Nutritional and Anti-nutritional Evaluation of Selected Sorghum Varieties and Sorghum – Pigeon Pea Flour Blends for Ready to Eat Complementary Food Product Development

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DECLARATION

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

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DEDICATION

This thesis is dedicated to my parents and siblings for their support.

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
Cu	Copper
$CuSO_4$	Copper sulphate
FAO	Food Agriculture Organization of the United Nation
Fe	Iron
$FeCl_3$	Iron (III) chloride
G	Grams
HCl	Hydrochloric Acid
H_2SO_4	Sulphuric acid
HPLC	High performance liquid chromatography
KALRO	Kenya Agricultural and Livestock Research Organization
K_2SO_4	Potassium sulphate
mg	Magnesium
mg	Milligram
ml	Milliliter
NaOH	Sodium hydroxide
N	Normal
nm	Nanometer
ppm	Parts per million
µl	Micro litre
%	Percent
UN	United Nations

WHO	World Health Organization
Zn	Zinc

ABSTRACT

Sorghum and pigeon peas are important crops in semi-arid regions of Kenya. Improved varieties of these crops that are adapted to these regions have been developed, however a study to determine the nutritional value has not been done. These crops are good sources of protein and minerals but they have some limitations, the presence of anti-nutrients in the grains reduces the bioavailability of these nutrients to the human body. These limitations have been overcome by processing with fermentation and malting the most common. The nutritional composition of two improved sorghum varieties (Seredo and Gadam), two improved pigeon pea varieties (KAT 60/8 and Mbaazi II) and three local varieties of each crop were determined. The effects of fermentation, germination and dehulling techniques on nutritional composition of the food were determined. Malted sorghum, fermented sorghum and steam cooked dehulled pigeon pea flours were prepared by appropriate processes. The flours were blended at three different ratios of 1:1, 2:1 and 5:1 (w/w, sorghum: pigeon pea). Untreated flour of both crops was also prepared and blended at the same ratios and used as control. The nutritional composition varied among the varieties. Seredo exhibited higher levels of anti-nutrient (phytates and tannins) than Gadam and the local variety while local variety exhibited the highest levels of phytates among pigeon peas varieties. The phytates content ranged from 237.4 mg/100 g – 318.8 mg/100 g in sorghum varieties and 149.4 mg/100 g – 208 mg/100 g in pigeon pea varieties. The tannin content ranged from 3.18 mg/100 g – 5.36 mg/100 g in sorghum varieties and 5.11 mg/100 g – 6.05 mg/100 g in pigeon pea varieties. The macro elements (Ca and mg) were generally high while the microelements (Cu and Zn) contents were low for both crops, Seredo and local variety of pigeon pea showed high levels of Fe. From the study KAT 60/8 variety of pigeon pea had the highest protein content and was recommended for improving protein quality of the three sorghum varieties. Results also indicated that Seredo, Gadam and local varieties of sorghum from Tharaka Nithi, Machakos and Makueni Counties respectively had the highest crude protein value hence were recommended for product development. Ready to eat complementary food product was developed by blending flours from the three sorghum varieties and the pigeon pea variety. Fermentation and malting increased the crude protein content of the ready to eat complementary food significantly ($p<0.05$) compared to control.

Fermented sorghum flour blend exhibited the highest levels of crude protein. Crude protein content differed significantly ($p<0.05$) among the formulations, flour blended at the ratio of 1:1 had the highest with 14.91%. The anti-nutrients content of the ready to eat complementary food was significantly reduced by fermentation and malting. The tannin and phytate content was least in fermented and malted food which had 2.25 mg/100 g and 207.5 mg/100 g respectively. Blending flours at the ratio of 1:1 had the anti-nutrients content compared to flour ratios of 2:1 and 5:1. The macro elements (Ca and mg) were found to be highest in formulation 1:1 which had 29.81 mg/100 g and 44.86 mg/100 g respectively. Micro element (Fe) Iron was found to be highest in food formulated at the ratio of 5:1 which had 11.87 mg/100 g. The results suggest that fermentation, as a processing technique and blending sorghum and pigeon pea flours at the ratio of 1:1 using Gadam variety, can be used to effectively enhance the nutritional value of sorghum- pigeon pea ready to eat complementary food with concomitant reduction of its anti-nutritional factors. This may be recommended as desirable for solving the problem of protein deficiency among infant in developing countries.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

The Kenyan economy largely depends on the agricultural sector. About 75% of Kenyans owe their livelihood to agriculture. The agro-grain processing sub sector is one of the leading and well-established industries and it includes major cereal foods such as maize, wheat, rice, sorghum, millet and barley among others.

In many regions of Africa where the climatic conditions are unfavorable for the growth of other crops, sorghum is the major staple food (Eugene *et al*., 2011).Due to high cost and unavailability of animal products such as milk, legumes and cereals are largely used as alternative sources of protein in Eastern semi-arid region of Kenya. However, due to lack of proper processing techniques to increase the nutritional value of the crops infant malnutrition rates are high. Identification of the best varieties of sorghum and pigeon peas in terms of their nutritional value grown in this region and use of appropriate food processing techniques can be used in developing complementary food and help solve malnutrition.

Pigeon pea (*Cajanus cajan* L.) accounts for about 5% of the world's pulse production (Opoku *et al*., 2003). It's an important pulse in the farming systems adopted by small holder farmers in semi-arid regions of Kenya. With an average of 24 g protein per 100 g and about 7 g lysine per 100 g protein (USDA, 2008), pigeon pea is a major source of protein. Due to its high level of protein compositing pigeon pea with sorghum flour improves its protein quality and could help alleviate protein energy malnutrition in infants.

Sorghum and pigeon peas have some limitations, due to the presence of anti-nutritional factors, such as trypsin and amylase inhibitors, phytic acid, and tannins. These compounds are known to interfere with protein, carbohydrates and mineral metabolism. Processing techniques such as fermentation, malting and dehulling techniques have been

used to improve nutritional value of ready to eat weaning mixes. Fermentation increases the protein content of sorghum flour after 48 hours (Mihiret, 2011). Malting has been shown to improve sorghum flour digestibility and quality of the protein, which generally increased with increased malting time (Dewar *et al*., 1997). Pigeon pea is dehulled to improve cooking and nutritional qualities and to reduce cooking time. Dehulling legume grains lowers the anti-nutrients content.

Complementary foods are usually introduced between the ages of six months to three years, this is done with gradual withdrawal of breastfeeding. Infants are able to maintain adequate growth until the age of six months when additional nutrients are required to complement breastfeeding. During this transitional phase protein-energy malnutrition occurs (Ijarotimi *et al*., 2006). It's therefore required to feed infants with highly nutritious food during this period.

There is limited research work on nutritional value for sorghum and pigeon pea in Eastern region of Kenya and value addition options for the crops. This is so because most farmers possess limited information on the nutritional value and processing techniques of both crops. For instance, ready to eat complementary food is a potential value added product of sorghum using pigeon pea. However, there is no proper documentation on information regarding the technological aspects of its production. This study explores nutritional value of sorghum and pigeon pea varieties grown in Eastern region of Kenya and how scientific and technological approaches can be used for improving the nutritional value of sorghum.

1.2 Problem statement

Various researchers in the country have done a lot of research on sorghum and pigeon pea and new varieties of the crops adapted to the climatic conditions of Eastern semi-arid region of Kenya have been released to farmers. Although development of these new varieties has been achieved a study has not been done on their nutritional and sensory characteristics in order to determine their nutritional and anti-nutritional content.

Emergency food aid interventions for infants have been on-going in the semi-arid Eastern region of Kenya, it has not solved infant malnutrition and will not solve it alone. Stunting rates are highest in 18-23 month olds (46%) and lowest in children younger than 6 months (11%). In Kenya 26% of children under 5 are stunted (KDHS, 2014). Stunting rates are extremely high in Eastern Province (42%) (KNBS & ICF Macro, 2010). The period during which complementary food is introduced is critical in a child's life since it poses the greatest danger to the child's growth and development. If the nutritional needs of the child are not met, serious consequences follow for growth, resistance to diseases, intellectual development and survival hence it is the most critical period in life of infants.Many babies of this age often do not grow well. This is because the diet changes from clean breast milk, which contains anti-infective factors, to foods that are often prepared, stored and fed in unhygienic ways. The introduction of such foods into the child's diet often results in increased ingestion of food poisoning organisms that result in sickness and diarrhea. In addition, these foods are often quite bulky (thick) and of very low energy and nutrient density. Further, such complementary foods, which are normally drawn from adult family-pot, are high in fibre and hence not easily digestible by the child. Malnutrition is likely to occur in infants due to these problems.

1.3 Justification

By identifying the best varieties of traditional crops in terms of nutritional value grown in Eastern semi-arid region of Kenya and the processing methods, a nutritious ready to eat complementary food can be developed. This information can be used by agricultural research institutions to promote use of the best varieties in terms of nutritional value. Microprocessors and farmers can also the information to develop home based nutritious

ready to eat complementary food. Food processing techniques such as fermentation, malting and dehulling are applied to improve nutritional quality of foods. Fermentation reduces viscosity of cereal products making them easier to digest. It improves starch and protein availability and also increases acidity of the food thus preventing the growth of pathogenic organisms thus increasing the safety and shelf life of the food products.

1.4 Objectives

1.4.1 Overall objective

- To establish the nutritional and anti-nutrient content of selected sorghum and pigeon pea varieties grown in Eastern region of Kenya and their potential in developing ready to eat complementary food product**.**

1.4.2 Specific objectives

- To determine the nutritional and anti-nutrient content of the selected sorghum and pigeon pea varieties grown in Machakos, Makueni and Tharaka Nithi Counties.
- To evaluate the effects of fermenting and malting sorghum flour on the nutritional and anti-nutrient content of the ready to eat complementary food.
- To determine the effect of varying blending ratios on the nutritional and anti-nutrient content of the ready to eat complementary food.
- To determine shelf life and sensory characteristics of the ready to eat complementary food.

1.5 Hypothesis

- There is no difference in the nutritional and anti-nutrient content of the three sorghum and pigeon pea varieties among the three Counties.
- There is no difference in the nutritional and anti-nutrient content for fermentation and malting processing technique on the three sorghum varieties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sorghum: Importance, Agronomy and Utilization

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop and is the dietary staple of more than 500 million people in 30 countries. It is grown on 40 million hectares in 105 countries of Africa, Asia, Oceania and the Americas. The USA, India, México, Nigeria, Sudan and Ethiopia are the major producers. Other sorghum producing countries include Australia, Brazil, Argentina, China, Burkina Faso, Mali, Egypt, Niger, Tanzania, Chad and Cameroon. Grain is mostly used as food (55%), in the form of flat breads and porridges (thick or thin) in Asia and Africa, and as feed (33%) in the Americas. Its stover is an increasingly important source of dry season fodder for livestock, especially in Asia. Sorghum provides the staple food of a large population in Kenya and it's well adapted to the Eastern semi-arid parts. Its adaptation to the semi-arid regions is due to its C_4 photosynthetic nature, extensive root system, waxy leaves and its ability to stop growth in periods of drought (Muu *et al*., 2013). More than 35% of sorghum in the world is grown directly for human consumption. The rest is used primarily for animal feed, alcohol production and industrial products (FAO, 1995; Rooney, 2004).

The main component of sorghum grain is starch followed by proteins, non-starch polysaccharides (NSP) and fat. Processed sorghum seeds or flour is an important sources of calories and proteins to the vast majority of the population as well as for poultry and livestock (FAO, 2006).

More than 7000 sorghum varieties have been identified (Kangama & Rumei, 2005), therefore there is a need of their further characterization in terms of their nutritional value. It is estimated that more than 300 million people from developing countries essentially rely on sorghum as source of energy (Mamoudou *et al.,* 2006).

Whole sorghum grain flour has an average energy value of 356 kcal/100 g, however, it contains resistant starch which impairs its digestibility especially for infants (Mamoudou *et al.,* 2006). The protein content in whole sorghum grain is in the range of 7 to 15% (FAO, 1995).

Processing methods, such as soaking, sprouting and cooking has been reported to improve the nutritional and functional properties of plant seeds. There are a number of roles that microorganisms can play in food processing, either positive or negative. The positive effect is generally regarded as part of the fermentation processing namely product preservation, flavor development and reduction of anti-nutrients. Furthermore, fermentation enhances vitamins, essential amino acids and protein, by improving proteins and fiber digestibility. Processing of sorghum (boiling, germination, fermentation and cooking) greatly improved its nutritive value (Inyang & Zakari, 2008). However, combination of these processes further improved the quality of sorghum as a food by removing the anti-nutritional factors as well as by alleviating the effect of heating.

2.2 Pigeon pea: Importance and Nutritional content

The world production of pulses is estimated to be 57.5 million metric tonnes annually. Pigeon pea (*Cajanus cajan*) accounts for about 5% of the world's pulse production (Opoku *et al*., 2003). It finds an important place in the farming systems adopted by small holder farmers in a large number of developing countries. It is ranked sixth in area and production in comparison to other grain legumes such as beans, pea and duck peas (Fasoyiro *et al*., 2005).

Pigeon pea is well adapted to the tropical regimes. One of the best solutions to protein energy malnutrition in developing countries is supplementing cereals with protein rich legumes. Pigeon pea flour has been tested and found to be suitable as a protein source for supplementing cereal food products due to its high level of protein, iron and phosphorus (Harinder *et al*., 1999). It has therefore been recommended for school feeding programs and vulnerable sections of the populations in developing nations

Typically, the average nutritional composition of pigeon pea is 19.2% protein, 57.3% carbohydrates, 1.5% fat, 8.1% fiber and 3.8% ash (Smartt,1976), and that of mung bean is 22.9% protein, 61.8% carbohydrate, 1.2% fat, 4.4% fibre and 3.5% ash (Duke, 1981). Pigeon pea and mug bean can be consumed as dehulled splits, whole, canned, boiled, roasted or ground into flour to make a variety of desserts, snacks and main dishes. Mature and dried pigeon peas require long cooking hours. The hard pigeon peas seed coat contributes to the long cooking hours. Dehulling hence reduces the cooking time. The seeds coats are high in fibre, low in nutrients and digestibility. Anti-nutrients are also a limiting factor to the utilization of pigeon peas. Cotyledons of dry seeds are rich in carbohydrates (66.7%) and the major portion of protein (50%) is located in embryo. Sulfur-containing amino acids such as methionine and cysteine range around 1% and are present in cotyledons and embryo (Saxena *et al*., 2010). Pigeon peas native in Africa are largely creamy or white with relatively low anti-nutritional factors (Damaris, 2007). Green pigeon pea seeds (vegetable pigeon pea) are considered superior to dry splits in nutrition. They have a higher crude fibre, fat and protein digestibility. The green peas are better in phosphorus by 28.2%, potassium by 17.2%, zinc by 48.3%, copper by 20.9%, and iron by 14.7% than dried pigeon peas (Saxena *et al*., 2010)

2.3 Anti-nutrients in cereals and legumes

2.3.1 Phytates

Phytate are the principal storage form of phosphorus and are particularly abundant in cereals and legumes (Reddy *et al*., 1989). These chelate divalent cations such as calcium, magnesium, zinc and iron, thereby also reducing their bioavailability (Sandberg, 2002). Germination has been an effective treatment to remove anti-nutritional factors in cereals e.g. phytate. These are the mobilizing secondary metabolic compounds which are thought to function as reserve nutrients (Reddy *et al*., 1978). The phytic acid serves as an important reserve of phosphate generated by the action of phytase during seed germination for the developing seedling. However, this conversion depends upon the germinating conditions. As the phytate cannot be absorbed and humans have a limited

ability to hydrolyze this molecule, an adverse effect of the phytic acid on the bioavailability of minerals is predicted (Pawar & Ingle, 1988). In many plant species, 90% of phytic acid is localized in the aleurone layer and only 10% in the embryo. There are many factors, such as genetics, environmental fluctuations, location, irrigation conditions, type of soils, year and fertilizer application that can effect the phytic acid content and phosphorus availability in cereal grains. During germination, phytate salt is degraded by the action of phytase enzymes which provides the growing seedling with phosphate. Phytic acid has long been considered as an anti-nutrient because of its strong ability to complex multi-charged metal ions, especially Zn (II), Ca (II) and Fe (III) (Harland & Oberkas, 1987). In consequence, the consumption of great quantities of food containing high phytic acid levels could produce a deficit in the absorption of some dietary minerals (Reddy & pierson, 1994).

2.3.2 Tannins

Oligomers of flavan-3-ols and flavan-3,4-diols, called condensed tannins, occur widely in cereals and legumes (Haard & Chism 1996). These compounds are concentrated in the bran fraction of cereals (Salunkhe *et al*. 1990). Tannin-protein complexes can cause inactivation of digestive enzymes and reduce protein digestibility by interaction of protein substrate with ionizable iron (Salunkhe *et al*. 1990). The presence of tannins in food can therefore lower feed efficiency, depress growth, decrease iron absorption, damage the mucosal lining of the gastrointestinal tract, alter excretion of cations, and increase excretion of proteins and essential amino acids (Reddy & Pierson 1994). Dehulling, cooking and fermentation reduce the tannin content of cereals and other foods.

2.4 Processing methods of sorghum and pigeon peas

2.4.1 Malting

Malting is among the traditional processing method that is widely used as a major functional component of traditional African food, beers, lagers and stouts, non-alcoholic malt beverages and porridges (Taylor, 2008). In Kenya, the production of malted products

is getting commercialized though it is not fully industrialized (Wambugu *et al*., 2003). Malting is the controlled germination followed by controlled drying of the kernels. The main objective of malting is to promote the development of hydrolytic enzymes, which are not present in non-germinated grain (Dewar *et al*., 2003). Malting increases the level of crude protein and crude fibre but reduces the ash and fat content (Oluwole *et al*., 2012). The reduction in fat and ash might probably be attributed to malting losses incurred as a result of dry matter loss, mainly due to the growth and respiration of the embryo and the enzymatic activities in the grains (Abiodun *et al*., 2000). Malting also degrades starch leading to reduction in gruel thickness and increases bio-availability of nutrients in cereal-based foods (Onyango *et al.,* 2003). Malting has produced improvement in flavor profile and color (Rooney & Waniska, 2004; Gordon, 2001) of sorghum. Research conducted on the improvement of the protein quality of sorghum and its introduction into staple food products for southern and Eastern Africa showed that malting, in addition to improving the malt quality characteristics, also improved the digestibility and quality of the protein, which generally increased with increased malting time (Dewar *et al*., 1997).

The process of malting comprises three unit operations: steeping, germination and drying. During steeping, kernels are immersed in water until imbibed with sufficient water to start the metabolic processes of germination and at the same time dirt, chaff and broken kernels are removed by washing and flotation. Soaking generates softening and increases water availability (Enwere *et al*., 1998).

The germination phase begins after the kernels have absorbed enough water to start enzyme production and starch hydrolysis. Conditions that are necessary during the germination phase are moisture content, temperature, length of germination time, and oxygen availability. Germination takes about 4-6 days and occurs rapidly between 20°C and 30°C with an optimum temperature of 25°C to 28°C (Hoseney, 1994).The enzymes produced during germination leads to the hydrolysis of starch and proteins with release of sugar and amino acids. These are then transported into the germ where they are further metabolized by the growing seedling (Hoseney, 1994; Leder, 2004). Proteolytic enzymes

improve amino acid availability particularly lysine, methionine and tryptophan that are lacking in cereals. This may be responsible for the progressive increase in the crude protein in sorghum.

Drying is the final stage of the malting process and is required for stopping further growth of the kernels, reducing the moisture content and water activity, hence producing a shelf-stable product with active enzymes (Hoseney, 1994). Kernels are dried at a temperature of about 50°C for 24 hours (Hoseney, 1994). After drying the roots and shoots are removed and the kernels milled into malted flour ready for use in the preparations of different food products. Elaboration of amylases during malting has been taken advantage of in the development of complementary food and different infant and young child formulations.

2.4.2 Fermentation

Fermented foods are those foods which have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food. However, to the microbiologist, the fermentation describes a form of energy-yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is incompletely oxidized, and an organic carbohydrate acts as the electron acceptor. Lactic, yeast, and mixed fermentations are old methods for food processing and preservation (Jay *et al*., 2000; Holzapfel, 2002). Today, defined starter cultures and controlled conditions are frequently used. Because of the production of lactic acid and other organic acids, the pH is lowered and the phytase activity increased (Jay *et al*., 2000; Shimelis & Rakshit, 2008). Studies have shown that combined germination and lactic fermentation of white sorghum and maize gruels can yield an almost complete degradation of phytate and antioxidant potentials (Fagbemi *et al*., 2005; Janiszewska *et al*., 2007). Moreover, traditional fermentation processes are increasingly attracting the attention of scientists and policy makers as a vital part of food security strategies (Abegaz *et al*., 2002).

The majority of traditional cereal-based foods consumed in Africa are processed by natural fermentation. Fermented cereals are particularly important as complementary foods for infants and as dietary staples for adults (FAO,1999). Fermentation increases the protein content of sorghum flour after 48 hours (Mihiret, 2011).The increase can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth circles. Fermentation was also found to increase vitamin B content particularly thiamine and improve mineral availability (Mungula *et al*., 2003). It can improve nutrient density and increase the amount and bioavailability of nutrients through degradation of anti-nutritional factors, pre-digestion of certain food components, synthesis of compounds that improve absorption and by influencing the uptake of nutrients in the intestine (Leder, 2004). Using different combinations of starter organisms fermentation reduces polyphenol, tannins and phytic acid content with increase in fermentation time (Wakil *et al*., 2012). The reduction is as a result of exogenous and endogenous enzymes such as phytase enzyme formed during fermentation. Processing has been reported to increase the bioavailability of minerals especially iron and zinc (El-moneim *et al*., 2012). Fermentation causes reduction of total carbohydrates in sorghum (Mihiret, 2011). The decrease in starch content is expected to occur because starch and soluble sugars are principal substances for fermenting microorganisms; (Ejigui *et al*., 2005). Fermentation gradually reduces the fat and fibre content of sorghum. Decrease in fibre content is due to partial solubilization of cellulose and hemicellulose type of material by microbial enzymes (Mihiret, 2011). Indigestion in infants can be caused by food with high fibre content. Crude fibre content is expected to be low after fermentation (Olorunfemi *et al*., 2006). Fermentation hence is a recommended process to meet crude fibre standards in the preparation of complementary foods from locally available cereals.

2.4.3 Dehulling

After harvest, pigeon pea is dehulled to improve cooking and nutritional qualities and to reduce cooking time. Structurally, whole legume grain may consist of the seed coat (hull), embryo and the cotyledons. The process of removing the seed coat (hull) is referred to as dehulling. Dehulling plays a significant part in processing and utilization of

legume grains. Dehulling legume grains may lower the tannin content and improve their digestibility. Several methods of pre-dehulling processes that have been used include toasting, boiling, soaking, frying and pressure–steaming. The other processes apart from pre-dehulling are drying, splitting and grinding. The oldest and most common home scale technique for hulling pigeon pea is to pound them in a mortar with a pestle either by spreading the grains in the sun for few hours or after mixing them with a little water (Ogunji *et al*., 2005). Due to the long process pigeon peas must undergo before the husks are removed affects the flavor and odor of the ground pea flour (Kamath & Belavady, 2003).

2.5 Complementary foods from cereal-legume blends

Beyond the age of four to six months complementary foods are required to supplement breast milk in infants. If the nutritional needs of the child are not met, serious consequences follow for growth, resistance to diseases, intellectual development and survival hence it is the most critical period in life of infants. Traditional complementary foods in Kenya and other parts of Africa are mostly made from cereals, starchy roots and tubers that provide mainly carbohydrates and low protein quality (Charles *et al*., 2008). Such foods are the leading cause of protein energy malnutrition in infants and preschool children in Africa (Atinmo *et al*., 1982). Although nutritious and safe complementary foods produced by food multinationals are available in developing countries, they are far too expensive for most families. The economic situation in these countries necessitates the adoption of simple, inexpensive processing techniques that result in quality improvement and that can be carried out at household and community levels for the production of nutritious, safe and affordable complementary foods.

The principle of complementing cereals with legumes has been used in the production of high protein-energy complementary foods using locally available food crops. Cereals are limiting in some essential amino acids, notably lysine and tryptophan and low in protein content. The protein quality of cereals can be improved by supplementing with locally available legumes that are high in protein and lysine, although often limiting in sulfur amino acids through mutual complementation of their individual amino acids (Charles *et*

al., 2008). Using locally available cereals and legumes, community-based complementary food production using 4:1 ratio has proved successful in many African countries. 'Weanimix', a complementary food made from a cereal-legume blend, developed by the Nutrition Division of the Ministry of Health in Ghana was introduced on a large scale in the country in 1986 (Sosi *et al*., 1991). In a study cereals mixed with groundnuts produced the poorest quality blend due to the relative inadequacy of groundnut protein in complementing cereal amino acids. Soybean and winged bean produced the best

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design

The first part of the study was carried out to determine the best sorghum and pigeon pea varieties in terms of their nutritional composition. The nutritional content for the varieties was compared among three Counties (Tharaka Nithi, Machakos and Makueni) and among the three varieties of each crop. One pigeon pea variety KAT60/8 and three sorghum varieties Seredo, garden and local were then used in developing ready to eat complementary food product. The varieties were found to contain the highest level of protein and least levels of phytates and tannins. Two Improved sorghum varieties Seredo, Gadam and two pigeon pea varieties KAT 60/8, Mbaazi II were collected from each of the three semi-arid regions of Eastern Kenya namely Tharaka Nithi, Machakos and Makueni Counties, three local varieties of each crop were also collected. KAT 60/8 and Gadam varieties from Machakos County were collected from researchers at KALRO-Katumani and the rest from farmers' stores from the harvest of the year 2012 (About two kilograms of each sample was collected). Each variety was collected three times from each County. Identification of both the improved and local varieties was done with the help of KALRO researchers working on the crops. A total of fifty four samples were collected for each crop. Grains of Seredo, Gadam and local sorghum varieties are shown in plate 1, 2 and 3 respectively. Grains of KAT 60/8 pigeon pea variety are shown in plate 5.

The second part of the study examined the effect of three components of ready to eat complementary food preparation on nutritional and anti-nutrient composition: The variety of sorghum (Gadam, Seredo, and Local), the food preparation technique or treatment (fermentation, malting and control), and the formulation ratios of sorghum-to-pigeon pea flours (1:1: 2:1 and 5:1). Using all combinations of these three factors, a total of twenty-seven formulas of ready to eat complementary food were prepared and analyzed for their chemical composition, mineral profile and anti-nutrient levels.

Seredo and the Local variety sorghum were obtained from farmers in Tharaka Nithi and Makueni Counties respectively. Gadam sorghum and KAT 60/8 pigeon pea varieties were collected from KARI-Katumani in Machakos County.

Samples analyses were done in triplicates to determine nutritional value of the sorghum and pigeon pea varieties. Factorial arrangement was used to determine effects of three regions (Machakos, Tharaka Nithi and Makueni) on nutritional value of the varieties; three treatments at three levels (three grain varieties with three different regions). Samples analyses were done in triplicates to determine effects of treatments and formulations on the nutritional value of the ready to eat weaning product. The treatments (fermentation, malting and control) and formulations (1:1, 2:1 and 5:1) were carried out in a factorial arrangement; three treatments each at three levels (three grain varieties each having three treatments and three formulations)

3.2.1 Germinated sorghum flour

Selected clean grains of sorghum weighing 500 g were steeped in boiled cooled water (1:3 w/v, grain: water) for 4 h. The steeped grains were then transferred to a wide container with cotton wool to allow for germination at room temperature (27ºC) for 5 days (plate 5). The washed germinated seeds were dried in the oven at 35ºC for a total of about 10 h. The grains were then cleaned of sprouts and hulls by hand rubbing and winnowing, after which they were toasted in a shallow pan at 80ºC to a uniform light brown color. The dried grains were ground using hammer mill and the flour passed through a 1 mm aperture size laboratory test sieve (Endecotts Ltd., London England) to obtain a fine powder (Elemo, 2011).

3.2.2 Steam cooked pigeon pea flour

Cleaned pigeon pea grains weighing 500g were soaked in water (1:2 w/v, grain: water) for 1 h and dehulled by hand rubbing. The dehulled seeds were steamed for 45 min until tender. The seeds were then dried in an oven at 56ºC for 10 h. The dried seeds were ground using hammer mill and the flour passed through a 1 mm aperture size laboratory

test sieve (Endecotts Ltd., London England) to obtain a fine powder(Elemo, 2011). Dehulled steam cooked pigeon pea grains are shown in plate 6.

3.2.3 Fermented sorghum flour

To prepare the starter culture 300 g of finely milled sorghum flour was mixed with 375 ml of boiled and cooled water to a thick paste, covered and left to ferment at room temperature (25ºC) for 2 days until it tasted sour (pH approximately 3.6). The starter culture was then used to prepare a larger amount of fermented sorghum flour. Half of the culture was mixed with 225 g of finely milled sorghum flour, 300 ml of boiled cooled water and left to ferment at room temperature (25ºC) for 2days, plate 7 shows the fermented sorghum flour. The slurry was transferred to a metal pan and spread into a thin layer. The pans were put into a oven at 65°C for 24 hours. The dried material in the form of fermented cakes (plate 8) was allowed to cool before breaking into small pieces and milling using a hammer mill, it was then passed through a 1 mm aperture size laboratory test sieve (Endecotts Ltd., London England) to obtain a fine powder(Taylor, 1999).

3.2.4 Formulation of the ready to eat complementary food

The germinated sorghum flour and steamed cooked pigeon pea flour was blended at ratios of 1:1, 2:1 and 5:1 (w/w, sorghum: pigeon pea).The fermented sorghum flour and steam cooked pigeon pea flour were also blended at the same ratios. Processed sorghum and pigeon pea flour is shown in plate 9.

3.2.5 Preparation of untreated formula (control)

This was prepared from untreated sorghum and pigeon pea flour and blended in ratios of 1:1, 2:1 and 5:1 (w/w, sorghum: pigeon pea) and labeled control.

3.2.6 Product preparation

Fermented Gadam variety flour and dehulled steam cooked pigeon pea flour KAT 60/8 blended at three different ratios of 1:1, 2:1 and 5:1 (w/w, sorghum: pigeon pea) was used in making the final product for sensory evaluation. About 4 table spoons (50 g) of the

food were mixed with 100 ml of boiled hot milk to get a thick consistency. A tea spoon of sugar was added to increase the energy content.

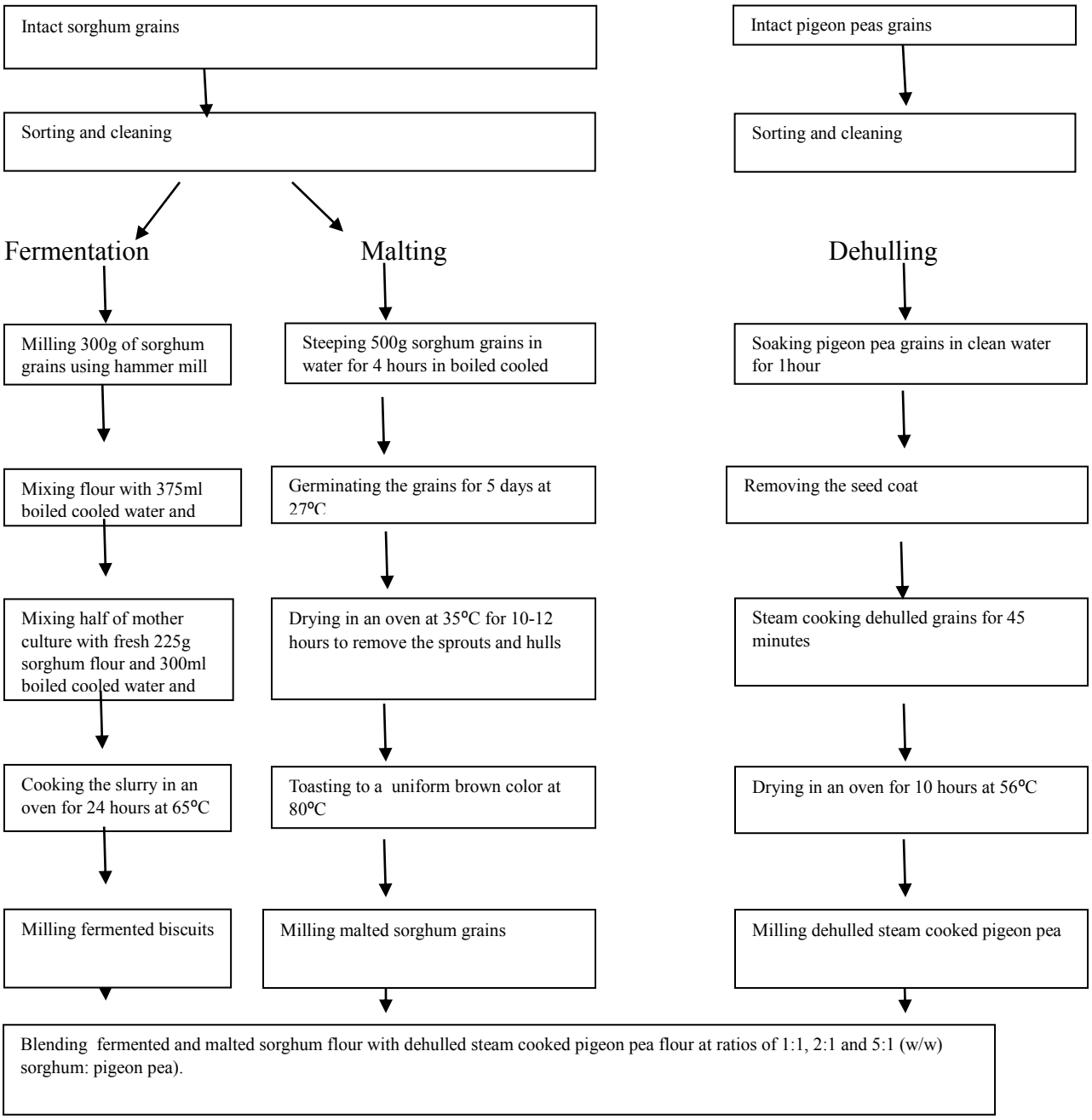

Figure 3.1: Flow diagram of fermentation, malting and dehulling processes

3.3 Proximate Analysis

Moisture, protein, fat, crude fibre and ash were determined according to AOAC methods specification 950.46 (AOAC, 1995)

3.3.1 Moisture Content

About 2 g of sample was accurately weighed into a moisture dish and transferred to an oven previously heated to temperatures of 105^0C and drying done for 1 hour. The final weight of the sample was taken after the drying period and cooling in a desiccator. The flour residue was then reported as total solids and loss in weight as moisture by formula given below (AOAC, 1995, method 925. 10).

$$\text{Moisture}(\%) = \left(\frac{\text{W1} - \text{W2}}{\text{W1}}\right) \times 100$$

Where

W_1= Weight of sample before drying

W_2 = Weight of sample after drying

3.3.2 Crude protein

About 1 g of sample was weighed into a digestion flask together with a catalyst composed of 5 g of K_2SO_4 and 0.5 g of $CuSO_4$ and 15ml of concentrated H_2SO_4. The mixture was heated in a fume hood till the digest colour turned blue signifying the end of the digestion process. The digest was cooled, transferred to a 100 ml volumetric flask and topped up to the mark with distilled water. A blank digestion with the catalysts and acid was also made. Ten (10) ml of diluted digest was transferred into a distilling flask and washed with about 2 ml distilled water. 15 ml 0f 40% NaOH was added and this was also washed with about 2 ml distilled water. Distillation was done to a volume of about 60 ml distillate. The distillate was titrated using 0.02N-HCl to an orange colour of the mixed indicator which signified the end point (AOAC, 1995, Method 20.87-32.1.22).

Calculations were done using the formula below

Nitrogen%= $(V_1 - V_2) \times N \times f \times 0.014 \times 100/V \times 100/S$

Where V_1=Titer for the sample (ml); V_2= Titer for blank (ml)

N= Normality of standard HCl solution (0.002);

F= Factor of standard HCl solution

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

S= Weight of sample taken (g)

Protein %= Nitrogen × protein factor

3.3.3 Crude fat

The soxhlet extraction method which gives intermittent extraction of oil with excess of fresh organic solvent was used. About 5 g of samples were weighed into extraction thimbles and the initial weights of the extraction flasks taken. Fat extraction was done using petroleum ether in soxhlet extraction apparatus for 16 hours. The extraction solvents were evaporated and the extracted fat dried in an oven for about 15 min before the final weights of the flasks with extracted fat were taken (AOAC, 1995, Method 920.85-32.1.13).

Calculations were done using the formula below;

$$\text{Fat (\%)} = \left(\frac{W1 - W2}{W1}\right) \times 100$$

Where

W_1= Weight of sample before extraction

W_2 = Weight of sample after extraction

3.3.4 Crude fibre

Approximately 2 g of the sample was weighed into a 500 ml conical flask. About 200 ml of boiling 1.25% H_2SO_4 was added and boiling done for 30 min under reflux condenser. Filtration was done under slight vacuum with Pyrex glass filter 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 min. 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 an oven at 105^0C in a porcelain dish to a constant weight (W_1). Incineration was done in a muffle furnace at 550^0C for 3 hrs, the dish was then cooled in a desiccator and the final weight (W_2) taken (AOAC, 1995, Method 920.86-32.1.15)

Calculations were done as shown below

$$\text{crude fibre}(\%) = \left(\frac{\text{w1} - \text{w2}}{\text{w}}\right) \times 100$$

Where

W_1= Weight of acid and alkali digested sample

W_2 = Weight of incinerated sample after acid and alkali digestion

W= Weight of sample

3.3.5 Ash

Sample weights of between 2-5 g were weighed in pre-conditioned crucibles. The samples were first charred by flame to eliminate organic material before being incinerated at 550^0C in a muffle furnace to the point of white ash. The residues were cooled in desiccators and the weights taken (AOAC, 1995, Method 925.03-32.1.05).

Calculations were done as shown below

$$\text{Crude ash}\% = \left(\frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}}\right) \times 100$$

3.3.6 Carbohydrates

The contents of total carbohydrates were calculated by subtracting the sum of moisture, protein, fat, ash and crude fibre from 100 (AOAC, 1995)

3.3.7 Determination of Tannins

Flour samples of about 5 g each were put inside a volumetric flask and 50 ml of distilled water was dispensed inside the volumetric flask, shaken for 30 min and filtered. About 2 ml of the filtrate was measured into 50 ml volumetric flask, Similarly, 2 ml of standard tannic acid solution and 2 ml of distilled water were measured with separate flasks to serve as standard and blank respectively. 2 ml of Follins-Dennis reagent was added to each of the flask followed by 2.5 ml of saturated sodium carbonate solution. The content of each flask was made up to 50 ml with distilled water and incubated for 90 min at room temperature. The absorbance of the developed colour was measured at 760 nm wavelength with reagent blank as zero, Mehrotra (1993)

3.3.8 Determination of Phytates

For phytate determination, HPLC analysis according to Camire and Clydesdale (1982) was used. 50 mg of each sample being utilized. Extraction was carried out with 25 ml of 3% H_2SO_4 for 30 minutes on a shaker bath at medium speed for 30 min at room temperature. The slurry was filtered through fast filter and rinsed using a fine jet stream from a squeeze bottle, with a small volume of extracting solvent. The filtrate was transferred to 50ml centrifuge tubes and placed in boiling water bath for 5 min before addition of 3 ml of $FeCl_3$. The tubes were heated in boiling water bath to allow for the complete precipitation of the ferric phytate complex. Centrifugation was done at 2,500 rpm (Japan model H 2000C) for 10 min and the supernatant discarded. The precipitates were washed once with 30 ml distilled water, centrifuged and the supernatant discarded again. Three (3) ml of 1.5N NaOH and a few ml of distilled water were added to the contents of the tubes. The volume was then be brought to approximately 30 ml with distilled water and heated in boiling water bath for 30 minutes to precipitate the ferric hydroxide. The cooled samples were centrifuged and the supernatant transferred to 50ml volumetric flasks. The precipitate was then rinsed with 10 ml distilled water, centrifuged and the supernatant added to the contents of the volumetric flask. Samples of 2 µl of the supernatant were injected into a HPLC (Shimazu model C-R7A plus) fitted with a 50377RP-18 (5 µl) column Cat. at an oven temperature of 30°C and RID-6A detector

model. A stock solution of the standard containing 10 mg/ml of sodium phytate was prepared. Serial dilutions were made for the preparation of the standard curve. Results of the phytate content were obtained as per the calculations of (Vohraet, 1965).

3.3.9 Minerals

Five grams of sample were weighed in crucibles and transferred to hot plates in the fume hood chamber where they were charred to clear all the smoke from the carbonatious material before transferring them to the muffle furnace. The charred materials were then incinerated at 550ºC until they were reduced to white ashes. The ashes were cooled, 15 ml of 6N HCl was added to each of them in the crucibles before transferring them to 100 ml volumetric flasks. Distilled water was used to top them up to the mark before mineral analysis (AOAC, 1995). Atomic Absorption Flame Emission Spectrophotometer was used for the samples analysis (Model A A-6200, Shimadzu, Corp., Kyoto, Japan). The various mineral standards were also prepared to make the calibration curve.

3.3.4.1 Shelf life analysis

Ready to eat weaning mix was prepared from fermented sorghum and dehulled steam cooked pigeon peas, blended at ratios of 1:1, 2:1 and 5:1 (w/w, sorghum: pigeon pea). Samples of 200g of the products (stored in Kraft paper packages at room temperatures of 25^0C and 80% RH in a cupboard for 120 days) were used for analysis. The total plate count (TPC), yeast and mold counts were determined after every 14 days to determine how long the products would store and still be suitable for consumption.

3.3.4.2 Total bacterial counts

Total bacterial counts were done according to AOAC, (1995). Initial product sample homogenates were prepared in sterile diluents in ratios of 1:10. For each homogenate, 1 ml was aseptically diluted through a series of tubes containing 9ml sterile diluents. Approximately 1 ml of diluents of each tube were spread plated on to plate count agar (PCA) and incubated for 48 hrs at 35^0C. Plates with less than 300 colonies were counted and the rest autoclaved. The number of bacterial colonies was expressed as colony

forming units per gram (CFU/g) of the sample using the formula from International Dairy Federation method (IDF, 1996) as follows

$\text{Log } C=\sum x/n_1+ (0.1n_2)^x d$

Where;

C= Count CFU/g

x= Total number of colonies in all plates

n_1= Number of plates from initial dilution where counts were made

n_2= Number of plates from second dilution from where counting was done

d= Initial dilution of counting

3.3.4.3 Yeast and mould count

The mould count was carried out using potato dextrose agar (PDA) AOAC (1995). Initial product sample homogenates were prepared in sterile dilutes in ratios of 1:10. 1ml of each homogenate was then aseptically diluted in series up to a dilution of 10-3. The dilutes were then pour plated in duplicates. Incubation of the plates was done at 25^0C for 72 hrs. The number of yeast and molds were expressed as colony forming units per gram (CFU/g) using the formula in TPC determination.

3.4 Sensory test

Fermented sorghum (Gadam) flour and dehulled steam cooked pigeon pea flour blended at three different ratios of 1:1, 2:1 and 5:1 (w/w, sorghum: pigeon pea) was used in making the final product for sensory evaluation. Four table spoons (50 g) of the food was mixed with 100 ml of boiled hot milk to get a thick consistency. A tea spoon of sugar was added to increase the energy content. The following scale was used to express people's attitude towards the products color, texture, appearance, taste, flavor and general acceptability. Like extremely 9, like very much, 8, like moderately 7, like slightly 6 neither like nor dislike 5, dislike slightly 4, dislike moderately 3, dislike very much 2 and

dislike extremely 1. A panel of twelve mothers with infants in the complementary feeding age range was used in sensory evaluation of the product. Each sample was given three times to each mother.

3.5 Statistical Analysis

Data were assessed using Analysis of Variance (ANOVA) with Statistical Analysis Software (Genstat, 14th Edition). Duncan's Multiple Range Test (DMRT) was applied to assess the differences between the means (Duncan, 1955) and significance defined at $p<0.05$ (Steel R *et al*., 1997). The mean values were displayed with standard deviation (SD) of the means.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Proximate, mineral and anti-nutrient composition of sorghum and pigeon peas varieties among regions

4.1.1 Chemical composition of the sorghum varieties

The means for the proximate composition (moisture, ash, fat, protein and fibre) of the sorghum varieties from different Counties are presented in Table 1. The ash content varied significantly ($p<0.05$) in all the varieties across the three regions, the local, Gadam and Seredo varieties from Machakos County had the highest ash contents of 1.7%, 1.7% and 1.4% respectively. The data obtained showed that the protein values were significantly different ($p<0.05$) for all the varieties across the three Counties, the local variety from Makueni County had the highest crude protein content of 18.5%.The level of protein was also recorded highest in Gadam and Seredo varieties from Machakos and Tharaka Nithi Counties which had 13.4% and 9.9% respectively and they were used in development of aready to eat complementary food. Protein content was found to be least in the local variety from Tharaka Nithi County which had 4.2%. The fat varied significantly for the three varieties across the Counties. Gadam, local and Seredo from Makueni County had the highest fat content with 5.8%, 8.3% and 7.6% respectively. The three sorghum varieties differed significantly in their crude fiber content. Fiber content was found to be highest in Seredo variety from Makueni which had 3.7% and least in the same variety from Machakos County which had 1.3%. Carbohydrates level was highest in local variety from Tharaka Nithi County which had 78.9%. Protein content and composition varies due to genotype, and water availability, temperature, soil fertility and environmental conditions during grain development, thus explaining the differences.

Table 4.1: Proximate composition (%) in the sorghum varieties across the Counties (fresh weight)

Variety/ County	Moisture	Ash	Fat	Protein	Fibre	Carbohydrates
Gadam						
Machakos	11.1±1.47[a]	1.7 ±0.19[b]	4.1±0.22[a]	13.4±0.24[c]	1.6±0.26[a]	67.8±1.71[a]
Tharaka Nithi	10.8± 0.3[a]	0.8±0.32[a]	3.6±0.17[a]	8.7±0.54[a]	1.4±0.31[a]	74.6±0.59[b]
Makueni	11±0.15[a]	1.3±0.59[ab]	5.8±0.58[b]	10.4±0.43[b]	3±0.62[b]	70.6±3.41[b]
Local						
Machakos	10.5±1.93[a]	1.7±0.55[b]	2.5±0.13[a]	11.9±0.32[b]	2.1±0.23[a]	71.1±1.29[b]
Tharaka Nithi	10.0±1.05[a]	1.2±0.04[ab]	3.4±0.15[b]	4.2±0.13[a]	2±0.22[a]	78.9±1.4[a]
Makueni	14.6±3.43[a]	1.±0.03[a]	8.3±0.44[c]	18.5±2.47[c]	3.5±0.81[b]	53.8±2[c]
Seredo						
Machakos	15.2±5.48[a]	1.4±0.23[b]	2.8±0.54[a]	7.0±0.72[a]	1.3±0.16[a]	71.6±5.65[a]
Tharaka Nithi	11.1±0.11[a]	1.1±0.17[ab]	3.1±0.70[a]	9.9±0.34[b]	2.4±0.85[a]	71.9±1.01[a]
Makueni	11.1±0.20[a]	1±0.21[a]	7.6±1.41[b]	7.8±1.81[ab]	3.7±0.53[b]	69.9±2.12[a]

Values are means ± SD. Means sharing a common superscript letter in a column are not significantly different ($p < 0.05$)

4.1.2 Proximate composition of the pigeon peas varieties

Table 2 shows the values of proximate composition of the three pigeon pea varieties across the three Counties. The moisture content in KAT 60/8 variety varied significantly across the Counties, the variety from Machakos County had the highest moisture content of 16.3%. There were significant differences ($p<0.05$) in crude protein values for the three varieties across the Counties, KAT 60/8 and Mbaazi II varieties from Machakos had the highest crude protein value of 23% and 22.2% respectively and KAT 60/8 was then used to improve protein value of the ready to eat complementary food using the three varieties of sorghum. Similar findings were recorded by (Kul Bhushan Saxena *et al*., 2010) who reported pigeon pea seeds contain about 22% crude protein and is among the main constituents of the seeds. Pigeon pea varieties KAT 60/8, Mbaazi II and the local from Makueni County had the highest fat content with 3%, 3.7 and 7.9% respectively. Crude fibre value in Mbaazi II ranged between 5.4%-9.9% and varied significantly, significant differences in crude fibre content were also observed in the local variety and ranged between 4.6%-7.7%. Carbohydrates level was highest in local variety from Machakos County which had 63.37%.

Table 4.2: Proximate composition (%) in the pigeon pea varieties across the Counties (fresh weight)

Variety/ County	Moisture	Ash	Fat	Protein	Fibre	Carbohydrates
KAT 60/8						
Machakos	16.3±4.23[b]	2.7±0.95[a]	1.0± 0.37[a]	23±1.03[b]	7±0.16[a]	49.85±4.81[a]
Tharaka Nithi	9.3±0.97[a]	2.5±0.19[a]	1.5±0.08[a]	16.9±0.09[a]	6.7±0.86[a]	62.89±2.03[b]
Makueni	12.0±2.36[a]	2.9±0.88[a]	3±0.29[b]	20±1.54[b]	7.7±0.79[a]	54.18±1.62[a]
Mbaazi II						
Machakos	14.5±0.85[a]	3.2±0.23[a]	1±0.02[a]	22.2±1.08[c]	6.4±0.72[ab]	54.26±3.18[a]
Tharaka Nithi	10.1±0.23[a]	2.9± 0.46[a]	1.4± 0.28[a]	19.1±1.02[b]	5.4± 1.73[a]	54.26±3.85[a]
Makueni	13.3±5.83[a]	3.2± 0.26[a]	3.7±0.66[b]	15.4±0.48[a]	9.9± 3.28[b]	55.63±3.85[a]
Local						
Machakos	10.6±0.32[a]	3.1± 0.29[a]	1.4 ±0.48[a]	13.6±0.25[a]	7.7± 0.91[b]	63.37±1.40[b]
Tharaka Nithi	10.6±1.87[a]	3.2± 0.51[a]	1.3± 0.13[a]	19± 0.84[b]	4.6± 0.28[a]	60.98±2.04[b]
Makueni	10.5±0.06[a]	3.6± 0.07[a]	7.9 ±3[b]	17.8±1.54[b]	5.6±0.49[a]	54.38±3.98[a]

Values are means ± SD. Means sharing a common superscript letter in a column are not significantly different ($p < 0.05$)

4.1.3 Mineral composition of the sorghum varieties

Mineral composition of the sorghum varieties across the three Counties are shown in table 3. There were significant differences ($p<0.05$) in the iron content for all the varieties. Gadam, local and Seredo from Makueni had the highest amounts of iron of 13.1 mg /100 g, 11.2 mg /100 g and 9.3 mg /100 g respectively. The variation among plants in their ability to absorb iron is not always consistent and is affected by changing conditions of soil and climate and by the stages of plant growth. However, where iron is easily soluble, plants may take up a very large amount of iron. Magnesium levels differed significantly across the three Counties for Seredo, samples from Machakos County had the highest mg level while Seredo from Makueni had the least with 9.82 mg/100 g and 15.7 mg/100 g respectively. Lower amount of magnesium averagely in Seredo variety from Makueni County may be due to the fact that divalent cations such as mg may be present as mineral phytate chelates which may explain the lower availability of these minerals (Mamiro *et al*., 2001). Copper ranged between 0.12 mg/100-0.9 mg/100 g and 0.22 mg/100 g-0.46 mg/100 g for Gadam and Seredo respectively across the Counties and varied significantly. There were significant differences in Zinc content and ranged between 0.17 mg/100 g-0.78 mg/100 g, 0.09 mg/100 g-0.69 mg/100 g and 0.08 mg/100 g-0.88 mg/100 g for Gadam, local and Seredo varieties across the Counties. The local variety from Makueni was shown to have the highest level of calcium while the local variety from Machakos had the least levels of 9.98 mg/100 g and 3.19 mg/100 g respectively.

Table 4.3: Mineral composition (mg/100 g) in the sorghum varieties across the Counties (fresh weight)

Variety/ County	Mg	Fe	Zn	Cu	Ca
Gadam					
Machakos	14.83±3.45[a]	0.77±0.25[a]	0.17±0.14[a]	0.12±0.11[a]	3.14 ±0.72[a]
Tharaka Nithi	17.65±2.86[a]	1.08±0.09[a]	0.63± 0.05[b]	0.15± 0.1[a]	4.44±0.25[a]
Makueni	16.77± 0.81[a]	13.1± 2.76[b]	0.78± 0.11[b]	0.9± 0.13[b]	8.18±1.27[b]
Local					
Machakos	14.26±2.75[a]	0.33 ± 0.05[a]	0.09± 0.04[a]	0.19 ±0.12[a]	3.19±0.24[a]
Tharaka Nithi	18.85 ±3.20[a]	1.39± 0.13[a]	0.69± 0.07[c]	0.27± 0.08[a]	5.81 ± 1.82[ab]
Makueni	14.88 ±0.46[a]	11.26±1.39[b]	0.58±0.02[b]	0.49± 0.3[a]	9.98 ±3.44[b]
Seredo					
Machakos	19.82± 1.20[b]	0.78± 0.22[a]	0.08±0.04[a]	0.29±0.13[ab]	3.61±0.42[a]
Tharaka Nithi	19.04±2[b]	0.85 ±0.66[a]	0.88±0.16[c]	0.46± 0.08[b]	5.07 ±0.27[b]
Makueni	15.7± 1.41[a]	9.39±2.74[b]	0.43±0.19[b]	0.22±0.08[a]	5.34± 0.69[b]

Values =Mean ± SD. Means sharing a common superscript letter in a column are not significantly different ($p < 0.05$)

4.1.4 Mineral composition of the pigeon peas varieties

Mineral composition of the pigeon pea varieties across the three counties are presented in table 4. Significant differences were observed in the iron content for all the varieties across the regions. Pigeon pea varieties from Makueni County were generally rich in iron compared to Machakos and Tharaka Nithi, KAT 60/8, Mbaazi II and local had 6.55 mg/100 g, 7.3 mg/100 g and 8.69 mg/100 g respectively. Activity of nutrients in soil solution is affected by high concentrations of salt ions, usually Na and Cl, resulting in a nutritional disorder in plants. Interactive effect of salinity and nutrients on the plant growth may be associated with the nutrient status in plant tissues. Nutrient uptake and accumulation by plants is often reduced under saline conditions as a result of competitive process between the nutrient and a major salt species. However, this depends on the type of nutrients and composition of soil solution (Esmaili *et al*., 2008). Significant differences were observed in Ca content for KAT 60/8 and Mbaazi II. Calcium content was found to be highest in KAT 60/8and Mbaazi II variety from Makueni County with 13.38 mg/100 g and 11.7 mg/100 g respectively. Calcium value was least in KAT 60/8 and Mbaazi II from Machakos County which had 8.34 mg/100 g and 8.56 mg/100 g respectively. Copper content was found to range between 0.12 mg/100 g-0.98 mg/100 g and 0.12 mg/100 g-1.19 mg/100 g in KAT 60/8 and Mbaazi II varieties across the regions. The three pigeon pea varieties differed significantly in the levels of zinc, KAT 60/8, Mbaazi II and the local zinc content ranged between 0.56 mg/100 g-0.86 mg/100 g, 0.29 mg/100 g-0.76 mg/100 g and 0.2 mg/100 g-0.83 mg/100 g respectively. No significant differences were observed among the three varieties across the Counties for mg content.

Table 4.4: Mineral composition (mg/100 g) in the pigeon pea varieties across the Counties (fresh weight)

Variety/ County	mg	Fe	Zn	Cu	Ca
Kat 60/8					
Machakos	13.68±1.73[a]	0.6± 0.32[a]	0.56±0.07[a]	0.98 ±0.26[b]	8.34±0.69[a]
Tharaka Nithi	15.86±1.32[a]	0.83±0.09[a]	0.86± 0.06[b]	0.86± 0.43[b]	12.27± 0.86b
Makueni	14.11± 0.49[a]	6.55±1.54[b]	0.64± 0.09[a]	0.12± 0.03[a]	13.38±2.04[b]
Mbaazi II					
Machakos	17.06±3.44[a]	0.46±0.3[a]	0.53±0.05[ab]	1.03±0.2[b]	8.56±1.53[a]
Tharaka Nithi	14.2 ± 0.62[a]	0.32± 0.42[a]	0.76±0.1[b]	1.19±0.04[b]	10.47±0.47[ab]
Makueni	15.35±0.26[a]	7.3±0.96[b]	0.29± 0.19[a]	0.12± 0.15[a]	11.7± 1.28[b]
Local					
Machakos	16.7± 5.34[a]	0.74±0.19[a]	0.69±0.15[b]	0.86±0.09[a]	10.6±1.33[a]
Tharaka Nithi	15.23 ±0.61[a]	0.7± 0.16[a]	0.83±0.07[b]	0.55 ±0.08[a]	10.8±0.56[a]
Makueni	15.07±0.98[a]	8.69±0.72[b]	0.2± 0.05[a]	0.57± 0.71[a]	12.26± 0.87[a]

Values are means ± SD. Means sharing a common superscript letter in a column are not significantly different ($p < 0.05$)

4.1.5 Anti-nutrient content in the sorghum varieties

Tannin and phytate concentration in the sorghum varieties across the three Counties is shown in figure 1 and 2 respectively. Tannin was generally found to be highest in varieties from the Makueni County, for Gadam, local and Seredo which had 5.15 mg/100 g, 8.64 mg/100 g and 8.98 mg/100 g respectively. Gadam and local variety from

Machakos County both had the least levels of tannins with 2.3 mg/100 g each. Red sorghum genetically has higher condensed tannin compared to white sorghum and pearl millet. Tannins impart a bitter taste to the grains making them unpalatable and also interfere with protein digestibility. Before ripening the tannin content of grain is always higher than after ripening. The tannin content of dark grains is always higher than that of pale grains. The phytate amounts were also noted to be elevated in the red sorghum generally compared to the white sorghum.

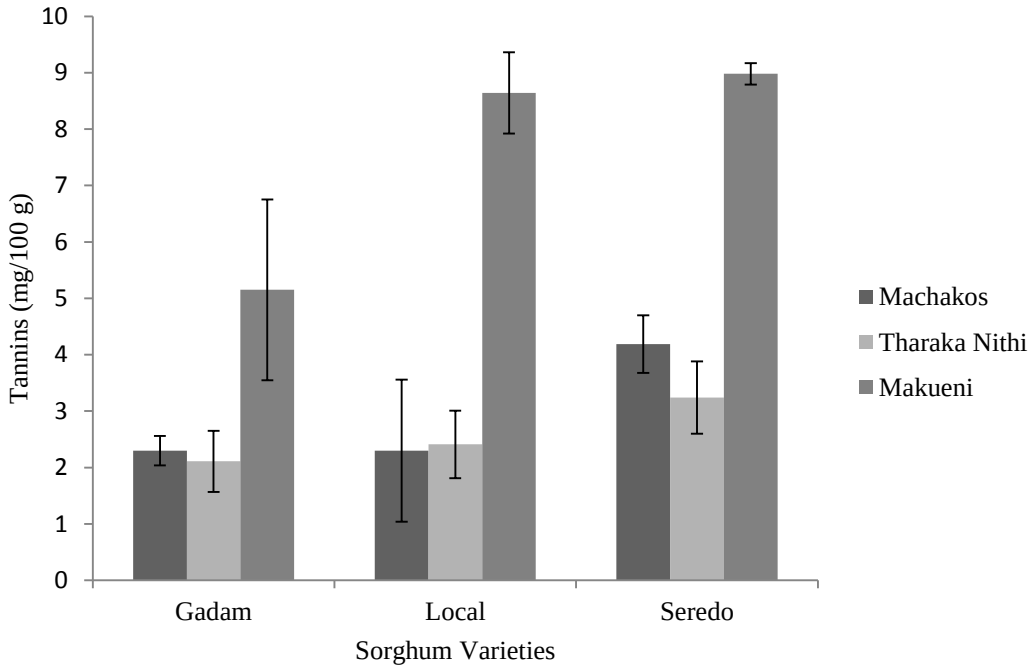

Figure 4.2: Tannin content (mg/100 g) in the sorghum varieties across the counties (fresh weight)

Phytate content ranged between 212.6 mg/100 g-282.5 mg/100 g in Gadam variety across the Counties, and variety from Makueni had the least levels of phytates. The local and Seredo variety from Makueni County had the highest amounts of phytates of 283.2 mg/100 g and 410.7 mg/100 g respectively. The least amount of phytates was observed in local and Seredo variety from Tharaka Nithi and Machakos Counties with 244.7 mg/100 g and 234.1 mg/100 g respectively.

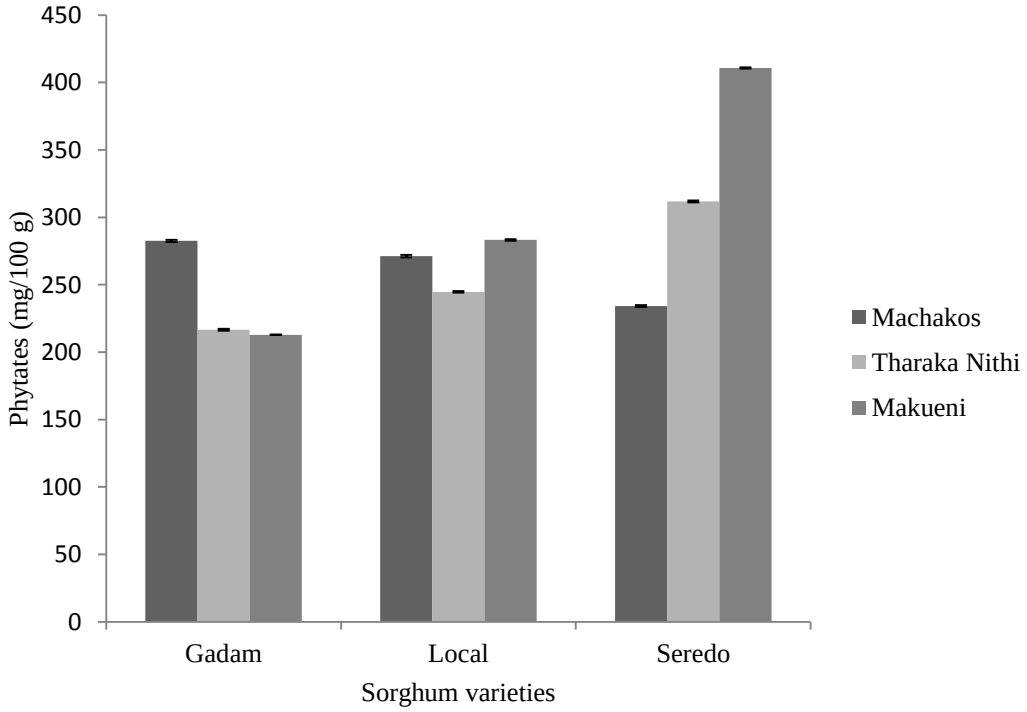

Figure 4.3: Phytates content (mg/100 g) in the sorghum varieties across the counties (fresh weight)

4.1.6 Anti-nutrient content in the pigeon peas varieties

Tannin and phytate contents (figure 4 and 5 respectively) were noted to be higher in dark seeded pigeon pea varieties compared to pale seeded varieties. Tannins content was generally highest in varieties obtained from Makueni County. The level of tannins varied significantly for all the varieties across the Counties, Gadam, local and Seredo varieties from Machakos, Tharaka Nithi and Makueni Counties tannins level ranged between 3.80 mg/g-7.22 mg/100 g, 3.73 mg/100 g-7.83 mg/100 g and 5.17 mg/100 g-7.7 mg/100 g.

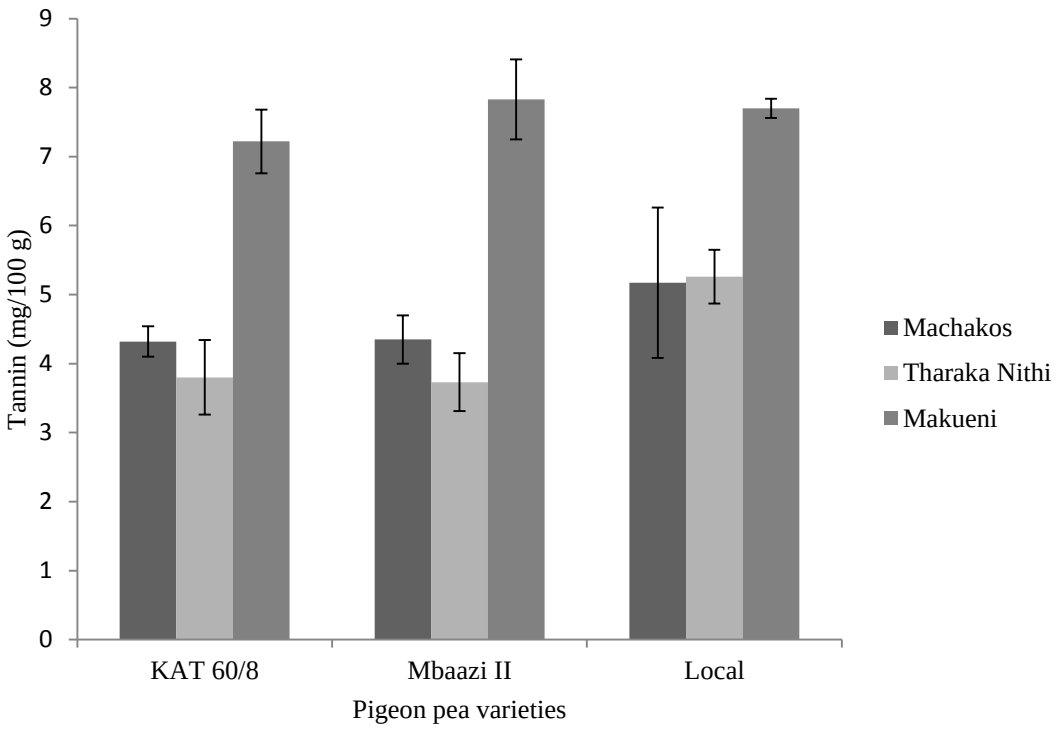

Figure 4.4: Tannin content (mg/100 g) in the pigeon peas varieties (mg/100 g) across the Counties (fresh weight)

The amount of phytates for KAT 60/8 variety differed significantly ($p < 0.05$), variety obtained from Makueni County had the least with 155.9 mg/100 g. Mbaazi II and local variety phytate level ranged between 142.3 mg/100 g-153 mg/100 g and 123 mg/100 g-313.8 mg/100 g respectively.

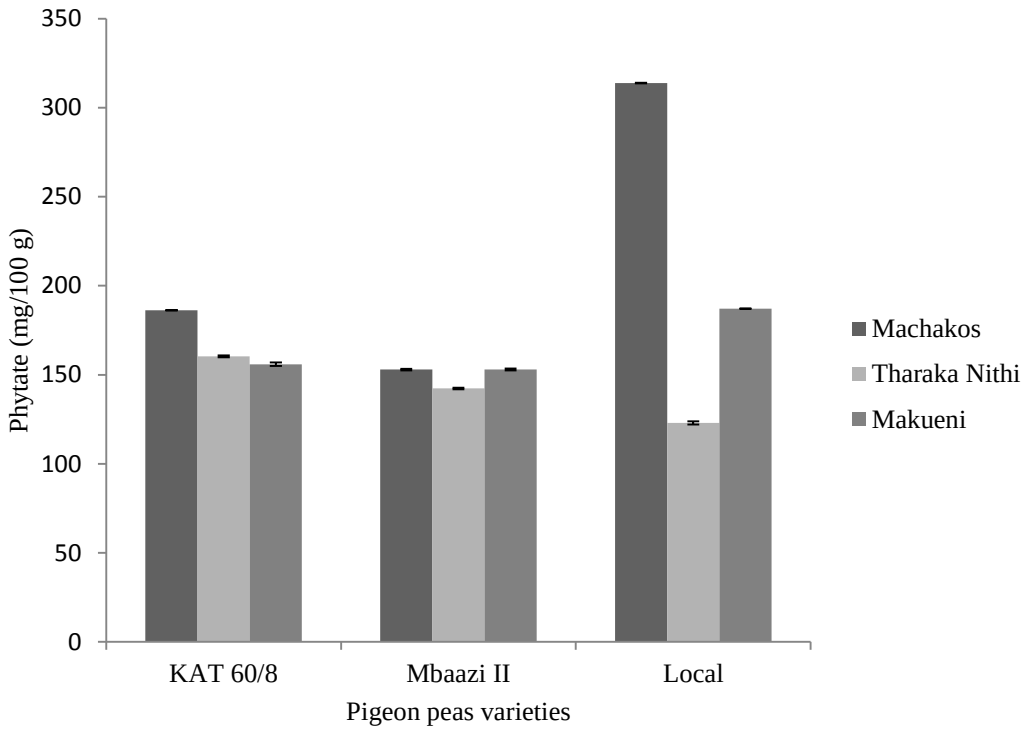

Figure 4.5: Phytates content (mg/100 g) in the pigeon peas varieties (mg/100 g) across the Counties (fresh weight)

4.2 Proximate, mineral and anti-nutrient composition of the ready to eat complementary food

4.2.1 Chemical Composition

Proximate composition (%) among the sorghum varieties, treatments and formulations are shown in table 5. The moisture content was found to be highest in control which had 11.34%. Crude protein content differed significantly ($p < 0.05$) among the three treatments and formulations. Malting and fermentation increased protein content of the complementary food. Protein content for fermented and malted ready to eat complementary food was found to be 14.1% and 13.8% respectively. These results are in agreement with previous studies (Carnovale *et al.,* 1988; Dewar *et al.,* 1997) which indicated that malting, in addition to improving the malt quality characteristics, also improved the quality of the protein, which generally increased with increased malting time. Various studies have also shown that fermentation can increase the concentrations of vitamins, minerals and protein (Taylor *et al*., 2003). The increase in protein content for fermented food can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth circles. Fermentation was then use in developing the final ready to eat complementary food. The ash content ranged between 1.6% - 2.7% and differed significantly among the three formulations, flour blended at the ratio of 2:1 had the highest ash content. The fiber content differed significantly ($p < 0.05$)and ranged between 2.8% - 3.9% and 2.7% - 4.5% among the treatments and formulation respectively. Ready to eat complementary food blended at the ratio of 2:1 had

significantly higher amounts of ash, fat and fibre but lower amounts of carbohydrates than formulations of 1:1 and 5:1. The lower carbohydrate content is due the greater proportion of fat and fibre of formulation 2:1 compared with formulations 1:1 and 5:1. Protein was found to be highest in food blended at the ratio of 1:1 which had 14.9% hence used in the preparation of the final product.

Table 4.5: Proximate composition (%) among the sorghum varieties, treatments and formulations (fresh weight)

	Moisture	Ash	Fat	Fibre	Protein	Carbohydrates
Variety						
Seredo	5.1±4.83^{a}	2.2±0.93^{a}	3.8±0.91^{a}	3.4±1.29^{a}	13.2±3.22^{a}	71.2±5.73^{a}
Gadam	5.1±4.80^{a}	2±1.12^{a}	4±1.62^{a}	3.4±1.44^{a}	11.8±4.81^{a}	73.4±4.66^{a}
Local	4.7±4.82^{a}	2.2±0.69^{a}	3.5±1.26^{a}	3.2±1.32^{a}	12.9±3.44^{a}	73.3±5.77^{a}
Treatment						
Fermentation	2±1.29^{a}	2±0.75^{a}	3.8±1.20^{a}	2.8±1.38^{a}	14.1±3.84^{b}	75±4.23^{b}
Malting	1.7±1.40^{a}	1.8±0.77^{a}	3.7±1.51^{a}	3.3±a1.3^{b}	13.8±2.76^{b}	75.3±4.37^{b}
Control	11.3±2.89^{b}	2.5±1.02^{a}	3.7±1.38^{a}	3.9±1.12^{b}	10±3.47^{a}	67.5±3.62^{a}
Formulation						
1:1	6.1±4.26^{a}	2±0.68^{a}	3.7±1.32^{b}	2.7±1.25^{a}	14.9±3.82^{b}	70.3±4.26^{a}
2:1	4.5±4.59^{a}	2.7±1.78^{b}	4.6±1.18^{c}	4.5±0.90^{b}	11.5±3.27^{a}	71.8±4.36^{a}
5:1	4.3±4.46^{a}	1.6±1.50^{a}	2.9±0.76^{a}	2.9±0.99^{a}	11.5±2.80^{a}	75.8±5.94^{b}

Values =Mean ± SD. Means sharing a common superscript letter in a column are not significantly different ($p < 0.05$)

4.2.2 Mineral composition

Table 6 revealed that the complementary food differed significantly ($p< 0.05$) in their iron (4.37-11.87 mg/100 g), zinc (1.02-2.34 mg/100 g), copper (0.28-0.61 mg/100 g), calcium (19.36 29.81 mg/100 g) and magnesium (33.21-44.86 mg/100 g) among the formulations. Significant differences were observed among the varieties in calcium level, Seredo had the highest with 30.64 mg/100 g and Gadam the least with 21.01 mg/100 g. No significant differences were observed in all minerals for fermented food. Similar observations were made by (Ejigui *et al*., 2005) that fermentation does not have an overall effect on the contents of total minerals.

Table 4.6: Mineral composition (mg/100 g) among the sorghum varieties, treatments and formulations (fresh weight**)**

	Fe	Zn	Cu	Ca	Mg
Variety					
Seredo	8.03±4.39[a]	1.76±1.07[a]	0.50±0.20[a]	27.90±9.55[a]	39.29±17.92[a]
Gadam	6.72±2.81[a]	1.37±0.47[a]	0.42±0.22[a]	21.01±6.86[a]	36.66±10.48[a]
Local	8.43±4.11[a]	1.83±0.72[a]	0.55±0.44[a]	24.24±13.02[a]	43.08±13.78[a]
Treatment					
Fermentation	8.44±4.75[a]	1.65±0.66[a]	0.53±0.46[a]	23.31±13.6[a]	42.25±16.84[a]
Malting	6.89±2.97[a]	1.86±0.89[a]	0.48±0.22[a]	23.62±8.75[a]	38.56±13.88[a]
Control	7.86±3.96[a]	1.44±0.68[a]	0.45±0.23[a]	26.21±8.04[a]	38.22±13.76[a]
Formulation					
1:1	6.95±2.92[b]	1.59±0.30[b]	0.61±0.44[b]	29.81±11.62[b]	44.86±16.81[b]
2:1	4.37±1.62[a]	1.02±0.58[a]	0.28±0.15[a]	19.36±8.49[a]	33.21±12.57[a]
5:1	11.87±3.36[c]	2.34±0.75[c]	0.57±0.18[b]	23.98±18.23[ab]	40.97±a12.92[ab]

Values =Mean ± SD. Means sharing a common superscript letter in a column are not significantly different ($p < 0.05$)

4.2.3 Anti-Nutrient profile

Tannins and phytate content were found to be reduced significantly ($p<0.05$) in fermented and malted ready to eat complementary food compared with the control. Fermented food had the least while untreated food had the highest tannin content with 1.85 mg/100 g and 4.59 mg/100 g respectively (figure 6). Malted food had the least phytate content which found to be 207.5 mg/100 g. The results of this study are in agreement with those

reported by (Abdelhaleem *et al*., 2008) and (Makokha *et al*., 2002), who reported that fermentation of sorghum, produces significant loss in phytate. Reduction in tannin contents due to fermentation might have been caused by the activity of polyphenol oxidase present in food grain or microflora (Fagbemi *et al*., 2005). The results revealed that fermentation enhances the removal of the anti-nutritional factors which are believed to be responsible for unavailability of both proteins and divalent minerals.

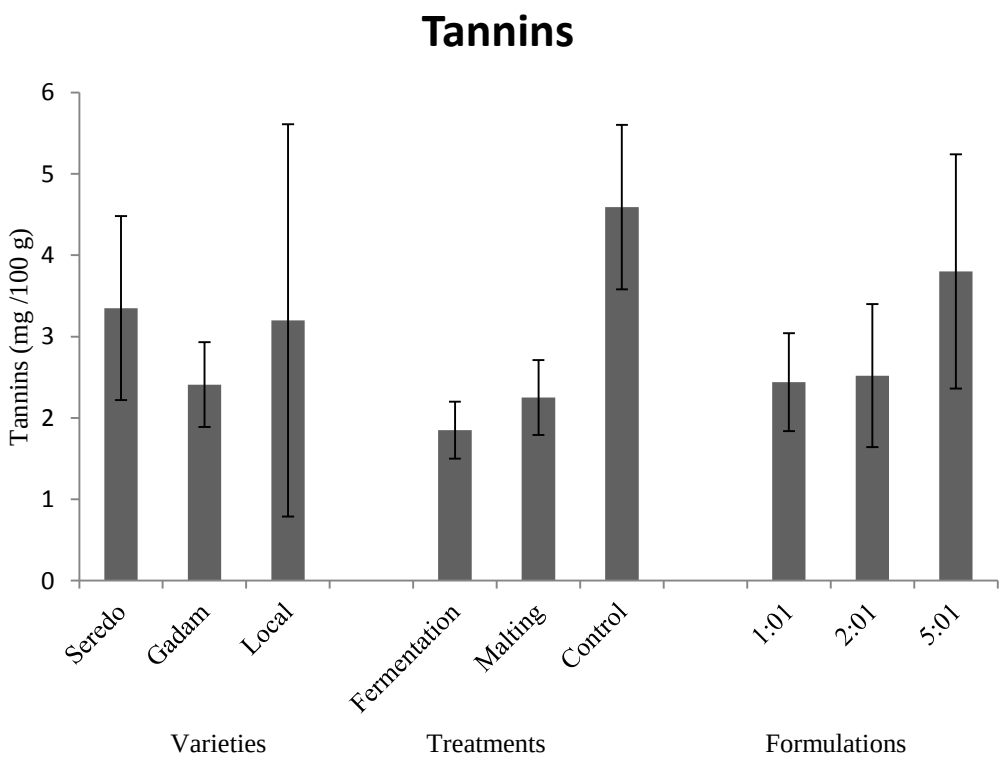

Figure 4.6: Tannins (mg /100 g) among the sorghum varieties, treatments and formulations (fresh weight)

Various studies have shown that malting reduces phytate content in sorghum. According to (wisal *et al*., 2005) the reduction is due to the action of endogenous phytases obtained during germination that degrade the phytate into organic phosphorus and inositol and its intermediate forms. The rate of reduction depends upon the age as well as the amount of

malt. Significant differences were observed among the formulations and varieties for phytate content. Gadam and formulation 1:1 had the least phytate content with 207.7 mg/100 g and 202.4 mg/100 g respectively (figure7). Tannin content was least in food formulated at the ratio of 1:1 which had 2.44 mg/100 g. Gadam was then used in developing the final ready to eat complementary food.

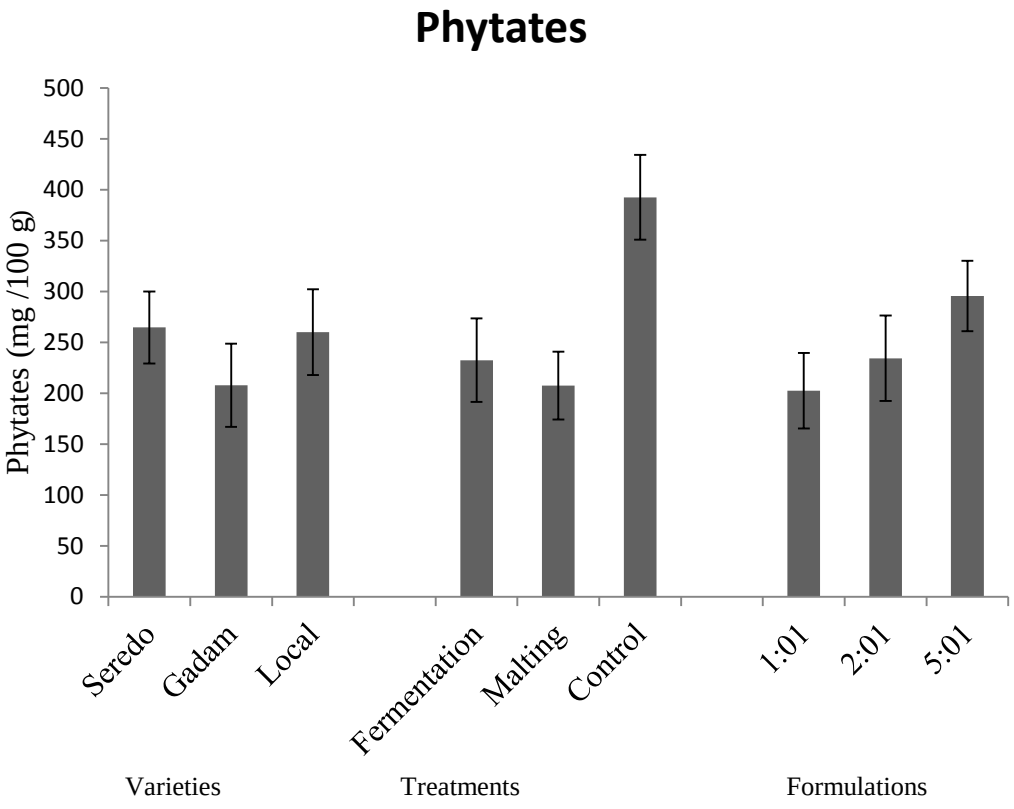

Figure 4.7: Phytates (mg /100 g) among the sorghum varieties, treatments and formulations (fresh weight)

4.3.1 Changes in the microbial, yeast and moulds load of the ready to eat complementary food blends during storage

Standard plate count of the three ready to eat complementary food blends were and the results are presented in table 7. Total microbial counts were undetectable from week 0 to week 8. This was as a result of drying during the food preparations hence the flour was low in moisture which supports growth of microorganisms. Complimentary food ratio 2:1 had detectable microbial loads after week 10. From week 12-18 there was steady increase in the total number of microorganisms. The levels were within the acceptable limits of microbial standards on cereal products (ICMSF, 1996).

Table 4.7: Standard plate count of the ready to eat complementary food blends during storage (Log10cfu/ml)

Time (week)	1:1	2:1	5:1
0	*ND	*ND	*ND
2	*ND	*ND	*ND
4	*ND	*ND	*ND
6	*ND	*ND	*ND
8	*ND	*ND	*ND
10	*ND	0.02	*ND
12	0.02	0.12	0.04
14	0.02	0.14	0.04
16	0.12	0.21	0.18
18	0.14	0.24	0.24

Values= Mean of 2 replicates *($Log_{10}cfu/ml$)*ND= Not detected

Table 4.8: Yeast and molds count

Time (weeks)	1:1	2:1	5:1
0	*ND	*ND	*ND
2	*ND	*ND	*ND
4	*ND	*ND	*ND
6	*ND	*ND	*ND
8	*ND	*ND	*ND
10	*ND	*ND	*ND
12	*ND	*ND	*ND
14	0.12	0.12	0.14
16	0.14	0.12	0.24
18	0.20	0.24	0.40

Values= Mean of 2 replicates *($Log_{10}cfu/ml$)*ND= Not detected

Yeast and moulds were not detected from week 1-12 (table 8). Yeast and molds in the complementary food were detected from week 14 and increased for the remainder of the storage period. The levels remained within the acceptable standard levels. Fermentation improves starch increases acidity of the food thus preventing the growth of pathogenic organisms thus increasing the safety and shelf life of the food products.

4.3.2 Sensory properties of the three ready to eat complementary food blends

Mean sensory scores for the three blends of ready to eat complementary food are shown in table 9. Significant differences were observed in the scores for colour, texture, taste, flavor, appearance and general acceptance. Ready to eat complementary food blended, at the ratio of 1:1 had the preferred taste texture and the overall acceptance. It was stated ready to eat complementary food blended at the ratio of 5:1 was thick for infants and had the least overall acceptability.

Table 4.9: Mean sensory scores for the three ready to eat complementary food blends

Sensory parameters	Formulations		
	1:1	2:1	5:1
Color	6.83±0.98[b]	7.16±0.98[b]	5.33±1.09[a]
Texture	7.50±1.37[b]	7.33±1.96[b]	5.33±0.81[a]
Taste	8.5±0.54[b]	7.33±1.21[ab]	5.66±2.33[a]
Flavor	7.5±0.54[b]	7.5±0.83[b]	5.50±1.87[a]
Appearance	7.66±1.03[b]	7.33±1.21[b]	5±1.41[a]
General acceptance	8.33±0.81[b]	7.66±1.21[b]	5.33±1.63[a]

Values are means ± SD. Means sharing a common superscript letter in a row are not significantly different ($p < 0.05$)

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The significant mean square values obtained from the analysis of variance for the nutritional composition suggests that differences exist between same varieties of sorghum and pigeon pea from different regions of Eastern Kenya, indicating that they are highly variable. KAT 60/8 pigeon peas and local sorghum variety from machakos and Makueni County had the highest levels of protein. The white Gadam variety of sorghum and Mbaazi II variety of pigeon pea had the least anti-nutrient factors and therefore the best with respect to nutrient availability since low levels of anti-nutrients in both crops are shown to increase the utilization of nutrients by the body.

Fermentation and malting of sorghum as well as dehulling pigeon peas has reduced tannin and phytate content. It has also improved the protein content of the weaning blends compared to the untreated. Blending Gadam and KAT 60/8 flour at the ratio of 1:1 improved the protein content of sorghum and was effective at reducing the anti-nutrients content.

Total microbial counts were detected from week 8 for complementary food blended at the ratio of 1:1 and 5:1. Complementary food blended at the ratio of 2:1 had detectable microbial loads after week 10. From week 14 yeast and molds were detected in all the ratios of the complementary food and increased steadily for the reminder of storage time. The levels were within the acceptable limits of microbial standards on cereal products (ICMSF, 1996). The ready to eat complementary food is safe for human consumption up to 18 weeks at room temperature of $25\ ^{0}$ C. Complementary food blended at the ratio of 1:1 was the best in terms of taste, texture and was the most accepted for feeding to infants.

Fermenting Gadam sorghum variety, dulling KAT 60/8 Pigeon pea variety from Machakos County and blending them at the ratio of 1:1 was the best at improving protein content and reducing anti-nutrients. This may be recommended as desirable for solving the protein deficiency among infants in developing countries.

5.2 Recommendations

i. Blending fermented sorghum with dehulled pigeon pea should be adopted by the ministry of health as an appropriate pre-processing technique as a way to increase the protein level and reduce anti-nutrients of the traditional crops. The information should be passed at the health centers to mothers and guardians with infants in the complementary feeding stage.

ii. Scaling up is needed by involving processors for commercialization purposes to enable full utilization of the complementary food product.

iii. Clinical trials on the complementary food should be conducted to determine its efficacy in maintaining infant nutritional status.

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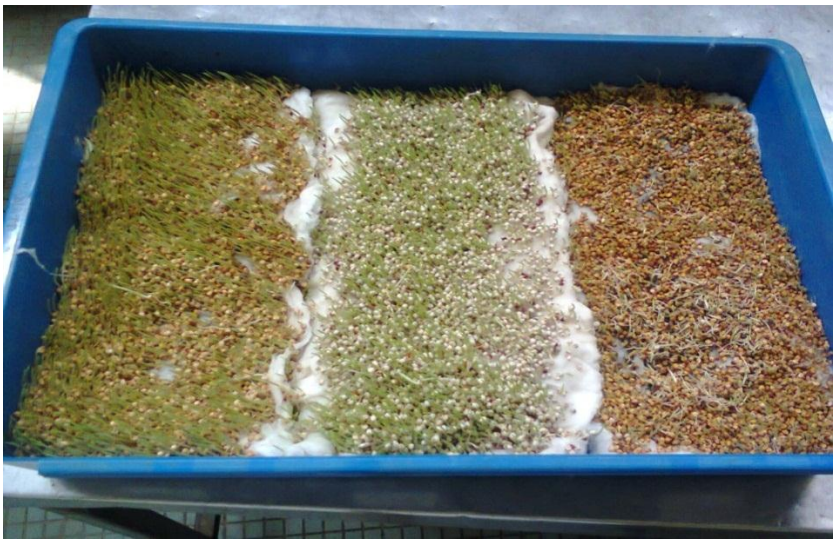
APPENDICES

Appendix 1: Images of sorghum, pigeon pea grains and the processing techniques

Grains of Seredo sorghum variety

Grains of Gadam sorghum variety

Grains of pigeon pea variety KAT 60/8



Germinated sorghum grains

Dried dehulled and steam cooked pigeon pea before milling

Fermented sorghum flour

Dried fermented biscuits before milling

Processsed sorghum and pigeon pea flour before blending

Appendix 2: Sensory evaluation form

The scale below was used to express people's attitude towards the products color, taste, flavor, texture, appearance and general acceptability.

Description Score

Like extremely...……..…….9

Like very much…….…8

Like moderately …….…..……7

Like slightly ……..….....6

Neither like nor dislike..……...…….…5

Dislike slightly…….…..4

Dislike moderately …….....3

Dislike very much …….……2

Dislike extremely …….….1

Attribute			
Samples	1:1	2:1	5:1
Appearance: Color			
Texture			
Taste			
Flavour			
General Acceptability			