DEVELOPMENT OF IRON AND ZINC OPTIMIZED COMPOSITE FLOURS USING SELECTED RAW FOOD MATERIALS FROM MALAWI

BY

CHIKONDI MEMORY LIOMBA (A56/87035/2016)

BSc. Nutrition and Food Science (Lilongwe University of Agriculture and Natural Resources)

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Signature………………………………….. Date:..26 SEPTEMBER 2018

Chikondi Memory Liomba

This thesis was submitted for examination with our approval as University Supervisors:

Signature…………………………….. Date:..26/09/2018

Dr. Catherine Kunyanga, PhD

Department of Food Science, Nutrition and Technology, University of Nairobi

Signature…………………………….. Date:..26/09/2018

Dr. Angela Andago, PhD

Department of Food Science, Nutrition and Technology, University of Nairobi

UNIVERSITY OF NAIROBI

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DEDICATION

This thesis is dedicated to my late mother, Maureen Martha Malanda who could not see me going further with my education. Thank you dear mum for the sacrifices you made so that I get quality education. My success in life today is attributed to your moral support, endless love, and your pieces of advice. These gave me strength to reach for the stars and chase my dreams. Though I cannot see you I know you always walk close by me, unseen and unheard and I believe you are proud of the woman I have become. Thank you beautiful mum and may your soul continue resting in peace.

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ABBREVIATIONS AND ACRONYMS

FAO	Food and Agriculture Organization of the United Nations
MDHS	Malawi Demographic and Health Survey
MICS	Micronutrient Indicator Cluster Survey
PEM	Protein Energy Malnutrition
RDA	Recommended Dietary Allowance
SPSS	Statistical Package for the Social Science
UNICEF	United Nations Children Fund
WHO	World Health Organization
CPV	Cereal-pulse-vegetable
AOAC	Association of official analytical chemists
PEM	Protein energy malnutrition
NSO	National Statistical Office
IDA	Iron deficiency Anaemia
ID	Iron deficiency
SCN	Standing nutrition committee
AOAC	Association of official analytical chemist
RDI	Recommended daily intakes
RDA	Recommended dietary allowance
RNI	Recommended nutrient intakes
CF	Complementary foods
MSB	Malawi Bureau of standards
CHO	Carbohydrate

PKd	Pumpkins
MR_w	Raw Maize
PPR_w	Raw pigeon peas
FMR_w	Raw finger millet
MSd	Soaked and dried maize
PPR_s	Roasted pigeon peas
FMG_d	Germinated finger millet

ABSTRACT

Child malnutrition is among the major problems in low income countries such as Malawi. The most common forms of malnutrition are protein-energy malnutrition, vitamin A deficiency, zinc deficiency and iron deficiency anemia and are mostly caused by dependence on monotonous cereal based weaning foods which do not provide enough nutrients for rapid growth and development. This study aimed at improving the contents and bioavailability of iron and zinc of the cereal based complementary porridge currently used in Malawi.

Raw materials used in the formulation were pigeon peas (*Cajanus cajan*), finger millet, pumpkins (fresh and seeds) and maize. These ingredients underwent simple inexpensive household level food processing technologies such soaking, roasting and germination. The control complementary flour was prepared from 100% raw maize flour. Four complementary porridge flours named cereal-pulse-vegetable flours (CPV1, CPV2, CPV3, and CPV4) were developed from blends of the selected ingredient in varying amounts. Sensory evaluation of the developed products was done using 10 untrained panelist to assess the attributes of taste, smell, mouthfeel, colour and general acceptance. The most preferred flour was evaluated for keep quality using accelerated shelf life testing in three different storage containers namely gunny bag, kraft paper and plastic container. Microbial analysis (coliforms and yeast and molds) and chemical parameters (moisture and peroxide value) were monitored every 24 hours. The effect of processing on iron and zinc bioavailability was also analyzed using in vitro enzymatic digestion on CPV1, which was a blend made of unprocessed foods and CPV2 that was developed from processed foods.

The developed flours provided significantly ($P \leq 0.05$) higher amounts of Iron (17.3-21.2 mg/100g), zinc (4.3-5.2 mg/100g), protein (11.9-14.1 g/100g) and energy (387.6-398.6 Kcal) as compared to the control. All the four flour blends developed met over 80% recommended nutrient intakes for energy, protein, iron and zinc. The best flour blend was CPV3 because it provided the highest amount of iron, zinc and protein. Sensory analysis of the porridges formulated flours conducted by untrained caregivers (panelist) showed that CPV2 (70 g finger millet + 15 g roasted pigeon peas + 8 g pumpkins + 7 g soaked maize) was the most preferred composite flour. The shelf evaluation of CPV2 was longest in kraft paper and plastic container which was significantly safe for consumption up to four months. In vitro enzymatic digestions for CPV1 and CPV2 showed that CPV2 had significantly high amount of bioaccessible iron and zinc (7.14 and 1.8 g respectively) against CPV1 which had 2.8 and 1.1 g for iron and zinc respectively. The study recommends germination as the best processing method for cereals because it significantly increased the amount of nutrients of interest. It also recommends the use of roasted legumes for they impart desirable sensory attributes in complementary foods. The use of all these simple food processing methods should be promoted because they have proved to increase the iron and zinc bioavailability.

CHAPTER 1: GENERAL INTRODUCTION

1.1. Background information

In the sub-Saharan region of the tropics, protein deficiency in diets is common and it is usually associated with deficiencies in energy leading to endemic protein malnutrition with its attendant health consequences in children. Despite abundant global food supplied, widespread malnutrition persists in many developing countries. The World Health Organization (WHO) and UNICEF have been concerned about this trend, particularly of Protein Energy Malnutrition (PEM) and micronutrient deficiencies (hidden Hunger) among infants, children and pregnant women. The United Nations' Standing Committee on Nutrition (SCN, 2004) pointed out that malnutrition is directly and indirectly associated with more than 50% of all children mortality, is the contributor to disease in developing world.

Micronutrients play important roles in the immune system functioning, reproduction, growth and hormonal regulation (Hutchinson, 2016). During first years of childhood and other periods of rapid growth and development such as pregnancy and breastfeeding, adequate micronutrient intake is very crucial (UNICEF, 1998). Micronutrient deficiencies are mostly caused by inadequate dietary intake, malabsorption, low bioavailability, excessive losses, or a combined causes (ILSI, 1996).

The United Nations (UN) sustainable development goals have been developed to assist with improving the nutritional status of individuals by 2030. The second sustainable developmental goal (SDG) is to end hunger, achieve food security and improve nutrition, and promote sustainable agriculture. It was agreed that by 2030 all countries end all forms of malnutrition, including achieving by 2025 the internationally agreed targets on stunting and wasting in children under five years of age, and address the nutritional needs of adolescent girls, pregnant and lactating women, and older persons (United Nations Inter Agency and Expert group, 2014). Malawi is very far from achieving these goals. To achieve these goals, children's nutritional status must be improved by both prevention and treatment of micronutrient and macronutrient deficiencies; but there is slow progress towards achieving these goals (MDHS, 2015).   Even though the Malawian government has implemented various strategies to alleviate iron and zinc deficiencies, there are still major health problem.

Globally, the prevalence of anaemia is highest in Africa among preschool children (0-5 years) with the prevalence rate of 64.6% (McLean *et al*., 2009). There is limited information on prevalence of zinc deficiency but it was discovered that where there is persistence of iron deficiency the deficiency of zinc also occurs (Ramakrishnan, 2002). In Malawi, zinc deficiency was identified to be the leading micronutrient deficiency followed by iron deficiency (MDHS, 2016; WHO, 2013b). The research conducted by Malawi demographic and health survey (2016) found that the prevalence of iron and zinc deficiency were still high at 22% and 60% respectively in preschool children. The problem is even worse in peri urban and rural areas.

In first years of life, chronic micronutrient deficiencies like vitamin A, iron and zinc deficiencies may lead to an increased susceptibility to infections, blindness, stunting and impaired cognitive development (Black *et al*., 2007). These nutritional deficiencies lead to growth faltering, death and may also limit a person from developing to their full potential during childhood. Black and others, (2008) found that among all vitamins and minerals, zinc and vitamin A deficiencies had the largest burdens of disease.

In Africa, most households where iron and zinc deficiencies are prevalent, their source of energy and micronutrients are monotonous cereal based diets (Oniango *et al*., 2003). Such diets generally contain antinutrients such as phytates, tannins in high amounts which reduce the absorption of the already limited bioavailable non heme iron and zinc in the diet (Hun, 2003). Anigo and others (2009) found that legumes increase the nutritive value of cereal based food by improving the protein and mineral content of the diet.

Preparation of legumes however needs a lot of time and requires much labour, and this eventually limits their consumption (Dos Santos *et al*., 2013) .Due to urbanization, there is an increase of women from low social economic and rural areas working outside the home (Tacoli, 2012). Therefore, there is an increased need for development of convenient complimentary foods which are culturally acceptable (Kennedy *et al*., 2004).

Food-based interventions have been identified as the best approach for ending under five malnutrition in developing countries (Campaore *et al*., 2011). Incorporating high zinc and iron foods like pumpkin pulp, its seeds and finger millet respectively is forming a low food-based approach to increase intake of protein, energy, iron and zinc in young children (Hashim and Pongjata, 2000; Berteram and Bortkiewicz, 1995). Dhiman *et al.*, (2009) reported that complementary mix supplemented with pumpkin flour resulted in a nutritious food with highly acceptable sensory qualities. Therefore utilizing a locally produced plant based foods high in energy and rich in iron and zinc in formulating complementary foods could offer an opportunity of ending these deficiencies (Noorfarahzilah *et al.*, 2014).

1.2. Problem Statement

Inadequate complementary feeding both in quantity and quality is a major contributor of the wide spread malnutrition among low social economic status communities in Malawi. A majority of these generally consume a diet made of predominately starchy staples (Faber and Wenhold 2007; Gibson and Mtimuni 2004). These foods have high bulk density and high anti-nutrient content and therefore do not offer adequate nutrient density and bioavailable minerals notably iron, zinc and calcium. Weaning children require high nutrient density complimentary foods. Commercial fortified complementary foods intended for young children are mostly nutritionally adequate in terms of quality and the quantities per serving as they are fortified by adding minerals and vitamins. However, the cost of such products may be generally out of reach for people in developing countries, more especially those in rural communities. With civilization, indigenous processing methods which are affordable and likely to enhance nutrient density and bioavailability have been neglected.

Malawi has legumes and vegetables rich in proteins, iron and zinc. For instance pigeonpeas and pumpkins are an inexpensive protein source and zinc sources respectively but their use for protein and mineral complementarity in weaning foods in Malawi is minimal (Adebooye and Singh, 2007). Pigeon peas are locally produced and at low cost and are rich in glutamic acid, aspartic acid and lysine, but low in sulfur containing amino acids (Hallén *et al.*, 2004).pumpkins are rich sources of zinc, calcium and vitamin A. This makes pigeonpeas an excellent choice to enhance protein quality when combined with cereal grains that have proteins low in lysine but rich in sulfur containing amino acids (Hallén *et al*., 2004).

A strategy to combine cereals with pumpkins and pigeonpeas that have undergone indigenous traditional processing to remove phytates in development of complementary foods will result in adequate nutrient density and high mineral bioavailability.

1.3. Justification for the Study

In Malawi, the strategies to address iron deficiency include food fortification of certain foods during commercial processing and zinc supplementation and promotion of dietary diversity (NSO, 2016). However, fortified foods are not always accessible to individuals who are at risk of malnutrition. WHO and USAID (2011) recommend use of indigenous foodstuffs in complementary feeding. Malawi has diverse indigenous plant foods most of which are nutrient rich. Legumes, cereals and vegetables have high mineral content though with high amount of antinutrients. Appropriate indigenous processing techniques will reduce anti-nutrients thus improving mineral bioavailability. The foodstuffs are culturally acceptable and affordable in most parts of the country and uptake of the developed foods will be high.

1.4. Objectives

1.4.1. Main Objective

To develop complementary food with enhanced protein, energy, iron and zinc from locally available foods of Malawi for children

1.4.2. Specific Objectives

1. To develop multimix composite complementary flour from combinations of pigeon peas, maize, pumpkins and millet.
2. To evaluate the acceptability of the developed flour as consumed
3. To determine the shelf stability of the most preferred formulation
4. To determine the effect of traditional processing methods on in vitro bioavailability of iron and zinc of the most acceptable complementary flour.

1.5. Hypotheses

1. Nutritious complementary food high in iron, zinc, protein and energy cannot be made from maize, millet and pigeon peas and pumpkins.
2. Complementary food developed from maize, pigeon peas, millet and other locally produced ingredients has low consumer acceptability due to the undesirable sensory properties.
3. Complementary food developed from maize, pigeon peas, millet and pumpkins has low shelf life.
4. The developed complementary food will not have high bioavailable iron and zinc

CHAPTER 2: LITERARTURE REVIEW

2.1 Micronutrient malnutrition in Malawi

Micronutrient deficiencies are caused by consumption of undiversified diets which are also of poor quality. Iron, iodine, zinc and vitamin A are micronutrient deficiencies mostly experienced in developing countries (Bain *et al.*, 2013; Smuts *et al.*, 2005b). Some of the effects of micronutrient deficiencies include delayed development and retardation of growth (Bain *et al*, 2013). The MDHS (2015) found that preschool children in Malawi were deficient in vitamin A, calcium, iron and zinc.

Iron is an essential component of hemoglobin and enzymes and is very important for transportation of oxygen and respiration of the cells (Bailey *et al*., 2015). Iron also is critical for optimal growth and cognitive function. Iron deficiency anaemia (IDA) in infancy is associated with significant loss of cognitive abilities, reduced disease resistance and physical activity. In school-age children, IDA is linked to poor performance in school while in adulthood, it can cause fatigue and reduced work capacity (Slingerland *et al*, 2006; Ruel, 2006).Globally, IDA affects 22% of under five children in Malawi in (MDHS, 2016).

The main causative factors of iron deficiency in Malawi and is inadequate dietary intake. Complementary foods for poor communities are mostly plant based such as porridge prepared from maize, and a blend of maize and legumes particularly soy beans and common beans. These are minimally processed and contain high amounts of phytates and polyphenols which bind iron in the gastro intestinal tract and lead to iron malabsorption (Mendoza, 2001).

Zinc is an essential mineral that is involved in several aspects of cellular metabolism (King, 2011). Zinc plays vital role in cell division, synthesis of proteins and growth such that its deficiency places children, pregnant women and adolescents at risk when the intake is low. Zinc contributes to reproduction, growth, taste, night vision, appetite, and the immune system functioning (Mofokeng, 2013).

The main causes of zinc deficiency are inadequate dietary zinc intake, inhibitors of zinc absorption, high zinc losses due to diarrhea or both. Zinc deficiency arises occurs mostly due to limited bioavailability of dietary zinc, mostly attributable to the high phytate content in the diets (Melaku *et al.,* 2005 and WHO, 2000). Zinc deficiency is associated with poor growth, impaired immune function, increased susceptibility and severity infections and poor pregnancy outcome. In most developing countries for instance Malawi, zinc deficiency is mainly caused by low dietary intake of foods from animal sources rich in zinc as well as high intake of plant based foods with highest levels of phytates (Romana *et al.,* 2003).

Good plant sources of zinc are pumpkin seeds, legumes, moringa leaves and seeds, millet and Amaranth seeds. However these contain high amount of anti-nutritional factors which can be reduced by simple traditional food processing techniques including fermentation, germination and roasting.

The MICS (2015) survey showed that 22% of preschool children suffered from iron deficiency (ID), 28% IDA in Malawi. The MICS (2015) also indicated that 60 % of preschool children had Zinc deficiency. Previous surveys conducted by MICS study further indicated that the highest numbers of micronutrient deficiencies were seen in the rural communities of Malawi. This is contrary to recent survey results of 2016 (MDHS) which has revealed the shift in trends. According to MDHS (2016) it showed that prevalence of IDA anaemia was 23.7% in urban areas while in rural area was much lower at 7.7%. Similarly for Zinc deficiency for preschool children in urban areas was 64.7% while that for rural areas was 59.9%. This means that development of high micronutrient rich foods and other nutrition interventions should target both the rural and the urban areas because they could be equally at risk of micronutrient deficiencies.

2.2. Iron and zinc requirements

Recommended daily intake of dietary iron for normal infants are 1 mg iron per kg per day and for children, male and female adolescents are 10, 12 and 15 mg per day respectively, and adult men and postmenopausal women require only 10 mg per day (Martinez-Navarrete *et al.*, 2001). At birth, most term infants have 75 mg of elemental iron per kilogram of body weight, found primarily as hemoglobin (75%), body storage (15%) and tissue protein (10%) (Oski, 1982). Infants of mothers with poorly controlled diabetes and small-for-gestational-age infants have approximately 10% and 40% of normal storage iron, respectively, meaning that they may have less of a buffer for protection from postnatal iron deficiency (Georgieff *et al.*, 1995 and Petry *et al.*, 1992). The magnitude of the decline in zinc concentrations in human milk as lactation progresses is notable relative to the longitudinal changes in the concentrations of other nutrients (Casey *et al.*, 1983). Zinc for the first 6 months is adequate from breast milk alone, but is inadequate for the older infant. In addition to breast milk or infant formula, complementary food sources of zinc, such as meats or fortified infant cereal, help meet an infant's zinc needs after 6 months of age (Institute of Medicine, Food and Nutrition Board 2001). The recommended dietary allowance (RDA) for infants 6-24 months of age is 2.8 mg/day (Dewey and Brown, 2002).

2.3. Strategies employed to combat iron and zinc deficiencies in Malawi

2.3.1. Dietary diversification

Dietary diversity is viewed as a long-term strategy to combat iron and zinc deficiencies in Malawi (Gibson and Mtimuni, 2005). A diversified diet comprises of a variety of foods, such as fruits and vegetables, legumes, starch and animal products. Unfortunately, fruits and animal products are not often consumed by poor communities, due to the high cost of these foods (Faber *et al.,* 2002). Homestead food gardens are becoming popular in Malawi. This trend is as a result of community initiatives and/or the initiatives and support from the government and development partners (FAO, 2014; MoAIW, 2012).

2.3.2. Iron and zinc supplementation

Diarrhea, pneumonia and malaria are the most common causes of death among children in developing countries. Sazawal, (2011) found that zinc supplementation reduced diarrhoea, pneumonia and malaria incidences. These diseases are responsible for high mortality rates in Africa. Supplementation is another strategy that was implemented by the Malawi government through ministry of health in order to alleviate iron and zinc deficiencies. According to the Ministry of health regulations, all infants and under five children are to be supplemented with routine and therapeutic doses of zinc. Iron supplementation is mostly done to routinely primary school going children. In countries with known widespread iron and zinc deficiencies and high levels of stunted children, preventive iron and zinc supplementation has a small but significant positive effect on linear growth (WHO, 2013)

2.4. Challenges of the employed strategies to combat iron and zinc deficiencies

2.4.1. Dietary diversification

A cross-sectional study conducted by Smuts *et al.,* (2008) indicated that 37% of households in Malawi had their own homestead gardens. However, Iron and zinc rich foods and vegetables were only eaten by a few individuals. Iron and zinc status could be improved if a greater quantity of these vegetables were eaten (Gibson and Mtimuni, 2005).

Despite the use of food gardens to alleviate iron and zinc deficiencies, there are many problems limiting their effectiveness. These include inadequate low purchasing power for seeds and pesticides, insects and plant diseases as well as inadequate knowledge on how they can manage the garden (Faber *et al.*, 2013a; Faber *et al.*, 2013b). Dietary diversification can be seen as a long-term goal to alleviate iron and zinc deficiencies. However, this strategy requires significant economic resources and hence not appropriate for poor communities of Malawi. This is the same in almost all other countries in sub Saharan Africa (Faber *et al.*, 2002).

2.4.2. Iron and zinc supplementation

Although zinc and iron supplements are available at clinics and hospitals, many infants hardly access them. This is partly due to poor utilization of, health services by the target population; inadequate training and motivation of frontline health workers; inadequate counselling and low adherence of the of the caregivers (Bhutta *et al.*, 2013). Many poor rural communities rely on home remedies to cure illnesses/sicknesses such that they do not like taking children to clinics unless the sickness becomes critical (Faber and Benadé, 2007).

2.5. Locally produced plant based foods as potential sources of iron and zinc

2.5.1. Cereals and legumes as potential source of iron and zinc

Cereals are the most important source of food for the world population. Cereal products comprise more than 80%, 50%, and between 20-25% of the average diet in India and Africa, central and Western Europe, and USA, respectively. In addition, cereals are the main source of nutrients for weaning children in developing countries (Dicko, 2005; Malleshi *et al.*, 1998). Legume refers to the edible seeds of leguminous plants belonging to the leguminous family and they constitute an important source of dietary protein especially for most people in developing countries (Siegel and Fawcet, 2006). The short fall in the production of animal proteins and wide prevalence of protein malnutrition in developing countries of the world have refocused the importance of legumes as source of protein in human diets, especially in those countries where the consumption of animal protein is limited by its low availability with its consequent high cost, cultural or religious habits (FAO, 2002). Legumes also have low sodium and high potassium contents, more complex carbohydrates and high in fibre and can be made into diverse foods (Sathe, 20066).

2.5.2. Finger millet as potential source of iron

Millet is among the most important drought-resistant crops and the 6th cereal crop in terms of world agriculture production (Yibeltal *et al,* 2016). With appropriate processing and value adding technologies, the millet grains can be used in the preparation of several weaning foods and health food –products (Mal *et al.,* 2010). Finger millet (*Eleusine coracana)* is also known as African millet, is and indigenous crop and is widely grown in Malawi and it has an orange-red colour. It is used in production of local beer and nonalcoholic beverage known as *Thobwa.*

Shimelis and others (2009) found that finger millet was significantly rich in resistant starch, soluble and insoluble dietary fibers, minerals, and antioxidants. It contains 6.7% crude protein, 2.8% ash, 26.6 mg/100g iron and 1.57 mg/100g. Because of its high iron contents as compared to other commonly consumed cereal based crops like maize, finger millet can be used as a cheap source of iron in developing complementary foods.

Table 2. 1: Nutrient composition of millets and other cereals (per 100 grams edible portion: 12% moisture)

Food	Protein	Fat	Ash	Fibre	CHO	Kcal	Cal*	Iron*	Thiamine
Brown rice	7.9	2.7	1.3	1.0	76.0	362	33.0	1.8	0.41
Wheat	11.6	2.9	1.6	2.0	71.0	348	30	3.5	0.41
Maize	9.2	4.6	1.2	2.8	73.0	358	26	2.7	0.38
Sorghum	10.4	3.1	1.6	2.0	70.7	329	25	5.4	0.38
Pearl millet	11.8	4.8	2.2	2.3	67.0	363	42	11.0	0.38
Finger millet	7.7		2.6	3.6	72.6	336	350	3.9	0.42
Foxtail millet	11.2	4.0	3.3	6.7	63.2	351	31	2.8	0.59

*Mg/100 g
CHO= Carbohydrate
Cal= Calcium

Source: Hulse and others (1980); United States National Research Council/National Academy of Sciences (1982); USDA/HNIS

2.5.3. Pumpkins as potential source of Zinc

Pumpkin (*Cucurbita maxima*) is an important traditional plant food of indigenous communities. Pumpkins produce high yield, have high amount of minerals (especially the seeds) and long storage life (Dhiman *et al.,* 2009). Pumpkins are available in Malawi almost throughout the year. Pumpkins are rich in carotene, pectin, minerals, vitamins and dietary fibers and phenolic phytochemicals that are beneficial to health (Kwon *et al.,* 2007; Dhiman *et al.,* 2009). Pumpkin seeds are rich in zinc and oil which also makes them the best ingredient in zinc deficient diets.

One of the processed products of pumpkin fruit is pumpkin flour which can be easily stored for a long time and conveniently used in manufacturing a number of foods. Pumpkin flour could be used to supplement cereal flours in complementary porridge and bakery products to improve nutritional and sensory qualities of such products (Lee *et al.,* 2002; See *et al.,* 2007).

2.6. Iron and zinc bioavailability

Bioavailability is defined as the amount of zinc or iron in a food or a diet that is digested, absorbed and metabolized by normal pathways (Pressmen, 2017). There are predominant factors affecting iron bioavailability and these include amount of dietary heme and non heme iron, dietary factors that influence iron bioavailability and iron status of the individual. In the same way zinc bioavailability is also affected by the presence of anti-nutritional factors in the diet as well as the zinc source whether plant based or animal based (UNICEF, 2010).

2.6.1. Inhibition of mineral bioavailability by antinutrients

Plant-based complementary foods are often characterized by high levels of phytate which is a potent inhibitor of bioavailability of zinc, iron and calcium. Bioavailability of minerals from indigenous cereals and legumes used in preparation of complementary foods is generally low due to the presence of the phytates (Cook *et al.,* 1997; Egli *et al.*, 2002; Davidsson, 2003). Recent research findings on *in vivo* isotope in adults (Egli *et al.,* 2004; Hambige *et al.*, 2004 Mendoza *et al.,* 1998) and infants (Davidsson *et al.,* 2004) have reported an increase in absorption of iron, zinc, and calcium in cereal-based foods prepared with a decreased phytate content.

2.7. Indigenous strategies for reducing antinutritive factors in plant foods

2.7.1. Germination

Germination has been an indigenous technique practiced in most rural areas of Malawi. Though the locals attribute this practice to improved beverage taste from geminated grain, the practice also improves their nutritional value of the foods. During germination, enzymatic activity and bioactive compounds increase within the seed (Dicko, 2006). It was reported that due to degradation of phytic acid, iron and zinc absorption increase(Egli *et al.*, 2004; Davidsson *et al.*, 2004; Sandberg *et al.,* 2000; Hurrell *et al.*, 2000).

Nutritional and functional qualities of millet can be improved through germination by modifying chemical compositions and eliminating antinutrients. Effect of germination and fermentation of millet on proximate, chemical, and sensory properties of instant *fura* (a Nigerian cereal food) was examined. It was discovered that germination was a promising food processing technique for improving the nutrient and energy densities of *fura* and, when combined with fermentation, reduced phytic acid significantly (Inyang and Zakari, 2008).

Germination can reduce dietary bulk and paste viscosity of the gruel (Melaku *et al.,* 2005). It also decreases the levels of condensed tannins content present in cereals which results in the formation of polyphenol complexes with protein and the gradual degradation of oligosaccharides. Such reductions in polyphenols may facilitate mineral and protein absorption (Inyang *et al.*, 2008).

2.7.2 Soaking

Soaking has been an indigenous technique practiced in rural communities of Malawi especially for cereal grains and legumes before cooking (Gibson and Mtimuni, 2005). It said to soften the grain and therefore cooks faster. The practice has other advantages. Soaking cereal in water can result in leaching of water-soluble phytates, which can then be removed by discarding the water (Hortz and Gibson, 2007). The amount of phytates removed depends on length and soaking conditions, species and the PH. Some polyphenols and oxalates that inhibit iron and calcium absorption may also be removed by soaking (Erdman and Pneros-Schneier, 2004).

2.7.3. Roasting

Legumes are sometimes roasted, toasted or heated to improve their nutritional value and taste/acceptability (Salunkhe *et al.,* 2005; Kurien, 2001). Due to the formation of pyrazine compounds in the roasted food, roasting brings nutty, burnt and coffee like aromas in the food. It was reported (Powrie and Nakai, 2001) that the level of pyrazine compounds also related to the extent of browning. Roasting time is very important in enhancing protein efficiency ratio (PER) in legumes, about 15 minutes of roasting at 200 ºC was the optimum time to maintain maximum protein quality considering available lysine.

2.8. Malawian weaning practices and weaning Foods

In developing countries such as Malawi, the foods that are commonly used for weaning are prepared from flours of starchy staples, cereals and legumes, such maize, rice (but rarely) and soya beans. Cereals and legumes are the most common first weaning foods because they are locally produced and are inexpensive (Lalude & Fashakin, 2006). Gruels/thin porridges prepared from cereals and legumes (commonly soy beans for Malawi) play an important role as a weaning food but their nutrient density is often low, especially deficient in essential nutrients (Gibson *et al.,* 2000). Cereals have low content of proteins and fat while legumes are low in fat. The presence of high concentration of crude fiber and absorption inhibitors (antinutritional factors like phytic acid and condensed tannin) are major factors reducing their nutritional benefits (Mariam, 2005; Gibson *et al.,* 2000).

During cooking/reconstitution process of staple-based weaning foods, the starch granules absorb a large amount of water and swell, resulting in high viscosity gruels. More solids cannot be added in the gruel because it becomes too thick, viscous and do not cook well and a small child cannot easily eat the gruel. This high viscosity characteristics of weaning foods referred to as dietary bulk, is responsible for the occurrence of malnutrition in Malawi where cereals and starchy staples are the major foods (Manary *et al.,* 2002).

2.9. Viscosity of weaning foods

During weaning inadequate intake of energy and protein occurs due to traditionally prepared weaning foods, which are bulky in nature (have high viscosity but low energy density) and limit the infant's ability to eat enough (Walker, 2000). Low energy density weaning foods, caused by dietary bulkiness, contributes to PEM. When a weaning food (gruel) contains 100g cereal flour/liter of the gruel, then only 20 grams maybe eaten by an infant at each feeding time. Many infants in the developing world are only fed two or three times daily. So, the maximum quantity of flour eaten would be 60 gram/day. Assuming that the flour average energy content is 14,640kJ/kg and the protein content is approximately 80 g/kg, then an infant eating only gruel would be receiving 878 kJ/day and 4.8 gm protein/day (Walker, 2000).

2.10. Sensory properties of weaning foods

Sensory evaluation is a scientific method used to evoke, measure, analyze and interpret responses to products as perceived through the senses of sight, smell, touch, taste, and hearing (Meilgaard *et al.,* 1991). It is an irreplaceable tool in food industry while interacting with the key sectors in developing new foods, improving existing new foods by adding or removing an ingredient or cost reduction.

In sensory evaluation, judges are asked to rate the products for appearance, color, flavor, taste and overall acceptability using a scorecard of hedonic rating scale. This test relies on people's ability to communicate their feelings of like or dislike. Hedonic testing is popular because it may be used with untrained people as well as with experienced panel members (Bruce, 2010). A minimum amount of verbal ability is necessary for reliable results (Sadana and Chabra, 2004; Meilgaard 2000).

In a study by Muhimbula *et al.,* (2011) on a complimentary porridge made from germinated and spatially roasted maize, millet, sorghum, cowpeas and green peas he found that all formulations were organoleptically accepted using 5 point hedonic scale by untrained and semi trained panelist. Addition of sugar and oil was found to improve the sensory attribute of the formulated foods contributed to their higher acceptability (Muhimbula *et al.*, 2011).

2.11. Gaps in knowledge

This review shows that there is lack of nutritious complementary foods in resource poor settings in Malawi due to inadequate knowledge and over dependency on traditional maize based complementary foods (Dewey, 2005). Cereal based gruels predominantly used in resource constrained settings do not meet nutrition requirements for rapid growth and development (WHO, 1998; Owino *et al.*, 2007).

One of the pillars of optimal infant and young child feeding (IYCF) is provision of safe and nutritionally adequate complementary foods for infants together with exclusive and continued breastfeeding. Improved breastfeeding and complementary feeding are the most effective nutrition interventions in reducing under-five mortality, and play a crucial role in growth, development, long-term health and a nation's economic development and productivity. The development and promotion of complementary food using locally available foods processed using recommended indigenous processing is therefore of paramount importance, considering the nation's high under-5 mortality rate, the double burden of stunting and overweight/obesity, as well as the presence of micronutrient deficiencies in young children, which are attributable in part to poor breastfeeding and complementary feeding practices.

2.12. Study Setting

The study took place in Agriculture Research Trust laboratories (Lilongwe district), Kasungu district of the central region of Malawi and the University of Nairobi Department of Food Science (Kenya). Kasungu district was targeted for sensory analysis of the developed porridge flour.This site was chosen because it is located in an area reported to have high incidence of child malnutrition.The major crops produced in this district include maize, sorghum, millet, groundnut, cowpea, soybean, gram beans, common beans, pigeon peas and sweet potatoes. Some of the produce mentioned was used in the formulation of the porridge flour that was developed. University of Nairobi, Department of Food Science, Nutrition and Technology Laboratories is where bioavailability and shelf life studies were carried out.

2.13. Thesis Layout

The study was organized as 6 chapter, which for purposes of presenting the thesis are organized in 6 chapters as follows:

Chapter 1: General introduction extensively describing the background, the problem that needs to be addressed and the objectives.

Chapter 2: Review of literature for the study

Chapter 3: Simple low cost processing of millet, maize, pigeon peas and pumpkins and formulation of the complementary flours using blends of millet, maize, pigeon peas and pumpkin flour.

Chapter 4: Sensory evaluation of the developed products (CPV1, CPV2, CPV3 and CPV4) by caregivers and the shelf stability of the most preferred formulation.

Chapter 5: Analysis of bioavailability of iron and zinc in CPV2 and the control using in-vitro methods.

Chapter 6: General conclusion and recommendations.

CHAPTER 3: OPTIMIZATION AND DEVELOPMENT OF A COMPLEMENTARY FLOUR WITH HIGH NUTRIENT DENSITY

3.1. ABSTRACT

Child malnutrition is one of the biggest problems affecting about 195 million under five children in low income countries, such as Malawi. Complementary foods are generally cereal based and do not meet the nutrient requirement recommended by the World Health Organization. This study aimed at improving iron and zinc contents of the usual traditional maize-based complementary flour by utilizing high energy, iron and zinc locally available raw foods (Finger millet, pigeon peas, pumpkin fresh and seeds). The raw materials were divided into two potions of which one was processed other portion was unprocessed. Pigeon peas were roasted on varying times for 15 minutes and 40 minutes at 160 ^{0}C, finger millet was germinated for 48 hours and 72 hours at room temperature and maize was soaked for 24 hours at room temperature. Nutritional analysis showed that pumpkins had significantly high amount of iron (68 mg/100g) and energy (460.03 Kcal), zinc and protein were significantly high in roasted pigeon peas roasted at 160 ^{0}C for 40 minutes (7.25 mg/100g for zinc and 21.25 g/100g for protein). Four blended complementary porridge flours named cereal-pulse-vegetable (CPV1, CPV2, CPV3, CPV4) guided by linear programming of Nutrisurvey and they met iron, zinc, protein, and energy recommended nutrient requirements for infants. However formulation CPV3 was the best for it contained the highest amount of protein, iron and zinc which are nutrients of interest in this study. Therefore based on these results it can be concluded that complementary flour made from maize alone can be improved by incorporating millet, pigeon peas and pumpkins since they have shown to improve the amount of zinc, iron and protein which may in turn help reduce protein energy malnutrition and iron and zinc deficiencies.

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3.2. INTRODUCTION

In developing countries malnutrition is widely prevalent and begins during infancy (UNICEF/WHO, 2012). This is typically due to low food intake, poor dietary quality, low bioavailability and frequent infections. Micronutrient malnutrition also known as hidden hunger can affect health outcomes such as child survival, growth and development (SCN, 2004).

Iron, Vitamin A, Iodine and zinc deficiencies are among major nutritional concerns in lower income countries. In infancy iron deficiency anaemia (IDA) is associated with significant loss of cognitive abilities, reduced disease resistance and physical activity. On the other hand Zinc deficiency is associated with poor growth, impaired immune function, increased susceptibility and severity to infections and poor pregnancy outcome.

Among the deficiencies caused by lack of vitamins and minerals examined globally, the largest disease burdens were attributed to vitamin A and zinc deficiencies. Worldwide, zinc deficiency is responsible for approximately 16% of lower respiratory tract infections, 18% of malaria and 10% of diarrhoea disease (WHO, 2017). The highest attributable fractions for lower respiratory tract infection, malaria and diarrhoea (18-22%) occurred in WHO AFR-E regions in which Malawi is in the list (WHO, 2017). In total, 1.4% (0.8 million) of deaths worldwide were attributable to zinc deficiency. Micro indicator cluster survey, (2018) reported that 60% of preschool children in Malawi had zinc deficiency.

Iron deficiency is also one of the most prevalent nutrient deficiencies in the world, affecting an estimated two billion people (WHO, 2016). Evidence has indicated that iron deficiency anaemia in early childhood reduces intelligence in mid-childhood. In its most severe form, this will cause mild mental retardation. In total, 0.8 million (1.5%) of deaths worldwide are attributable to iron deficiency (Lancet, 2017). A report by the Malawi demographic and health survey (2016) indicated that 22% of preschool children had Iron deficiency and 28% had iron deficiency anaemia.

Food-based approaches have been recognized as one of the ways of combating these nutritional problems although some other forms of intervention could serve as a complement (Lucretia *et al.*, 2017). The introduction of high bioavailable iron and zinc rich foods like meat, fish, poultry and eggs in the diet of infants and young children is late and only few consume them due to cost implication. At the age 6-23 months, only one in ten children consume meat, fish, shellfish, poultry or eggs (Gibson *et al.*, 2005).

The three most common strategies for combating Iron and zinc deficiencies are distribution of iron and zinc supplements, food fortification, and food-based approaches that aim to increase access to and intake of iron and zinc rich foods (Underwood, 2004). Among these strategies, dietary diversification/modification may be more sustainable, economically feasible, and culturally acceptable than supplementation or fortification and can be used to alleviate several micronutrient deficiencies simultaneously without risk of antagonistic interactions (Gibson and Hotz, 2001).

Pumpkins (*Curcubita maxima*), millet and pigeon peas have emerged as some of the most promising plant sources of zinc and iron. They are also cheaper and a complementary source of iron and zinc to the rural and urban poor families (Dhiman *et al.*, 2009). Studies conducted in other countries have given evidence that pumpkins could play an imperative role in mitigating the impacts of iron and zinc deficiencies in populations where the problem is considered to be a significant public health importance (See *et al.*, 2007).

However, though nutritious, millet and most cereals are often characterized by high levels of anti-nutrients such as phytates and oxalates which are potent inhibitors of bioavailability of zinc, iron and calcium (Soetan and Oyewole, 2009). Soaking, germination and roasting are important household level food processing methods that have been used to eliminate antinutritional factors in food (Olanipekun *et al.,* 2015). Germination and soaking have been indigenous technique practiced in most rural areas of Malawi. The locals attribute germination to improved beverage taste from geminated grain. Soaking is mostly done on cereal grains and legumes and the locals practice it because it softens the legumes and grains thereby reducing cooking time and saves fuel and labour (Olanipekun *et al.,* 2015). Soaking also helps in reducing phytic acid in cereals because phytic acid is water soluble and it leaches out in the soaking water (Paragya *et al.*, 2012; Manary *et al.*, 2004).

Millet and pumpkins have not been incorporated in the standard complementary feeding recipes recommended for children 6-23 months old recommended by the Ministry of Health of Malawi. The present study was, therefore, one of the first of its kind in assessing the utilization of millet and pumpkins in formulating high iron and zinc plant based complementary flour for Malawian infants and young children.

3.3. MATERIALS AND METHODS

3.3.1. Study Setting

The study took place in Agriculture Research Trust laboratories in Lilongwe district. This is where processing of ingredients, proximate composition and formulation of the products was done. Kasungu district local market was where the raw ingredients were sourced. The major crops produced in this district include maize, sorghum, finger millet, groundnut, cowpea, soybean, gram beans, common beans, pigeon peas, pumpkins and sweet potatoes. Some of the produce mentioned was used in the formulation of the porridge flour that was developed.

3.3.2. Ingredients used for processing of complimentary foods

Maize *(Zea mays*), finger millet *(Eleusine coracana),* pigeon peas *(Cajanus Cajan)* and pumpkins *(Cucurbita maxima)* were purchased from the local market in Kasungu district in Malawi. These raw materials are locally produced, accessible and are inexpensive.

3.3.3. Experimental Design

The experimental design of this study is shown in Table 3.1. This was a 2x3 factorial in completely randomized design. There were a total of 6 treatments. Soaking and germination was done as described by Okorie (2016), roasting as described by Sefa (2009).

Table 3. 1: Experimental design

Roasting	**Germination (Room Temperature)**	**Soaking (Room Temperature)**	**Drying (<8% moisture)**
1. Pigeon peas $_{40\ min/160}$ ^{0}C	Millet $_{48\ hours}$	Maize $_{24\ hours}$	Pumpkins
2. Pigeon peas $_{15\ min/160}$ ^{0}C	Millet $_{72\ hours}$	Maize $_{24\ hours}$	Pumpkins
3. Pigeon peas $_{40\ min/160}$ ^{0}C	Millet $_{72\ hours}$	Maize $_{24\ hours}$	Pumpkins

* Roasting at 160 ^{0} C for 15 minutes and 40 minutes have an effect on antinutritional factors and the protein quality
* Germination time influences the amount of nutrients and antinutrients

3.3.4. Product formulation

The ingredients were divided into two portions. The first portions of maize grains, finger millet and pigeon peas were not subjected to various forms of processing treatment apart from milling and were used as control. The second portions underwent various processing methods such as roasting, germination and soaking (Figure 3.1). Drying was done using cabinet solar dryer.

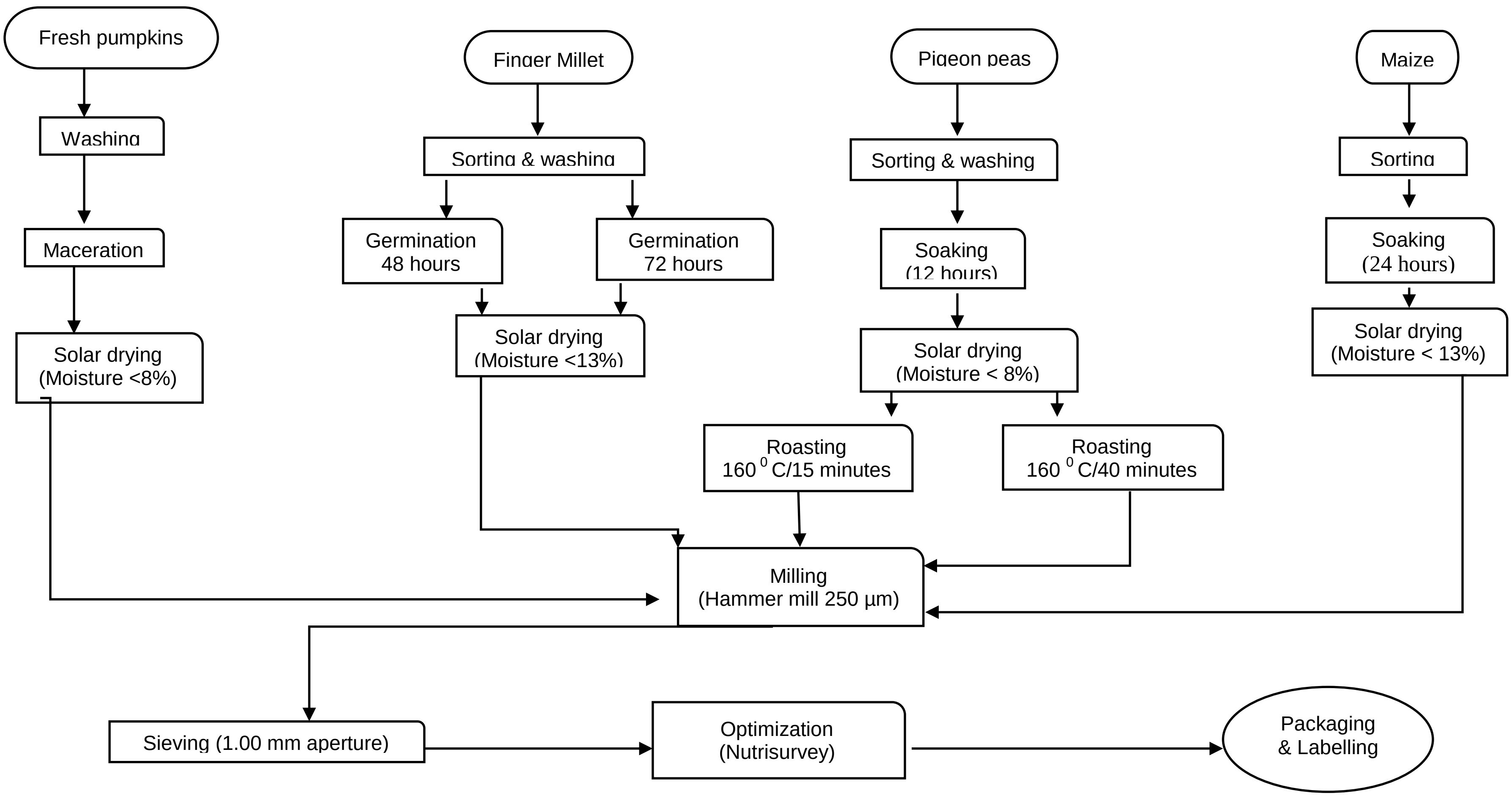

Figure 3. 1: Flowchart diagram for preparation of multimix complementary flour

3.2a **3.2b** **3.2c** **3.2d**

Figure 3. 2: Complementary flour ingredients used in production of the composite flours.

*3.2a = Pumpkin flour, 3.2 b= finger millet flour, 3.2 c= Maize flour, 2.2 d= Pigeon peas flour

3.3.5. Optimization and product formulation

Maize, millet, pigeon peas and pumpkins blends in optimized proportions formed the basis of the formulations of complementary foods. Nutrient content of each ingredient obtained from proximate assessment results (see Tables 3.2 and 3.3) was entered in Nutrisurvey (2010 version) to modify the food data base to suit the composition of Malawian foods. To optimize iron, zinc, protein and energy contents of the flour to be formulated linear programming of Nutrisurvey (2010 version) was used. The proportions of maize flour, millet flour, pigeon peas flour and pumpkin flour were determined on the basis of the recommended nutrient intakes (RNI) for iron, zinc, energy and protein for infants 6-23 months old (WHO/FAO 2004). Five foods, Control (traditional maize flour) were formulated (refer to table 3.6).

3.3.6. Chemical analysis

Pumpkin flour, unprocessed finger millet flour (control), germinated finger millet flour, unprocessed pigeon peas (control), roasted pigeon peas flour, soaked (processed) maize and unprocessed maize flour (control) were analyzed for proximate composition, zinc and iron contents in triplicates using the standard methods.

3.3.6.1. Determination of moisture

The moisture content of the samples was measured according to the AOAC Official Method 934.01 (AOAC 2003).

3.3.6.2. Determination of crude protein

The protein content was determined according to Kjeldahl procedure according to the AOAC official method 990.03 (AOAC 2003).

3.3.6.3. Determination of crude Fat

The fat content of the samples was determined according to the Soxhlet procedure, using a Büchi 810 Soxhlet Fat extractor (Büchi, Flawil, Switzerland) according to the AOAC Official Method 920.39 (AOAC 2003).

3.3.6.4. Determination of crude rude fiber

Fibre was determined as described by AOAC official methods 985.29 (2003).

3.3.6.5. Determination of Total ash (Mineral Content)

The total mineral content of the samples was determined as ash according to the AOAC Official method 942.05 (AOAC 2003).

3.3.6.6. Determination of Carbohydrates

Total carbohydrate was determined by difference according to AOAC official method 931.02c (AOC, 2003).

3.3.6.7. Iron (Fe) and Zinc determination

Using the Association of Analytical Chemist method (AOAC, 2003), Iron and zinc was determined. Iron and zinc were estimated using the atomic absorption spectrophotometer (Varian AA 20 Leicestershire, England).

3.4. Data Analysis

Data was analyzed using Statistical Package for Social Sciences (IBM SPSS 25 statistics).The mean scores were analyzed using analysis of variance (ANOVA) method and difference separated using Dunnet test. A p-value of ≤ 0.05 was regarded as being statistically significant.

3.5. RESULTS AND DISCUSSION

3.5.1 Proximate composition of the food ingredients

The moisture content of finger millet was significantly ($P < 0.05$) higher (10.84 %) than that of all raw materials (Table 3.2). Isingoma and others found similar results with moisture content of finger millet at 10.44 %. There was no significant difference in moisture content for maize, pigeon peas and pumpkin flour. The moisture content for maize (7.43%) agrees with that of (Jemberu *et al.*, 2016) who found that the moisture content of maize flour that was used during the formulation of maize based complementary food was 7.82%. Low moisture content in complementary foods is important to prevent nutrient losses and to ensure prolonged shelf life of the product (Amankwuah *et al.*, 2009). This implies that households may prepare these flours in the quantities that can be easily consumed over a period of time since they will be less susceptible to microbial spoilage due to low moisture levels. In this way it will ensure safety and prevent the child from diarrhoea and other infections.

The carbohydrate content of the raw materials were 78.32 g/100g for maize, 76.2 g/100g for finger millet, 72.92 g/100g for pigeon peas and 38.6 g/100g for pumpkins (Table 3.2). The amount of carbohydrate was significantly higher in maize and finger millet as compared to pigeon peas and pumpkins. Results on carbohydrate content for maize compare closely with findings by Govender (2015). The high amount of carbohydrate found in maize flour gives it an upper hand to improve the carbohydrate content of complementary foods for children. Ogbe and Affiku (2010) reported 6.0 mg of zinc and 1440 kcal energy against 2.1 mg zinc and 382.7 kcal energy observed in the present study. Muhimbula *et al.*, (2011) also reported protein content of 31.62-35.59 g and iron content of 20.34-33.68 mg which are much higher than what was found in this study. This difference cannot be explained and may need further research

Table 3. 2: Nutrient composition of raw maize, pigeon peas, finger millet and pumpkin fresh and seed

Samples*	Moisture	CHO	Protein	Fat	Ash	Fibre	Energy
MRw	7.43 ± 0.13^{a}	78.32 ± 0.23^{a}	9.34 ± 0.18^{a}	3.56 ± 0.06^{a}	1.35 ± 0.01^{a}	1.00 ± 0.00^{ab}	382.70 ± 1.07^{a}
PPRw	7.56 ± 0.17^{a}	72.92 ± 0.29^{b}	14.69 ± 1.43^{b}	0.95 ± 0.36^{b}	3.90 ± 0.02^{b}	1.52 ± 0.28^{b}	359.0 ± 1.00^{b}
FMRw	10.84 ± 2.23^{b}	76.20 ± 1.65^{a}	8.13 ± 0.18^{a}	3.87 ± 0.02^{a}	0.96 ± 0.56^{a}	0.54 ± 0.10^{a}	372.15 ± 7.02^{a}
PKd	7.14 ± 0.05^{a}	38.59 ± 0.45^{c}	17.40 ± 0.38^{c}	26.23 ± 0.052^{c}	10.64 ± 0.05^{c}	3.50 ± 0.21^{c}	460.03 ± 0.17^{c}

**Values are mean (n=3) ± standard error on dry weight basis, values with different superscript in the same row significantly different (Dunnet test, P= 0.05)*

MR_w= Raw maize, PPRw= Raw pigeon peas, FMRw= Raw finger millet, PKd= Dried pumpkin flour, CHO= Carbohydrate

The values of crude protein were found to be 9.34 g/100g for maize, 14.69 g/100g for pigeon peas, 8.13 g/100g for finger millet and 17.4 g/100g for pumpkins (table 3.2). Pumpkins and pigeon peas had significantly high amount of protein and they met over 100% of the recommended protein intake (10.9 g) for children 6-24 months.

Anju and others (2009) reported that seeds of pumpkin representing 3.1% of total pumpkin fruit weight are rich in protein (33 g/100g). These values are slightly higher than those of the present study and the reason may be attributable to varietal differences and that in this study the whole pumpkin (both fresh and seed) flour was used which means the high amount of protein in seed was diluted by the low protein content of the fresh there by lowering the protein content. Analysis of ‘Lady Godiva’ variety indicated that <400 g of pumpkin seeds could supply the total daily protein and mineral requirements other than Ca and Na for an adult person (Sharma, 2009).

Nutritionally, protein from pigeon peas is an excellent complement to lysine-limited cereal protein, as such it justifies the use of pigeon peas flour as an economical protein supplement in cereal based complementary foods (Fasoyiro *et al.,* 2010). The protein content of pigeon peas in this study is within the reported range of 5.6-12.7 g/100g by Paragya and Raghuvanshi, (2012). Pigeon pea is a high protein legume and incorporation of pigeon peas flour in infant foods has a greater potential of reducing PEM in the in developing countries (Hegstad, 2008).

Though maize provided more protein of 9.34 g/100 g, white maize is known to have low levels of the essential amino acids lysine, methionine and tryptophan (FAO, 1992). A study conducted by Scott *et al.,* (2006) found that maize contain limiting amounts of the essential amino acids lysine and tryptophan. Pillay and others (2013) found that white maize had lower levels of the essential amino acids histidine and lysine. In the present study, the amino acid profiles of the raw materials were not determined and the protein quality of these raw materials is thus not known.

In Malawi, protein-energy malnutrition is a major problem among under five children and is responsible for a high percentage of deaths. The vicious cycle of malnutrition is endemic, due to poverty, food insecurity and diseases. The higher protein content of pigeon peas and pumpkins, relative to white maize and finger millet, found in this study suggests that pumpkins and pigeon peas could be used to improve the nutritional status of infants in Malawi and other developing countries. Apart from the protein content of the pigeon peas and pumpkins, however, it is important to assess its protein quality of these two. Crude fibre content, which represents the amount of indigestible sugar falls within the acceptable range of 0.5 to < 5 g/100g (Akubor, 2007) amongst all raw materials. The importance of fibre in the diet of humans cannot be over emphasized. Fibre assists in maintaining gastro intestinal health and also prevents cancer of the colon (Amadou, *et al*, 2013).

Whole Pumpkin flour (PKd) had a significantly ($P \leq 0.05$) high amount of energy (460.03 Kcal) than the rest of the ingredients (Table 3.2). The high amount of fat present in pumpkins (26.23 g/100g) was the reason why pumpkins had the high amount of energy because fat contains double as much kilocalories (9 kcal per gram) compared to protein and carbohydrate (Compaore *et al*, 2011). In developing countries, malnutrition is common during the weaning period and one of the reason is the low energy density weaning foods (Gibson, 2005). Hence infants need more amount of foods. Energy density plays a critical role in energy intake. The energy intake/kg body weight is maintained by increasing the energy density of diet and not by an increase in intake volume (WHO/UNICEF 2009). Feeding energy dense food provides children with required energy for proper growth and development and also daily requirements will be fulfilled in fewer meals. Therefore this justifies the importance of using pumpkins in formulation of high energy density complementary foods.

3.5.2 Iron and Zinc

The Iron content for all ingredients is shown in Table 3.3. Pumpkins had a significantly ($P \leq 0.05$) high amount of iron (68.32 mg/100g) and zinc (6.11 mg/100g) as compared to pigeon peas, maize and finger millet. Among the cereals finger millet had a significantly ($P \leq 0.05$) high amount of iron (7.6 mg/100g) than maize which had the lowest amount of iron (2.01 mg/100g) among all ingredients. Pigeon peas also provided significant high amount of zinc (4.31 mg/100g) compared to maize and pigeon peas which had 3.81 mg/100g and 3.49 mg/100g. These study results are very close to those of Bachar (2013) who reported iron content of 9.9 mg/100g in finger millet and iron content. The low amount of iron, 2.01 mg/100 and zinc, 3.81 mg/100 in maize provides evidence on why communities that depend on maize alone as a complementary food for children have high micronutrient deficiencies.

Table 3. 3: Micronutrient contents of raw maize, pigeon peas, finger millet and pumpkin fresh and seed

Samples*	Iron (mg/100g)	Zinc (mg/100g)	Ca (mg/100g)	Vit A (µg/100g)	Vit C (mg/100g)
MRw	2.01±0.07^{a}	3.81±0.10^{a}	14.9^{a}	0.04^{a}	0.04^{a}
PPRw	3.45±0.3^{b}	4.31±0.05^{b}	168.02^{b}	27.8^{b}	0.01^{a}
FMRw	7.60±0.73^{c}	3.49±0.05^{a}	289.7^{c}	0.0^{a}	0.02^{a}
PKd	68.32±0.45^{d}	6.11±0.18^{c}	37.78^{d}	397^{c}	14.5^{b}

**Values are mean (n=3) ± standard error on dry weight basis, values with different superscript in the same row significantly different (Dunnet test, P= 0.05)*

MR_w= Raw maize, PPRw= Raw pigeon peas, FMRw= Raw finger millet, PKd= Dried pumpkin flour

3.5.2. Nutrient composition of the processed raw materials used in the formulation

3.5.2.1 Iron and Zinc

The total iron and zinc contents of the raw materials increased with processing time as shown in Table 3.5. The amount of iron for millet germinated for 72 hours was significantly ($P \leq 0.05$) higher (13.57 mg/100g) than that of finger millet germinated for 48 hours (11.57 mg/100g). These results are in agreement with Inyang and Zakari (Inyanga, 2008) who reported that the amount of iron increased from 3.3 mg/100 g (ungerminated) to 4.5 mg/100 g (germinated). This indicates that germinating millet for a longer period than 48 hours increases iron content in the millet. Therefore, germination could be an appropriate food- based strategy to maximally derive iron and other minerals from food grains (Platel *et al.*, 2010). The high iron content observed in finger millet could help address the iron deficiencies that are common among infants and preschool children in Kasungu and the whole Malawi. On the other hand, roasted pigeon peas roasted provided highest amount of zinc compared to all ingredients in this study and the values were 6.74 and 7.5 mg/100g for $PPRs_{15}$ and $PPRs_{40}$ respectively. Therefore roasted pigeon peas can also be used to complement low zinc content of cereal flours such as maize in production of complementary flour for weaning children.

Table 3. 4: Micronutrient contents of processed maize, pigeon peas and finger millet

Samples	Iron (mg/100g)	Zinc (mg/100g)	Ca (mg/100g)	Vit A (µg/100g)	Vit C (mg/100g)
MSd	2.85±0.14^{a}	3.34±0.11^{a}	9.1^{a}	0.03^{a}	0.0^{a}
PPRs15	6.02±1.10^{b}	6.74±2.13^{b}	171.4^{b}	26.9^{b}	0.0^{a}
PPRs40	7.25±2.37^{b}	7.5±2.75^{b}	169.01^{b}	27.03^{b}	0.0^{a}
FMGd48	11.57±0.24^{c}	3.10±0.05^{a}	264.2^{d}	0.0^{a}	0.0^{a}
FMGd72	13.57±0.49^{d}	4.11±0.17^{c}	257.3^{e}	0.0^{a}	0.0^{a}

Values are mean (n=3) ± standard error on dry weight basis, values with different superscript in the same row significantly different (Dunnet test, P= 0.05)

MSd= Soaked maize, PPRs15= pigeon peas roasted for 15min/160 ^{0}C, PPRs= Pigeon peas roasted for 40 min/160 ^{0}C, FMGd48= finger millet germinated for 48 hours, FMGD72= Finger millet germinated for 72 hours

3.5.2.2 Proximate composition and energy content

The total amount of gross energy was significantly (*P*≤0.05) different among all processed ingredients with pigeon peas roasted for 40 minutes having the highest amount (402.13 kcal). The amount of energy increased with processing time for roasting due to an increase in fat content (2.27-4.17 g/100g) while for germinated finger millet the fat content decreased with processing time (3.81-2.67 kcal). The higher the amount of fat in a product the higher the energy because fat provides 9 kcal/g and this should have contributed to the highest amount of energy to the pigeon peas roasted for 40 minutes which had highest amount of fat among the processed ingredients. On the contrary, the amount of crude fat decreased with germination time. The fat content in millet germinated for 48 hours was significantly higher (*P≤0.05*) at 3.81 g/ 100 g as compared to 2.67 g/ 100 g for millet germinated for 72 hours. Chaudhary and Vyas (2011) also noted similar results on crude fat concentration during a study on the effect of germination time on finger millet on nutrient composition. They found that finger millet germinated for 24 hours had a high amount of crude fat (2.03 g/100 g) while that germinated for 48 hours had a low amount (1.23 g/ 100 g) of crude fat and this difference was significant (*P≤0.05*). Several researches have attributed the reduction in fat content during germination to total solid loss during soaking prior to germination or use of fat as an energy source in germination process as the major source of carbon for seed growth. However, the amount of fat observed in all processed low materials in this study was very therefore these ingredients alone cannot be used as a good source of fat and energy when formulating infant foods.

Table 3. 5: Proximate composition and energy of processed maize, pigeon peas and finger millet

Samples	Moisture	CHO	Protein	Fat	Ash	Fibre	Energy
MSd	4.94 ± 0.12^{a}	82.00 ± 0.15^{a}	8.65 ± 0.10^{a}	3.39 ± 0.08^{a}	1.02 ± 0.01^{a}	1.05 ± 0.046^{a}	393.11 ± 0.86^{a}
PPRs15	5.07 ± 0.06^{a}	68.17 ± 1.34^{b}	20.62 ± 0.32^{b}	2.67 ± 0.07^{a}	3.47 ± 0.06^{b}	1.16 ± 0.10^{a}	379.20 ± 1.84^{b}
PPRs40	1.38 ± 0.11^{b}	69.90 ± 0.57^{b}	21.25 ± 0.18^{b}	4.17 ± 0.43^{b}	3.3 ± 0.10^{b}	0.42 ± 0.04^{b}	402.13 ± 2.02^{c}
FMGd48	10.86 ± 1.83^{c}	74.34 ± 0.97^{c}	8.75 ± 0.18^{a}	3.81 ± 0.03^{a}	2.24 ± 0.14^{a}	1.16 ± 0.10^{a}	366.65 ± 3.94^{d}
FMGd72	11.76 ± 1.88^{c}	74.09 ± 1.75^{c}	8.96 ± 0.21^{a}	2.67 ± 0.82^{b}	2.52 ± 0.09^{ab}	1.59 ± 0.03^{a}	356.23 ± 8.17^{e}

Values are mean (n=3) ± standard error on dry weight basis, values with different superscript in the same row significantly different (Dunnet test, P= 0.05)
MSd= Soaked maize, PPRs15= pigeon peas roasted for 15min/160 ^{0}C, PPRs= Pigeon peas roasted for 40 min/160 ^{0}C, FMGd48= finger millet germinated for 48 hours, FMGD72= Finger millet germinated for 72 hours, CHO= Carbohydrate

The roasted pigeon peas contained significantly high amounts of protein than germinated finger millet and soaked maize because pigeon peas belong to leguminous family that naturally contain high amounts of protein than cereals. The protein values of the flour from roasted pigeon peas flour were slightly different but the difference was not significant ($P \leq 0.05$). Protein content of pigeon peas roasted for 15 minutes was 20.62 g/100g while that of pigeon peas roasted for 40 minutes was 21.25 g/100g. The slight increase in the protein value of the processed pigeon peas may be due to break down of crude protein to amino acids during processing, Oboh (UNICEF 2012). The high values obtained for protein in this research (20.62% and 21.25%) indicates that pigeon peas is a good source of protein and compares favorably with values obtained for other legumes like cowpea (WHO 2007) and kidney beans (FAO/WHO 2004). It has been reported that when food is subjected to roasting, the activity of proteolytic enzymes is increased (Chaudhary *et al.*, 2014). The higher protein value obtained for the roasted sample is due to the increased activity of proteolytic enzymes, which hydrolyzed inherent proteins to their constituent amino acids and peptides.

In the present study, maize flour that had been soaked for 24 hours (MSRs) (Table 3.4) had slightly lower crude protein (8.65 g/100g) as compared to raw unprocessed maize (Table 3.2). These were higher than those indicated in available literature for the maize flour, which was 7.7g/100g (Dewey and Brown, 2003). The difference could be attributable to variety differences and geographical variation. The protein content of white maize is generally low (Johnson, 2000). White maize is known to have low levels of the essential amino acids lysine, methionine and tryptophan (Dewey and Brown, 2003). A study by Scott *et al.*, (2006) found that lower density maize plants contain limiting amounts of the essential amino acids lysine and tryptophan. These limiting amino acids are high in pulses and legumes such as pigeon peas therefore this gives a basis of complementing maize flour with pulses and legumes.

3.5.3. Nutrient contents of optimized cereal–pulse-vegetable blends using linear programming model

Table 3.6 shows nutrient contents of optimized cereal–pulse-vegetable composite flours. It was found that it is possible to achieve optimal complementary foods that meet energy, protein, iron and zinc requirements through blending different local ingredients to make one product. In this study, improvements in energy balance was achieved by the addition of whole pumpkin flour (seed and fresh) which had the highest amount of fat (26.23%) than the rest of ingredients, while improvements in protein quantity were achieved by the addition of pigeon peas which had the highest amount of protein of (14.69-21.25%).

Optimization of macronutrients (Fat, protein, carbohydrate, energy) and micronutrients (iron and zinc) ratios was achieved in all the cereal-pulse-vegetable combinations of the developed flours. Each flour blend met over 80% of the RDA for iron, zinc, protein, carbohydrate and energy and they costed less than 1 $ per day as calculated in the linear programming (Table 3.7).

Improvement of dietary protein quality through the introduction of higher-quality protein varieties of maize has contributed to positive effects on growth in children in Africa, Asia, and Latin America (Amuna *et al.*, 2000).

A diet that is moderately deficient in energy (5% below requirement) could increase protein needs by 10% (NSO, 2016). Therefore, a complementary food blend that helps a child meet her daily energy requirements and has additional lysine will improve utilizable protein to meet increased needs. The macronutrient balance achieved in the optimized food blends in this study may be important in the prevention of wasting and underweight among children.

Table 3. 6: Nutrient contents (g/100 g) of optimized cereal–pulse-vegetable composite flours

Formulation	Protein	CHO	Energy	Fat	DF	Zinc (mg/100g)	Iron (mg/100g)	Cal (mg/100g)	Vit A (µg/100g)	Vit C (mg/100g)
CPV1	12.8	67.1	391.8	8.0	2.1	4.5	20.5	19.0	640	25.6
CPV2	11.9	70.2	392.6	7.1	1.8	4.3	17.3	21.5	267.6	17.6
CPV3	14.1	66	387.6	7.5	1.8	5.2	21.2	20.1	841.4	47.6
CPV4	12.4	69.5	398.6	7.9	1.5	4.7	19.3	20.8	793.2	46.8

Values are mean (n=3) ± standard error on dry weight basis, values with different superscript in the same row significantly different (Dunnet test, P= 0.05)

CPV1= unprocessed finger millet, pigeon peas, pumpkin flour and maize (70:10:10:5)

CPV2 = Germinated finger millet (48 hours), roasted pigeon peas (40 minutes), pumpkin flour and soaked maize (70:15:8:7)

CPV3 = Germinated finger millet (72 hours), roasted pigeon peas (15 minutes), pumpkin flour and soaked maize (50:20:20:10)

CPV4 = soaked maize Germinated finger millet (72 hours), pumpkin flour and roasted pigeon peas (15 minutes), (60:15:15:10)

DF= Dietary fibre, CHO= Carbohydrate

3.5.4. Expected nutrient intake from cereal-pulse-vegetable porridges compared to maize porridge (100%) and WHO guidelines

Table 3.7 shows the number of servings, amount per serving, amount of solids in the serving, cost of the porridges per day and the % recommended nutrient intake (RNI) expected to be met by the porridges made from these flour formulations. After adjusting for expected number of porridge servings per day basing on WHO guidelines for infant feeding, traditional maize porridges (control) and all formulations (CPV1, CPV2, CPV3, CPV4) met the RNI for providing energy for children aged 7-24 months. Foods for infants should be energy dense because of the limited gastric capacity in young children coupled with the need for increased nutrient intake (Isingoma *et al,* 2015). The formulated cereal-pulse-vegetable porridges if integrated in the feeding practices of local communities and combined with nutritional education could address children's energy needs (Gibson, 2005).

However, for the maize porridge there was a very huge gap for iron for it only catered for less 30% of the RNI for all the age groups of infants. MDHS (2016) reported that among children aged 6-24 months Kasungu district, central region of Malawi plain maize porridge and sometimes maize-soy porridges were the main complementary foods and only 5.3% of under five children were fed with minimum acceptable diet. Prolonged consumption of maize porridge by infants and young children as their primary food puts them at high risk for developing ID and IDA (Hotz and Gibson, 2007). This could be the reason why iron deficiency anaemia prevalence rate is still high in Kasungu at 60% among under five children (NSO, 2016).

Table 3.7: Nutrient intake from cereal-pulse-vegetable porridges compared to the control and WHO guidelines

Variable	7-8 months	9-11 months	12-23 months
Serving			
Number of servings	1	1	1
Amount of serving	150 ml	200 ml	300 ml
Amount of solids in porridge	75 g	100 g	150 g
Cost per day (MWK)			
Maize porridge (control)	11.25	15.0	22.5
CPV1	35.8	47.7	71.55
CPV2	20.33	27.1	40.65
CPV3	22.77	30.3	45.45
CPV4	28.65	37.4	56.1
Energy (RNI)	**200 Kcal**	**300 Kcal**	**500 Kcal**
Maize porridge (control)	287.025 (144)	382.7 (128)	574.05 (115)
CPV1	264.15 (132)	353.2 (118)	528.3 (106)
CPV2	293.85 (147)	352.2 (117)	528.3 (106)
CPV3	294.45 (147)	392.6 (131)	588.9 (118)
CPV4	290.7 (145)	387.6 (129)	581.4 (116)
Protein (RNI)	**9.1 g**	**9.6 g**	**10.9 g**
Maize porridge (control)	7.01 (77)	9.34 ((97)	14.1 (129)
CPV1	7.73 (85)	10.3 (93)	15.45 (142)
CPV2	9.6 (105)	12.8 (133)	19.2 (176)
CPV3	8.93 (98)	11.9 (124)	17.85 (164)
CPV4	10.58 (116)	14.1 (147)	21.15 (194)
Iron	**11 mg**	**11 mg**	**11 mg**
Maize porridge (control)	1.51 (14)	2.01 (18)	3.02 (27)
CPV1	13.13 (119)	17.5 (159)	26.25 (239)
CPV2	15.4 (140)	20.5 (186)	30.75 (280)
CPV3	12.8 (116)	17.3 (157)	25.95 (236)
CPV4	15.9 (145)	21.2 (193)	31.8 (289)
Zinc	**2.8 mg**	**2.8 mg**	**2.8 mg**
Maize porridge (control)	2.9 (104)	3.81 (136)	5.72 (204)
CPV1	2.8 (100)	3.8 (136)	5.7 (204)
CPV2	3.38 (121)	4.5 (161)	6.75 (241)
CPV3	3.23 (115)	4.3 (154)	6.45 (230)
CPV4	3.9 (139)	5.2 (186)	7.8 (279)

Iron Assuming medium iron bioavailability (10%). Zinc** Assuming moderate bioavailability (30%) References: World Health Organizations Recommendations (Dewey and Brown 2002; Ruel, Loechl, and Pelto 2004).Numbers in brackets represent % RNI met.*

On the other hand the rest of the formulations (CPV1-CPV4) met the recommended RNI for iron and zinc but CPV3 was the best because it provided the highest amounts of protein, iron and zinc which nutrients of interest in this study. This is because high iron (millet and pumpkin) flours were blended with the low iron maize flour. Despite adding high fat food (pumpkin) the fat gaps still remained for all children for it was below 50% of the RNI. Maize flour porridges fortified with pigeon peas, pumpkins and millet have the potential of meeting RNIs for energy, protein, iron and zinc. In this study, improvements in energy balance and iron were achieved by the addition of pumpkin flour which had the highest amount of fat (26.23%) and iron (68.32mg/100 g) than the rest of ingredients, while improvements in protein quantity were achieved by the addition of pigeon peas which had the highest amount of protein (up to 21.25%). All the porridges costed less than 1$ per day as calculated using the linear programming of Nutrisurvey.

CHAPTER 4: STORABILITY AND SENSORY QUALITY OF COMPLEMENTARY FLOURS DEVELOPED FROM CEREAL-PULSE-VEGETABLE BLENDS

4.1. ABSTRACT

High nutritional and sensory quality commercial complementary foods are available but often expensive for low income households. Food based approaches that offer households low cost high quality complementary foods utilizing indigenous processing technologies need to be employed. This study aimed at testing the preference and acceptability of cereal-pulse-vegetable flour porridges (CPV1, CPV2, CPV3 and CPV4) and the control porridge (100% maize flour). The porridges were formulated by reconstituting the flour with water in the ratio of 1:2 while for control the flour was reconstituted with water in a ratio of 1:3 and heated for 20 minutes while stirring. All the porridges prepared were evaluated for their acceptability by 10 untrained panelists using a five point hedonic scale. Generally, formulations CPV1, CPV2 and the control were accepted but CPV2 was the most preferred with a score of 4.7. The most preferred formulation (CPV2) was evaluated for shelf stability under elevated temperatures of 55 ^{0} C (accelerated shelf life testing) and the parameters used to test shelf life were yeast and mold count, coliform count and peroxide value. Yeast and molds increased steadily in the flour stored in gunny bags and the shelf life ended at three months when yeast and molds grew beyond acceptable levels of 19.2×10^{2}. On the other hand the shelf life of flour stored in kraft paper and plastic container ended at four months. The results obtained showed that the best packaging material that can be adopted for longer shelf life of the stored flour could be Kraft paper and plastic container because they provided longest shelf life than gunny bags.

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4.2. INTRODUCTION

More than 10 million children die each year from malnutrition and infectious diseases and the majority of children who die are from poor countries (GNR, 2016).Nutritionally adequate complementary foods are of high priority in developing countries including Malawi. After 6 months of age, breast milk is no longer nutritionally sufficient for infants, and nutrient-dense complementary foods must be introduced (WHO, 2009). Inadequate energy intake of infants in developing countries may be due to household food insecurity, infrequent feeding and undesirable sensory properties of plant based foods (Bennet *et al.*, 2009).

Food processing techniques, storage, socio-economic status, organoleptic properties, cultural and religious factors and food quality and safety issues should be put into consideration when formulating weaning foods (Yewelsew *et al.*, 2006; Amuna *et al.*, 2000).

Complementary foods that form a very viscous paste during cooking will require excessive dilution with water before they become suitable for infant feeding (Amaglo *et al.,* 2012). However, diluting the paste to reduce viscosity leads to energy and nutrient thinning. Therefore, simple inexpensive household level food processing methods such as soaking and sprouting have been suggested for maize and millet to reduce bulkiness and increase nutrient and energy density.

The physical and sensory properties of plant based complementary foods for infants are indirectly of nutritional significance as they impact on the quantities of food eaten and invariably, nutrient intake (Muhimbula *et al.,* 2011). The participation of young children’s mothers in complementary food formulation and acceptability testing encourages them to gain nutrition knowledge and positive attitudes towards dietary improvements (Pelto *et al.*, 2003).

Due to its high protein, iron and zinc contents its consumption can thus contribute immensely in reducing protein-energy and micronutrient malnutrition among older infants and young children (Dewey and Brown, 2003; WHO/UNICEF, 1998) in Malawi and other developing countries.

Employing household level food processing technologies during formulation of CPV3 as a strategy to further improve bioavailability of the iron and zinc and to improve palatability is likely to affect storage stability of the product due to combination of flour blends from foods of varying storage stabilities. During formulation of any complementary foods made from locally available raw materials, the storage and food quality and safety issues should be taken in to account (Amuna *et al.*, 2000; Yewelsew *et al.,* 2006). The foods should also be free from pathogenic microorganisms and free from any substances originating from microorganisms in amounts which may represent a hazard to health. They should also not contain any other poisonous or deleterious substances in amounts which may represent a hazard to health of the infants or young children (Codex, 1991).

Therefore, the present study evaluated the acceptance of complementary porridge flour (refer to table 2.6,) formulated from blends of millet, maize, pigeon peas and pumpkin flour locally available in in most communities in Malawi. The most accepted blend was also evaluated for storage stability using parameters like moisture content, peroxide value and microbial count.

4.3. MATERIALS AND METHODS

3.3.1. Sample Collection and preparation

Complementary flours used in this study were CPV1, CPV2, CPV3 and CPV4. CPV1 comprised of unprocessed finger millet, maize, pigeon peas and pumpkin fresh and seed while CPV2, CPV3 and CPV comprised of the same food materials but were processed (refer to Table 3.6).

4.3.2. Preparation of porridges

The porridges from each of the 4 formulations including the control were prepared by reconstituting 1 part of flour to 2 parts of water (2:1) while that for the control (100% maize) was prepared by reconstituting 1 part of flour to 3 parts of water (3:1). After reconstitution the mixture was boiled for 20 minutes while stirring occasionally. The porridges were prepared by a black African woman living in a rural area in Lisasadzi area of Kasungu district with experience in cooking porridge. This ensured that the porridges were culturally acceptable to the participants. The porridges were prepared at the research site, Lisasadzi rural training Centre.

4.3.3. Sensory evaluation

The porridges prepared from section 3.3.2 were subjected to sensory evaluation. This was to test their acceptability for taste, mouthfeel, odour, colour and general acceptability using a five point hedonic scale, where 5= Very good, 4 = Good, 3 = Average, 2 = Bad and 1 = Very bad. Ten untrained female caregivers participated in the sensory evaluation. The use of adult females instead of the target recipients, children, was necessary because of their ability to objectively evaluate the sensory characteristics of the formulations. Before the study, all panelists were briefed about the procedure and each had to verbally consent to participation. All Participants were none smokers, non-English speakers and they self-reported to have normal taste or smell sensitivity. Panelists were requested to refrain from eating or drinking for at least 1 hour prior to their scheduled session when tasting was involved (ASTM E2263, 2013).

Figure 4. 1: Porridge made from CPV1, CPV2, CPV3, and CPV4

4.3.5. Shelf life evaluation

The most preferred flour from sensory results was subjected to shelf life evaluation to establish how long it will keep before it declared unsafe for consumption. The parameters used to determine shelf life were moisture, peroxide value, coliforms and yeast and molds.

4.3.5.1. Packaging and accelerated shelf study

The flour was divided into 18 portions of 200g each and packaged plastic containers (Figure 4.2 a), kraft paper (Figure 3.2 b) and ganny bags (Figure 3.2 c) (a total of 6 for each storage container). The plastic container and ganny bags are household level storage containers in Malawi (personal observation). An incubator was used in this study to subject the packaged containers to elevated temperatures for rapid deterioration. The shelf stability was determined using accelerated shelf life study at 55 ^{0}C (1 day= 1 month) for period of 5 days.

4.2 a **4.2 b** **4.2 c**

Figure 4. 2: Storage containers: 4.2 a= Plastic containers, 4.2 b= Kraft paper, 4.2 c= Ganny bag

4.3.5.2. Chemical and microbial analysis

After every 24 hours (1 day) the contents from each storage container were analyzed for coliforms count, *Eschelericia Coli*, yeast and molds, moisture and peroxide value according to ISO (6579 and 4833-2:2013) and AOAC methods (2003). For the analysis about 100 grams from each storage container was taken and the remainder from the container was destroyed.

4.3.6. Chemical analysis

4.3.6.1. Determination of peroxide value (PV)

To determine peroxide value, 5g of the sample from each storage container was put in a conical before adding 25ml of solvent mixture of acetic acid and chloroform. Saturated potassium iodide (1 g) was added to the mixture, shaken and then placed in the dark for 1 minute. Then the mixture was removed from the dark and 30 ml of distilled water was added against a standardized 0.001M of sodium thiosulphate using starch solution as an indicator until blue black color disappeared. The Peroxide value was calculated using the following equation:

$$\text{Peroxide value (meq/Kg)} = \frac{(A-B) \times N \times 1000}{\text{Sample weight (g)}}$$

Where; A= Volume of Na2S2O3 used for sample, B= Volume of Na2S2O3 used for blank C= Normality of $Na_2S_2O_3$.

4.3.6.2. Determination of moisture content

Moisture content was determined by drying method (AOAC 934.1, 2003). 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 (w0). 2g of ground sample was weighed into the moisture dish in triplicates and the weight of the dish, lid and the sample taken (W1). The samples were dried in an air oven (Mermet Laoding Modell 100-800) at 105 ^{0} C for 3 hours. Moisture content was calculated as follows:

$$\text{Moisture Content (\%)} = \frac{\text{Weight before drying (w1) – weight after drying (w2) x 100\%}}{\text{Sample wt +dish+lid before drying (w1) – moisture dish + lid (w0)}}$$

4.3.6.3. Microbial analysis

The yeast and mold count was carried out using potato dextrose agar (PDA) ISO 2171 (2006). Initial product sample homogenates were prepared in sterile dilutes (Sodium Chloride) in ratios of 1:10. 1ml of each homogenate was then aseptically diluted in series up to a dilution of 10-3. The dilutes were then pour plated in duplicates. Incubation of the plates was done at 30 ^{0} C for 48 hours. The number of yeast and molds were expressed as colony forming units per gram (CFU/g) using the formula in TPC determination. For total bacterial count sample preparation was the same but Hicrome media was used. A 0.1 ml homogenate was used for spread plating and the incubation was done at 37 ^{0} C for 24 hours. The number of bacterial colonies was expressed as colony forming units per gram (CFU/g) of the sample using the formula from International Dairy Federation method (IDF, 1996) as follows:

$$\text{Log } C = \sum x/n1 + (0.1n2)^x d$$

Where; C= Count CFU/g, x= Total number of colonies in all plates, n1= Number of plates from initial dilution where counts were made, n2= Number of plates from second dilution from where counting was done, d= Initial dilution of counting

4.4. Statistical Analysis

The data of the sensory evaluation of porridge from CPV flour formulations was analyzed using statistical package for social sciences (SPSS IBM STATS Version 25) to determine the most desirable formulations and the shelf life. Statistical significance was set at a level of 95% confidence interval. Shelf life data were also analyzed using statistical package for social sciences (SPSS, IBM STATS Version 25). Means were analyzed by ANOVA and were separated using Dunnet test. Statistical significance was set at ($P<0.05$).

4.5. RESULTS AND DISCUSSION

4.5.1. Sensory evaluation

The results of the sensory evaluation of the nutritionally optimized cereal-pulse-vegetable (CPV) complementary flour porridge and the control (maize flour porridge) are presented in Figures 4.3 to 4.8.

4.5.1.1. Colour

The Tukey test indicated that formulations CPV1, CPV2, CPV3, CP4 and the control (100% maize) scored 3.15, 3.36, 3.18, 3.56 and 3.8 respectively (figure 4.3). CPV4 and the control were highly scored in terms of colour and these score were significantly different ($P\leq0.05$) from CPV1, CPV2 and CPV3. There was no significant difference between scores for CPV1, CPV2 and CPV3. Formulation CPV4 and the control were ranked as good in terms of colour while the other formulations were ranked as average.

Generally, CPV4 and the control were formulated using high percentage of maize (60% and 100% respectively) which were found to be more appealing and liked by majority of the panelists. The panelists stated that CPV4 porridge had a nice brown colour, the control porridge was the colour of porridge that they are used to while CPV1, CPV2 and CPV3 the colour was too dark and children may not like foods with very dark colors. CPV1, CPV2 and CPV3 were formulated using a high percentage of millet and pigeon peas which contributed to the darkening of the colour of the porridge. Fikiru, (2017) found similar results on maize-roasted peas-barley blends. The formulation that had 55% scored highly in terms of colour. The color acceptance of this study corresponds with Marcel, (2015) that showed that complementary foods that were substituted with higher amount of maize (55%) and roasted peas (35%) had higher colour acceptability. Colour is important attribute in food choice and can have a major effect on its acceptability (Kikafunda *et al*, 2006). If the colour of the food is not appealing like in the case of CPV1, CPV2 and CPV3, the brain will naturally tell an individual to not eat the food and this may make the child reject the nutritious food. On the contrary, if the food has a good colour, the brain believes it will taste as great as it looks and this entices the child to eat the food (Spence *et al.*, 2010).

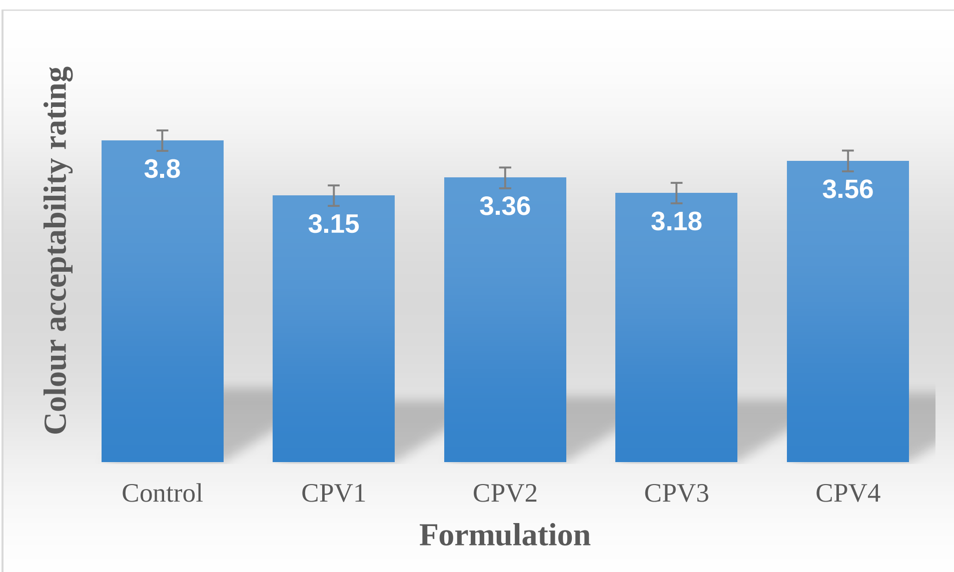

Figure 4. 3: Colour acceptability score of formulated complementary flour porridges

4.5.1.2. Smell

Results of sensory evaluation indicated that odour of certain samples varied significantly ($P < 0.05$) from others. Formulation CPV3 was rated significantly higher ($p<0.05$) in terms of odour (4.82= very good) than the control (2.3=bad), CPV1 (2.92=good), CPV2 (2.36= Bad) and CPV4 (2.6=Average). The burnt odour of the pigeon peas roasted for 15 minutes at 160 ^{0}C which was added at levels of 20% in CPV3 might have contributed to the high scores. Another study also reported the increase in sensory rating for flavor with increase in the proportions of roasted pigeon peas flour incorporated (Fasoyiro *et al.*, 2010). Smell is an integral part of taste and general acceptance of the food before it is put in the mouth. It is therefore an important parameter when testing acceptability of formulated foods (Edelstein, 2013).Roasting of samples done for the legumes had an important improvement on the flavor of the formulations (Mitzner *et al.*, 2004). Although all formulations contained roasted pigeon peas, formulations containing high amount of pumpkin flour (20%) were significantly not liked because of the bitter after taste they imparted in the formulations. The product might be appealing and having high energy density but without good smell, such a product is likely to be unacceptable (Awasthi *et al.*, 2000).

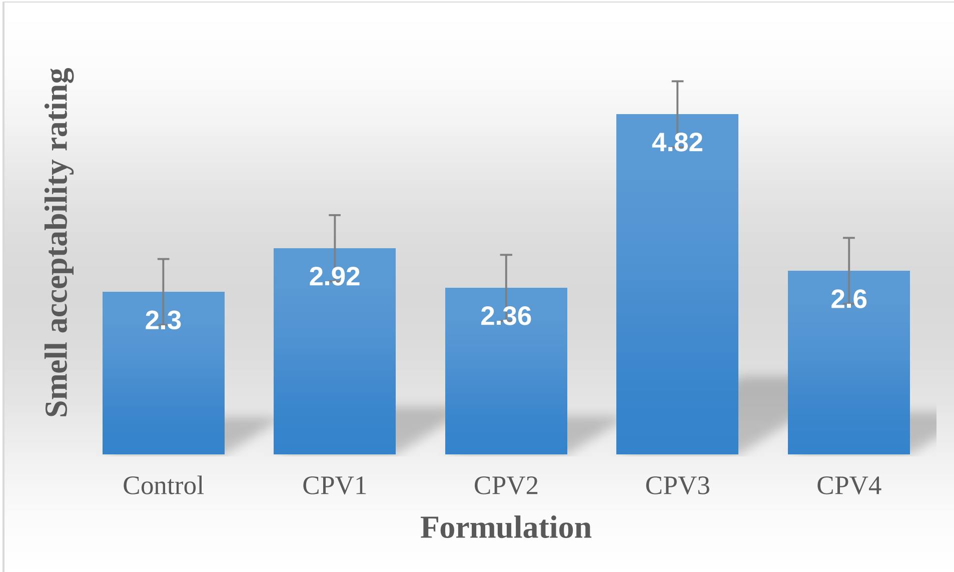

Figure 4. 4: Smell acceptability score of formulated complementary flour porridges

4.5.1.3. Mouthfeel

The results revealed that no significant differences ($P \geq 0.05$) were observed by the panelist between CPV2 and CPV3 in terms of mouth feel and both were highly liked as indicated by higher scores of 3.7 and 3.8 respectively (Figure 4.5). The mean scores for CPV2 and CPV3 were significantly higher than the rest of the samples. These two had higher substitution levels of pigeon peas flour amongst all formulations and thus 15% for CPV2 and 20% for CPV3. This shows that pigeon peas imparts a good texture in complementary foods and can be used to improve acceptability of complementary foods. However, the control, CPV2 and CPV4 were ranked significantly lower (2.5 to 2.82). Based on this test parameter these formulations were liked by the panelists in terms of mouth feel. Denhere, (2016) found that the mean values for mouthfeel acceptability of the composite complementary instant porridges increased with an increase in the concentration of Bambara groundnuts. It is reported that the mean sensory scores for weaning porridges prepared with either roasted or improved flour indicates very much liking for the product, whereas those prepared with raw flour indicates slight preference in all attributes (Mbata, 2007).

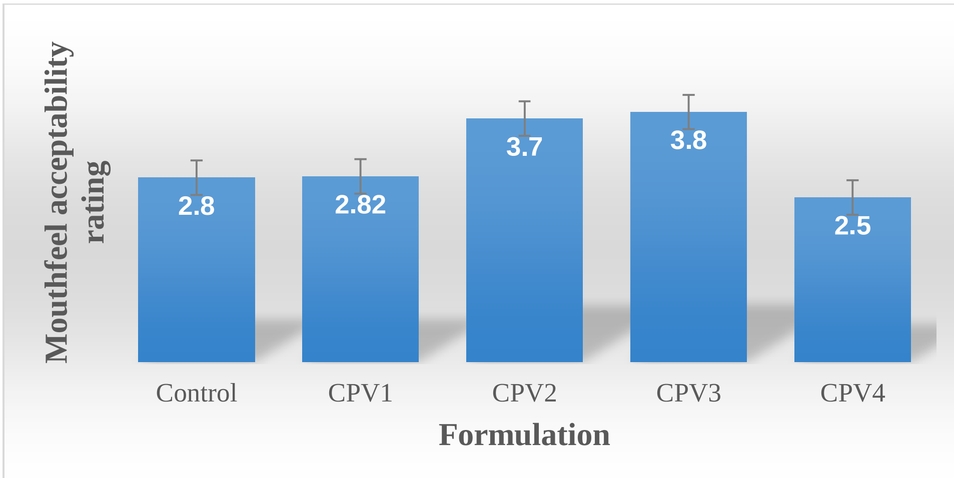

Figure 4. 5: Mouthfeel acceptability score of formulated flour porridges

4.5.1.4. Taste

Taste is an important parameter when evaluating sensory attributes of food. The product might be appealing and having high energy density but without good taste, such a product is likely to be unacceptable. The panelists rated formulation CPV2, CPV1 and the control (100% maize) with a score of 4.6 (very good), 4.1 (good) and 4.3 (good) respectively. These were significantly higher ($P < 0.05$) than the rest of tested formulations in terms of their taste. The caregivers reported that the taste of the control is what they were used to as it is consumed everyday while CPV1 and CPV2 had a slightly sweet taste than the rest of the porridges. This could be because these two formulations had high amounts of finger millet added to them and it is reported that millet has a sugary taste. In a study on complementary flour blends of millet, soybean and plantain, Islamiyat and others (2016) found that the formulation that had highest amount of millet (50%) was rated the sweetest. This shows that sweetness is a desirable attribute in determining acceptability of complementary foods. Sweetness is by far the most important attribute to complementary foods and sugar is commonly added to improve the flavour and to encourage infants to eat. CPV3 and CPV4 were scored poorly (2.2 and 2.3 respectively) because the care givers indicated that they had a bitter after taste. These two formulations had the highest levels of pumpkin flour added to it (15% and 20% respectively). These results are in agreement with Kiharason et.al (2017) who found that the taste acceptability of bakery products reduced with the increase in substitution level (≤20%) of wheat flour with pumpkin flour as the panelist reported that such bakery products had a pungent taste. The bitter taste in pumpkin flour is due to presence of cucurbitacins in the pumpkin fruit which are extremely bitter with a disagreeable taste (Gry *et al.*, 2006).

Generally, the mean scores of all formulations with high amount of roasted pigeon peas and millet were accepted, showing that judged by this sensory attribute, addition of oil and sugar is very important in the acceptability of product by target groups. Samples formulated using more roasted pigeon peas and millet were found to be more appealing and were liked by majority. Similar results were reported by Martin *et al.,* (2010) in study assessing nutrient content and acceptability of soybean based complementary foods. Although, formulations CPV3 and CPV4 (with less millet and pigeon peas) gave adequate protein, energy and micronutrients for infants 6-24 months, they were generally not well accepted in terms of taste, mouthfeel and odour as compared to the rest of formulations.

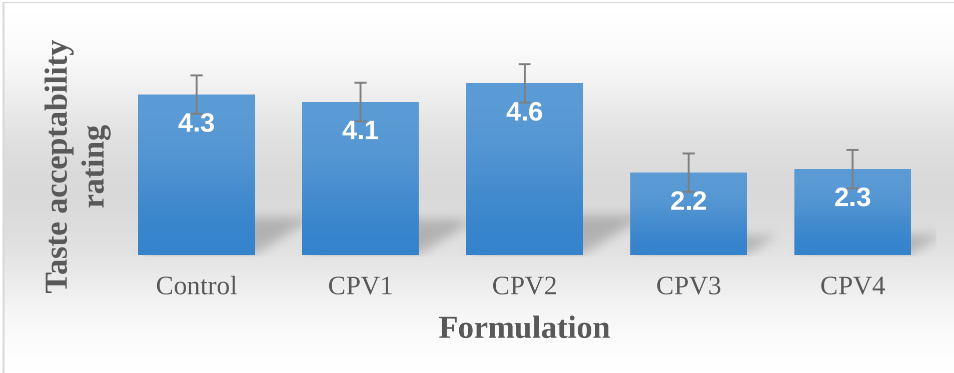

Figure 4.7: Taste acceptability score of formulated complementary flour porridges

4.5.1.5. General acceptability

Generally, formulated complementary food CPV2 was more acceptable with the mean score of 4.7 (Very good) followed by control (4.2) and CPV1 (3.9) amongst all the porridges. CPV3 scored 3.2 (Average) and CPV4 was by the panelists as they scored below average (<3). Complementary food formulations with addition of millet and more roasted pigeon peas were found to be tastier, appealing, and having good mouthfeel than those with less roasted pigeon peas flour and millet, indicating that inclusion of sugar and oil not only increased the energy density and protein content of the porridge but enhanced the taste and characteristic improved flavor thus differentiating them from other formulations. The sweet taste and the burnt flavor from millet and pigeon pea respectively could have contributed to CPV2 being the most liked amongst all the porridges. The bitter after taste of CPV3 and CPV4 contributed to its poor scores in overall acceptability. The results are similar to those by Kiharason (2017) who reported that trained and untrained panelists, observed that addition of 5% and 20% pumpkin flour into the recipes produced desirable effects, while addition of too much pumpkin flour spoiled the products. Inclusion of roasted pigeon peas and millet into the formulation may improve the consumer preference as evidenced by the increase in percentage for CPV1 and CPV3. Though

CPV3 and CPV4 had high levels of millet they were least preferred because they also contained highest amounts of pumpkin flour (20% each) which in turn made the porridges bitter. This means that pumpkin flour needs to be added at low level of ≤ 10% or it needs to be processed to remove the bitter taste.

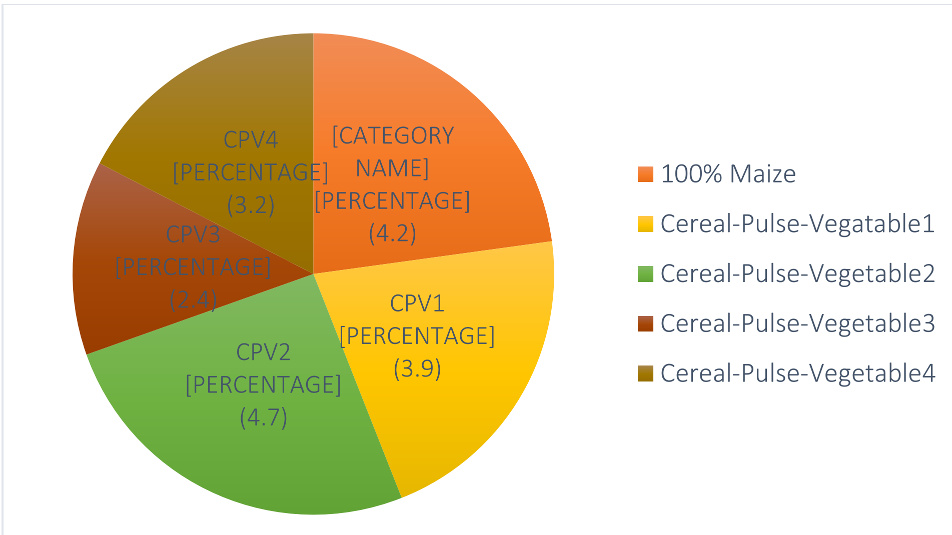

Figure 4.8: Consumer preference for complementary foods

4.5.2. Shelf Life Stability Evaluation

4.5.2.1. Changes in moisture content

The initial moisture content of CPV2 was 10.2% (Figure 4.9). This means that at the beginning of shelf life the moisture content was within the recommended range of below 12% (WHO, 2007). The result obtained for the moisture content of the flour sample during storage shows that there was a gradual loss of moisture in Kraft paper (10.2%-6.7%) and gunny bags (10.2-6.3%) throughout the storage period. On the contrary moisture content in plastic containers significantly (*P*<0.05*)* increased (10.2%-10.8%) from day zero to day two and gradually started decreasing from day three and the increase was significant (p <0.05) as compared to Kraft paper and ganny bags. The increase in moisture of CPV2 stored in plastic container with time (Figure

3.2a) indicated the transfer of water vapor through the packaging, which may have occurred due to sealing faults.

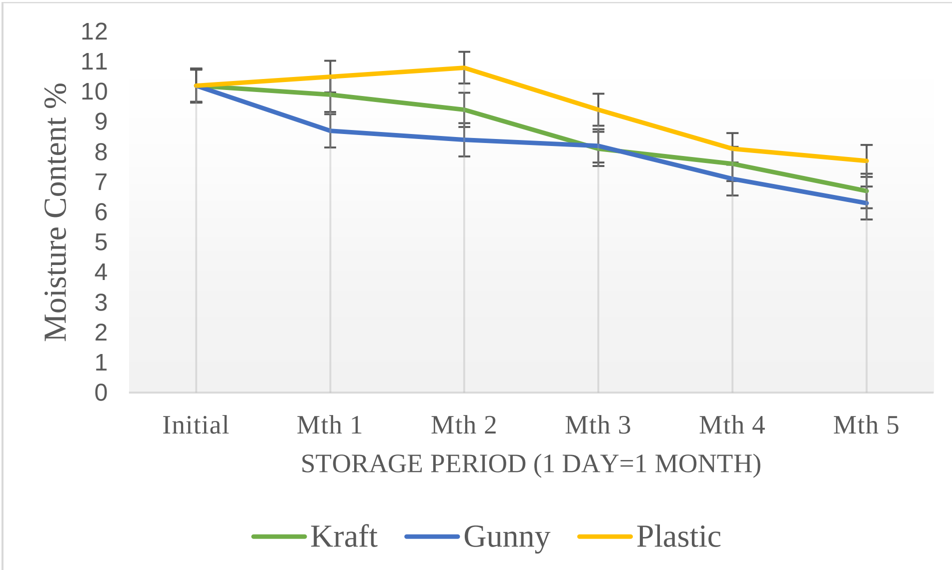

Figure 4.9: Changes in moisture content of CPV2 flour during storage under accelerated temperature of 55 ^{0}C

4.5.2.2. Changes in peroxide value

Peroxide value (PV) is the most useful method to determine the degree of oxidation in free fatty acids and production of hydro peroxides. It reflects the storage quality of the product. At certain level these hydro peroxides break down into volatile products that are responsible for a variety of undesirable odour and flavour known as oxidative rancidity. The PV in the product CPV2 during the 5 months accelerated storage was 0.0 meq / kg. The absence of peroxides implies that there was no lipid oxidation over the entire storage period. The absence of PV in CPV2 after 6 days storage in the product could be attributed to low amount of fat (7.5%) in the product. This result indicated that the product had acceptable peroxide values based on industrial standards. This implied that CPV2 would have a longer keeping quality because long shelf life is associated with low PVA of a food/product (Food Chain, 2001).

4.5.3. Changes in total coliform count

The initial total coliform count at zero time was 6.9 x 10^2 cfu/ml (Table 4.1) which is within the acceptable range of <100 cfu/g (MSB, 2007). The total coliform count in plastic container and Kraft paper decreased gradually with increase in time to 2.3 × 10^2 and 2.1 × 10^2 cfu/g on the fifth day respectively. According to FAO and WHO recommendations (FAO/WHO, 20044) related to the microbiological qualities of microbial proliferations in foods need certain conditions - namely available water, proper pH, right temperature and nutrients and time. The decrease in coliform population could be attributable to decrease in any of those conditions of growth mentioned and the toxins produced by the coliforms themselves leading to their death (Mbata *et al.*, 2009). The presence of members of coliform group are indicator microorganisms which provide evidence about the hygiene standard maintained during production. Presence of coliforms in food sample suggests the presence of other enteric pathogenic bacteria samples contaminated and may pose risk to public health (Paragya, 2012). Detection of coliforms in the product could be attributed to the water used during processing of the ingredients in CPV2, the cabinet solar dryer contamination and the slow sun drying rate (3 days) which could allow microbial growth of some of the ingredients that were used in formulating CPV2. These results were also in accordance with Osundahunsi and Aworh, (2002) who found total plate counts in the range of 3.2 x 10^2- 4.3 x 10^2 cfu/g for the formulated weaning foods. The composite flour tested negative for *Escherichia coli* showing that the composite flour was safe throughout storage.

4.5.2.4. Changes in yeast and molds

The results for yeast and molds counts are shown in Table 4.1. Total yeast and mold count at zero time was 4.1 x 10^2 cfu/g. This count is within the safe limits specified by the Malawi bureau of standards on complementary flours made of a mixture of cereals and pulses. There was a significant difference ($P<0.05$) in yeast and mold counts in different packaging materials. Gunny bag showed a steady growth of yeast and molds and the shelf life of the flour stored in gunny bags ended on third day. The increase could be because the gunny bags were already contaminated by spores of fungi from processing equipment or from the environment by the time they were used for storage. This means that when CPV2 if stored in gunny bags it can safely be consumed within three months from the day of production. On the contrary, yeast and mold count in plastic container and kraft paper decreased in the first two days. For kraft paper, yeast and mold count decreased from 4.1×10^2 to 3.5×10^2 cfu/g on third day and it started increasing from fourth day and in fifth month it grew beyond the maximum limit to 1.1×10^3 cfu/g. In the same way yeast and mold count in plastic container decreased from the initial 4.1×10^2 to 3.3×10^2 cfu/g on third day. The molds and yeast started increasing again from fourth day which registered 9.3×10^2 cfu/g. This means that for plastic containers and kraft paper CPV2 can be stored and safely consumed within a period of 4 months from the day of manufacturing. The gradual increase in yeast and molds in storage containers could be because yeast and molds survive and grow in a wide range of environments including high and low temperatures, low and high PHs. The high temperature in an incubator could not stop yeast and mold growth. These results agree with what Andago *et al.*, (2015) found in the study of composite flour made from cereals and bovine blood. The decrease in the yeast and mold counts in these packaging materials is attributable to anaerobic conditions created in plastic bottles and Kraft packaging materials due to tight closing of the bottle lid and impermeability of the Kraft materials (Andago *et al.*, 2015). Yeasts are commonly present as contaminants in cereals and can probably be attributed to the low value of the pH which creates ideal conditions for yeast growth (Serna-Saldivar and Rooney, 20055).

For plastic container and the Kraft paper the shelf life ended on day 4 when the yeast and mold count reached the maximum levels of 9.2 x 10^2 and 9.3 x 10^2 cfu/ml respectively. This means that CPV2 can be stored in plastic containers and Kraft paper for 4 months; and for ganny bags it can be stored to 3 months at ambient temperatures. Sanitation is a factor in the control of microbial growth. The flours should be prepared under hygienic conditions and stored in air-tight container and in clean and dry pantry that is not dark to discourage growth of mold. Also, porridge made from the flour should be well cooked to further ensure safety. Therefore it can be concluded that Kraft paper and plastic containers will be the best choice for storing CPV2.

Table 4. 1: Microbial count in different storage containers

Packaging	**Period (days)**	**Coliform count (cfu/g)**	***E.Coli* count**	**Yeast and mold count (cfu/g)**
Recommended Value		**<10^3**	**<10**	**<10^3**
	0	6.9^a x 10^2 ±1.22	nd	4.1^a x 10^2±0.03
Kraft Paper	1	8.2^b x 10^2±1.01	nd	3.3^a x 10^2 ±0.00
	2	6.4^a x10^2 ±1.11	nd	3.1^a x 10^2 ±0.0
	3	3.8^c x 10^2 ±0.04	nd	3.5^a x10^2±0.01
	4	2.9^c x 10^2 ±0.03	nd	7.2^b x10^2 ± 1.25
	5	2.1^{cd} x 10^2 ±0.06	nd	10.7^c x10^3 ±0.07
Gunny Bag	0	6.9^a x10^2 ±1.22	nd	4.1^a x 10^2 ±0.03
	1	7.1^a x 10^2 ±1.21	nd	3.4^a x 10^2 ±0.01
	2	8.5^b x 10^2 ±1.27	nd	7^b x 10^2 ±1.21
	3	12.8^e x 10^2 ±0.07	nd	13^d x 10^2 ±0.89
	4	7^a x 10^2 ±1.21	nd	19.2^e x 10^2 ±0.82
	5	4.4^c x 10^2 ±0.38	nd	20.6^f 10^2 ±0.76
Plastic containers	0	6.9^a x 10^2 ±1.22	nd	4.1^a x 10^2 ±0.03
	1	7.9^b x 10^2 ± 0.87	nd	3.5^a x 10^2 ±0.01
	2	6^a x 10^2 ±1.9	nd	3.4^a x 10^2 ±0.01
	3	3.2^c x 10^2 ±0.01	nd	3.7^a x 10^2 ±0.01
	4	2.7^c x 10^2 ±0.02	nd	9.3^g x 10^2 ±0.08
	5	2.3^c x 10^2 ±0.04	nd	13.9^d x 10^2 ±0.87

nd= Not detected

Values are mean (n=3) ± standard error on dry weight basis, values with different superscript in the same row significantly different (Dunnet test, P= 0.05)

CHAPTER 5: EFFECT OF PROCESSING ON IRON AND ZINC BIOAVAILABILITY OF COMPLEMENTARY FLOUR BLENDS

5.1. ABSTRACT

Micronutrient deficiencies especially iron and zinc deficiencies are problems in developing countries. The diets of these countries, which mainly consist of cereal products, do not contain sufficient minerals such as iron and zinc. These minerals have a low bioavailability owing to the presence of inherent antinutrients. Pretreatment of food using low-cost technologies such as germination can help reduce the anti-nutrients and enhance bioavailability of micronutrients. In this study, bioavailability of iron and zinc was determined in cereal-pulse-vegetable complementary (finger millet, maize, pigeon peas and pumpkins) composite flours, using *in vitro* enzymatic digestion method. One complementary flour blend namely CPV2 (finger millet= 70%, roasted pigeon peas= 15%, soaked maize= 7%, pumpkins= 8%) was formulated from ingredients that had undergone various processing technologies such as soaking, germination and roasting. The other complementary flour (CPV1) was formulated from unprocessed (untreated) ingredients (Finger millet= 70%, pigeon peas= 10, maize= 10%, pumpkins= 10%) and was treated as control. Processing of ingredients through germination, soaking and roasting caused significant (P<0.05) increase in vitro iron and zinc bioavailability of formulation CPV2 compared to unprocessed CPV1 (control). The amount of bioaccessible iron and zinc were 41.3% and 51.1% respectively; CPV1 had low bioaccessibility of iron (28.3%) and zinc (37.3). Preprocessing did not have any effect on bioaccessible calcium because the results showed that CPV2 had a slight increase of 25.6% while CPV1 had 24.4% and this difference was not significant (P = 0.05). From the study it can be concluded that preprocessing improves

bioavailability of Iron and zinc and such technologies should be promoted to contribute to reduction of iron and zinc deficiencies in developing countries.

5.2. INTRODUCTION

Plant based food products especially cereals, pulses and legumes are considered as good sources of dietary minerals (Wakil *et al.*, 2012). Deficiencies of iron and zinc are extremely prevalent in developing countries affecting mostly young children. The children are fed on cereal and legume based complementary foods (CF) such as millet, maize, beans and sorghum which have low bioavailability of such minerals (Gibson *et al.*, 2010).

The low bioavailability of nutrients, arising from the presence of phytate, polyphenols, and oxalate, that limits the quality of predominantly plant-based diets used for complementary feeding (Nuss and Tanumihardjo, 2016; Gibson *et al.*, 2010) .The total iron, zinc and calcium contents in a food product are not the only criteria determining its nutritional quality, bioavailability of these minerals must also be determined. In order to determine whether a product is a good source of a particular mineral, it is important to determine the amount of that mineral released from the food matrix and absorbed by the human organism (Gibson *et al.*, 2010).

The Food and Agriculture Organization (2009), has encouraged the use of affordable processing techniques such as soaking, roasting, germination and fermentation to increase nutrient bioavailability in plant- based CFs. Traditional processing technologies (soaking, fermentation and germination) have always been widely used in Malawi especially rural areas for making local beer and *thobwa* (non-alcoholic beverage made from germinated cereal). Soaking and germination is carried out at low cost without the use of any sophisticated and expensive equipment. Germination, soaking and roasting may help enhance iron and zinc bioavailability in food by reducing the amount of phytates, oxalic acids and fibre that lowers the release of iron and zinc from the food matrix (Coulibaly *et al.,* 2011). In an experiment by Towo *et al.,* (2003), the effect of soaking and germination on phenolic content of sorghum and finger millet was investigated. After soaking in water for 24 hours, the phenolic content was reduced by 23 % and after the subsequent germination, a total reduction of 48 % was detected in sorghum. In the finger millet samples, the reduction was 19 % (soaking) and 21 % (germination). How this reduction of phenolic compounds influenced the mineral bioavailability, was not further investigated (Towo *et al.*, 2003).

The simplest and low cost *in vitro* method to determine bioavailability of minerals are available (Skibniewska *et al.,* 2002). These involve the enzymatic digestion of a product under physiological conditions of the stomach and intestines (Krejpcio *et al.*, 2009; Suliburska *et al.*, 2009).

Therefore, the objective of the study was to determine the influence of soaking, roasting and germination on the iron, zinc and calcium bioavailability of two cereal-pulse-vegetable complementary flours made from blends of finger millet, maize, pigeon peas and pumpkins.

5.3. MATERIALS AND METHODS

5.3.1. Sample Collection

The samples were complementary flours made from blends of finger millet, maize, pigeon peas and pumpkins with known quantifiable content of iron and zinc (Table 5.1). There were two samples of which one was formulated from unprocessed ingredients (CPV1) and the second one from processed ingredients (CPV2) was selected as the most preferred flour (refer to Chapter 4).

Table 5. 1: Multimix complementary flours used in the experiment

Formulation	MR_W	PPR_W	FMR_W	MSD	PPR_{40}	FMG_{d48}	Pumpkins
CPV1 **Iron 20.5 mg/100g** **zinc 4.5 mg/100g**	10	10	70	0	0	0	10
CPV2 **Iron 17.3 mg/100g** **zinc 4.3 mg/100g**	0	0	0	7	15	70	8

MR_w= Raw maize, PPRw= Raw pigeon peas, FMRw= Raw finger millet, PKd= Dried pumpkin flour,

MSD = Soaked maize

PPR_{40} = Roasted pigeon peas (40 minutes 160 ^{0} C)

FMG_{D48} = Germinated finger millet (48 hours)

5.3.2. Enzymatic digestion

In vitro enzymatic digestion was performed according to the method developed by Skibniewska et al. (2002). Two grams (2 g) of each flour were weighed in conical flasks and treated with deionized water (20 ml) and shaken for 10 minutes. In order to create suitable conditions for pepsin action, pH was brought to 2 using 0.1 M HCl aqueous solution (Sigma Aldrich), then pepsin solution (0.5 ml/100 ml) was added to the homogenate. Subsequently samples were placed in a thermostat shaker (37°C) for 2 h. During the incubation process, pH was maintained by an addition of 6 M HCl aqueous solution, as necessary. After 2 hours digested samples were treated with 6% $NaHCO_3$ aqueous solution to bring pH to 6.8–7.0, and subjected to pancreatin solution (10 ml/40 ml of homogenate), and placed in a thermostatic shaker (37°C) for 4 hours. Then, digested samples were centrifuged for 30 minutes (4000 rpm/min), and the supernatant was quantitatively transferred to quartz crucibles, and treated with a mixture of concentrated nitric (65% w/w) and perchloric (70% w/w) acids (2:1 v/v) (Suprapure, Merck). Samples were placed in a thermostatic block and heated until complete mineralization (Skibniewska *et al.*, 2002)

5.3.3. Determination of minerals

The content of iron and zinc *in vitro* digested samples was determined by atomic absorption spectrometry (AAS-3, Zeiss spectrometer), after an appropriate dilution with deionized water (AOAC, 2003). The degree of a mineral released (bioavailability) was expressed as the amount of mineral (mg) liberated during the enzymatic digestion *in vitro* from 100 g of product and a percentage of a mineral released vs. its total content. The content of minerals in food products was expressed in mg/100 g dry mass.

5.4. Statistical analysis

The statistical analysis was carried out using the statistical package for social sciences (IBM SPSS Version 25). The mean and standard error of means were analyzed using analysis of variance (ANOVA). Dunnet test was used to separate means. Significance level was tested at $P \leq 0.05$.

5.5. RESULTS AND DISCUSSION

5.5.1. Zinc

According to the results (Figure 5.1) CPV2 had significantly ($P \leq 0.05$) much higher bioaccessible zinc 1.8 mg/100g (41.9%) compared to CPV1 which had 1.1 mg/100g (24.4%). These results are in agreement with Hemalatha *et al.,* (2007) who observed low zinc bioaccessibility from flour blends that were developed from unprocessed finger millet, rice, maize and mung beans. In finger millet, zinc bioaccessibility of complementary foods improved with germination of the ingredients by 24% (Mamiro *et al.,* 2001). Hurrell (2003), reported that cereal porridges that were made from germinated and roasted grains had a significant reduction in phytic acid and showed an increased zinc bioavailability as compared to cereal porridges that were made from unprocessed ingredients. This current study clearly shows that promoting household level food processing technologies maybe the best and low cost approach to increase zinc bioavailability in pant based complementary foods.

WHO recommends 2.8 mg/100g RNI of iron for children 6-24 months. Consumption of CPV2 will provide 41.9% of the RNI while on the other hand CPV1 will provide 24.4% of the RNI. This is evidence enough to show how important it is to pretreat the cereals and legumes used in preparation of complementary foods.

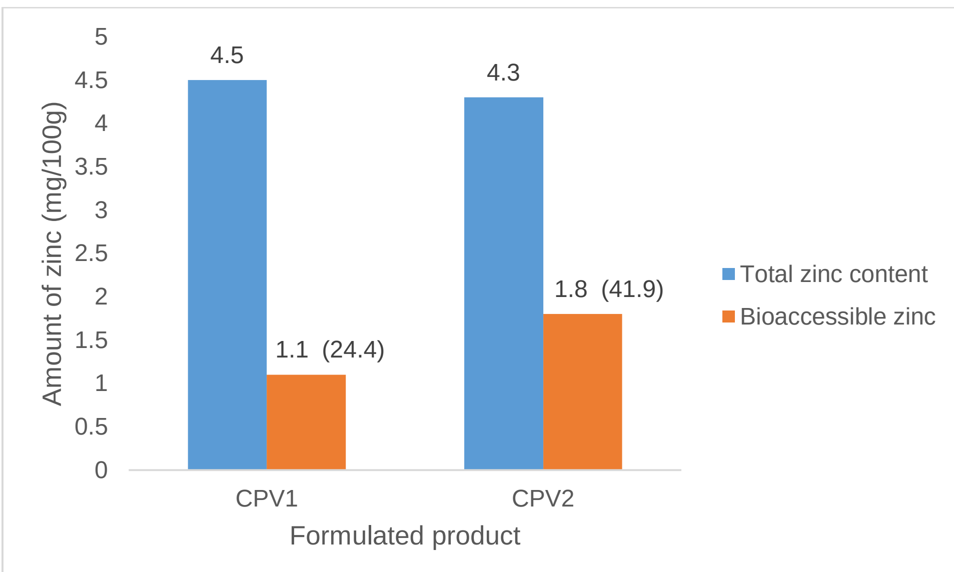

Figure 5. 1: Released zinc after *in vitro* enzymatic digestion of food products

5.5.2. Iron

Figure 2.2, shows that the amount of bioavailable Iron was significantly higher ($P \leq 0.05$) in CPV2 41.3% (7.14) as compared to CPV1 which registered a bioavailability of 28.3% (2.8 mg). Amongst all inhibitors, phytates, tannins and dietary fibre are the most potent (Sandberg, 2002).

The low bioavailability of iron in CPV1 could be attributable to the higher tannin and phytic acid content coming from unprocessed finger millet and maize respectively. CPV2 had high amount of bioavailable iron because it was made from blends of millet, maize, pigeon peas and pumpkins that had undergone various household level food processing technologies such as germination, soaking and roasting that are known to reduce antinutrients associated with low absorption of iron. Suma and Urooj (2014) have reported drastic reductions, approximately 50% of phytate in pearl millet upon germination which resulted in an increase of bioavailable iron as compared to pearl millet which was not germinated (unprocessed). Germination/malting increases endogenous phytase activity in cereals, legumes, and oil seeds through de novo synthesis and this process reduces the amount of phytates in the food and hence increase iron bioavailability. These results are also in agreement with Hamalatha (2007) who observed that germination of finger millet for 24 and 48 hours significantly increased the bioavailability of iron by 38% in finger millet based composite flour.

Considering the RNI of 11 mg for iron (WHO/Dewey, 2004) for infants it translates that if infants were to be fed with CPV2 they will able to meet 41.3% of the RNI of iron while with CPV1 they will be able to meet only 13.7% of the RNI. Therefore, indigenously processed locally available foods should be promoted because indigenous technologies have shown improve the amount of bioavailable iron in plant based complementary foods.

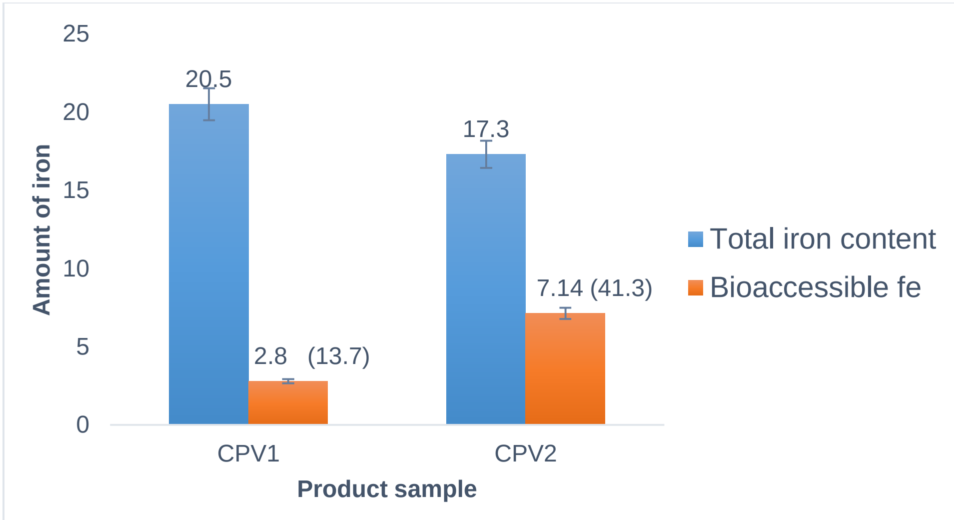

Figure 5. 2: Released iron after *in vitro* enzymatic digestion of food products

CHAPTER 6: GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

It can be concluded that, it is possible to formulate a nutrient dense product high in iron and zinc from locally available foods which can meet over half of the recommended nutrient requirements of children aged 6-24 months. Based on the nutrients of interest in this study, all ingredients used had a cooperative contribution in improving the nutritional quality and sensory properties of the formulated product. Pigeon peas and pumpkins contributed to protein densities of the formulations. Pumpkins also had a great contribution on the iron, Zinc, vitamin A, energy and fat while finger millet and maize had a greatest contribution of carbohydrates. The formulation which was the most accepted and with a shelf life of four months was able to contribute over 100% of the RNI for children 6-24 months. Therefore its adoption for complementary feeding is of great importance since it seems to be good enough to help in reducing malnutrition contributed by nutritionally poor quality complementary foods.

In adopting this formulation, great care should be taken on the implications of excess intake of proteins, iron and zinc which are associated with health dangers to infants like chances of diabetes, obesity, kidney failure injury to internal organs and even death. This can be done by following recommended feeding frequency and portions. Despite the contribution of iron, zinc, vitamin A, energy and protein from pumpkins, it also decreased the acceptability of the product due to its bitter after taste.

It is also clear that the traditional processing technologies of soaking and germination, which are commonly used most local communities of Malawi for making local beer could be utilized to enhance the nutritional properties of complementary foods used by infants and young children. These technologies are low cost and does not require any sophisticated and expensive equipment.

6.2. Recommendations

Since results from this study have revealed the significant contribution of the developed product to the nutrient requirements of children aged 6-24 month, it is therefore recommended that utilization of pumpkins, germinated finger millet and roasted pigeon peas in complementary feeding should be promoted in the communities. Since the panelist recommended a bitter after of the porridges with high amounts of pumpkin flour (< 15%) future studies should consider pretreating the pumpkin fresh and seeds by soaking and roasting them during the formulation of the products.

The mineral content of water may be determined in future studies because there are expectations that the water used during soaking and germination contains iron and zinc which increases the amount of these minerals in the formulated flours. In shelf life another study should be carried out to determine the stability of iron and zinc during storage. Further research should also be carried out to determine the protein quality of the composite flour. An awareness and nutrition education program to inform community members about transferring the low cost technologies used in this study and its usefulness to complementary feeding is recommended.

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APPENDICES

APPENDIX 1: PREFERENCE TEST-RANKING (ENGLISH)

Complementary porridge

Name of panelist (optional) ________________________ Date _____________

Instructions:

1. Please rinse your mouth with water before starting. You may rinse again at any time during testing if you need to.

3. Please taste the samples of the porridge in the order presented, from left to right.

4. You may retest the samples once you have tried them all.

5. Rank the samples from most preferred to least preferred in terms of taste, texture, smell, colour and overall acceptability.

1= most preferred, 5= Least preferred

Sample code	Colour	Smell	Taste	Texture	Overall Acceptability

APPENDIX 2: PREFERENCE TEST-RANKING (CHICHEWA)

Phala

Dzina ________________________ **Tsiku _____________**

Zoyenera Kuchita:

Chonde tsukani mkamwa mwanu ndi madzi omwe mwapatsidwa musanayambe. Mungatheso kuchukuka mkamwa ndi madzi nthawi iriyonse yomwe mukufuna .

Musanalawe kapu iri yonse mukuyenera kutsuka kaye mkamwa mwanu.

Mulawe mundondomeko mmene makapu ayikidwira kuchokera ku manzere kupita ku manja.

Sankhani mmene mwakondera kakomedwe, kamvekedwe mkamwa, kanunkhiridwe, mtundu komanso phala lomwe mwakonda kwambiri.

Mukhoza kulawa kawiri ngati mukufuna.

1= kukonda kwambiri, 5= Simunakonde

Dzina la phala	**Mtundu**	**Kununkhira**	**Kukoma**	**Kamvekedwe**	**Phala lomwe mwakonda kwambiri**