^{d}± 0.10	2.15^{d} ±0.09
LSD 5%	2.458	0.585	0.141
Grand Mean	110.13	4.41	9.09
P value	0.001	0.001	0.001

Means within the same column with different superscripts were significantly different at (P=0.001), Values are presented as means ± standard error, n=3. RMF- Refined Maize flour; GAF- Grain Amaranth flour; RA1- 80:20 (RMF: GAF); RA2-70:30 (RMF: GAF); RA3- 60:40 (RMF: GAF); LSD – Least Significance Difference

4.3.1 Calcium

GAF was significantly (p=0.001) higher in calcium (190.13 mg/100 g) as compared to RMF (2.46 mg/100 g) and WMF (6.94 mg/100 g). The content of calcium in grain amaranth is slightly higher than reported values (189 mg/100 g) by Mburu *et al*., (2011). Significant differences (p=0.001) were observed in the blends. The calcium content of the refined maize flour blends and the whole meal flour blends ranged from 57.1 - 114.8 mg/100 g and 63.11 - 13.82 mg/100 g respectively. Calcium plays a vital role in bone and tooth development, blood clotting and maintenance of healthy nerves and muscles (Ishida *et al*., 2000), thus important to have its daily requirement met.

4.3.2 Iron

Iron is a hemoglobin component which transports oxygen to body tissues. It is also a component of proteins and enzymes. Iron deficiencies especially in infants, has been associated with cognitive impairment and mental development (Andraca *et al*., 1997). GAF was significantly (p=0.001) higher in iron (9.58 mg/100 g) as compared to RMF (0.72 mg/100 g) and WMF (2.38 mg/100 g). Significant differences (p=0.001) were observed in the blends. The calcium content of the refined maize flour blends and the whole meal flour blends ranged from 57.1 - 114.8 mg/100 g and 63.11 – 113.82 mg/100 g, respectively.

Table 10: Mineral composition of raw materials and whole meal flour blends (mg/100 g)

Samples	Calcium	Iron	Zinc
WMF	6.94^{a}±0.20	2.38^{a}± 0.33	1.36^{a}± 0.08
GAF	290.13^{e}±1.30	9.58^{d}± 0.14	4.38^{d} ±0.03
WA1	63.11^{b}± 0.44	3.81^{b}± 0.10	2.02^{b} ±0.10
WA2	84.22^{c}± 1.14	4.65^{b}± 0.13	2.22^{c}± 0.07
WA3	113.82^{d}± 1.29	5.26^{c}± 0.10	2.53^{c} ±0.08
LSD 5%	4.052	0.2370	0.64
Grand Mean	111.64	2.50	5.14
P value	0.001	0.001	0.001

Means within the same column with different superscripts were significantly different at (P=0.001), Values are presented as means ± standard error, n=3. WMF- Whole Meal Maize flour; GAF- Grain Amaranth flour; WA1- 80:20 (WMF: GAF); WA2-70:30 (WMF: GAF); WA3- 60:40 (WMF: GAF); LSD – Least Significant Difference

4.3.3 Zinc

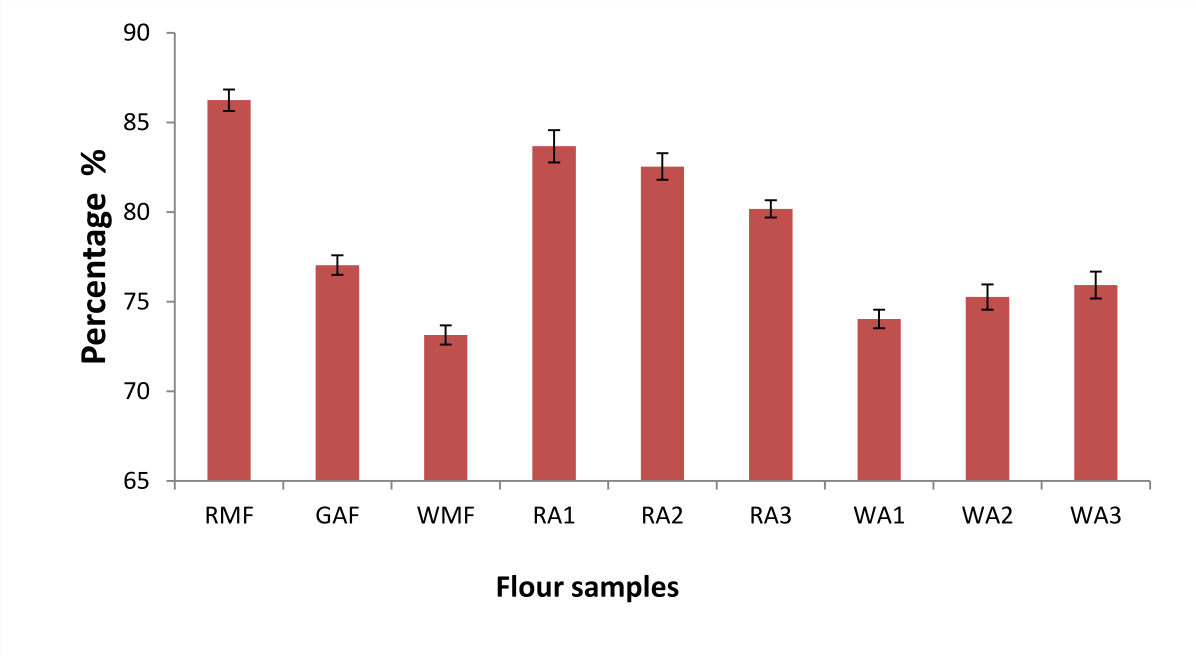
Zinc is an essential component of most enzymes in the body. Zinc boosts immunity and also helps the body heal wounds and maintain normal blood glucose levels. Research suggests that zinc also has a role in improving recall skills, reasoning and attention (Krebs *et al*., 2006). GAF was significantly (p=0.001) higher in zinc (4.38 mg/100 g) as compared to RMF (0.67 mg/100 g) and WMF (1.36 mg/100 g). The content of zinc in grain amaranth is slightly higher than reported values (3.6-4mg/100 g) by Kariuki *et al*., 2013. Hassan *et al*., (2009) reported 0.5 mg/100 g of maize varieties grown in Sudan which is slightly lower than the reported values in this study. Significant differences (p=0.001) were observed in the blends. The zinc content of the refined maize flour blends and the whole meal flour blends ranged from 1.41 - 2.15% mg/100 g and 3.81 - 5.26 mg/100 g respectively.

4.4 Protein digestibility

The protein digestibility of raw materials and the formulated blends are shown in Figure 3. RMF had significantly (p=0.001) higher protein digestibility (86.23%) than GAF (77.04%) and WMF (73.14%). *In vitro* digestibility of 61 - 76% has previously been reported for raw grain amaranth proteins (Correa *et al.*, 1996). The relatively low protein digestibility of raw grain amaranth compared to refined maize flour may be attributed due to the influence of anti-nutrients such as enzyme inhibitors, lectins, phytates, tannins and dietary fiber, which inhibits protein digestion (Nestares *et al.*, 1993). These anti nutrients interact with protein to form complexes that decrease protein solubility (Alonso *et al*., 2000). Also the protein structures are not denatured thus less protein are accessible for enzyme digestion (Fennema, 1996). The *in vitro* protein digestibility values recorded from grain amaranth were higher than reported digestibility values for whole raw maize (66.6%) and sorghum (55.8 - 59.1%) (Duodu *et al*., 2002). The digestibility of RMF was higher than that reported of 84.5% (Malomo *et al*., 2013). This could be attributed to the removal of the bran during milling resulting in reduction of anti-nutrients.

Significant differences (p=0.001) were observed in the blends. The protein digestibility of the refined maize flour blends and the whole meal flour blends ranged from 80.17 - 83.67% and 74.04 - 75.92% respectively. The protein digestibility of the refined maize flour blends decreased with increase of GAF with sample RA1 being the highest. The decrease could be attributed to the high content of anti-nutrients in grain amaranth flour. The protein digestibility of the whole meal flour blends increased with increase of GAF with sample WA3 being the highest.

The biological utilization of protein is majorly dependent on its digestibility (Cruz *et al*., 2003). Products with high protein digestibility are considered nutritionally better for they provide higher amino acid amounts during absorption. Low protein digestibility in the diet affects the bioavailability of the amino acids and also the protein quality of the food (Gilani *et al.*, 2005). However, the higher protein content of GAF will compensate for the reduced digestibility in the blends.



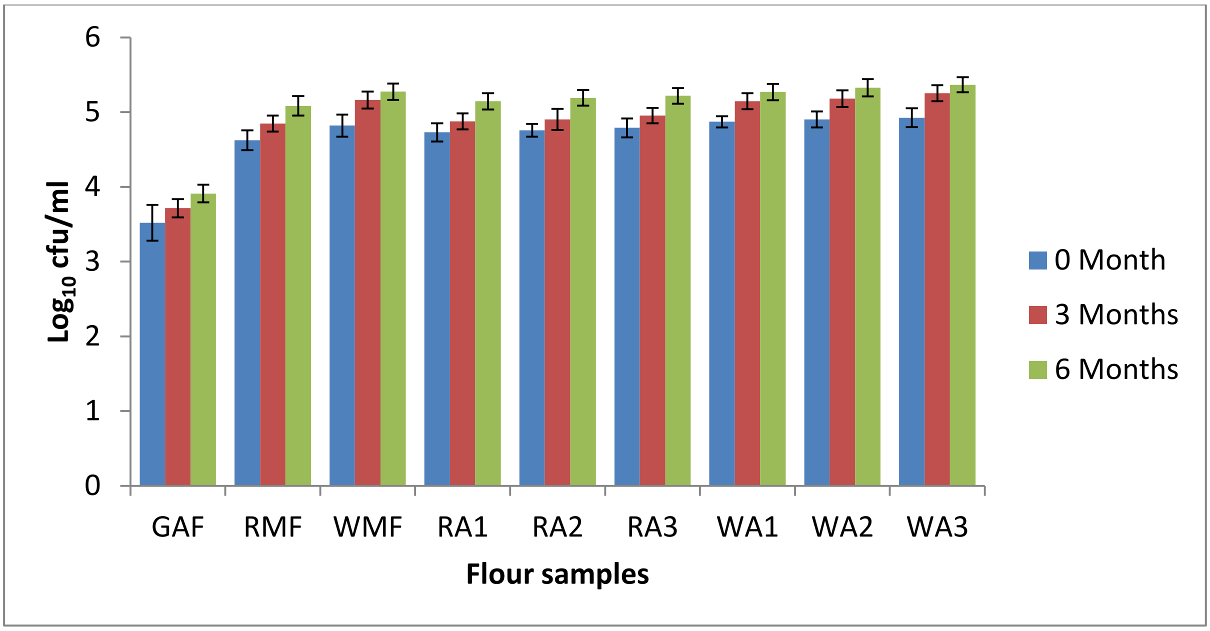
Values are presented as means ± standard error, n=3. RMF - Refined Maize flour; GAF - Grain Amaranth flour; WMF - Whole Meal Maize flour; RA1 - 80:20 (RMF: GAF); RA2 - 70:30 (RMF: GAF); RA3 - 60:40 (RMF: GAF); WA1 - 80:20 (WMF: GAF); WA2 - 70:30 (WMF: GAF); WA3- 60:40 (WMF: GAF).

Fig 3: Protein digestibility of the different flour blends

4.5 Microbiological quality of the flours

Generally, flour is considered a microbiologically safe product due to its low water activity. The results in Figure 4, 5 and 6 indicate the rate of microbial growth during storage at 0, 3 and 6 months.

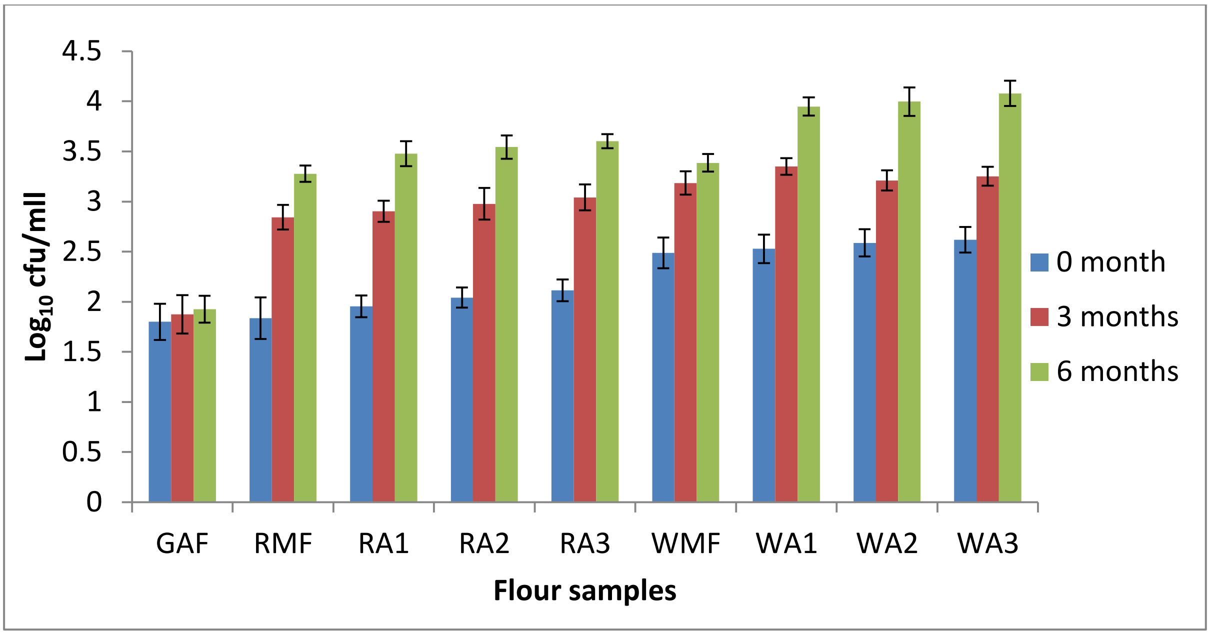
Total plate count(TPC) and coliform count indicate the effectiveness and efficiency of the food chain process and also information on shelf life and organoleptic changes of the food stuff (Batool *et al*., 2012). Higher coliform count, TPC and *E. coli* counts more than the legal limits indicate poor sanitation and/or problems with the process control and handling of the raw materials and their products.



Values are presented as means ± standard error, n=3. RMF - Refined Maize flour; GAF - Grain Amaranth flour; WMF - Whole Meal Maize flour; RA1 - 80:20 (RMF: GAF); RA2 - 70:30 (RMF: GAF); RA3 - 60:40 (RMF: GAF); WA1 - 80:20 (WMF: GAF); WA2 - 70:30 (WMF: GAF); WA3- 60:40 (WMF: GAF).

Fig 4: Total plate count of the different flour blends

GAF had significant (p=0.002) lower microbial counts as compared to RMF and WMF. This might be due to the fact that the grains had been toasted for 5 minutes at 100ºC and this may have reduced the microbial load. High microbial counts found in refined maize flour and whole meal flour may be attributed to microbes already present in the cereal before milling or the milling method used. Build-up of residues may also be a source of contamination (Berghofer *et al*., 2003). There was an increase in microbial load of the blends during the storage period. This could be attributed to the blends having a better nutrient base than the unfortified flour, thus offering better growth conditions for the microbes. Similar to this, Bothast *et al*., (1981) found that both bacteria and mold counts in maize meal increased during the storage period

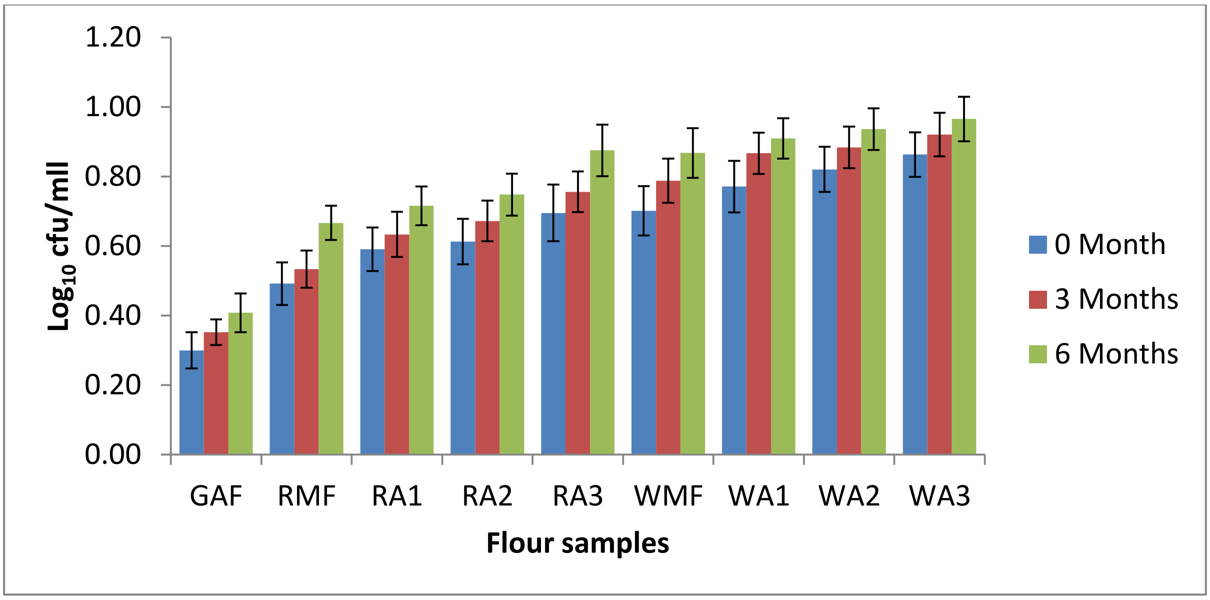


Values are presented as means ± standard error, n=3. RMF - Refined Maize flour; GAF - Grain Amaranth flour; WMF - Whole Meal Maize flour; RA1 - 80:20 (RMF: GAF); RA2 - 70:30 (RMF: GAF); RA3 - 60:40 (RMF: GAF); WA1 - 80:20 (WMF: GAF); WA2 - 70:30 (WMF: GAF); WA3- 60:40 (WMF: GAF).

Fig 5: Yeast and mould counts of the different flour blends

The level of contamination of flour by yeasts and moulds is of paramount importance when considering the quality and safety of food. Yeast and moulds in the blends increased with increased storage time. This deterioration could lead to moulds producing enzymes which begin to degrade the lipids. Consequently, this affects the quality parameters of the flour during storage. The maximum legal limit for fungi in flour is $\log_{10}$ 3 cfu/g (WFP, 2012). Yeasts and moulds from the results were within recommended limits. Higher level of fungi more than the legal limits deteriorates the quality of food and causes food borne diseases.

E. coli was detected in all the samples at very low levels and the results were within the WFP legal limits ($\log_{10}1$ cfu/ml). All samples were free from *Salmonella*. The results obtained were consistent with WFP (2012) up to 3 months. Studies have shown few cases of food borne disease outbreaks caused by flours (Batool *et al*., 2012).



Values are presented as means ± standard error, n=3. RMF - Refined Maize flour; GAF - Grain Amaranth flour; WMF - Whole Meal Maize flour; RA1 - 80:20 (RMF: GAF); RA2 - 70:30 (RMF: GAF); RA3 - 60:40 (RMF: GAF); WA1 - 80:20 (WMF: GAF); WA2 - 70:30 (WMF: GAF); WA3- 60:40 (WMF: GAF).

Fig 6: Escherichia coli counts of the different flour samples

4.6 Sensory analysis

The sensory attributes of *"ugali"* from the different blends are shown in Tables 11 and 12. Generally, the different variations of *"ugali"* samples were all acceptable. There were significant differences ($p \leq 0.05$) among the products. Incorporation of grain amaranth flour to refined maize flour and whole meal flour significantly ($p<0.05$) reduced the attributes of appearance, colour, mouth feel, texture, taste and overall acceptability. In addition, there were no significant differences ($p \leq 0.05$) in all the sensory attributes between RA2 and RA3 and WA2 and WA3.

The colour of the 'ugali' is dependent on the colour of the flour used to make it. The results showed that for the refined maize flour blends, the colour of the 'ugali' prepared of RMF was most preferred followed by RA1 and RA2. In fact, this was similar to whole meal flour blends whereby the most preferred was WMF followed by WA1 and WA2. Notably, the preference for RMF could be due to its light colour in comparison to the rest of the blends. Increasing the proportion of grain amaranth flour resulted in a darker colouration of the blends.

Table 11: Sensory evaluation scores for '*ugali*' from the refined maize flour blends

Samples	Appearance	Colour	Mouth feel	Texture	Taste	Overall Acceptability
RMF	$8.17^{c}\pm0.14$	$8.00^{c}\pm0.16$	$7.63^{c}\pm0.18$	$7.57^{c}\pm0.23$	$7.80^{c}\pm0.19$	$8.00^{c}\pm0.17$
RA1	$6.37^{b}\pm0.26$	$5.93^{b}\pm0.35$	$6.37^{b}\pm0.35$	$6.20^{b}\pm0.31$	$6.27^{b}\pm0.36$	$6.27^{b}\pm0.29$
RA2	$5.03^{a}\pm0.36$	$4.97^{a}\pm0.35$	$4.50^{a}\pm0.39$	$4.73^{a}\pm0.39$	$4.53^{a}\pm0.40$	$4.93^{a}\pm0.38$
RA3	$4.40^{a}\pm0.33$	$4.93^{a}\pm0.30$	$4.30^{a}\pm0.44$	$4.33^{a}\pm0.41$	$4.20^{a}\pm0.43$	$4.43^{a}\pm0.33$
LSD 5%	0.771	0.825	0.945	0.962	0.959	0.804
Grand Mean	6.08	5.98	5.69	5.79	5.75	6.00
P value	0.003	0.003	0.003	0.003	0.003	0.003

Means within the same column with different alphabetic superscripts were significantly different at (p=0.003). Values are presented as means ± standard error, n=30. RMF- Refined Maize flour; GAF- Grain Amaranth flour; RA1- 80:20 (RMF: GAF); RA2-70:30 (RMF: GAF); RA3- 60:40 (RMF: GAF); LSD – Least Significance Difference

Table 12: Sensory evaluation scores for '*ugali*' from the whole meal flour blends

Samples	Appearance	Colour	Mouth feel	Texture	Taste	Overall Acceptability
WMF	$6.43^{b}\pm0.23$	$6.07^{b}\pm0.28$	$5.63^{b}\pm0.28$	$6.10^{ab}\pm0.26$	$5.97^{b}\pm0.34$	$6.27^{b}\pm0.22$
WA1	$5.30^{a}\pm0.39$	$5.60^{ab}\pm0.32$	$5.50^{b}\pm0.33$	$5.37^{b}\pm0.30$	$5.27^{ab}\pm0.34$	$5.83^{b}\pm0.26$
WA2	$4.80^{a}\pm0.41$	$5.00^{a}\pm0.38$	$4.50^{a}\pm0.32$	$4.73^{a}\pm0.40$	$4.80^{a}\pm0.39$	$4.83^{a}\pm0.32$
WA3	$5.20^{a}\pm0.32$	$4.93^{a}\pm0.36$	$4.30^{a}\pm0.41$	$4.60^{a}\pm0.41$	$4.87^{a}\pm0.35$	$4.67^{a}\pm0.33$
LSD 5%	0.927	0.948	0.978	1.037	0.956	0.801
Grand Mean	5.43	5.40	4.98	5.20	5.22	5.40
P value	0.008	0.008	0.008	0.008	0.008	0.008

Means within the same column with different alphabetic superscripts were significantly different at (p=0.008). Values are presented as means ± standard error, n=30. WMF- Whole Meal Maize flour; GAF- Grain Amaranth flour; WA1- 80:20 (WMF: GAF); WA2-70:30 (WMF: GAF); WA3- 60:40 (WMF: GAF); LSD – Least Significant Difference

The mouth feel, texture and taste from RMF and WMF were most preferred. Addition of grain amaranth flour resulted in the ‘ugali’ obtaining a ‘grainy’ texture, thereby affecting the mouth feel and taste of the blends. The results show that the most acceptable refined maize flour blend and whole meal flour blend was RA1 and WA1 respectively. Further increasing the grain amaranth flour resulted in progressive decline in acceptability of the blends. This could be due to the reason that respondents are more familiar to ‘ugali’ made solely from maize flour.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMENDATIONS

5.1 Conclusion

Grain amaranth flour and its wide array of products are sold in relatively large amounts in the supermarkets. This is advantageous as supermarkets can be used as distribution platforms of new developed products, such as in this study.

According to the study, grain amaranth flour is highly nutritious particularly with respect to proteins, fat and minerals as compared to refined and whole meal maize flour. Blending refined and whole meal maize flour with grain amaranth flour significantly increases the nutritional quality. Samples with the highest nutritional profile were the blends with 40% grain amaranth flour while the most acceptable blends were with 20% grain amaranth flour.

The protein digestibility of refined maize flour blends decreased with increase of grain amaranth flour while that of whole meal flour blends decreased. Nutritionally, flours with higher levels of protein digestibility are preferred due to the increase in protein digestion. The shelf life of the blends was acceptable up to 3 months of storage after which they were not generally regarded as safe to consume due to unacceptable levels of microbes.

Therefore, this study confirms that the approach of food to food fortification can provide a sustainable alternative to current chemical fortification approaches if indigenous food crops of high nutrient content are incorporated in common staple diets. Strategies such as nutrition education can be explored in order to enhance acceptability of blends with more than 20% inclusion of grain amaranth flour. Overall, grain amaranth can successfully be used in mitigating nutrition insecurity and reducing malnutrition levels in Kenya if used in blended maize recipes.

5.2 Recommendations

From the study, recommendations include to determine the effect of other processing techniques (malting, fermentation) on the nutrients and anti-nutrient content of grain amaranth flour, in particular digestibility of proteins. Also, to carry out *in vivo* tests in order to determine the bioavailability of nutrients such as starch.

CHAPTER SIX

6.0 REFERENCES

A.O.A.C. (1995). Official methods of analysis. Association of Analytical Chemists, Washington, D.C.

Abiose, S. H. and Ikujenlola, A.V. (2014). Comparison of chemical composition, functional properties and amino acids composition of quality protein maize and common maize (Zea may L). *African Journal of Food Science Technology*, *5*(3), 81-89.

Abuya, B. A, Ciera J. and Kimani-Murage E. (2012). Effect of Mother's Education on Child's Nutritional Status in the Slums of Nairobi. *BMC pediatrics, 12*(1), 80.

Adeyeye, S. A. and Akingbala, J. O. (2014). Evaluation of Nutritional and Sensory Properties of Cookies Produced from Sweet Potato- Maize Flour Blends. *Researcher*, *6*(9), 61-70.

Aisha, S. M. F. and El-Tinay, A. H. (2004). Effect of genotype, malt pretreatment and cooking on in vitro protein digestibility and protein fractions of corn. *Food Chemistry, 84*, 613-619.

Akpagu, F.C., Nnamani, E. V. and Eze C. G. I. (2015). Estimation of Protein Content in Soyabeans Consumed In Abakaliki, Ebonyi State, Nigeria. *IOSR Journal of Pharmacy and Biological Sciences, 1* (1), 08-12.

Alais, C. and Linden G. (1991). Food Biochemistry. Ellis Horwood Limited, New York.

Alemu, J. (2005). A Plant full of Medicinal Values.

Allen, L. H., Peerson, J. M. and Olney, D. K. (2009). Provision of multiple rather than two or fewer micronutrients more effectively improves growth and other outcomes in micronutrient deficient children and adults. *Journal of Nutrition,* 139, 1022–1030.

Alonso, A. A. and Marzo, F. (2000) Effect of extrusion and traditional processing methods on anti-nutrients and in vitro digestibility of proteins and starch in kidney beans. Food chem, 68, 159-165.

ALRMP (2008). Arid Lands and Resource Management Project. Monthly Bulletin Kajiado District.

Aluko, O., Brai, M. R. and Adelore, A. O. (2013) Evaluation of Sensory Attributes of Snack from Maize-Moringa Seed Flour Blends. *International Journal of Biological, Food, Veterinary and Agricultural Engineering*, 7 (10).

Andraca, I., Castillo, M. and Walter, T. (1997). Psychomotor development and behavior in iron-deficient anemic infants. Nutrition Revision; 55, 125–132.

Aquino, P., Carrión, F., Calvo, R. and Flores, D. (2000). Selected maize statistics. CIMMTY World Maize Facts and Trends.

Arêas, J. A. (1996). Starch molecular interactions in the extrusion process. Critical Revision on Food Science and Nutrition, 30, 28–29.

Batool, S. A, Rauf, N., Tahir, S. S. and Kalsoom, R. (2012) Microbial and Physico-chemical contamination in the wheat flour of the twin cities of Pakistan. *International Journal of Food Safety 14*, 75-82.

Bahilgwa, G. (2006). Access of Eastern African farmers to domestic and international markets: opportunities and constraints. Gold Coast, Australia.

Berganza, B. E., Moran, A. W., Rodrigez, G. M, Coto, N. M., Santamaria, M and Bressani R. (2003). Effect of Variety and Location on the Total Fat, Fatty Acids and Squalene Content of Amaranth. Plant Foods Hum.Nutr. 58, 1-6.

Berghofer, L. K., Hocking A. D., Miskelly, D. and Jansson, E. (2003) Microbiology of wheat and flour milling in Australia. *International Journal of Food Microbiology, 85*, 137-49.

Berkelaar, D. and Alemu, J. (2006). Grain amaranth. Christian Reformed World Relief, Nairobi.

Black, R. E., Allen, L. H., Bhutta, Z. A., Caulfield, L. E. and de Onis M. et al. (2008) Maternal and child undernutrition: global and regional exposures and health consequences. Lancet 371: 243–260.

Bothast, R. J., Anderson, R. A., Warner, K. and Kwole, W. F. (1981). Effects of moisture and temperature on microbiological and sensory properties of wheat flour and corn meal during storage. Cereal Chemistry, 58: 309 –311.

Bressani, R and Elías, L. G. (1984).Development of 100% amaranth foods. In: *Proceedings Third Amaranth Conference*. Emmaus, USA: Rodale Press.

Bressani, R. and Colmenares, D. R. (1990).Effect of Germination on the Chemical Composition and Nutritive Value of Amaranth Grain. Cereal Chemistry 67 CRC Press, Boca Raton, FL.155-84.

Brewbaker, J. L. (2003). Corn production in the tropics. The Hawaii experience. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa.

Cameron, M. and Hofvender, Y. (1983). Infants and Young Children. 2nd Edition. Oxford University Press. Washington DC, USA.

Caulfield, L. E., Richard, S. A., Rivera, J. A., Musgrove, P., and Black, R. E. (2006). Stunting, wasting, and micronutrient deficiency disorders. Disease control priorities in developing countries, 551-567.

Chávez-Jáuregui, R. N., Silva, M. E. M. P. and Arĕas, J. A. G. (2000), Extrusion Cooking Process for Amaranth (*Amaranthus caudatus* L.). *Journal of Food Science, 65*, 1009–1015.

Colmenares De Ruiz, A.S. and Bressani, R. (1990). Effect of Germination on the Chemical Composition of Amaranth Grain. Cereal chem. 67: 519-522.

Correa, A. D., Jokl L. and Carlsson R. (1986). Chemical constituents, in vitro digestibility, and presence of antinutritional substances in amaranth grains. Arch. Latinoam. Nutr. 36:319–326.

Cruz, G. A., Oliveira, M. G., Pires, C. V., Costa, N. M., Brumano, M. H., Gomes, M. R. and Moreira, M. A. (2003). Protein Quality and in vivo Digestibility of different bean varieties. *Braz. J. Food Technol. 6* (2): 157-162

Cynthia, A., (2009). Increasing drought in Kenya. In Understanding Climate Change Adaptation: Lessons from community-based approaches, pp 101-114.

De Groote, H. and Kimenju, S. (2012). Consumer Preferences for Maize Products in Urban Kenya. Food and nutrition bulletin. 33. 99-110.

Duodu, K. G., A. Nunes, I. Delgadillo, M. L., Parker, E. N. C., Mills, P. S. and Belton, et al. (2002). Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *J. Cereal Sci,. 35,* 161–174.

Elmadfa, J., Anklam, E. and Koning, J. (2003). Modern Aspect of Nutrition. Proceedings of the 17th International Congress of Nutrition. Austria.

Escudero, N. L., De Arellano, M. L., Luco, J. M., Gime´nez, M. S. and Mucciarelli, S. I. (2004). Comparison of the chemical composition and nutritional value of Amaranthus cruentus flour and its protein concentrate. *Plant Foods for Human Nutrition, 59*(1), 50-59.

Ezzati, M., Lopez, A. D., Rodgers, A. and Murray, C. J. (2004). Comparative quantification of health risks: The global and regional burden of disease attributable to selected major risk factors. Geneva: World Health Organization.

Faber, M., Kvalsvig, J. D., Lombard, C. J. and Benadé, A. J. S. (2005). Effect of a fortified maize-meal porridge on anemia, micronutrient status, and motor development of infants. *The American Journal of Clinical Nutrition, 82*, 1032–1039.

FAO, (2005). Nutrition country profile: Republic of Kenya. Rome. Pp 3-4; 9-15; 2127; 31-32.

FAO, (1997). Preventing Micronutrient Malnutrition. A Guide to Food Based Approaches. A Manual for Policy Makers and Programme Planers; International Life Science Institute, Washington.

Fennema, O.R. (1996). *Food chemistry* , (3rd ed., 321–425. NewYork, NY: Marcel Dekker.

Ferreira, T. A. (1999). Nutritional evaluation of amaranth (*Amaranthus caudatus* L.) extruded at distinct moisture contents. São Paulo.

Food and Nutrition Technical Assistance (FANTA) and FAO (2007). Guidelines for Measuring Household and Individual Dietary Diversity. (Version 3). Rome Italy

Funke, O. (2011). Evaluation of Nutrient Contents of Amaranth Leaves Prepared Using Different Cooking Methods. Food and Nutrition Sciences, 02, 249-252.

Gilani, G. M., Cockell, K.A. and Sepehr, E. (2005). Effects of antinutritional factors on protein digestibility and amino acid availability in foods. *Journal of AOAC International, 88*, 967-87.

GOK, (2008). The Kenya National Technical Guidelines for Micronutrient Deficiency Control. Ministry of Public Health and Sanitation. Nairobi. Kenya.

GoK, (2015) the Food, Drugs and Chemical Substances (Food Labelling Additives and Standards) (Amendment) (No. 2) Regulations, 2015.

Goldberg, G. (2003). Plants: Diet and Health. The Report of the British Nutrition Foundation Task Force. Blackwell, Oxford.

Grubben, G. J. H. and van Sloten, D. H. (1981). Genetic Resources of Amaranths. International Board for Plant Genetic Resources, FAO, Rome.

Haggblade, S. and Holzapfel, W. H. (1989). Industrialization of Africa's Indigenous Beer Brewing. In Keith H. Steinkraus (eds). Industrialization of Indigenous Fermented Foods, (Pp.191-283). Marcel Dekker, Inc.

Hamaker B. R, Kirleis L. G, Butler A. W, Axtell J. D and Mertz E. T (1987). Improving the in vitro protein digestibility of soghurm with reducing agents. Proceedings of the National Academy of Sciences of the United States of America, 84: 626-628.

Hassan, A. B., Osman G. A. M. and Rushdi, M. A. H. (2009). Effect of Gamma Irradiation on the Nutritional Quality of Maize Cultivars (Zea mays) and Sorghum (Sorghum bicolor) Grains. *Pak. J. Nutr., 8*, 167-171.

Hefle, L. (1996). Allergenic foods. Critical review in Food Science and Nutrition; 36S: 69-90.

Holzapfel, W. H. (1989). Industrialization of Mageu Fermentation in South Africa. In Keith H. Steinkraus (eds). Industrialization of Indigenous Fermented Foods, (Pp.285-328). Marcel Dekker, Inc.

Horton S., Mannar V. and Wesley A. (2008) Micronutrient Fortification (Iron and Salt Iodization). Best Practice Papers from Copenhagen Consensus.

Hotz, C. and Gibson R. S. (2001).Assessment of home-based processing methods to reduce phytate content and phytate/zinc molar ratios of white maize (Zea mays). *J Agric Food Chem*. 49:692–8

Huffman, S. L., Oniang′o, R. and Quinn, V. (2000). Improving young child feeding with processed complementary cereals and behavioural change in urban Kenya. Food Nutrition Bulletin, 21: 75– 81.

Hurrell, R. F., Reddy, M., Dassenko, S. A. and Cook, J. D. (1991). Ferrous fumarate fortifcation of a chocolate drink powder. *British Journal of Nutrition, 65*, 271-283.

Ijabadeniyi, A. O. and Adebolu, T. T. (2005). The effect of processing methods on the nutritional properties of ogi produced from three maize varieties. *J. Food, Agric. and Environ., 3,* 108-109.

Ikujenlola, A. V., Oguntuase, S. O. and Omosuli S. V. (2013) Physico-Chemical Properties of Complementary Food from Malted Quality Protein Maize (*Zea mays L.*) and Defatted Fluted Pumpkin Flour (*Telfairia occidentalis Hook, F*). *Food and Public Health, 3*(6), 323-328.

Ishida, H., Suzuno, H., Sugiyama, N., Innami, S., Todoro, T. and Maekawa, A. (2000). Nutritional evaluation of chemical components of leaves, stalks and stem of sweet potatoes (Ipomea betatas poir).Food Chem. 68:359-367.

Jayne T. S., Rubey L., Tschirley D., Mukumbu M., Chisvo M., Santos A. P., Webber M. T., and Diskin P. (1996). Effects of market reform on access to food by low-income households: Evidence from four countries in Eastern and Southern Africa. Technical Paper No. 25. Bureau for Africa/USAID.

Jayne, T. S. and Argwings-Kodhek, G. (1997). Consumer response to maize market liberalization in urban Kenya. Food Policy, 22, 447-458.

Johnson, L. (2000). Corn: The major cereal of the Americas. In: Kulp, K., & Ponte-Jr, J. G. (eds.). Handbook of Cereal Science and Technology (pp. 31-80). New York: Marcel Dekker, Inc.

Kariuki, S., Sila, D. And Kenji, G. (2013). Nutrition Profile of Amaranth Grain Varieties Grown in Kenya. *Journal of International Institute for Science, Technology and Education, 17,* 19-25

Kauffman, C. S., and Weber, L. E. (1990). Grain amaranth. Advances in new crops. Timber Press, Portland ,pp 127-139.

KDHS. (2008-2009) Kenya Demographic and Health Survey. National Council for Population and Development, Central Bureau of Statistics and Ministry of Planning and National Development, Nairobi. Kenya.

Kenya National Bureau of Statistics, Ministry of Health/Kenya, National AIDS Control Council/Kenya, Kenya Medical Research Institute, and National Council for Population and Development/Kenya (2015). Kenya Demographic and Health Survey 2014. Rockville, MD, USA.

Kim, H. K., Kim, M. J., Cho, H.Y., Kim, E. U. and Shin, D. H. (2006a). Antioxidative and anti-diabetic effects of amaranth (Amaranthusesculantus) in streptozotocin-induced diabetic rats. Cell Biochemistry and Function, 24:195–199.

Kim, H. K., Kim, M.J. and Shin, D.-H. (2006b). Improvement of lipid profile by Amaranth(Amaranthus esculantus) supplementation in Streptozotocin-induced diabetic rats. *Ann. Nutr. Metab., 50*, 277-281.

Krebs, N. F., Westcott, J. E., Butler, N., Robinson, C., Bell, M. and Hambidge, K. M., (2006). Meat as a first complementary food for breastfed infants: Feasibility and impact on zinc intake and status. *Journal of Pediatric Gastroenterology and Nutrition, 42*, 207-214.

Kulp, K. and Ponte, J. G. (2000). Handbook of cereal science & technology (pp. 43 1-438). New York, NY: Marcel Dekker.

Laleye, L. and Wesley, A. (2001). Small Scale Fortification, Draft of paper presented at the 2001 AACC Annual Meeting, Charlotte, NC Symposium: Enhancing the Micronutrient Content of Cereal Grains and Product, Micronutrient Initiative, Ottawa, Canada

Lartey, A., Manu, A., Brown, K. H., Peerson, J. M. and Dewey, K. G. (1999). A randomized, community-based trial of the effects of improved, centrally processed complementary foods on growth and micro-nutrient status of Ghanian infants from 6 to 12 mo of age. *American Journal of Clinical Nutrition, 70,* 391– 404.

Legacy (2003). The Official Newsletter of the Amaranth Institute.Volume XV

Malomo O, Alamu E. A. and Oluwajoba S. O. (2013). Chemical evaluation of protein Quality of Sprouted Maize and Cowpea. *International Journal of Nutrition and Food Sciences, 2*(5), 254-260.

Mamabolo, R. L., Alberts, M., Mbenyane, G. X., Steyn, N. P., Nthangeni, N. G., Delemarre-van De Waal, H. A. and Levitt, N. S. (2004). Feeding Practices and Growth of Infants From Birth to 12 Months in the Central Region of the Limpopo Province of South Africa. Nutrition, 20, 327–333.

Martinez-Bustos, F., Chang, Y. K., Bannwart, A. C., Rodriguez, M. E., Guedes, P. A. and Gaiotti, E. R. (1998). Effects of calcium hydroxide and processing conditions on corn meal extrudates. Cereal Chemistry, 75, 796-801.

Martirosyan, D.M., Miroshnichenko, L.A., Kulakova, S. N., Pogojeva, A. V. and Zoloedov, V.I. (2007). Amaranth oil application for coronary heart disease and hypertension.*Lipids in Health and Disease, 6*(1).

Masarirambi, M., Mavuso, V., Songwe, V., Nkambule, T., and Mhazo, N. (2010). Indigenous Post-Harvest Handling and Processing of Traditional Vegetables in Swaziland: A Review. *African Journal of Agricultural Research, 5* (24), 3333-3341.

Mburu, M., Gikonyo, N., Kenji, G and Mwasaru, A. (2011). Properties of a complementary food based on Amaranth grain (*Amaranthus cruentus*) grown in Kenya. *Journal of Agric, Food Tech., 1*(9) 153-178.

McLaren, D., and Frigg, M. (2001). The Sight and Life Manual of Vitamin A Deficiency Disorders (VADD). 2nd Edition. Basel: Sight and Life.

Mendoza, C. and Bressani, R. (1987). Nutritional and Functional Characteristics of Extrusion-Cooked Amaranth Flour. Cereal Chemistry, 64: 218-222.

Mlakar, S.G., Turinek, M., Jakop, M., Bavec, M. and Bavec, F. (2009). Nutrition value and use of grain amaranth: potential future application in bread making. Agricultura 6: 43-53.

Modi, A. T. (2006). Growth temperature and plant age influence on nutritional quality of Amaranthus leaves and seed germination capacity. Revised version. Originally presented at the International Symposium on the Nutritional Value and Water Use of

Indigenous Crops for Improved Livelihoods held on 19 and 20 September 2006 at the University of Pretoria in Pretoria, South Africa.

Mugalavai, V. K. (2013). Effect of amaranth: maize flour ratio on the quality and acceptability of ugali and porridge (kenyan cereal staples). *ARPN Journal of Agricultural and Biological Science 8*(1), 6-12

Mukumbu, M. and Jayne, T. S. (1995). Urban Maize Meal Consumption Patterns Strategies for Improving Food Access for Vulnerable Urban Households in Kenya, Paper presented at the Symposium on Agricultural Policies and Food Security in Eastern Africa, May 19–20, 1994, SD Publication Series Office of sustainable Development Bureau for Africa, Technical Paper No. 8, USAID.

Muller, H. G, and Tobin, G. (1980). Nutrition and Food Processing Croom Helm. London.

Mulokozi, G., Hedren, E., and Svanberg, U. (2004). In Vitro Accessibility and Intake of Beta- carotene from Cooked Green Leafy Vegetables and their Estimated Contribution to Vitamin A Requirements. *Journal of Plant Foods for Human Nutrition; 59*(1), 2-9.

Muriuki, E. N., Sila, D.N. and Onyango, A. (2014). Nutritional diversity of leafy amaranth species grown in Kenya. *Journal of Applied Biosciences*, *79*, 6818–6825.

Muthayya S., Rah J. H., Sugimoto J. D., Roos F. F., Kraemer K. and Black E. R. (2013). The Global Hidden Hunger Indices and Maps: An Advocacy Tool for Action. PLoS ONE 8(6).

Muyonga, J. H., Nabakabya, D., Nakimbugwe, D. N. and Masinde, D. (2008). Efforts to promote amaranth production and consumption in Uganda to fight malnutrition. Using Food Science and Technology to Improve Nutrition and Promote National Development, Robertson, G.L. & Lupien, J.R. (Eds). International Union of Food Science & Technology.

Mwangi, D. (2003). Introduction to Amaranth. 2nd Edition Joypet Services and Printers Nairobi - Kenya. Pp 51-56.

Myers, R. L. (1996). Amaranth: New crop opportunity. p. 207-220. In: J. Janick (ed.), Progress in new crops. ASHS Press, Alexandria, VA.

Myers, R. L. and Putnam, D. H. (1988). Growing Grain Amaranth as a Specialty Crop. Communication and Educational Technology Services, University of Minnesota Extension.

National Academy of Sciences (2006). Lost Crops of Africa: Volume II: Vegetables. Descriptions and assessments of individual species. National Academy of Sciences, 500 Fifth St. N.W., Washington, D.C. 20001.

Nestares, T., M. Lopez-Jurado, A. Sanz, and M. Lopez-Frias. (1993). Nutritional assessment of two vegetable protein concentrates in growing rats. *J. Agric. Food Chem., 41*, 1282– 1286.

Njoki, J.W., Sila, D. N, and Onyango, A.N (2014). Impact of processing techniques on nutrient and anti-nutrient content of grain amaranth (A. albus). Food Science and Quality Management, 25, 10-17.

Nuss, E. T. and Tanumihardjo, S. A. (2011). Quality Protein Maize for Africa: Closing the Protein Inadequacy Gap in Vulnerable Populations. *Adv. Nutr. 2,* 217–224. doi:10.3945/an.110.000182.

O' Brien, G. K. and Price, M. L. (1983). Amaranth: Grain & Vegetable Type. Echo Technical Note. ECHO. 17391 Durrance Rd, North Ft. Myers, FL 33917, USA.

Ofitserov, N. E. (2001). Amaranth: Perspective raw material for Food-processing and Pharmaceutical Industry. Chemistry and computational simulation, Tatarstan. Pp 1-4.

Oniang'o, R. (2001). Enhancing Peoples Nutritional Status through Revitalization of Agriculture and Related Activities. *African Journal of Food and Nutritional Sciences, 1*(1).

Onyango, C. (2014). Physical properties of dry-milled maize meals and their relationship with the texture of stiff and thin porridge. *African Journal of food science, 8* (8), 435-443

Onwulata, C. I., Smith, P. W., Konstance, R. P. and Holsinger, V. H. (2001b). Incorporation of whey products in extruded corn, potato or rice snacks. Food Research International, 34, 679–687.

Pedersen B., Knudsen K. E. and Eggum B. O. (1990). The nutritive value of amaranth grain (*Amaranthus caudatus*). Energy and fibre of raw and processed grain. Plant-Foods-Hum-Nutr. 40 (1), 61-71.

Pedersen, B., Kalinowski, L. S. and Eggum, B. O. (1987). The nutritive value of amaranth grain (Amaranthus caudalus). Protein and minerals of raw and processed grain. *Plant Foods Human Nutrition, 36.*

Plate, A. Y. A. and Arêas, J. A. G. (2002). Cholesterol-lowering effects of extruded amaranth (Amaranthus caudatus L.) in hypercholesterolemic rabbits, Food Chemistry, 76. 1–6. Price

Railey, K. (1993). Amaranth: A Healthy Grain for Vegetarian Recipes. Health & Beyond Online

Rangana, S. (1994). Manual Analysis of fruits and vegetables production, 2nd Edition, Tata McGraw Hill Co. Ltd., New Delhi, India.

Sammon, A. M. (1999). Maize meal, non-esterified linoleic acid, and endemic cancer of the esophagus— preliminary findings. Prostaglandins & other Lipid Mediators, 57, 167–171.

Schnable, P.S., Ware, D. and Fulton, R.S. (2009). The B73 maize genome: complexity, diversity and dynamics.

Shukla, R. and Cheryan, M. (2001). Zein: the industrial protein from corn. Industrial Crops and Products 13, 171– 192.

Silva-Sánchez, C., Barba de la Rosa, A. P., León-Galván M. F., Lumen B. O., León-Rodríguez, A. and González, E. (2008). Bioactive Peptides in Amaranth (Amaranthus hypochondriacus) Seed. *Journal of Agricultural and Food Chemistry*, *56* (4), 1233-1240.

Singhal, R. S. and Kulkarni P. R. (1988). Composition of the seeds of some Amaranthus species.*Journal of Science, Food and Agriculture, 42*.

Singhal, R. S. and Kulkarni, P. R. (1990a). Utilization of Amaranthus paniculatas (Rajgeera) starch in salad dressing. Starch/Starke 42:52-53.

Singhal, R. S. and Kulkarni, P. R. (1990b). Studies on applicability of Amaranthus paniculatas (Rajgeeraa) starch for custard preparation. Starch/Starke42:102-103.

Spetter, J. and Thompson, L. (2007). The revival of an ancient crop. LEISA Magazine: (23) 3.

Steel, R. G. D and Torrie, J. H (1980). Principles and Procedures of Statistics, 2nd Edition, New York: McGraw-Hill.

Steinkraus, K.H. (editor) (1996) Handbook of Indigenous Fermented Foods, 2nd edn., Marcel Dekker, New York, pp. 111-347; 363-496.

Stephens J.M. (2009). Amaranth- Amaranthus spp. IFAS, University of Florida, Florida. Pp 1-2.

Stratton, R. J., Green, C..J and Elia, M. (2003). Scientifc criteria for defning malnutrition Disease-Related Malnutrition. An Evidence Based Approach to Treatment. CABI Publishing Wallingford.

Sustainable Rural Livelihoods Program (SRLP) (2005). Annual Report 2004-2005.

Tagwira, M., Tagwira, F. Dugger, R. and Okum, B. (2006). Using grain amaranth to fight malnutrition. RUFORUM working document No. 1. Pp. 201-206.

Tembo, M. S. (2007). Nshima and Ndiwo are the Most Important Pair of Staple Foods among Zambians in Southern Africa.

Terblanche, N.S. and Boshoff, C. (2004). The in-store shopping experience: A comparative study of supermarket and clothing store customers. *South African Journal of Business Management*, *35*(4), 1-10.

Thanapornpoonpong, S. (2004). Effect of nitrogen fertilizer on nitrogen assimilation and seed quality of amaranth (Amaranthus spp.) and quinoa (Chenopodium quinoa Willd). Doctoral Dissertation submitted for the degree of Doctor of Agricultural Sciences of the Faculty of Agricultural Sciences Georg-August-University of Göttingen.

Ullah, I., Ali, M. and Farooqi, A. (2010). Chemical and Nutritional Properties of Some Maize (Zea mays L.) Varieties Grown in NWFP, Pakistan. *Pakistan Journal of Nutrition. 9,* 1113-1117.

Van der Merwe, B., Erasmus, C. and Taylor, J. R. N. (2001). African maize porridge: a food with slow in vitro starch digestibility. Food Chemistry, 72, 347-353.

Watson, S. A. and Ramstad, P. E. (1987). Corn: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, MN.

Weber, E. (1987). Amaranth Grain Production Guide. Rodale Research Center Rodale Press, Inc.

Wen, L-F., Rodis, P. and Wasserman, B. P. (1990). Starch fragmentation and protein in solubilization during twin screw extrusion of cornmeal. Cereal Chemistry, 67, 268-275.

West, C., Pepping, F., Scholte, I., Schultink, W., Jansen, W., and Albers, H. (1987). Food Composition Table, Wageningen: Wageningen Agricultural University

World Health Organization (WHO), (2002). World Health Report 2002: Reducing risks, promoting healthy life: Overview. Geneva: World Health Organization.

World Health Organization (WHO), (2004). Iodine status worldwide. WHO Global Database on Iodine Deficiency. Geneva: World Health Organization.

World Health Organization (WHO) and FAO (2006). Guidelines on food fortification with micronutrients.

World Health Organization (WHO), (2008). Worldwide prevalence of anaemia 1993-2005. WHO Global Database on Anaemia. Geneva: World Health Organization.

World Health Organization (WHO), (2009). Global prevalence of vitamin A deficiency in populations at risk 1995–2005. WHO Global Database on Vitamin A Deficiency. Geneva: World Health Organization.

World Food Program (WFP) (2012). Food quality control. www.wfp.org (Last Accessed: 15 November 2015).

World Food Summit (1996). Rome Declaration on World Food Security.

World Health Organization (WHO), (2009). Complementary feeding of children in Developing Countries: A review of current Scientific Knowledge. WHO/NUT/98.1

Yadav S. K. and Sengal S. (1995). Effect of home processing on ascorbic acid and betacarotene content of spinach (Spinacea oleracia) and amaranth (Amaranth tricolor). *Plant Foods Hum. Nutr., 47,* 125-131.

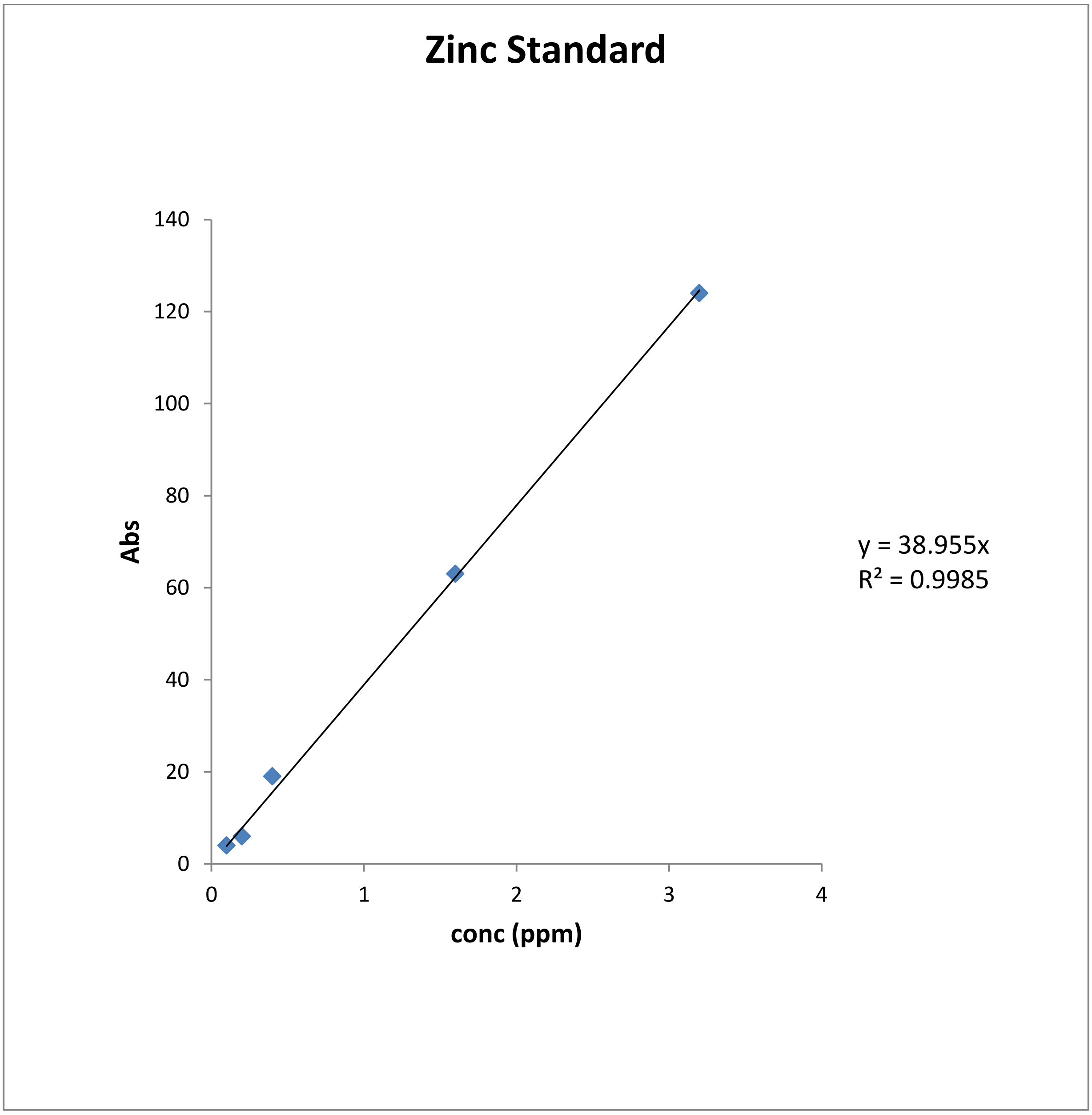
Yarger, L. (2008). Amaranth: Grain and Vegetable Types. Echo Technical Note, Florida. Pp 1-14.

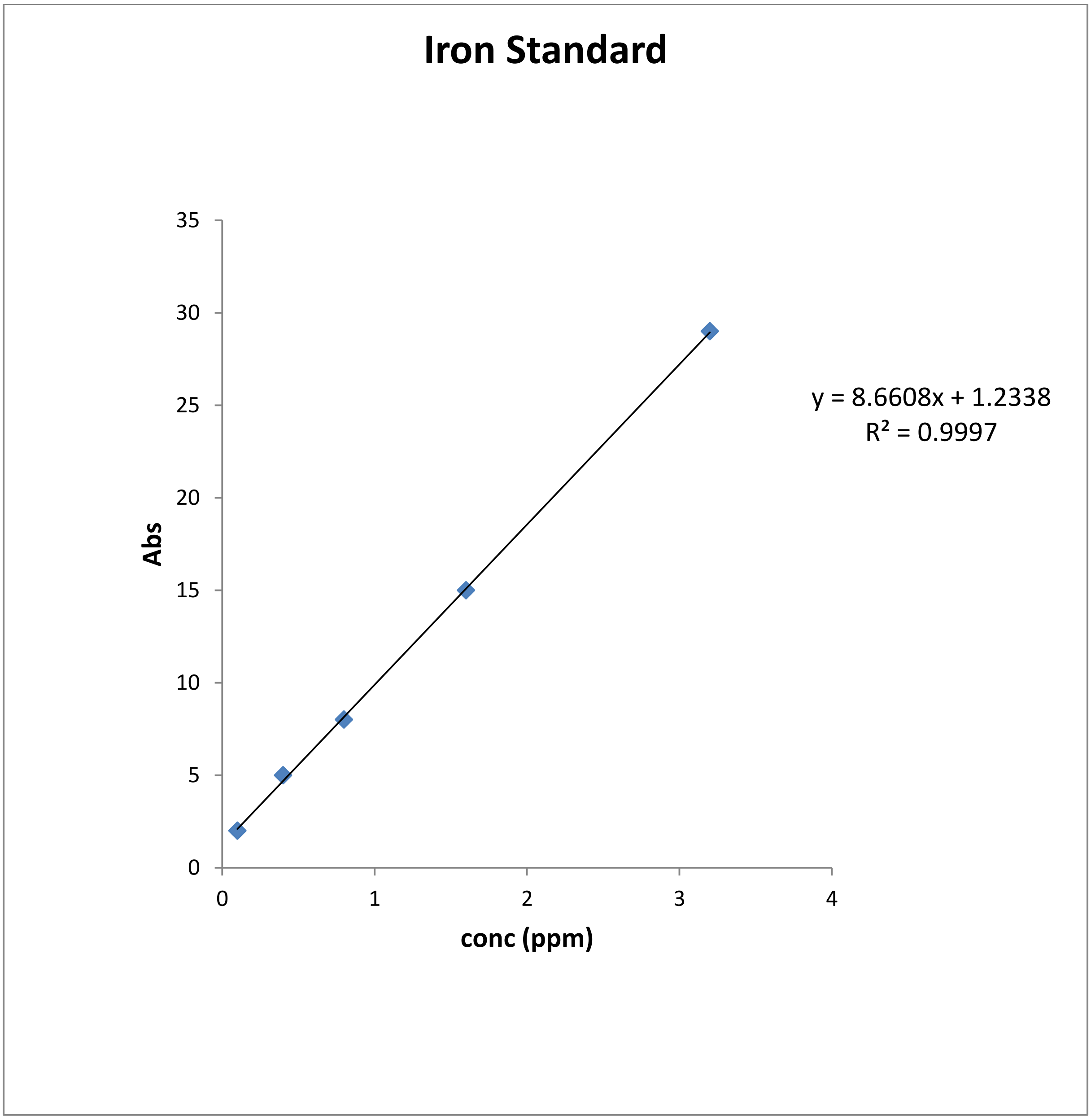
Yongo, G. C. (2009). Grain Amaranth Production. Ndhiwa Community Knowledge Centre, Ndhiwa, Homa Bay, Kenya.

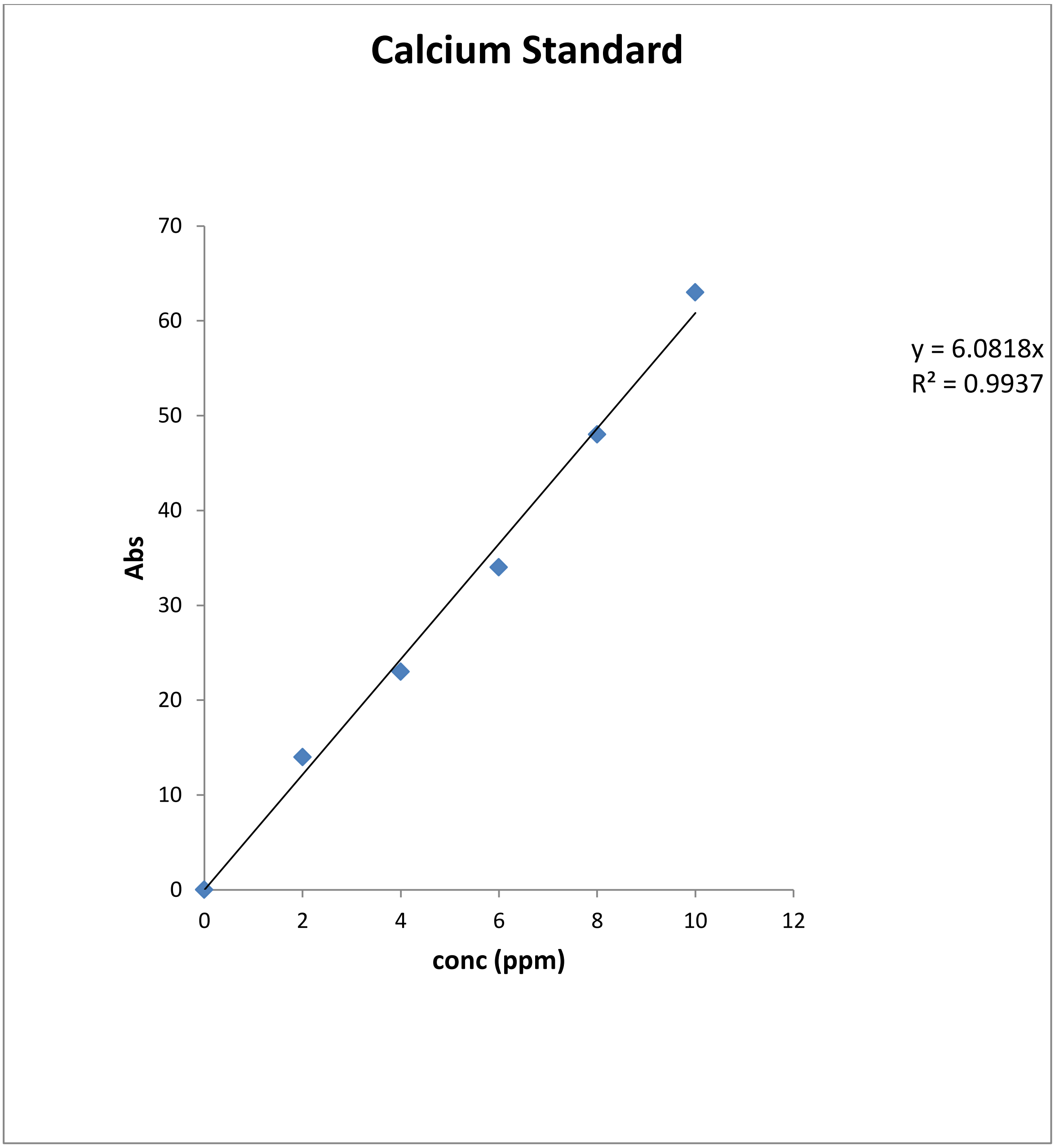
Zhang, W. and Hoseney, R. C. (1998). Factors affecting expansion of corn meals with poor and good expansion properties. Cereal Chemistry, 75, 639-643.

APPENDICES

Appendix 1: Standard curves for mineral composition




Iron Standard
35
30
25
20
15
10
5
0
Abs
0
1
2
3
4
conc (ppm)
y = 8.6608x + 1.2338
R² = 0.9997


Calcium Standard
Abs
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
conc (ppm)
y = 6.0818x
R² = 0.9937

Appendix 2: Supermarket Survey questionnaire

Name of supermarket………………………….. Date…………………………………..

Product name	Package unit	Price (KES)	Average sales March	Average sales April

Thank you for your time

Appendix 3: industrial Survey questionnaire

Date of interview: ………………….....

Name of the industry

…………………………………………………………………………………………

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Where do you source your raw materials from?

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At what price per kg do you buy amaranth grain?

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What is the amount of amaranth grain used per month?

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What are the post-harvest practices carried out after receiving grain amaranth? …………………………………………………………………………………………………
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…………………………………………………………………………………………………

Which are the common processing methods applied on grain amaranth? Please mention in order of the highest to lowest
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In the case of processing, what are the time temperature combinations used? …………………………………………………………………………………………………
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What are the blending ratios of grain amaranth flours with other flours? …………………………………………………………………………………………………
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…………………………………………………………………………………………………
…………………………………………………………………………………………………

Thank you for your time

Appendix 4: Sensory evaluation form

SENSORY EVALUATION FORM

The sensory evaluation being carried out is of 'ugali'. Different flour mixing ratios have been used in preparation. The key flour includes Grain amaranth flour, refined maize flour and whole meal flour. The scale used is the 9 point hedonic scale whereby;

1 Dislike extremely
6 Like slightly

2 Dislike very much
7 Like moderately

3 Dislike moderately
8 Like very much

4 Dislike slightly
9 Like extremely

5 Neither like nor dislike

	SAMPLES							
	F	M	H	N	G	S	J	A
Appearance								
Colour								
Taste								
Mouthfeel								
Texture								
Overall Acceptability								

THANK YOU FOR YOUR TIME

Appendix 5: Published paper

BIOFORTIFICATION OF MAIZE FLOUR WITH GRAIN AMARANTH FOR IMPROVED NUTRITION

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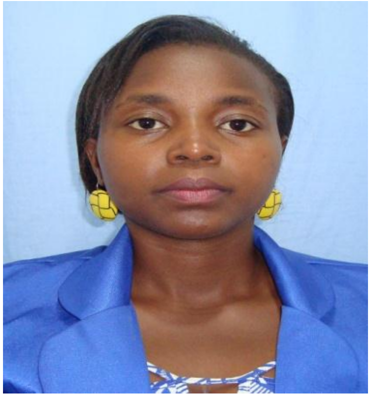

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BIOFORTIFICATION OF MAIZE FLOUR WITH GRAIN AMARANTH FOR IMPROVED NUTRITION

ABSTRACT

Food insecurity and malnutrition remain two major problems in Kenya that causes under-nutrition (protein-energy malnutrition) and nutrient deficiencies. In a bid to combat the problem, the government has targeted increased production of the micronutrient (minerals, vitamins) fortified maize flour. This is mostly based on chemical fortificants which is a short term measure that targets reducing the level of malnutrition while creating better livelihoods. However, there is need to think about long term and sustainable strategies. Among the causes that have led to food and nutrition insecurity in the country, there is the over reliance of maize as the staple food with others being sorghum, rice and millet. This study aimed at providing a better and more sustainable approach by fortifying maize based diets with grain amaranth. Maize was procured from National Cereals and Produce Board and processed to refined flour while amaranth grain that had been toasted at 100ºC for 5 minutes and milled into flour was obtained from Annicos Limited. Complementary formulations were then prepared by blending the flours. The refined maize flour was mixed with varying ratios of 0, 20, 30 and 40% of the grain amaranth flour. The proximate composition, mineral content and microbiological analysis of the raw materials and the blends were determined using recognized standard methods while protein digestibility was determined after enzyme digestion. Nutritional composition of grain amaranth flour differed significantly ($p \leq 0.05$) as compared to refined maize flour; amaranth flour was found to be superior in proteins (15.82%), lipids (7.61%), ash (2.54%) and fibre (4.39%) as compared to proteins (6.29%), lipids (1.92%), ash (0.55%) and fibre (0.76%) in refined maize flour. Adding grain amaranth flour to refined maize flour at the different ratios increased the nutrient density significantly

($p \leq 0.05$), particularly protein, iron, calcium and zinc. However, it decreased the digestibility of protein significantly ($p \leq 0.05$). The results indicate that although adding 40% grain amaranth gave the best results, the most acceptable blend was the 20% grain amaranth addition. They also indicate that "food to food" fortification can be an approach that can be adapted towards meeting the nutrition requirements of our society.

Key words: Nutrition security, biofortification, amaranth flour, maize flour, optimal blend

INTRODUCTION

Amaranth is an annual herb and is a pseudo cereal native to South and Central America [1]. There are about 60 amaranth species and between 4000 – 6000 varieties [2]. These species are cultivated in the world as cereals, leafy vegetables, and ornamental plants, while others occur naturally as weeds. Grain amaranth is mainly characterized by large flower heads which produce thousands of dicot seeds. It thrives mostly in temperate and tropical regions and also in poor soils and arid conditions [3]. In Kenya, the crop is mainly grown in Central, Western and Nyanza regions naturally in open fields especially in the rural areas. The average yield in fields is highly dependent on the weather patterns and agronomic practices [1]. This means a lot of arid and semi-arid areas can be converted to amaranth producing zones without compromising maize production. Amaranth grain contains significantly higher protein than most other cereal grains. In particular, it has a relatively high proportion of lysine, an essential amino acid, compared to maize, leading to its effective utilization as a protein source [4]. It is also a good source of fats [5], carbohydrates, fibre, sugars, vitamins (A, B, C, E) and minerals (iron, calcium and zinc) [6].

Malnutrition is a widespread problem in Kenya mainly manifested through under-nutrition (protein-energy malnutrition) and micronutrient deficiency. On a national level, 35% of children under 5 years are stunted, 16% are underweight and 7% are wasted [7]. Prevalence of under-nutrition is high in urban slums where stunting among children under 5 years is more than 40% [8]. Food and nutrition insecurity aggravated by the over reliance on staple foods especially highly refined maize is the main cause of under-nutrition in Kenya. Besides children, the other most vulnerable individuals are pregnant and lactating mothers, the elderly and refugees [9].

Maize is a staple food in many African countries thus increased possibilities for nutritional interventions, hence the main vehicle in fortification programs. Currently, maize flour is being fortified with vitamins A, B_1, B_2, B_6, niacin and folate; iron and zinc. However, concerns on bioavailability, lipid oxidation and sensory quality resulting from fortification have previously been raised [10]. In Kenya, it is now government policy to fortify all flours. This is geared towards mitigating malnutrition within the vulnerable groups. The use of chemical fortificants is a short term measure which targets reducing the level of malnutrition while creating better livelihoods. However, there is need to think about long term and susainable strategies. In this project, other than using chemical fortificants, a food diversification approach is applied in which maize flour is fortified using grain amaranth. This approach has been previously recommended for unfermented porridge where it was shown that, inclusion of 70% amaranth flour in porridge will increase dietary iron intake among children in Kenya [11]. It is anticipated that this will be more sustainable since amaranth is locally available and the nutritional outcomes are better.

MATERIALS AND METHODS

Sample preparation

Maize grain was provided by the National Cereals and Produce Board and processed to refined flour while amaranth grain which had been toasted at 100ºC for 5 minutes and milled into flour was obtained from Annicos Limited, Nairobi. The refined maize flour was mixed with inclusions of 0% (RMF), 20% (RA1), 30% (RA2) and 40% (RA3) of the grain amaranth flour as shown in table 1. Preliminary studies indicated that with above 40% of grain amaranth, the product losses its cooking-pasting properties. Complementary meals were then prepared from the refined maize flour and the different blends of the flours.

Determination of proximate composition

Proximate composition of the raw materials and blends were determined as follows: Moisture content was determined by the oven drying method according to AOAC [12] method 930.04; Crude protein (N x 6.25) was determined using semi-microkjeldal method according to the AOAC [12] procedure 978.04; Crude fat was determined by the Soxhlet extraction according to the AOAC [12] method 920.85; Crude ash was determined by ashing according to AOAC [12] method 923.05; Crude fibre was determined by the Hennenberg-Stohman according to AOAC [12] method 920.86. An amount of 5 grams of the samples was used for each replicate. Total carbohydrates content of the samples was determined by difference [13]. Nutritional analysis was carried out in triplicate for the raw materials (refined maize flour (RMF) and grain amaranth flour (GAF)) and the different blends (RA1, RA2, RA3).

Determinations of mineral composition

The ash that was previously determined was cooled and dissolved in 15 ml 10% HCl in a volumetric flask which was then topped up to 100 ml mark with distilled water. This was used for mineral determination according to the AOAC method [13]. Iron, calcium and zinc were determined by Atomic Absorption Flame Spectrophotometer (Model A. A-6200, Shimadzu., Kyoto, Japan).

Determination of Protein Digestibility

Protein digestibility was determined according to the method by Hamaker *et al* [14] whereby 1 g samples were digested in 2ml of pepsin for 2 hours at 37ºC. The residual protein was then determined using Semi-MicroKjeldal method according to the AOAC [12] procedure 978.04.

Microbiological analysis

Microbial analysis was done for samples kept for 0, 3 and 6 months. Total plate counts were determined on Plate Count Agar pour plates and enumerated after an incubation period of 48–

72 hours at 37°C. *E. coli* was enumerated on Violet Red Blue Agar pour plates after an incubation period of 24hrs at 37ºC. Yeast and Moulds enumerated on Potato Dextrose Agar pour plates after an incubation period of 120 hours at 25ºC [15].

Sensory analysis

Thirty semi-trained panellists consisting of staff members and graduate students from Jomo Kenyatta University of Agriculture and Technology evaluated 'ugali' made from the different blends. All samples were prepared and cooked in clean drinking water boiled for 10 minutes and the flour blends added and mixed to make 'ugali'. Characteristics evaluated were: a) Appearance, b) Colour, c) Mouth feel, d) Texture, e) Taste and f) Overall preference. A 9-point hedonic scale [16] was used to measure the consumer acceptability of the products. The relative importance of each factor was compared numerically on a scale of 9 to 1 (9 = like extremely, 1 = dislike extremely). Each panellist gave a score. The average score for each sample was then calculated.

Data analysis

Each determination was carried out in triplicate; the figures were averaged and standard error calculated. Data was subjected to ANOVA ($p \leq 0.05$) using GenStat 14th edition (VSN international, UK). Comparison of treatment means was done using Duncan's Multiple Range Tests [17].

RESULTS

The nutritional composition of the raw materials and the formulated blends are shown in table 2. There were significant differences ($p \leq 0.05$) in the moisture content of the flours. The moisture content of grain amaranth flour (GAF) was found to be lower than that of the refined maize flour (RMF), and all the maize-amaranth flour blends (RA1, RA2 and RA3). The moisture content of the flour blends was within the range of 11.11 - 12.57%.

Grain amaranth flour (GAF) had significantly ($p \leq 0.05$) higher lipid content than refined maize flour (RMF). Significant differences ($p \leq 0.05$) in lipid content were observed among the blends. The lipid content of the blends ranged from 3.75 - 4.63% with RA3 being the highest. Similarly, GAF had significantly ($p \leq 0.05$) higher protein content than RMF. Significant differences ($p \leq 0.05$) in protein content were observed among the blends. The protein content of the blends ranged from 6.62 – 8.80% with RA3 being the highest. Crude fibre was significantly ($p \leq 0.05$) higher in GAF as compared to RMF. Significant differences ($p \leq 0.05$) were observed among the blends. The fibre content of the blends ranged from 0.83 - 2.99% with RA3 being the highest. Grain Amaranth flour had significantly ($p \leq 0.05$) higher ash content than RMF. Significant differences ($p \leq 0.05$) were observed among the blends. The ash content of the blends ranged from 0.83 - 1.10%. Sample RA3 had the highest nutritional composition. Refined maize flour had significantly ($p \leq 0.05$) higher carbohydrate content than GAF. Significant differences ($p \leq 0.05$) were observed in the carbohydrate content of the blended flours. The carbohydrate content of the blended flours ranged from 71.38 - 77.44%.

The mineral composition of the raw materials and the formulated blends are shown in table 3. The results indicate that there was a significant difference ($p \leq 0.05$) between GAF and RMF. GAF was higher in calcium, iron and zinc as compared to RMF. Significant differences ($p \leq 0.05$) were also observed in the mineral content of the blended flours. Sample RA3 had the highest levels of the minerals analysed; calcium, iron and zinc.

Figure 1 shows that the protein digestibility of the different flours and blends ranged from 77.04 - 86.24%. RMF had significantly ($p \leq 0.05$) higher protein digestibility than GAF. There were significant differences ($p \leq 0.05$) observed among the blends with RA1 being the highest.

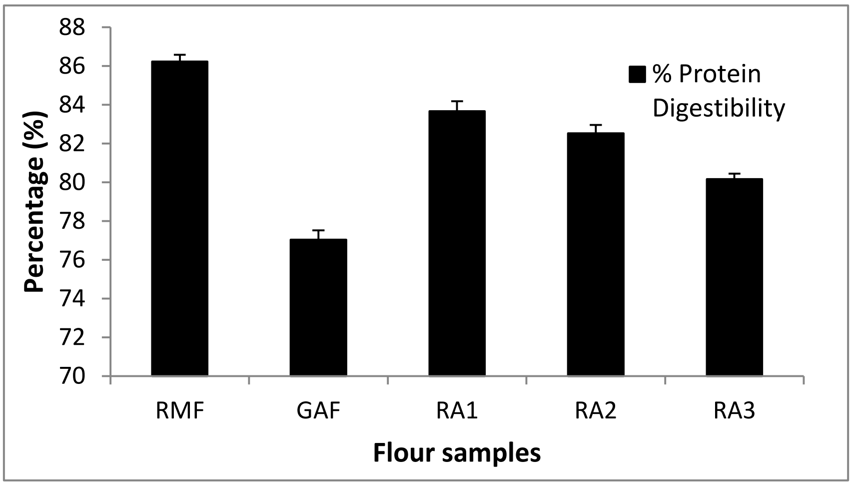

Figure 1: Protein digestibility of the different flour samples. RMF- Refined Maize flour, GAF- Grain Amaranth flour, RA1- 80:20 (RMF:GAF) RA2- 70:30 (RMF:GAF), RA3- 60:40 (RMF:GAF)

The results in Figure 2 indicate the microbial counts during storage. GAF had significant ($p \leq 0.05$) lower microbial counts (Total plate count (A), *E. coli* (B) and yeasts and moulds (C)) as compared to RMF during the storage interval. Significant differences ($p \leq 0.05$) were also observed among the blends. All samples followed an increasing trend as the storage months progressed.

The sensory attributes of 'ugali' made from the different blends are shown in Table 4. Generally, the different variations of "ugali" samples were either acceptable or neutral with significant differences ($p \leq 0.05$) among the products. However, there was no significant difference ($p \leq 0.05$) in all the sensory attributes between RA2 and RA3. The colour of the 'ugali' prepared from RMF was most preferred followed by RA1 and RA2. The mouth feel, texture and taste from RMF were most preferred. The results show that the most acceptable blend was RA1.

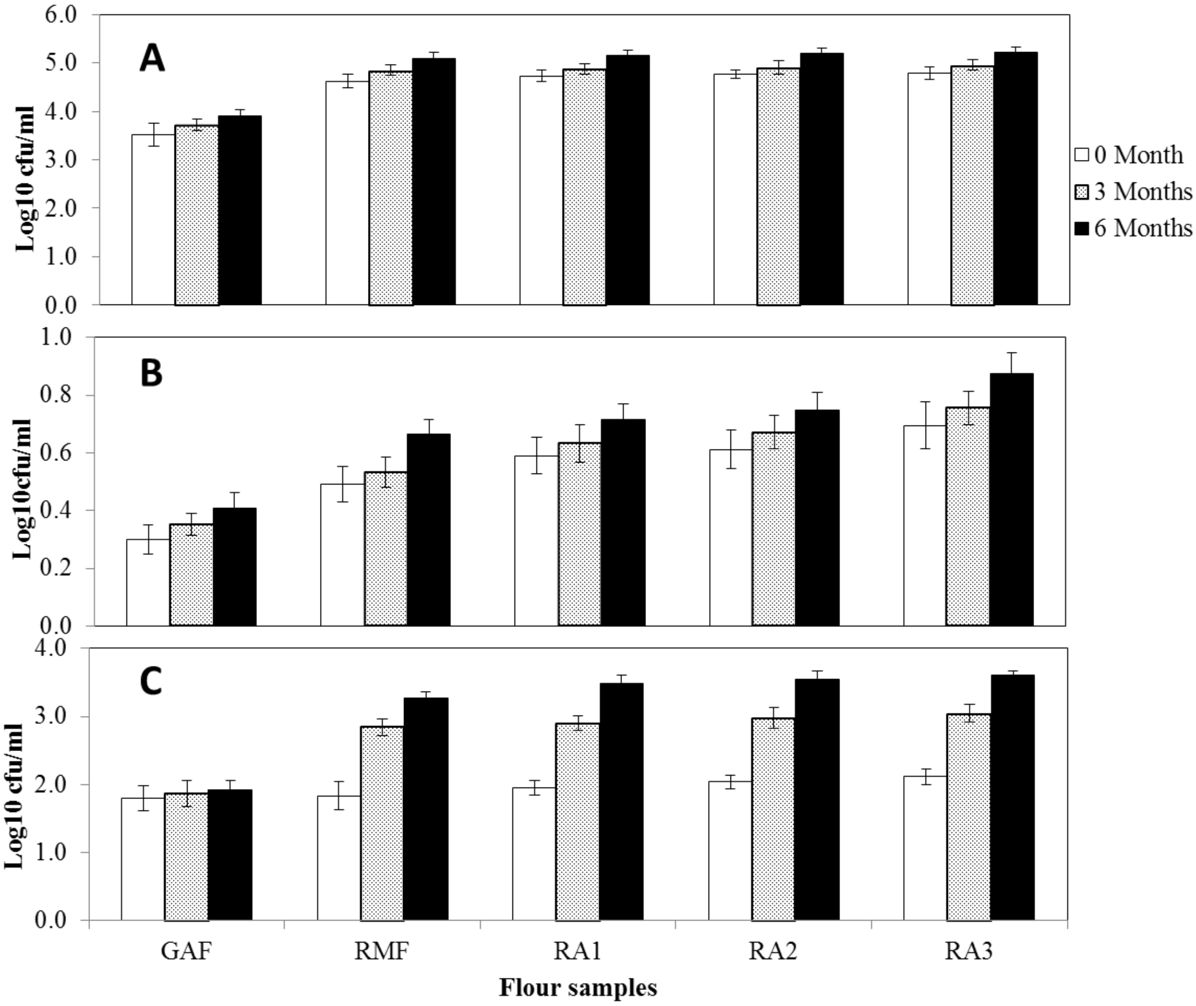

Figure 2: Total plate count (A), *E. Coli* (B), and fungal (yeasts and moulds) count (C) of the different flour samples. GAF- Grain Amaranth flour, RMF- Refined Maize flour, RA1- 80:20 (RMF:GAF), RA2- 70:30 (RMF:GAF), RA3- 60:40 (RMF:GAF)

DISCUSSION

The moisture content of grain amaranth flour (GAF) was lower than refined maize flour (RMF) and all the maize-amaranth flour blends (RA1, RA2 and RA3). The moisture content of all the flours was within the recommended limit of 15.5% [18]. The low moisture contents observed in this study is an indicator of longer shelf life of the products. Moisture content is highly dependent on the duration of the drying process thus an index of storage stability of the flour.

Increase in the proportion of GAF in the blends resulted in increase of protein, lipid, fibre and ash content. The protein content of GAF was similar to that reported values [6]. Therefore, the use of grain amaranth flour in blending RMF increases the biological value of the blends [19]. Increasing the refinement degree of maize flour eliminates much of the germ portion. This results in removal of most of the oil which is found mainly in the germ. Ash content is an indication of the total mineral matter in the flour. All the samples were within the acceptable range of up to 3% ash [18]. Generally, removal of the outer skin of the maize during the milling process reduces the ash, protein and fat content [20]. The high carbohydrate content (61.19-77.75%) observed in this study indicates that the samples are energy dense foods which thus can be incorporated in addressing energy malnutrition.

From the studies, sample RA3 had the highest amounts of the minerals analysed. This indicates that grain amaranth can successfully be used to improve the micronutrient content of refined maize flour. Calcium plays a key role in bone and tooth development, nerve transmission and muscular contractions. Iron is a haemoglobin component which transports oxygen to body tissues. It is also a component of proteins and enzymes. Iron deficiencies especially in infants, has been associated with cognitive impairment and mental development [21]. Zinc is an essential component of most enzymes in the body [22]. The increased popularity and use of highly milled maize meal as opposed to traditionally ground or lightly milled maize results in deficiency of such micronutrients. The mineral constituents lost in milling may be replaced in maize meal, as in other cereal flours, by fortification with grain amaranth flour as indicated by the results.

The protein digestibility of RMF was higher than that reported of 84.5% [23]. Higher protein digestibility in refined maize flour was probably due to the reduction of the anti-nutrients level during milling [24]. Reported values on protein digestibility of grain amaranth range between 74-80% [7]. In vitro digestibility of 61 - 76% has previously been reported for raw grain amaranth proteins [25]. The in vitro protein digestibility of grain amaranth in this study were higher than reported values for whole raw maize (66.6%) and sorghum (55.8 - 59.1%) [26].

The relatively low protein digestibility of the GAF as compared to RMF in this study may be attributed to the influence of anti-nutrients such as phytates, tannins, enzyme inhibitors, lectins and dietary fibre. These anti nutrients interact with protein to form complexes that decrease protein solubility [24]. Increase of GAF in the blend resulted to reduced protein digestibility. The decrease could be attributed to the high content of anti-nutrients in grain amaranth flour. Low protein digestibility in the diet affects the bioavailability of the amino acids and also the protein quality of the food. However, the higher protein content of GAF will compensate for the reduced digestibility in the blends.

Generally, flour is considered a microbiologically safe product due to its low water content [27]. The results in Figure 2 indicate the rate of microbial growth during storage interval. GAF had significantly ($p \leq 0.05$) lower microbial counts as compared to RMF. This may be due to the fact that the grains were toasted for 5 minutes at 100ºC before milling therefore reduction in the microbial load. Studies have shown few cases on food borne disease outbreaks caused by flours [28]. High microbial counts found in refined maize flour may be attributed to microbes already present in the cereal before milling, and/or the milling method. Build-up of residues at the storage and handling points may be a source of contamination [28]. Increase in microbial load of the blends during storage could be attributed to the blends having a better nutrient base than refined maize meal. Therefore, the blends offered better growth conditions for microbes.

The level of contamination of fungi (yeasts and moulds) is highly important as it affects the quality and safety of the flour. The maximum legal limit for fungi in flour is $\log_{10}$ 5 cfu/g

[18]. Yeasts and moulds from the results were within the recommended limits. Yeast and moulds in the blends increased with increased storage time. This could be as a result of favourable conditions of the microbes to grow. Fungi levels beyond the maximum limit lead to deterioration of foods and causes food borne diseases. Moulds may produce enzymes which begin to degrade the lipids consequently affecting the quality parameters of the maize meal during storage. Bothast *et al*. [29] found that both bacteria and mould counts in maize meal increased during the storage period before finally decreasing.

E. coli and total plate count (TPC) indicate the hygienic properties and gives information on shelf life of the food. Higher counts beyond the maximum limit indicate poor sanitation in the processing methods as well as mishandling of the raw products. *E. coli* was detected in all the samples at very low levels and the results were within the WFP maximum limits of $\log_{10}1$ cfu/ml. The maximum legal limit for total plate count is $\log_{10}$ 5 cfu/ml [18]. The results obtained were therefore consistent with WFP recommendations [18] up to 3 months. However, the shelf life of the blends can be improved by applying Good Agricultural Practices (GAP).

Incorporation of grain amaranth flour to refined maize flour significantly ($p \leq 0.05$) reduced the attributes of appearance, colour, mouth feel, texture, taste and overall acceptability. However, there was no significant difference ($p \leq 0.05$) in all the sensory attributes between RA2 and RA3.The colour of the ‘ugali’ prepared from RMF was most preferred followed by RA1 and RA2. The preference for RMF could be due to its lightest colour. Increase of grain amaranth flour resulted in darker colouration. The mouth feel, texture and taste from RMF were most preferred. Addition of Grain amaranth flour resulted in the product obtaining a ‘grainy’ texture. The results show that the acceptable blend was RA1. Further increase of the grain amaranth flour in the blends resulted in progressive decline in acceptability. This can be explained by familiarity of solely maize flour.

CONCLUSION

According to the study, grain amaranth flour is highly nutritious particularly with respect to proteins, fat and minerals as compared to refined maize flour. Blending refined maize flour with grain amaranth flour significantly increases the nutritional quality of the refined maize flour. The sample with the highest nutritional profile was the blend with 40% grain amaranth flour while the most acceptable blend was the 20% grain amaranth addition. The approach of food to food fortification can provide a sustainable alternative to current chemical fortification approaches if indigenous food crops of high nutrient content are incorporated in common staple diets. Grain amaranth can successfully be used in mitigating nutrition insecurity and reducing malnutrition levels in Kenya if used in blended maize recipes.

ACKNOWLEDGEMENT

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TABLES

Table 1: Formulation of maize and amaranth blends

	RMF	RA1	RA2	RA3	GAF
Refined Maize Flour	100	80	70	60	0
Grain Amaranth Flour	0	20	30	40	100
Total Weight (g)	100	100	100	100	100

RMF- Refined Maize flour, GAF- Grain Amaranth flour, RA1- 80:20 (RMF: GAF) RA2-70:30 (RMF: GAF), RA3- 60:40 (RMF: GAF)

TABLE 2: Proximate composition of the raw materials and the blends

Samples	Moisture	Crude fat	Crude Protein	Crude Ash	Crude Fibre	CHO
RMF	$12.73^{d}\pm0.09$	$1.92^{a}\pm 0.27$	$6.29^{a}\pm 0.17$	$0.55^{a}\pm 0.05$	$0.76^{a}\pm 0.10$	$77.75^{c}\pm0.26$
GAF	$8.34^{a}\pm0.02$	$7.61^{d}\pm 0.21$	$15.82^{e}\pm0.22$	$2.54^{d}\pm0.02$	$4.50^{d}\pm 0.13$	$61.19^{a}\pm0.57$
RA1	$12.57^{d}\pm 0.02$	$3.75^{b}\pm 0.19$	$6.62^{b}\pm0.22$	$0.83^{b}\pm 0.01$	$1.78^{b}\pm0.19$	$77.44^{d}\pm0.51$
RA2	$12.15^{c}\pm 0.03$	$4.11^{bc}\pm 0.08$	$7.92^{c}\pm 0.24$	$1.04^{c}\pm0.01$	$2.15^{b}\pm0.26$	$72.63^{c}\pm 0.24$
RA3	$11.11^{b}\pm 0.11$	$4.63^{c}\pm 0.13$	$8.80^{d}\pm0.12$	$1.10^{c}\pm 0.03$	$2.99^{c}\pm0.18$	$71.38^{b}\pm 0.22$
LSD 5%	0.377	0.585	0.141	0.121	0.7077	1.179
Grand Mean	11.38	4.41	9.09	1.21	2.433	71.48

Means within the same column with different alphabetic superscripts were significantly different at ($p\leq0.05$), values are presented as means ± standard error, n=3
CHO – Carbohydrates, LSD – Least Significant Difference

TABLE 3: Mineral composition of raw materials and the blends

Samples	Calcium	Iron	Zinc
RMF	$2.46^{a}\pm0.15$	$0.72^{a}\pm 0.08$	$0.67^{a}\pm 0.02$
GAF	$290.13^{e}\pm1.30$	$9.58^{e}\pm 0.14$	$4.38^{e}\pm0.03$
RA1	$57.1^{b}\pm 0.53$	$2.21^{b}\pm 0.21$	$1.41^{b}\pm0.10$
RA2	$88.8^{c}\pm 1.22$	$3.06^{c}\pm 0.25$	$1.77^{c}\pm 0.07$
RA3	$114.8^{d}\pm 1.13$	$4.05^{d}\pm 0.10$	$2.15^{d}\pm0.09$
LSD 5%	0.377	0.585	0.141
Grand Mean	11.38	4.41	9.09

Means within the same column with different superscripts were significantly different at ($P\leq0.05$), values are presented as means ± standard error, n=3, LSD – Least Significance Difference

Table 4: Sensory evaluation scores for 'ugali' from the blends

Samples	Appearance	Colour	Mouth feel	Texture	Taste	Overall Acceptability
RMF	8.17^{c}±0.14	8.00^{c}±0.16	7.63^{c}±0.18	7.57^{c}±0.23	7.80^{c}±0.19	8.00^{c}±0.17
RA1	6.37^{b}±0.26	5.93^{b}±0.35	6.37^{b}±0.35	6.20^{b}±0.31	6.27^{b}±0.36	6.27^{b}±0.29
RA2	5.03^{a}±0.36	4.97^{a}±0.35	4.50^{a}±0.39	4.73^{a}±0.39	4.53^{a}±0.40	4.93^{a}±0.38
RA3	4.40^{a}±0.33	4.93^{a}±0.30	4.30^{a}±0.44	4.33^{a}±0.41	4.20^{a}±0.43	4.43^{a}±0.33
LSD 5%	0.771	0.825	0.945	0.962	0.959	0.804
Grand Mean	6.08	5.98	5.69	5.79	5.75	6.00

Means within the same column with different alphabetic superscripts were significantly different at ($p \leq 0.05$), values are presented as means ± standard error, n=30, LSD – Least Significant Difference

REFERENCES

1. **O'Brien GK and ML Price** Amaranth: Grain & Vegetable Type. Echo Technical Note. ECHO. 17391 Durrance Rd, North Ft. Myers, FL 33917, USA, 1983.
2. **Yarger L** Amaranth: Grain and Vegetable Types. Echo Technical Note, Florida, 2008; 1-14.
3. **Kauffman CS and LE Weber** Grain amaranth. Advances in new crops. Timber Press, Portland,1990; 127-139.
4. **Saunders RM and R Becker** *Amaranthus*. **In**: Pomeranz Y (ed). Advances in cereal science and technology. St. Paul, Minn, USA: American Association of Cereal Chemistry, 1983; **6:** 6.
5. **Njoki JW, Sila DN and AN Onyango** Impact of Processing Techniques on Nutrient and Anti-Nutrient Content of Grain Amaranth (*A. albus*). *Journal of Agriculture and Food Technology,* 2014; **25:** 10-17.
6. **Kariuki SW, Sila DN and GM Kenji** Nutritional Profile of Amaranth Grain Varieties Grown in Kenya**.** *Journal of Agriculture and Food Technology*, 2013; **17**:19-25.
7. **KDHS.** Kenya Demographic and Health Survey. National Council for Population and Development, Central Bureau of Statistics and Ministry of Planning and National Development, Nairobi, Kenya 2008-2009.
8. **Abuya BA, Ciera J and E Kimani-Murage** Effect of Mother's Education on Child's Nutritional Status in the Slums of Nairobi. *BMC pediatr*, 2012; **12**: 80.
9. **FAO**. Nutrition country profile. Republic of Kenya. FAO, Rome, 2005; 3-4, 9-15, 21-27, 31-32.
10. **Hurrell RF, Reddy M, Dassenko SA and JD Cook** Ferrous fumarate fortification of a chocolate drink powder. *British Journal of Nutrition*, 1991; **65**: 271-283.

11. **Macharia-Mutie CW, Van de Wiel AM, Moreno-Londono AM, Mwangi AM and ID Brouwer** Sensory Acceptability and Factors Predicting the Consumption of Grain Amaranth in Kenya. *Ecology of Food and Nutrition*, 2011; **50**: 375-392.
12. **AOAC.** Official methods of analysis of the Association of *Official Analytical Chemists,* I6th Edition. Volume II, Washington DC, 1995.
13. **Pearson D** The Chemical Analysis of Foods, 7th edition. Churchill Livingstone, Edinburg, 1976.
14. **Hamaker BR, Kirleis LG, Butler AW, Axtell JD and ET Mertz** Improving the in vitro protein digestibility of sorghum with reducing agents. *Proceedings of the National Academy of Sciences of the United States of America*, 1987; **84**: 626-628.
15. **Batool SA, Rauf N, Tahir SS and R Kalsoom** Microbial and Physico-chemical contamination in the wheat flour of the twin cities of Pakistan. *Int J. Food Safety*, 2012; **14**: 75-82.
16. **Rangana S** Manual Analysis of fruits and vegetables production, 2nd Edition, Tata McGraw Hill Co. Ltd., New Delhi, India, 1994.
17. **Steel RGD and JH Torrie** Principles and Procedures of Statistics, 2nd Edition, New York: McGraw-Hill, 1980.
18. **WFP.** World Food Programme. Food Quality Control. www.wfp.org, 2012. Last Accesed:15 November 2012.
19. **Bressani R and DR Colmenares** Effect of Germination on the Chemical Composition and Nutritive Value of Amaranth Grain. *Cereal Chemistry*, 1990; **67**: 155-84.
20. **Fandohn P, Hell K, Marasa WFO and MJ Wingfield** Infection of Maize by Fusarium species and contamination with fumonisins in Africa. *African Journal of Biotechnology*, 2003; **2**: 570-579.

21. **Andraca I, Castillo M and T Walter** Psychomotor development and behavior in iron-deficient anemic infants. *Nutrition Revision*, 1997; **55**: 125–132.

22. **Krebs NF, Westcott JE, Butler N, Robinson C, Bell M and KM Hambidge** Meat as a first complementary food for breastfed infants: Feasibility and impact on zinc intake and status. *Journal of Pediatric Gastroenterology and Nutrition*, 2006; **42**: 207-214.

23. **Malomo O, Alamu EA and SO Oluwajoba** Chemical evaluation of protein Quality of Sprouted Maize and Cowpea. *International Journal of Nutrition and Food Sciences*, 2013; **2** (**5**): 254-260.

24. **Alonso, Aguirre A and F Marzo** Effect of extrusion and traditional processing methods on anti-nutrients and in vitro digestibility of proteins and starch in kidney beans. *Food chem*, 2000; **68**: 159-165.

25. **Correa AD, Jokl L and R Carlsson** Chemical constituents, in vitro digestibility, and presence of anti-nutritional substances in amaranth grains. *Arch. Latinoam. Nutr*., 1986; **36**: 319–326.

26. **Duodu KG, Nunes A, Delgadillo I, Parker ML, Mills ENC and PS Belton** Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *J. Cereal Sci*., 2002; **35**:161–174.

27. **ICMSF.** Microorganisms in Foods: Microbial Ecology of Food Commodities. Blackie Academic and Professional, London, 1998; **6**: 313-346.

28. **Berghofer LK, Hocking AD, Miskelly D and E Jansson** Microbiology of wheat and flour milling in Australia. *Int J Food Microbiol*, 2003; **85**:137-49.

29. **Bothast RJ, Anderson RA, Warner K and WF Kwole** Effects of moisture and temperature on microbiological and sensory properties of wheat flour and corn meal during storage. *Cereal Chemistry*, 1981; **58**: 309 –311.