DEVELOPMENT OF A NUTRITIOUS COMPOSITE FLOUR FROM PEARL MILLET (*pennisetum glaucum*) AND PUMPKIN FRUIT (*cucurbita pepo*-variety *styriaca*)

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DECLARATION

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

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This work has been submitted with our approval as the supervisors

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DEDICATION

This work is dedicated to my family comprising of my husband, Warren Mithanga, sons Cephas and Dan and daughter baby Angel.

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I would like to express my sincere gratitude to those who made major contributions to the development of this work.

First, I would like to thank the lord God Almighty who gave me the strength and wisdom to come up with the best ideas. My sincere gratitudes also goes to my family who were always there for me, even when sometimes, nothing seemed to work out. Thank you so much.

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ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
AAS	Atomic Absorption Spectrophotometry
ANOVA	Analysis of Variance
ASALs	Arid and Semi Arid Lands
BHT	Butylated Hydroxytoluene
CSB+	Corn Soya Blend plus
CSB++	Corn Soya Blend plus plus
FAO	Food and Agriculture Organisation
HPLC	High Performance Liquid Chromatography
JKUAT	Jomo Kenyatta University of Agriculture and Technology
LSD	Least Significant Difference
RDA	Recommended Daily Allowance
RAE	Retinol Activity Equivalent
UNICEF	United Nations Children's Fund
WFP	World Food Programme
WHO	World Health Organisation
RPM	Revolutions per minute

ABSTRACT

Malnutrition continues to be a serious problem in Kenya today among children, the elderly, as well as expectant and lactating mothers, especially in resource poor settings including the urban poor, arid and semi-arid areas. These groups normally do not have access to a wide variety of foods, and the bulk of their diets consists of cereal staple foods especially maize, which mainly provides starch. This is due to their low purchasing power, high reliance on maize as a staple and lack of awareness of importance of drought tolerant crops. Therefore, the development of nutritious composite flours from cereal and non-cereal foods using cheap techniques may greatly improve the nutritional status of these vulnerabe groups. For children, such composite flours may be good for complementary feeding.

The aim of this study was to develop a nutrious composite flour from pearl millet and pumpkin fruit. Germination, fermentation and roasting of pearl millet grains, as well as soaking, preboiling and roasting of pumpkin seeds were used to enhance the nutrition and sensory properties of the product. Effects on nutritional and sensory properties of germination of pearl millet grains at various time- temperature combinations followed by fermentation and roasting were studied. Also the effects of soaking, preboiling and roasting of pumpkin seeds were evaluated.A composite flour was developed from germinated, fermented and roasted pearl millet grains, dried pumpkin flesh and roasted pumpkin seeds. The treated pearl millet flour gave porridge of superior nutritional and sensory quality than flour from untreated pearl millet. Roasting of pumpkin seed enhanced development of a desirable nutty flavour in the porridge. The treated pearl millet had significantly lower phytic acid and calcium levels ($p< 0.05$) and higher crude protein, total ash, crude fiber, iron and zinc levels than untreated millet flour. The pumpkin flesh enhanced the flour's nutritional quality in terms of beta carotene which is a major precussor of vitamin A. The composite flour was developed from the treated flours that exhibited superior nutritional based on recommended daily allowances for

infants aged 7-12 months as well as well as based on their sensory qualities. This group (7-12 months old infants) was considered because it is a critical window period of growth where proper nutrition is key.

The developed composite flour had a pearl millet: pumkin flesh: pumpkin seed ratio of 7: 1: 2. It contained 11.4 % protein, 5.2 % fat, 4.7 % crude fiber, 3.6 % crude ash, 67.0% carbohydrate, and 8.0 % moisture. It had 3 mg/100g zinc, 9.8 mg /100g iron, 78.8 mg/100g calcium and 131.3µg/100g retinol activity equivalent. The phytate content was 175mg/100g. The product had a phytic acid: zinc, phytic acid: iron, and phytic acid: calcium molar ratios of 6.5, 1.5, 0.15 respectively. Phytate:zinc, phytate: iron, and phytate: calcium rations of <18, <1, and < 0.17 have been suggetsed as desirable for proper mineral bioavailability. Therefore this product may have acceptable bioavailability of zinc a nd calcium, and a recommendation of a little supplementation with an animal food such as milk may help towards achievement of adequate levels of iron.

CHAPTER ONE
INTRODUCTION

1.1Background Information

The national trends in childhood undernutrition in Kenya for the period between 1993 to 2008-09 showed significant declines in underweight, but trends in wasting and stunting were stagnant (Matanda, Mittelmark & Kigaru, 2014). However Comparison of Kenya Demograghic and Health Survey [KNDH] data over time indicates an overall improvement in children's nutritional status in Kenya. Since 1998, stunting has declined from 38 percent to 26 percent, wasting has declined from 7 percent to 4 percent, and the proportion of underweight children has declined from 18 percent to 11 percent (Kenya Demographic health survey [KDHS], 2014). Although the Kenya under-5 mortality rate had declined signiicantly between 2003 and 2009, it increased again in 2013, (Mohajan, 2014)

Kenya is home to more than 40 million people, 80% of whom live in rural areas and rely mainly on agriculture. More that 10 million people are chronically food insecure (Republic of Kenya 2011; FAO 2011; Republic of Kenya 2008). Malnutrition is the single greatest contributor to child mortality at 53%. Some elementary causes of malnutrition are inadequate food intake and disease while the underlying causes include poor maternal and child care practices, household food insecurity and inadequate health services (Mohajan, 2014). The country's food and nutrition status is often attributed to the performance of agriculture sector. The sector's strategies have not led to full food security for the country and micronutrient-rich foods have been insufficiently promoted (Republic of Kenya, 2008). Kenya's 2011 National food and nutrition security policy shows that in the past years, per capita food availability has declined. About 84% of the total land in Kenya is classified as arid and semi-arid, mainly in the northern and eastern regions. It is estimated that the arid and semi-arid areas support about 25% of the

nation's human population and slightly over 50% of its livestock. Only 0.97% land of Kenya is used for the production of permanent crops, (Mohajan, 2014).

Malnutrition exists in various forms, including acute and chronic undernutrition, micronutrient deficiencies as well as overweight and obesity. These conditions primarily affects expectant and lactating women and children under five years. This contributes to their morbidity and mortality (Ministry of Public Health and Sanitation [MoPHS], 2011). Micronutrient deficiencies are highly prevalent among children under-5 and women. According to 1999 national micronutrient survey in Kenya, the most common deficiencies include vitamin A deficiency (under-5, 84.4%), iron deficiency anemia (6–72 month olds 69% and pregnant women 55.1%), iodine deficiency disorders (36.8%) and zinc deficiency (mothers 52% and children under-5, 51%). Poor nutrition in infancy and early childhood increases the risk of infant child morbidity and mortality; diminished cognitive and physical development marked by poor performance in school and also impacts on productivity later in life (National Nutrition Action Plan 2012-2017, Ministry of Public Health and Sanitation, Republic of Kenya, 2012.)

Kenya Agricultural Research Institute Food security report of 2012 on policy response to food crisis in Kenya, shows that the current food insecurity problems are attributed to several factors which includes frequent droughts in most parts of the country, high costs of domestic food production due to high costs of inputs especially fertilizer, high global food prices and lowpurchasing power for most people due to high poverty levels. The report also indicates as part of policy response to food insecurity the need for encouraging diversification of crops planted and encouraging the citizens to diversify their eating habits to other foods and avoid high reliance on maize. Cereals such as sorghum and millet are known to be more tolerant than maize, to adverse climatic conditions, and their increased production and consumption may contribute to better food security. Unlike soghurm millet has got sweet and pleasant nutty flavour thus more palatable (Regassa & Wortmann, 2014).

In addition pearl millet is easy to grow. It suffers less from diseases than sorghum, maize, or other grains. According to export processing zones authority, grain production in Kenya report of 2005, The types of millet grown in Kenya include bulrush, finger, foxtail, and proso. These are grown mainly for domestic consumption. Recent statistics indicate that 50% of the total millet grain production is pearl millet, 30% proso/golden and fox tail millet and 10% finger millet. The remaining 10% is accounted for by other varieties.

Many of the nutritionally vulnerable individuals mostly consume cereal foods with very little or same type of vegetables, fruits or animal products (Bwibo, 2003). Such individuals might benefit from affordable composite flours containing both cereal and vegetable food components. In this regard, a composite flour produced from pearl millet and pumpkin seeds and/or pumpkin flesh is expected to offer better nutrition than pearl millet alone, because pumpkin flesh is rich in carotenoids (United States Development Authority [USDA] National nutrient data base) and will thus contribute vitamin A, while the pumpkin seed is rich in fats, protein and minerals including zinc, iron and calcium (William Peterman, 2015). There is a need to develop such flours by using methods that improve both nutritional value and sensory acceptability.

1.2 Problem Statement

Malnutrition leads to major health problems to the affected populations, including susceptibility to various illnesses and poor immune response. Many people in resource-poor settings in Kenya highly rely on maize as a staple food, with very low consumpton of fruits, vegetables and animal products. Although composite flours made from drought tolerant cereals such as pearl millet and nutrient rich vegetables such as pumpkin flesh and pumpkin seeds could improve the nutritional status of such people, these cereal and vegetable resources remain underutilized. However, cereals such as pearl millet have a high content of phytates which bind to the minerals and make them unavailable..

1.3 Justification

This development if adopted by relevant government agencies will improve utilization of pearl millet and pumpkin fruit to produce a nutritionally superior product for nutritionally vulnerable groups in the soceity. It is easy to cultivate these two food crops since they do well in arid and Semi-arid lands [ASALs]. In addition to addressing nutrition status of the population, the development can also improve diversification of eating habits. Various processing techniques can be applied on pearl millet to reduce the phytate content and thus increase the bioavailability of the minerals. The processes being applied are cheap, therefore can be administered even in resource – poor communities.

1.4 Objectives

Overall objective

To develop a nutritious composite flour from pearl millet and pumpkin fruit.

Specific objectives

1. To determine the effects of germination followed by fermentation and roasting on the nutrient composition, phytates content and sensory acceptability of pearl millet grain flour
2. To determine the effects of soaking, pre-boiling and roasting on the nutrient composition and phytates content of pumpkin seed flour.
3. To determine optimum blending ratios of pearl millet flour, pumpkin flesh flour and pumpkin seed flour for high nutritional value and sensory acceptability of the formulated composite flour.

1.5 Hypothesis

1. Germination at different time-temperature combinations followed by fermentation and roasting will not affect phytates content, nutritional and sensory properties of pearl millet flour.
2. Soaking, preboiling and roasting will not have any effect on the nutritional attributes of pumpkin seed flour.

CHAPTER TWO
REVIEW OF LITERATURE

2.1 Composite Flour

Composite flour is a product obtained by blending of flour prepared from food plants and/or their products. It may also be obtained by blending grains/seeds before milling (East African Standards [EAS] 782, 2012). Composite flour technology initially referred to the process of mixing wheat flour with cereal and legume flours for making bread and biscuits. However, the term can also be used in regard to mixing of non-wheat flours, roots and tubers, legumes or other raw materials (Dendy, 1992). One example is the mixture of sorghum and maize flour for tortillas. Diluting wheat flour with locally available cereals and root crops was found to be desirable to encourage the agricultural sector and reduce wheat imports in many developing countries.

In Africa there has been an ever-increasing demand for wheat products such as bread. Africa is not a major wheat-growing region, but it produces large quantities of other cereals such as sorghum and millets. It has been reported that replacing wheat with 20% non-wheat flour for the manufacture of bakery products would result in big savings in foreign currency annually (Food and Agricultural Organization [FAO], 1982). Thus composite flour technology holds excellent promise for developing countries. Although actual consumer trials have been rare, products made with composite flour have been well accepted in Colombia, Kenya, Nigeria, Senegal, Sri Lanka and the Sudan (Dendy, 1992).

Sorghum flour milled at 80 percent extraction rate could be blended with white wheat flour for bread-making without any adverse effect (Rao & Shurpalekar, 1976). Acceptability studies conducted at the Food Research Centre in Khartoum, Sudan, indicated that breads made with composite flour of 70 percent wheat and 30 percent sorghum were acceptable. Milling at 72 to 75 percent extraction rate yielded fine sorghum flour that is more suitable for bread-making. A combination of 80 percent non-

wheat cereal and 20 percent wheat can be used to produce biscuits with acceptable quality. Sorghum and pearl millet flour blended with wheat flour can be used to make biscuits (Badi & Hoseney, 1976, 1977). Olatunji, Adesina and Koleoso (1989) reported that a proportion of 55% sorghum could be used for biscuits without adversely affecting biscuit quality. Pearl millet could replace 50 percent of the wheat for cake and 80 percent for biscuits (Thiam, 1981). In Senegal, traditional foods such as faux, conus conus and beignets (fritters) are prepared by mixing millet flour with rice, maize or wheat flour (Thiam, 1981).

Sorghum and millets can be adopted for other food products by using appropriate processing methods like dehulling and milling practices to improve the quality of foods made from them. It may be possible to select grain types with improved milling quality that will make these crops competitive with other cereals in terms of utilization. Wheat milling technology with suitable modification can be effectively used for grinding sorghum and millets. Although bread can be produced from whole sorghum flour, the quality of the bread can be improved by using sorghum flour from which the bran fraction has been removed by passage through sieves (Caster et al., 1977).

Kulkarni, Parlikar and Bhagwat (1987) reported that sorghum malt could be used to make biscuits, weaning foods and beer wort. Addition of up to 40% sorghum malt in biscuits caused reduction in stack height and increase in spread because of increased water absorption. Agricultural research and development must focus on finding new and improved ways to produce more diverse, balanced, and healthy diets that include more nutrient-rich foods and to support farmers in fostering local biodiversity and diversified farming systems.

2.2 Pearl Millet

2.2.1 Production of pearl millet

The name given to millet is a broad term applied to several small seeded grains from several genera of Poaceae family of grasses. There are many species and varieties of millet grown around the world. These include proso millet *(Panicum miliaceum)*, foxtail millet *(Setaria italica)*, finger millet *(Eleusine coracana)*, kodo millet *(Paspalum scrobiculatum)*, little millet (*Panicum sumatrense*) and pearl millet (*Pennisetum glaucum*) (Singh & Raghuvanshi, 2012).

Millets are some of the oldest crops and have been cultivated for thousands of years. Approximately 90% of the world's millet production is being used in the developing nations. The remaining 10% is being used by developed countries mostly as livestock feed (FAO, 1995). India is the largest producer of millet with 38.6% of the world's millet being grown. Pearl millet accounts for two-thirds of this production.

Millet is mostly grown as a subsistence crop for the local market, with some produced for the commercial market in India (FAO, 1995). It ranks as the sixth most important cereal crop in terms of production, worldwide (Saleh, Chen, & Shen, 2013). Millet is a staple in the diets of those from poorer regions of the world. It is deemed "the poor man's grain" and is often stigmatized for this reason. However, it has played a major role in food security in Africa and Asia. Millet is a short season crop that is usually ready to harvest in 60 to 90 days. Millet is adaptable to various types of soil and climates and requires little water and tending for optimal growth. This ability to grow in dry and unfertilized soil conditions makes millet relatively drought resistant.

Interest in drought resistant crops is growing as water scarcity and climate change becomes an issue (Ashraf, Ashfaq & Ashraf, 2002; Baltensperger, 2002; Saleh et al., 2013). They can survive with less than 300 mm of rainfall compared to corn, which needs a minimum of 600 mm (Léder, 2004). Millet is considered to be more nutrient dense than more popular grain varieties available in North America, such as rice, wheat and corn (Asharani Jayadeep & Malleshi, 2010).

2.2.2 Physiology of pearl millet

There are many varieties of millet found around the world. The basic structure of the grain is similar among all millet types, including a pericarp, endosperm and germ. The pericarp is the outermost layer of the grain and aids in the control of water loss during growth. It is the protective layer that forms around the endosperm and germ of the seed (Bradbury, MacMasters & Cull, 1956). Fibres are mainly concentrated into the outer layers of the grain. The endosperm is made up of two layers, the starchy endosperm and the aleurone layer. The starchy endosperm is the largest component of the grain and consists of peripheral, floury and corneous zones. It is mostly comprised of starch granules within a matrix of protein bodies. The aleurone layer surrounds the starchy endosperm, with concentrations of protein, lipids, vitamins and minerals. The germ of the grain includes the embryo and scutellum. The germ is rich in lipids, proteins and minerals (Evers & Millar, 2002).

The size, structure and shape of the grain can vary depending on the type of millet and where it was grown. Within millet types, cultivars can differ based on production region, breeding and genetics (Colosi & Schaal, 1997). Pearl millet is about 2 mm in length and has a 1000 kernel average weight of 8 grams. They tend to have an ovoid shape. The seed coat of this millet is 0.4 mm in thickness. In pearl millet, the pericarp is caryopsis type, meaning the pericarp is completely bound to the endosperm. Pearl millet characteristically has a larger germ (Belton & Taylor, 2004). The endosperm to germ ratio of pearl millet is 4.5:1. Pearl millet has a larger floury than corneous zone which creates a softer texture. The starch granules in the corneous zone of the endosperm are polyhedral in shape and about 6.4 μm in size. The starch granules found in the floury zone are spherical and larger than those found in the corneous at 7.6 μm. Starch that is larger is hydrolyzed more slowly (FAO, 1995).

2.2.3 Nutritive value of pearl millet

Pearl millet has been used in developing nations to solve problems of food insecurity and malnutrition. Along with its appealing drought resistance and short growing season, millet is also a nutritionally dense food (Saleh et al., 2013). It is often ground into flour and used to make porridge, *roti* and beverages. The nutrient composition of pearl millet can vary by type and variety. Nutritionally, it has been found to be superior when compared to other common grains (Parameswaran & Sadasivam, 1994). It is a good source of protein, fat, fibre and many micronutrients (Léder, 2004). The use of pearl millet worldwide is important as it feeds many low income families and provides proper nutrition to those in need.

Protein content of millet ranges from 7.7-12.5% in varieties of proso, pearl and finger. Kumar and Parameswaran, (1998) found that pearl millet contains five protein fractions. These include Fraction I: albumin+globulin, Fraction II: true prolamin, Fraction III: prolamin-like, Fraction IV: glutenin-like, and Fraction V: true glutenin. Prolamin is considered to be the fraction directly associated with protein quality and is the most prevalent fraction in pearl millet (FAO, 1995). The albumin+globulin content ranges from 22.6-26.6% and 17.3-27.6% in pearl millet and finger millet, respectively. Prolamin ranges from 22.8-31.7% in pearl millet and is the highest in finger millet with 24.6-36.2%. Glutenin content is the lowest protein fraction with 16.4-19.2% in pearl and 12.4-28.2% in finger millet (FAO, 1995). Pearl millet has been found to contain high levels of essential amino acids, particularly sulphur containing amino acids of methionine and cysteine (Amadou, Gounga, & Le, 2013). In comparison to sorghum and maize, pearl millet contains higher amounts of lysine (Ejeta et al., 1987).

Lipids are concentrated in the germ, pericarp and aleurone layers of the millet grain. Free lipids in many varieties of millet have been found to range from 2.8-8.0% (Rooney, 1978). Pearl millet has a free lipids content range of 5.6-7.1% and bound lipids range of 0.57-0.90% (Lai & Varriano-Marston, 1980). The free lipid fraction is comprised of hydrocarbons, triglycerides, monoglycerides, diglycerides and free fatty acids, while the

bound lipid fraction is made up of lecithin and other components but no free fatty acids (Rooney, 1978). The free lipid content of pearl millet is high in unsaturated fatty acids, accounting for 70.3% of the total free lipid content (Lai & Varriano-Marston, 1980). The main fatty acids found in free lipids are linoleic, oleic and palmitic (Lai & Varriano-Marston, 1980; Rooney, 1978).

The dietary fibre in pearl millet is found mostly in the outer pericarp and decreases in quantity to the endosperm of the grain. In comparison to many other cereal grains, the soluble dietary fibre content of pearl millet has been shown to be 1.45% and an insoluble fibre content of 13.5% (Ragaee, Abdel-Aal & Noaman,., 2006). The total dietary fibre of millet is higher than that of wheat; however it is lower than the values of rye and sorghum grains (Ragaee et al., 2006).

2.2.4 Antinutrients in pearl millet

Pearl millet contain antinutrients, including polyphenolics, phytic acid and tannins, that can interfere with the bioavailability of some nutrients and minerals. Polyphenolics are located in the pericarp and endosperm of all pearl millet. Ferulic acid is the major phenolic acid found in all millets (Dykes & Rooney, 2006). Phenolic content is higher in pearl millet than in wheat. Total phenolic content of wheat ranges from 500-560 μg/g, whereas millets contain approximately 1387 μg/g phenolic content (Ragaee et al., 2006). Phenolics also have an antioxidative capacity. They help prevent free radical formation in the body and can prevent undesirable changes in flavour and nutrients (Pushparaj & Urooj, 2014; Subba Rao & Muralikrishna, 2002).

Phytic acid is present in all cereal grains. However, all grains contain different levels of phytates. These phytates tend to bind to certain minerals, including iron, decreasing their absorption by the body. Although phytic acid levels are slightly higher in wheat, millets still contain levels that are high enough to decrease mineral bioavailability (Simwemba, Hoseney, Varriano-Marston, & Zeleznak, 1984).

2.2.5 Inhibition of mineral bioavailability by phytates

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

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

2.3 Pumpkin Fruit

2.3.1 Crop description

The pumpkin is an angiosperm belonging to the Cucurbitaceae family that is characterized by prostrate or climbing herbaceous vines with tendrils and large, fleshy fruits containing numerous seeds (Acquaah, 2004). Pumpkin of the genus C. maxima is also called squash guard. The common names are Chinese pumpkin or crookneck squash and winter squash (Tindall, 1983). Its centre of origin is South America, possibly Peru,

now widely distributed throughout the tropics. The areas of cultivation are tropical Asia (India, Indonesia, Malaysia, and the Philippines), tropical Africa, Central and South America, particularly Mexico and the Caribbean.

Botanically the squash guard can be described as an annual herb, rarely upright, generally trailing, and vines up to 3 m in length. Stems are slightly hairy, soft, and cylindrical in cross-section and tendrils are branched. Leaves are dark green mainly reniform, cordate, and rarely lobed, sometimes with white markings, 15-30 cm in diameter. Flowers are monoecious, female flowers are 15cm in diameter, yellow-orange; male flowers are smaller, 8.5cm in diameter with long upright thin pedicels, calyx of 5 sepals, fused at base, 3 stamens that are short and fused, and stigmas are small and yellow. The fruits are large, variable in shape, round or oblong, covered with small raised spots, weighing 2-5 kg. The rind may be soft or hard, sometimes brightly coloured. The flesh is yellow. Seeds are white or brown, ovoid, 1.3cm x 0.9cm, flattened, narrow towards point of attachment, (Rice, 1973; Tindall, 1983).

The environmental response of the crop is varied. Plants require a fairly high temperature, above 25-27^{0}C during the growing period with fairly low humidity (Tindall, 1983). Soils with a high organic content are preferable, but in general, pumpkins are tolerant to a wide range of soil conditions. Fruits may be harvested 80-140 days from sowing. The optimum harvesting stage is before the seeds ripen and when the skin of the fruit begins to harden. Maturity indices include a well corked stem, development of abscission layer and subtle changes in rind colour depending on variety (Cantwell & Suslow, 2002). Yields are 3-6 fruits per plant and individual fruits may weigh 2-5 kg.

Cucurbita moschata is closely related to *C. maxima*. Its other names are winter squash or butternut squash. Its origin is tropical South or Central America. It is the most widely grown species of the *Cucurbita*. It is an annual vine; stems grow up to 3 m. It is generally lacking in bristly hairs on leaves and stems, which mainly distinguishes it from *C. maxima* (Rice, 1973; Tindall, 1983). The flesh of the fruit is yellow to orange. The

seeds are either white or brown in colour. The environmental response is such that *C. moschata* is more tolerant than most species of *Cucurbita* to high temperatures and is well adapted to the environment of the lowland tropics. The cultural requirements and growth period are similar to those for *C. maxima.*

2.3.2 Importance of pumpkin in the human diet.

2.3.2.1 Starch

Although pumpkin is botanically classified as a fruit vegetable, it is consumed by many communities as a staple, providing a substantial amount of calories on consumption. Pumpkin as a fruit is a storage organ and has been found to contain starch in amounts that may go up to 60% (Corrigan et al., 2001).

2.3.2.2 Vitamins

Pumpkin fruit pulp contains beta-carotene, a pro-vitamin A, which plays a major role in human nutrition. Beta-carotene has been used for many years as a food colouring agent, pro-vitamin A in food and animal feed, an additive to cosmetics, multivitamin preparations, and in the last decades as a health food product under the claim 'anti-oxidant' (Ben, Amotz &Fishler, 1997). Murkovic, Mülleder and Neunteufl (2002) reported that β-carotene content in pumpkins ranged between 0.06 and 7.4 mg per 100 g. Vitamin A occurs only in animal tissues such as fish liver oil, livers of animals, milk fat and in egg yolk (Belitz & Grosch, 1985). Plants are devoid of vitamin A. Vitamin A in nature originates from carotenes, which are the yellow and red pigments responsible for colour of many vegetables and fruits. Of the 500 or so naturally occurring carotenoids, about 60 posses the vitamin A activity in varying degrees but only 5 or 6 of these are commonly found in food. The major carotenoids are β- carotene, α-carotene, cryptoxanthin, lutein, zeaxanthin, and lycopene. The most common and active of the pro-vitamins is β-carotene, found in fruits and vegetables. It exists naturally as the all–trans isomer (Frigg, 1999). One of the most important features of carotenoids is that they are organic compounds with long unsaturated chains. These chains are responsible for their bright colour. The unsaturated property is easily destroyed by oxidation in air or by

hydrogenation (Ihekororonye and Ngoddy, 1985). The carotenoids yield vitamin A by cleavage of the centrally located double bond (Belitz & Grosch, 1986).

The carotenoids which have the unsubstituted β-ionone ring can be cleaved oxidatively to yield retinaldehyde which is reduced to retinol (Bender, 1992). Once ingested, β-carotene and other pro-vitamin A carotenoids are cleaved in the intestinal mucosa by carotene dioxygenase yielding retinaldehyde (Combs, 1992). Central oxidative cleavage of β-carotene gives rise to two molecules of retinaldehyde. The retinaldehyde is reduced to retinol, which is esterified and enters the circulation in chylomicrons. Animals are unable to biosynthesize carotenoids, but assimilate them through their diet in the form of vitamin A (Woolfe, 1992).

Pro-vitamin A accounts for 60-90% of vitamin A intake. Populations depending on it as dietary source of vitamin A are in South East Asia, Africa and West Pacific, where animal sources of vitamin A are out of reach. The biological value of dietary carotene varies widely depending on the efficiency of absorption but it is taken as an average of one-sixth that of all trans-retinols (Woolfe, 1992). This disparity in biological activity is primarily due to inefficiency of carotene absorption and subsequent bioconversion to retinol. The bioavailability is greatly influenced by the nature of the embedding matrix (fibre) and the composition of the accompanying meal (Bender, 1992).

2.3.2.3 Mineral content and availability

In addition to protein, fat, and carbohydrates the body requires inorganic nutrients such as sodium, calcium, potassium, and phosphorus in available form. Also, other constituents are required in much smaller amounts such as copper, zinc, manganese, cobalt, and iron known as trace element (Mohamed, 2003). Studies involving seeds from eight lines of naked seed squash (*Cucurbita pepo*.L) found that the endogenous mineral contents varied significantly among samples with the exception of magnesium and manganese, whereas Ca (243-2377 μ*g*/g), K (20692-28384 μ*g*/g), Zn (197-266 μ*g*/g), Fe (164-231 μ*g*/g) and Mn (105-117μ*g*/g), also found that potassium, magnesium, and

calcium were the most prevalent minerals, respectively, so naked squash seeds appeared to be a good source of macro- and micro elements (Idouraine et al., 1996).

El-Adawy and Taha (2001) reported that the mineral content of pumpkin seed kernel was 1.7 mg/100g Co, 8.2 mg/100g Zn, 10.9 mg/100g Fe, 8.9 mg/100g Mn, 38 mg/100g Na, 130 mg/100g Ca, 982 mg/100g K and 1090 mg/100g P. The minerals content of pumpkin seed flour (defatted) was 74.9 mg/100g, 20.5 mg/100g, 1379 mg/100g, 4.5 mg/100g, and 148.9 mg/100g for calcium, sodium, potassium, iron, and phosphorus, respectively,(Giami *et al*., 2005). Minerals are important for enzymatic activity and normal physiological function in the human body. Calcium is a macro- mineral that is required in amounts of more than 1mg per day. The recommended daily allowance (RDA) is 1000-1200 mg for adults and 1300mg for those aged between 9-18 years. Calcium is important in the formation and maintenance of strong bones and teeth throughout the life cycle. It is also involved in blood clotting and aids in nerve impulse transmission, muscle contractions and contributes to cell permeability (Fawzi and Hunter, 1998). Calcium deficiency can cause osteoporosis. Good sources of dietary calcium are dairy products, fish, leafy vegetables and nuts.

Phosphorus is a micro-mineral that participates in the energy cycle that turns the food we eat into energy in form of Adenosine Triphosphate (ATP), for use by the body. Like calcium, phosphorus is also involved in muscle contraction and nerve transmission, and it is needed in protein synthesis. Phosphorus is part of deoxyribonucleic acid (DNA) which controls heredity. Phosphorus is required for bone and tooth strength. As part of cell membranes, phosphorus plays a protective role by regulating what comes in and goes out. The RDA for phosphorus is 700 mg for those aged above 18 and 1250 mg for those aged between 9-18 years. Phosphorus is found in nearly all foods and dietary deficiency is unknown, though too much phosphorus can cause calcium deficiency, leading to osteoporosis. Besides carbonated drinks, phosphorus is found in milk and milk products, meat, fish, nuts and legumes (Wardlaw & Kessel, 2002)

Zinc is significant in the human diet since it is required in more than 100 enzymes involved in growth, immunity, alcohol metabolism, sexual development and reproduction. The human body contains 2-3 g of zinc (Gibson, 2003). There are no specific storage sites for zinc hence a regular supply in the diet is required. Zinc is found in all parts of the body, 60% is found in muscle, 30% in bone and about 5% in our skin (Gibson, 2003). Particularly high concentrations are in the prostate gland and semen. The RDA for zinc is 11 mg for men and 8 mg for women (Wardlaw and Kessel, 2002). The first signs of zinc deficiency are impairment of taste, a poor immune response and skin problems. Other symptoms of zinc deficiency include hair loss, diarrhoea, fatigue, delayed wound healing, and decreased growth rate and mental development in infants (Wardlaw & Kessel, 2002).

Zinc supplementation can help skin conditions such as acne and eczema, prostate problems, anorexia nervosa, alcoholics and those suffering from trauma or post surgery. Zinc is present in a variety of foods, particularly in association with protein foods. A vegetarian diet contains less zinc than a meat based diet and so it is important for vegetarians to eat plenty of foods that are rich in this vital mineral (Gibson, 2003). Good sources of zinc for vegetarians include dairy products, beans, sesame, lentils, whole grain cereals and green vegetables. Pumpkin seeds provide one of the most concentrated vegetarian food sources of zinc. All meats and sea foods are rich sources of zinc (Wardlaw & Kessel, 2002).

Iron is a functional component of haemoglobin and other key compounds used in respiration, immune function and cognitive development. Iron plays an important role in changes in some neurotransmitters in the brain and brain development (Latunde-Dada, 2000). People most at risk are infants, preschool children, adolescents and women in child bearing age (Dallman, 1992). The RDA for men is 8 mg and 18 mg for women Nutrient dense dietary sources include meats, sea food, broccoli, peas, pumpkin seeds, bran and enriched breads. Vitamin C helps in iron absorption. Deficiency symptoms

include fatigue, small pale, red blood cells and low blood haemoglobin values **(**Lewis, Towey, Bruinvels, Howatson & Pedlar, 2016).

Effects of exercise on alterations in redox homeostasis in elite male and female endurance athletes using a clinical point-of-care test. *Applied Physiology, Nutrition, and Metabolism*, *41*(10), 1026-1032.The trace element copper is important in iron metabolism, works with many antioxidant enzymes and those involved in protein metabolism and hormone synthesis. Deficiency symptoms are anaemia, low white blood cell count and poor growth. The RDA for humans is 900 μg. Dietary sources are liver, cocoa, beans, whole grains and dried fruits. Manganese is a micro- mineral that is a co-factor of some enzymes such as those involved in carbohydrate metabolism. There are no known deficiency symptoms in man. The RDA or adequate intake is 1.8-2.3 mg. Rich dietary sources are nuts, oats, beans and tea. The mineral content of the fruits, seeds and seed kernels of the two of pumpkin species were analysed in this study. Comparison was made between the elemental mineral content of fruit and seed flours for each of the pumpkin species (Wardlaw & Kessel, 2002).

2.3.2.4 Chemical composition of pumpkin seeds

Pumpkin seed was considered as a good source of oil and protein as stated by Cirrilli (1971), who found the oil and protein content of Italy variety to be 45.35% and 41.85%, respectively. He also stated that the moisture, fiber, and ash content were 7.1%, 1.95%, and 4.7%, respectively.

Kamel (1982) reported that the composition of seed kernel of pumpkin from Canada (*Cucurbita mixta*) as moisture content 5.1%, and the nutrient composition on dry basis was 41% fat, 34% protein, 4.13% ash, 3.74% fiber, and 7.5% carbohydrate. Sharama *et al*. (1986) reported that after oil extraction of pumpkin seed flour the content of protein increase over 70% of dry matter.

2.3.3 Anti-nutritional factors in pumpkin seeds

Anti- nutritional factors such as the oxalate, tannins, cyanide and phytic acid found in pumpkin seeds interferes with mineral element absorption and utilization and react with

proteins to form complex products which have inhibitory effect on peptic digestion. However heat treatment will effectively eliminate most of these undesirable substances. The application of other process such as soaking, cooking, steeping, decorticating, or germination have also been effective in reducing antinutritional factors (Fadul, 1998). Phytic acid content of pumpkin seed kernel flour was 2.37 g/100g as reported by El-Adawy and Taha (2001). Goldstein and Swain (1965) reported that tannins are phenolic polymers which through hydrogen bonding with peptide linkages, precipitate protein from aqueous solutions, rendering plant protein relatively indigestible and reducing or inhibiting enzymes activity. Removal of tannin through chemical or physical treatment was found to improve *in vitro* protein digestibility and weight gain, but removal or lowering the content of tannin through genetic means is an important goal in both cereal and legumes (FAO, 1981). El-Adawy and Taha (2001) found that the tannin content of pumpkin seed kernel was 0.17 g/100g.

2.4 Indigenous processing methods to reduce phytates in cereals

There are many traditional processing methods that have been used to change the nutritional value and sensory properties of cereals. They aim to increase nutrients, decrease antinutrients and make micronutrients more bioavailable within the body. Soaking, fermentation, germination and decortication are some traditional pre-processing methods that have been used for many years (Saleh et al., 2013).

2.4.1 Germination

Germination has been shown to improve the nutritional content of grains and decrease the antinutrients that may be present (Mbithi-Mwikya et al., 2000). It is a natural processing method that has gained new interest as a practical way of increasing the health benefits of whole grains, while improving palatability. Germination can change the physicochemical properties of grains. However these changes can vary between seed varieties and the germination conditions the grains are exposed to (Nelson et al., 2013).

The first phase of germination is breaking the seed's dormancy. About 30-35% moisture content in the millet grain is required for this to occur (Malleshi & Desikachar, 1986). Steeping the mature seeds in water is essential for them to be able to imbibe water and break dormancy. The grain then undergoes many processes and resumes metabolic activity and respiration during phase two. Many enzymes that are present within the dry seed become activated, including α-amylase which hydrolyzes starch (Helland et al., 2002). Germination is completed after radicle elongation occurs in phase three (Bewley, 1997).

Germination is a method performed at household and industrial level in India and African nations. Generally, steeping occurs for 24 hours in order to imbibe enough water to increase moisture content. The seeds are then laid in a single layer in controlled conditions for at least 24 hours. They are then dried at 50°C and the root growth is removed manually by gentle brushing (Swami et al., 2013).

According to Garcia-Huidobro et al., (1982), the leading factor that determines how well germination occurs is temperature. It has been shown that the rate of germination increases with temperature, however there is a narrow range of optimal temperature. Their results show that sprouting conditions are best between the temperatures of 18-38 °C (Garcia-Huidobro et al., 1982). The second most important variable for germination is time. The yield of viable germinated seeds decreased with increased sprouting time (Badau et al., 2006). Those seeds that require an increased amount of time to germinate will usually not survive as a crop (Garcia-Huidobro et al., 1982). The least amount of sprouting occurs at the 24 hour mark as pearl millet has insufficient time to fully germinate. (Badau *et al*., 2006)

2.4.1.1 Effects of germination on nutritional properties of millet

Germination has been suggested to alter alter the nutritional properties of millet, specifically protein, carbohydrate and antinutrient content. Protein content of millet has

been shown to increase after germination. This could be attributed to dry matter loss that occurs during germination. Parameswaran & Sadasivam (1994) saw an increase of protein content in proso millet from 12.32% to 14.30% after seven days, while Swami *et al*., (2013) also found protein content to increase from 14% to 17.5% after 24 hours of germination of finger millet. During this process, degradation of the protein matrix that surrounds starch granules occurs. The protease enzyme becomes activated after imbibition and protein changes follow through proteolysis and transamination (Belton & Taylor, 2004). The conversion to soluble peptides and amino acids can help to synthesize proteins within the embryo (Swami et al., 2013).

The carbohydrates in millet are mainly starch. Starch is a storage polysaccharide that is made up of two parts, amylose and amylopectin. During germination, α-amylase is synthesized by a plant growth hormone called gibberellic acid (GA3) that becomes activated during this process (Muralikrishna & Nirmala, 2005). Gibberellic acid is released into the endosperm and new enzymes are synthesized in the aleurone layer (Zarnkow et al., 2007). Once α-amylase is synthesized, it works to breakdown starch polymers. The enzyme attacks the surface of starch and begins to tunnel inside the granule. Then, α-amylase hydrolyzes the starch from the inside (Zarnkow et al., 2007). It randomly hydrolyzes α-1,4 linkages of amylose and amylopectin to release monosaccharides and polysaccharides. Glucose is the major sugar released, along with maltose and α-limit dextrins. This action degrades starch granules, thereby reducing water binding capacity and leading to low viscosity (Helland et al., 2002).

Germination has been shown to have contradictory effects on the antinutrients present in grains. Many studies on wheat, barley and rice have found that germination can both increase and decrease antinutrient values (Nelson et al., 2013). The reduction of antinutrients, such as phenolic acids, tannins and phytates due to germination increases the bioavailability of minerals. Phenolics and phytates are known to bind minerals and as their concentration decreases, minerals become more readily available. Micronutrient deficiencies are prevalent in developing countries, especially in iron, zinc and calcium,

and germination has been utilized as a method of increasing the bioavailability of these nutrients in plant foods.

2.4.1.2 Effect of Germination on Sensory Properties of Millet

There are few studies examining the effect of germination of millet on its sensory properties and those tests that have been conducted have only examined consumer liking. Inyang & Zakari (2008) germinated pearl millet for two days and made a traditional Nigerian cereal called instant *fura*. Various sensory attributes were tested using a 7-point hedonic scale. The germinated *fura* sample was rated higher than native millet in colour, texture and taste. It was rated highest amongst all samples for overall acceptability of the product and indicated its preference. Balasubramanian, Kaur, & Singh (2014) prepared a weaning formula using variations of pearl millet and barley extrudates in combination with pearl millet and barley malted flour. A 9-point hedonic scale was used to test for overall acceptability of the product. Acceptability ranged from like moderately to like very much and those formulas using a higher concentration of malted pearl millet flour were rated on the higher end of the scale.

2.4.2 Soaking

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

2.4.3 Fermentation

Fermentation is an age old practice in Kenya and the world over. Fermentation induces phytate hydrolysis via the action of microbial phytase enzymes, which hydrolyze phytate to lower inositol phosphates which do not inhibit nonheme iron absorption (Sandberg et

al., 1999; Hurrel, 2004). Microbial phytases originate either from the microflora on the surface of cereals and legumes or from a starter culture inoculate (Sandberg & Svanberg, 1991). The extent of the reduction in higher inositol phosphate levels during fermentation varies; sometimes 90% or more of phytate can be removed by fermentation of maize, soy beans, sorghum, millet, cassava, cocoyam, cowpeas, and lima beans. In cereals with a high tannin content (e.g., bulrush millet and red sorghum), phytase activity is inhibited, making fermentation a less-effective phytate-reducing method for these cereal varieties (Sandberg & Svanberg, 1991).

2.4.4 Mechanical processing

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

2.4.5 Combined strategies

These effects emphasize that an integrated approach that combines a variety of the indigenous/traditional food processing and preparation practices discussed above, including the addition of even a small amount of animal-source foods, is probably the best strategy to improve the content and bioavailability of micronutrients in plant-based diets in resource-poor settings (Gibson., et al 2010). Use of such a combination of strategies can almost completely remove phytate. This is important because phytic acid is a potent inhibitor of iron absorption, even at low concentrations (Hurrel, 2004). Sprouting releases vitamins and makes grains and seeds more digestible.

2.5 Complementary Feeding in the Developing Countries

According to the Kenya Demographic Health Survey of 2008 – 2009, 72% of infants are introduced to complementary feeding using foods made from cereal grains (KNBS, 2014). Sometimes the grains are substituted with foods made from roots and tubers while legumes are introduced at a much later age. This is the case in most developing countries where complementary foods are made of a mixture of flour from cereals mostly rich in carbohydrates. The foods are sometimes fortified with some proteins, with a little oil and sugar added (Walker, 1990). Foods rich in protein such as powdered milk, fish, peanut butter or soya are out of reach of the poorest people in some of these countries.

Most of the foods used during the complementary feeding stage are frequently characterized by low nutrient density and high bulk, which can adversely affect infants' health (Owino, kala, Amadi, Tomkins & Filteau, 2007). Infants are usually either weaned directly onto the family diet early in life or continue to be exclusively breastfed, both resulting in suboptimal nutrient intake with adverse impact on optimum growth. The high cost of commercial complementary foods adds to the difficulty of providing good nutrition to infants (Asma,, 2006). In resource poor settings such as most rural parts of Kenya, preparation of a complementary food should be based on foods available locally. The food habitually eaten by the family (family meal) can also be used as the basis to make a fluid complementary food by adding water and enriching it during the cooking (Lung'aho & Glahn, 2009).

2.6 Consumer Acceptability of Complementary Foods

Many factors affect acceptance of complementary foods both by mothers and infants including culture and food properties like taste, color and consistency (Codex 1991). Traditional foods are locally accepted and therefore pose no challenge to consumers. If a food is to be cooked to gruel, consistency has been found to affect acceptability (Owino et al., 2007). Some studies have found that complementary food formulations with

addition of sugar were found to be more tasty and appealing than those without sugar by mothers and infants (Martin, Laswai, & Kulwa, 2010; Muhimbula 2011). Formulation and sensory evaluation of complementary foods., 2011).

2.7 Current Trends in Development of Complementary Foods

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

CHAPTER THREE
METHODOLOGY

3.1 Study Design

Raw materials, Pearl millet and pumpkin fruit, were purchased from 3 markets, Githurai, Nyamakima and Mlolongo. From every market 10 kgs of pearl millet (*penisetum glaucum*), and two pieces of pumpkin fruit (*Curbeta pepo var. styriaca*) were purchased randomly, taken to Kenya Bureau of Standards food and agriculture laboratory for treatments and analysis as described in section 3.2. Sensory evaluation was done on the treated pearl millet flour against untreated pearl millet flour prior to formulating the composite flour as it formed the bulk of the formulated composite flour. A composite flour was formulated using the batches of treated pearl millet and pumpkin treated pumpkin seed flour with best nutritinal and sensory attributes. Pumpkin flesh flour was added so as to the β carotene content of the product. A comparative nutritional and sensory analysis was done on the treated formulated composite flour against an untreated composite flour formulated in the same ratio.

3.2 Materials and Methods

3.2.1 Production of pearl millet flour

3.2.1.1 Sample preparation

All the pearl millet samples were blended and cleaned to remove stones and other foreign matter.

3.2.1.2 Malting at different temperature-time combinations

The grains were subdivided into four batches into large nylon bags and steeped in water for 8 hours. The nylon bags were covered with wet cloths to maintain hydration during germination for 1 to 5 days at different temperatures of 20^0C, 30^0C, 40^0C, and 50^0C. After each set of germination time and temperature condition, the grains were prepared

for fermentation. The germination process varied in time and temperature for the four batches as follows:

Batch No 1- 20^0C for 1day, 2days, 3days, 4days and 5days.

Batch No 2- 30^0C for 1day, 2days, 3days, 4days and 5days.

Batch No 3- 40^0C for 1day, 2days, 3days, 4days and 5days.

Batch No 4- 50^0C for 1day, 2days, 3days, 4days and 5days.

3.2.1.3 Natural fermentation of germnated grains

Each portion of each batch of the germinated grains were mixed with excess water, and allowed to ferment at 37°C for optimal growth of most naturally occuring microorganisms eg *lactobacillus plantarum* and *saccharomyces cerevisiae*, in an incubator for 24 hours and then drained of excess water. The grains were then mildly dried at 60°C to maintain enzyme activity.

3.2.1.4 Roasting of fermented grains

Each portion of each batch of pearl millet grains was roasted in an open pan until brown colour was developed. This took about 10 minutes and aimed to develop colour and improve the nutty flavor in pearl millet.

3.2.1.5 Grinding

The grains were ground into flour using a laboratory mill (Zetsc xm 200)

3.2.1.6 Analysis

Pearl millet flour from each portion of each batch was analysed for Moisture, protein, fat, fibre, ash, calcium, iron, zinc and phytates as described in section 3.4

3.2.2 Production of Pumpkin Flesh Flour

3.2.2.1 Peeling the Pumpkin

Pumpkins were randomly obtained from various retail markets (Nyamakima, Githurai and Mlolongo). The raw pumpkins were peeled all round then halved. The seeds were

scooped out and reserved for further treatments and flour production as shown in section 3.3.

3.2.2.2 Slicing the Pumpkin flesh

The pumpkins were diced into small cubes of about 2cm to allow heat to penetrate during drying to avoid the risk of moisture sealing inside the dices.

3.2.2.3 Dehydrating the Pumpkin Slices

The dices were arranged on the racks of an air oven and dehydrated at 100^0C until the slices became brittle.

3.2.2.4 Grinding the Dried Pumpkin slices into flour fineness

A laboratory mill (Zetsc xm 200) was used for grinding the slices to pumpkin flesh flour.

3.2.2.5 Analysis

Pumpkin flesh flour was analysed for Moisture protein, fat, fibre, ash, calcium, iron, zinc and beta carotene content as described in section 3.4.

3.2.3 Processing of pumpkin seed flour

3.2.3.1 Cleaning of pumpkin seeds

After extraction of seeds from the pumpkins, they were cleaned by washing in water.

3.2.3.2 Treatment of seeds

The seeds were subjected to different processes as follows:

Batch No 1- soaking overnight (12 hrs) in water at room temperature, followed by drying at 100^0C for 8 hours in an air oven.

Batch No 2- Pre-cooking in excess water for 10 minutes, followed by drying at 100^0C for 8 hours in an air oven.

Batch No 3- Drying in an air oven at 100^0C for 8hrs, followed by roasting.

3.2.3.3 Grinding

The pumpkin seeds were ground to pumpkin seed flour using a laboratory mill (Zetsc xm 200)

3.2.3.4 Analysis

The flours from each batch were analysed for protein, fat, fibre, ash, calcium, iron, zinc and phytates as described in section 3.4.

3.2.4 Procedures for product analysis

3.2.4.1 Determination of moisture content

Moisture content was determined by drying method (AOAC, 14.003). Empty moisture dish with a lid was dried in an oven prior to sample addition. The dish with lid was then cooled to room temperature in a desiccator and weighed accurately (w_0). 5g of ground sample was weighed into the moisture dish in triplicates and the weight of the dish, lid and the sample taken (W_1). The samples were dried in an air oven (Mermet Laoding Modell 100-800). Temperature and time of drying varied within the samples whereby for pearl millet grains and pearl millet flour drying was at 105^0C for 5hrs, pumpkin seeds and pumpkin flesh were dried at 105^0C for 2hrs The samples were then cooled in a dessicator and weighed. They were redried for 30 minutes and the whole process repeated until the change in weight between successive drying cycles at 30 minutes interval was not >0.5g (W_2).

Moisture content was calculated as follows;

$$\text{Moisture Content (\%)} = \frac{\text{weight of moisture evaporated} \times 100\%}{\text{Weight of sample}}$$

$$= \frac{\text{Weight before drying } (w_1) - \text{weight after drying } (w_2) \times 100\%}{\text{Weight of sample+dish+lid before drying } (w_1) - \text{weight of moisture dish + lid } (w_0)}$$

3.2.4.2 Determination of crude fat content

Crude fat was determined using the Soxhlet method (AOAC, 7.056). Alluminium extraction cups containing 3-4 boiling chips were conditioned in an air oven at 105^0C, cooled in a dessicator and weighed ($W_{0)}$. 5g of each Sample were measured into extraction thimbles in triplicates, stoppered with a defatted cotton wool and adapters attached to the thimbles. Each thimble was placed in the corresponding cup and then inserted into the condensers. The cups were fixed using the cup holder to mate to the condensers. The cups were loaded with 70ml of petroleum ether using the connectors on top of the extraction unit and the sovvent addition kit connected to a dispenser. The system was started whereby all the stages (boiling, rinsing and drying) were done automatically as per the settings (Boiling- 15 mins, Rinsing- 45 mins, Drying - 10 mins) at 85^0C The cups were released, removed from the extraction unit and dried in an air oven (Mermet loading modell) at 105^0C for 30 mins, then cooled and weighed (W_1). The crude fat content was calculated as follows;

$$\text{Crude Fat (\%)} = \frac{\text{Weight of fat extracted x 100}}{\text{Weight of sample}}$$

$$= \frac{(W_1 - W_0) \text{ x } 100}{\text{Weight of sample}}$$

3.2.4.3 Determination of crude fibre content

Crude fibre was determined using ISO 5489, method. Glass crucibles with a porosity number 2 (40-90 microns) were conditioned by heating them in a muffle furnace (Nabertherm 30-3000^0C, German) at a temperature of $\leq 200^0$C and increasing the temperatures to 500^0C. They were heated at that temperature for 30mins. The muffle furnace was switched off to lower the temperature to $\leq 200^0$C. The glass crucibles were removed from the muffle furnace and cooled. A pinch of previously conditioned celite was added into each glass crucible. 1g of each sample was weighed into each glass

crucible (W_1) in triplicates and the crucibles were fixed into a holder then mounted onto a cold extraction unit. The samples were defatted using acetone in a spray bottle by gently spraying until acetone covered the whole sample then filtered. This was repeated twice.The samples were mounted onto the Foss fibretec^{TM} 2010.

Previously boiled 1.25% m/v sulphuric acid was poured through the columns to 150ml mark. The fibretec was switched on and the samples were digested for 30 minutes after which washing was done thrice using 50mls hot water per wash. The same was repeated using 1.25 m/v sodium hydroxide.

The samples were dried in an air oven at 105^0C for 2 hrs, cooled in a dessicator and weighed (W_2). They were then ransfered into a muffle furnace at $\leq 200^0$C and temperature increased slowly to 500^0C. Incineration was done for 3 hrs and furnace switched off to lower the temperatures to $\leq 200^0$C. The crucibles were removed cooled in a dessicator and weighed (W_3). The crude fibre content was calculated as follows;

$$\text{Crude Fibre}(\%) = \frac{(\text{Undigested matter+ash})- \text{ash} \times 100}{\text{Weight of Sample}}$$

$$= \frac{(W_2 - W_3) \times 100}{W_1}$$

3.2.4.4 Determination of crude protein content

Crude protein was determined using Kjeldhal method (AOAC,14.068) with modifications stipulated in the kjeltec operating manual and ISO 5983. After placing the digestion tubes on the rack, 0.3g of each sample was weighed into each tube in triplicates and 2 kjeltabs (copper II Sulphate catalyst) added into each tube. 10ml of conc. Sulphuric acid were added into each tube and mixed carefully by swirling.. The rack containing the digestion tubes was placed beside the digester and the exhaust maniflod fitted on top. The vacuum source (scrubber) was turned on to maximum air

flow. The tubes and the manifold were then placed in the preheated digester at 420^0C and the heat shields were hooked on the stand. The samples were digested for 5 mins with maximum airflow through the manifold then the flow adjusted until the fumes were contained. Digestion was continued until all samples were clear with blue/ green solution. This took about 30 mins. The stand with tubes and exhaust manifold was removed and placed on the cooling stand. The nitrogen in samples was then distilled into 1% boric acid and titrated with 0.5N HCL one by one starting with the blank while recording the results. This was done using Gerhardt Kjedahl vapodest 50^S equipment. Protein content was calculated as follows;

$$\text{Crude Protein (\%)} = \frac{1.401X(V_2-V_1)XMXA}{W}$$

Where

V_1- Volume of hydrochloric acid required for blank

V_2- Volume of hydrochloric acid required for

M- Normality of hydrochloric acid used in titration

Kjedahl conversion factor (5.83 for pearl millet, 5.71 for pumpkin seeds, 6.25 for pumpkin flesh)

W- Weight of sample

3.2.4.5 Determination of crude ash content

The crude ash was determined by sample incineration in a muffle furnace (AOAC, 14.006). Crucibles were conditioned in a muffle furnace (Nabertherm 30-3000^0C, German model) for 30 mins at 550^0C cooled in a dessicator and weighed (W_0). 5g of each sample was weighed into the crucible, charred on a hot plate until they stopped producing smoke. They were then transfered into the muffle furnace and incinerated for

6 hours at 550^0C after which they were cooled in a dessicator and weighed (W_1). Crude ash content was calculated as follows;

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

$$= \frac{(W1 - W_0) \times 100}{\text{Weight of sample}}$$

3.2.4.6 Determination of calcium iron and zinc content

The ash obtained from section 3.2.4.5 was dissolved in 15 ml 10% HCl in a volumetric flask which was then topped up to 100 ml mark with distilled water to be used for mineral determination according to the AOAC method (1995).

Iron and zinc was determined by Atomic Absorption Spectrophotometer using the respective cathode lamps. The individual mineral element composition was calculated from the AAS spectrophotometer readings obtained for both the blank and the test solution. All determinations were done in triplicate and reported in mg/100g sample.

3.2.4.7 Determination of phytates content

Estimation of phytates was done by HPLC analysis of phytic acid according to Camire and Clydesdale (1982). 50mg of sample was weighed into flasks in triplicate, 10ml of 3% sulphuric acid added and shaken (German model KS 250 basic) for 30min at 1500 rpm. This was followed by filtration using Whatman no. 1 filter paper. The filtrate was transferred to a boiling water bath for 5 min then 3ml of $FeCl_3$ solution (6mg iron per ml in 3% H_2SO_4) added. A second boiling water bath heating was done for 45min to complete precipitation of the ferric Phytate complex. Centrifugation followed at 2500 rpm for 10 min and supernatant discarded. The precipitate was washed with 30ml distilled water, centrifuged, and the supernatant discarded. 3ml of 1.5N NaOH was added to the residues and volume brought up to 30ml with distilled water. Heating was

done for 30 min to precipitate ferric hydroxide. Cooled samples were centrifuged and supernatant transferred into 50ml volumetric flasks. The precipitate was rinsed with 10ml distilled water, centrifuged and supernatant added to the contents of the volumetric flask. 20µl of the supernatant was injected into HPLC. A stock solution of the standard containing 10mg/ml of sodium Phytate [Inositol hexaphosphoric acid $C_6H_6(OPO_3Na_2)6+H_2O$)] in distilled water was used.

Serial dilutions were made for the preparation of a standard curve. Results of phytate content was obtained as per the calculations of Vohra *et al* (1965). The equation of the standard curve line was used for calculating the phytate content.

3.2.4.8 Determination of beta carotene content

A sample of 2g was extracted of the colour using 10ml acetone by swirling until the residue was colourless. This was repeated severally while all extracts were combined into 100ml volumetric flask and then 25ml transfered into round bottomed flask and evaporated to dryness at about 60ºC. 1ml of petroleum ether was added to dissolve the contents which were eluted through a packed column using petroleum ether until the column was clear of colour. The elute was collected in a 25ml volumetric flask and topped up to the mark. The absorbance was read in a spectrophotometer at 450nm. A standard curve was developed using pure standards at concentrations of 0.4, 0.8, 1.2, 1.6 and 2.0. The concentration of β carotene was calculated as follows;

$$\beta \text{ carotene concentration}(\mu g/ml) = \frac{0.4 \times \text{absorbance} \times \text{final volume}}{0.119} \times \frac{100}{\text{Sampe weight}} \times \frac{}{25}$$

The amount of β carotene obtained was converted to retinol activity equivalent (RAE) using the following conversion factors:

1mg β carotene = 1667 IU

1 IU β carotene = 0.05 µg RAE

3.2.5 Sensory Evaluation

A panel of 10 untrained panellists with food technology background were used for sensory evaluation of the pearl millet flour porridge and composite flour porridge both against a control (untreated) sample. A 9 point hedonic scale was used (attached appendix) and the feed back obtained for the parameters tested (taste, colour, aroma and general acceptability), subjected to statistical analysis.

3.2.6 Formulation and Analysis of the Composite Flour

The composite flour was formulated in the ratio of 7:1:2 (pearl millet flour: pumpkin flesh flour: pumpkin seed flour) so as to try to meet the Recommended Daily Allowance for infants aged 7-12 months for all the nutrients and micro nutrients (carbohydrates- 95g/day, protein- 11g/day, Fat- 30g/day, zinc- 3mg/day, iron- 11mg/day, calcium- 270mg/day, vitamin A- 500µg RAE/day). The pearl millet flour and pumpkin seed flour used for formulation had the highest nutritional benefits after the treatments. Therefore pearl millet flour from grains germinated for 3 days at 30^0C and pumpkin seed flour from dried and roasted seeds were used. Amount of pumpkin flesh flour added was so as to help meet Recommended Daily Allowance of Vitamin A for infants aged 7-12 months. A control composite flour (from untreated flours) was prepared in similar ratios for comparison.

Both composite flours (from the treated flours and the untreated flours) were analysed for moisture content, protein content, fat content, fibre content, ash content, zinc content, iron content, calcium content, phytic acid content and β carotene content. Sensory evaluation was also carried out for the two composite flours.

This target group (7-12 months) was preffered since it is the period when children are introduced to complementary foods other than breast milk. Therefore it is a critical window where proper nutrition should be observed so as to curb malnutrition.

3.2.7 Statistical Analysis:

The analysis of the samples were done in triplicates for replication of the data. All data obtained were subjected to One- and Two- way analysis of variance (ANOVA), using GENSTAT statistical package. Means were separated by Duncan's Multiple Range tests (Steel and Torries, 1980) and significance was accepted at $p < 0.05$

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Chemical Composition of Untreated Pearl Millet Flour, Pumpkin Flesh Flour and Untreated Pumpkin Seed Flour.

Table 4.1 shows the chemical composition of the pearl millet flour, pumpkin flesh flour and pumpkin seed flour prior to any treatment. The raw material flours were analysed for proximate composition, mineral content, phytates content and β carotene content. Pearl millet flour had high amounts of phytates content (516.33 *mg/100g*). Abdalla (1998) dealing with 10 pearl millet genotypes reported phytic acid content in the range of 354-796 *mg/100g.* Calcium content of pearl millet flour was also within a similar range as reported by Abdalla (1998). Pumpkin seed flour contained high amounts of fat, protein, fibre and iron compared to other raw materials. Okonya, and Maass, (2014). Protein and iron composition of cowpea leaves: an evaluation of six cowpea varieties grown in Eastern Africa. *African Journal of Food, Agriculture, Nutrition and Development*, *14*(5), 2129-2140 working with pumpkin seeds from varios regions in Kenya reported similar results of iron content (13.71-35.65 *mg/100g*), protein content (14.05-33.29 %) and fibre content (11.69-22.79 *mg/100g*).

Table 4.1 Chemical compositions of pearl millet flour, pumpkin flesh flour and pumpkin seeds flour (% on dry weight basis)

	Pearl millet flour	Pumpkin flesh flour	Pumpkin seed flour
Fat (%)	3.42 ± 0.01	3.57 ± 0.02	30.51 ± 0.01
Protein (%)	9.11 ± 0.01	7.40 ± 0.02	29.05 ±0.01
Fibre (%)	2.59 ± 0.01	3.36 ± 0.01	18.54±0.57
Ash (%)	2.15 ± 0.02	4.42 ± 0.02	4.80± 0.01
Iron *(mg/100g*)	14.83 ± 0.51	14.70 ± 0.02	25.49 ± 15.54
Zinc (*mg/100g*)	4.45 ± 0.01	24.5 ± 3.02	0.58 ± 12.42
Calcium(*mg/100g*)	16.28 ± 0.01	334.57 ± 3.85	13.84 ± 0.06
Phytic acid*(mg/100g)*	516.33 ± 0.58	-	37.03 ± 0.01
Vitamin A Expressed as RAE(µg/100g)	-	279.33 ± 1.53	-

Values are mean ± S.D

4.2 Effect of Germination Followed by 24-hours Fermentation and Roasting on the Protein Content of Pearl Millet.

Table 4.2 shows results for protein contents for germinated, fermented and roasted pearl millet grains. Two- way analysis of variance of germinated then fermented samples showed there was significant interaction between different periods of germination and different germination temperatures. There was significant ($p< 0.05$) increase in the protein levels of pearl millet from a minimum of 9.11 - 9.12% in untreated (0-hours germination, no fermentation) samples to a maximum of up to 11.78% in samples that were germinated for five days (120 hours) at 30^{O}C then fermented.

Similar results were observed by Nnam (2000) and Akpapunam et al., (1996) who also reported an increase in protein content of various cereals and legumes during germination and fermentation. This increase during germination can be attributed to synthesis of enzymic protein by germinating seeds (WHO, 1998, Nzeribe & Nwasike, 1995). Marero, Payumo, Aguinaldo, Matsumoto and Homma, (1989) attributed the increase in protein during germination of cereals to the production of some amino acids during protein synthesis in excess of the requirement and accumulation in free amino acid pool. Fermentation may have also played a role in increase in protein content as it has been observed by other researchers (Alhag, 1999; Obizoba & Atii, 1994. However Abdalla (1996) observed a non-significant reduction in protein content of pearl millet during fermentation. Therefore protein increase was attributed to germination process while fermentation had no much influence on the protein content.

One- way analysis of variance showed there were significant differences in protein contents during different periods of germination and at different germination temperatures. Highest protein contents were obtained at germination temperature of 30^0C and during the fifth day (120 hours) of germination. The 30^0C is within the optimum range for millet germination (Mohamed, Clark & Ong, 1988a) and thus it promoted maximum protein synthesis as explained previously.

Table 4.2 Effect of germination at different time-temperature combinations, followed by 24 hours fermentation at 37^0C and roasting on the protein content (%) of pearl millet flour on dry weight basis.

Germination Time/ Temperature	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Room temperature	9.11 ± 0.01^{a1}	10.15±0.01^{b4}	10.92±0.01^{c45}	11.29± 0.01^{d3}	11.60±0.01^{e4}	11.75±0.01^{f45}
20^0C	9.11 ± 0.01^{a1}	10.08 ±0.01^{b3}	10.89 ± 0.01^{c4}	11.25± 0.01^{d3}	11.55±0.01^{e3}	11.73± 0.02^{f4}
30^0C	9.11 ± 0.01^{a1}	10.25 ±0.02^{b5}	10.96 ± 0.02^{c5}	11.36± 0.02^{d4}	11.66±0.04^{e5}	11.78± 0.01^{f5}
40^0C	9.11 ± 0.01^{a1}	10.03 ±0.01^{b3}	10.66 ± 0.02^{c3}	11.13± 0.02^{d2}	11.63±0.02^{e45}	11.66± 0.01^{e3}
50^0C	9.12 ± 0.01^{a1}	9.88 ±0.01^{b2}	9.94 ± 0.01^{b2}	10.16 ± .02^{c2}	10.28±0.02^{cd2}	10.31± 0.02^{d2}

Values are mean ± S.D

Values in the same row with different superscript letters are significantly different at 5% level and values in the same column with different superscript numbers are significantly different at 5% level.

4.3 Effect of Germination Followed by 24 hour Fermentation and Roasting on the Fat Content of Pearl Millet.

Germination, fermentation and roasting had significance influence on fat content of pearl millet as shown in table 4.3. Two- way analysis of variance of fermented samples showed there was significant interaction between different periods of germination and different germination temperatures which significantly ($p< 0.05$) decreased the fat content of pearl millet from a maximum of 3.39 - 3.42% in untreated (0-hours germination, no fermentation) samples to a minimum of 1.82% in samples that were germinated for 5 days (120 hours) at 30^0C. Many researchers (Malleshi and Desikachar, 1986; Dendy, 1995; Elmaki et al, 1999; and Shah et al, 2011) have attributed the observed reduction in fat content to germination. Obizoba and Atii, (1994) also reported that fermentation process leads to reduction in fat content due to the utilization of fat as energy source by the fermenting organisms.

One- way analysis of variance showed significant differences in fat contents during different germination periods and at different germination temperatures. The highest fat contents were obtained in untreated samples (no fermentation and no germination) and samples germinated at 50^0C respectively. At 50^0C, germination was limited by unfavorable condition (Soman, Stomph, Bidinger & Fussell, 1987) and thus fat content of pearl millet remained high as germination and subsequent fat reduction was minimal. Lowest fat contents were obtained during the fifth day (120 hours) of germination and at germination temperature of 30^0C respectively which as explained previously are conditions that supported maximum germination and significant reduction of fat content.

Table 4.3 Effect of germination at different time-temperaure combinations followed by 24 hour fermentation at 370C and roasting on the fat content (%) of pearl millet flour on dry weight basis

Germination Time/ Temperature	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Room temperature	3.42± 0.01^{c1}	2.58±0.01^{d12}	2.31± 0.01^{b2}	2.31± 0.01^{b2}	2.29± 0.01^{b2}	1.97± 0.01^{a2}
0^0C	3.42± 0.02^{e1}	2.87 ± 0.02^{d4}	2.67± 0.02^{c3}	2.41± 0.02^{b3}	2.36± 0.02^{b3}	2.13± 0.02^{a3}
30^0C	3.42± 0.01^{d1}	2.50 ± 0.01^{c1}	2.27± 0.01^{b1}	2.24± 0.01^{b1}	2.26± 0.02^{b1}	1.82± 0.02^{a1}
40^0C	3.42± 0.01^{d1}	2.63± 0.01^{c2}	2.62± 0.01^{c3}	2.52± 0.01^{b4}	2.28± 0.01^{a2}	2.31± 0.01^{a4}
50^0C	3.40± 0.02^{c1}	2.70± 0.02^{b3}	2.66± 0.01^{b3}	2.63± 0.02^{b5}	2.44± 0.18^{a4}	2.43± 0.02^{a5}

Values are mean ± S.D

Values in the same row with different superscript letters are significantly different at 5% level and values in the same column with different superscript numbers are significantly different at 5% level.

4.4 Effect of Germination Followed by 24 hour Fermentation and Roasting on the Fibre Content of Pearl Millet.

Results for fibre contents for germinated, fermented and roasted samples are shown in Table 4.4. The treatments affected the level of fibre in pearl millet. Two- way analysis of variance of fermented samples showed significant interaction between different periods of germination and different germination temperatures which significantly ($p< 0.05$)

increased the fibre content of pearl millet from a minimum of 2.58 – 2.59% in untreated (0-hours germination, no fermentation) samples to a maximum of 3.47% achieved after 120 hours of germination at 30^0C. Dendy (1995) and Malleshi and Desikachar (1986) reported a similar observation that recorded an increase in fibre content of pearl millet on malting. On the other hand, Abdelnour (2001) reported that fermentation reduced the fibre content of pearl millet but his finding was based on dehulling treatment which was absent in this study.

Optimum temperatures for germination vary little across pearl millet genotypes or races, averaging about 34^0C with a base temperature between 8 and 13.5^0C, and an upper limit of between 47 and 52^0C (Mohamed, Clark & Ong, 1988a). High temperatures (>45^0C) may result in poor crop establishment (Soman, et.al., 1987). The greater increase in fibre content after germination at 30^0C is consistent with greater depletion of carbohydrates and fats at this temperature.

Table 4.4 Effect of germination at different time-temperature combinations followed by 24-hours fermentation at 37^0C and roasting on the fibre content (%) of pearl millet flour on dry weight basis

GerminationTime/ Temperature	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Room temperature	2.59±0.01^{a1}	2.76±0.01^{b3}	2.98±0.01^{c4}	3.12±0.01^{d2}	3.22±0.02^{d2}	3.45±0.01^{e3}
20^0C	2.59±0.01^{a1}	2.69±0.03^{a2}	2.88±0.02^{b2}	3.05±0.01^{c2}	3.13±0.01^{c2}	3.42±0.02^{d3}
30^0C	2.59±0.01^{a1}	2.85±0.02^{b4}	3.07±0.01^{c3}	3.16±0.02^{c2}	3.42±0.02^{d3}	3.47±0.02^{d3}
40^0C	2.59±0.02^{a1}	2.66±0.03^{b12}	2.79±0.02^{c12}	2.93±0.02^{cd2}	2.95±0.02^{cd2}	3.10±0.02^{d2}
50^0C	2.58±0.01^{a1}	2.63± 0.01^{b1}	2.71± 0.02^{c1}	2.85± 0.02^{d1}	2.88± 0.01^{d1}	2.97±0.01^{e1}

Values are mean ± S.D

Values in the same row with different superscript letters are significantly different at 5% level and values in the same column with different superscript numbers are significantly different at 5% level.

4.5 Effect of Germination Followed by 24 Hours Fermentation and Roasting on the Ash Content of Pearl Millet.

Results for ash contents for germinated, fermented and roasted samples are shown in Table 5. Two- way analysis of variance of fermented samples showed there was significant interaction between different periods of germination which significantly ($p< 0.05$) increased the ash content of the pearl millet from 2.14 - 2.15% in untreated sample to a maximum of 2.85% achieved after 120 hours of germination at 30^0C and fermentation for 24 hours. Obizoba and Atii (1994) reported that fermentation at room temperature for 36, 48, and 72 hours increased the ash content of pearl millet. Similarly, Malleshi and Desikachar (1986) reported that germination at 25^0C for 48 h increased the ash content of pearl millet. Thus, a combination of germination and fermentation might result in greater increase in ash content than in individual treatments.

In this study the combined effects of germination followed by fermentation were studied, with varying germination conditions and constant fermentation conditions at 37^0C for 24 hrs. One- way analysis of variance showed germination at varying periods significantly ($p< 0.05$) influenced the level of ash of pearl millet. The ash content increased as germination progressed with time. However different temperature treatments at the same period of sprouting showed no significant difference in ash content. Germination at 30^0C produced the highest ash contents attributed to the optimum germination condition at about 30^0C (Mohamed, Clark & Ong, 1988a). Shah et al (2011) found an increase in ash content during the germination of two mung bean varieties and suggested that such an increase was as a result of the reduction in fat and carbohydrate contents. The same may be true in this study where fat content was also found to decrease with germination time (Table 4.3).

Table 4.5 Effect of germination at different time – temperature combinations followed by 24 hours fermentation at 37^0C and roasting on the ash content (%) of pearl millet flour on dry weight basis

Germination Time/ Temperature	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Room temperature	2.15±0.02^{a1}	2.24±0.01^{b1}	2.62±0.01^{c1}	2.79±0.01^{e1}	2.83±0.01^{d1}	2.84±0.01^{d1}
20^0C	2.15±0.01^{a1}	2.22 ± 0.01^{b1}	2.57± 0.01^{c1}	2.78±0.01^{e1}	2.83±0.01^{d1}	2.82±0.01^{d1}
30^0C	2.15±0.01^{a1}	2.25± 0.01^{b1}	2.64± 0.01^{c1}	2.8 ± 0.01^{e1}	2.84±0.02^{d1}	2.85±0.01^{d1}
40^0C	2.14±0.01^{a1}	2.24 ± 0.01^{b1}	2.60± 0.01^{c1}	2.79±0.02^{e1}	2.84±0.01^{d1}	2.84±0.01^{d51}
50^0C	2.14±0.01^{a1}	2.24 ± 0.01^{b1}	2.58± 0.01^{c1}	2.78±0.02^{e1}	2.83±0.02^{d1}	2.82±0.01^{d1}

Values are mean ± S.D

Values in the same row with different superscript letters are significantly different at 5% level and values in the same column with different superscript numbers are significantly different at 5% level.

4.6 Effect of Germination Followed by 24-hours Fermentation and Roasting on the Iron, Zinc and Calcium Content of Pearl Millet.

Germination, fermention and roasting processes can significantly influence the mineral contents of pearl millet. Table 4.6.1, 4.6.2 and 4.6.3 shows results for iron, zinc and cacium content, respectively. Two- way analysis of variance of fermented samples showed there was significant interaction between different periods of germination and different germination temperatures which significantly ($p< 0.05$) increased the iron and zinc contents of pearl millet (Tables 4.6.1 and 4.6.2). One-way analysis of variance showed that iron and zinc contents significantly increased as germination progresses with the maximum levels for every temperature treatment obtained during the fifth day (120 hours) of germination and minimum level at 0 hours of germination. The best temperature treatment was 30^0C which produced the highest iron and zinc contents. Nnam (2000) working with 'hungry rice-acha' reported related result of an increase in iron level of sprouted cereal of up to two folds. Obizoba and Atii reported similar

findings for zinc as they observed that sprouting for 24, 36, 48, 72 and 92 h significantly increased zinc content of pearl millet.

Calcium content of pearl millet ranged between 10.58-16.28 mg/100g which was within a range that was observed by Adam, Ahamed, Abdelsamd and El tinay, (2009). Two-way analysis of variance of fermented samples showed there was significant interaction between different periods of germination and different germination temperatures which significantly ($p< 0.05$) decreased the calcium contents of pearl millet (Table 4.6.3). One-way analysis of variance showed significant ($p< 0.05$) differences in calcium contents during different periods of germination and for different germination temperatures.

Highest iron and zinc contents (Table 4.6.1 6 and 4.6.2 respectively) in pearl millet flour were both obtained at temperature of 30^0C of sprouting which can be attributed to optimum germination condition of peal millet. Highest calcium contents were obtained in samples that were germinated at a temperature of 50^0C and samples that were not germinated at all. This was a deviation from the behavior of the latter two minerals (iron and zinc) and the contrary results may be attributed to minimal germination at this temperature thus calcium level almost remained unchanged. However there was significant ($p< 0.05$) differences in iron contents since millet is able to establish in difficult environmental condition of about 50^0C as reported by Mohamed, Clark & Ong, (1988) who observed an upper limit temperature of 52^0C for germination of pearl millet and recorded varying optimum level with pearl millet genotypes. Kumar et al (1998) working with green gram and cowpea also reported reduction of calcium content by 40% after 72 hours of germination which was attributed to leaching.

Table 4.6.1 Effect of germination at different time-temperature combinations followed by 24-hours fermentation at 37^0C and roasting on the iron content of pearl millet flour (*mg/100g*)

Germination Time/ Temperature	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Room temperature	14.83 ± 0.51^{a1}	16.98 ± 2.15^{b34}	17.93 ± 0.85^{c3}	19.20 ± 2.22^{d4}	19.58 ± 1.20^{d4}	20.64 ± 2.01^{e4}
20^0C	14.79 ± 1.19^{a1}	15.91 ± 2.06^{b1}	17.73 ± 1.35^{c3}	19.01 ± 1.02^{d4}	19.46 ± 1.32^{d4}	20.40 ± 0.88^{e4}
30^0C	14.85 ± 0.47^{a1}	17.27 ± 4.44^{b4}	18.18 ± 1.72^{c34}	19.30 ± 1.72^{d4}	19.67 ± 1.99^{d4}	20.82 ± 0.39^{e4}
40^0C	14.88 ± 0.16^{a1}	16.70 ± 1.81^{b3}	17.14 ± 0.93^{bc2}	17.75 ± 0.73^{c3}	18.29 ± 1.70^{cd3}	18.38 ± 3.54^{cd23}
50^0C	148.55 ± 0.23^{a1}	161.99 ± 1.26^{b2}	168.51 ± 0.68^{bc12}	172.90 ± 2.25^{c2}	175.23 ± 0.87^{cd}	176.48 ± 1.53^{cd12}

Values are mean ± S.D

Values in the same row with different superscript letters are significantly different at 5% level and values in the same column with different superscript numbers are significantly different at 5% level.

Table 4.6.2 Effect of germination at different time-temperature combinations followed by 24-hours fermentation at 37^0C and roastingon the zinc content of pearl millet flour (mg/100g)

Germination Time/ Temperature	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Room temperature	4.45±0.01a1	5.5±0.26b2	5.85±0.62b2	7.17±0.67c3	7.55±0.81cd3	7.65±1.22d
200C	4.48±0.20a1	5.50±0.35b2	5.68±0.36b2	6.74±0.49c2	7.08±0.37cd23	7.15±0.29d
300C	4.46±0.05a1	5.67±0.71b2	5.91±0.40b2	7.32±0.49c3	7.8± 0.39d34	7.95±0.20d
400C	4.47±0.17a1	5.15±1.28b12	5.53±0.95b2	6.46±0.34c2	6.65 ± 0.18c2	6.79 ± 39c
500C	4.48±0.22a1	4.80± 0.94a1	5.08±0.18ab1	5.23±0.11b1	5.12 ± 0.41b1	5.19±0.65b

Values are mean ± S.D

Values in the same row with different superscript letters are significantly different at 5% level and values in the same column with different superscript numbers are significantly different at 5% level.

Table 4.6.3 Effect of germination at different time-temperature combinations followed by 24-hours fermentation at 37^0C and roastingon the calcium content of pearl millet flour (mg/100g)

Germination Time/ Temperature	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Room temperature	16.28 ± 0.01^{f1}	14.53 ± 0.01^{e1}	13.49 ± 0.02^{d1}	13.44 ± 0.02^{c1}	12.70 ± 0.01^{b2}	12.38 ± 0.02^{a2}
20^0C	16.28 ± 0.01^{f1}	14.61 ± 0.02^{e1}	13.57 ± 0.02^{d1}	13.31 ± 0.02^{c1}	12.81 ± 0.02^{b2}	12.40 ± 0.01^{a2}
30^0C	16.27 ± 0.03^{f1}	14.48 ± 0.02^{e1}	13.42 ± 0.01^{d1}	13.16 ± 0.01^{c1}	12.63 ± 0.01^{b2}	12.11 ± 0.02^{a2}
40^0C	16.29 ± 0.02^{f1}	15.68 ± 0.01^{e2}	14.93 ± 0.02^{d2}	14.85 ± 0.02^{c2}	10.58 ± 0.02^{a1}	10.58 ± 0.01^{a1}
50^0C	16.28 ± 0.01^{f1}	15.72 ± 0.03^{e2}	15.08 ± 0.03^{d3}	14.91 ± 0.01^{c2}	14.69 ± 0.03^{b3}	14.51 ± 0.02^{a3}

Values are mean ± S.D

Values in the same row with different superscript letters are significantly different at 5% level and values in the same column with different superscript numbers are significantly different at 5% level.

4.7 Effect of Germination at time – temperature combinations followed by 24-hours Fermentation and Roasting on the Phytic Acid Content of Pearl Millet.

Germination and fermention processes significantly ($p<0.05$) influenced the phytic acid content of pearl millet. Table 4.7 shows results for phytic acid contents for germinated, fermented and roasted pearl millet flour. Two-way analysis of variance of fermented samples showed significant interaction between different periods of germination and different germination temperatures which significantly ($p< 0.05$) decreased the phytic acid levels of pearl millet (Table 4.7) from maximum amounts obtained before germination to lower levels obtained during day 5 (120 hours) of germination. The decrease in phytic acid levels with germination and fermentation can be attributed to increased activity of the enzyme phytase during germination and fermentation (Watchararparpaiboon, Laohakunjit, & Kerdchoechuen, 2010).

Sutardi and Buckle (1985) also reported similar result of reduced phytic acid during germination and fermentation of soybean.

One- way analysis of variance showed different germination periods and temperatures significantly ($p< 0.05$) influenced phytic acid contents of pearl millet. Highest phytic acid contents were obtained in samples that were germinated at a temperature of 50^0C and samples that were not germinated respectively due to low activity of the enzyme phytase. This may be attributed to minimal germination at this condition (Mohamed, Clark & Ong, 1988a). Khetarpaul and Chauhan (1990) reported that phytic acid was reduced during fermentation due to the action of the phytase released by micro-organisms during fermentation.

Table 4.7 Effect of germination at different time-temperature combinations followed by 24-hours fermentation at 37^0C and roasting on the phytic acid content (mg/100g) of pearl millet flour .

Germination Time/ Temperature	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Room temperature	516.33±0.58^{f1}	369.33±1.16^{e1}	316.33±0.58^{d2}	282.67±1.16^{c12}	259.33±1.53^{b12}	248.33±0.58^{a2}
20^0C	516.33±0.58^{f1}	387.67±1.16^{e3}	329.67±0.58^{d23}	304.67± 0.58^{c3}	281.67± 1.53^{b3}	267.67± 1.44^{a3}
30^0C	515.67±1.16^{f1}	360.67±1.53^{e1}	302.33±1.53^{d1}	271.33 ±0.58^{c1}	252.67± 2.08^{b1}	236.33± 1.16^{a1}
40^0C	516.33±1.16^{f1}	378.00±1.00^{e2}	321.33±0.58^{d2}	289.67± 1.53^{c2}	269.33±0.577^{b2}	257.00±1.00^{a23}
50^0C	515.42±0.16^{f1}	383.73±2.01^{e3}	338.84±3.83^{d3}	316.08±2.11^{c34}	296.67± 2.50^{b4}	274.91 ± 1.24^{3}

Values are mean ± S.D

Values in the same row with different superscript letters are significantly different at 5% level and values in the same column with different superscript numbers are significantly different at 5% level.

4.8 Effect of Processing on the Proximate Composition of Pumpkin Seed Flour

Statistical analyses of results showed that different treatments of pumpkin seed caused significant ($p< 0.05$) differences in protein, ash and fiber contents but insignificant difference in fat content as shown in Table 4.8. The seeds had significantly high crude fat and crude protein levels. Previous studies indicated that pumpkin seeds are excellent sources of both oil and protein (Lazos, 1986). The highest protein level was found in

samples that were soaked overnight then oven dried. This may be due to a possible initial process of sprouting which involves synthesis of enzymic protein (WHO, 1998) and increased activities of protease during germination (Nzeribe & Nwasike, 1995). There was also slight reduction in protein content in precooked and roasted samples which could not be explained as it was in contrary to most findings by other researchers.

The fiber content of the samples was affected by roasting procedure. Soaking treatment had no significant ($p<0.05$) difference in fiber content of the samples. Vidal-Valverde et al., (1992) working with lentils soaked at room temperature for 9 hours found that soaking had no sigificant difference on fiber content. Azizah and Zainon (1997) while studying the effect of processing on dietary fiber contents of selected legumes and cereals also found that soaking caused no significant differences ($p<0.05$) in most samples.

Precooking treatment also caused no significant ($p<0.05$) difference in fiber content though the level showed slight increase when compared to control sample. Azizah and Zainon (1997) however found contradicting results when working with barley and rice boiled for 10 mins at 100^0C as they reported significant ($p<0.05$) reduction and increase in fiber content of barley and rice respectively.

Roasting treatment caused significant ($p<0.05$) increase in fiber content (Table 4.8) which can be attributed to the production of Maillard reaction products. Previous studies including Azizah and Zainon (1997) reported that thermal treatments of cereals and legumes samples with high protein content increased both insoluble dietary fiber and soluble dietary fiber. Roasting treatment significantly ($p<0.05$) increased fiber content of different samples (wheat, rice, mung bean and soya bean) but decreased significantly ($p<0.05$) that of ground nut. The untreated (control) samples had a mean fiber content of 18.54 ± 0.57% which was within previously reported range (Kamel, Davidsson, Juillerat, Barclay & Hurrell 1982).

The crude ash content ranged between 4.61 - 4.80% (Table 4.8), which was within the previously reported range (Lazos, 1986). Each treatment significantly ($P< 0.05$) reduced the amounts of crude ash as shown in the Table 4.8. The precooked samples had the least ash content (4.61%) while the untreated (control) sample had the highest ash content (4.80%). The loss in ash content can be attributed to leaching of both minor and major elements during soaking treatment (Vijayakumari, Siddhuraji, Janardhanan ,1996).

Table 4.8 Proximate composition of treated pumpkin seed flours versus the untreated flour (%) on dry weight basis

Composition	Crude fat	Crude protein	Crude fiber	Crude ash
Control (untreated)	30.51± 0.01[a]	29.05 ±0.01[c]	18.54±0.57[a]	4.80± 0.01[c]
Soaked overnight then dried	30.50± 0.01[a]	29.29±0.03[d]	18.55 ±0.01[a]	4.63 ± 0.01[a]
Precooked for 10 min then dried	30.51± 0.02[a]	28.29±0.02[b]	18.59 ±0.01[a]	4.61± 0.01[a]
Oven dried then roasted	30.53±0.02[a]	27.97 ±0.02[a]	18.67±0.01[b]	4.74 ± 0.01[b]

Values are mean ± S.D

Values in the same column with different superscripts are significantly different at 5% level.

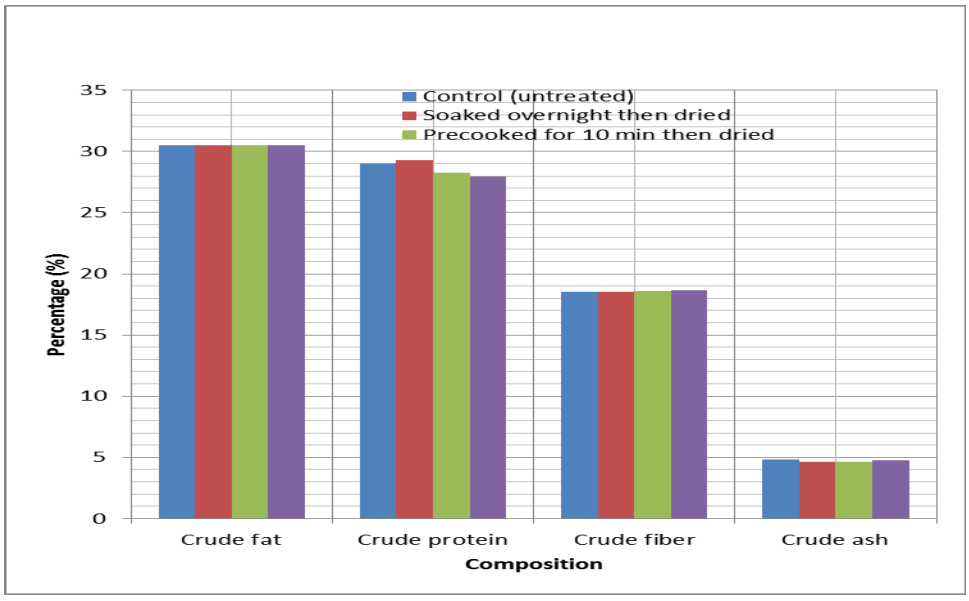


Figure 4.1 Comparison for proximate composition (%) of pumpkin seed flours after different treatments versus untreated pumpkin seed flour

4.9 Effect of Processing on Iron, Zinc, Calcium and Phytic Acid Composition of Pumpkin Seed Flour

Table 4.9 shows important mineral composition of pumpkin seeds. Results obtained showed that pumpkin seed contained moderate concentrations of minerals which concur with Lazos (1986) and El-Adawy and Taha (2001) reports. One- way analysis of variance showed that all the treatments significantly ($p< 0.05$) affected the amounts of zinc, iron and phytic acid. However there was no significant difference in the amounts of zinc in samples that were soaked and untreated (control) samples as shown in the table. Also amounts of zinc in precooked samples and dried and roasted samples showed insignificant differences. Iron content in all treated and control samples showed significant ($p< 0.05$) differences. The phytic acid content of pumpkin seeds were also significantly ($p< 0.05$) affected by the applied treatments with the lowest level obtained from precooked samples and the highest level obtained from untreated (control) samples. Heat treatment of pumpkin seeds significantly ($p<0.05$) reduced phytic acid

content as evident in the table 4.9. Similar results was observed and reported by Hassan, Osman and Babiker (2005) while working with lupin seeds indicating that processing techniques such as cooking have been effective in reducing antinutritional factors.

From the results obtained, it can be deduced that the treatments significantly ($p< 0.05$) increased iron and zinc contents which can be attributed to increase in extractability of these minerals after processing. These minerals are bound by phytic acid which may be responsible for their lower extractability. Reduction of phytic acid as a result of processing may explain higher HCL-extractability of minerals (Duhan, Khetarpaul & Bishnoi, 2002). Related results was reported by Gupta and Sehgal (1991) who observed significant reduction of phytic acid content of cereal grains during soaking and germination. Alono, Aguirre, and Marzo (2000) working with faba bean seeds reported that soaking in water treatment significantly reduced phytic acid content. This could be attributed to leaching out in soaking water under concentration gradient (Kataria, Aguirre & Marzo, 1989

Table 4.9 Zinc, calcium, iron and phytic acid composition of treated pumpkin Seed Flour

Composition	**Zn** *(mg/100g)*	**Calcium** *(mg/100g)*	**Fe** *(mg/100g)*	**Phytic acid** *(mg/100g)*
Control (untreated)	0.58 ± 0.01^{a}	13.84 ± 0.06^{d}	25.49 ± 0.02^{c}	37.03 ± 0.01^{d}
Soaked overnight then dried)	0.58 ± 0.01^{a}	12.66 ± 0.06^{b}	25.13 ± 0.02^{b}	34.07 ± 0.04^{c}
Precooked for 10 min then dried)	0.61 ± 0.01^{b}	12.12 ± 0.08^{a}	25.94 ± 0.01^{d}	31.49 ± 0.82^{a}
Oven dried then roasted)	0.60 ± 0.01^{b}	13.35 ± 0.01^{c}	24.97 ± 0.02^{a}	33.13 ± 0.05^{b}

Values are mean ± S.D

Values in the same column with different superscripts are significantly different at 5% level.

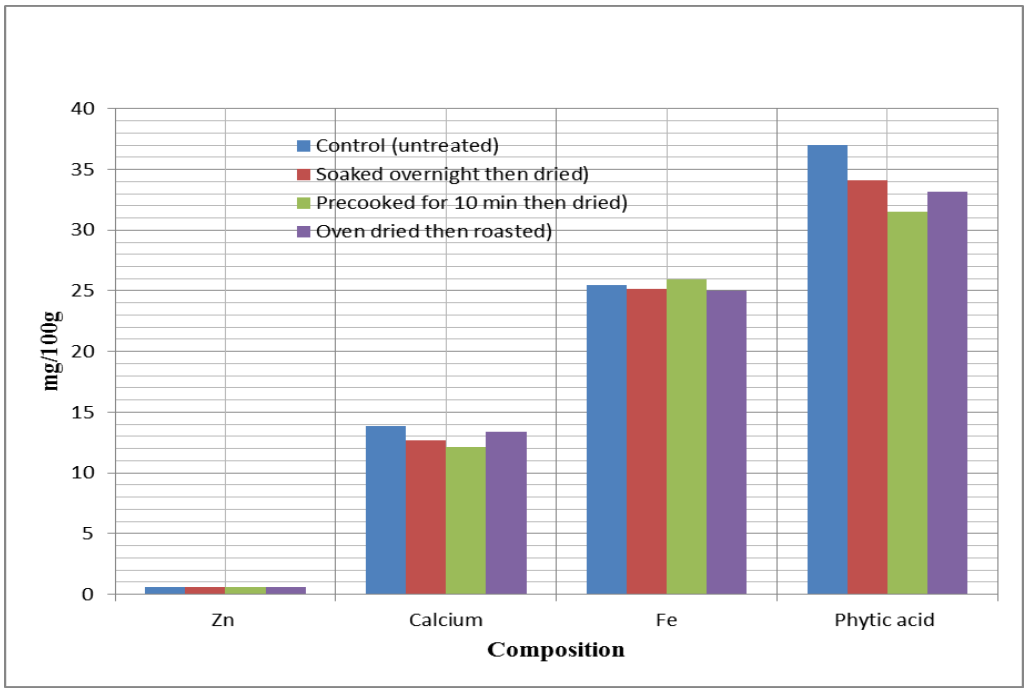


Figure 4.2 Comparison for mineral and phytic acid composition (mg/100g) of pumpkin seed flours after different treatments versus untreated pumpkin seed flour

4.10 Sensory evaluation of pearl millet flour

Flour derived from treated pearl millet (germination at 30^{O}C for 3 days followed by fermentation for 24 hours at 37^{0}C and subsequent roasting) had higher nutritional benefits and was evaluated for sensory acceptability. It scored highly in all sensory attributes; colour, taste, aroma, texture and overall acceptability (table 4.10) as evaluated using 9- point hedonic scale, as opposed to samples derived from untreated pearl millet (no germination, no fermentation, no roasting) samples which scored relatively lower in all sensory attributes with statistical analysis showing significant difference($p< 0.05$) in all attributes except texture. The result indicates clearly that treatments significantly imparted desirable sensory characteristics with regard to colour, taste, aroma and general characteristics. Germination, fermentation and roasting of pearl millet could have

contributed majorly to the high preference of treated samples due to the developed aroma.

Table 4.10 Sensory evaluation of pearl millet flour (evaluated as porridge)

Characteristic	Colour	Taste	Aroma	Texture	General acceptability
Treated pearl millet flour	8.60±0.54[a]	8.40±0.34[a]	8.00±0.501[a]	8.10±0.41[a]	8.20± 0.66a
Untreatedpearl millet Sample	7.70±0.32[b]	6.30±0.10[b]	6.70± 0.23[b]	8.00±0.23[a]	5.90 ± 0.80b

Values are mean ± S.D

Values in the same column with different superscripts are significantly different at 5% level.

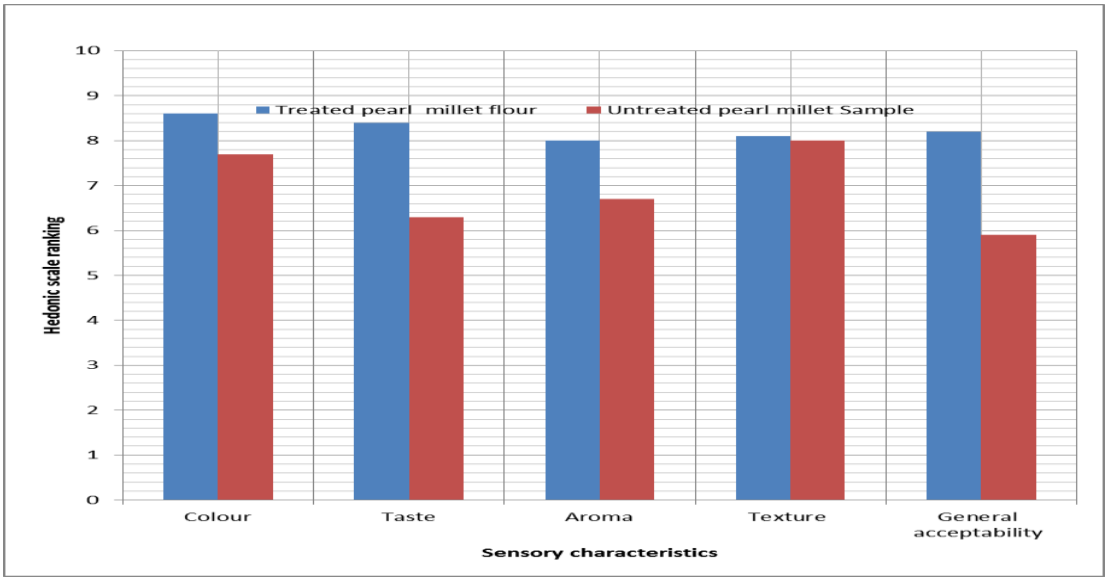


Figure 4.3 Sensory evaluation comparison between tre ated and untreated pearl millet flours using 9- point hedonic scale

4.11 Sensory Evaluation of Formulated Composite Flours

Sample A derived from blending treated pearl millet flour, pumpkin flesh flour and roasted pumpkin seed flour scored highly in all sensory attributes; colour, taste, aroma, texture and overall acceptability (table 4.11) as evaluated using 9- point hedonic scale compared to sample B derived from blending untreated samples of pearl millet, pumpkin flesh flour and untreated pumpkin seed flour which scored relatively lower in all sensory attributes with statistical analysis showing significant difference($p< 0.05$) in all attributes except texture. The results indicates clearly that treatments significantly imparted desirable sensory characteristics with regard to colour, taste, aroma and general characteristics.Germination, roasting and fermentation of pearl millet could have contributed majorly to the high preference of treated samples due to the developed aroma. Roasting of pumpkin seed also contributed to the acceptability and palatability of the treated flour.

Table 4.11: Sensory evaluation of formulated composite flours from the treated and untreated flours (evaluated as porridge)

Characteristic	Colour	Taste	Aroma	Texture	General acceptability
A (Treated Blend)	8.40 ± 0.76^{a}	8.30 ± 0.61^{b}	8.00 ± 0.56^{b}	8.00 ± 0.62^{a}	8.20± 0.66a
B (Untreated Blend)	8.60 ± 0.59^{b}	6.30 ± 0.51^{a}	6.70 ± 0.70^{a}	8.00 ± 0.54^{a}	5.00 ± 0.59^{a}

Values are mean ± S.D

Values in the same row with different superscripts are significantly different at 5% level.

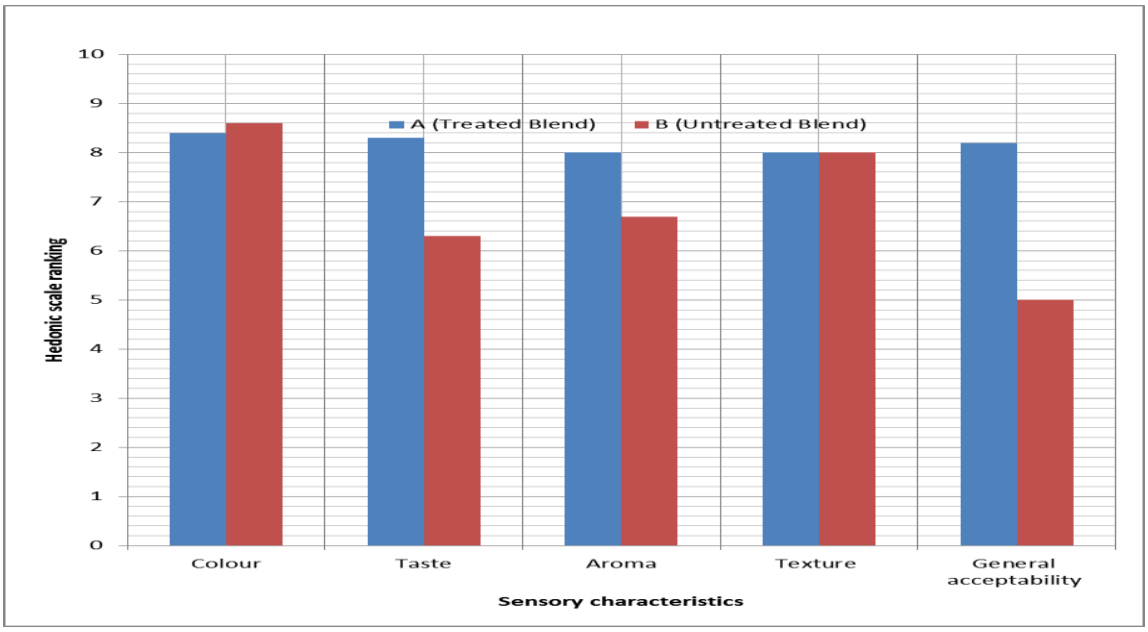


Figure 4.4 Sensory evaluation comparison between formulated treated blend and the formulated untreated blend

4.12 Nutrient Composition of Composite Flours

Table 4.12 shows the composition of the composite flours formulated from pumpkin flesh, pumpkin seeds and pearl millet flours (both the treated flour and the untreated flour) compared with recommended daily allowance or adequate intakes for infants between 7-12 months. One- way analysis of variance showed significant ($P< 0.05$) differences in all constituents of the formulated composite flour in the table except for calcium which showed no significant ($P<0.05$) difference. Blend A derived from treated samples possessed superior quality attributes compared to blend B derived from untreated samples with regard to fat, fiber, ash, protein, zinc, iron and vitamin A which showed significant($P< 0.05$) increments. The phytic acid content of treated blend A reduced to more than half compared to untreated blend B. This finding was important as phytic acid is known to bind important minerals like calcium and iron leading to lower extractability of these minerals (Duhan et al., 2002).

Table 4.12 Nutrient composition of the fomulated composite flours versus the recommended dietary allowances for infants between 7-12 months

Composition	BLEND A *(derived from treated samples)*	BLEND B *(derived from untreated samples)*	RDA
Ash (%)	3.6 0± 0.02[a]	3.24 ± 0.03[b]	-
Fibre (%)	4.70 ± 0.02[a]	3.94 ± 0.01[b]	ND
Fat (%)	5.20 ± 0.02[b]	5.67 ± 0.01[a]	30* g/d
Protein (%)	11.40 ± 0.02[a]	10.76 ± 0.04[b]	11 g/d
Carbohydrate (%)	67.10 ± 0.05[b]	69.83 ± 0.03[a]	95*g/d
Zn (mg/100g)	3.00± 0.02[a]	2.40± 0.04[b]	3mg/d
Fe (mg/100g)	9.80± 0.03[a]	7.50± 0.07[b]	11mg/d
Calcium (mg/100g)	78.80 ± 0.62[a]	75.78 ± 1.34[a]	260*(mg/d)
Phytate/phyticacid (mg/100g)	174.9± 2.54[b]	389.82 ± 6.82[a]	-
Vitamin A (µg/100g) Expressed as RAE	131.30± 2.25[a]	88.75 ± 1.08[b]	500*µg/d

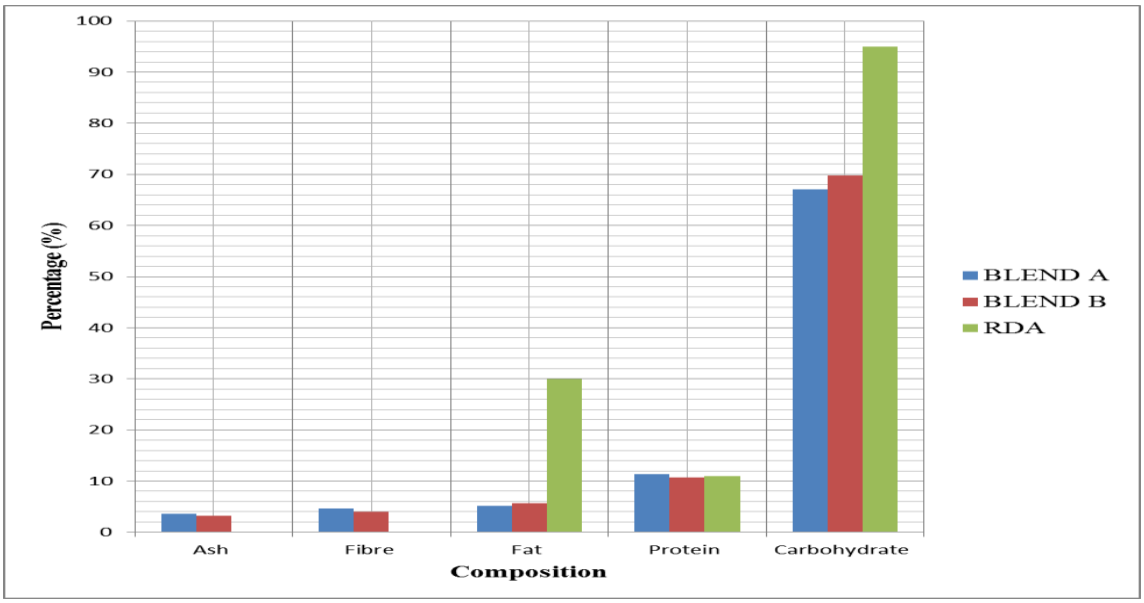
ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts.

RDA are given in ordinary type and adequate intakes are given in ordinary type followed by an asterisk(*)

Values are mean ± S.D

Values in the same row with different superscripts are significantly different at 5% level.

SOURCE: *Dietary Reference Intakes for Carbohydrate. Fiber, Fat,, Protein,etc (2002/2005). Dietary Reference Intakes for Vitamin A, Iron and Zinc (2001); and Dietary Reference Intakes for Calcium (2011)* .**www.nap.edu.**



Figur4.5 Comparison for proximate composition of formulated treated and untreated composite flours versus the RDI for infants aged 7–12 months

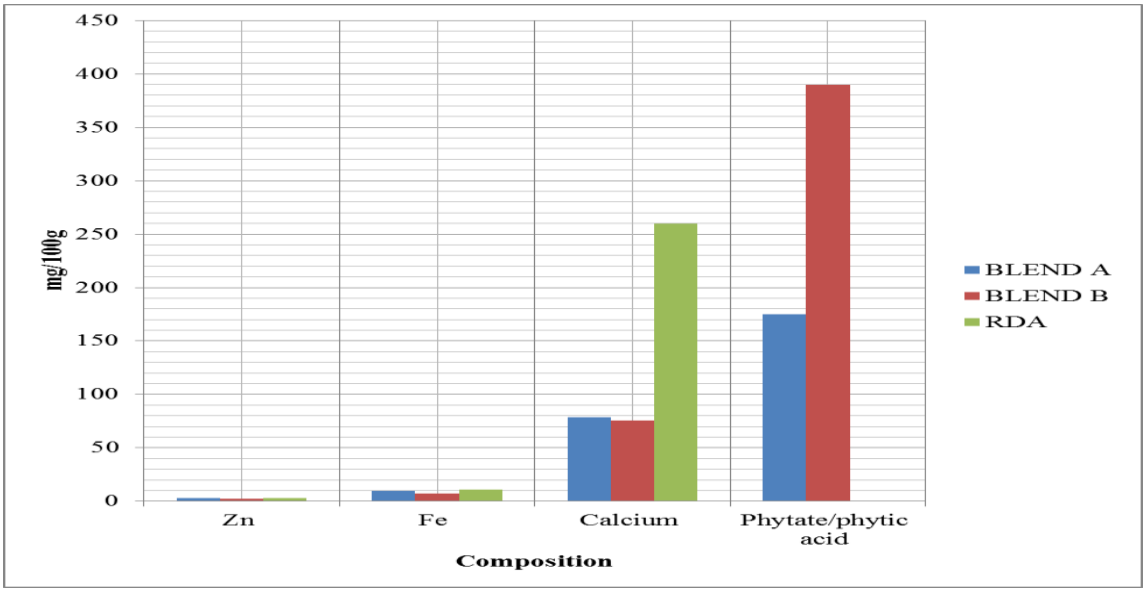


Figure 4.6 Comparison for micronutrient composition of formulated treated and untreated composite flours versus the RDI for infants aged 7-12 months

CHAPTER FIVE
CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Germination, fermentation and roasting processes which were applied on pearl millet grains improved its nutritive value and palatability. The combined treatments led to an increase in protein, fibre, iron, zinc and calcium contents. This increase was gradual upto an optimum temperature of 30^0C during the third day of germination after which the increase was minimal. The phytate content was also reduced in pearl millet grain flour after the treatments.

Precooking and roasting of pumpkin seeds reduced the protein levels. The precooked samples had the least ash content, calcium content and phytate content. Roasting increased the fibre content of pumpkin seeds but precooking caused no difference in fibre content. Roasted samples had highest crude fat content, crude fibre content, zinc content and calcium content. Soaking gave the highest protein content.

The optimum blending ratio for the formulated composite flour was 7:1:2 (pearl millet flour: pumpkin flesh flour: pumpkin seed flour). The pearl millet germinated for 3 days at a temperature of 30^0C was found to exhibit high quality attributes both nutritionally and sensory and was therefore used for formulation of the blend. Roasted pumpkin seeds were used for the formulation since they exhibited best nutrional attributes. Pumpkin flesh flour was also incorporated in the blend due to its high beta carotene levels so as to acheive the RDI/AI for the target group (infants aged 7-12 months).

5.2 Recommendation

Clinical trials of the product were lacking in this study and are therefore recommended on infants aged 7-12 months as this group's RDA was reffered to in this study.

The formulated composite flour was able to meet the recommended daily intake of protein for infants between the ages of 7-12 months in just 100g serving. Though the recommended daily intake of vitamin A, calcium, fat and carbohydrate contents for infants (7-12 months) is not met in 100g serving it is recommended that the infants receive an adequate serving. Four servings of 100g each are able to meet all the RDA/AI.

Studies with animal source supplementation are also recommended.

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APPEDICES

APPENDIX I: 9-Point Hedonic Scale

Kindly fill the scale below

Degree of liking	Rate	Results
Like Extremely	**9**	
Like Moderately	**8**	
Like Very Much	**7**	
Like Slightly	**6**	
Neither Like nor Dislike	**5**	
Dislike Slightly	**4**	
Dislike Moderately	**3**	
Dislike Very Much	**2**	
Dislike Extremely	**1**	