BIOACCESSIBILITY OF IRON AND ZINC FROM MICRONUTRIENT POWDER ADDED IN GERMINATED PORRIDGES AMONG CHILDREN AGED 6-23 MONTHS IN HOMABAY COUNTY, KENYA

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DECLARATION

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

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DEDICATION

Earning a PhD is a lengthy and grueling process. I would like to devote this work and achievement to my husband and family for putting up without a caring wife and mother during the process. Dr. Mokaya has been consistently supportive and has endured the burden of nurturing as I spent my time and energy pursuing goals that took me away from him and my family. Without the relentless support of my family, encouragement and understanding, it would have not been possible to achieve my academic goals. I wish there was room in my PhD certificate to write the names of my husband and children: Dr. Evans Mokaya, Brandy Mokaya and Adrian Mokaya.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AoR	Absolute Odds Ratio
AR	Absolute Requirements
BMI	Body Mass Index
CI	Confidence Interval
cm	Centimeters
CoR	Crude Odds Ratio
CRP	C- Reactive Protein
DALYS	Disability Adjusted Life Years
DDS	Dietary Diversity Score
DON	Division of Nutrition
EBF	Exclusive Breast Feeding
EFA	Explorative Factor Analysis
ENA	Emergency Nutrition Assessment
FFQ	Food Frequency Questionnaire
GAIN	Global Alliance for Improved Nutrition
HAZ score	Height for Age
HINI	High Impact Nutrition Intervention
HIV	Human Immune Deficiency Virus
HPLC	High Performance Liquid Chromatography
ID	Iron Deficiency
IDA	Iron Deficiency Anemia

IFAD	International Fund for Agricultural Development
IYCF	Infant and Young Child Feeding
KDHS	Kenya Demographic and Health Survey
KIRDI	Kenya Industrial Research and Development Institute
KEMRI	Kenya Medical Research Institute
LLOQ	Lower Limit of Quantification
mg	Milligrams
MICS	Multi Indicator Cluster Survey
mls	Milliliters
MNP	Micro Nutrient Powder
MOH	Ministry of Health
MUAC	Mid Upper Arm Circumference
NaFeEDTA	Ferric Sodium Ethylenediaminetetraacetate
PCA	Principal Component Analysis
RCT	Randomized Controlled Trial
RDA	Recommended Dietary Allowance
RNI	Recommended Nutrient Intake
SPSS	Statistical Package for Social Sciences
ULOQ	Upper Limit of Quantification
UNICEF	United Nations Children Education Fund
WAZ score	Weight for Age
WHO	World Health Organization
WHZ	Weight for Height

DEFINITION OF OPERATIONAL TERMS

Anthropometric: The measurement of weight for age, height for age, weight for height and mid-upper arm circumference.

Anti-nutrients: Natural and synthetic compounds found in complementary food that interferes with the absorption of nutrients [iron and zinc].

Bioaccessibility: The quantity or fraction of iron and zinc released from the food matrix in the gastrointestinal tract that is available for absorption.

Bioavailability: The proportion of iron and zinc that is digested and metabolized through normal pathways.

Biochemical assessment: Laboratory procedure used to check the levels of iron and zinc in children's blood.

Complementary feeding: Feeding a child with other foods and liquids alongside breast milk when breast milk alone is no longer sufficient to meet the nutritional requirements of infants aged 6- 24 months.

Food pattern 1: Ugali, vegetable [Jute mallow], porridge and tea

Food pattern 2: Liver, red meat, chicken, eggs and soda

Nutrient pattern 1: Carbohydrates, vitamin B1, magnesium, and phosphorus

Nutrient pattern 2: Vitamin B2, zinc and calcium

Nutrient pattern 3: Vitamin A, vitamin C and folic acid

Micronutrient powder: A single-dose packet of iron and zinc and other vitamins and minerals in powder form that can be sprinkled onto any ready to eat semi-solid food consumed at home or at any other point of use.

Processing: is soaking and germination of cereals thus, processing refers to germination.

ABSTRACT

Iron and zinc deficiency remains a burden among Kenyan children. Point-of-use fortification of complementary foods using micronutrient powder [MNP] containing vitamins and minerals has been recommended to improve the health and nutrition of children aged 6-23 months. Evidence-based information on different food vehicles for optimum bioavailability of micronutrients from the MNP is lacking. The main objective of this study was to determine the bioaccessibility of iron and zinc from micronutrient powder added in germinated porridges in children aged 6-23 months in West Kwabwai location, Homabay County, Kenya. Phase 1 of the study was the *in vitro* bioaccessibility of iron and zinc from germinated and non-germinated maize, millet and sorghum porridges with MNP. Non-germinated or germinated cereals were milled to flour. Porridge was cooked following the local known procedures. One-gram sachet of MNP [10mg iron as NaFeEDTA and 4.1mg zinc] was added to a 250ml cup of cooked porridge. Iron and zinc bio-accessibilities [%] were measured using *in vitro* dialysability methods. Factorial ANOVA was used to determine the effect of germination and cereal type on iron and zinc bioaccessibility. Phase 2 was conducted to establish the acceptability of different germinated porridges with MNP among children. The trial was single blind cluster randomized parallel study for 8 weeks with four sub-locations being randomized. A total of 200 children were selected by simple random sampling. A questionnaire and a morbidity data sheet were used to collect data on the amount of porridge consumed and health status of children, respectively. The acceptability trial data was analyzed using ANOVA. Phase 3 determined the anthropometric, iron and zinc nutritional status, and dietary intake of children. A descriptive cross sectional survey was done in the location. Using a population weighted simple stratified sampling method, 314 children were selected in the three sub-locations. Structured questionnaires were used to collect data on sociodemographic status, and anthropometric measurements of children. Food frequency questionnaire and a 24-hour dietary recall questionnaire were used to identify the complementary foods and to assess the dietary adequacy, respectively. ENA for SMART software was used for analysis of anthropometric data while Nutri-survey was used for analysis of nutrient intake. Serum content of iron and zinc were determined by spectrophotometric method. Binary logistic regression models were computed to identify the association of food and nutrient patterns as independent variables with wasting, stunting, underweight, anemia, iron deficiency, iron deficiency anemia and zinc deficiency as the dependent variables. The association was considered statistically significant when the p-value obtained was less than 0.05. Both, germination and cereal type significantly affected iron and zinc bioaccessibility. Germination significantly increased bioaccessibility of added iron in maize [1.3 to 2.7%] and millet [1.8 to 5.5%] porridges, but not in sorghum [5.3 to 4.5%; p=0.192]. Germination significantly increased bioaccessibility of added zinc in all three cereal porridges [maize: 12.6 to 30.7%; millet: 10.6 to 33.7%; sorghum: 15.5 to 38.8%]. The mean amount of germinated porridges [millet 241.46ml, maize 238.69ml, sorghum 230.29ml] consumed during the trial was more than the non-germinated porridges. The survey revealed that, at <-2SD, 17.5% of children were stunted, 13.4%; underweight, 6.4%; wasted. The prevalence of anemia, iron and zinc deficiency is 50.6%, 39.5% and 43.6%, respectively. These results indicate that germination of cereal-based porridge can significantly improve the bioaccessibility of added iron and zinc from MNP.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Child malnutrition remains a public health problem in the world, contributing to a high burden of morbidity and mortality (Ahmed, Hossain, & Sanin, 2012). Worldwide in 2017, more than 150 million and nearly 50 million children were estimated to be stunted and wasted, respectively (United Nations Children's Fund, 2018). Asia and Africa are home to the majority of the malnourished children. Although the report indicated a decline in the prevalence of stunting, Africa reported an increase in the number of stunted children. According to the Kenya Demographic Health Survey [KDHS] of 2014, 26% of children less than five years were stunted, 4% were wasted, and 11% were underweight in Kenya (Kenya National Bureau of Statistics, 2014). Even though the KDHS report shows an improvement trend in stunting, wasting and underweight in children over the last few years, the prevalence rates are still alarmingly high in Homabay County, where stunting is 18.7%, wasting 2.3% and underweight 5.4% (Kenya National Bureau of Statistics, 2014).

Micronutrient deficiencies of iron and zinc coexist and remain a burden in children, especially in the developing world during the first 1000 days. The two micronutrients deficiencies account for 33% of total disability-adjusted life years [DALYs] in pre-school children worldwide (De-Regil, Suchdev, Vist, Walleser, & Peña-Rosas, 2013). Most of these DALYs occur in developing countries. Iron deficiency is the most common micronutrient deficiency in the world, affecting more than 30% of the world's population, an estimated 2 billion people (Petry et al., 2016). In the developing countries, iron deficiency contributes to the most significant documented disease burden among micronutrients and accounts for about 50% of the anemia cases (Black et al., 2008). It is estimated that 273.2 million preschool children are anemic, and of this, 9.6 million have severe anemia (WHO, 2015). Africa and Southeast Asia have the highest burden of anemia in preschool children and pregnant women (Pasricha, Armitage, Prentice, & Drakesmith, 2018). Although there are not many studies that have estimated the global burden of zinc deficiency, it is estimated that 17% of the entire population globally and 50% of the entire population in Sub-Saharan Africa are at risk of zinc deficiency (Wessells & Brown, 2012). Pregnant females and their young children in Africa and Asia are the highest-risk groups for zinc deficiency (Bailey, West Jr, & Black, 2015). In Kenya, 83.3% of children, 6-59 months have low plasma zinc that is an indication of zinc deficiency, while 26.3% are anemic (Kenya National Bureau of Statistics, 2011).

Recently, deaths caused by undernutrition in children below the age of two years have been reported warranting the shifting of focus to child nutrition and health during the 1000 days of life. This window period is considered critical since malnutrition of any form at this stage is known to cause irreversible consequences to these children later in their lives. Evidence shows that undernutrition in children causes severe cognitive and behavioral disabilities right through life if not managed in infancy (Prado & Dewey, 2014).

Although the predictors of child malnutrition have been documented based on the UNICEF conceptual framework, these factors tend to vary regionally. These determinants have been associated with anthropometric or biochemical nutritional status or both. It has been reported that children from households with low socioeconomic status lack access to sufficient food of adequate quality, essential health care services, and have a higher risk of infection as a result of compromised immunity due to poor nutrition and suboptimal living conditions (Akombi et al., 2017). Child morbidity is also a causality for undernutrition and vice versa. This is because malnourished children are likely to die from infections and illnesses such as diarrhea and pneumonia (WHO, 2002). Then again, such mortalities are bound to plummet because of nutrient loss caused by vomiting and diarrhea, malabsorption of nutrients, increased nutrient need and high energy expenditure, reduced appetite, poor digestion and altered metabolic process (Asfaw, Wondaferash, Taha, & Dube, 2015).

Poor infant and young child feeding practices significantly contribute to child undernutrition. According to the WHO and UNICEF recommended indicators for infant and young child feeding practices, early initiation of breastfeeding within an hour after birth, exclusive breastfeeding for the first 6 months during infancy with timely and appropriate initiation of complementary feeding of at least 4 food groups, and continued breastfeeding up to 24 months and beyond [including HIV exposed children] are recommended (WHO, 2016a; WHO, 2018). However, recent studies are reporting adverse effects of undernutrition in spite of the set WHO recommendations on IYCF (Biks, Tariku, Wassie, & Derso, 2018; Habtewold et al., 2018). The dwindling adherence to IYCF recommendations could be attributed to low education levels, as well as lower socioeconomic status of caregivers, among other factors (Manikam et al., 2018).

The frequency of feeding infants and young children, coupled with continued breastfeeding are two critical determinants of their nutritional status. Meal frequency and breastfeeding have been reported previously, and in recent studies to be associated with malnutrition (Dhanalakshmi & Selvaraj, 2019; Garg & Chadha, 2009). In poor settings, dietary diversity scores may be used to predict the micronutrient adequacy of diets, as well as the biochemical status of individuals. Consumption of assorted foods ensures acceptable nutritional needs are achieved (Korkalo et al., 2017). In addition to that, evidence indicates that inadequate dietary diversity is associated with low serum zinc, especially during the hunger period (Korkalo et al., 2017). Significant relationships have been shown between a lower risk of nutritional anemia and a higher dietary diversity among preschoolers (Gashu et al., 2016). In contrast, zinc deficiency has been reported to be associated with severe stunting, low serum zinc levels, episodes of persistent diarrhea, a diet that is low in animal products and based on high phytate cereals and legumes, especially during the period of complementary feeding (Allen, 1998).

Significant steps are being made to address malnutrition. While there has been progress, it has been slow and patchy (United Nations Children's Fund, 2018). Nutrition education, supplementation of micronutrients such as micronutrient powders, food fortification programs, and therapeutic zinc programs are some high impact interventions that are recommended to address malnutrition. However, these interventions are yet to be scaled up and yield results.

Diets in developing countries are primarily plant-based, and the presence of anti-nutrients hinders the bioavailability of iron and zinc (Roos et al., 2013). A study conducted in Kenya found porridge made from cereals, especially maize as the most prevalent complementary food for children aged 6-23 months (Macharia-Mutie et al., 2012). Maize, millet, and sorghum cereal-based complementary foods in the form of soft and stiff porridge are insufficient to provide adequate iron and zinc. The frequency of feeding is also low because these porridges usually are administered to children 2–3 times a day (Gabaza, Shumoy, Muchuweti, Vandamme, & Raes, 2016). Furthermore, these non-processed cereal-based porridges cannot provide the expected daily requirement for iron and zinc, which is 9.3 mg/day for infants aged 9 months and 3.0 mg/day for those aged 11 months (WHO, 2002).

In populations where micronutrient deficiencies including nutritional anemia are a public health problem, point of use fortification of complementary foods with micronutrient powder [MNP] has been recommended in children aged 6-23 months (WHO, 2016b). Point-of-use fortification with multiple micronutrient powders refers to the addition of powders containing vitamins and minerals to energy-containing foods like porridge, at home or in any other place where meals are to be consumed, such as schools, nurseries and refugee camps (Suchdev et al., 2016). It is particularly encouraged because it allows families to continue using home-prepared foods, and it is relatively cheap compared to processed foods (Dewey, Zhenyu, & Boy, 2009).

The iron and zinc bioaccessibility from MNP may be dependent on many factors. The food matrix used as a vehicle for fortification determines the efficacy of nutrient fortification. Experimental results in animal and human models demonstrated that, no matter how good the iron fortificant maybe, its intake/bioaccessibility in combination with enhancers and inhibitors determines the final effect (Shilpashree, Arora, Sharma, & Singh, 2015). Complementary foods prepared solely from maize, sorghum, and millet are considered less bioavailable since the levels of anti-nutrients in them are more compared to the reduced levels of promoters (Gabaza et al., 2016). Nutrients in different complementary foods, as well as the nutrient interactions in MNP, may affect the amount of bio-accessible iron and zinc contained in MNP. Furthermore, a broad diversity in the contents of intrinsic nutrients and antinutrients in different cereals based porridges may also influence the bioavailability differently in these cereals. The phytate: mineral molar ratio is a useful indicator of mineral availability (Lazarte, Carlsson, Almgren, Sandberg, & Granfeldt, 2015). The maximum desired molar ratios of phytate: calcium, phytate: iron, and phytate: zinc is 0.17, 1 and 15, respectively, and the mineral availabilities are severely reduced when the molar ratios are higher than these values (Hurrell, 2004; Umeta, West, & Fufa, 2005a).

Soaking, germination, and fermentation of cereals are some of the processing methods that reduce antinutrients resulting to improved mineral bioavailability (Chauhan, 2018; Hemalatha, Platel, & Srinivasan, 2007; Luo, Xie, Jin, Wang, & He, 2014; Ojha et al., 2018; Platel, Eipeson, & Srinivasan, 2010). When cereals are soaked in water for some days, spontaneous fermentation begins aided by the presence of low sugar levels in the grain. The moist and controlled conditions during germination activate the hydrolytic enzymes in grains, which promotes the germination and fermentation process (Ayernor & Ocloo, 2007; Taylor & Dewar, 2001). The combined endogenous hydrolytic activities such as amylosis, lipolysis and proteolysis together with physiological activities of microorganisms bring about fermentation (Achi & Asamudo, 2018). This is due to respiration, which lowers the pH. The cooking of these processed cereals further improves the availability of nutrients as well as reducing phenolics, tannins, and phytates (Hambidge, 2010; Kumar, Sinha, Makkar, & Becker, 2010; Matuschek, Towo, & Svanberg, 2001). Besides, when complementary foods are prepared from germinated cereals, the palatability is improved, and the calorie density is increased as a result of reduced viscosity (Platel et al., 2010). Although there are many processing techniques, fermentation resulting from germination of cereals is an old method that has been practiced in Africa, since it can be done in any setting and it is simple.

To maximize the bioavailable iron and zinc in MNP, it is essential that more evidence on the appropriate complementary foods to serve as vehicles for micronutrient powder to improve on bioavailability is adduced. An improvement in bioavailability may reduce the potential risks of unabsorbed iron from micronutrient powders, increasing gastrointestinal morbidities that have recently been reported (Paganini, Uyoga, & Zimmermann, 2016).

Although it is evident that the addition of micronutrient powder in porridge does not affect the organoleptic properties of these porridges (de Pee et al., 2008; WHO, 2016b), use of germinated porridges may interfere with the organoleptic properties caused by the effects of processing (Cui, Li, & Liu, 2012). These changes are related to the colour, texture, flavor, and taste of the porridge, which may determine whether the porridge is consumed/accepted or not.

The global nutrition report recommends more studies be conducted at sub-national levels to understand the local dynamics for effective planning and implementation of interventions (United Nations Children's Fund, 2018). As Kenya plans to take this intervention to scale, the findings of this study are essential in informing the choices of complementary foods that promote the bioavailability of iron and zinc from micronutrient powder, since it remains unknown. For this reason, the objective of this study was first, to determine the effects of processing on mineral binders and nutrients in cereal-based porridges and secondly, to compare the iron and zinc bioaccessibility from added micronutrient powder in processed versus non-processed cereal-based porridges typically consumed by Kenyan children. Thirdly, to test the sensory acceptability of the foods that were found to enhance the bioavailability of MNP. Lastly, to determine the prevalence of undernutrition as well as the iron, zinc status, and identify the socioeconomic, morbidity and dietary factors related to malnutrition among of children aged 6-23 months in Homabay County, Kenya.

1.2 Problem statement

According to the Kenya National Micronutrient Survey conducted in 2011, 21.8% and 13.3% of children aged 6–59 months suffer from iron deficiency and iron deficiency anemia respectively. Iron deficiency affects more than one-quarter of infants 6–11 months of age, while iron deficiency peaks at 12–23 months of age affecting 34.6% of them (Kenya National Bureau of Statistics, 2011). According to the data by Hidden hunger index in preschool children, the prevalence of zinc deficiency in Kenya is alarmingly high, considering the range of 45-100% (Muthayya et al., 2013). This is in line with the results from the micronutrient survey conducted eight years ago in Kenya where 85.3% of children aged 6-23 months were found to have low serum zinc levels (Kenya National Bureau of Statistics, 2011). Micronutrient deficiency is directly related to under-nutrition. Children who suffer from malnutrition are most likely to suffer from micronutrient deficiencies. Although we did not find any literature on iron and zinc deficiency in Homabay County, the Kenya Demographic and Health survey revealed high rates of malnutrition in the County.

There still exist challenges in reducing micronutrients deficiency rates, despite decades of research to develop effective interventions to reduce its prevalence and consequences. Many studies have opined that food-based approaches through dietary diversification and modification are sustainable strategies to improve on the consumption of micronutrients (Gibson & Hotz, 2001; Keding & Krawinkel, 2008; Keding, Msuya, Maass, & Krawinkel, 2012; Keding, Schneider, & Jordan, 2013; Oniang'o, 2001). However, Ferguson, Chege, Kimiywe, Wiesmann, and Hotz (2015) using linear programming analysis [LPA] confirmed that dietary adequacy for iron, zinc, and calcium would be challenging to ensure using only local foods as consumed by rural Kenyan children. The study recommends alternative strategies to achieve dietary adequacy for the three micronutrients. Point of use fortification of complementary foods with MNP that contain essential micronutrients is recommended. However, evidence of the best complementary foods that enhance the bioavailability of the essential micronutrients is lacking. Also, there is not much literature on the consumption of processed porridges and plasma zinc status among children 6-23 months in Kenya. It is with this view that this study is assessing the impact of germination of porridges on the amount of iron and zinc bio-accessible from MNP, evaluating the acceptability of the germinated porridge and determining the magnitude of zinc deficiency among children since theirs lack of documentation and scanty data on *in vitro*-dialysability.

1.3 Justification of the study

In Kenya, porridge is the most common complementary food. The porridge is made from cereals that are known to have low levels of micronutrients. Though point of use fortification of porridge with micronutrient powder [MNP] has been recommended to achieve diet adequacy for iron and zinc (WHO, 2016b), the presence of anti-nutrients in plant-based foods hinders the bioavailability of iron and zinc (Roos et al., 2013).

A study by Hotz and Gibson (2007), found that processing of cereal-based porridges reduced the phytic acid content by as much as 90% improving the bioavailability of iron and zinc. Promotion of processing of porridges could significantly improve the dietary adequacy for zinc and iron among Kenyan children who are fed on cereal-based porridges.

1.4 Purpose of the study

The purpose of this study was to determine the effectiveness of germinated porridge on bioaccessibility of iron and zinc from micronutrient powder in children aged between 6 – 23 months and the acceptability of germinated porridges in Homabay County, Kenya.

1.5 Objectives of the study

The objectives of this study were to:

1. Determine the percentage of iron and zinc bio-accessible from micronutrient powder added to germinated porridges in Homabay County, Kenya.
2. Establish the acceptability of germinated porridges among children aged between 6 – 23 months in Homabay County, Kenya.
3. Determine the anthropometric, iron and zinc nutritional status of children aged between 6 – 23 months in Homabay County, Kenya.
4. Establish the commonly used complementary foods and feeding practices among children aged between 6 – 23 months in Homabay County, Kenya.

1.6 Hypotheses of the study

The study tested the following null hypotheses;

1. Ho1: There is no significant relationship between germinated porridge and bioaccessibility of iron and zinc from the micronutrient powder in porridge.
2. Ho2: There is no significant difference in the acceptability of germinated versus non-germinated maize, sorghum and millet porridges.

3. Ho3: There is no significant relationship between complementary foods and the anthropometric, iron, and zinc nutritional status of children aged between 6 – 23 months.

1.7 Significance of the study

This study has provided evidence that germination of porridge when used as a vehicle for MNP, significantly improves the amount of iron and zinc bio-accessible. Further, the study has demonstrated the acceptability of germinated porridges among children and their caregivers. These two findings provide programs an opportunity to increase the effectiveness of MNPs in addressing iron and zinc deficiencies that have remained a chronic public health problem in Kenya.

Besides, this study provides further supportive evidence, that undernutrition remains a weighty public health challenge in Homabay County and identifies vital determinants of malnutrition and iron and zinc deficiencies. The findings of this study underscore the unique challenges and local dynamics at the sub-national level that are essential for effective planning and implementation of nutritional interventions to address zinc and iron deficiencies. The Global nutrition report is advocating for similar studies to unlock the slow pace of progress in addressing malnutrition (United Nations Children's Fund, 2018).

1.8 Assumptions of the study

The study assumed that there would be no significant change in food consumption patterns among the study group during the study period.

1.9 Limitation of the study

1. In vitro dialysability gives a fair estimate of the mineral availability for absorption in vivo. For this reason, our results should be interpreted prudently in implementing interventions.
2. Findings on the acceptability of the porridges solely depended on caregivers' judgement as young children and infants could not give us their formal opinion regarding the sensory tasting of food.
3. DDS and amount of nutrients were based on 1-day 24-hour recall as opposed to 3-day 24-hour recall and therefore not allowing us to capture the day to day variation of diets in the community.

1.10 Conceptual framework

Figure 1.1 illustrates the conceptual framework and selected indicators for this study. The bioaccessibility of micronutrients from the micronutrient powder is influenced by the presence of anti-nutrients in complementary foods resulting in malnutrition.

The study focused on iron and zinc in micronutrient powder and different cereal based porridges as the complementary food. Anti-nutrients [phytates, tannins, and total phenolic content] were analyzed in porridge given to children aged 6-23 months old.

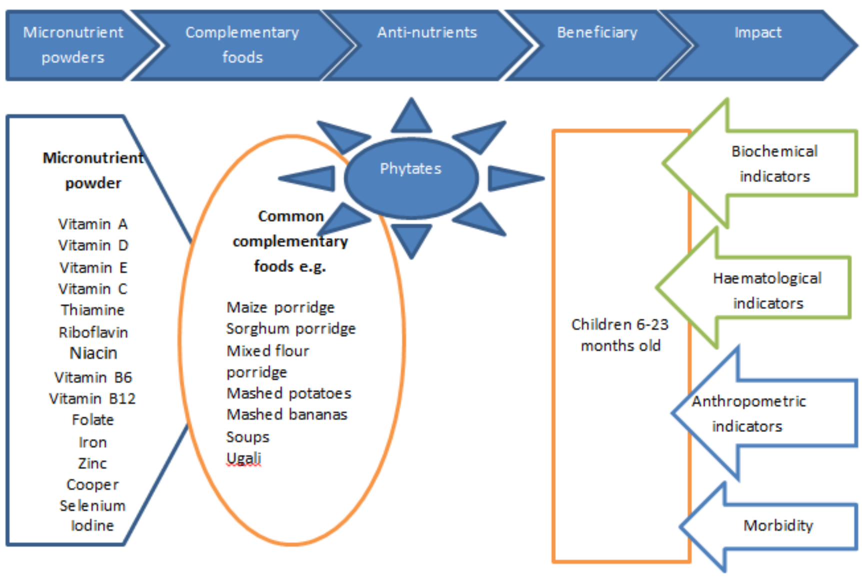

Figure 1. 1: Conceptual framework of bioaccessibility of iron and zinc from micronutrient powder in complementary foods (Das, Salam, Kumar, & Bhutta, 2013).

CHAPTER TWO: LITERATURE REVIEW

2.1 Point of use fortification with micronutrient powder

Food fortification [point-of-use or industrial mass] is among other approaches for intensification of iron and zinc intake in children. While nutrient supplementation, dietary diversification and plant bio-fortification are other strategies used, they will not be discussed in detail because they are considered outside the scope of this thesis compared to food fortification.

Point-of-use fortification with multiple micronutrient powders refers to the addition of powders containing vitamins and minerals to energy-containing foods, at home or in any other place where meals are to be consumed, such as schools, nurseries and refugee camps (Suchdev et al., 2016). The use of multiple micronutrient powders for point-of-use fortification of foods has been suggested by the World Health Organization [WHO] as an alternative to overcome the constraints associated with supplementation and mass fortification (WHO, 2016b). The recommendation by WHO was based on two Cochrane systematic review done on clinical trials conducted in several developing countries which concluded that micronutrient powders are effective (Bhutta et al., 2013; De-Regil et al., 2013).

The micronutrient powder contains vitamin A, vitamin D, vitamin E, vitamin B1, vitamin B2, vitamin B6, vitamin B12, niacin, folate, vitamin C, iron, zinc, copper, selenium and iodine in a one-gram sachet. They are intended to increase the vitamin and mineral intake of infants and young children aged 6 to 23 months (WHO, 2016b). They are not food or a breast-milk substitute and do not mix well with liquids (WHO, 2016b), therefore, children under the age of 6 months should be exclusively breastfed and then have timely introduction of adequate and safe complementary foods, while continuing breastfeeding up to 2 years of age (WHO, 2016a). One gram of micronutrient powder is added to cooked and cooled, complementary food that the child is able to finish based on their age just before feeding the child, it should be mixed well and it should not be added to hot liquids. Therefore, the cereal based porridge is a preferred food because it is the commonest complementary food for children aged 6-23 months in many parts of Kenya.

According to recommendation by WHO (2016b), the suggested scheme for point-of-use fortification of foods with multiple micronutrient powders consumed by infants and young children aged 6–23 months is; the composition of nutrients per sachet is Iron [10 to 12.5 mg of elemental iron], Vitamin A [300 μg retinol], Zinc [5 mg elemental zinc], with or without other micronutrients to achieve 100% of the Recommended Nutrient Intake [RNI]. Considering the diet in low and middle income countries, a total of 90 sachets/doses should be given over a 6-month period [15 sachets per month or a sachet on alternate days] in settings where the prevalence of anemia in children aged under 2 years or under 5 years is 20% or higher (WHO, 2016b). On the contrary, UNICEF suggests one dose [i.e. one sachet] each day to be given to children aged 6-23 months. Based on these recommendations the need for Kenya to adopt this undertaking lies on the 26.3% prevalence of anemia in preschool children. Notably, the specifications for zinc deficiency are not as clear as for iron probably because zinc deficiency is difficult to determine save for the manifestations associated with it compared to iron.

The 12.5 mg of elemental iron equals 37.5 mg of ferrous fumarate or 62.5 mg of ferrous sulfate heptahydrate or equivalent amounts in other iron compounds. In children aged 6–12 months, sodium iron EDTA [NaFeEDTA] is generally not recommended and if selected as a source of iron, the EDTA intake [including other dietary sources] should not exceed 1.9 mg EDTA/kg/day (WHO, 2016b). Multiple micronutrient powders can be formulated with or without other vitamin and minerals in addition to iron, vitamin A and zinc to achieve 100% of the RNI based on WHO/FAO (2004), and also taking into consideration the technical and sensory properties. Where feasible, likely consumption from other sources, including home diet and fortified foods, should be taken into consideration for establishing the composition of the sachet.

Zinc sulfate [ZnS], zinc gluconate [ZnG], and zinc-enriched yeast [ZnY] are commonly used in zinc supplements, and considered three types/generations of zinc sources, that is, inorganic, organic, and biologically organic zinc (Zhang et al., 2018). Zinc sulphate has a strong metallic, bitter, astringent, unpalatable taste that needs to be masked, and causes nausea and vomiting (Zhang et al., 2018), while zinc gluconate with the low zinc content load tends to be more expensive (Bel-Serrat et al., 2014). According to Rider, Davies, Jha, Clough, and Sweetman (2010), currently, any supplementation of commercial and experimental diets is largely from inorganic sources, namely zinc sulphate [ZnSO4], zinc oxide[ZnO], and sodium selenite [Na2SeO3].

Organically chelated minerals are becoming increasingly favoured trace element supplements over inorganic sources because of their increased bioavailability. The chelation of trace elements by organic compounds may protect metal ions against antinutritional factors present in practical diets. Chelated zinc sources are increasingly thought to be more bioavailable than inorganic zinc sources because of the protection of the zinc ion from the formation of insoluble complexes in the digestive tract, thus facilitating absorption from the intestinal lumen (Ashmead, 1993).

2.2 Bioaccessibility of iron and zinc

2.2.1 Difference between bioaccessibility and bioavailability

Bioaccessibility refers to the amount of nutrients released from the food matrix and accessible for absorption (La Frano, de Moura, Boy, Lönnerdal, & Burri, 2014; van Lieshout et al., 2001). It can be measured by in vitro methods like solubility and dialysability. In contrast, bioavailability refers to the ingested nutrient that is absorbed and metabolized through normal pathways, and that is available for utilization in normal physiological functions and / or for storage (La Frano et al., 2014; van Lieshout et al., 2001). Bioavailability can be measured by in vivo methods using animals or humans. Consequently, an in vitro method using CaCo2 cell model can also be used for bioavailability if it is coupled with simulated digestion or dialyzability testing (Etcheverry, Grusak, & Fleige, 2012). Therefore, bioaccessibility is a prerequisite for bioavailability.

2.2.2 Factors that inhibit the bioaccessibility and bioavailability of iron and zinc

Phytic acid

Phytic acid referred to as Myo-inositol 1,2,3,4,5,6-hexakisphosphate [InsP6], is made up of an inositol ring with six phosphate ester groups, and is the principle storage form of phosphorus [P] in cereal grains, and may account for 65%–85% of the total seed phosphorous (Perera, Seneweera, & Hirotsu, 2018; Raboy et al., 2000). It is mainly found in plant tissues especially the bran and seeds, cereals and grains. The six inositol phosphate forms are named according to the number of phosphate groups attached to the inositol ring which are IP1 to IP6. Phytic acid is negatively charged and, thus, strongly chelates cations such as calcium [Ca], magnesium [Mg], potassium [K], iron [Fe] and zinc [Zn] and usually exists as mixed salts referred to as phytate or phytin (Perera et al., 2018; Raboy et al., 2000). These salts have been identified in deterring the absorption of iron and zinc in the human intestine. However, only Inositol hexaphosphates [InsP6] and pentaphosphates [InsP5] have a major inhibitory effect on mineral bioavailability (Sandberg et al., 1999). Therefore, the "total phytate" of a meal or a diet may not be a measure when evaluating zinc bioavailability; instead, methods that specifically quantify the various forms of inositol phosphates are needed when assessing the effects on zinc absorption (Sandberg & Ahderinne, 1986; Zhang et al., 2018). The phosphorus and inositol in phytic acid are generally not bioavailable to non-ruminant animals because they lack the digestive enzyme phytase that hydrolyzes phosphorous from the phytic acid molecule (Marounek, Skřivan, Rosero, & Rop, 2010; Mroz, Jongbloed, & Kemme, 1994; Perera et al., 2018). In addition, phytic acid inhibits enzymes needed for protein degradation in the stomach and small intestine (Kies, De Jonge, Kemme, & Jongbloed, 2006). In spite of the negative effects on human nutrition, phytic acid is also reported to be beneficial as a natural plant antioxidant and as a protector against oxidative stress in seeds (Doria et al., 2009).

Phenolic compounds [phenolic acid, flavonoids, condensed tannins]

Polyphenols are naturally occurring compounds found in fruits, vegetables, cereals and beverages. More than 8,000 polyphenolic compounds have been identified in various plant species. Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another (Spencer, El Mohsen, Minihane, & Mathers, 2008). Polyphenols are classified as flavonoids and non-flavonoids and are found in two major structural formats: attached to sugars which increase its solubility, known as glycosides, or as a single compound known as aglycones (Bravo, 1998; Del Rio et al., 2013; Santhakumar, Battino, & Alvarez-Suarez, 2018). Phenolic acids are the most common non flavonoid phenolic in the human diet followed by the hydrolysable tannins which are divided into two subclasses: gallotannins and ellagitannins (Gabaza, Muchuweti, Vandamme, & Raes, 2017; Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Other tannins are in condensed forms for example the proanthocyanidins.

Phenolic acids are found abundantly in foods and divided into two classes: derivatives of benzoic acid and derivatives of cinnamic acid. Insoluble phenolics are found in cell walls, while soluble phenolics are present within the plant cell vacuoles (Pandey & Rizvi, 2009; Wink, 1997). The outer layers of plants contain higher levels of phenolics than those located in their inner parts (Fernandez de Simon, Perez-Ilzarbe, Hernandez, Gomez-Cordoves, & Estrella, 1992; Pandey & Rizvi, 2009). While most cereals contain approximately 1% of phenolic compounds, sorghum has close to 10% (Bravo, 1998; Gabaza et al., 2017).

Polyphenols, like phytate, form insoluble complexes with metal cations that inhibit intestinal absorption of non-heme iron (Bravo, 1998; Gibson, 2007), and zinc (Coudray, Bousset, Tressol, Pépin, & Rayssiguier, 1998; Gibson, 2007), but not calcium (Van Leeuwen, Koninkx, Jansman, & Mouwen, 1993).

Dietary Fiber

Dietary fibers are mainly indigestible complex carbohydrates in plant cell walls [cellulose, hemicellulose, and pectin] including lignin which is a non-carbohydrate component (Thebaudin, Lefebvre, Harrington, & Bourgeois, 1997). These fibres can be divided into soluble and insoluble. Soluble fibres dissolve in the stomach, resulting in the formation of a gel which entraps minerals hindering the cell wall absorption in the intestine. Dietary fibre inhibits iron and zinc bioavailability probably due to the presence of phytate in the same foods.

Reducing the phytate content of bread by leavening considerably increased zinc absorption to a degree similar to that from white bread [low fiber], suggesting that fiber in itself has no or little effect on zinc absorption (Lonnerdal, 2000). Studies on isolated fiber components such as a-cellulose (Lonnerdal, 2000) show no significant inhibitory effect on zinc absorption. It is apparent that fibre may not have an inhibitory effect in zinc absorption.

Calcium

Calcium intake affects both the absorption of non heme and heme iron (Etcheverry et al., 2012). The effect on zinc absorption could be due to the calcium content of the diet from phytate containing meals. The reason for this is that calcium is able to form insoluble complexes with phytate and zinc affecting zinc absorption (Lonnerdal, 2000). Zinc and calcium combined forms a stronger bond with phytates compared to other single minerals (Coulibaly, Kouakou, & Chen, 2011; Gabaza, Shumoy, Muchuweti, Vandamme, & Raes, 2018; Greiner & Konietzny, 2006). It is still not clear whether the adverse effect of inositol phosphates on calcium absorption is restricted to the higher inositol phosphates, such as IP5 and IP6 (Gibson, Bailey, Gibbs, & Ferguson, 2010; Gibson R. S., Raboy, V., & King, J. C., 2018).

Although the critical molar ratio above which calcium absorption is compromised by phytate is uncertain, the phytate-to-zinc molar ratio has been suggested and used because of its better predictability than the ratio that includes calcium as a variable (Lonnerdal, 2000). In spite of this, some investigators have suggested a phytate: calcium molar ratios of less than 0.17 (Gibson et al., 2010; Gibson R. S. et al., 2018; Umeta, West, & Fufa, 2005b).

Notably, from most studies, the effect of calcium on zinc absorption seem to be understood better compared to iron. An interaction between calcium and food components that affect iron bioavailability, or an effect of calcium on the luminal surface receptors that mediate iron uptake into enterocytes might explain the inhibitory effect of calcium since iron and calcium are absorbed by independent cellular mechanism (Lynch, 2000). A CaCo-2 cell study sought to find out whether the interaction between calcium and iron was a lumenal event, affecting iron uptake (Lönnerdal, 2010). From that study calcium did not affect iron uptake or divalent metal transporter 1[DMT1] expression at 1.5 hours, but serosal exporter ferroportin [FPN] abundance at the basolateral membrane decreased, resulting in increased cellular iron retention and decreased iron efflux. After 4 hours, DMT1 and FPN expression increased and there was increased FPN at the membrane, suggesting a rebound effect. Thus, the effect of calcium on iron absorption may be of short duration and adaptation may occur with time (Lönnerdal, 2010). However, it has been suggested, when considering the effects of calcium on iron absorption the relative doses and sources of calcium and iron should be considered (Beck & Coad, 2017). This could be due to the fact that the inhibitory effect of calcium on iron absorption from complete diets appears to be less than that from single meals, and long term calcium intake over several weeks does not appear to impact on an individuals' iron status in healthy people consuming a varied diet (Beck & Coad, 2017; Lönnerdal, 2010). Additionally, a randomised controlled trial found no differences in either heme iron or non-heme iron bioavailability either at baseline or after treatment when calcium supplements were given to non-pregnant women for one month (Ríos-Castillo, Olivares, Brito, Romaña, & Pizarro, 2014).

Other factors that have been found to inhibit iron and zinc bioavailability that are nod discussed in this thesis are, oxalic acid, zinc and iron interactions, other nutrients like cadmium, copper, phosphitin, and the effects of low molecular weight ligands and chelators in food.

2.2.3 Factors that promote the bioaccessibility and bioavailability of iron and zinc [emphasis on germination]

Soaking, germination, malting and fermentation

Soaking and germination precedes the process of malting and fermentation. Germination not only improves sensory properties of foods but also their nutritional content and the amounts available for absorption. Although several techniques are available, germination is commonly used since it has been existing for a while (Singh, Rehal, Kaur, & Jyot, 2015). This method has been used for a long time probably because they can be available at any setting, it is relatively simple and cheap.

Fermentation can be natural [spontaneous] or controlled. When cereals are soaked in water for days [depending on the desired product] Similar to spontaneous fermentation is back-slopping which is mostly practiced in households due to its simplicity. Since spontaneous fermentation can result to undesirable dangerous microorganism (Galati, Oguntoyinbo, Moschetti, Crescimanno, & Settanni, 2014), controlled fermentation seeks to select strains and starter cultures [for example LAB and yeasts] that are safe.

Dry grains are metabolically inactive but with the addition of water, fermentation process begins aided by the presence of low sugar levels in the grain. According to Achi and Asamudo (2018), the addition of water to the grains make them absorb water which ultimately stimulates the enzyme into action and growth of microorganisms which triggers the process of fermentation. This is due to respiration which lowers the redox potential and pH. The breakdown of starch to simple fermentable sugars such as maltose and glucose is supported by cereal endogenous enzymes, selected enzymes or added malt (Achi & Asamudo, 2018). Malting and germination of cereals promote the growth of molds which also produce amylolytic enzymes powerful enough for hydrolysis and liquefaction. The combined endogenous hydrolytic activities such as amylosis, lipolysis, proteolysis and physiological activities of microorganisms bring about fermentation (Achi & Asamudo, 2018).

Several studies around the world have shown the effects of fermentation in reducing antinutrients and improving the availability and bioaccessibility of iron and zinc. According to Cui et al. (2012), fermentation caused significant increase in protein [43.5%], amino acid [131.5% in lysine content] and a reduction in total phenolic content [23.4%] and phytic acid content [24.3%] of four maize varieties in China. In the same study fermentation caused colour changes [increase in a-values and reduction in L-values] in two maize varieties (Cui et al., 2012). In India, germination of food grains improved the bioaccessibility of iron but not that of zinc. In that study, zinc bioaccessibility was significantly decreased in finger millet by 38% when geminated for 48 hours, while iron bioaccessibility was increased by 20% in relation to a reduction in tannin content (Hemalatha et al., 2007). Similar results were reported in the same country when malting increased the bioaccessibility of iron by >3-fold from the two varieties of finger millet, but reduced the bioaccessibility of zinc (Platel et al., 2010). Fermentation effects varies based on the type of grain and even the location where it is grown as reported in Zimbabwe where fermentation caused a reduced phytic acid by 20-88%, and iron and zinc bioaccessibility ranging from 2.77 and 26.1% respectively, in fermented maize, sorghum and millets from different locations (Gabaza M. et al., 2018). In Kenya, fermentation resulted in a mean decrease of phytic acid by 64.8% after 96 hours and 39.0% after 72 hours in sorghum grain, while in finger millet, there was a mean decrease of 72.3% and 54.3% after 96 and 72 hours, respectively. Malting also resulted in a mean decrease of 23.9 and 45.3% after 72 and 96 hours, respectively. There was also an increased rate of available iron, manganese, and calcium in both sorghum and finger millet, with the available minerals being higher in finger millet than in sorghum after fermentation. The same study showed, the effectiveness of fermentation compared to malting in reducing phytic acid in sorghum and finger millet (Makokha, Oniang'o, Njoroge, & Kamar, 2002).

2.2.4 Methods used to estimate iron and zinc bioaccessibility and bioavailability

The methods for in vitro bioaccessibility [solubility and dialysability], in vitro bioavailability [CaCo-2 cell models] and in vivo bioavailability [animal models and human studies] are used to estimate iron and zinc bioaccessibility or and bioavailability. Bioavailability of iron and zinc in humans is determined by these sequential six stages; digestion and release of nutrients from food matrix into the gastro intestinal tract [nutrient availability], transport into intestinal enterocytes [nutrient uptake], efflux across the basolateral membrane of enterocytes into the circulation [nutrient absorption], retention or endogeneous excreation in urine and faeces [nutrient retention], transport to tissues for use in normal body functions [nutrient utilization], and transport to storage sites [nutrient body stores] (Fairweather-Tait et al., 2005). In vitro models are designed to predict nutrient availability [dialysability], nutrient availability and uptake [dialysability combined with CaCo-2 cells] and, nutrient absorption [when CaCo-2 cells are grown on transwell membranes in different iron concentrations]. Contrary, in vitro models cannot be used to measure nutrient retention, utilization or storage (Fairweather-Tait et al., 2005). In spite of this, algorithms may be used to predict the composite effect of all the six stages in population-based data (Fairweather-Tait et al., 2005). In this thesis all the methods will be described briefly except for dialyzability which is within the scope of this study.

In vitro models

Dialysability is used to estimate non- heme iron and zinc availability from the food matrix during simulated digestion (Fairweather-Tait et al., 2005). The stages involved in the simulated digestion are gastric and intestinal stage. At the gastric stage, the sample is weighed, mixed with deionized water, and the pH is adjusted to 2.0 by adding 6 M HCL

(Kruger Johanita et al., 2013; Miller, Schricker, Rasmussen, & Van Campen, 1981). Then freshly prepared pepsin solution is added and the sample incubated for 2 hours in a shaking water bath at 37^0C. The titratable acidity is measured from the gastric digests by adding freshly prepared pancreatic mixture, then the pH is adjusted to 7.5 with 0.5 M NaOH and the titratable acidity is defined as the amount [moles] of 0.5 M NaOH required to attain pH of 7.5 (Kruger Johanita et al., 2013; Miller et al., 1981). At the intestinal stage, deionized water and $NaHCO_3$ [equivalent in moles to the NaOH used to determine the titratable acidity] are added to the 12.5cm dialysis tube, then put to the Erlenmeyer flask containing the gastric digest to incubate in a shaking water bath for 2 hours at 37^0C, coupled with the addition of pancreatic mixture to the digest in the first 30 minutes of the incubation. The dialysis bag is rinsed, dried and the contents transferred into sterile/acid washed tubes. The contents [dialysate] is added with 0.03ml concentrated nitric acid and analysed for iron and zinc content by ICP-OES. The bioaccessibility is the percent of mineral available when compared to the total mineral (Kruger Johanita et al., 2013; Miller et al., 1981).

The dialysis membrane [tube] used has a defined molecular weight capacity cut-off. The proportion of the element diffused through a semi-permeable membrane during the intestinal stage represents the dialysability of the element after an equilibration period and is used as an estimate of the proportion of the element that is available for absorption (Kruger Johanita et al., 2013). The assumption in this model [simulated digestion and dialysis tube] is that the membrane will filter out large-molecular-weight complexes likely to be unavailable for absorption while allowing more bioavailable small-molecular-weight complexes to penetrate the membrane (La Frano et al., 2014).

Dialysability is used for screening iron-containing foods for differences in micronutrient accessibility by taking into account diet composition, the chemical form of iron, and food-processing effects (La Frano et al., 2014). There is evidence that in vitro iron and zinc dialyzability strongly correlates with in vivo human data (Chiplonkar, Agte, Tarwadi, & Kavadia, 1999). However, for dialysability results to make sense in real life setting, foods need to be made in the manner in which they are normally consumed (Fairweather-Tait et al., 2005). In this study all the foods analysed by dialysability were prepared and cooked as they are consumed by children in Homabay County, Kenya.

In vitro simulated models like solubility and micellerization, simulate oral, gastric, and small intestine digestion by applying a series of digestive enzymes and pH conditions to assess the effects of the food matrix and processing on the digestive stability and bioaccessibility of a nutrient (Failla & Chitchumronchokchai, 2005). This method has been used alone extensively to evaluate the bioaccessibility of carotenoids from foods, meals, and supplements (Lipkie et al., 2013). However, results from this method vary from different studies because of factors like Ph and incubation period during the experiment, thus, simulated digestion studies cannot always be depended on as substitutes for human studies (La Frano et al., 2014). This method can only be used for iron and zinc estimation if it is combined with dialysability and Caco2 cell model (Lipkie et al., 2013).

Human intestinal Caco-2 cells are colonic carcinoma cells that display morphological and functional characteristics similar to those of differentiated epithelial cells from small intestinal mucosa (Sambruy, Ferruzza, Ranaldi, & De Angelis, 2001). Iron uptake is estimated through either ferritin formation or the use of a radioisotope [^{59}Fe] while zinc uptake is usually estimated by using a radioisotope [^{65}Zn] (Etcheverry et al., 2012). In the case of iron, CaCo-2 cells are grown on trans-well membranes to study various systematic effects on iron metabolism [absorption] by growing the cells in different iron concentrations and /or manipulating the media in the basolateral well to simulate the effect of high stores (Fairweather-Tait et al., 2005). CaCo-2 cells predict the correct direction of response for all major iron absorption modifiers and zinc (Fairweather-Tait et al., 2005). However, CaCo-2 cell methodologies vary significantly between laboratories, and it requires good quality cell culture facilities to obtain reliable and reproducible results (Fairweather-Tait et al., 2005). Essentially, Caco-2 cell models can assess in vitro bioavailability when combined with simulated digestion and/or dialyzability.

In vivo models

Animal models are used for in vivo bioavailability because they can be dissected and individual tissues analyzed for absorption. However, their physiological response in absorption and metabolism is different from humans. Rodents are commonly used in iron studies but they are known to synthesize ascorbic acid leading to an overestimation of iron bioavailability, while the rat pup model is used for zinc because they do not have intestinal phytase (Bates, 1997). Although experimental animals can have physiological responses similar to those in humans and can allow for the analysis of specific tissues, no animal model can be considered to digest and absorb nutrients exactly the same as humans do (La Frano et al., 2014).

The use of radioactive and stable isotopes in human studies allows for the discrimination between the dose of micronutrient provided and endogenous forms of the micronutrient, allowing for a more accurate measurement of bioavailability. Micronutrients have been labeled both intrinsically and extrinsically. Intrinsic labeling is the biological incorporation of an isotope into the plant or the animal during its growth, while extrinsic labeling is the addition of an isotope to food prior to ingestion (Fairweather-Tait et al., 2005; La Frano et al., 2014). It has been shown that extrinsic labeling can be used for non-heme iron [as in plant foods] and zinc absorption studies in humans (Flanagan, Cluett, Chamberlain, & Valberg, 1985; La Frano et al., 2014).

To estimate iron and zinc bioavailability, mathematical models have been developed. One such model is the algorithms which has been developed to predict the bioavailability of iron and zinc from mixed diets. These equations are based either on human radioisotope studies measuring iron absorption or on food recalls and dietary records that are compared with iron status biomarkers from sample populations (La Frano et al., 2014). In order for algorithms to be considered useful predictors of bioavailability, they must consistently predict iron or zinc absorption in study populations of similar physiological status and across a wide range of combinations of food components. Algorithms derived from isotopic absorption studies provide semi-quantitative information such as the classification of diets into high, moderate or low bioavailability (Fairweather-Tait et al., 2005). Unfortunately, a single algorithm to predict iron absorption has not been validated (La Frano et al., 2014). Nevertheless, for zinc, two commonly used models are the International Zinc Nutrition Consultative Group [IZiNCG] (Hotz & Brown, 2004a) model and the Miller, Krebs, and Hambidge (2007) model.

2.2.5 Bioaccessibility of iron and zinc in human

Iron

Absorption of dietary iron depends on whether it is haem iron or non-haem iron. Haem is a component of haemoglobin and myoglobin and haem iron is complexed as ferrous iron [Fe2+] in the haem form, which is present in animal tissues such as meat, poultry, fish and shellfish. Non-haem iron [Fe3+ or ferric iron] is mostly provided by the plant based diets for example cereals and vegetables. In as much as haem iron is better absorbed compared to non-haem iron, it only accounts for less than 15% of total iron intake (Lopez, Cacoub, Macdougall, & Peyrin-Biroulet, 2016). Majority of diets in developing countries are mostly plant based despite the low absorption of non- haem iron. Iron absorption could be enhanced by vitamin C, organic acids and consuming flesh foods like meat. However, antinutrients, calcium and zinc have been known to inhibit iron absorption (Zimmermann, Chaouki, & Hurrell, 2005).

Dietary iron is absorbed by enterocytes of the duodenal lining. The first step to absorb iron is that the insoluble ferric iron [Fe3+] has to be converted into the ferrous form [Fe2+] by a brush border ferric reductase [duodenal cytochrome B, DCYTB] in the duodenum and upper jejunum. The ferrous iron is then transferred across the enterocyte membrane into the cell by divalent metal transporter 1 [DMT1]. Afterwards, the iron is stored in these intestinal lining cells as ferritin which is accomplished by Fe3+ binding to apoferritin, or could be released into the body via the only known iron exporter ferroportin. Cooperated with either of the ferroxidases hephaestin [enterocytes] or ceruloplasmin [other cell types] that facilitate iron extraction from the ferroportin channel, ferroportin at the basolateral membrane transports Fe^{2+} that is subsequently loaded onto plasma transferrin [Tf] (Hentze, Muckenthaler, Galy, & Camaschella, 2010).

In blood, plasma iron-loaded transferrin [Tf-Fe^{2+}] transports iron to all cells in a transferrin receptor-mediated endocytotic process. Iron-loaded Tf binds to transferrin receptor 1 [TfR1], and their complex internalizes and enters into cells, wherein iron releases by pH-dependent mechanism. As it provides most of the iron required for various functions of an organism, plasma transferrin plays a crucial role in iron metabolism. TfR1 is highly expressed on haemoglobin-synthesizing erythroblasts. Most of plasma iron is used by bone marrow to synthesize haemoglobin in red blood precursors. Several other cell types, such as enterocytes, hepatocytes and reticuloendothelial macrophages, are considered to be major iron storage sites. Iron release from or store in enterocytes, hepatocytes and macrophages depends on plasma iron levels, to meet the physiological demand. Figure 2.1 summarizes this mechanism. Iron balance in the body is regulated to avoid iron deficiency and excesses. Hepcidin is a core regulator of the entry of iron into the circulation, which is a peptide hormone synthesized mainly in the liver, which was discovered in 2000 (Khan, 2018).

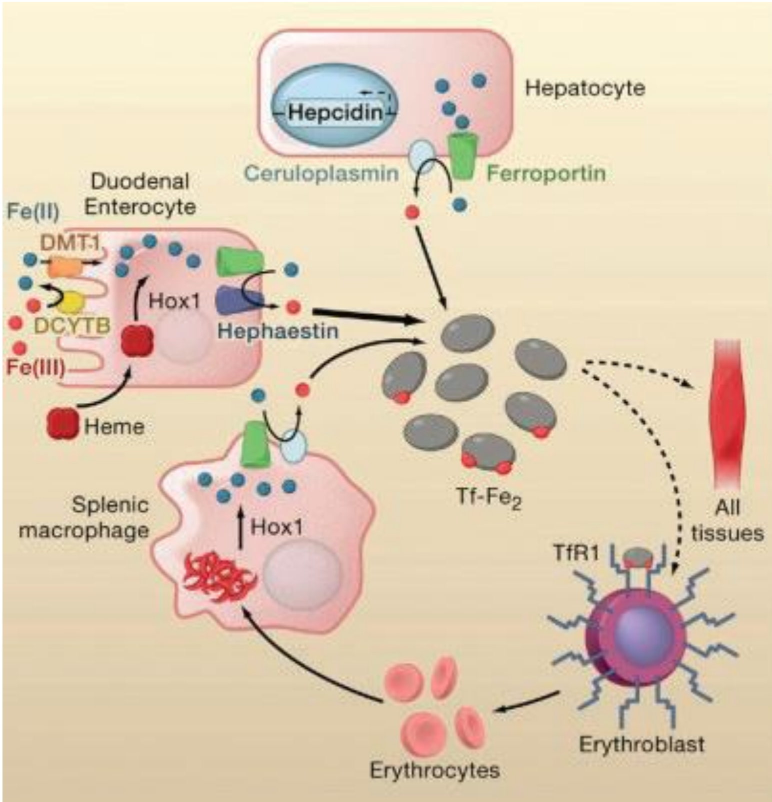

Figure 2. 1: Mechanism of iron metabolism (Hentze et al., 2010).

Iron overload and anaemia are disorders of iron metabolism with the latter being the most prevalent. Iron deficiency anaemia affects children, adults and the elderly in different ways. In pregnancy, iron deficiency anemia results to perinatal mortality, pre-term birth, low birth weight, poor offspring intellectual development and motor development. In one study prenatal supplementation with iron-folic acid was associated with a reduction in early neonatal mortality compared with prenatal folic acid supplementation only (Zeng et al., 2008). For non-pregnant women, the effects are mainly on cognitive ability, physical performance and mood. One study conducted in the United States reported iron status was significantly associated with cognitive ability in women of reproductive age. The iron-sufficient women completed the cognitive tasks faster than women with iron deficiency anaemia, and got higher accuracy of cognitive function over a broad range of tasks (Murray-Kolb & Beard, 2007).

In infants, the effects are on social-emotional, cognitive and motor development. An observational study conducted in African-America found poorer motor function in iron deficiency infants (Shafir et al., 2008). Another longitudinal study found lower motor scores in infants with chronic iron deficiency anaemia, and iron treatment at the age of 12–23 months did not prevent long-term effect of iron deficiency in infancy on motor development (Shafir et al., 2008). A study conducted in the United States showed positive effects of iron status of infants at 9 months on gross motor development and motor coordination or sequencing (Shafir et al., 2008). Among the elderly, the effects are on cardiovascular disease, all-cause mortality, physical functioning impairment, increasing risk of death from ischemic heart disease and symptoms of depression. A cohort study conducted in Taiwan reported iron deficiency was significantly associated with cardiovascular disease and all-cause mortality in elderly (Hsu et al., 2013). Iron overload on the other hand results to tissue damage caused by the formation of free radicals (Lieu, Heiskala, Peterson, & Yang, 2001).

Zinc

The amount of zinc in the human body is about 2-3 g with 90% of it found in muscle or bone (Wastney, Aamodt, Rumble, & Henkin, 1986).Oral uptake of zinc leads to absorption throughout the small intestine and distribution subsequently occurs via the serum, where it exists bound to several proteins such as albumin, α-microglobulin, and transferrin (Scott & Bradwell, 1983).To avoid accumulation of zinc in the body cells, the cellular homeostasis of zinc is mediated by two protein families; the zinc-importer family, containing 14 proteins that transport zinc into the cytosol, and the zinc transporter family, comprising 10 proteins transporting zinc out of the cytosol (Lichten & Cousins, 2009). Zinc plays an important role in signal transduction; gene expression; regulation of apoptosis; metabolism of carbohydrates, proteins, and lipids; maintenance of structures of over 300 enzymes; proper growth; sensory organ adjustment; and reproductive function (Khan, 2018).

An inverse relationship between dietary zinc quantity and the amount of zinc absorbed is evident. Increasing amount of zinc intake from diet results to decreased percentage of fractional zinc absorption(Lonnerdal, 2000). It is likely that the reduced fractional absorption of zinc at higher doses is due to saturation of the transport mechanisms for zinc (Lonnerdal, 2000). The type of diet determines the bioaccessibility of zinc where plant based diets have 10-15% of zinc absorbed (Fairweather-Tait & Hurrell, 1996). The dietary factors that influence zinc absorption are; amount of zinc in a meal, amount of protein in a meal, cadmium, copper, calcium, antinutrients [phytates and fiber], interaction between iron and zinc, low molecular ligands and chelators (Lonnerdal, 2000).

Zinc disorders

Zinc disorders due to excessive zinc intake or zinc deficiency affects different organ systems in the body. Zinc deficiency unlike many other deficiencies is difficult to diagnose. It is associated with numerous manifestations, such as diarrhea, pneumonia, malaria, growth retardation, hypogonadism in males, eye and skin lesions, impaired immunity and appetite, neuro-sensory disorder, and cognitive impairment (Zhang et al., 2014). In contrast excess zinc causes neural deficits in the brain, metal fume fever in the respiratory tract, nausea and vomiting in the gastro intestinal tract and risk of prostate cancer (Plum, Rink, & Haase, 2010).

2.3 Acceptability of MNPs in germinated porridges

As reviewed literature suggests, it is evident that the addition of micronutrient powder in porridge does not affect the organoleptic properties of these porridges (de Pee et al., 2008; WHO, 2016b). However, use of germinated porridges may interfere with the organoleptic properties caused by the effects of processing (Cui et al., 2012). These changes are related to the colour, texture, flavour and taste of the porridge which may determine whether the porridge will be consumed/accepted or not.

Sprouted cereals when cooked have been reported to cause colour changes [more reddish and yellowish] in the final product (Baranzelli et al., 2018). These change in colour is most likely non-appealing resulting to the product not being acceptable. Longer sprouting time has also been reported to interfere with the flavor of germinated products due to increased lipase and lipoxygenase action (Dong et al., 2013). These may further negatively alter the taste and texture of the germinated food since it is likely to be rancid, fatty and have undesirable bean-like flavours (Wu, Yang, Chen, Jin, & Xu, 2011). Notably, germination has different effects on different cereals making the final product either to be accepted or not. In view of this, it is important to assess whether germinated porridges will be acceptable among children.

2.4 Nutrition assessment: Anthropometric and biochemical

When children from the ages of 0 to 59 months are below 2 standard deviations from the median weight for height, height for age and weight for age as determined by the World

Health Organization child growth standards, they are wasted, stunted and underweight, respectively (UNICEF; WHO, 2006). Wasting is low weight for height, generally due to weight loss associated with a recent period of starvation. Wasting measures acute malnutrition. Severe acute malnutrition is WHZ score <-3 SD, moderate acute malnutrition is WHZ score -3 to <-2 SD, and global acute malnutrition is a combination of both severe and moderate forms of acute malnutrition. Stunting is low height for age, reflecting a sustained past episode or episodes of undernutrition. Stunting measures chronic malnutrition. Severe chronic malnutrition is HAZ score <-3 SD, moderate chronic malnutrition is HAZ score -3 to <-2 SD, and global chronic malnutrition is a combination of both severe and moderate forms of malnutrition. Underweight is low weight for age, reflecting a current condition resulting from inadequate food intake, past episodes of undernutrition or poor health conditions i.e. measures wasting and stunting combined. Severe undernutrition is WAZ score <-3 SD, moderate chronic undernutrition is WAZ score -3 to <-2 SD, and global undernutrition is a combination of both severe and moderate forms of undernutrition.

Anemia, which is defined as a decreased amount of red blood cells or hemoglobin in the blood, is an indicator of poor nutrition and health. According to the (WHO, 2007), the cut-off value to be used to define anemia in infants 0.5 to 1 year is a hemoglobin level less than 110mg/dl.

Zinc deficiency may occur when any or a combination of these factors are present; severe stunting, low serum zinc levels, episodes of persistent diarrhea, a diet that is low in animal products and based on high phytate cereals and legumes, especially during the period of complementary feeding (Allen, 1998). Zinc and iron deficiencies usually coexist since diets that provide inadequate amounts of zinc are likely to provide inadequate amounts of iron as well, because animal products are the best sources of both nutrients (Allen, 1998). Because diets in developing countries are predominantly based on plants and often high in phytates, which inhibit zinc absorption strongly, it can be difficult for children in these countries to obtain their recommended intake of zinc from their usual diet. Zinc requirements for infants and children aged 6-24 months is estimated to be 2.8mg/day and complementary food must provide 84-89% since breastmilk does not provide an abundant amount of zinc (Allen, 1998). The recommended cutoff among those less than 10 years of age for samples collected in the morning hours in a non-fasted state is 65µg/dl (Hess, Peerson, King, & Brown, 2007; Hotz & Brown, 2004b).

2.5 Macronutrient and micronutrient deficiencies [wasting, underweight, stunting, iron and zinc]

According to the Joint child malnutrition estimates 2018, globally, 22.2% of children under five are stunted while 7.5% are wasted (United Nations Children's Fund, 2018). Asia and Africa still bear the greatest burden of malnutrition in the world with the prevalence of stunting being 55% and 39%, respectively. While for wasting is 69% in Asia and 27% in Africa. In Eastern Africa where Kenya is situated, the prevalence of stunting is rated among the highest globally which is at 35.6%. Whereas there is a reduction in the number of stunted children in the world, Africa is the only region with increased number of stunted children since 2000 to 2017. The prevalence of wasting in Eastern Africa is 6% which is considered medium level. However, more than half of the wasted children globally live in Southern Asia region. According to the KDHS 2014, 26% of children less than five years are stunted, 4% are wasted and 11% are underweight in Kenya (Kenya National Bureau of Statistics, 2014). In Nyanza where Homabay County is located, the prevalence of stunting, wasting and underweight is 22.7%, 2% and 7.4%, respectively. In Homabay County, the prevalence of stunting is 18.7%, wasting 2.3% and underweight 5.4%.

Globally 273.2 million preschool children are anemic and of this 9.6 million have severe anemia (WHO, 2015). South-East Asia, Mediterranean and Africa regions have the highest prevalence levels worldwide. In Africa the prevalence of anemia and severe anemia among children 6-59 months is 62.3% and 3.6%, respectively. Based on the global estimates data, the prevalence of anemia among infants and children in Kenya is between 40.0% to 59.9%. In 2011, the prevalence of anaemia and severe anemia in Kenya was estimated at 46% and 2.0%, respectively, which was considered severe (WHO, 2015). In a recent study in 3 counties in Western Kenya, the results showed that the prevalence of anaemia in 6-24 months children was 48.8% (Stewart et al., 2019). Using only the ferritin cutoff of<12µg/L, the prevalence of iron deficiency in their study was 29.9%. In Maasai, Southern part of Kenya, the prevalence of anaemia among children 3-5 years was 38% (Houghton et al., 2019). According to the Kenya National Micronutrient Survey conducted in 2011, 21.8% of children 6–59 months of age suffer from iron deficiency, whereas iron deficiency anaemia affects 13.3% of children . Iron deficiency affects more than one quarter of infants 6–11 months of age, while iron deficiency peaks at 12–23 months of age 34.6%; (Kenya National Bureau of Statistics, 2011).

An estimated 17% of the entire population globally and 50% of the entire population in Sub-Saharan Africa is at risk of zinc deficiency (Wessells & Brown, 2012). According to the data by Hidden hunger index in preschool children, the prevalence of zinc deficiency in Kenya is alarmingly high considering the range of 45-100% (Muthayya et al., 2013). This is in line with the results from the micronutrient survey conducted eight years ago in Kenya where 85.3% of children aged 6-23 months were found to have low serum zinc levels (Kenya National Bureau of Statistics, 2011). However, we never found any documented data on the prevalence of zinc deficiency in Homabay county.

2.6 Factors associated with malnutrition: socioeconomic, morbidity and dietary

According to the UNICEF 1990 conceptual framework on the causes of malnutrition, inadequate dietary intake and disease are the immediate causes of malnutrition, while insufficient health services and unhealthy environment, inadequate care for mothers and children, and inadequate access to food are the underlying causes. Underlying causes lead to immediate causes. Generally, child health, food selection and socioeconomic factors are common predictors to malnutrition.

Malnutrition in children 6-24 months is caused by poor infant and young child feeding practice. The WHO and United Nations International Children's Emergency Fund recommends early initiation of breastfeeding within an hour after birth, and exclusive breastfeeding for the first 6 months during infancy with timely and appropriate initiation of complementary feeding (WHO, 2010). Worldwide, only a few children acquire adequate nutrition along with proper complementary feeding which is appropriate for their age group they belong to (WHO, 2010). In developing countries, these sub-optimal feeding practices of infants and young children contribute to the prevailing burden of malnourishment (Akber et al., 2019). In South Asia, lack of complementary food knowledge, low maternal education, socio-economic status and cultural beliefs were reported influencing factors for infant and young child complementary feeding practice among children less than 2 years old (Manikam et al., 2018).

Micronutrient deficiencies of iron and zinc among children is a consequence of complex interactions of several factors. A number of studies have shown that iron deficiency anemia is associated with low birth weight, sex, age, rural residence, infectious disease like malaria, tuberculosis, intestinal parasitic infestation, and HIV/AIDS, undernutrition; stunting, wasting, and underweight, poor socioeconomic status, household food insecurity, duration of lactation and poor dietary iron intake, poor maternal educational status, and maternal anemia (Ngesa & Mwambi, 2014; Pasricha et al., 2010; Pollitt, 1999; Shet et al., 2009; Villalpando, Shamah-Levy, Ramírez-Silva, Mejía-Rodríguez, & Rivera, 2003). Nutritional anemia is a majorly caused by inadequacy in diets. A range of foods when consumed regularly improves nutrient intake (Korkalo et al., 2017). Besides, greater dietary diversity has been shown to reduce the chances of having nutritional anaemia among pre-schoolers (Gashu et al., 2016). On the other hand, zinc deficiency has been reported to be associated with severe stunting, low serum zinc levels, episodes of persistent diarrhea, a diet that is low in animal products and based on high phytate cereals and legumes, especially during the period of complementary feeding (Allen, 1998). Notably, iron and zinc deficiencies coexist because animal products are rich sources of both nutrients.

2.7 Summary of literature review and research gap

Reviewed literature reveals that micronutrient deficiencies of iron and zinc in children coexist and the prevalence rate is alarmingly high. Insufficient nutrient intake from diets and poor feeding practices among other factors exacerbate child malnutrition. Therefore, point of use fortification of complementary foods with added micronutrient powder containing iron and zinc has been promoted to enhance child health and nutrition based on the efficacy studies cited. However, the common complementary foods such as porridges are prepared from cereals that have high levels of phytic acid, tannin or total phenolic compounds which are known to hinder the bioavailability of iron and zinc. In spite of this, germination of cereals like maize, sorghum and millet have been shown to reduce these inhibitors significantly, promoting nutrient bioavailability. Germinated porridges are also tastier and acceptable among children making it a suitable vehicle for fortification. While germination is evidently useful, safe, practical, sustainable and affordable, the *in-vitro* bioaccessibility of iron and zinc from added micronutrient powders in cereal-based porridges remains unknown. Theirs also lack of documentation and scanty data on *in vitro*-dialysability.

CHAPTER THREE: METHODOLOGY

3.0 Introduction

This study was conducted in three phases. Phase one included the laboratory analysis of germinated porridges to determine mineral contents, anti-nutrients levels and, *in vitro* bioaccessibility of iron and zinc from MNP. Phase two was the controlled trial done in the community to establish the acceptability of different germinated porridges with added MNP among children, while phase three was the cross sectional nutrition survey to establish the anthropometric, iron and zinc nutritional status of children.

3.1 Phase one: Bioaccessibility of iron and zinc from MNP in porridges

3.1.1 Materials and methods

Laboratory experimental design was adopted to determine the bioaccessibility of iron and zinc from micronutrient powder in either germinated or non-germinated maize, millet and sorghum porridges. Therefore, the analyzed samples in this study were six in total. They are cooked germinated maize, millet and sorghum porridges with added micronutrient powder and non-germinated maize, millet and sorghum porridges with added micronutrient powder. Dry white maize, finger millet, and red sorghum cereals were purchased from Ndhiwa open air market in Homabay County, Kenya.

3.1.2 Sample collection procedures and study site

West Kwabwai Division in Homabay County of Kenya was selected as the study site because of the high level of malnutrition among children (Kenya National Bureau of Statistics, 2014). A pilot study conducted in October 2016 identified cereal based porridges, specifically maize, as the common complementary food fed to children aged 6-23 months. To understand the different local methods of cooking porridge, 4 focus group discussions [involving 8-12 women per group] were conducted in the four locations [Kadhola, Lwandawiti, Wachara, Gotkojowi] in the study area. Later these women were tasked with the responsibility of cooking the porridge. The food was purchased by the women from the local market at Ndhiwa. To cater for seasonality, the three common cereals used for porridge were purchased [maize, millet, sorghum]. The cereals were milled at the local electric commercial mill as normally practiced. The foods were cooked in selected health facilities in the 4 locations using utensils the women had brought from their homes. The weighing scales, calibrated cups and utensils for weighing all the ingredients were provided by the investigator. The women came with their children aged 6-23 months and all the food served to children was weighed including the leftovers. The entire process of food preparation, the recipes used in the preparation of foods, the amount served to children and leftovers were recorded accurately with as much detail as possible. This information was instrumental in the preparation of samples for dialysability testing.

Notably, processing methods for porridge flour was not mentioned by anyone during the focused group discussions. However, when they were probed it was clear that processing is not practiced in the preparation of porridge for children unlike porridge for adults. Therefore, the cereal based porridges cooked were all non-processed and they included maize, millet and sorghum.

Sample collection and purchases were done in January 2017. Dry white maize, finger millet and red sorghum were purchased at Ndhiwa open air market. This market serves West Kwabwai Division which is the study site. The cereals were transported to East Africa Nutraceuticals Company in Nairobi, Kenya, for germination and milling [see more detail below, Figure 3]. Processed and non-processed flours [500g] were packaged in airtight polythene bags and refrigerated at 4^0C. All the samples were taken to South Africa, cooked from the industrial plant at University of Pretoria, micronutrient powder added, freeze dried and analyzed for color changes, phytate content, mineral content, total phenolic content, tannin, as well as the amount of iron and zinc available after in *vitro* bioaccessibility.

Therefore, the analyzed samples in this study are cooked processed and non-processed maize, millet and sorghum porridges with added micronutrient powder. Dry white maize, finger millet, and red sorghum cereals were purchased from Ndhiwa open air market in Homabay County, Kenya.

3.1.3 Germination steps of maize millet and sorghum

Germination of grains was done using the methods described by Hotz and Gibson (2001) with modifications. White maize, finger millet, and sorghum grains were prepared by soaking/steeping them in excess tap water at 22^{o}C for 12 h followed by draining the excess water as shown in Figure 3.1 below. The grains were put on perforated trays and covered with a cotton cloth at 28-35^{o}C for 48 hours with occasional turning for the first 24 hours, for sprouting/germination to occur. The germinated grains, including the vegetative portion, were then sun dried to a final moisture content of 12% at 25-28°C for 2 days. The grains were extruded at 105-110^{o}C to achieve a moisture content of 8% followed by electric disk milling with 0.8mm sieve size. The flour [500g] was packaged in air tight polythene bags and shipped to the University of Pretoria food science laboratory for porridge preparation and further analysis.

Maize, millet, sorghum grain

↓

Sorting and cleaning

↓

Soaking in tap water for at room temperature [22^{o}C] for 12 hours

↓

Draining off water

↓

Grains put on perforated trays and covered with a cotton cloth at 28-35^{o}C for 48 hours for Sprouting/germination to occur

↓

Occasional turning for the first 24 hours

↓

Sun drying for 2 days to achieve a moisture content of 12% at 25-28°C

↓

Extruding at 105-110^{o}C to achieve a final moisture content of 8%

↓

electric disk milling with 0.8mm sieve size

Figure 3. 1: Germination process of maize, millet, and sorghum grain

3.1.4 Micronutrient powder

The micronutrient powder is manufactured by DSM in South Africa and distributed and sold by Phillips Healthcare Services limited in Kenya. All the micronutrient powder used for this study was therefore purchased in Kenya. One gram of micronutrient powder sachet contains NaFeEDTA iron 10mg, zinc 4.1mg, vitamin A 400µg RE, vitamin D 5µg, vitamin E 5mg, vitamin C 30mg, vitamin B1 0.5mg, vitamin B2 0.5mg, vitamin B3 6mg, vitamin B6 0.5mg, vitamin B12 0.9µg, folate 150µg, copper 0.56mg, selenium 17µg and iodine 90µg (WHO, 2016b). Each gram of micronutrient powder was added to 250ml of cooked porridge.

3.1.5 Cooking of porridge

The cooking of porridge was done at the industrial food plant of the Food Science Department, University of Pretoria in South Africa. Stainless steel saucepans were used for cooking. Cooking in the laboratory followed the local procedures and recipe recorded in the field.

To make 500ml porridge, flour [50g] was mixed with cold drinking tap water [200ml] to make a slurry. It was then added to boiling tap water [300ml], stirred and left to boil in a saucepan for 8 minutes with continuous stirring to avoid sticking at the bottom of the pan and to form lumps. The porridge was left to cool at room temperature to serving temperature. This 500ml porridge was divided into two portions of 250ml each. One gram of micronutrient powder was added to one portion of 250ml porridge and mixed well with a spoon. Micronutrient powder was not added to the other 250ml porridge. The same was repeated to other processed and non-processed porridge types [n=12]. The porridges were frozen overnight at -20^{0}C, freeze dried for one week, and ground finely using a coffee grinder to fine powder for analysis.

3.1.6 Laboratory procedures

Figure 3.2 below depicts the laboratory procedures undertaken during sample analysis. Both germinated and non-germinated porridge were cooked, cooled and then micronutrient powder was added to them. Thereafter the analysis of nutrient content, anti-nutrient content, color determination and dialysability were repeated twice per sample. However, reproducibility was twice for anti-nutrients and color determination, six times for dialysability and only once for nutrient content. Notably, dialysability was only conducted in samples with added micronutrient powder.

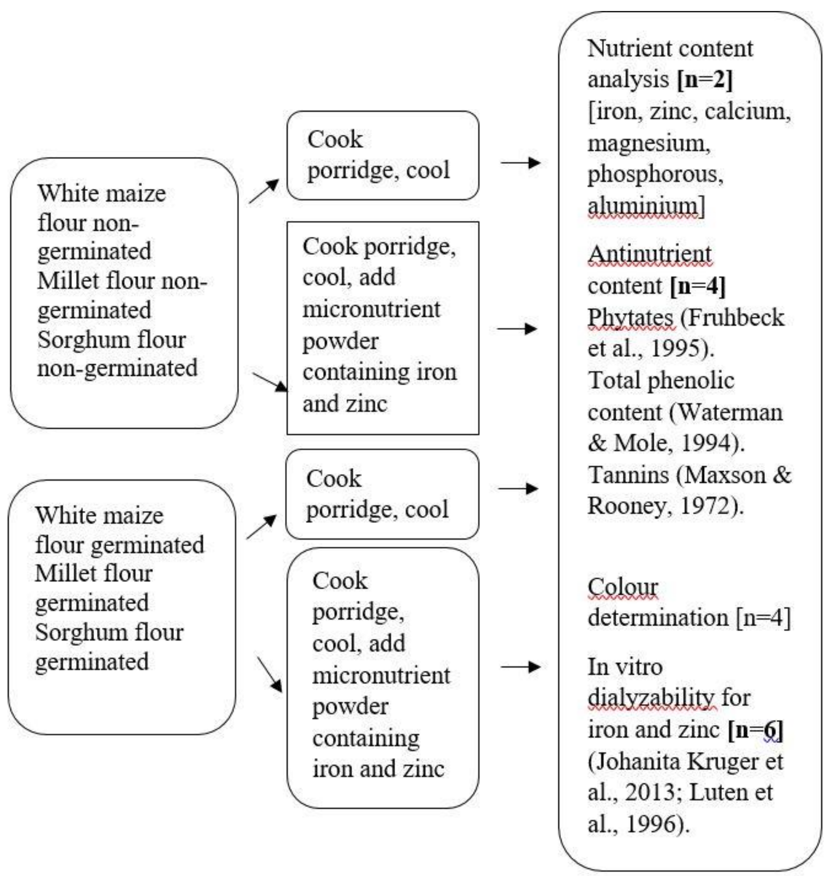

Figure 3. 2: Analysis study design indicating the sample types and analyses

3.1.7 Phytate content

Phytate was determined through anion exchange chromatography, indirect quantitative analysis by measuring the organic phytate phosphorous [inositol – 1 to 6- phosphates] (Fruhbeck, Alonso, Marzo, & Santidrián, 1995). The assay is used to determine the levels of phytate by extraction, and purification using anion-exchange and measurement of the phosphate content with spectrophotometry. The absorbance values of the standard solutions were used to determine the equation of the curve [expressed as mg phytic acid/100g dry weight] which determined the concentration [µg phytate/ml diluted purified sample]. The dilution factor was calculated and it was used to determine the concentration on phytate in the original sample.

3.1.8 Mineral content

Total iron, zinc, calcium, phosphorous, magnesium and aluminium were analysed using Inductively Coupled Plasma-Optical Emission Spectrometry method [ICP-OES, SPECTRO Analytical Instruments, and Kleve, Germany] after acid digestion (Kruger Johanita et al., 2013). This method involves calibration and extraction of the iron, zinc, calcium, phosphorous, magnesium and aluminum minerals. This was done at CAF, Stellenbosch University, South Africa.

3.1.9 Phytate mineral molar ratio

The mineral and phytate values previously determined as explained above were used to calculate the mole of phytate and minerals. This was done by dividing the weight of phytate and minerals with its atomic weight [phytate: 660g/mol; Fe: 56g/mol; Zn: 65g/ mol; Ca: 40 g/mol]. The molar ratio between phytate and mineral was obtained after dividing the mole of phytate with the mole of minerals.

3.1.10 Total phenolic content

Total phenolic content was determined using a modified Folin Ciocalteu method (Waterman & Mole, 1994). This is a spectrophotometric method that quantifies the total concentration of phenolic hydroxyl groups present in the sample. A standard catechin calibration curve is plotted against absorbance which is measured at 760 nm to show a high degree of linearity which is, $R^2 > 0.995$ using the Equation 3.1 below:

> Y = mx + c, where x is the mg catechin equivalents per 0.5 ml sample, y is the absorbance of the sample, m is the gradient and c is the y-intercept on the standard curve.

Equation 1: Total phenolic content calculation

3.1.11 Tannin content

Tannin content was determined by the modified Vanillin HCL assay (Maxson & Rooney, 1972; Price, Hagerman, & Butler, 1980). This method is used for the quantitative measurements of condensed tannins [or its monomeric components] in different types of grains. The formula below was used to calculate the tannin content of samples in mg Catechin equivalents [CE] per 100mg sample for both the Extract determination and the Color blank determination, see Equation 3.2 below.

$$CE^{*} = \frac{A_{500}{}^{**}\text{Test} - A_{500}\text{Colour Blank}}{A_{500}\text{ Standard} - A_{500}\text{ Reagent Blank}} \times 1.5 \text{ mg/ml} \times 5$$

Where:

*CE = mg Catechin Equivalence per 100mg sample [as is basis]

**A_{500} = Absorbance read at 500 nm

Equation 2: Tannin content calculations

3.1.12 *In vitro* bioaccessibility of iron and zinc

In vitro iron and zinc bioaccessibility was determined according to dialysis method described by Luten et al. (1996) with minor modifications given by Kruger Johanita et al. (2013). The simulated digestion with pepsin and pancreatic bile extracts mimics the human digestion and releases the soluble minerals as in the human body [gastric stage] from the sample into the supernatant while the dialyses tubing estimates how much of the minerals is available for human absorption [intestinal stage] (Miller et al., 1981). The collected digested sample [dialysate] was acidified with 0.002 ml 65% nitric acid/ml dialysate, to ensure all minerals remained in solution and analysed by Inductively Coupled Plasma-Optical Emission Spectrometry [ICP-OES]. Results are presented as the percentage of iron and zinc in the dialysate to the total iron and zinc content as shown in Equation 3.3 below. Enzymes and reagents used were porcine pepsin and pancreatin [Sigma, Johannesburg, South Africa] in dialysis tubing [Spectra/Por 7{Ø=20.4 mm} with a molecular mass cut-off of 10000 Da [GIS, Johannesburg, South Africa].

$$\%\text{ Bioaccessible iron or zinc} = \frac{\text{Dialysable Iron or Zinc [µg]}}{\text{Total Iron or Zinc [µg]}}$$

Equation 3: *In vitro* bioaccessibility of iron and zinc calculation

3.1.13 Quality control and validity

The total phenolic content, tannin content and phytate content were analyzed in duplicate on two individually prepared samples [n=4]. The mineral content was analysed in duplicate on one prepared sample [n=2]. The iron and zinc dialysability was done in triplicate on two individually prepared samples [n=6].

The R^2 of more than 0.996 from the standard curves generated were considered for quality. The coefficient of variation curves was also calculated. All the standards were prepared according to the protocol set for each analysis [mineral, phytate, tannin, total phenolic content], thereafter sample readings were compared to the standards after analysis to identify any variations. All sample analysis was repeated on a different day and outstanding variations of $\geq$20% on the final set of data generated was not included in the final analysis. Pepsin, pancreatin and other enzymes, as well as other unstable reagents like vanillin were freshly prepared just before use.

All glassware used were thoroughly washed, rinsed and dried according to the standard laboratory procedures. However, those used for dialysability were soaked overnight in nitric acid, rinsed in deionized water and oven dried before use. Gloves were used to handle all glassware during cleaning and use.

3.2 Phase two: Acceptability trial of germinated porridges with MNP in children

3.2.1 Materials and methods

The purpose of this study was to determine whether germinated porridge has adequate acceptability for the use as a complementary food to Kenyan children. An acceptability trial was conducted in the health centers/chief's camp in Homabay County. The study products were germinated maize, germinated millet, and germinated sorghum porridges with added micronutrient powder, whereas the non-germinated porridges of maize, millet and sorghum with added micronutrient powder served as the control. In total, six samples were used in this study.

3.2.2 Research design

A single blind cluster randomized parallel controlled trial to determine the acceptability of germinated maize, millet and sorghum porridge [the experiment], against non-germinated maize, millet and sorghum porridge [a comparator] on bioaccessibility of iron and zinc from MNP for a period of 8 weeks.

3.2.3 Study area

This study was conducted in West Kwabwai location, Homabay County, Kenya. The area is mainly occupied by the Luo community whose main livelihood is fishing and sugarcane farming. The recent commissioning of Sukari Sugar Company in the area has persuaded farmers to commit more land acreage to sugarcane farming at the expense of nutritious food crops. The location has a malnutrition rate of 27.5%, total population of 14,210, life expectancy of 46.5 years and food poverty level of 62.78% (Kenya National Bureau of Statistics, 2014).

3.2.4 Target population

The target population in this study were children aged between 6-23 months and their caregivers in Homabay County.

3.2.4.1 Inclusion criteria: Caregivers with children aged between 6-23 months who consent to participate in this study, lived in the area for at least 6 months prior to the study and planning to live there for at least 7 months after recruitment to the study. Also they had to be currently breastfed children who were receiving semi-solid or solid complementary foods at least once per day for at least two weeks before the start of the study.

3.2.4.2 Exclusion criteria: Caregivers with children who are eligible and decline to participate, those involved in other research trials, those on other supplementary feeding programs and those with chronic debilitating illness.

Children with height-for-age [HAZ], weight-for-age [WAZ] or weight-for-height [WHZ] z-scores <-3 with respect to World Health Organization growth standards (De Onis et al., 2006), presence of edema, diarrhea or other ailments that may have affected appetite during the previous seven days, congenital abnormalities and current severe systemic illness were excluded.

3.2.5 Sampling techniques

West Kwabwai location in Homabay County was purposively selected because of the high malnutrition rate.

3.2.5.1 Randomization: four sub-locations were randomly assigned on a 1:1 ratio to the four study groups: germinated maize, millet and sorghum porridge with MNP and non-germinated maize, millet and sorghum porridge with MNP. The randomization was computer generated using the Microsoft Excel 2016 software and it was conducted by a biostatistician who was not part of the research team. Sub-locations were randomized instead of children to prevent contamination.

3.2.5.2 Blinding: This was a double-blind study where only the investigator was aware of the food given to the experimental group. The hypotheses of the study were concealed from the participants and research assistants.

3.2.5.3 Recruitment: The study participants were recruited at the sub-locations. All children aged 6-23 months were first screened at the community level by community health workers. A listing of all households with children aged 6-23 months in each sub-location was compiled of those found eligible for the study. Simple random sampling was done to select and enroll the study participants based on the sample size required.

3.2.6 Sample size

A sample size for the randomized controlled trial was calculated using this formula (Chan, 2003). $m = [c / \delta^2]2$ where, m =total sample size for control and intervention, c = 7.9 for 80% power, δ =30% standardized effect size. Based on previous studies in Kenya, standard deviation=9, design effect=1.5, attrition=14% (Macharia-Mutie et al., 2012; Suchdev et al., 2012).

$$m= [7.9/ \{0.3 \times 0.3\}]\ 2=175 \text{ then, } 175x14\% =200$$

A total sample size of 200 children with 50 per group, Figure 3.3 was adopted for our study since a minimum of 50 judgments [preferably independent individual] is required to provide sufficient statistical power (De Kock, Zandstra, Sayed, & Wentzel-Viljoen, 2016).

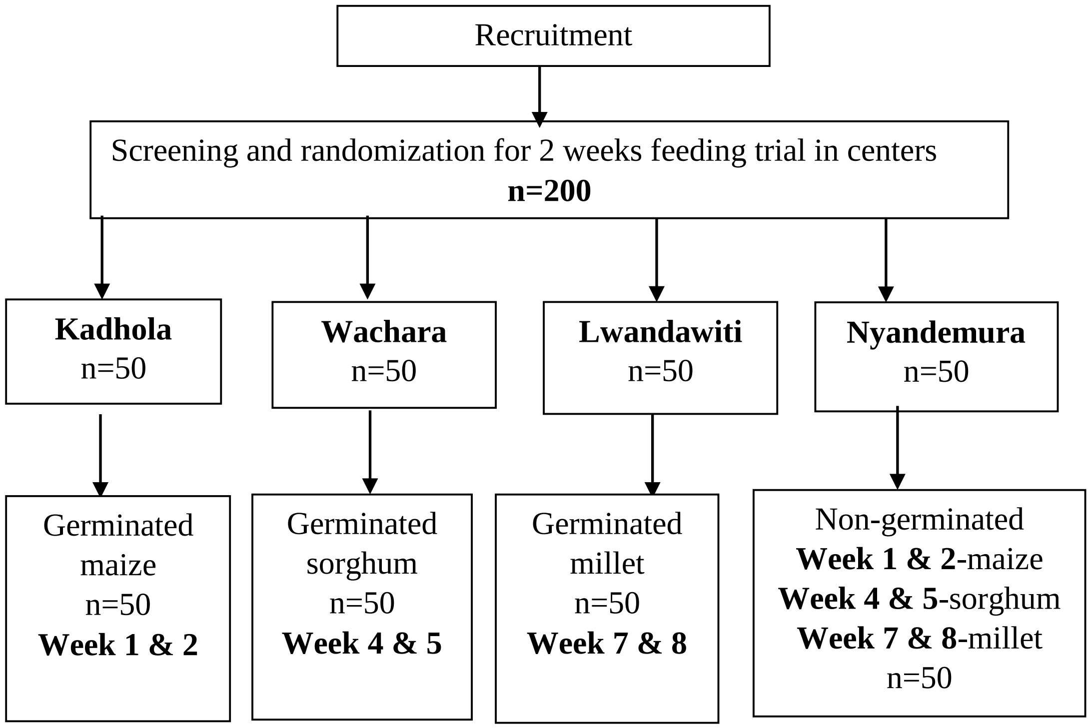

Figure 3. 3: Representation of the research approach for acceptability trial

3.2.7 Product development

We purchased the cereals from the study area and germinated in Kenya Industrial Research Development Institute [KIRDI]. The flour was packaged and labeled with codes that only the researcher understood. Porridge was prepared in the study site following the local procedure, cooled to room temperature, served in identical cups and then a sachet of micronutrient powder added to it, mixed thoroughly using a spoon before being fed to the child.

3.2.8 Feeding trial

Different mothers and their children were recruited and randomized to participate in the 2-week feeding trial. The mother-infant pairs were randomly assigned to one of the three different trial groups: germinated maize porridge [Kadhola center], germinated millet porridge [Lwandawiti center], germinated sorghum porridge [Wachara center], and one control group for each of the non-germinated porridges [Nyandemura center]. Four feeding centers for the four study groups were identified, and caregivers informed to bring their children for feeding every day for two weeks. While there was only one control group for the three germinated porridges. To minimize the carryover effects, the control group had a one-week break between the tests. Consequently, the study lasted for 8 weeks.

Each day, the porridge was prepared following a local recipe commonly consumed in the study area. The porridge was served in pre-weighed cups, and then one gram of micronutrient powder was added and mixed well. Before feeding the child, mothers completed a brief morbidity questionnaire on child illness symptoms during the past 24 hours. The age of the child determined the amount of porridge offered and what mothers would offer at home to ensure that the portion size is similar.

The caregiver was asked to feed the child until either the child finished or refused further porridge. In cases when the child does not want to eat more, the researchers instructed the mother to wait for 30 seconds and then offer the porridge a second and final time. The feeding was supervised by study personnel, and the total amount of porridge consumed was determined by reweighing the feeding cup [to 1 g precision] and calculating the difference from the initial weight. The amount of leftovers was also recorded. Any amounts spilled or regurgitated were measured by mopping the spillage on pre-weighed towels, and the amounts lost were considered in the calculations. The identity of the germinated porridge was concealed from the mothers and the study personnel.

3.2.9 Data collection tools and procedures

3.2.9.1 Acceptability trial questionnaire

The acceptability trial questionnaire [see *Appendix C*] was used to ascertain the daily amount of porridge consumed by each child aged 6-23 months. The cup used by the child was weighed before serving 250ml of porridge. The cup was weighed again after consumption to record the amount of porridge consumed and leftovers. The duration taken to consume the porridge was also recorded daily.

3.2.9.2 The daily symptoms of the child data sheet

The daily symptoms/morbidity data sheet of the child [*see Appendix D*] was used to record the daily health status of the child aged 6-23 months. The information recorded included whether the child was healthy, had diarrhea, fever, vomiting, coughing or rash.

3.3 Phase three: Nutrition survey to determine the nutritional status of children

3.3.1 Research design

A descriptive cross-sectional study was conducted to assess the anthropometric nutritional status, as well as the iron and zinc status of children aged 6-23 months in Homabay county, Kenya.

3.3.2 Sampling techniques

This study was conducted among children aged between 6 and 23 months in three sub-locations of West Kwabwai location in Homabay County using a simple random sample of 314 children. Based on facility data in Homabay County, West Kwabwai location reported the highest prevalence of malnutrition among children aged under five years. Consequently, this study was conducted in West Kwabwai location. Got Kojowi, Lwandawiti and Wachara sub-locations among five sub-locations in West Kwabwai were randomly selected for this study. Using community health volunteers [CHV], children aged 6-23 months in each of the three sub-location were enlisted. To ensure the study sample was representative with respect to age distribution and gender, a population weighted simple stratified sampling method was applied to identify the study subjects in each of the sub-locations.

3.3.3 Sample size

A sample size was calculated using the EPI info StatCalc [statistical calculator] software for cross sectional study (Dean, Sullivan, Zubieta, & Delhumeau, 2000; Sullivan, Dean, & Soe, 2009). The confidence level and the power were set at 95% and 80% respectively. The ratio of children with malnutrition to those without malnutrition was set at 30% and 70% respectively. Other values like the odds ratio and risk ratio were generated automatically and the results were presented using the Kelsey, Fleiss and Fleiss with a continuity correction. Therefore, a sample size of 314 children was used in this study.

3.3.4 Data collection tools and procedures

3.3.4.1 Structured household questionnaire

A structured questionnaire was developed to collect demographic, socioeconomic, morbidity and dietary data. The questionnaire was uploaded to ODK [Open Data Kit], an open source software for offline collection of data in the field and submitting it when internet is available. The questionnaire consisted of six sections used to collect data on six different themes as described below.

Socioeconomic status and demographic

Part A of the questionnaire consisted of the demographic information. These were the general characteristics of the child like age and sex. Part B of the questionnaire comprised of socio-demographic characteristics of the study participants which included age and education level of the caregivers, number of children, family size, property ownership, monthly household income, access to utilities and infrastructure, and housing characteristics.

Morbidity status

Part C of the questionnaire comprised of the health status of the child, as reported by the caregiver and/or verified from the caregiver and child health book for the last 2 weeks prior to the study. Information regarding whether the child was sick and the type of ailment was reported.

Feeding practice

Part D of the questionnaire consisted of the study variables related to feeding practices regarding infant and young child feeding [IYCF]. These were timely initiation of breastfeeding after birth, duration of breastfeeding and exclusive breastfeeding, complementary feeding initiation, and practices related to pre-lacteal feeding like age of introduction and type of food.

Food frequency

Dietary intake was assessed using a validated food frequency questionnaire [FFQ], with caregivers as the proxy for children in part E of the questionnaire. The FFQ consisted of 41 foods. These foods were grouped into carbohydrates, plant proteins, animal proteins, vegetables, fruits, dairy products, eggs, beverages, spreads and seasonings. The frequency categories of intake for the last one week were; never, once a week, most days in a week and daily.

24hour dietary recall

In the last section, "E" of the questionnaire, dietary data was collected by a means of 24-hour dietary recall method once per participant. Various food models, common utensils used in the households, food photo album and the actual food measurements were used to determine the portion sizes. The energy and nutrient intake were converted using the Nutri-Survey software program and each child's daily intake was calculated thereafter.

Dietary diversity assessment

Foods were categorised into the following groups: 1) grains, roots and tubers; 2) legumes and nuts; 3) dairy products; 4) flesh foods; 5) eggs; 6) vitamin A rich fruits and vegetables; 7) other fruits and vegetables [WHO, indicators for assessing infant and young child feeding practises, 2008]. A score of "1" was assigned to each food group if at least one food item within the specific food group was consumed during the 24-hour recall reference period. A score of "0" was assigned if the child did not consume any food item from a given food group. The DDS for each child was calculated as the sum of the scores, with a maximum possible score of "7". For the purpose the study, low dietary diversity was defined as DDS $\leq$ 4 (Gashu et al., 2016).

3.3.4.2 The laboratory data sheet

The laboratory data sheet was used to record the hemoglobin values of each child aged 6-23 months. The time when blood was drawn from the child was also recorded.

3.3.5 Determination of patterns

Dimension reduction for socio-economic status, food frequency, 24-hour dietary and morbidity data using the explorative factor analysis technique in the Statistical Package of Social Sciences [SPSS] for windows version 25 [Inc., Chicago, IL, USA] was used to determine the different patterns. Explorative factor analysis technique was used to organize socioeconomic, morbidity, food frequency, and 24-hour recall dietary data to reduce its dimensionality with as little loss of information as possible in the total variation these variables explain (Moskal et al., 2014).

Using the factor scores as weights, an individual child score was derived. The score indicated the degree to which each child's score conformed to the identified patterns. For each factor children were grouped into four categories according to quartiles of factor scores. Distribution of the children across the quartiles of each pattern according to the anthropometric and biochemical indices was determined.

3.3.6 Participants and recruitment

A total of 314 children aged 6-23 months participated in this study. Caregivers with sampled children were invited to a recruitment centre accompanied by the community health volunteer working in their villages. Children who met the eligibility criteria and whose caregiver agreed to participate in the study and signed an informed consent letter were included in the study. A trained study assistant fluent in the local language [dholuo] gave a full explanation of the study protocol and written informed consent was obtained from participating caregivers. Inclusion criteria for the children were 1) Not severely sick on the day of the study 2) aged between 6 and 23 months. Severely sick children were excluded and referred to the nearest health centre accompanied by the community health volunteer.

3.3.7 Setting

In Lwandawiti and Got Kojowi, the study was carried out in the compound of the health center. A compound of a local community based organization was used as a study centre in Wachara sub-location. A maximum of 50 children were mobilised and escorted by the community health volunteer to the study centre each day. After undergoing registration, anthropometric measurement on each child was conducted by two trained nurses. Thereafter, the children blood samples were collected by four trained phlebotomists. Lastly, individual caregivers were interviewed on the socioeconomic status, morbidity status and dietary intake of his/her child by six trained field officers.

3.3.8 Anthropometric Assessments

Standardized anthropometry measurements were performed on each child participating in the study. Children were weighed without clothes using a digital baby scale. The length of each child was measured using a height board with the head supported at one end, the torso and lower limbs extended, and feet flexed to 90◦ and supported by the lower-end stopper, to the nearest 0.1 cm. Head circumference was measured with a non-elastic measuring tape, fixed on the occiput and passing around the head and above the supraorbital ridges. Mid - Upper Arm Circumference [MUAC] was measured at the mid-point between the acromion and the olecranon with non-elastic MUAC tape. Measurements were performed twice, rated, and repeated if inconsistencies were identified. Weight for age z scores, length/ height for age z scores and BMI for age z scores [BAZ] of the infants were calculated using the WHO 2007 references [WHO Anthro software]. Those found with malnutrition were referred to the nutritionists in the nearest health facilities.

3.3.9 Biochemical Assessments

Blood collection and processing

Blood samples were collected from infants and children by trained research phlebotomist from the Kenya Medical Research Institute [KEMRI]. A 2 ml sample of non-fasting venous blood was drawn from the infants and children through antecubital venepuncture. Standard methods for pediatric blood collection were followed. Blood were drawn into trace element free tubes heparin tubes [Sarstedt] and then centrifuged within 1 hour from collection to obtain heparin plasma. Two plasma aliquots were stored at -20°C and shipped to the North-West University, Centre of Excellence for Nutrition in South Africa on dry ice using a specialized courier service [World Courier]. At the Centre of Excellence for Nutrition the samples were stored at -80°C until analysis.

Biochemical analysis

Haemoglobin was measured in whole blood by using HaemoCue® [HemoCueAngelholm, Sweden] on site. The iron status indicators ferritin and transferrin receptor, as well as the inflammation markers alpha-1-acid glycoprotein [AGP] and C-reactive protein [CRP] were analysed in plasma using the multiplex micronutrient immunoassay from Quansys Bioscience at the North-West University, Center of Excellence for Nutrition, South Africa (Brindle et al., 2017). The acute phase proteins AGP and CRP were used to identify subjects with infection and inflammation, which could confound measures of iron [especially ferritin].

Plasma zinc concentrations were measured by atomic absorption spectrometry at CAF in Stellenbosch University, South Africa.

Cut-off limits to assess anemia, iron deficiency, iron deficiency anemia and zinc deficiency

The following cut-offs were used for the different iron and anaemia indicators for infants and children: Hb <110g/L was used to define anaemia, ferritin <12µg and TfR >8.3 mg/L were used to define iron deficiency [ID] (WHO, 2011a; WHO, 2011b). Iron deficiency anemia [IDA] was defined as the co-existence of anemia with ID. Correctional factors were used to adjust ferritin concentrations since they can be affected by the presence of infection, which was identified by the analysis of the acute phase proteins AGP and CRP. In this study, CRP concentrations above 5mg/L were used to identify subjects with acute inflammation and AGP > 1 g/L to identify subjects with both acute and chronic inflammation.

Three correctional factors were used as suggested to adjust serum ferritin values: 1) for subjects with CRP > 5 mg/L and AGP ≤ 1 g/L [incubation phase] a CF of 0.77 was used; 2) for subjects with CRP > 5 mg/L and AGP > 1 g/L [early convalescent phase] a CF of 0.53 and 3) for subjects with CRP ≤ 5 mg/L and AGP > 1 g/L [late convalescent phase] a CF of 0.75 was used (Thurnham et al., 2010). The seemingly healthy subjects without inflammation were defined by CRP ≤ 5 mg/L and AGP ≤ 1 g/L and in those subjects no CF was used.

To assess zinc status, the recommended cut off for children under 10 years is 65µg/dl [9.9µmol/L] for samples collected in the morning in a non-fasted state was used (De Benoist, Darnton-Hill, Davidsson, Fontaine, & Hotz, 2007). Zinc measurements were adjusted in the presence of raised CRP: Zinc was multiplied by a factor of 1·2 (Thurnham et al., 2010).

3.4 Pilot study for pretesting of the instruments

For phase two and three of the study, a pilot study was done on 10% of the sample from the study area but in a different sub-location [Kasirime Kawanga] which was not targeted for the main study. The purpose of this was to test the reliability and validity of the tools as well as test response rate. During pre-testing, the same measuring equipment were used to take three measurements for reliability test. The reliability of the instrument was tested using the Cronbach's coefficient test. For this study, a reliability of 0.70 was used (Fraenkel, Wallen, & Hyun, 2011). After testing the instruments were modified accordingly.

3.5 Study variables

Laboratory bioaccessibility study: The variables for the bioaccessibility study were, the amount and percentage of iron and zinc from MNP bio-accessible; amount of phytates, tannins and total phenolic; amount of iron, zinc, calcium, magnesium, phosphorous, aluminum; in either germinated or non-germinated maize, millet and sorghum porridges with MNP, Table 3.1.

Acceptability trial study: The amount of porridge intake and the consumption day [1-14] of germinated maize, millet and sorghum porridge with MNP versus the non-germinated porridges.

Nutrition survey study: The variables for the nutrition survey were, complementary foods, feeding practice, dietary diversity score, nutrient intake in relation to the wasting, stunting, underweight, anemia, iron deficiency, iron deficiency anemia, zinc deficiency.

Table 3. 1: Study variables

	Independent variables	**Dependent variables**
Bioaccessibility laboratory study	amount and percentage of iron and zinc from MNP bio-accessible amount of phytates, tannins and total phenolic amount of iron, zinc, calcium, magnesium, phosphorous and aluminium nutrients	germinated or non-germinated maize, millet and sorghum porridges with MNP.
Acceptability trial	amount of porridge intake, day [1-14] of porridge intake,	germinated or non-germinated maize, millet and sorghum porridges with MNP.
Nutrition survey	complementary foods, food frequency, food patterns, nutrient patterns, feeding practice, dietary diversity score, iron and zinc nutrient intake	Nutritional status-wasting, stunting, underweight, anaemia, iron deficiency, iron deficiency anaemia, zinc deficiency

3.6 Statistical analysis

Statistical Package of Social Sciences [SPSS] for windows version 25 [Inc., Chicago, IL, USA] was used for statistical analyses.

The laboratory bioaccessibility data: Two-way ANOVA was used to determine the effect of porridge type [maize, millet, sorghum] and processing method [non-processed, processed], and their interaction, on iron and zinc bioaccessibility. In the presence of significance in main effects, comparisons of means between groups with one-way ANOVA was done using the Fisher's least significant difference [LSD] at [p=0.05].

Acceptability trial data: The information on the acceptability trial was analyzed using ANOVA. The analysis of the amount of porridge consumed per day compared to the control for each center was performed using the General Linear Model Univariate procedure using repeated measures, for interactions between the porridge type and the day.

All adjustments of *p*-Values for multiple comparisons were made using Bonferroni adjustment. Significant differences were considered at $p \leq 0.05$.

Nutrition survey data: ENA for SMART software with Epi info was used to compute nutrition indices of weight for age z scores, length/ height for age z scores and BMI for age z scores [BAZ] of the infants using the WHO 2007 references. Nutri-survey software was used for analysis of nutrient intake. This data was then imported to SPSS for analysis with other variables [demographic, socioeconomic, morbidity, dietary, and iron and zinc biochemical indices].

Binary logistic regression models were computed to identify the association of socioeconomic status, morbidity, food, and nutrient patterns as independent variables with wasting, stunting, underweight, anemia, iron deficiency, iron deficiency anemia and zinc deficiency as the dependent variables. Ordinal regression was performed to assess the strength of association between an ordinal dependent variable with more than two categories and an independent variable.

3.7 Logistical and ethical considerations

Authenticity to conduct the study was sought from Kenyatta University graduate school and the Kenyatta University ethical review committee. A research permit was obtained from the National Commission for Science, Technology and Innovation. The respondents were caregivers of the children as the children of this age are too young to respond. An informed and signed consent was sought before the study. The research purpose and protocols were explained in detail to the local administration, community leaders and the caregivers. All the children aged 6-23 months from selected sub-locations, even those excluded from the study were provided with porridge. The caregivers were informed of the benefits of the study which includes improved health status of children. The anticipated risks were also discussed with the caregivers such as the likelihood of a child having diarrhea or no change in serum levels may be due to other underlying factors. The respondents were assured of confidentiality during and after the study.

CHAPTER FOUR: RESULTS

4.1 Phase one: *in vitro* bioaccessibility of iron and zinc

4.1.1 Mineral contents in porridge

Iron content in non-processed and processed porridges without micronutrient powder ranged between 0.24 to 2.09 mg/100g and 1.86 to 3.91 mg/100g respectively, Table 4.1. The highest iron content among non-processed and processed porridges was recorded for finger millet and red sorghum respectively. Before adding MNP, zinc content in porridges ranged between 0.21 to 0.4 mg/100g in non-processed porridges, and 0.21 to 0.3 mg/100g in processed porridges. Non-processed finger millet and processed sorghum had the highest zinc contents [Table 4.1].

Processing of porridge caused a significant [$p<0.001$] effect on phosphorous, magnesium and aluminum mineral contents but not on iron, zinc and calcium [Table 4.1]. On the other hand, the porridge type showed significant differences [$p<0.001$] on the levels of calcium, phosphorous magnesium and aluminum but not on iron and zinc. However, the interaction between porridge type and processing caused a significant effect [$p<0.001$] on calcium and magnesium only.

Figures 4.1 and 4.2 shows the levels of iron in zinc in various porridges with or without micronutrient powder. It is evident that when micronutrient powder is added to porridges the levels of these nutrients increase tremendously. The porridge/cereal type, processing and their interaction [porridge type x processing] were all significant [$p<0.001$] for zinc content of porridges without and with added micronutrient powder, and for iron content for only porridges without micronutrient powder. However, processing did not have any effect on iron for porridges with added micronutrient powder [$p<0.001$].

Table 4. 1: Mineral contents [mg/100g] in different porridges without micronutrient powder

		Maize	Millet	Sorghum	Porridge P value	Processing P value	Porridge x Processing P value
Fe	Non-Processed	0.24 [0.005]	2.09 [0]	1.16 [0.005]	0.699	0.560	0.907
	Processed	1.86 [0.005]	3.17 [0.005]	3.91 [0.005]			
Zn	Non-Processed	0.21 [0.005]	0.40 [0]	0.31 [0]	0.998	0.553	0.973
	Processed	0.30 [0.005]	0.27 [0]	0.30 [0]			
Ca	Non-Processed	3.90 [0.05] ^{B}	43.70 [0.05] ^{A}	7.20 [0.005] ^{B}	<0.001	0.771	<0.001
	Processed	6.60 [0.05] ^{c}	29.00 [0.005] ^{a*}	20.00 [0.005] ^{b*}			
P	Non-Processed	22.90 [0.05] ^{C}	35.43 [0.005] ^{B}	38.15 [0.005] ^{A}	<0.001	<0.001	0.289
	Processed	30.38 [0.005] *	29.80 [0.005] *	31.18 [0.005] *			
Mg	Non-Processed	9.61 [0.005] ^{C}	23.36 [0.005] ^{A}	19.88 [0.005] ^{B}	<0.001	<0.001	<0.001
	Processed	13.09 [0.005] ^{c*}	17.30 [0.005] ^{a*}	16.28 [0.005] ^{b*}			
Al	Non-Processed	0.00 [0.00] ^{B}	2.07 [0] ^{AB}	0.73 [0.005] ^{AB}	<0.001	<0.001	0.370
	Processed	0.52 [0.005] ^{c*}	1.74 [0] ^{a}	1.46 [0.005] ^{b*}			

Values expressed as means [n=2] and Standard Error Mean in parenthesis
Values with different small/capital superscripts letters within rows are significantly different according to Bonferroni values from main effects ANOVA.
Values with asterisks superscripts within columns are significantly different.

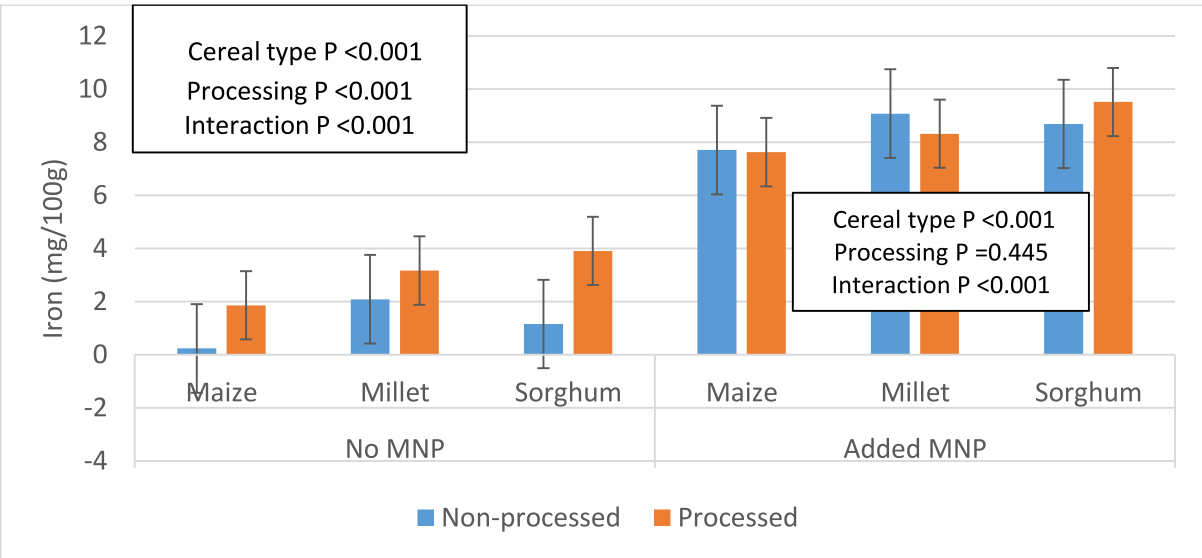

Figure 4. 1: Iron content of porridges without and with added MNP

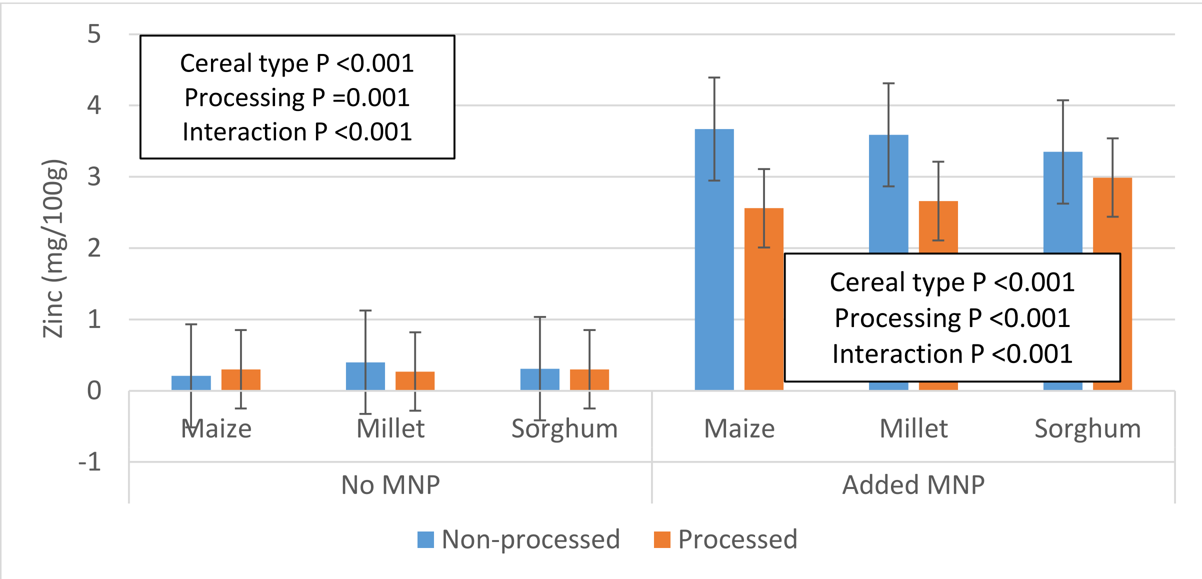

Figure 4. 2: Zinc content of porridges without and with added MNP

4.1.2 Total phenolic, tannin and phytate contents in porridge

Total phenolic, tannin and phytate content decreased significantly in all the porridges with processing [Table 4.2]. Before adding MNP, the total phenolic content of non-processed porridges and processed porridges ranged from 1.23 -15.91 mg CE/100g and 1.25-4.45 mg CE/ 100g respectively. Tannin content was between 0.53 -8.70 mg CE/100g in non-processed porridges and 0.53- 3.34 mg CE/100g in processed porridges with no MNP. Processed and non- processed red sorghum had the highest total phenolic and tannin content. Lower levels of tannin and undetectable levels of phenolics were observed in maize. Phytate content was between 302-2230 mg/100g in non-processed porridges and 154-1090 mg/100g in processed porridges. Maize porridge whether processed or not had the highest levels of phytates followed by millet then sorghum [Table 4.2]. The porridge type, processing, and porridge type x processing interaction had a significant [$p<0.001$] effect on the levels of tannins, total phenolic content and phytate content in all porridges.

Based on the mineral and phytate levels presented in Tables 4.1 and 4.2 respectively, the phytate: iron **molar ratios** ranged between 22-788 in non-processed porridges and 3-50 in processed porridges [Table 4.3]. The phytate: zinc molar ratio in non-processed porridges was between 96-1046 and 51-358 in processed porridges. Both processed and non-processed maize porridge had the highest phytate to iron and zinc molar ratios with processed sorghum having the least. The phytate: calcium molar ratio ranged between 1.1 to 34.6 and 0.4-10.0 in non-processed and processed porridges respectively. Both processed and unprocessed maize had higher levels while processed sorghum had the lowest. The phytate x calcium: zinc molar ratio was between 17-224 in non-processed porridges and 25-122 in processed porridges [Table 4.3]. Both processed and non-processed millet had the highest phytate x calcium: zinc molar ratios compared to maize and sorghum.

Table 4. 2: Tannin [mg CE/100g] Phenolic [mg CE/100g] and Phytate [mg/100g] content in different porridge without micronutrient powder

		Maize	Millet	Sorghum	Porridge P value	Processing P value	Porridge x Processing P value
Tannin	Non-Processed	0 [0] ^{C}	1.48 [0.07] ^{B}	8.70 [0.23] ^{A}	<0.001	<0.001	<0.001
	Processed	0 [0] ^{c}	2.00 [0.07] ^{b*}	3.34 [0.07] ^{a*}			
Phenolic	Non-Processed	0 [0] ^{C}	1.23 [0.06] ^{B}	15.91 [0.14] ^{A}	<0.001	<0.001	<0.001
	Processed	0 [0] ^{c}	1.25 [0.09] ^{b}	4.45 [0.09] ^{a*}			
Phytate	Non-Processed	2230 [75.05] ^{A}	834 [9.81] ^{B}	302 [5.77] ^{C}	<0.001	<0.001	<0.001
	Processed	1090 [5.77] ^{a*}	461 [6.35] ^{b*}	154 [3.17] ^{c*}			

[]-Values represent the Standard Error Mean
Values with different small/capital superscripts letters within rows are significantly different according to Bonferroni values from main effects ANOVA.
Values with asterisks superscripts within columns are significantly different.

Table 4. 3: Iron, zinc, calcium and Phytate contents [mg/100g], and Phytate: mineral molar ratios of porridge without micronutrient powder

		Iron	Phytate: Iron	Zinc	Phytate: Zinc	Calcium	Phytate: Calcium	Phytate x Calcium: Zinc	Phytate
Maize	Non-processed	0.24 [0.005]	788	0.21 [0.005]	1046	3.90 [0.05]	34.6	102	2230 [75.05]
	Processed	1.86 [0.005]	50	0.30 [0.005]	358	6.60 [0.05]	10.0	59	1090 [5.77]
Millet	Non-processed	2.09 [0]	34	0.40 [0.005]	205	43.70 [0.05]	1.1	224	834 [9.81]
	Processed	3.17 [0.005]	12	0.27 [0]	168	29.00 [0.005]	0.9	122	461 [6.35]
Sorghum	Non-processed	1.16 [0.005]	22	0.31 [0]	96	7.20 [0.005]	2.5	17	302 [5.77]
	Processed	3.91 [0.005]	3	0.30 [0]	51	20.00 [0.005]	0.4	25	154 [3.17]

[]-Values represent the Standard Error Mean

4.1.3 Iron and zinc bioaccessibility in porridge

Processing had a significant effect on the **percent** bioaccessibility of iron and zinc [Table 4.4]. Percentage bioaccessibility of iron and zinc from the micronutrient powder added in non-processed and processed porridges ranged between 1.3-5.29% and 2.7-5.48% for iron and 10.6-15.48% and 30.68-38.79% for zinc respectively. Although non-processed sorghum had the highest percentage bioaccessibility for iron and zinc, processed millet and processed sorghum had the highest percentage bioaccessibility of iron and zinc respectively [Table 4.4].

Table 4. 4: Bioaccessibility [%] of Iron and Zinc in different porridges with micronutrient powder

		Maize	Millet	Sorghum	Porridge P value	Processing P value	Porridge x Processing P value
Iron	Non-Processed	1.3 [0.13] ^{B}	1.8 [0.16] ^{B}	5.29 [0.62] ^{A}	<0.001	<0.001	0.020
	Processed	2.7[0.37] ^{b*}	5.48 [0.20] ^{a*}	4.46 [0.43] ^{a}			
Zinc	Non-Processed	12.6 [1.31]	10.6 [1.76]	15.48 [2.43]	0.232	<0.001	0.694
	Processed	30.68 [5.28] *	33.71 [2.57] *	38.79 [5.15] *			

[]-Values represent the Standard Error Mean
Values with different small/capital superscripts letters within rows are significantly different according to Bonferroni values from main effects ANOVA.
Values with asterisks superscripts within columns are significantly different.

4.2 Phase two: Acceptability trial of germinated porridge

4.2.1 Sociodemographic characteristics and different centres

Demographic characteristics of children and caregivers who participated in the study are shown in Table 4.5. An equal number of children [n=50] were enlisted in the three intervention arms of the study with another 50 children enrolled into the control arm of the study. These children were used as control group for the three intervention groups with a wash out period of one week between the tests. Children who participated in the intervention were from three different sub-locations whilst children in the control were all from Nyandemura sub-location.

Both control and the intervention arms of the study had representation among the three age groups and sexes. More male children than females participated in both the control and the intervention groups.

All the caregivers were the mothers of the index children. Demographic characteristics were nearly distributed across the control and intervention groups. Approximately 50% of the caregivers in the intervention and control groups had attained incomplete primary level of education with less than 10% attaining secondary school level of education. Over 40% of the caregivers were young mothers aged between 15-24 years in both the control and intervention groups. In both the control and intervention groups, 60% of the caregivers reported a monthly earnings of less than Kenya shillings 4,000 [USD 40].

Table 4. 5: Sociodemographic characteristics of study participants for the acceptability trial

	Germinated porridge			**Non-germinated porridge**		
Sub - location	Maize Kadhola [n=50]	Millet Lwandawiti [n=50]	Sorghum Wachara [n=50]	Maize Nyandemura [n=50]	Millet	Sorghum
Children characteristics						
Age of children						
06-11months	[15] 30%	[17] 34%	[13] 26%	[20] 40%	[20] 40%	[20] 40%
12-17 months	[21] 42%	[15] 30%	[22] 44%	[12] 24%	[12] 24%	[12] 24%
18-23 months	[13] 26%	[18] 36%	[15] 30%	[18] 36%	[18] 36%	[18] 36%
Sex of children						
Male	[27] 54%	[26] 52%	[24] 48%	[26] 52%	[26] 52%	[26] 52%
Female	[23] 46%	[24] 48%	[26] 52%	[24] 48%	[24] 48%	[24] 48%
Caregiver characteristics						
Age of caregiver						
15-19	[9] 18%	[10] 20%	[9] 18%	[13] 26%	[13] 26%	[13] 26%
20-24	[11] 22%	[13] 26%	[13] 26%	[12] 24%	[12] 24%	[12] 24%
25-29	[11] 22%	[10] 20%	[11] 22%	[10] 20%	[10] 20%	[10] 20%
30-34	[7] 14%	[4] 8%	[5] 10%	[4] 8%	[4] 8%	[4] 8%
35-39	[6] 12%	[7] 14%	[6] 12%	[4] 8%	[4] 8%	[4] 8%
40-44	[5] 10%	[4] 8%	[5] 10%	[5] 10%	[5] 10%	[5] 10%
45+	[1] 2%	[2] 4%	[3] 6%	[2] 4%	[2] 4%	[2] 4%
Education status of caregiver						
None	[3] 6%	[1] 2%	[4] 8%	[0] 0%	[0] 0%	[0] 0%
Primary incomplete	[24] 48%	[27] 54%	[24] 48%	[28] 56%	[28] 56%	[28] 56%
Primary complete	[10] 20%	[13] 26%	[12] 24%	[10] 20%	[10] 20%	[10] 20%
Secondary incomplete	[6] 12%	[5] 10%	[4] 8%	[6] 12%	[6] 12%	[6] 12%
Secondary complete	[5] 10%	[3] 6%	[5] 10%	[4] 8%	[4] 8%	[4] 8%
Tertiary	[2] 4%	[1] 2%	[1] 2%	[2] 4%	[2] 4%	[2] 4%
Monthly income status of caregiver						
<2000	[10] 20%	[12] 24%	[11] 22%	[11] 22%	[11] 22%	[11] 22%
2001-4000	[23] 46%	[19] 38%	[24] 48%	[21] 42%	[21] 42%	[21] 42%
4001-6000	[8] 16%	[11] 22%	[8] 16%	[9] 18%	[9] 18%	[9] 18%
6001-8000	[4] 8%	[3] 6%	[3] 6%	[5] 10%	[5] 10%	[5] 10%
8001-10000	[4] 8%	[4] 8%	[3] 6%	[4] 8%	[4] 8%	[4] 8%
>10,000	[1] 2%	[1] 2%	[1] 2%	[0] 0%	[0] 0%	[0] 0%

4.2.2 Comparing the consumption and acceptability of porridge types at different centres

The mean amount of consumed germinated millet [241.46ml] was the highest followed by maize [238.69ml] then sorghum [230.29ml] in that order. At Wachara center where germinated sorghum was consumed, the mean amount of consumed porridges was 220.89mls and 230.29mls for non-germinated and germinated sorghum respectively. While the amount of consumed germinated porridge progressively increased, the amount of non-germinated porridge remained constant over the 2-week period, Figure 4.3.

The mean amount of consumed non-germinated and germinated millet porridge was 238.20mls and 241.46mls respectively at Lwandawiti center. The amounts of consumed porridge were relatively similar for all the days except the first day when children consumed more germinated millet porridge compared to the non-germinated millet porridge, Figure 4.4.

For maize porridge that was consumed at Kadhola center, the mean amount of non-germinated and germinated maize porridge consumed was 235.07mls and 238.69mls respectively. As from the fourth day, the amount of porridge consumed in both groups plateaued and minimal differences were noted, Figure 4.5.

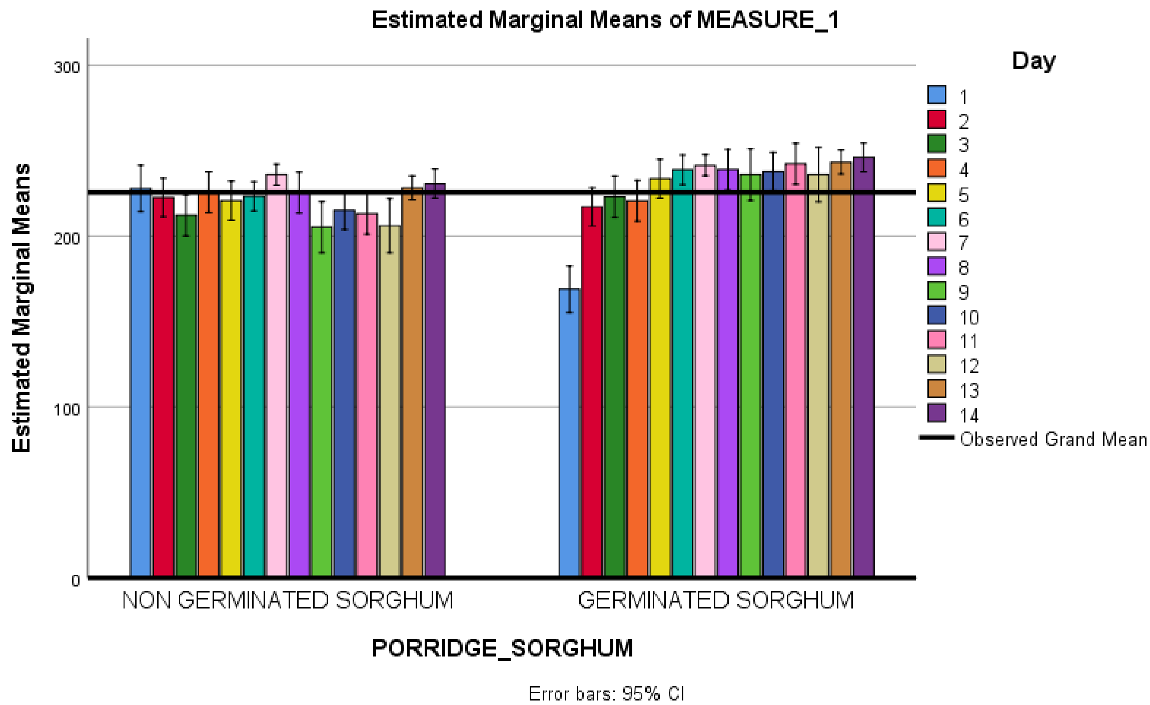

Figure 4. 3: Daily consumption of sorghum porridge at Wachara center.

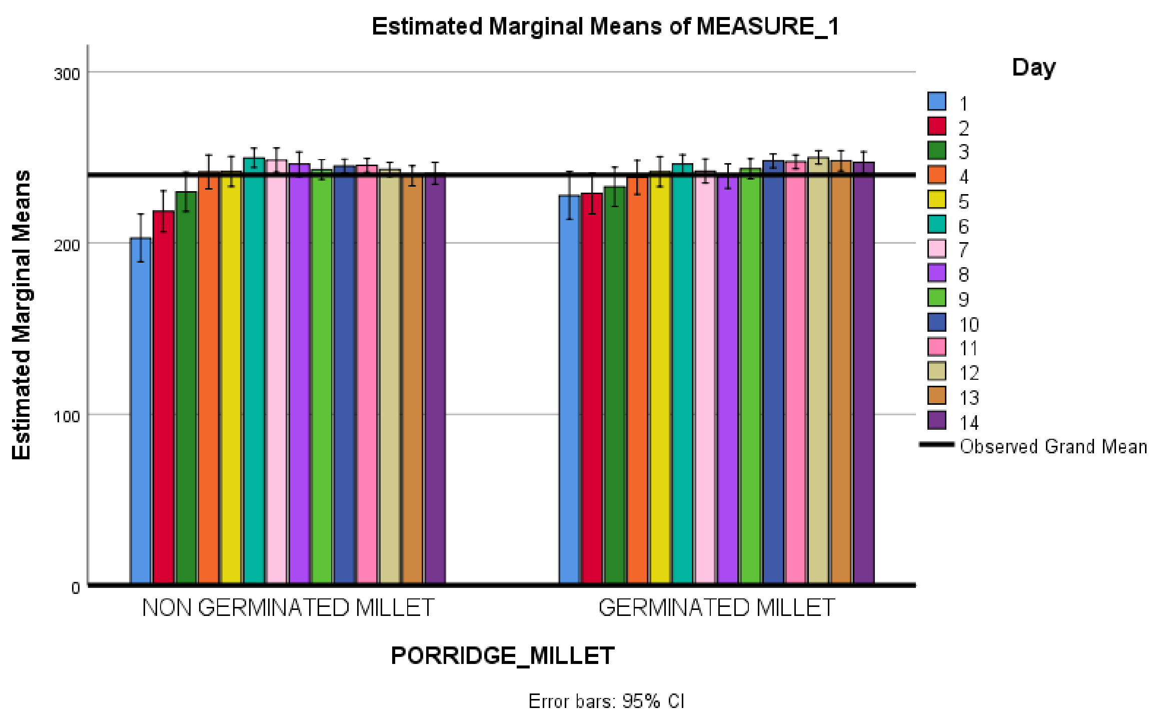

Figure 4. 4: Daily consumption of millet porridge at Lwandawiti center.

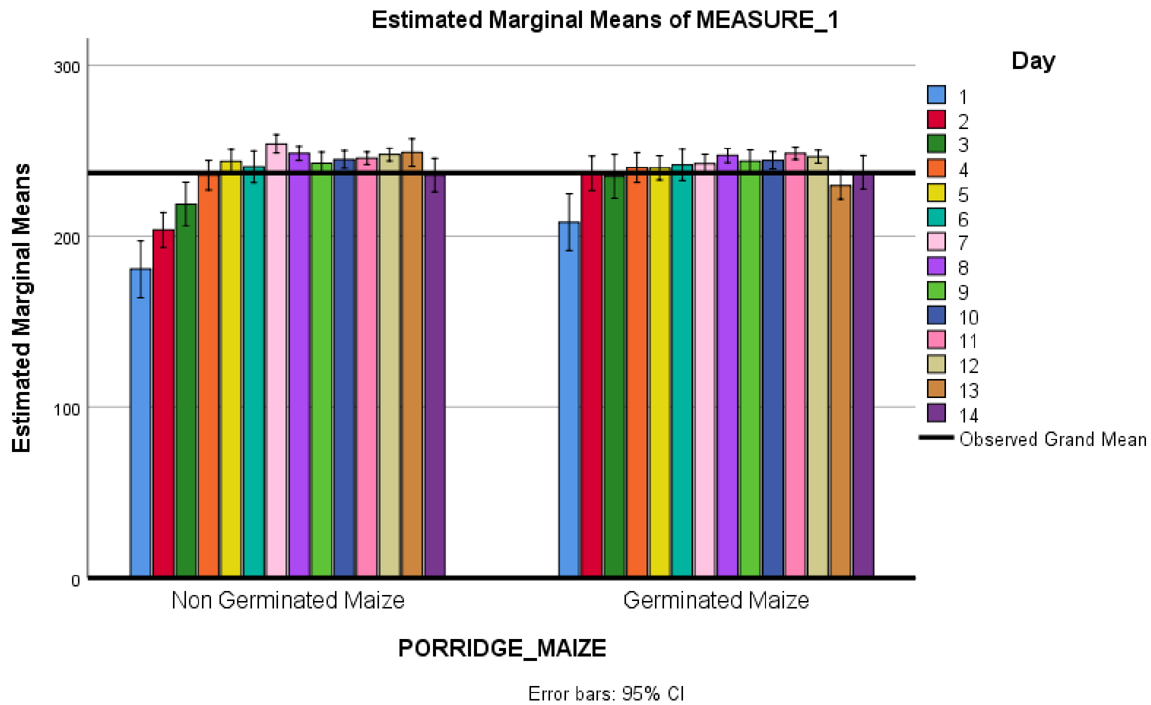

Figure 4. 5: Daily consumption of maize porridge at Kadhola center.

4.2.3 Overall consumption of trial and control porridges

Overall, the amount of germinated porridges consumed was more than the non-germinated porridges for the three cereals, Figure 4.6. However, only the difference between germinated and non-germinated sorghum was statistically significant [p=0.003].

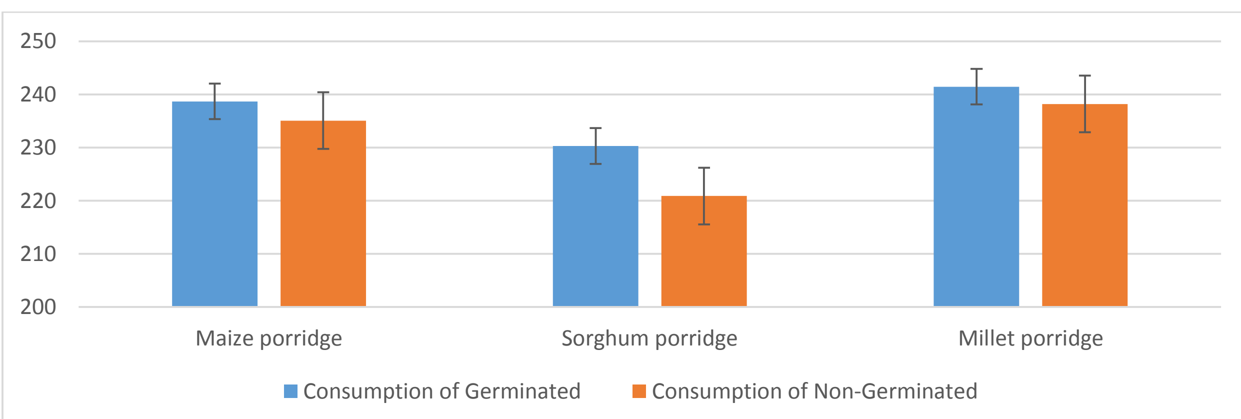

Figure 4. 6: Overall consumption of porridges in different centers

4.2.4 Acceptability of different germinated versus non-germinated porridges during the 2-week trial period

A significant [p=0.003] difference was observed between the amount of germinated and non-germinated sorghum porridge consumed over the 2-week period, unlike maize [p=0.127] and millet [p=0.073], Table 4.6. The day the germinated versus non-germinated porridges consumed was also significant [p<0.001] for all the three porridge types in reference to the amount. There was also a statistically significant [p<0.001] interaction between the amount of maize and sorghum porridge consumed and the day of consumption. Furthermore, when the last day [14] was compared to all the other days, a significant difference was observed between the amount of germinated versus non-germinated porridge consumed on day 2, 3 and 4 for sorghum and day 2 and 3 for maize and millet, Table 4.6.

Table 4. 6: Acceptability of different germinated versus non-germinated porridges during the 2-week trial period

Day	**Maize**	***p*-Value**			**Millet**	***p*-Value**			**Sorghum**	***p*-Value**		
		Germinated Maize	**Day**	**Germinated Maize X Day**		**Germinated Millet**	**Day**	**Germinated Millet X Day**		**Germinated Sorghum**	**Day**	**Germinated Sorghum X Day**
1	194.42*	0.127	0.000	0.000	215.38*	0.073	0.000	0.201	198.39*	0.003	0.000	0.000
2	220.15*				223.76*				219.92*			
3	226.88				231.34				217.66*			
4	237.89				239.97				223.20			
5	241.88				241.74				227.20			
6	241.20				247.89				231.08			
7	248.25				245.18				238.76			
8	247.81				242.54				232.13			
9	243.33				243.13				220.63			
10	244.59				246.46				226.36			
11	248.40				246.37				227.72			
12	246.50				246.49				221.05			
13	229.60				243.61				235.79			
14	237.30*				243.81*				238.43*			

Asterisk shows Pairwise Comparison Significance of day 14 [last day] in relation to all the other days.

Generalized Linear Model; Univariate analysis using repeated measures

4.3 Phase three: Nutrition survey results

4.3.1 Sociodemographic characteristics

Table 4.7, below shows the sociodemographic characteristics of children and their caregivers who participated in the cross-sectional nutritional survey in Homabay County. More than half of the children, 55.7% were male. Children were almost evenly distributed in the three age groups. Lwandawiti location had the highest number [28.9%] of all children amongst the three locations in the study. The majority of the caregivers [64%] were young mothers aged between 15-24 years. The majority of these caregivers were married [86.6%] and had attained an incomplete primary education level [50.6%]. Table 10 shows the household characteristics. Seven out of every ten households reported more than five members [76.1%]. They practice farming [53.2%], and their source of drinking water is from a communal well [44.3%]. Forty-two percent of households reported having the traditional type of latrine [42.0%]. The majority of them own a cellphone [91.4%] and a radio [76.1%].

Table 4. 7: Sociodemographic characteristics of children and caregivers

Indicators	Frequency [Percentage] n=314
Child characteristics	
Sex	
Male	175 [55.7%]
Female	139 [44.3%]
Age group	
6-11 months	112 [35.7%]
12-17 months	105 [33.4%]
18-23 months	97 [30.9%]
Location	
Got-Kojowi	85 [27.1%]
Lwandawiti	122 [38.9%]
Wachara	107 [34.0%]
Maternal characteristics	
Age	203 [64.6%] 15-24 years
Education level	159 [50.6%] Primary incomplete
Marital status	272 [86.6%] married
Household characteristics	
Household size	239 [76.1%] ≥5 members
Occupation	167 [53.2%] Farmer
Monthly income	130 [41.4%] KES 2001-4000
Income source	200 [63.7%] Agriculture
Wall of the house	291 [92.7%] Mud
Source of drinking water	139 [44.3%] Communal well
Latrine use	230 [73.2%] own latrine
Type of latrine n=230	132 [42.0%] Traditional latrine
Source of lighting	197 [62.7%] Kerosene 110 [35.0%] Solar
Ownership of property	
Own a radio	239 [76.1%]
Own a cellphone	287 [91.4%]
Bicycle	60 [19.1%]
Wheelbarrow	66 [21.0%]
Sofa set	115 [36.6%]
Television	33 [10.5%]
Solar	109 [34.7%]

4.3.2 Morbidity status

More than half [60.2%] of the children were sick 2 weeks before the study with many [37.9%] of them seeking medical attention, as shown in Table 4.8, below. Almost half of the children had fever [49.4%] followed by cough at [29.9%], with the least ailment being wheezing [6.1%]. Of note is that children often presented with a combination of the signs and symptoms presented in this study. It is therefore essential to assess the impact of a combination of the illnesses in an individual child on the nutritional status.

Table 4. 8: Morbidity status of children in the last 2 weeks prior to the survey

Indicator	Yes	No	Mean ±SD Number of Days
Sick in the last 2 weeks n=314	189 [60.2%]	125 [39.8%]	0.60±0.49
Sought treatment in health facility n=189	119 [37.9%]	70 [22.3%]	0.63±0.48
Diagnosis n=189			
Fever	155 [49.4%]	34 [10.8%]	3.32±2.23
Cough	94 [29.9%]	95 [30.3%]	5.32±5.39
Fast breathing	40 [12.7%]	149 [47.5%]	3.00±1.56
Wheezing	19 [6.1%]	170 [54.1%]	4.26±3.01
Vomiting	60 [19.1%]	129 [41.1%]	1.95±0.89
Diarrhea	61 [19.4%]	128 [40.8%]	3.13±1.92
Skin Rash	66 [21.0%]	123 [39.2%]	6.68±6.46
Lack of appetite	64 [20.4%]	125 [39.8%]	3.39±1.93

4.3.3 Anthropometric nutritional status

Table 4.9 shows the indices of malnutrition by gender, age group, and location. Overall, at <-2SD, 17.5% of children were stunted, 13.4% underweight, and 6.4% were wasted. Male children compared to females were more affected by stunting [21.7%], underweight [14.9%], and wasting [7.4%]. Compared to 6-11 and 12-17 months' age groups, children aged 18-23 months had a higher prevalence of underweight [19.6%], stunting [14.4%], and wasting [7.2%].

Stunting compared to underweight and wasting was prevalent in all the locations. However, stunted children were more in Lwandawiti location [22.1%]. On the other hand, Got-

Kojowi location had the highest proportions of underweight [17.6%] and wasted [8.2%] children.

Table 4. 9: Prevalence of wasting, stunting, and underweight by gender, age group, and location

	Wasting z-scores [percentages]		Stunting z-scores [percentages]		Underweight z-scores [percentages]	
	<-2	<-3	<-2	<-3	<-2	<-3
Overall [n=314]	6.4	1.9	17.5	7.6	13.4	4.1
Gender						
Male [n=175]	7.4	1.7	21.7	10.3	14.9	5.1
Female [n=139]	5	2.2	12.2	4.3	11.5	2.9
Age group						
6-11 [n=112]	5.4	0.9	8.9	2.7	8.9	1.8
12-17 [n=105]	6.7	2.9	12.4	6.7	12.4	4.8
18-23 [n=97]	7.2	2.1	33	14.4	19.6	6.2
Location						
Lwandawiti [n=122]	4.9	1.6	22.1	9.0	15.6	4.9
Wachara [n=107]	6.5	0.9	11.2	3.7	7.5	1.9
Got-Kojowi [n=85]	8.2	3.5	18.8	10.6	17.6	5.9

Figure 4.7 below indicates the prevalence of moderate [-3 to <-2SD] and severe [<-3SD] forms of malnutrition in male and female children. Male children had a high prevalence of both moderate and severe forms of malnutrition than females. Stunting was the most prevalent form of moderate and severe malnutrition in both males and female children, followed by underweight. Moderate stunting in males was higher [22%] than severe [10%], which was almost similar to moderate stunting in females [12%].

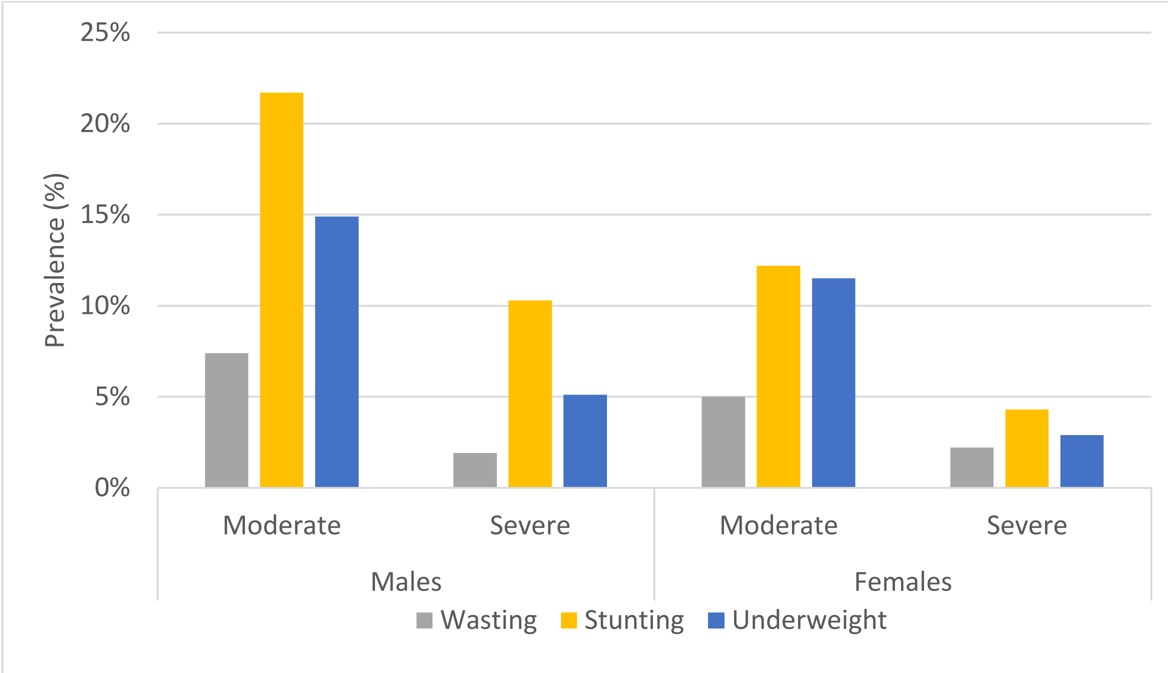

Figure 4. 7: Prevalence of moderate and severe malnutrition

4.3.4 Biochemical nutritional status

Figure 4.8 depicts iron indices, zinc, and CRP concentrations. Of the 314 infants enrolled in the study, we obtained blood from all of them for iron assessment but from 283 infants for zinc assessments. There was a lower prevalence of infection among the infants [4.5% with elevated CRP]. Using the WHO cut-offs 11.0g/dL of hemoglobin levels for infants (WHO, 2007), the prevalence of anemia was 50.6%. The prevalence of iron deficiency within the study population was 39.5% as reflected by low ferritin concentrations below a cut-off point of <15µg/L. On the other hand, the prevalence of Iron Deficiency Anemia was 19.1% among the infants as reflected by a combination of anemia and iron deficiency (WHO, 2007). Zinc deficiency was at 43.6% among the infants based on the recommended cut-off point of children less than 10 years which is <65µg/L (Hess et al., 2007; Hotz & Brown, 2004a).

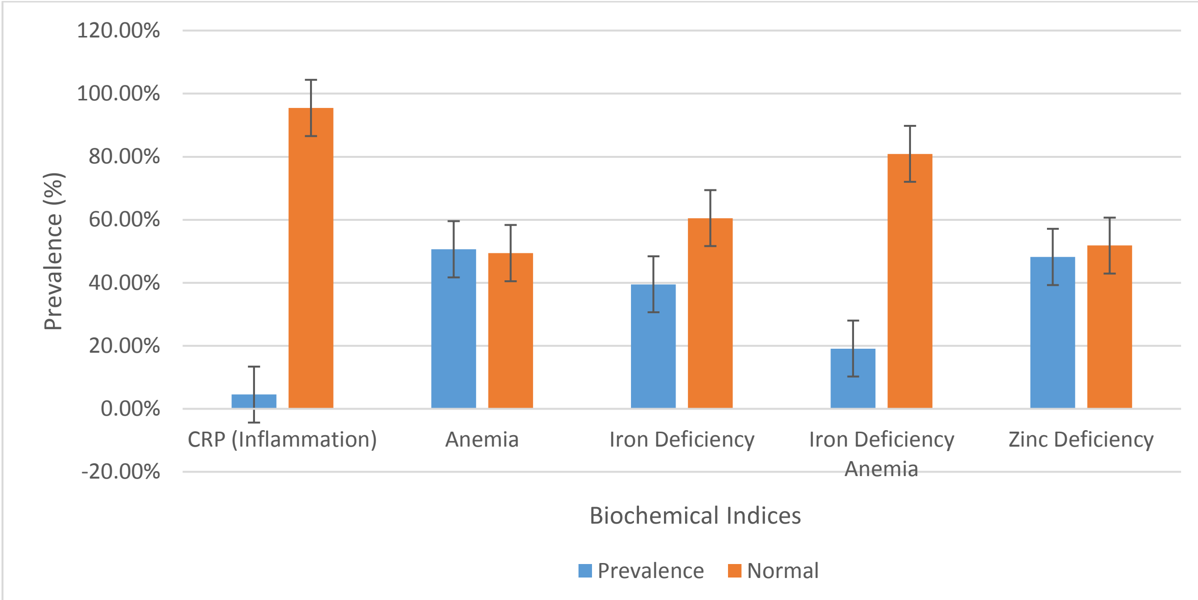

Figure 4. 8: Prevalence of Anemia, Iron deficiency, Iron deficiency Anemia, and Zinc deficiency

4.3.5 Associations of nutritional status of children by age group, sex and location

Table 4.10 below shows the z-scores by age group, sex, and location/cluster. Male children 18-23 months were statistically stunted compared to male children 6- 11 and 12- 17 months [p=<0.001]. Female children, 18-23 months were significantly stunted compared to female children 6-11 months [p=0.038]. On the other hand, male children aged 12-17 months were significantly underweight when compared with male children aged 6-11 and 18-23 months [p=0.046].

Stunting is significant between males and females in Lwandawiti [p=0.019] and Wachara [p=0.005] locations but not in Got-Kojowi [p=0.144]. However, stunting within the different locations was only significant in females [p<0.001] in all the locations. Underweight within the different locations was also significant [p=0.001] in only females of Lwandawiti location compared to Wachara and Got-Kojowi. Notably, no significant differences were observed between sex and within different age groups and locations for wasting.

Anemia is significant within age groups in males [$p<0.001$] of 18-23 months but not in 6-11 and 12-17 months' age group. Iron deficiency anemia is significant between males and females of age group 6-11 months [$p=0.014$] only but not in 12-17 and 18-23 months' age groups. However, iron deficiency anemia within the different age groups was not significant in both males [$p=0.069$] and females [$p=0.490$].

Iron deficiency anemia within the different locations was significant [$p=0.05$], though borderline, in only males of Got-Kojowi location. Zinc deficiency within the different locations was significant between males and females [$p=0.001$] in Got-Kojowi location.

Table 4. 10: z-scores by age group, sex, and location

Nutrition Index	Sex	Age Groups Mean ± SD			Age p	Locations in Homabay Mean ± SD			Location p
		6-11	**12-17**	**18-23**		**Lwandawiti**	**Wachara**	**Got-Kojowi**	
Stunting	Male	-0.50 ± 1.20^{a}	-0.58 ± 1.69^{a}	-1.57 ± 1.61^{b}	**<0.001**	-0.69 ± 1.59	-0.82± 1.57	-1.09 ± 1.60	0.408
	Female	-0.28 ± 1.95^{a}	-0.45 ± 1.11	-1.05 ± 1.46^{b}	**0.038**	-1.37 ± 1.46^{a}	0.04 ± 1.51^{b}	-0.61 ± 1.38^{c}	**<0.001**
	Sex p	0.511	0.659	0.088		**0.019**	**0.005**	0.144	
Underweight	Male	-0.48 ± 1.13^{a}	-0.74 ± 1.28	-1.08 ± 1.29^{b}	**0.046**	-0.75 ± 1.17	-0.64 ± 1.33	-0.89 ± 1.31	0.622
	Female	-0.64 ± 1.34	-0.61 ± 0.98	-0.68 ± 1.04	0.952	-1.08 ± 1.15^{a}	-0.27 ± 0.98^{b}	-0.60 ± 1.06^{b}	**0.001**
	Sex p	0.519	0.578	0.084		0.131	0.102	0.278	
Wasting	Male	-0.21 ± 1.18	-0.61 ± 1.01	-0.43 ± 1.07	0.130	-0.53 ± 1.09	-0.29 ± 1.15	-0.44 ± 1.02	0.460
	Female	-0.47 ± 1.24	-0.55 ± 0.88	-0.23 ± 0.93	0.271	-0.43 ± 1.16	-0.38± 0.88	-0.37 ± 1.04	0.954
	Sex p	0.299	0.746	0.303		0.647	0.623	0.751	
Anemia	Male	1.68±0.46^{a}	1.59±0.49^{a}	1.31±0.469^{b}	**<0.001**	1.55±0.50	1.57±0.49	1.46±0.50	0.483
	Female	1.57±0.50	1.43±0.50	1.44±0.50	0.328	1.43±0.50	1.55±0.50	1.44±0.50	0.416
	Sex p	0.253	0.162	0.197		0.199	0.818	0.851	
ID	Male	0.40±0.49	0.48±0.50	0.37±0.48	0.492	0.49±0.50	0.27±0.44	0.50±0.50	0.416
	Female	0.26±0.44	0.43±0.50	0.38±0.49	0.260	0.43±0.50	0.25±0.44	0.41±0.49	0.146
	Sex p	0.145	0.621	0.923		0.487	0.880	0.414	
IDA	Male	0.30±0.46*	0.25±0.43	0.12±0.32	0.069	0.32±0.46^{a}	0.14±0.35^{b}	0.20±0.40^{ab}	**0.058**
	Female	0.10±0.29	0.14±0.35	0.18±0.38	0.490	0.16±0.37	0.12±0.32	0.15±0.36	0.796
	Sex p	**0.014**	0.170	0.361		0.060	0.703	0.619	
ZN	Male	0.43±0.49	0.49±0.50	0.43±0.50	0.712	0.58±0.49^{a}	0.47±0.50^{a}	0.23±0.42^{b}	**0.001**
	Female	0.46±0.50	0.66±0.48	0.45±0.50	0.109	0.63±0.49^{a}	0.65±0.48^{a}	0.27±0.45^{b}	**0.001**
	Sex p	0.775	0.097	0.783		0.775	0.097	0.783	

The Bonferroni test was used to identify which groups differed in the analysis of variances [ANOVA]

4.3.6 Feeding practices and World Health Organization [WHO] indicators

Figure 4.9 shows the common complementary foods fed to children. Almost half [41.7%] of the children were fed porridge as their first drink beginning from 5 months of age, but the mean age was 7 months. The porridges commonly served in this region were white maize porridge [14%], sorghum [10.5%] and millet porridge [9.9%], in that order. Notably, cassava was added in about a quarter of the porridges [26.4%]. However, less than 3% of caregivers processed or fermented these porridges.

More than half [53.5%] of the children were not given milk feeds. However, the mean age for starting milk feeds for those who received [46.5%] was 6 months. While diluted fresh cow's milk was the commonest milk feed served to 27.1% of the children, infant formula was the least [0.3%]. The majority of the children [88.2%] consumed solid and semi-solid foods. The mean age for starting these foods was 7.5 months. Among the solid foods, ugali, jute mallow, and rice topped the list by 58.0%, 55.4% and 34.1%, respectively.

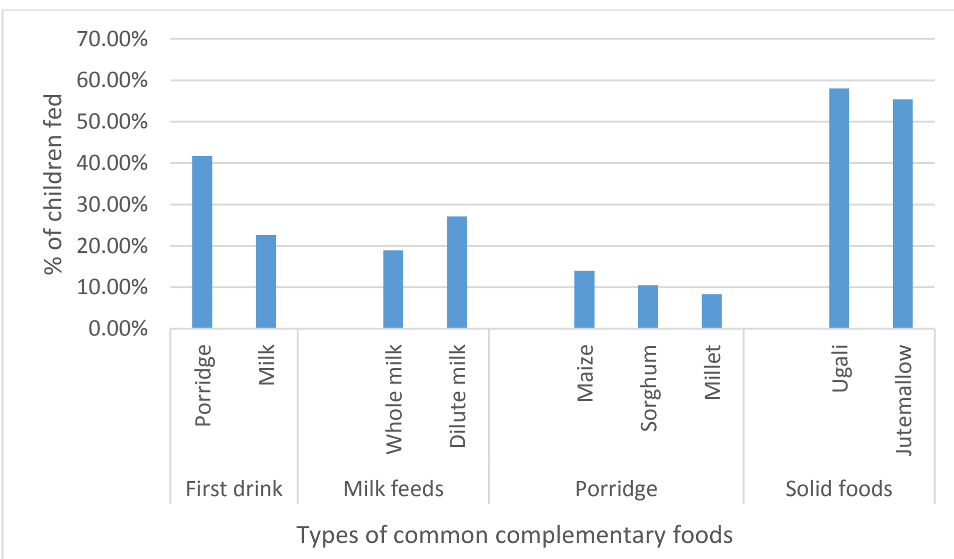

Figure 4. 9: Complementary foods fed to children 6-23 months

Figure 4.10 shows the WHO infant and young child feeding indicators. Sixty-two percent of children aged 6-8 months had received solid, semisolid, or soft foods. Whereas 30.3% of breastfed and non-breastfed children 6–23 months of age received solid, semi-solid, or soft foods [including milk feeds for non-breastfed children] the minimum number of times [frequency] or more, only 14.6% received foods from four or more food groups. Only 4.8% of the children 6-23 months received solid, solid foods or soft foods the minimum number of times and had at least the minimum food diversity.

All the children had ever been breastfed. More than three quarters [72.6%] of the children studied were still breastfeeding. However, only 38% of these caregivers initiated breastfeeding immediately after birth. Six out of 10 children [61.8%] were exclusively fed on breast milk for the first 5 months. Eighty-five percent continued breastfeeding after one year while 33.9% continued breastfeeding into the second year of life. Although the duration of breastfeeding varied from one caregiver to the other, the median age of stopping breastfeeding was 14 months.

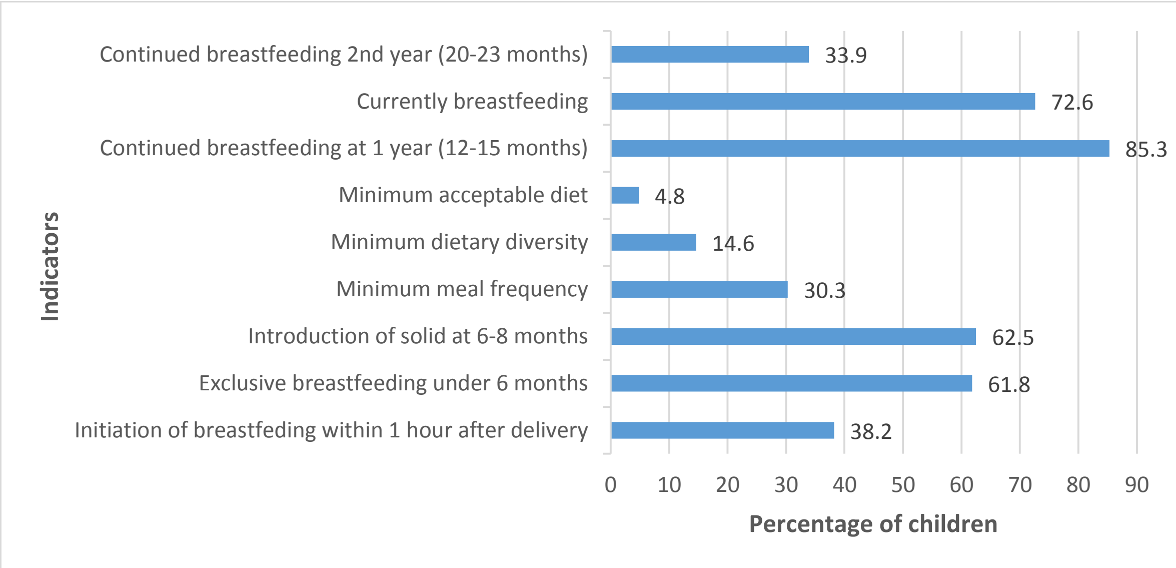

Figure 4. 10: WHO indicators for infant and young child feeding

4.3.7 Frequency of consumption of foods

Table 4.11 represents the frequency of feeding the children on various foods [every day, most days, once a week and never] based on the usual intake in the last week. Daily, 7 in 10 surveyed children were breastfeeding [70.7%], 7 in 10 children were drinking porridge [71.7%], and 6 in 10 children received ugali [60.2%]. Half of the children [53.5%] were feeding on vegetables daily. About 3 in 10 children fed on cow's milk [27.7%] and tea [29%] every day. In most days in a week, nearly a third of the children fed on sardines [34.4%] and legumes [36.6%]. Twenty percent of children consumed fish once a week. Notably, 8 in 10 children never fed on eggs [83.1%], red meat [91.7%], chicken [90.4%], and liver [97.1%] the entire week.

Table 4. 11: Association of foods frequency and nutrition status

Parameter	Description Frequency [Percentage] n=314				Mean±SD
	Everyday	Most days	Once a week	Never	
Breastmilk	222 [70.7%]	5 [1.6%]	1 [0.3%]	86 [27.4%]	1.84±1.34
Cow milk	87 [27.7%]	44 [14.0]	18 [5.7%]	165 [52.5%]	2.83±1.32
Porridge	225 [71.7%]	36 [11.5%]	11 [3.5%]	42 [13.4%]	1.59±1.06
Vegetable	168 [53.5%]	87 [27.7%]	11 [3.5%]	48 [15.3%]	1.81±1.07
Ugali	189 [60.2%]	67 [21.3%]	8 [2.5%]	50 [15.9%]	1.74±1.09
Eggs	2 [0.6%]	19 [6.1%]	32 [10.2%]	261 [83.1%]	3.76±0.59
Red meat	0 [0%]	6 [1.9%]	20 [6.4%]	288 [91.7%]	3.90±0.36
Chicken	0 [0%]	3 [1.0%]	27 [8.6%]	284 [90.4%]	3.89±0.34
Liver	0 [0%]	1 [0.3%]	8 [2.5%]	305 [97.1%]	3.97±0.19
Fish	1 [0.3%]	42 [13.4%]	63 [20.1%]	208 [66.2%]	3.52±0.73
Sardines	9 [2.9%]	108[34.4%]	80 [25.5%]	117 [37.3%]	2.97±0.91
Legumes	7 [2.2%]	116[36.9%]	74 [23.6%]	11 7[37.3%]	2.96±0.91
Tea	91 [29.0%]	81 [25.8%]	25 [8.0%]	117 [37.3%]	2.54±1.26

4.3.8 The food patterns identified by factor analysis

Often children do not consume one food in a diet. This study, therefore, tested the relationship between consuming a combination of foods and nutritional indicators. In this study, children consumed 41 different foods, albeit at varying frequencies. We employed the Explorative Factor Analysis [EFA] method in SPSS, to define the dietary patterns among the study population. The sample size adequacy and significance of correlation of factors were determined using the Kaiser Meyer Olkin test [0.735] and the Barttletts test of sphericity [0.000] respectively. The variables were found to be orthogonally related. We selected components where the associated eigenvalue was greater than one. The four components with an eigenvalue of greater than one accounted for 51% of the total variation in the data explained. Factors with loadings 0.4 and above were considered correlated to the other factors in the component. However, after testing for the reliability of the components using the Cronbach's alpha test [≥ 7], two components were found to be reliable. The two components accounted for 35% of the total variation in the data explained. The factor loadings did not change when the variables [foods] that did not have high loadings in the food patterns and did not show correlations with food patterns were excluded in the analysis.

The names given to the food patterns were based on the first three foods with the highest factor loadings value. The first food pattern was predominantly ugali, vegetable [Jute mallow], porridge and tea while the second component comprised of liver, red meat, chicken, eggs and soda.

Food intake [determined by individual child score] among children in the different quartiles[Q] varied. Intake of the dominant foods in the Q1 was significantly lower compared to Q2, Q3, and Q4 [$p = <0.001$]. Children were stratified based on the status of their anthropometric and nutritional biochemical indices in this study. We then compared the distribution of children in the quartiles for each of the indices.

The results showed that the frequency of consuming foods in the second food pattern was negatively associated with anemia [all causes] [p=<0.001], iron deficiency anemia [p=<0.001] and underweight [p=0.046]. On the other hand, the frequency of consuming the foods in the first food pattern negatively correlated with anemia [all causes] but the result was not significant [p=0.055] Table 4.12.

Table 4. 12: Correlations of food, nutrient with nutritional status of children

Components/Patterns	**p-value***				
	Anemia	**IDA**	**Zinc**	**Stunting**	**Underweight**
Food patterns					
Ugali, jute-mallow, porridge and tea	**0.055**	<0.981	0.288	0.188	0.934
liver, red meat, chicken, eggs	**<0.000**	**<0.000**	0.311	0.261	**0.046**
Nutrient patterns					
Carbohydrates, fat, dietary fiber, and iron	0.800	0.759	0.288	**0.027**	**0.011**
Zinc, B vitamins, cholesterol, calcium, potassium and sodium	0.173	0.896	**0.012**	0.431	0.4
Vitamin C, Vitamin A and folic acid	0.398	**0.011**	0.401	0.522	0.622

*Spearman's correlation

A logistics regression model conducted with anthropometric and biochemical indices as dependent variable and the quartiles of the first and second food patterns as independent variables are presented as adjusted odds ratio, Table 4.13.

Overall the odds of being anemic is inversely related to the frequency of consuming the foods in the "liver, red meat, chicken, eggs" pattern. Children with the least frequency of consuming foods in the second food pattern [Q1] are three times more likely to be anemic as compared to those who consumed the same foods with the most frequency [p=0.001]. Similarly, the odds of being anemic due to iron deficiency [IDA] is negatively related to the frequency of consuming foods in the "liver, red meat, chicken, eggs" pattern. Children who consumed the least [Q1] and the second least [Q2] frequency of "liver, red meat, chicken, eggs” pattern were four and three times more likely to be IDA compared to children in Q4 respectively [p=0.005, p= 0.019]. The odds of being underweight was higher among children who consumed Q1 frequency compared to those in Q4. However, this trend is not significant.

4.3.9 Nutrient patterns from the explorative factor analysis

Using the 24-hr dietary recall method, 23 nutrients [equal to the number of nutrient variables] were studied. Using the explorative factor analysis method, the 23 nutrients were reduced to three nutrient patterns. The adequacy of the sample of this study and the correlation of nutrients was tested and found to be adequate [Kaiser Meyer Olkin test = 0.735] and correlated [Barttletts test of sphericity <0.000]. After performing a varimax rotation of the nutrients, four components [nutrient patterns] accounted for 83% of the total variance explained and had eigenvalues equal to or greater than one. However, only three components were found to be reliable, based on Cronbach’s alpha test [≥ 7]. The three nutrient patterns were named based on the three dominant nutrients with the highest factor loadings in the nutrient pattern. The first nutrient pattern comprised of carbohydrates, vitamin B1, magnesium, and phosphorus. Other nutrients in this group included iron, fat, and dietary fiber. The second nutrient pattern comprised of vitamin B2, zinc, and calcium. Other nutrients in the second pattern were VitaminB6, PUFA, and cholesterol. The third nutrient pattern consisted of vitamin A, vitamin C, and folic acid. We then stratified the children into quartiles based on the amounts of these nutrients consumed.

A comparison of the proportions of children based on the anthropometric and biochemical indices were compared with proportions of children in the quartiles for each of the nutrient patterns. Table 4.13 shows the results. Intake of nutrient pattern 1 “carbohydrates, vitamin

B1, magnesium, and phosphorus" was negatively correlated with being underweight [p=0.011]. On the other hand, consumption of nutrients pattern 2 "vitamin B2, zinc and calcium" and pattern 3 "vitamin A, C and folic acid" were negatively correlated with zinc deficiency and iron deficiency anemia respectively [p=0.012, p=0.011]

Table 4.13 shows the results of a binary logistic regression performed on underweight, zinc deficiency, and IDA as outcome variables and consumption of the nutrient patterns as independent variables. The results show that children who consumed the least amounts of the nutrients "Carbohydrates, fat, dietary fiber, and iron" had a lower odd of being underweight as compared to those who consumed the highest amounts of the nutrients in this nutrient pattern [Adjusted odds ratio 0.385 [0.181-0.817], p=0.013]. On the contrary, children who consumed the least amounts of nutrients "vitamin B2, zinc and calcium" had a higher odd of being zinc deficiency [AOR 2.326[1.108 -4.883] P=0.026] as compared to those who consumed higher amounts of the same nutrients. Children who consumed the least of "vitamin A, C and folic acid" were 3.3 times more likely to suffer from anemic due to iron deficiency [AOR 3.391[1.154 – 9.961] p=0.026] respectively.

We observed no significant associations between the nutrient patterns and the odds of being stunted, wasting, and anemia [all causes].

Table 4. 13: Logistics regression for nutritional status versus food patterns, nutrient patterns, and dietary diversity score

Patterns and diversity	**Anemia**		**IDA**		**Zinc**		**Stunting**		**Underweight**	
	p value	AOR	p value	AOR	p value	AOR	p value	AOR	p value	AOR
Food patterns										
Liver, red meat, chicken, eggs [Q1]	**0.001**	3.111 [1.545-6264]	**0.005**	3.986 [1.529-10.393]	0.381	1.388 [0.666 - 2.892]	0.274	1.554 [0.705 - 3.426]	0.182	1.680[0.785 - 3.599]
Liver, red meat, chicken, eggs [Q2]	0.085	1.789 [0.924-3.463]	**0.019**	3.130 [1.210-8.095]	0.965	0.984 [0.479-2.021]	0.320	1.468 [0.689 -3.127]	0.340	1.440[0.680 - 3.046]
Liver, red meat, chicken, eggs [Q3]	0.326	1.394 [0.718-2.707]	0.371	1.595 [0.574-4.438]	0.652	1.181 [0.572-2.438]	0.989	0.995 [0.449 - 2.204]	0.918	0.959(0.433 - 2.125]
Nutrient patterns										
Carbohydrates, fat, dietary fiber, and iron [Q1]	0.325	0.711 [0.361 - 1.402]	0.796	1.123 [0.465 - 2.714]	0.180	0.609 [0.295 - 1.258]	0.107	0.535 [0.250 - 1.145]	**0.013**	0.385 [0.181 - 0.817]
Carbohydrates, fat, dietary fiber, and iron [Q2]	0.903	1.041 [0.543 - 1.999]	0.223	1.653 [0.736 - 3.713]	0.384	0.733 [0.364 - 1.476]	0.131	0.579 [0.285 - 1.176]	0.065	0.524 [0.264 - 1.041]
Carbohydrates, fat, dietary fiber, and iron [Q3]	0.896	0.958 [0.500 - 1.832]	0.586	1.262 [0.547 - 2.911]	0.443	0.758 [0.373 - 1.539]	0.161	0.600 [0.293 - 1.227]	**0.006**	0.356 [0.172 - 0.739]
Zinc, Vitamin Bs, cholesterol, calcium, potassium and sodium [Q1]	0.671	1.154 [0.597 - 2.230]	0.075	3.625 [0.879 - 14.940]	**0.026**	2.326[1.108 - 4.883]	0.256	1.545[0.730 - 3.270]	0.330	1.433 [0.695 - 2.953]

Zinc, Vitamin Bs, cholesterol, calcium, potassium and sodium [Q2]	0.078	1.811 [0.935 - 3.509]	0.065	3.851 [0.918 - 16.152]	**0.031**	2.239 [1.077- 4.654]	0.758	1.124 [0.535 - 2.359]	0.942	1.027 [0.493 - 2.141]
Zinc, Vitamin Bs, cholesterol, calcium, potassium and sodium [Q3]	0.507	1.248 [0.649 - 2.401]	0.152	2.871 [0.679 - 12.148]	0.646	1.182 [0.579 -2.415]	0.550	0.792 [0.369 - 1.701]	0.868	0.940 [0.449 - 1.965]
Vitamin C, Vitamin A and folic acid [Q1]	0.929	1.046 [0.389 - 2.810]	**0.026**	3.391[1.154 - 9.961]	0.111	0.434 [0.156 - 1.211]	0.183	2.186 [0.692 - 6.907]	0.306	0.588 [0.213 - 1.625]
Vitamin C, Vitamin A and folic acid [Q2]	0.709	0.829 [0.309 - 2.220]	**0.013**	3.918 [1.334- 11.511]	0.178	0.483 [0.168 - 1.393]	0.828	1.141 [0.346 - 3.766]	0.526	0.718 [0.258 - 1.999]
Vitamin C, Vitamin A and folic acid [Q3]	0.986	0.990 [0.346 - 2.834]	0.117	2.418 [0.802- 7.292]	0.779	0.852 [0.279 - 2.601]	0.536	1.480 [0.427 - 5.128]	0.455	0.658 [0.219 - 1.974]
Dietary diversity score										
Dietary diversity score <4	0.362	1.360 [0.702 - 2.634]	0.827	0.913 [0.403 - 2.066]	0.332	1.426 [0.696 - 2.919]	0.976	0.989 [0.471 - 2.077]	**0.029**	2.773 [1.109 - 6.936]

4.3.10 The energy and nutrient intake of children

Table 4.14 presents the nutrient adequacy and the quality of the diet consumed by children in the study.

Overall, seven out of 10 children [73.6%] met the Estimated Energy Requirements [EER]. Although children in the three age groups met the recommended percent of total calories from proteins and fats, the average percentage of proteins and fats is just above the minimum range. Less than 30% of children met the EAR for Zinc [14%], Vitamin B6 [27.4%], Vitamin B2[27.4%], folic acid [1.3%], potassium [7%], and calcium [6.4%]. Four in every 10 children did not meet the EAR for iron.

Overall, the results showed a positive correlation between the adequacy of nutrients and total calories with age groups. Children aged 18-23 months were more likely to meet both the EER and EAR for most nutrients. However, fat, carotene, vitamin B2, folic acid, and cholesterol adequacy showed an inverse relationship with age groups with children 6-11 months meeting the EER and EAR as compared to the children 12-17 and 18-23 months. Sex, cluster, and socio-economic status of the children had no association with the adequacy of energy [EER] and nutrients [EAR].

Table 4. 14: Energy and nutrient adequacy for children of different age groups

		Age group			
Nutrient/energy	Pooled group	6-11 months	12-17 months	18-23 months	p-value[2]
Total energy, kcal [TE][1]	1348.6 [772.2; 2106.8]	1092.7 [395.02;1833.9]	1483.3 [1006.8;2152.4]	1408.7 [952.2;2280.2]	
≥ EER, %	73.6	62.7^{b}	81.7^{b}	75.5^{a}	0.007
Protein ≥ EAR, %	88.3	75.8^{b}	92.7^{a}	95.3^{b}	<0.000
Protein, % TE	12.6	13.1	12.6	12.3	0.124
Fat ≥ EAR, %	15	15.2^{a}	15.6^{a}	14.2^{a}	0.838
Fat, % TE	13.8	17.8	12.3	11.4	<0.000
Carbohydrates ≥ EAR, %	83.4	67.7^{a}	89.9^{b}	91.6^{b}	<0.000
Carbohydrate, % TE	73.6	68.9	74.9	76.4	<0.000
Dietary fiber < EAR, %	88		10.3^{a}	13.3^{a}	0.526
VitaminB1 ≥ EAR, %	86	75.8^{b}	90.9^{a}	90.6^{a}	0.003
Magnesium < EAR, %	92	81.8^{a}	96.3^{b}	97.2^{b}	<0.000
Phosphorus < EAR, %	82.8	76.8^{a}	86.2^{a}	84.9^{a}	0.152
Iron < EAR, %	59.6	30.3^{a}	71.6^{b}	74.5^{b}	<0.000
Zinc ≥ EAR, %	14	8.1^{b}	21.1^{b}	12.3^{a}	0.021
Vitamin B6 ≥ EAR, %	27.4	26.3^{a}	33^{a}	22.6^{a}	0.222
PUFA ≥ EAR, %	1	0^{a}	1.8^{a}	0.9^{a}	0.503
Cholesterol ≥ EAR, %	1.3	3^{a}	0.9^{a}	0^{a}	0.102
Vitamin A ≥ EAR, %	51.9	39.4^{b}	56^{a}	59.4^{a}	0.009
Carotene ≥ EAR, %	99.4	100^{a}	99.1^{a}	99.1^{a}	0.629
Vitamin B2 ≥ EAR, %	27.4	29.3^{a}	27.5^{a}	25.4^{a}	0.828
Total Folic Acid ≥ EAR, %	1.3	2^{a}	0^{a}	1.9^{a}	0.956
Vitamin C ≥ EAR, %	45.5	30.3^{b}	50.5^{a}	54.7^{b}	0.001
Sodium ≥ EAR, %	90.8	85.9^{b}	89^{a}	97.2^{b}	0.015
Potassium < EAR, %	7	3^{a}	8.3^{a}	9.4^{a}	0.163
Calcium ≥ EAR, %	6.4	8.1^{a}	9.2^{a}	1.9^{b}	0.037

EER [Estimated Energy Requirements]; EAR [Estimated Average Requirements]; TE [Total Energy]. [1]reported as median [25th; 75th percentile]. [2]spearman's rho test for categorical data, [3] negative correlation
a, b - the values with different letters in superscript differed significantly

4.3.11 Dietary diversity score

Figure 4.11 indicates the dietary diversity scores of these children. From the seven food groups, most of the children fed on grains, roots, and tubers [93.0%] followed by 62.4 % on other fruits and vegetables. However, only two children out of the 314 consumed eggs. The mean DDS was 2.47. A majority of the children received food from two food groups [37.3%] followed by three food groups [32.5%]. Notably, only 1% of all the children studied were given foods from five food groups.

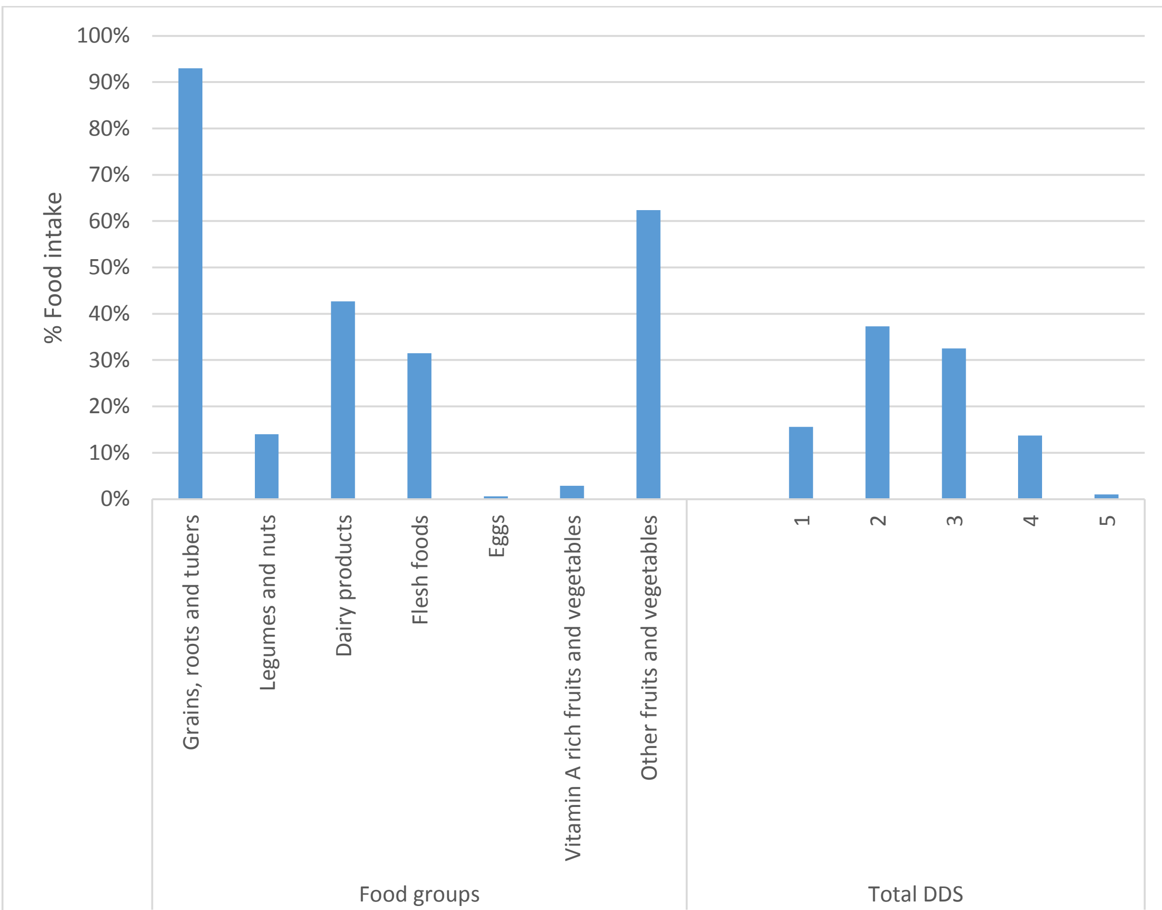

Figure 4. 11: Dietary diversity score and total dietary diversity score [TDDS]

The results of Table 4.15 depict the correlation between food groups, dietary diversity score with the nutritional indices. Intake of foods from the grains, roots, and tubers food group was associated with zinc deficiency in children [p=0.031]. Foods from the dairy products group were associated with stunting [p=0.043] and underweight [p=0.005]. On the other hand, we revealed an association between the intake of flesh foods and underweight [p=0.020] Additionally, the total dietary diversity score was associated with underweight [p=0.023].

The intake of foods from the grains, roots, and tubers food group was associated with zinc deficiency in children [p=0.031]. Foods from the dairy products and flesh foods groups were associated with stunting [p=0.003]. On the other hand, a low intake of eggs among children was associated with stunting [p=0.023]. Additionally, the total dietary diversity score intake based on the food groups was associated with underweight [p=0.026] and zinc deficiency [p=0.022] in children.

Table 4. 15: Associations of dietary diversity score with nutritional status

	p-value						
Dietary Diversity Score	**Wasting**	**Stunting**	**underweight**	**Anemia**	**ID**	**IDA**	**ZN**
Grains, roots and tubers	0.584	0.288	0.364	0.206	0.445	0.656	**0.031**
Legumes and nuts	0.728	0.547	0.215	0.286	0.647	0.069	0.149
Dairy products	0.747	**0.043**	**0.005**	0.379	0.985	0.861	0.424
Flesh foods	0.089	0.428	**0.020**	0.084	0.822	0.980	0.916
Eggs	0.864	0.084	1.000	0.243	0.521	1.000	0.499
Vitamin A rich fruits & vegetables	0.428	0.720	0.772	0.292	0.317	0.271	0.413
Other fruits and vegetables	0.893	0.618	0.205	0.861	0.418	0.306	0.788
Total Dietary Diversity Score < 4	0.234	0.733	**0.026**	0.171	0.785	0.932	**0.022**

Binary logistics regression with nutritional indices as dependent variable and food groups and total dietary diversity as the independent variables was conducted. The results are presented as adjusted odds ratio with 95% CI after controlling for potential confounding factors are presented in Table 4.16. Children who consumed grains, roots, and tubers had a lower odd of being zinc deficiency compared to those who not fed from the same food group [p=0.049]. Consumption of dairy products and flesh foods was associated with lower odds of being underweight [p=0.010, p=0.027]. Consumption of dairy foods was associated with lower odds of being stunted. However, the AOR was not significant [p=0.087]. The DDS≥4 was associated with a lower odd of being underweight [p=0.023] and zinc deficiency [p=0.016].

Table 4. 16: Regression model on dietary diversity score and nutritional indices

Food group	CoR	95% C.I	P value	AoR	95% C.I	P value
Stunting						
Dairy products	1.690	1.013-2.820	0.044	1.623	0.931-2.828	0.087
Zinc deficiency						
Grains, roots & tubers	0.33	0.118-0.944	0.039	0.319	0.104-0.983	0.049
Underweight						
Dairy products	2.126	1.246-3.628	0.006	2.069	1.189-3.599	0.010
Flesh foods	1.993	1.108-3.586	0.021	1.981	1.082-3.626	0.027
DDS ≥ 4	0.372	0.152-0.913	0.031	0.350	0.141 - 0.873	0.024
DDS ≥ 4	0.446	0.220-0.901	0.024	0.400	0.190 - 0.844	0.016

CoR-Crude odds ratio, AoR-Adjusted Odds ratio

CHAPTER FIVE: DISCUSSION

5.1 Phase one: *In vitro* bioaccessibility of iron and zinc

The objective of this study was to determine the effect of processing of maize, millet and sorghum porridge on the bioaccessibility of iron and zinc from added micronutrient powder. The effect of germination on phytates, tannin, total phenolics and minerals were evaluated using appropriate methods. Additionally, the influence of germination [processing] on iron and zinc bioaccessibility was determined using an *in vitro* dialysability assay.

5.1.1 Effects of processing on iron and zinc and other nutrients

The reported iron and zinc contents in unprocessed porridges without MNP ranges between 1.1–8.7 and 1.5–4.9 mg/100 g for maize (Ortiz-Monasterio et al., 2007), 2.17–6.52 mg/100 g and 1.66-2.53 mg/100 g for millet (Upadhyaya et al., 2011) and 1.1 and 6.5 mg/100 g for sorghum (Hemalatha et al., 2007), respectively. As expected, our results showed similar trends where iron contents were higher than zinc, but our values were lower than what was reported for all cereals. The amount of extrinsic iron in soil, [from grain threshing on surfaces exposed to soil and storage of grains on the mud floor], as well as zinc, has been found to influence the amount of these nutrients in cereals grown in such areas (Gabaza M. et al., 2018). It can be inferred, that the soils in Kenya are deficient in both iron and zinc. Furthermore our results showed aluminium contents greater than 1 mg/100 g which is indicative of soil contamination (Kang & Priyadarshan, 2008). Nonetheless, since the nutrients were analyzed in cooked porridges, the levels varied with what has been reported in flours, slurries and porridges cooked under controlled environment. In agreement to what has been previously reported (Devi, Vijayabharathi, Sathyabama, Malleshi, &

Priyadarisini, 2014), this study showed that finger millet had the highest levels of calcium, magnesium, aluminum, iron, and zinc compared to maize and sorghum.

Soaking and germination has been reported to cause a modest reduction of minerals (Platel et al., 2010), due to the leaching of ions into the soaking water through a concentration gradient (Lestienne, Icard-Vernière, Mouquet, Picq, & Trèche, 2005). A similar trend was observed in our findings where the levels of nutrients except iron reduced upon processing in millet and sorghum, but not in maize. Longer soaking [24 hours] and germination [>48 hours] as well as, the grain water ratios [1:5] increases the losses (Aparna et al., 2017). These explains possibly, the slight decrease of these nutrients in our study attributable to the shortened soaking [12 hours] and germination [48 hours] time, with 1:3 grain water ratio. Furthermore, the reduced processing time has been recommended since undesirable microorganism have been found to develop during longer germination periods (Adebiyi, Obadina, Adebo, & Kayitesi, 2018; Frančáková, Líšková, Bojňanská, & Mareček, 2012). The observed increase of iron levels in processed cereals without MNP is attributable to the drinking water used in a commercial pilot plant during processing which may contain iron. Our results supports the fact that, higher mineral contents observed after processing, is linked to the additional minerals from the processing water (Wu, Ashton, Simic, Fang, & Johnson, 2018). Although cooking increases the availability of nutrients (Hambidge, 2010), it may further reduce iron and zinc when the mineral inhibitors are released due to their chelating effects. Despite MNP added in porridges after processing and cooking, our results showed minimal reduction in iron and zinc. The unexpected reduction was perhaps due to the binding effects of mineral inhibitors to these nutrients during the cooling process of the porridge after MNP is added, and previous losses of the intrinsic zinc and iron caused by processing and cooking respectively. The observed higher losses in iron compared to zinc, is consistent to what was reported recently (Aparna et al., 2017), which could be due to the different locations of these nutrients in the grains, as well as the molecules linked to them (Lestienne et al., 2005). Likewise, this explanation may justify the different observations with cereals in our study, where the levels of intrinsic nutrients decreased and varied in millet and sorghum respectively, but increased in maize upon processing and cooking. Therefore, processing of maize can be beneficial in Kenya for improved nutrient content since it is a staple. Further to that, when complementary foods are prepared from germinated cereals, the palatability is improved and the calorie density is increased as a result of reduced viscosity (Platel et al., 2010).

5.1.2 Effects of germination on phenolics and tannins

Similar to what was reported in Zimbabwe (Gabaza M. et al., 2018), we found lower levels of both phenolic and tannins content in unprocessed maize porridge without MNP compared to millet and sorghum. Although, the observations were similar, the values in our study were lower probably because we used cooked porridge as opposed to their uncooked porridge slurries. A decrease in phenolics during cooking could be due to thermal degradation, changes in the chemical reactivity, or formation of insoluble complexes with food components such as proteins (Matuschek et al., 2001). Since tannins in sorghum grain occur primarily in forms bound to other components of the grain matrix, additional bonding by the phenolic hydroxyl groups to the matrix to form non-extractable complexes may occur during cooking (Kruger, Taylor, & Oelofse, 2012). Moreover, much lower levels than ours have been reported in studies using cooked porridge (Kruger, 2016; Kruger, Mongwaketse, Faber, van der Hoeven, & Smuts, 2015), suggesting other underlying factors like processing/fermentation methods used, cooking conditions, temperature and duration, grain variety and location, and flour texture.

With germination, this study found that the total phenolic content was significantly reduced in sorghum but slightly increased in millet. Likewise, tannins were significantly reduced in sorghum, increased significantly in millet and no changes occurred in maize. Our findings were consistent to what has been reported recently (Gabaza M. et al., 2018). In another study the same was observed for phenolics, but not tannins which reduced in sprout cooked finger millet (Hithamani & Srinivasan, 2014). The reduction of tannins observed in finger millet in that study may have been caused by the longer cooking duration and high temperatures unlike ours. The changes occurring to phenolics after germinating can be as a result of the low pH which caused the abstraction of hydride ions with consequential reorganization of phenolics and also possible reduced extractability of phenolics due to self-polymerization and interaction with macromolecules (Towo, Matuschek, & Svanberg, 2006). Furthermore, depolymerization of phenolics into low molecular weight phenolics can occur, increasing the soluble fraction of the phenolics (Taylor & Duodu, 2015). The production of enzymes with ability to metabolize phenolics is responsible for changes in phenolics during fermentation (Svensson, Sekwati-Monang, Lutz, Schieber, & Gänzle, 2010). The difference observed in the level of both tannins and phenolics in sorghum and millet upon processing, suggests differences in the structure of tannins in the two cereals. Sorghum tannins bind with macromolecules such as proteins after processing such that they become less extractable and assayable (Dlamini, Taylor, & Rooney, 2007). On the other hand, catechin reacts positively in the vanillin assay, such that, most of what is measured as tannins in finger millet is catechin and its oligomers, as up to 84% of the soluble phenolics in finger millet has been found to be mainly catechin (Chandrasekara & Shahidi, 2011; Gabaza et al., 2016).

In agreement with published data, our results showed that non-germinated maize porridge without MNP had the highest phytate levels than millet and sorghum in that order. As expected, germination, significantly reduced phytate, by increasing phytase activity which is responsible for phytate breakdown similarly to what has been previously reported (Kruger, 2016). The reduction in phytate content caused by soaking may be due to solubilisation of phytic acid salts in water because it is stored in relatively water soluble form such as sodium and potassium phytate (Afify, El-Beltagi, El-Salam, & Omran, 2011). The losses may be more in cereals where the phytate are stored primarily in the aleurone layer of the grain. During germination endogeneous phytases are activated depending on the cereal/legume, pH and temperature (Bartnik & Szafrańska, 1987; Kumar et al., 2010; Towo et al., 2006). Since phytate is also heat stable, substantial thermally induced degradation during cooking is not expected (Kumar et al., 2010). This is a possible explanation why the phytate levels in our study ranged between the values of processed maize, millet and sorghum products which are either raw or cooked (Gabaza Molly et al., 2018; Kruger et al., 2015; Kruger et al., 2012). Even though the phytates were reduced in all cereals, it seems the microorganisms involved in the processing/fermentations may differ in different cereals, because the percentage reduction of phytates was higher in millet [55.2%], followed by sorghum [50.9%] and maize [48.8%] in that order.

5.1.3 Effect of germination on iron and zinc bioaccessibility from the micronutrient powder

According to our findings, iron and zinc bioaccessibility from porridges containing MNP depends on the intrinsic and extrinsic levels of these nutrients, nutrient interactions, effects of food processing techniques, type of cereal, components in MNP, the presence of antinutrients, and the molar ratios of phytate: mineral. Differences in bioaccessibility was observed between processed and unprocessed porridges with MNP. These differences were also evident between porridges with high phenolics, tannins, phytates, calcium and vice versa.

Processing methods such as milling, sprouting, enzymatic digestion and fermentation, are effective in the release of food compounds by increasing their surface area ratio, inducing the activity of endogenous enzymes and bioconversion of the bioactive compounds to more active compounds.(Henry Okwudili, Kwaku Gyebi, & Afam Israel Obiefuna, 2017). Soaking and germination has been found to cause phytate-mineral complex which may increase bioaccessible iron (Platel et al., 2010). The increase could result from the activation of endogenous enzymes such as esterases and phytase which consequently acts on polyphenols or phytate-mineral complexes, resulting in the release of the mineral (Platel et al., 2010). As a result, phenolics, tannins and phytates are also reduced in processed porridges as reported in this study, and elsewhere (Hithamani & Srinivasan, 2014). In contrast, no changes in phytates levels were observed in a different recent study on maize, millet and sorghum (Gabaza M. et al., 2018), possibly because they omitted the soaking and germination stage prior to fermentation in their study. The combined effect caused by soaking and germination has been shown to reduce phytates significantly (Aparna et al., 2017).

The phytate: mineral molar ratio determines the mineral bioavailability, where a lower molar ratio is indicative of higher mineral bioavailability and vice versa. Our results showed a reduction of phytate: iron, phytate: zinc, and phytate: calcium molar ratios upon processing. Contradicting literature is available on the phytate: iron molar ratios and iron bioavailability whereby, both ≥1 (Hurrell & Egli, 2010) and ≥10–14 (Saha, Weaver, & Mason, 1994) have been reported to impair iron bioavailability. In our study, the phytate: iron molar ratio for all porridges without MNP was >3, suggesting the low iron bioaccessibility observed in processed [3-6%] and non-processed [1-5%] porridges. However, the low iron bioaccessibility result is consistent to what is expected in non heme foods (Turhan, Altunkaynak, & Yazici, 2004). The International Zinc Nutrition Consultative Group [IZiNCG] estimates zinc absorption to be 18% and 25% for adult males and females, for diets with phytate: zinc molar ratios >18, for example unrefined cereal-based diets (Group, 2004). This is similar to the findings of our study where the phytate: zinc molar ratio in all porridges was >18 and the percent zinc bioaccessibility in processed and non-processed porridge ranged between 31%-39% and 11%-16%, respectively. Some discrepancy is evident between our values and IZINCG considering the effects of host factors in real human absorption studies versus dialysability. According to our findings, maize porridge whether processed or not had both low iron and zinc bioaccessibility because of the high phytate: mineral molar ratios. There is also evidence of reduced zinc bioavailability in maize due to the presence of insoluble dietary fibre (Luo et al., 2014). However, in spite of sorghum having the lowest phytate: zinc molar ratios, millet had the highest amount of dialyzable zinc because of the highest reduction in phytates [55.2%] and phytate plus calcium: zinc molar ratio. It is speculated that high dietary calcium may impair zinc absorption, but probably only in the presence of high intakes of phytate. Our results also showed a higher phytate: calcium molar ratio in all porridges based on the recommended ratio of 0.17 (Umeta et al., 2005a). Similar trends have been observed by other authors on phytate: mineral molar ratio (Kruger, 2016; Vilakati, Taylor, MacIntyre, & Kruger, 2016; Wu et al., 2018).

Despite non-germinated sorghum having relatively high amounts of iron in our study, the percentage bioaccessibility and the amount bioaccessible was not significant because of the high tannin and phenolic contents. Although, the iron bioaccessibility was generally improved with processing, sorghum was not the best of all porridges. These observations were consistent to another study where sorghum with the highest levels of tannins and phytate showed the lowest values of in vitro mineral availability (Wu et al., 2018). Therefore, these findings strengthen the fact that tannins have been found to be extremely potent iron bioavailability inhibitors (Santos-Buelga & Scalbert, 2000). In contrast, we observed high zinc bioaccessibility in processed and unprocessed sorghum, probably because of the unnoticed changes to the intrinsic zinc levels upon processing plus the significant reduction of mineral inhibitors. It has also been reported that zinc absorption is not related to dietary phytates (Miller, Hambidge, & Krebs, 2015). Our study also found that germinated and non-germinated sorghum had high phosphorous contents compared to maize and millet, yet high phosphorous in rice grains have been reported to influence iron and zinc bioavailability (Su, Zhou, Zhao, Pan, & Cheng, 2018). When foods are grown using high phosphate fertilizer, the phosphorous and phytate contents will increase

(Coulibaly et al., 2011), resulting to an increase in the phytate: iron and phytate: zinc molar ratios that inhibit bioavailability as reported in the rice study. In view of these differences on iron and zinc bioavailability with different porridges, our results may suggest that different mineral inhibitors have different chelating effects, on different nutrients in different cereals upon processing.

Conflicting findings have been reported on the effects of calcium and iron bioavailability where it is overestimated in single meal studies compared to multiple-meal studies where calcium has a small effect on iron absorption (Lönnerdal, 2010; Lynch, 2000). This discrepancy supports the relevance of calcium levels and iron bioavailability. Intrinsic calcium in all processed and unprocessed porridges for all cereals in our study were lower than 50mg/100g. Although these levels may not inhibit iron bioavailability, compared to where more than 75mg/100g calcium has been reported (Roughead, Zito, & Hunt, 2005), we observed low iron bioavailability in unprocessed millet and processed sorghum with more than 20mg/100g. These observations may suggest that the direction or significance of calcium increment may be more important than merely the levels, as we observed in processed sorghum. In addition to that, processed millet had the highest iron bioaccessibility contrary to the unprocessed suggesting the impact of increased intrinsic iron and reduced calcium levels in respect to bioaccessibility.

In contrast, the effect of iron and calcium on zinc bioavailability is more pronounced in the presence of phytate, as calcium and iron would bind to phytate, improving zinc bioavailability (Miller, Krebs, & Hambidge, 2013). In view of this, we observed high percentage zinc bioaccessibility with sorghum despite it having high levels of phytate, tannins and phenolics, probably because of the significant reduction of calcium levels after processing, and or the effects of NaFeEDTA on intrinsic zinc compared to other porridges. Furthermore, the magnitude observed in phytate: zinc molar ratios compared to phytate: iron molar ratios supports what other authors found in sorghum where they attributed the magnitude to the double decrease in the phytate: zinc and phytate x calcium: zinc ratios (Vilakati et al., 2016). Therefore, this phenomenon may as well apply to high calcium foods, even though conclusions from their study were solely based on sorghum.

Iron in form of NaFeEDTA in MNP has been found to significantly increase iron and, importantly zinc availabilities in fermented thick maize porridge (Kruger, 2016) and whole wheat bread (Rebellato et al., 2017). Even if these authors did not compare different food vehicles, their findings agree with ours where percentage zinc bioaccessibility was higher compared to iron in all processed porridges with added micronutrient powder. The increase could be possibly due to some of the NaFeEDTA complexes perhaps partially dissociated because of pH changes during simulated digestion, binding with zinc and increasing its availability (Kruger, 2016). In fact, an EDTA : Zinc molar ratio greater than one may enhance zinc absorption (Moretti Diego, Biebinger, Ralf, Bruins, Maaike J, Hoeft, Birgit, & Kraemer, Klaus, 2014), whereas a higher molar ratio of 2:1, decreases zinc bioavailability (Brnić, Wegmüller, Zeder, Senti, & Hurrell, 2014). Unfortunately, these molar ratios were not known in our study since we used an already manufactured/formulated MNP product. Similarly, iron bioaccessibility was improved because EDTA may have protected the minerals from the anti-nutrient influence of phytate during the simulated digestion (Bothwell & MacPhail, 2004). Additionally, the increased vitamin C content of grains during germination (Hemalatha et al., 2007), plus the 30mg of vitamin C in our MNP enhanced iron absorption as it is widely known. An ascorbic acid:

iron molar ratio of at least 2:1 in foods, and 4:1 in phytic rich foods is recommended as it has been shown to increase iron bioavailability by at least twofold in single-meal studies (Davidsson, 2003; Fidler, Davidsson, Zeder, Walczyk, & Hurrell, 2003).

EDTA is added in micronutrient powder as an absorption enhancer to iron-fortified foods (Moretti D., Biebinger, R., Bruins, M. J., Hoeft, B., & Kraemer, K., 2014). It is a hexacoordinating complexing agent, binding to almost any metal [at different stability constants] with four carboxylate, and two tertiary amine groups (Bothwell & MacPhail, 2004). The stability constants for EDTA with ferric iron [25.1] and zinc [16.1] have been found to be high, resulting in stable bioavailable complexes. The complex stability is, however, pH dependant with the optimal pH for complexing at 1 and 4, for ferric iron and zinc, respectively (Bothwell & MacPhail, 2004). Consequently, fermentation produces acids which form stable soluble complexes with iron and zinc (Teucher, Olivares, & Cori, 2004). Therefore, the change of ph during digestion in the stomach and intestines as well as in fermented porridges, makes some of the EDTA to dissociate and form stable complexes with zinc (Kruger, 2016). These further explains the reason for higher zinc bioavailability observed in our study, suggesting the significance of fermented foods in fortification programs for optimum mineral bioavailability. The clear difference observed in the bioavailability of iron and zinc may be as a result of them linked to different constituents, possibly proteins.

Even though our study focused on the bioaccesibility of only porridges with added micronutrient powder, adding the fortification mix without any additional iron has been shown to increase the availabilities of iron from fermented maize porridges perhaps due to the enhancement effect of the multi-nutrient fortificants on the availability of the intrinsic iron independently (Kruger, 2016). A concept that ranks the use of micronutrient powder highly because of the enhancing effect of these nutrients compared to single nutrient fortification strategies. Likewise, the absorption of iron compounds like NaFeEDTA in fortificants has been found to improve the bioaccesibility of intrinsic zinc in whole wheat bread (Rebellato et al., 2017) suggesting a competitive interaction on the minerals affecting their absorption. Importantly, zinc naturally occurring in food can be positively influenced by the absorption of iron compounds used in fortification (Moretti D. et al., 2014). In this study, added nutrient levels from MNP did not vary much after bioaccessibility compared to the levels at which they were added. Possibly because MNP is added to porridges just before consumption, compared to commercial fortification of flours where these losses may occur in mixing, storage and transportation.

Intrinsic and extrinsic nutrient interactions seem to influence iron and zinc bioaccessibility. One study on rice grains reported, that more phosphorous caused an evident decrease in grain zinc and iron concentrations on dry matter basis [mg/kg] which also increased phytate and reduced mineral bioavailability (Su et al., 2018). The intrinsic iron: zinc molar ratio [0.29-0.70] in food grains does not influence the iron and zinc dialyzability, unlike when calcium is present (Cilla, López-García, & Barberá, 2016). Both processed and non-processed millet had the highest levels of intrinsic calcium which could explain the high iron and low zinc percent bioaccessibility observed in this study. The presence of exogenous iron levels at a zinc: iron ratio of 1:7 does not interfere with the bioaccessibility of the minerals, but a zinc: iron ratio of 1:30 has a negative effect (Tripathi, Platel, & Srinivasan, 2012). In this study the zinc: iron ratio from the added 10mg of iron and 4.1mg of zinc was 1:0.4 and less than 1: 6 in all porridges with MNP, which did not affect bioaccessibility negatively based on reported evidence.

5.1.4 Effects of germination in bioaccessibility of iron and zinc

According to our results, the improved bioaccessibility of iron and zinc from processed finger millet compared to maize and sorghum is novel. It can be inferred therefore, that the highest intrinsic iron and zinc contents in processed and unprocessed millet influenced bioaccessibility. Furthermore, the lower phytate, tannins, phenolics and calcium levels in millet after processing compared to maize and sorghum was also important. In fact, millet ranked two for all these mineral inhibitors, where maize had the highest phytates while sorghum had the highest phenolics and tannins. Besides, the amount of iron in millet increased to the level of iron in unfermented sorghum which did not change after processing, because of the high phenolics and tannins. The percent mineral bioaccessibility gives a good indication of the sum effect of mineral bioaccessibility inhibitors and enhancers and the total amount of the specific mineral in the food. As regards dialysability, while processed millet is the best for percentage of iron dialyzable, processed millet and sorghum [in that order] are the best for percentage of zinc dialyzable. Clearly, millet is the best provider for the percent of dialyzable iron and zinc. These results further supports the fact that the presence of bioactive compounds like ferulic acid, caffeic acid, and quercetin have been found to enhance bioaccessibility in the finger millet grain (Henry Okwudili et al., 2017). Likewise, germination of finger millet increases its protein content making it more nutritious as it also enhances iron absorption (Chauhan, 2018).

5.1.5 Strength of the *in vitro* bioaccessibility study

This is the only study that used cereals locally grown from the region, employed spontaneous natural fermentation, cooked porridge using the local procedures, using drinking water and adding MNP to the quantity of porridge as it is recommended and consumed. Therefore, our results may reflect the reality, because iron and zinc bioaccesibility when assessed on cooked foods is more relevant than using raw foods. Likewise, for dialysability to be more relevant to human bioavailability, foods need to be made in the manner in which they are normally consumed (Fairweather-Tait et al., 2005). In contrast, most published data assessed mineral bioaccessibility either in cereals grown in controlled conditions, used fortified flours or slurries fermented artificially, or cooked porridges under controlled conditions in the laboratory using distilled/deionised water: procedures which are not practical and sustainable in real community setting. Moreover, this study compared three cereals unlike most of the studies which studied these cereals separately.

5.1.6 Limitation and recommendation of the *in vitro* bioaccessibility study

Though our findings strengthen the existing evidence of improved bioavailability with processing on phytate, tannin and phenolics reduction (Gabaza M. et al., 2018; Hemalatha et al., 2007; Kruger J. et al., 2013), the dialysability method alone is not useful to estimate the uptake and absorption of nutrients, unless it is combined with the CaCo-2 cell method followed by human trials. Furthermore, results from this method vary from different studies because of factors like Ph and incubation period during the experiment, thus, simulated digestion studies cannot always be depended on as substitutes for human studies (La Frano et al., 2014). For this reason, our results should be interpreted prudently in implementing interventions, because *in vitro* dialysability, as used here, gives a fair estimate of the mineral availability for absorption *in vivo*.

The use of micronutrient powder has been recommended with complementary foods, which may include stiff porridge, vegetable soup/broths and milk which are commonly consumed in this population. Therefore, further research may be done on these mixed foods [cereal vegetable relish] as they are normally eaten, to determine mineral bioavailability from MNP in these combinations as done by other authors (Kruger et al., 2015; Vilakati et al., 2016). The different formulations of MNP could also be studied further, to promote the use of MNP in milk which is currently discouraged by UNICEF as a vehicle for MNP, yet milk is a common complementary food in this community. Future researches could also consider combining germination and lactic acid fermentation which has been found to completely degrade phytates in white sorghum and maize gruels (Kumar et al., 2010).

Literature on iron and zinc dialysability from micronutrient powder in food is limited/lacking. No published research could be found on the effect of processing on the bioaccessibility of iron and zinc from micronutrient powder. However, our findings can be compared to work done on processed maize, millet and sorghum without the addition of MNP to discuss the inhibitors and promoters that may influence the bioavailability of intrinsic iron and zinc; which may also affect the extrinsic iron and zinc in the case of our study. Our findings can also be compared with work done on the addition of single/multiple nutrients in food to compare the effects of these additions regarding the iron and zinc bioaccessibility.

5.1.7 Conclusion of the *in vitro* bioaccessibility study

To our knowledge, no previous research finding has been reported on the iron and zinc bioaccessibility from micronutrient powder in processed maize, millet and sorghum porridges. This new information will be useful to guide policy on the appropriate foods for micronutrient use for optimum iron and zinc bioaccessibility from MNP. Furthermore, this study has emphasized the importance of processing/fermentation compared to non-processing in improving both iron and zinc bioaccessibility in white maize, finger millet and red sorghum cereals. Percentage zinc bioaccessibility was higher in all cereals compared to iron because of the effect of processing, EDTA in MNP, and the use of zinc gluconate on zinc bioaccessibility. Processed sorghum and millet had the highest percent bioaccessibility of zinc and iron, respectively. However, the high intrinsic iron and zinc, low levels of phenolics, tannins, phytates and significant reduction of calcium following processing, makes millet the best porridge for bioaccessibility of both iron and zinc from the micronutrient powder.

5.2 Phase two: Acceptability trial of germinated porridges with MNP

5.2.1 Preference of millet porridge compared to maize and sorghum

Millet porridge was preferred than maize and sorghum based on the acceptability results in this study. Similar findings were reported in Nigeria where sensory evaluation showed that the germinated millet grain product was highly rated for all the parameters investigated (Inyang & Zakari, 2008). This is because germinated finger millet exhibited high α-amylase activity within 2–3 days of germination, while maize and sorghum, showed high enzyme activity after 4–5 days of germination. Germination drastically lowered the paste viscosity of cereal flours especially millet. In addition to that, germinated finger millet has been long known to also have desirable flavour and taste besides high amylase activity (Malleshi & Desikachar, 1986). Furthermore, a recent study showed that processing imparts specific flavor, improve texture, taste and shelf life of millets based food products (Kaur, Purewal, Sandhu, Kaur, & Salar, 2019). Similar to our findings, germinated as compared to non-germinated millet porridge, was highly acceptable probably because of its reduced viscousity that had a positive impact on the general colour, flavor and consistency as reported in a study done in Nigeria (Inyang & Idoko, 2006).

5.2.2 Acceptability of germinated porridges versus non-germinated porridges

Although both germinated and non-germinated millet porridge ranked the best, our results revealed that children preferred the germinated porridges more than the non-germinated. A possible reason for this is related to the effects germination has on the viscousity of porridges which could influence the organoleptic properties. An earlier study found that the viscousity of non-germinated gruels of maize, millet and sorghum have similar concentration viscousity relationships unlike the large differences observed in germinated gruels (Mosha & Svanberg, 1983). For example, the amount of flour in germinated sorghum and maize gruels were increased considerably i.e. two to three times, before the viscosity became unacceptably high. Viscousity occurs as a result of starch degradation caused by the action of the alpha and beta amylases that have developed during the germination procedure, thus hydrolyzing some of the starch into limit dextrin and maltose, which do not swell when cooked (Gernah, Ariahu, & Ingbian, 2011). Similar to our results, a study in Nigeria (Inyang & Zakari, 2008) and South Africa (Nefale & Mashau, 2018) showed no significant differences in color changes between germinated and non-germinated cooked product, but germinated product was preferred in terms of the organoleptic properties. The sensory properties of germinated cereals may differ depending on the type of cereal. As one study reported, the maximum moisture contents of the grains on prolonged steeping/soaking were lower in corn and sorghum than in millet (Banigo & Muller, 1972). A close visual examination of the three cereals after steeping/soaking revealed, that the seed coats of corn and sorghum were essentially intact, while those of millet had ruptured extensively (Banigo & Muller, 1972). The decrease in flour viscousity induced by germination is desirable for preparing weaning foods as it also makes infants consume food more easily (Nefale & Mashau, 2018). This could explain possibly, the reason germinated millet was rated higher than maize and sorghum in our study.

Germinated maize flour has been found to have reduced bulk density due to the softened seeds, reduced swelling index which makes it sticky, increased water absorption capacity that makes the flour require more water during mixing, and decreased viscousity (Gernah et al., 2011). It may be speculated that the reduced bulk density and swelling index softened the porridge and made it sticky thus enhancing its taste and texture. The increased water absorption capacity and decreased viscousity of the germinated porridge might have affected its texture and colour since more water was required for mixing resulting to a thin porridge. These change in properties of germinated maize could explain why children liked germinated maize porridge in our study. Furthermore, unlike sorghum, germination of maize for more than 2 days has been reported to significantly cause a reduction in viscosity (Helland, Wicklund, & Narvhus, 2002). Similarly, germination has no effect on the colour of millet porridge as reported by a recent study in South Africa (Nefale & Mashau, 2018).

Although sorghum porridge was the least preferred based on the amount consumed compared to millet and maize, the acceptability results indicated children’s preference of germinated sorghum porridge to non-germinated. It can therefore be inferred that different indicators and participants could somewhat result to bias which may influence the outcome. In view of this, a study conducted in a natural feeding environment concluded that the differences observed between acceptability ratings and food intake suggest that environmental factors also have an important role in determining intake and choice (de Graaf et al., 2005). In spite of this, the degree of processing on cereals could have varied effects on the outcome as demonstrated in another study where, the acceptability of germinated sorghum was low as compared to other processing techniques used on sorghum (Mathew, Adebowale, & Oladayo, 2018). For instance, upon soaking sorghum for 96 hours, a small reduction in viscousity in high tannin sorghum variety and a bitter tasting porridge has been reported, since the amylolytic enzymes activity in the seed develops more slowly because of the inhibitory effect of the tannins (Mosha & Svanberg, 1983; Nefale & Mashau, 2018). Furthermore, germination of maize for more than 2 days has been reported to significantly cause a reduction in viscosity unlike sorghum (Helland et al., 2002). Even though our data might have suggested the negative effects germination had in sorghum, the assumption was based on the comparison with maize and millet. This is because the amount of sorghum porridge consumed was low. However, the amount of germinated porridge consumed was significant which is a clear fact of their preference.

5.2.3 The influence of the day the porridge was consumed during the feeding trial

Subsequently, the day the porridge was consumed during the 2-week feeding trial was significant for all porridges which may be indicative of the effect food has on the palate on subsequent exposure to it. This could also infer to the danger of assuming the acceptability of food during a one feeding stance for example, when using a tasting panel, as compared to several time exposures like a 2-week feeding trial. Previous research has found that 10–15 exposures to a novel food found can increase liking and consumption (Williams, Paul, Pizzo, & Riegel, 2008). In one study where infants were exposed to a diet for 8 days, it was reported that not only can infants clearly discriminate flavors but repeated opportunities to taste a particular or a variety of foods may promote willingness to consume that food resulting to its acceptability in the pediatric population (Mennella, Nicklaus, Jagolino, & Yourshaw, 2008). Yet again, in another study where the rating of food was low when tasted once observed that during the 5-day eating period, this was changed and the investigators concluded that with repeated exposure, the desire to eat a less preferred food could increase over time (Zandstra, De Graaf, Mela, & Van Staveren, 2000).

Interestingly, we found a significant interaction between the day the porridge was given and the porridge type for both maize and sorghum porridges but not millet porridge during the 2-week feeding trial. The same trend confirms the speculated effect germination process has on these cereals which may influence palatability. Again, this may indicate the effect food has on the palate on subsequent exposure to it. Furthermore, this result confirmed what we also observed where both germinated and non-germinated millet ranked first than maize and sorghum. Since palatability has a positive relationship with food intake, a laboratory study conducted to ascertain whether this relationship holds over repeated exposures in non-laboratory environment, found out that the likelihood of choosing a meal for the second time was positively related to the acceptability rating of the meal when it was consumed for the first time (de Graaf et al., 2005). These findings together with ours strengthen the importance of liking in food choice and food intake/choice behavior. In addition to that, our data showed a steady increase on consumption of germinated porridges as the day progressed. This could be due to improved palatability as reported by reviewed studies which have shown increased intake as palatability increased, for example, subjects either feel more hungry and less full after a palatable meal compared to a less palatable meal (Sørensen, Møller, Flint, Martens, & Raben, 2003). Since germinated porridges were new foods among these children, the increased acceptance intake could be as a result of monotony of the non-germinated porridges as demonstrated in another study where acceptance and intake of the meal declined with monotony (Meiselman, degraaf, & Lesher, 2000). Obviously, these children found their usual non-germinated porridge less pleasant than the new germinated porridge which they preferred. These results are similar to earlier findings from Ethiopian refugees which indicated that monotony in the diet could develop a long-term form of sensory-specific satiety (Rolls & De Waal, 1985).

5.2.4 Conclusion of the acceptability trial study

Germinated porridges were preferred as compared to non-germinated porridges with millet porridge being ranked first over the rest i.e. maize and sorghum. The day germinated maize and sorghum porridges were consumed during the 2-week feeding trial was significant suggesting the effect the palate has regarding taste on food acceptability based on the length of food exposure to it.

5.2.5 Strength of the acceptability trial study

In addition to the usual organoleptic properties of porridges, this study considered other parameters like the amount of porridge consumed, and the day the porridge was consumed to ascertain the acceptability of germinated porridges in the community. Different children were used for different porridges during the acceptability trial to prevent the cross over effect. Although the control group was the same, a one-week washout period was done between each porridge type.

5.2.6 Limitation of the acceptability trial study

Children were fed from an informal outdoor environment which may have influenced their intake, unlike the indoor trained panelist in most acceptability studies. Since we studied young children and infants who could not give us their formal opinion regarding the sensory tasting of food, all the data in our study depended solely on the observations and caregivers' judgements which may otherwise be biased.

5.3 Phase three: Nutrition survey

This cross-sectional study examined the prevalence of malnutrition, anemia, iron deficiency, iron deficiency anemia and zinc deficiency among children aged 6-23 months in Homabay County, Kenya. Based on our data, the prevalence -2SD of stunting, underweight and wasting was 17.5%, 13.4% and 6.4%, respectively. Half of the children surveyed had anemia, 39.5% were iron deficient, and 19.1% had iron deficiency anemia. Further, 43.6% of the children had zinc deficiency. These results also showed that a child's age, sex, morbidity status two weeks prior to the survey, as well as dietary intake were associated with their nutritional status.

5.3.1 Anthropometric status

According to the Joint child malnutrition estimates 2018, globally, 22.2% of children under five are stunted while 7.5% are wasted (United Nations Children's Fund, 2018). Asia and Africa still bear the greatest burden of malnutrition in the world with the prevalence of stunting being 55% and 39%, respectively. The prevalence of wasting in Asia and Africa is 69% and 27%, respectively. Also, more than half of the wasted children globally live in Southern Asia region. In Eastern Africa where Kenya is situated, the prevalence of stunting is rated among the highest globally which is at 35.6%. Whereas there is a reduction in the number of stunted children in the world, Africa is the only region with increased number of stunted children since 2000 to 2017 (United Nations Children's Fund, 2018). The prevalence of wasting in Eastern Africa is 6% which is considered medium level. According to Kenya Demographic Health Survey of 2014, 26% of children less than five years are stunted, 4% are wasted and 11% are underweight in Kenya (Kenya National Bureau of Statistics, 2014). In Nyanza where Homabay County is located, the prevalence of stunting, wasting and underweight is 22.7%, 2% and 7.4%, respectively. In Homabay County, the prevalence of stunting is 18.7%, wasting 2.3% and underweight 5.4%. Our study showed a prevalence of 17.5% in stunting, 6.4% in wasting and 13.4% in underweight. The slight disparity could be because of the differences in sampling methodology, differences in the sample size or an indication of worsening malnutrition status in Homabay.

Studies have consistently shown that male children are more affected by malnutrition especially stunting compared to female children (Dhanalakshmi & Selvaraj, 2019; Kavosi et al., 2014). The results of this study revealed a similar trend. Whereas 21.7% of male children were stunted, 12.2% female children were stunted. The observed biological difference in linear growth was expected as it is widely documented that female children grow faster during early childhood and infancy than male (Galloway, 2007; WHO, 2006). This study found out that older children aged 18-23 months in Homabay county compared to those 6-17 months suffered higher rates of malnutrition. This finding is consistent to what was reported recently in three countries in East Africa (Agho, Akombi, Ferdous, Mbugua, & Kamara, 2019). This could be due to increased need of energy expenditure as the child grows older coupled by the inadequacy to access nutritious foods at this stage. Likewise, as the child grows older the interaction of the child to the environment could subject the child to more infections through unsafe water and poor sanitation which could otherwise result to malnutrition.

The interactions between episodic and prolonged infections and malnutrition are complex and bidirectional (Walson & Berkley, 2018). The association between acute infections and loss of weight has been reported by several studies (Alam, Marks, Baqui, Yunus, & Fuchs, 2000; Briend, Aziz, Hasan, & Hoque, 1989; Moy, Choto, McNeish, & Booth, 1994). On the other hand malnourished children are likely to die from infections and illnesses such as diarrhea and pneumonia (WHO, 2002). Malnutrition secondary to infections is thought to result from nutrient losses caused by vomiting and diarrhea, malabsorption of nutrients, increased nutrient need and high energy expenditure, reduced appetite, poor digestion and altered metabolic process (Asfaw et al., 2015). In Malawi, an acute infection caused malnutrition in children due to weight loss, anorexia, increased nutrient needs and poor absorption (Weisz et al., 2011).

In a study conducted among children in 2 slums in Kenya, almost all children had ever been breastfed i.e. 99%, more than a third were not breastfed in the first hour following delivery, and 40% were given something to drink other than the mothers' breast milk within 3 days after delivery (Kimani-Murage et al., 2015). About 85% of infants were still breastfeeding by the end of the eleventh month and only about 2% of infants were exclusively breastfed for six months in that same study. Our study findings in a rural area in western Kenya showed almost similar results. All children had ever been breastfed and 85.3% of children aged 12-15 months were still breastfeeding. In our study only 38% of the newborn were initiated on breast milk within one hour after delivery and 61.8% of children aged 0-5 months received only breast milk. It is important to note that our findings on initiation of breastfeeding and exclusive breastfeeding are not consistent with results reported in the 2 slums in Kenya. Whereas about two thirds of children in the 2 slums were initiated on breast milk one hour following delivery, only one third of the children in our study met this important indicator. On the other hand, our study reported over half of the children were exclusively breastfed compared to only 2% in the two slums. Possible explanations for the differences could be the place of delivery and economic activities by the mothers that differed in these studies. Delivery in a health facility by 71% of mothers was a positive indicator for early initiation of breastfeeding (Kimani-Murage et al., 2015). It is therefore not surprising that in our study where 66% of the mothers delivered at home, early initiation of breastfeeding was a low 38%. A recent study found a similar association between childbirth location and early breastfeeding practices in 22 Sub-Saharan Africa countries (Bergamaschi, Oakley, & Benova, 2019). Their study found out that compared with home births, facility deliveries had higher adjusted odds of early initiation of breastfeeding. On the other hand, the high proportion of the mothers in our study who were either homemakers or subsistence farmers accounting for 72%, gave more time with their children explaining the relatively higher proportion of children who were exclusively breastfed in our study compared to the other study.

In this study, infants who were not exclusively breastfed for 5 months, did not continue breastfeeding through the second year, were not introduced to solid and semi solid foods at 6-8 months, did not meet minimum dietary diversity and minimum meal frequency were likely to suffer from either stunting, undernutrition or wasting. Similar results have been reported in India, Zambia and elsewhere, where lack of exclusive breastfeeding as well as not breastfeeding has been associated with increased risk of malnutrition among children (Ansuya et al., 2018; Aprameya et al., 2015; Irarrázaval et al., 2018; Katepa-Bwalya et al., 2015; Rasania & Sachdev, 2001). Timely introduction of complementary foods is essential to meet the increased nutrient-energy demands of growing children that breast milk alone cannot fulfill. Unfortunately, often the introduction of these foods is delayed and when introduced the quality is low. Complementary feeding practices have been reported previously and in recent studies to be associated with malnutrition (Dhanalakshmi & Selvaraj, 2019; Garg & Chadha, 2009).

Additionally, our study found out that children who received a diet with at least four food groups were less likely to be underweight, p= 0.026. This finding is consistent with evidence from a community-based, cross-sectional study which was carried out in ten states of India, that found an association between minimum dietary diversity with undernutrition among 12–23-month-old children (Meshram et al., 2019). Complementary foods in our study were predominantly plant based. Seventy-one percent and 60.2% of children were fed on porridge and ugali on daily basis. About half of the children were feeding on vegetables on a daily basis. On the contrary 8 in 10 children were never fed on eggs, red meat, chicken, and liver the entire week. A third of the children received legumes on more than 2 times in a week. That complementary foods in most developing countries are plant based and dominated by starchy staple foods is a finding that has been reported in several old and recent studies (Afify et al., 2011; Gabaza M. et al., 2018; Lonnerdal, 2000).

Similarly, it has been reported in studies that, unlike in the developed countries, nutrient-dense animal source foods are often unavailable or unaffordable in most developing countries (Ruel, Harris, & Cunningham, 2013). Although our study revealed that a third of the children were receiving plant based proteins like legumes, it is important to note that the plant-based diets have been known to have lower bioavailable nutrients as compared to the animal based foods. This is explained by the high content of phytic acid and associated magnesium, potassium, and calcium salts by plant based foods. Together, the acid and its salts are termed “phytate” and are potent inhibitors of iron and zinc absorption (Gibson Rosalind S, Raboy, Victor, & King, Janet C, 2018).

The above evidence would explain the results of this study on the associations found between food patterns and nutritional status of the children. In this study, the frequency of eating foods from the second food pattern consisting of ‘liver, red meat, chicken, eggs’ was inversely related to underweight. Children who frequently ate the food pattern were found to be less likely to be underweight. This is similar to recent results from Cambodia which showed that the degree of satisfaction of proteins and zinc requirements in children were positively associated with length-for-age, while having a higher degree of satisfaction of energy, protein, zinc, and iron requirements were associated to an improved weight-for-length (Blaney et al., 2019). Additionally, results from our 24-hour recall of foods consumed revealed that children who consumed the least amounts of the nutrient pattern consisting of ‘carbohydrates, fat, dietary fiber, and iron’ were less likely to be stunted and underweight as compared to those who consumed high amounts of the nutrients. This finding could be a reflection of the low mean dietary diversity score of <4 reported in this community. This result is similar to what has been reported in Bangladesh, Ethiopia and

Vietnam where the odds of being stunted or underweight were significantly higher for children in severely food-insecure households based on the dietary diversity score (Ali et al., 2013). A significant proportion of children exclusively consumed grains, roots and tubers denying them the benefits of the other foods groups. This further demonstrates the importance of feeding children with foods from 4 or more food group for optimum early stages of child development, particularly in the first 1000 days of life.

5.3.2 Biochemical status

Globally 273.2 million preschool children are anemic and of this 9.6 million have severe anemia (WHO, 2015). South-East Asia, Mediterranean and Africa regions have the highest prevalence levels worldwide. In Africa the prevalence of anemia and severe anemia among children 6-59 months is 62.3% and 3.6%, respectively. A systematic review performed to evaluate anemia and iron status in 4 countries in Africa [including Kenya], reported a prevalence of anemia at 38 - 72% among children less than 5 years old in Kenya (Harika et al., 2017). Several other studies conducted in Kenya have reported a high prevalence of anemia and severe anemia among children aged 6-23 months. In 2011, the prevalence of anemia and severe anemia in Kenya was estimated at 46% and 2.0%, respectively, which was considered severe (WHO, 2015). In a recent study in 3 counties in Western Kenya, the results showed that the prevalence of anemia in 6-24 months children was 48.8% (Stewart et al., 2019). Using only the ferritin cutoff of <12µg/L, the prevalence of iron deficiency in their study was 29.9%, whereas in our study using the cutoff of <15µg/L the prevalence was 39.5%. In Maasai, Southern part of Kenya, the prevalence of anemia among children 3-5 years was 38% (Houghton et al., 2019). Based on the global estimates data, the prevalence of anemia among infants and children in Kenya is between 40.0% to 59.9%

(United Nations Children’s Fund, 2018). Based on this study, the prevalence of anemia in Homabay County is 50.6%. This result is consistent with the global estimates for Kenya and the studies cited above.

According to the Kenya National Micronutrient Survey conducted in 2011, 21.8% and 13.3% of children aged 6–59 months suffer from iron deficiency and iron deficiency anemia, respectively . Iron deficiency affects more than one quarter of infants 6–11 months of age, while iron deficiency peaks at 12–23 months of age affecting 34.6% of them (Kenya National Bureau of Statistics, 2011). Harika et al. (2017), in a systematic review reported that 20-42% and 11-27% of children under 5 years in Kenya were iron deficient and suffered from iron deficiency anemia respectively. This study found out that 39.5% and 19.1% of children in Homabay were suffering from iron deficiency and iron deficiency anemia respectively. Further, it reveals that children aged 6-11 and 12-17 months suffered the highest prevalence of iron deficiency anemia; 21.2% and 21.1% respectively. Therefore, 36.2% of children 12-23 months of age were suffering from iron deficiency anemia. This result is consistent with the results from the Kenya National Micronutrient Survey . Based on the 24hours recall data, 41% of the children in our study did not meet the EAR for iron. Children 6-11 months were more likely not to meet the EAR for iron as compared to the 12-17 and 18-23 months’ children [p= <0.001]. The inadequacy of iron from the locally consumed complementary foods in our study is consistent with evidence from another study conducted in Kenya. Ferguson, Chege, Kimiywe, Wiesmann, Hotz, et al. (2015) reported similar nutrient inadequacies of diets consumed by children 6-23 months in two rural agro-ecological zones of Kenya. A systematic review of intake of iron involving Kenya, Ethiopia, Nigeria and South Africa revealed inadequate iron intake ranging 34-62% among under 5 years children in Kenya (Harika et al., 2017).

An estimated 17% of the entire population globally and 50% of the entire population in Sub-Saharan Africa is at risk of zinc deficiency (Wessells & Brown, 2012). According to the data by Hidden hunger index in preschool children, the prevalence of zinc deficiency in Kenya is alarmingly high considering the range of 45-100% (Muthayya et al., 2013). This is in line with the results from the micronutrient survey conducted eight years ago in Kenya where 85.3% of children aged 6-23 months were found to have low serum zinc levels (Kenya National Bureau of Statistics, 2011). However, findings from our study showed a prevalence of 43.6% which is considered severe based on a cutoff prevalence levels of 35-44.9%. It may be speculated that the prevalence of zinc deficiency has reduced due to many zinc fortification and supplementation strategies/programs currently in Kenya compared to eight years ago. Besides, we never found any documented data on the prevalence of zinc deficiency in Homabay county. This study revealed that only 14% of children 6-23 months met the EAR for zinc from the diet they consumed. Similar findings have been reported by other researchers in Kenya and in other African countries (Ferguson, Chege, Kimiywe, Wiesmann, Hotz, et al., 2015; Harika et al., 2017)

Our study showed that male children suffered from anemia as compared to female children. This finding is consistent with another study conducted in Ethiopia (Woldie, Kebede, & Tariku, 2015). An explanation to this result could be due to the rapid growth that males experience during the first months of life that increase micronutrient requirements needs which if not replenished may lead to malnutrition. Children of age groups 6-11 and 12-17 months were more likely to be anemic as compared to 18-23 months based on our study findings and another study (Woldie et al., 2015). This may be due to inadequate maternal reserve stores for iron during pregnancy resulting to children born anemic. Alternatively, it may be caused by prenatal depletion of iron which is highest beginning at six months of age (Black, Quigg, Hurley, & Pepper, 2011).

Loss of appetite and malabsorption of nutrients due to diarrhea and vomiting may easily lead to malnutrition. In addition to that, the reverses may happen where malnutrition doubles the risk of mortality for diarrheal and respiratory infections in children (Victora et al., 2008). The relationship between diarrhea with zinc deficiency has long been described. In six of nine trials that evaluated prevention of diarrhea, significantly lower incidence of diarrhea occurred in the zinc group than in the controls; a pooled analysis demonstrated 18% less diarrhea (Black, 2003).

The type, quality and frequency of complementary foods have been documented to be associated with anemia (Kejo, Petrucka, Martin, Kimanya, & Mosha, 2018; Woldie et al., 2015; Yang et al., 2012). This study showed that children who consumed food pattern ‘liver, red meat, chicken, eggs’ less frequently as compared to those who frequently ate the foods were 3.1 and 3.9 times likely to suffer anemia and iron deficiency anemia respectively. In line with our results, a recent study in Tanzania found out that poor feeding practices especially non consumption of iron-rich foods like meat, vegetables, and fruits were significantly associated with the presence of childhood anemia (Kejo et al., 2018). On the other hand, children who consumed the least amount of nutrient pattern “vitamin C, Vitamin A and folic acid” were 3.3 times likely to suffer from iron deficiency anemia.

Our result confirms what has been reported in the same community though among adults with an assumption that children from this community consume the same food as adults (Chege & Mokono, 2018). This finding is not surprising because it has long been known that vitamin C enhances absorption of iron and vitamin C deficiency has been linked with iron deficiency anemia, which is at times referred to as the anemia of scurvy (Cox, Meynell, Northam, & Cooke, 1967; Gosiewska, Mahmoodian, & Peterkofsky, 1996; Mettier & Chew, 1932). Ascorbate stimulated transferrin iron uptake via an intracellular reductive mechanism into the erythropoietic compartment (Lane, Chikhani, Richardson, & Richardson, 2013). A defect in this mechanism is known to contribute to ascorbate-deficiency-induced anemia. Based on the 24 hours recall of diet consumed, only 45.5% of the children 6-23 months consumed diet that met the EAR for vitamin C. Vitamin C inadequacy has also been reported in diets consumed by HIV infected persons living in Homabay County (Chege & Mokono, 2018). Although this finding was reported among adults, it is important to note that in most homes in the study area, children 6-23 months consumed the same diets as adults.

This present study revealed that children who ate less amounts of nutrients pattern 'zinc, B vitamins, cholesterol, calcium, potassium, and sodium' nutrient pattern, and those who received food from less than four food groups and did not consume adequate foods from to our findings, poor intake of grains has been recently reported as a potential risk factor for zinc deficiency (Sharma & Yadav, 2019). Lack of dietary diversity could be attributed to the majority of the children being fed on white maize porridge or ugali frequently which provided inadequate zinc resulting to deficiency. It seems also that these children had inadequate dairy intake since zinc deficiency has been previously reported among children with cow milk allergy (Canani et al., 2014). Additionally, a recent study in New Zealand and an earlier study in Sri Lanka observed that the zinc intake of children was low due to inadequate consumption of foods of animal origin (Daniels et al., 2018; Hettiarachchi, Liyanage, Wickremasinghe, Hilmers, & Abrams, 2008). Although the population that we studied was rural, it seems some locations like Lwandawiti and Wachara were hard to reach as compared to Got-Kojowi. Similar to our results, a recent study in India found out that zinc deficiency was prevalent in some rural settlements since those communities cannot access health facilities, markets and often experienced food insecurities (Sharma & Yadav, 2019).

5.3.3 Conclusion of the nutrition survey study

The prevalence of stunting, iron and zinc deficiencies are high in Homabay County. This study revealed that male and older children [18-23 months] were more likely to be malnourished as compared to female and younger children respectively. Adherence to IYCF practices is directly related to good nutritional status of the children. Children who received food/diet according to the recommended practices had a less chance of being malnourished. Specifically, this study found a significant association between dietary diversity and nutrition status of the children. The frequency of consuming animal based proteins [‘liver, red meat, chicken, eggs’] was a significant determinant of anemia, iron deficiency anemia and underweight. Consumption of foods rich in vitamin C, A and folic acid was associated with a less likelihood of being anemic due to iron deficiency. On the other hand, children who consumed diets rich in ‘zinc, vitamins B, cholesterol, calcium, potassium, and sodium’ were less likely to be zinc deficiency. Interestingly, this study found out that consumption of high amounts of 'carbohydrates, fat, dietary fiber, and iron' was positively correlated with stunting.

5.3.4 Limitations of the nutrition survey study

1. This was a cross sectional study therefore the results may change due to the dynamics of the community
2. 1-day 24-hour recall was used as opposed to 3-day 24-hour recall - This did not allow the study to capture the variation of diets in the community.

5.3.5 Strength of the nutrition survey study

1. Multiple factors likely to cause child malnutrition were put into considerations/were studied: child characteristics, complementary foods and feeding practices were considered in the study
2. EFA method was used to construct patterns/factors that reduced dimensionality with as little loss of information as possible in the total variation these variables explain. The patterns provide better information as compared to single variables.
3. Plasma zinc level was determined for each child. Very few studies have conducted similar investigations. Most studies deduce evidence of zinc deficiency from estimation of zinc in diet consumed by children [24-hour recall data].

Overall, the entire study tested the following null hypotheses which were all rejected based on the aforementioned results and discussion in this thesis;

1. Ho1: There is no significant relationship between germinated porridge and bioaccessibility of iron and zinc from the micronutrient powder in porridge.
2. Ho2: There is no significant difference in the acceptability of germinated versus non-germinated maize, sorghum and millet porridges.

3. Ho3: There is no significant relationship between complementary foods and the anthropometric, iron, and zinc nutritional status of children aged between 6 – 23 months.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

The objectives of this study were to:

1. determine the percentage of iron and zinc bio-accessible from micronutrient powder added to germinated porridges in Homabay County, Kenya.
2. establish the acceptability of germinated porridges among children aged between 6 – 23 months in Homabay County, Kenya.
3. determine the anthropometric, iron and zinc nutritional status of children aged between 6 – 23 months in Homabay County, Kenya.
4. establish the commonly used complementary foods and feeding practices among children aged between 6 – 23 months in Homabay County, Kenya.

This chapter presents the general findings and makes recommendations.

6.2 Main findings

1. This study confirmed that germination of porridges breaks down the anti-nutrients and therefore increasing the amounts of bioavailable zinc and iron. Germinated porridges enhance the bioaccessibility of iron and zinc that is contained in MNPs.
2. This study established that germinated porridges are accepted. Children aged 6-23 months favoured germinated millet, maize, and sorghum porridges in that order.
3. Cereal based porridges [71.7%] and ugali [60.2%] were the common complementary foods. Typically, none of the children received germinated porridge in the study population. Dietary diversity is very low and, 86% of children and 40% did not meet the EAR for Zinc and iron, respectively.

4. The prevalence of -2SD of stunting, underweight, and wasting was 17.5%, 13.4%, and 6.4%, respectively. Half of the children surveyed had anaemia, 39.5% were iron deficient, and 19.1% had iron deficiency anaemia. Further, 43.6% of the children had a zinc deficiency.

6.3 Conclusions

1. Germinated porridges improve the bioaccessibility of iron and zinc from micronutrient powder. Also germinated porridges are accepted among children and caregivers in Homabay County and would serve as very potent vehicles for the MNP.
2. Dietary inadequacy and, poor infant and young child feeding practices, are main contributors of malnutrition, iron and zinc deficiencies among children in Homabay County.

6.4 Recommendations for implementation

1. The community in Homabay County should be encouraged to feed children aged 6-23 months on germinated porridges with added MNP as one approach of addressing iron and zinc deficiency. Therefore, training of the community is paramount to enhance practice.
2. Concerted efforts to address malnutrition and iron and zinc deficiency are needed in Homabay County. A multipronged approach that includes; intensified nutritional education especially on IYCF practices, dietary diversification and zinc supplementation among sick children should be promoted in Homabay county. This is possible with the revision of the IYCF policy.

6.5 Suggested areas for further research

1. This study provides a background for further research in bioavailability since the dialysability method alone is not useful to estimate the uptake and absorption of nutrients. Therefore, dialysability method should be combined with the CaCo-2 cell method followed by isotope/human trials to determine the uptake and absorption of nutrients in future researches. In fact, this study has reported an alarmingly high malnutrition rate among children in Homabay County thus, the need for an urgent human trial to warrant appropriate interventions.
2. This study found out that complementary foods are consumed as mixed foods rather than single foods. Further research may be done on these mixed foods [cereal vegetable relish] as they are normally eaten, to determine mineral bioavailability from MNP in these combinations.
3. The different formulations of MNP could also be studied further, to promote the use of MNP in milk which is currently discouraged by as a vehicle for MNP, yet milk is a common complementary food in this community.
4. Future researches could also consider combining germination and lactic acid fermentation which has been found to completely degrade phytates in sorghum and maize porridges. Since germinated porridges were accepted based on the findings of this study, it is likely that porridges prepared from the aforementioned combination process will be accepted.

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APPENDICES

APPENDIX A: INFORMED CONSENT

My name is Susan Moraa Momanyi. I am a PhD student from Kenyatta University. I am conducting a study on “effectiveness of fermented porridge on bioaccessibility of iron and zinc from micronutrient powder in children 6-23 months in Homabay, Kenya”. The information will be used by the Ministry of Medical Services and Ministry of Public Health and Sanitation to promote home fortification of complementary foods with micronutrient powder to improve on child survival in this region as well as in other regions of Kenya.

Procedures to be followed

Participation in this study will require that I ask you some questions about your child and also examine your child in order to screen your child for iron and zinc status. Some specimen will be taken from the child for further tests. I will record the information from you about the child in a questionnaire.

You have the right to refuse participation in this study. Your child will get the same care and medical treatment whether you agree to join the study or not and your decision will not change the care your child receives from the clinic today or that you will get from any other clinic at any other time. Please remember that participation in the study is voluntary. You may ask questions related to the study at any time.

You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services your child receives from the clinic or any other organizations now or in the future.

Discomforts and risks

Some of the questions you will be asked are on intimate subject and may be embarrassing or make you uncomfortable. If this happens, you may refuse to answer these questions if you so choose. You may also stop the interview at any time. The interview may add approximately half an hour to the time you wait before you receive your routine services.

Benefits

If you participate in this study you will help us know the appropriate porridge to be used as vehicles for micronutrient powder to enhance the bioavailability of iron and zinc in children and reduce the risk of malnutrition. Your child will also benefit from being screened for malnutrition and if found to have a problem your child will be advised on treatment.

Reward

If you agree to participate in this study, your child will be provided with porridge three times a week.

Confidentiality

The interviews and examinations will be conducted in a private setting within the clinic. Your name will not be recorded on the questionnaire. The questionnaire will be kept in a locked cabinet for safe keeping at Kenyatta University. Everything will be kept private.

Contact information

If you have any questions you may contact Prof. Judith Kimiywe on 0722915459 or Prof. Hudson Nyambaka on 0721293140 or the Kenyatta University Ethical Review Committee

Secretariat on chairman.kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke, ercku2008@gmail.com.

Participant's statement

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that my records will be kept private and that I can leave the study at any time. I understand that I will still get the same care and medical treatment whether I decide to leave the study or not and my decision will not change the care I will receive from the clinic or that I will get from any other clinic at any other time.

Code of participant……………………………………………….

______________________________ ______________________

Signature or thumbprint Date

Investigator's statement

I, the undersigned, have explained to the volunteer in a language s/he understands the procedures to be followed in the study and the risks and benefits involved.

Name of interviewer………………………………….

______________________________ ______________________

Interviewer signature

APPENDIX B: STRUCTURED QUESTIONNAIRE

Data collector: ___________________________________

Participant no: | | | | **Date:** | |

Section A: Demographic and socio-economic status

1.Which Sub location and Village do you come from?

A. Wachara
   1. Rayudhi
   2. Nyamware
   3. Gingo
   4. Kodida
   5. Nyoniang
   6. Kanyobunga
   7. Nyarongi
   8. Ligongo
   9. Ngere
   10. Agulu
   11. Kamboga
   12. Wachara
   13. Nyonyangre
   14. Kotieno Lwanda
   15. Kiseke

B. Kadhola
   1. Osani
   2. Wayara
   3. Sibuoche
   4. Yathrateng
   5. Kanyalganda
   6. Kajuka
   7. Yago
   8. Nyabola
   9. Majita
   10. Ayiere
   11. Ombasa
   12. Kipingi
   13. Nyalhana

C. Kasirime Kawanga
1. Kandire
2. Kalwal
3. Koseso
4. Kamaina
5. Kokelo
6. Kachuth

D. Kasirime
1. Misita
2. Alara
3. Abura
4. Magawe
5. Omuga
6. Nyarandi
7. Got Kojowi
8. Otange
9. Nyaminabu
10. Nyandemra

2. Date of birth
   Age in months
   Category in months: 6-11[ ], 12- 18 [ ], 19- 23 [ ]

3. Sex: 1. Male 2. Female

4. Education level 1. None 2. Primary incomplete 3. Primary complete 4. Secondary incomplete 5. Secondary complete 6. Tertiary

5. Occupation 1. House wife 2. Formal employment 3 Business 4 Farmer, 5 Casual Laborer 6. Unemployed

6. What is your marital status?
   a) Unmarried
   b) Married
   c) Divorced
   d) Separated
   e) Widowed

7. How many people live in your household?

8. Please indicate the sources of income earned by the household.
   a) Agricultural farm produce
   b) Business
   c) Formal employment
   d) Casual labor
   e) Donations

9. Please indicate the level of income earned by the household in last one month.
   1. <2000
   2. 2001- 4000
   3. 4001- 6000
   7. >20000

4. 6001- 8000
5. 8001- 10000
6. 10001- 20000

11. Does the household own any of the following household items?

Item	1=Yes 2=No	Item	1=Yes 2=No	Item	1=Yes 2=No
Vehicle		Sofa set		Television	
Motorcycle		Computer		Refrigerator	
Bicycle		Telephone		Oven/cooker/meko	
Wheel barrow		Mobile phone		Solar panel	
Tractor		Radio			

11. Please indicate the materials the house is made up of

a) Walls ______________

1: Mud / Earth 2. Wood 3. Stones 4. Iron sheets 5. Wood off cuts

b) Roof________________

1: Iron sheets 2. Grass 3. Concrete 4. Tiles

c) Floor ______________________

1: Mud 2. Wood 3. Concrete 4. Tiles

12. **What is your main source of water**? __________________

1: Piped into the house 2. Piped to a tap on property outside the house 3. Rain tanks on property 4: Communal tap 5. Communal well 7. River 8. Stagnated water

13. **Do you have a toilet/latrine in your home or property**? ____1. Yes 2. No

If yes, specify _______1. Traditional latrine 2. Water Closet toilet 3. ordinary pit latrine 4. VIP latrines

14. **Please indicate your main source of fuel**

1. Firewood 2. Charcoal 3. Kerosene 4. Gas

15. **Please indicate your main source of lighting**

1. Electricity 2. Pressure lamp 3. Kerosene lamp 4. Solar

Section B: Morbidity of baby

Note: All questions are in reference to the last two weeks

1a. Was your child sick in the past 2 weeks? Yes _ No_ [if No the interview ends, go to next section infant feeding practices]

1b. Did the child have the following signs and symptoms

- Fever Yes _ No_ if yes for how long ----------days
- Cough Yes_ No_if yes for how long ----------days
- Fast breathing Yes_ No_if yes for how long ----------days
- Wheezing Yes_ No_if yes for how long ----------days
- Vomiting Yes_ No_if yes for how long ----------days
- Diarrhea Yes_ No_if yes for how long ----------days
- Skin rash [not nappy rash] Yes_ No_if yes for how long ----------days
- Refusal to breast feed or eat Yes_ No_if yes for how long ----------days

2a Did you take the child to be attended to in a health facility? Yes_ No_ [if No go to Q3]

2b Was the child admitted? Yes_No_

2c How long was the hospitalization -----days [In days]

2d What was the diagnosis of the child's illness? ____ [cross check from the Mother and Child book or any relevant documents]

2e what medications were given to the child? [copy the medications from prescription]

3a What do you think the child was suffering from? [tick what the caregiver tells you]

- Malaria Yes_ No_
- Common cold Yes_ No_
- Coughing Yes_ No_
- Pneumonia Yes_ No_
- Diarrhoea Yes_ No_
- TB Yes_ No_
- Don't know

4a Did you give the child any over the counter medication? Yes_ No_ [if No skip 4c]

4b What medications did you give the child? [caregiver to explain]

4c Did you give the child any home remedies Yes _ No_

Describe the home remedy________________

Any other information you would like to share with me regarding the health of your child? ____

Section C: Infant Feeding Practices Questionnaire

1.	How long after birth did you first put [NAME] to the breast?	Immediately 1 hour 2hours 3hours-6hours 6 hours-24hours After a day

2.	Are you currently breastfeeding your baby/Is the baby currently breastfed?	Yes ***[go to question 4]***	1	
		No	2	
		Don't know ***[go to question 4]***	3	

3a.	How old was the baby when breastfeeding was stopped? *[in months] options are 1month-12 months……………………*	

3b. Why did you stop breastfeeding your baby?

a) iIlness,
b) refusal,
c) use of medication,
d) cultural,
e) pregnant,
f) work,
g) death of mother,
h) mother left,
i) body image,
j) lack of breast milk,

…………………………………………………………………….

4a. How many times did your baby breastfeed yesterday during the day [24 hours]?

a) 1
b) 2
c) 3
d) 4
e) 5
f) frequently

4b. How many times did your baby breastfeed yesterday during the night?

a) 1
b) 2
c) 3
d) 4
e) 5
f) frequently

4c	Have you ever given the baby any drink, other than breast milk?	Yes	1
		No [go to question 5]	2
		Don't know [go to question 5]	3

4d What was the <u>first</u> drink other than breast milk that your baby was ever given to drink?

a) Water
b) Fresh cow milk
c) Fresh goat milk
d) Formula milk
e) Packet milk
f) Sweetened water
g) Lucozade/ribena
h) Soda
i) Fresh juice
j) Commercial juice

	k) Yoghurt l) Porridge m) Tea n) Soup o) Traditional herbs	
4.	How old was your baby when you gave this drink for the first time? *[in months] options 1-12 months*	

5.	At the moment, does your baby get any milk feeds other than breast milk?	Yes	1	
		No ***[go to question 8]***	2	
		Don't know ***[go to question 8]***	3	

6.	What type of milk, other than breast milk is your baby getting?	Fresh Cow's milk [whole]	1	
		Fresh Cow's milk [diluted]	2	
		Packet cow milk	3	
		Goat milk	4	
		Infant formula	5	

7.	How old was your baby when he/she was given this milk feed for the first time?*[in months] 1-24months*	

8.	At the moment, does your baby get any porridge?	Yes	1	
		No *[go to question 11]*	2	
		Don't know *[go to question 11]*	3	

9.	What type of porridge, is your baby getting?	a) White maize flour b) Millet flour c) Sorghum flour d) Maize flour + millet flour e) Maize flour + sorghum flour f) Millet flour + sorghum flour g) Maize flour + millet flour + sorghum flour h) Mixture flour	1
	Is cassava added in porridge flour	Yes/No	
	Is the porridge fermented	Yes/No	

10. How old was your baby when he/she was given this porridge for the first time?*[in months] 1-24months*	

11. At the moment, does your baby get any semi-solid or solid food [with a spoon]?	Yes	1	
	No *[go to next section food frequency questionnaire]*	2	
	Don't know *[go to next section food frequency questionnaire]*	3	

12. What was the first semi-solid or solid food [with a spoon] that your baby ate?

a) Mashed potatoes
b) Mashed bananas
c) Mixed mashed potato and banana
d) Ugali
e) Rice
f) Mashed bean/legume
g) Mashed vegetable
h) Mrenda/jutemallow
i) Pureed mixed fruit
j) Mashed pawpaw
k) Mashed avocado
l) Mashed ripe banana

13. How old was your baby when he/she ate semi-solid or solid food [with a spoon] for the first time? *[in months] 1-24 months*	

14a. How many times did your baby eat solid, semi-solid or soft foods [with a spoon] other than liquids yesterday during the day? Options 0-6	

14b. How many times did your baby eat solid, semi-solid or soft foods [with a spoon] other than liquids yesterday during the night? Options 0-6	

Section D: Infant food frequency questionnaire

Food item	Frequency of intake during the last week
Breastmilk	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Formula milk *If formula milk was used, please give name of the formula milk:*	*1___Every day* *2___Most days* *3___Once a week* *4___Never* ....
Cow's milk	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Goat milk	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Packet milk	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Yoghurt /Mala	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Infant cereals or commercial products e.g. Cerelac	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Porridge	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Ugali	*1___Every day* *2___Most days* *3___Once a week*

	4___Never
Rice	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Potatoes	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Cooked bananas	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Green maize	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Sweet potatoes	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Bread	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Pastries [chapatti, doughnut, pancake etc]	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Vegetables, any type [NOT potatoes] *If vegetables were eaten, please name the type of vegetables eaten mostly:*	*1___Every day* *2___Most days* *3___Once a week* *4___Never* a) Jute mallow [mrenda] b) Nightshade [manage] c) Spider plant [saga] d) Kales e) Cabbage f) Amaranth g) Cowpea leaves
Fruit juice [includes juice squeezed from the fruit]	*1___Every day* *2___Most days* *3___Once a week* *4___Never*

Fresh fruit [any type] *If fruit were eaten, please name the type of fruit eaten mostly:*	*1___Every day* *2___Most days* *3___Once a week* *4___Never* a) Oranges b) Mangoes c) Pineapple d) Ripe bananas e) Guavas f) Pawpaw g) Avocado
Eggs	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Red meat [beef, pork, mutton] / stew	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Chicken / poultry	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Liver e.g. chicken liver, beef liver, sheep liver etc	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Fish	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Sardines/Dagaa/Omena	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Beans/legumes/githeri	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Sweets/cakes/biscuits/chewing gum	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Soda	*1___Every day*

	2___Most days *3___Once a week* *4___Never*
Juice commercial e.g. Quencher, Afia, Delmonte etc	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Tea / Coffee /cocoa	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Sugar [any type], eaten as such, in drinks [e.g. tea] or added to food	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Salt [added to food]	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
How often did you use oil when preparing the <u>baby's food</u>?	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
How often did you use fat when preparing the <u>baby's food</u>?	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
How often did you use ghee/homemade fat when preparing the <u>baby's food</u>?	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
How often did you use margarine when preparing the <u>baby's food</u>? [any type of margarine]	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
How often did you use peanut butter when preparing the <u>baby's food</u>?	*1___Every day* *2___Most days* *3___Once a week* *4___Never*
Mention any other food your child has ever eaten …………………………………….	*1___Every day* *2___Most days* *3___Once a week* *4___Not Applicable*

12a). Did your baby drink anything from a bottle/cup with a teat yesterday during the day and/or the night?	Yes	1
	No	2
	Don't know	3
12b) If "Yes", what liquid did he/she drank? a) Water b) Fresh cow milk c) Fresh goat milk d) Formula milk e) Packet milk f) Sweetened water g) Lucozade/ribena h) Soda i) Fresh juice j) Commercial juice k) Yoghurt l) Porridge m) Tea n) Soup o) Traditional herbs		

13.	Does your baby get any dietary supplements [e.g. vitamin syrup, vitamin tablets]?	Yes, specify:	1
		No	2
		Don't know	3

Section E: Infant 24-hour recall dietary intake form
Please allow multiple selection of items from this section

1. What day was yesterday? [tick correct one]

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday

2. Would you describe the food that your baby ate yesterday as typical of your usual food intake?

Yes	1		No	2

Time of day	**Place**	**What drink and food?**	**How was it prepared?**	**What was added?**	**Amount Prepared in grams**	**Amount eaten in grams**
6-7 8-9 10-11 12-13 14-15 16-17 18-19 20-21 22-23 23-00 1-3 4-5	a) Home b) Church c) Clinic d) Market e) School f) Hotel g) Function place h) Journey [Safari]	a) Water b) Fresh cow milk c) Fresh goat milk d) Formula milk e) Packet milk f) Sweetened water g) Lucozade/ribena h) Soda i) Fresh juice j) Commercial juice k) Yoghurt l) Porridge m) Tea n) Soup o) Traditional herbs p) Ugali q) Rice r) Irish Potatoes s) Sweet potatoes t) Cooked bananas u) Green maize v) Bread w) Pastries x) Beef y) Lamb z) Chicken aa) Fish bb) Omena cc) Eggs dd) Liver ee) Beans/legumes ff) Jute mallow [mrenda] gg) Nightshade [manage] hh) Spider plant [saga] ii) Kales jj) Cabbage kk) Amaranth ll) Cowpea leaves mm) Oranges nn) Mangoes oo) Pineapple pp) Ripe bananas qq) Guavas rr) Pawpaw ss) Avocado tt) None	a) Ready to eat b) Raw c) Boiled d) Fried e) Stewed f) Bake g) Grill	a) Water b) Oil c) Fat d) Margarine e) Milk f) Cream g) Salt h) onions i) Tomatoes j) Capsicum [hoho] k) Parsley [dhania] l) Ginger m) Garlic n) Pepper o) Magadi / ash p) Sugar q) Eggs r) nothing	1000-2000 400-500 200-300 50-100 20-40 5-10	500 450 400 350 300 250 200 150 100 50 40 30 20 10 5

Section F: Anthropometry

WEIGHT [kg]						
1st Measurement				.		
2nd Measurement				.		
3rd [if needed]				.		

Oedema			
	Yes	1	
	No	2	

Length [cm]						
1st measurement				.		
2nd measurement				.		
3rd *[if needed]*				.		

MUAC [cm]				
1st Measurement			.	
2nd Measurement			.	
3rd [if needed]			.	

HC [cm]				
1st Measurement			.	
2nd Measurement			.	
3rd [if needed]			.	

APPENDIX C: ACCEPTABILITY TRIAL QUESTIONNAIRE

Data collector: ______________________________________

Participant no: Date:

Porridge Code: ____________________

Amount of porridge consumed

Weight of the cup	Amount of porridge	Weight of towel	Amount of spilled porridge	Amount of leftover porridge	Amount of porridge consumed	Total duration of feeding

APPENDIX D: DAILY SYMPTOMS OF THE BABY

Date	Sunday		Monday		Tuesday		Wednesday		Thursday		Friday		Saturday	
 Healthy		1		1		1		1		1		1		1
 Diahrrea		2		2		2		2		2		2		2
 Vomiting		3		3		3		3		3		3		3
 Runny nose		4		4		4		4		4		4		4
 Coughing		5		5		5		5		5		5		5
 Fever		6		6		6		6		6		6		6
Nappy rash		7		7		7		7		7		7		7
Rash [NOT nappy rash]		8		8		8		8		8		8		8
Did your baby go to the hospital, doctor or clinic? What did they say was wrong?		9		9		9		9		9		9		9
Did your baby get any medicine? Please write them down		10		10		10		10		10		10		10
Did your baby get any supplements [vitamins]? Please write them down		11		11		11		11		11		11		11

APPENDIX E: ETHICAL CLEARANCE BY KENYATTA UNIVERSITY

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Email: chairman.kuerc@ku.ac.ke
secretary.kuerc@ku.ac.ke
ercku2008@gmail.com
Website: www.ku.ac.ke

P. O. Box 43844 - 00100 Nairobi
Tel: 8710901/12
Fax: 8711242/8711575

Our Ref: KU/R/COMM/51/536

Date: 31st August, 2015

Momanyi Susan Moraa
Kenyatta University,
P.O Box 43844, Nairobi.

Dear Moraa,

RE APPLICATION NUMBER PKU/368/ I 341– "EFFECTIVENESS OF FERMENTED PORRIDGE ON BIOACCESSIBILITY OF IRON AND ZINK FROM MICRONUTRIENT POWDER IN CHILDREN IN HOMABAY COUNTY KENYA "– VERSION 2

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1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research **topic "Effectiveness of fermented porridge on bioaccessibility of iron and zink from micronutrient powder in children in Homabay County Kenya" – Version 2** dated 21st August, 2015.

2. APPLICANT

Momanyi Susan Moraa

3. STUDY SITE

Ndhiwa Sub-County,Homabay County, Kenya.

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 31st August, 2015.**

5. ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

PROF. NICHOLAS K. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

KENYATTA UNIVERSITY OFFICE OF THE CHAIRMAN 31 AUG 2015 ETHICS REVIEW COMMITTEE

I ..SUSAN..MORAA..MOMANYI.....accept the advice given and will fulfill the conditions therein.

Signature.............................. Dated this day of..04/09/15................ 2015.

cc. Vice-Chancellor