EFFECTS OF PHYTATE REDUCTION ON MAIZE/BEAN BLEND INFANT FOODS USED IN SOUTHERN ETHIOPIA

By

ALEMZEWED CHALLA ROBA

Bachelor of Science in
Rural Development and Family Sciences
Hawassa University
Awassa, SNNPR
2005

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2009

EFFECTS OF PHYTATE REDUCTION ON

MAIZE/BEAN BLEND INFANT FOODS USED IN

SOUTHERN ETHIOPIA

Thesis Approved:

Dr. Tay Kennedy

Thesis Adviser

Dr. Barbara J. Stoecker

Dr. Patricia Rayas-Duarte

Dr. A. Gordon Emsile

Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to my major advisor, Dr Barbara J. Stoecker, for her constructive guidance, encouragement and support throughout the course of my study and creating this education opportunity. Sincere appreciation is also expressed to my committee members: Dr. Tay Kennedy and Dr. Patricia Rayas-Duarte. I am grateful for their input to help improve my thesis. I wish to thank Dr. Donald Oberleas for his unlimited advice and technical assistance.

My genuine thanks go to Yeshewafanos Kibe, Meron Girma, Getahun Erisno, Afework Mulugeta, all RDFS staffs and all subjects who participated in this study. Your unlimited support throughout the course is so much appreciated.

Special thanks go to all my family and close friends. Mom, I know that you don't speak English, but for the record, it's all only because of you that I am here today. I am also blessed having my best friends Samson G/ Medhin, who always there no matter what and Rediet Bayu and Girum Zigale whom I totally depend on. Thanks guys.

Finally, there are countless people to whom I am grateful for their kindness. Indeed, I am humbled by few odds that I really am thankful and wish to give them credit. Their unfair challenge makes me stronger and more aspired to fill my own little shoes.

Thanks to NIH Grant R21 TW06729 (Fogarty International Center & the Office of Dietary Supplements) for supporting my assistantship and Hawassa University (my beloved home where I broke my tooth and grow) for all the support and belief.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER II

CHAPTER IV

CHAPTER V

CHAPTER I

INTRODUCTION

Proper nutrition is one of the major factors affecting physical growth, mental development and general health of infants and children. Chronic malnutrition arising from the interaction of inadequate dietary intake and morbidity has a major impact on growth and development of African children. As a result, large numbers of children in most countries of Africa suffer from malnutrition and its subsequent health complications.

Prevalence of malnutrition in some African countries has reduced slightly in recent years, but the magnitude yet remains high in many countries and is a major public health problem. Child stunting in Africa has declined from 41% in 1980 to 34% in 2005, but the problem increased in sub-Saharan countries from 47% to 49% and affected 24.4 million children (Mercedes de, Monika, Elaine, Edward, & Richard, 2004).

Similarly, high child malnutrition is reported in Ethiopia due to inadequate intakes of several nutrients. Demographic health survey of Ethiopia (CSA, 2007) reported 47% of under five years old children to be stunted, 11% wasted and 38% underweight. Such malnutrition is aggravated by predominant use of cereal based diets as staple food and very low animal source food intake (Abebe, 2003).

Beside protein-energy malnutrition, micronutrient deficiency also contributes to child morbidity and mortality in Ethiopia. Content and bioavailability of iron, zinc, vitamin A, vitamin B-12 and calcium in the diets are often low. Zinc deficiency affects tens of millions of children throughout the developing world (Sandstead, 1991). Zinc deficiencies result from inadequate intake of zinc-rich foods or inadequate utilization of available zinc in the diet due to inhibitors. In Ethiopia, plant-based foods, mainly unrefined cereals and legumes, are consumed as staple foods. Therefore, infants and under five years old children in this country are mainly fed cereal-based foods without animal source foods (Abebe, 2003). Because such foods have low zinc content or poor bioavailability due to inhibitors, zinc deficiency is a major phenomenon in this country (CSA, 2007; Melaku Umeta, 2000a, 2000b).

One of the factors that hinder bioavailability of zinc in cereal and legumes is phytate. In general, phytate ($C_6H_{18}O_{24}P_6$) is an anti-nutritional compound that exists in nature in the form of myo-inositol hexaphosphate (IP6) with a molecular weight of 660 (L. Bohn, Josefsen, Meyer, & Rasmussen, 2007; L. Bohn, Meyer, & Rasmussen, 2008). Cereals and legumes have high amounts of phytate in their bran or germ; and with moderate to high consumption, the phytate can bind trace minerals, form insoluble complexes and hinder their absorption in the human gut (Ferguson, Gibson, Thompson, & Ounpuu, 1989; Gibson, 1994; Sandstrom, 1987; Wise, 1995). As a result, phytate is a contributor to trace mineral deficiency in developing countries (Egli, Davidsson, Zeder, Walczyk, & Hurrell, 2004; Lonnerdal, 2000).

It is possible to predict zinc bioavailability from the phytate to zinc molar ratio present in foods (Morris & Ellis, 1989; Zhou et al., 1992). Molar ratios of phytate to Zn which are greater than 15 or Ca/phytate/Zn greater than 200 mmol/1000 kcal (4.2 MJ) are usually associated with increased risk of suboptimal zinc status (Gibson, Donovan, & Heath, 1997; Morris & Ellis, 1989). The mean dietary phytate-to-Zn and Ca/phytate/Zn molar ratios of most western diets are below the critical molar ratio (Abraham, 1982; Bindra & Gibson, 1986; Carnovale, Lombardi-Boccia, & Lugaro, 1987; W. P. Stephens, Klimiuk, Warrington, & Taylor, 1982). In contrast, high dietary phytate to Zn molar ratios (i.e. 24 - 40) were reported in West Africa and Iranian populations (Davies & Warrington, 1986; Reinhold, Nasr, Lahimgarzadeh, & Hedayati, 1973).

Diets with high phytate to Zn molar ratios increase the risk for zinc deficiency (Ferguson et al., 1989). Children who consume cereal-based foods are at greater risk for suboptimal zinc status than adults (Hambidge, 1981). Effects of zinc deficiency have been recognized globally in recent years (Hambidge, Krebs, & Miller, 1998). Human zinc deficiency was reported for the first time in 1960-70s, but was considered as a rare nutritional problem until the 1990s (Hambidge, 1981; Jameson, 1976; Mahloudji et al., 1975; Prasad, 1983; Sandstead, 1991). Since then, understanding of the prevalence of zinc deficiency has expanded and currently half of the world's population is estimated to be at risk for inadequate zinc intake (Chen, 2004). The problem is more severe in developing countries because unrefined cereals contribute a major portion of the dietary intake (Ferguson et al., 1989).

Basics about zinc biology and its importance in cellular growth and differentiation are well documented (N. E. Krebs & Hambidge, 2001). Zinc is a critical constituent of many enzymes needed in the body and is involved in a large number of metabolic processes. Young children require zinc for proper body functioning, rapid growth and good health and development (N. F. Krebs, 2000). Potential adverse effects of zinc deficiency on children include low birth weight, impaired growth, immune incompetence, cognitive impairment and increased morbidity and mortality (Gibson, Sazawal, & Peerson, 2003; Lonnerdal, 2000). Studies suggest that a mild to severe deficiency of zinc can disturb biological functions like gene expression, protein synthesis, skeletal growth and maturation, gonad development, pregnancy outcomes, taste perception, appetite, and cognitive impairment.

Zinc deficiency in developing countries arises mainly from impaired bioavailability of dietary zinc and can largely attributed to the high phytate present in the staple diets (Gibson, 1994). In rural areas of most developing countries, where predominantly cereal-based foods are consumed by children, zinc bioavailability is very poor (Melaku Umeta, 2000a) and hence, zinc deficiency becomes widespread among children (Ferguson et al., 1993; Hotz & Gibson, 2001b). In Ethiopia, staple diets of the population are predominantly cereal-based and low in animal source foods (Hambidge, Abebe et al., 2006). As a result, zinc deficiency in children is found to be very high (Melaku Umeta, 2000a). Thus reducing phytate from such cereal-based foods to improve zinc bioavailability is essential. The principal objective of this study is to determine how phytate can be reduced using home-based methods to improve zinc bioavailability with a goal of having a phytate: zinc molar ratio < 15.

Statement of the problem

Phytate is a potent inhibitor of zinc absorption in cereals and legumes (Morris & Ellis, 1989). It is present in high concentrations in maize (Ferguson et al., 1989). Maize is the predominant food source for many people in southern Ethiopia (Abebe, 2003; Hambidge, Abebe et al., 2006). Dietary zinc absorption was found to be poor in southern Ethiopia (Hambidge, Krebs, Westcott, & Miller, 2006). Zinc deficiency is considered as one contributing factor to infant and child morbidity in this area. Therefore, developing method reducing phytate from maize is potential strategy to improve zinc status in infants and children under five years old in southern Ethiopia.

Significance of the study

Adequate zinc absorption is required for early rapid growth and mental development of infants (Egli et al., 2004; Mazariegos et al., 2006). Zinc is limiting for many enzymatic activities and deficiency cause metabolic disorders in human body. Zinc deficiency can be caused by the chelating effect of phytate in cereal-based foods by forming insoluble complexes that hinder zinc absorption in the human gut (Mazariegos et al., 2006). A strategy to improve zinc absorption is therefore paramount to address this issue. Reducing phytate from diet can be achieved by novel precipitation methods during food processing, or by preparation methods that activate endogenous phytase (e.g., germination, fermentation, and hydrothermal processing), or addition of exogenous phytase (Lonnerdal, 2003). Many home-based food processing methods are used in Ethiopia; however, there are no reports indicating how they may facilitate phytate reduction in cereal-based foods.

Purpose of the study

The purposes of this study were to: evaluate phytate reduction potential of home-based food processing methods; develop low-phytate maize-based foods for infants and under five years old children using home-based food processing and assess sensory quality and acceptability of newly developed food and potential of adding meat to complementary foods.

Hypotheses

There is no significant difference between home-based food processing methods in reducing phytate. There is no significant difference between tasted foods in terms of sensory quality. There is no significant difference in acceptability between food analyzed sensory by staff/ students, mothers and 12-36 months children. There is no significant difference in sensory quality between tasted food prepared with or without meat.

Assumption

Subjects have evaluated each sample critically and objectively as instructed. Food samples were prepared in the same place under controlled condition, therefore the differences noted should be attributed to manipulated variables.

CHAPTER II

REVIEW OF LITERATURE

Description of Phytate

Plant-based foods, which are mainly composed of unrefined cereals, are major components of people's diets in many developing countries. Cereals are good sources of carbohydrate, protein, B vitamins, minerals, and dietary fiber. However, cereals also provide anti-nutritional factors, such as inhibitors and chelating agents which interfere with mineral absorption. The principal chelating agents found in cereals are dietary fiber and phytate (Egli et al., 2004).

Phytate ($C_6H_{18}O_{24}P_6$; $_{F.W.}$ 660.58) is a natural compound present in many plants as a primary storage form of phosphate. The amount present in plant-based foods is dependent on the part of the plant ingested and its stage of maturity. Mature fruits or seed of cereals like maize, wheat or rice are good storage sites for phytate (Oberleas, 1971). Phytate biosynthesis is initiated shortly after plant flowering and accumulates throughout seed maturation and desiccation. Phytate accounts for 60 to 90% of total phosphorus present in the kernel of mature cereal grains (de Boland, Garner, & O'Dell, 1975).

Occurrence of phytate

Myo-inositol hexaphosphate (phytate) is found abundantly in the germ or bran of whole cereal grains such as maize, wheat and rice ranging from 1.5 to 6.4%, predominantly located in globoids. Ninety percent of the globoids are present in the aleurone layer, while only 10% are in the embryo. However, wheat and rice endosperm are almost devoid of phytate as it is concentrated in the germ and aleuronic layers of kernel cells and bran or hull. About 87% of phytate in wheat and rice is present in bran; whereas 88% of the phytate in maize is present in the germ (Hotz & Gibson, 2001a) and aleurone (de Boland et al., 1975) compared to endosperm and hull.

Earley and colleagues followed phytate levels in maize from pollination to maturity and observed that phytate was not present in the leaves, stems, tassels, or cobs of the plant and that phytate began to increase in the kernels approximately 3 weeks after pollination and increased until maturity (Earley, 1944). Earley and DeTurk (1944) reported similar data in which, foods that incorporate maize germ can be high in phytate.

Structure of phytate

Phytate is the trivial name of myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate), which exists mainly as myo-inositol hexaphosphoric acid (calcium salt of phytic acid) or as phytin which is composed of calcium and magnesium salts of phytic acid (Lori Oatway, 2001). There are nine stereoisomers of inositol in nature, but only myo-inositol hexakis is nutritionally relevant form (Fig 2.1).

The myo-inositol 1,2,3,4,5,6-hexakis describes the exact structure and appearance of the molecule. The prefix "myo" refers to the conformation of the hydroxyl groups on the inositol ring. Whereas, “hexa*kis*” indicates that phosphates are not internally connected, and the compound as a whole is considered as ligand, which is a chelator that binds more than one coordination site of a metal atom (Johnson, 1969).

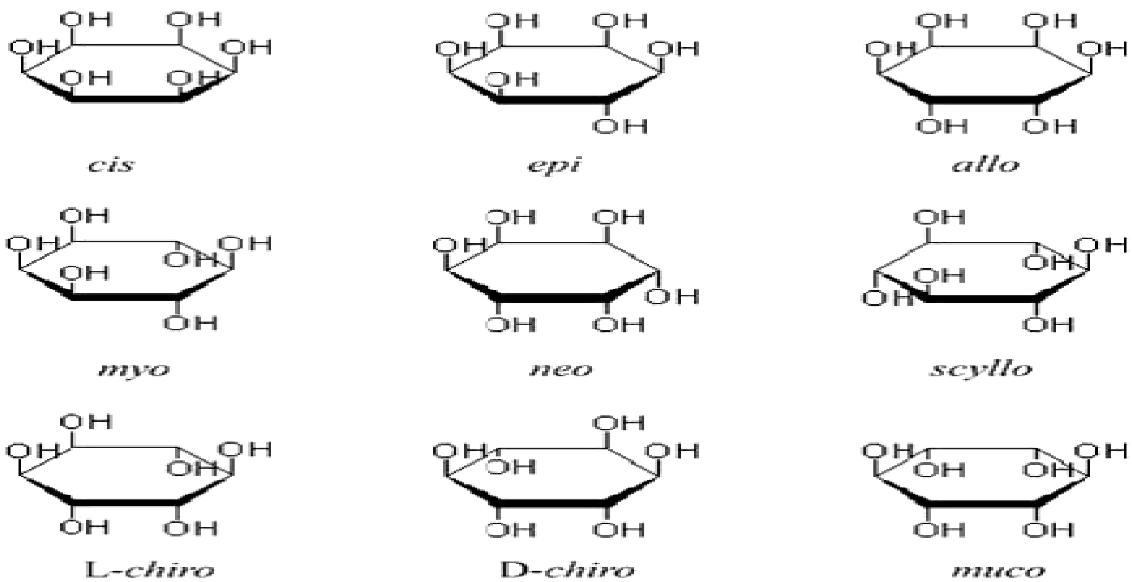

Figure 2.1 Nomenclature of the nine stereoisomers of inositol (Haworth projections). Seven have a mirror axis in the molecule and numbering of the carbon atoms can be performed either counterclockwise (D) or clockwise (L). Only the chiro form has specific D and L conformations (Software: ChemDraw 10.0, Cambridgesoft.com)

Hydrolysis of phytate can result in lower forms of inositol phosphates like mono, di-, tri-, tetra-, penta-phosphate and inorganic phosphate. Phytate normally has at least one phosphate group on its inositol ring. According to Blank and colleagues (1971), X-ray crystallographic analysis of sodium phytate reduced that the molecule exists in a chair conformation having five axial and one equatorial phosphate group. However, at pH 7 sodium phytate switched to different conformation than in strong alkali. Conformation of phytate and its salts depends on the type of cation, pH and physical state (Blank E. G., 1971) (fig.2.2).

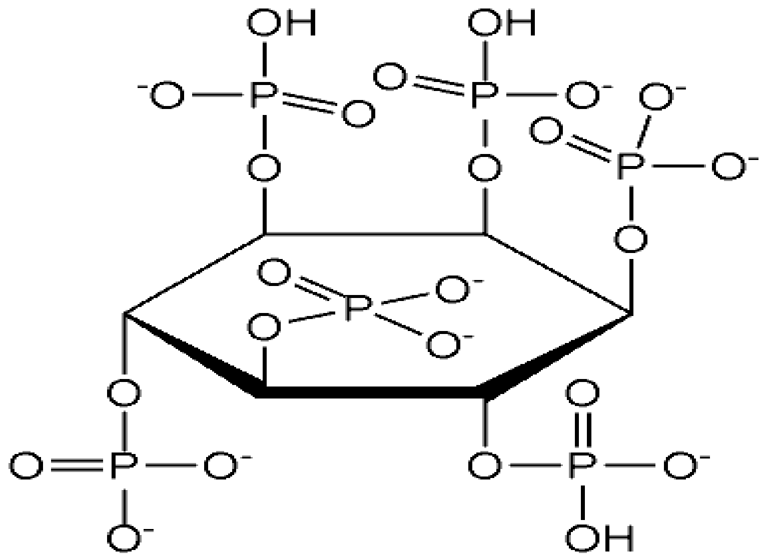

Figure 2.2 Myo-inositol (1,2,3,4,5,6) hexakisphosphate in boat formation showing the 5 equatorial and the 1 axial group. (Software: ChemSketch 10.0,ACDlabs.com)

Properties of phytate

Phytate is a principal chelating agent in cereal-based foods and is capable of impairing divalent mineral bioavailability through binding (Egli et al., 2004). Phytate has six phosphate groups attached to the inositol ring (Fig 2.2). Each phosphate group is esterified to the inositol ring and together they have 12 replaceable protons or reactive sites composed of six strongly acidic, with pK of 1.5 to 2.0, two weakly acidic with a pK of 6.0, and four very weakly acidic sites with pK of 9.0 to 11.0 (Costello, Glonek, & Myers, 1976).

Such unique structure enables phytate to carry a strong negative charge and actively bind divalent metal ions, to form insoluble complexes and to reduce in vivo bioavailability of divalent ions. The negatively charged property of phosphate in phytate allows phytate to interact with other positively charged compounds like protein, starch, and divalent metal ions like zinc (Bosscher et al., 2001), iron (Glahn, Wortley, South, &

Miller, 2002), magnesium (T. Bohn, Davidsson, Walczyk, & Hurrell, 2004), and calcium (Oatway Lori, 2001) and reduce their bioavailability in neutral pH range. Zinc is most affected as it forms very stable and insoluble complex when binding with phytate (Oatway Lori, 2001).

Solubility of the various divalent salts of phytate is strongly dependent on pH and synergistic effects between two divalent ions (Oberleas, 1971). Ferric phytate is least soluble in dilute acid, but dissolves in dilute alkali. On the other hand, calcium and magnesium phytates are least soluble under slightly alkaline conditions, whereas zinc and copper phytates are least soluble between pH 4 and 7. A mixture of zinc and calcium ions with phytate (molar ratio of 1:1:1) at pH 6 gives more precipitate than either metallic ion alone at similar concentration (Oberleas, 1971).

Effects of phytate on zinc bioavailability

Impacts of low zinc intake on health and development may be magnified by the presence of components that interfere with intestinal bioavailability. Phytate is a chelator that comprises 3% or more by weight of all seeds and is present in numerous foods. Excessive intake of phytate is believed to suppress bioavailability of zinc, by forming insoluble metal phytate complexes. Its high density of negatively charged phosphate groups causes phytate to form stable complexes with zinc and makes it unavailable for intestinal uptake (Lopez de Romana, Lonnerdal, & Brown, 2003; Lopez et al., 2003).

Accordingly, several animal and human studies reported a negative correlation between phytate and zinc bioavailability. For example, Lonnerdal found that feeding high phytate concentrations to suckling rat pups was inversely correlated with zinc absorption

(Lonnerdal, 1989). In another study, O'Dell and Savage reported reduction in growth of chicks after adding phytate to a casein-based diet at a level similar to that found in soy protein (O'Dell & Savage, 1960). Subsequent studies in pigs, chicks and rats also showed similar result of impaired zinc utilization when phytate-rich diets were fed (Likuski & Forbes, 1965; Oberleas & Harland, 1977). Lonnerdal and colleagues found an increase in zinc absorption from 22 to 36% after feeding infant rhesus monkeys a soy formula whose phytate concentration was reduced from 300 to 60 mg/l (Lonnerdal, Jayawickrama, & Lien, 1999).

Early findings of zinc deficiency in human subjects in the Middle East demonstrated that phytate can affect zinc status in humans as well (Mahloudji et al., 1975). Many studies have showed similar negative effects of phytate on zinc bioavailability. For example, Reinhold and colleagues have shown inhibitory effects of phytate on zinc absorption in humans (Reinhold et al., 1973). Greger and colleagues (1978) reported negative effects of phytate on zinc balance after substituting part of the meat in diets of adolescent women with soybean protein (Greger, 1978).

Subsequent single meal studies have shown negative correlations between the presence of phytate and zinc absorption in humans (Navert, Sandstrom, & Cederblad, 1985; Sandstrom, Almgren, Kivisto, & Cederblad, 1987; Sandstrom & Sandberg, 1992). (Fredlund et al., 2003) explored dose-dependent inhibition of zinc absorption by adding 4 to 250 mg phytate to a low phytate meal with constant amounts of zinc. Significant negative effects of phytate on zinc absorption were found when adding 50 mg phytate phosphorus and above, while the effect below this level of phytate was not significantly different from the control. Such results were in agreement with many other composite meal studies (Navert et al., 1985; Sandstrom, 1987; Sandstrom & Sandberg, 1992). High zinc absorption (21%) was observed from low phytate meals (lower than 50 mg), while zinc absorption was found to be very low (4-11%) in high phytate meals (100-280 mg phytate).

negative effects of phytate on zinc absorption have been confirmed by a stable isotope study in young men by Turnlund and colleague (1984) and adding phytate to a milk formula in a radioisotope study on human volunteers by Lonnerdal and colleague (1984) (Turnlund, King, Keyes, Gong, & Michel, 1984). In the latter study, phytate was added into the milk formula at a concentration similar to that of soy formula, and zinc absorption decreased from 32% to 16%, which was very similar to the absorption from soy formula.

Phytate to zinc molar ratio

Zinc bioavailability is usually predicted by phytate to zinc molar ratio of the food and by the amount of calcium in the foods (Davies & Olpin, 1979; Morris & Ellis, 1980; Simpson, Morris, & Cook, 1981). This index has been used widely (Bosscher et al., 2001; Fitzgerald et al., 1993; Mendoza et al., 2001) and is considered good estimate of zinc bioavailability by the International Zinc Nutrition Consultative Group (IZiNCG, 2004).

Davis and Olpin reported that a 10:1 or higher ratio decreased plasma zinc and reduced growth of young rats after feeding phytate: zinc ratios ranging from 0.1:1 to 40:1 (Davies & Olpin, 1979). Morris and Ellis (1980) showed that phytate: zinc molar ratios of 10:1 or higher had a negative effect on zinc absorption (Morris & Ellis, 1980). Ellis and colleagues and Lonnerdal reported 12.5:1 or higher phytate to zinc ratios reduced zinc bioavailability in a rat model (Ellis et al., 1987; Lonnerdal, 2000). Others suggested phytate: zinc ratio of 6:1 had an inhibitory effect in soy formula (Sandstrom et al., 1983).

Similarly Lonnerdal and colleagues reported a 6:1 phytate to zinc ratio in soy formula lowered zinc absorption (Lonnerdal, Cederblad, Davidsson, & Sandstrom, 1984). Significantly lower zinc absorption was observed at a ratio of 5.7:1 or higher in a rat model study by (Fredlund et al., 2003) who investigated phytate to zinc molar ratios that varied from 0.03:1 to 28.6:1. In general, adequate zinc bioavailability is associated with phytate to zinc molar ratios of 10 or below. Presence of calcium facilitates the formation of phytate: zinc: calcium molar ratios that further lower zinc bioavailability.

Function of Zinc in Humans

Zinc is an essential trace element in the human body that is needed for good health, proper development and functioning. It is the most abundant trace element in all cells of the body, except in red blood cells where iron predominates. The biological role of zinc is not limited to only a few functional roles. It is known to be a constituent of a large number of enzymes; to date more than 200 enzymes are known to be zinc dependent (Cousins, 1996). The role that zinc plays in enzymes can be either structural or catalytic. It plays a central role in gene replication, protein synthesis and cellular division and growth (Chesters, 1991)

Zinc deficiency arises when effective intake of zinc is lower than the body's requirement. Gibson and colleagues stated that zinc uptake is determined by zinc content in the food and its bioavailability (Gibson, Ferguson, & Lehrfeld, 1998). The staple foods consumed in developing countries are predominantly plant-based and mainly consist of cereal and legumes. These foods contain large amounts of zinc, but it is not all bio-available because of inhibiting substances that negatively affects its absorption (Brown, Wessells, & Hess, 2007). Phytate is a natural inhibiting substance that readily hinders proper absorption of zinc in the body. Inadequate intake of zinc consequently leads to a wide range of disturbances in hormones and enzymes, metabolic disorders, depression of bone growth and development, pronounced linear growth retardation or stunting, and increased risk of diarrhea and respiratory infections.

Strategies to Reduce Phytate

The human requirement for dietary zinc can be fulfilled in several ways. Dietary modification is one of the approaches that enhance availability, access, and utilization of foods with high content and bioavailability of zinc. This approach includes increased production of low phytate foods, increased intake of zinc-rich foods, use of food processing methods (soaking, fermentation and germination) to reduce levels of inhibitors, and increased consumption of absorption enhancers (ascorbic acid and flesh foods) with meals (Hotz & Gibson, 2001a).

Sandstrom and colleagues reported that zinc bioavailability can be increased by reducing dietary phytate (Sandstrom, Almgren, Kivisto, & Cederblad, 1989). Phytate in cereal is usually present in water soluble sodium or potassium phytate form and generally be reduced by using enzymatic and non-enzymatic methods (Hotz, Gibson, & Temple, 2001). Non-enzymatic methods involve extraction or thermal processing, whereas, enzymatic hydrolysis involves endogenous or exogenous phytase (Sandberg, 1991).

Phytate in cereals can be hydrolyzed to lower myo-inositol forms by activating endogenous phytase or by adding exogenous phytase (Davidsson, 2003).

Various food-processing techniques, traditionally used in meal preparation across many countries, have the potential to reduce phytate content. Enzymatic hydrolysis of phytate in cereals using soaking, germination or fermentation enhances the activity of endogenous or exogenous phytase (Aaron, Scott, & Kendall, 2008; Gibson & Ferguson, 1998; Sandberg & Svanberg, 1991). Germination consists of soaking seeds in water in the dark, usually for up to 3 days, to promote sprouting. Germination increases phytase activity, causing phytate to break down, and increases bioavailability of zinc (Sandberg, 1991).

Non-enzymatic methods, such as thermal processing, soaking and milling can reduce phytate content (Brunvand, Henriksen, Larsson, & Sandberg, 1995; Lee, Schroeder, & Gordon, 1988; Turk, Sandberg, Carlsson, & Andlid, 2000). Soaking reduces phytate content of cereals that contain water-soluble sodium or potassium phytate (Cheryan, 1980). Milling reduces phytate if it is localized within specific parts of the grain such as germ (corn) or aleurone layer (wheat, rice, sorghum, rye) (de Boland et al., 1975). Combining fermentation, soaking and germination techniques is highly efficient to activate endogenous phytase and to degrade phytate (Sandberg & Svanberg, 1991). Sour dough leavening can completely degrade phytate.

On a larger scale, phytate can be removed/reduced by novel precipitation methods applied during processing (Lonnerdal, 2003) or by genetic modification (plant breeding) to produce seeds with low phytate (Hambidge, Krebs et al., 2006; Mendoza et al., 1998). Reduction of the phytate content of maize by selective breeding had a significant positive effect on zinc absorption in human adults (Mendoza et al., 1998).

Effect of Home-based Food Processing on Phytate Reduction

Several studies have been carried out to develop food mixes using germination and malting techniques. When germinating grains at optimum conditions, the activity of endogenous phytase varied among cereals, rye being the highest, barley and wheat intermediate, and oats the lowest (Mosha, Gaga, Pace, Laswai, & Mtebe, 1995). Phytase is present prior to germination, but activated during germination. Germination in itself does not cause a substantial decrease in phytate, even after extended periods of time (Mosha et al., 1995).

Malting causes modest phytate reduction in grain. In malted grains which were ground and soaked for 2 hours, phytate was reduced by 95% from rye, wheat and barley, while it took considerably longer to achieve a similar reduction in oats (Sandberg, 1991). Malting barley-based breakfast cereals increased zinc bioavailability from 15 to 23% (Fredlund et al., 2003). Similarly, hydrothermal processing of barley porridge increased zinc absorption from 11 to 25%. Baking using yeast dough reduced phytate content in whole meal bread (Brunvand et al., 1995). A reduction of 66 to 97% of phytate was achieved in sour dough bread.

Adding exogenous phytase can also facilitate the degradation of phytate in foods. Commercial exogenous phytases, isolated from cereals or microbial/ fungal sources, have variable activities. *Aspergillus niger* phytase has been found to degrade phytate in oats completely, while wheat phytase decreased phytate by 89% and endogenous phytase reduced phytate by 70-80% (Sandberg, Hulthen, & Turk, 1996). Adding *A. niger* phytase to phytase inactivated wheat bran increased zinc absorption from 14 to 26% (Sandberg et al., 1996). In a novel approach to adding exogenous phytase, (Lucca, Hurrell, & Potrykus, 2002) inserted the gene for a heat-resistant phytase from *A. fumigatus* into rice in order to reduce phytate content. Although high expression levels were achieved, unfortunately, the phytase activity was significantly reduced by boiling the rice.

Home-based food processing can hydrolyze phytate to free inorganic phosphorus (P_i) and myo-inositol phosphates (IP5 - IP1), or leach phytate from cereals into the soaking medium (Hotz & Gibson, 2001a). Many studies showed that enzymatic and non-enzymatic processes facilitated by home-based methods have positive effects on reducing phytate from cereals. A study conducted in rural Malawi showed that soaking pounded maize for an hour reduced 49% of phytate from unrefined maize (Hotz & Gibson, 2001a). In another study, soaking whole millet for 24 hrs reduced 29% of phytate content; when soaking de-hulled millet, phytate reduced further by 39%. Reduction of phytate maximized to 52% when soaking flour (Duhan, Khetarpaul, & Bishnoi, 2002). Germinating cereals for 2-3 days increased endogenous phytase activity by 23-58% (Gibson & Ferguson, 1998) and reduced 21-28% of total phytate content from cereals (Gibson, Yeudall, Drost, Mtitimuni, & Cullinan, 1998)

Combined use of home-based food processing methods could be effective to reduce phytate from cereals (Gibson, Yeudall et al., 1998). Up to 90% phytate reduction was achieved using combined methods of soaking and germination at different stages and proportions (Hotz & Gibson, 2001b). Moreover, mixing germinated and un-germinated cereal flour was found to enhance the phytate hydrolysis process (Gibson, Yeudall et al., 1998). Adding 10% germinated maize flour to 90% un-germinated maize flour decreased 56% of phytate from tested food (Gibson & Ferguson, 1998).

Determination of phytate content

Quantification of phytate was first reported by Heubner & Stadler in 1914. The basic principle of this early method involved the formation of a stable complex between ferric ion and phytate in dilute acid (Oberleas & Harland, 2007). At that time, phytate was considered the only phosphate in plant seeds. The titration method used a standardized ferric chloride solution with the end point resulting from the formation of a flesh-pink colored complex with ammonium thiocyanate that persisted for 5 min. The end point was not easily detected because of the formation of white ferric phytate precipitate in the titrated solution. This initial method has been modified several times since, but various investigators used the same basic principles (Oberleas, 1971).

According to (Oberleas, 1971), measuring phyate involved extraction of phytate from finely ground food with aqueous acidic solution (such as 0.5M aqueous HCl, 1.2% HCl containing 10% sodium sulfate, or 3% trichloroacetic acid-TCA), precipitation of phytate with the ferric ion, centrifugation and washing of the precipitate, and quantitation of phytate by measuring iron or phosphorus content of the precipitate. There are no known specific reagents that can be used to identify and quantify phytate directly, nor does phytate have a unique ultra-violet or visible absorption spectrum (Harland & Oberleas, 1986). Moreover, lower inositol phosphates (IP_1 to IP_4) may interfere with phytate analysis.

(Oberleas, 1971) determined the ratio of iron to phosphorus in ferric phytate precipitated from dilute acid. A measured quantity of standardized ferric chloride solution was added to a known amount of sodium phytate (Oberleas, 1971). After phytate precipitation, the iron was determined in the supernatant and the amount in the ferric phytate was calculated by difference and reported based on an atomic ratio of 4:6 for iron to phosphorus in ferric phytate.

When iron is used to quantify the amount of phytate, the phytate content is usually calculated by assuming iron to phosphorus ratio of 4:6. However, this ratio is accurate only with a sufficient excess of ferric chloride and in the presence of 4% sodium sulfate. Thus, the composition of ferric phytate precipitate varies depending on precipitation conditions. For example, (Courtois, Malangeau, & Chabre, 1959) determined phytate in bread by extracting with 5% TCA and precipitating phytate by adding ferric ion, and measuring the iron in the precipitate using the color developed (520 nm) by the addition of sodium salicylate, ammonium hydroxide and acetic acid. An iron to phosphorus ratio of 3:6 was used to calculate the amount of phytate (Courtois et al., 1959).

Determining phosphorus in ferric phytate precipitate gives a more accurate measure of phytate than its iron content. This approach has been used by most investigators. Phosphorus is generally determined calorimetrically after ferric phytate is converted to orthophosphate by dry ashing or by wet ashing (Earley, 1944).

Earley and de Turk (1944) determined phytate in whole corn by extracting with 1.2% HCl containing 10% sodium sulfate and by precipitating phytate with 0.2% ferric chloride in 0.6% HCl (Earley, 1944). After dry ashing, phosphorus was determined phosphomolybdic acid is formed in hydrochloric acid, reduced to blue color with stannous chloride then measured in a photoelectric colorimeter.

(Oberleas, 1971) used the same extractant to isolate phytate as used by Early and de Turk (1944), but precipitated ferric phytate in 0.4% ferric chloride in 0.6% hydrochloric acid. Phosphorus was determined by wet ashing the precipitate in a mixture of sulfuric acid and 65% perchloric acid. Orthophosphate was determined by forming of phosphomolybdic acid followed by its reduction to a blue color using ascorbic acid. Color intensity was read at 650 nm.

In cereal grains and oilseed products, (de Boland et al., 1975) determined phyate by the method of Early and de Turk (1944), except that they oxidized phytate by wet ashing in a mixture of sulfuric and nitric acid. (Nahapetian & Bassiri, 1975) also oxidized ferric phytate from wheat by wet ashing in a mixture of sulfuric and nitric acid. They quantify phosphorus in ferric phytate using 0.75M sulfuric acid to convert phosphate to phosphomolybdic acid instead of acetate buffer (pH 4). The phosphomolybdic acid was reduced with ascorbic acid and the color intensity was measured at 660 nm.

Since then, numerous techniques have been developed to determine phytate content in cereals based on a wide variety of approaches. These techniques include ferric precipitation (Haug, 1983), colorimetric spectroscopy (Vaintraub & Lapteva, 1988), anion exchange (Harland & Oberleas, 1986; Oberleas & Harland, 2007), high performance liquid chromatography, inductively coupled plasma atomic emission spectroscopy (Plaami & Kumpulainen, 1991), and nuclear magnetic resonance (O'Neill, Sargent, & Trimble, 1980). In general, these techniques are time consuming, expensive, require use of harsh chemicals, and/or involve equipment not widely available in developing countries.

Determining phytate content by ferric precipitation involves extraction of phytate from food samples with hydrochloric acid and precipitation of the iron (III)-phytate complex on addition of ferric acid (Haug, 1983). Problems with the accuracy and precision of this method are evident as it relies upon a stoichiometric ratio of phosphorous to iron in the precipitate. This ratio is affected by different washing techniques (Thompson, Button, & Jenkins, 1987) and by the presence of different inositol forms (Harland & Oberleas, 1986). Colorimetric methods developed by (Vaintraub & Lapteva, 1988) are not widely used due to their qualitative rather than quantitative nature.

According to Oberleas and Harland (1977), phytate was extracted from samples with HCl and concentrated using anion exchange column (Oberleas & Harland, 1977). Then the phytate was wet digested in a sulfuric and nitric acid mixture and quantified by phosphorus determination using the colorimetric procedure. Theoretically, phytate is eluted from the column, separated from lower inositol phosphates and inorganic phosphates and the phosphorous released after acid hydrolysis of the eluate containing the phytate fraction is quantified. In this method phytate content is calculated from released phosphorus assuming phytate contains 28.2% phosphorus. This calculation is a problem when analyzing foods with a large proportion of inositol phosphates (IP_3, IP_4, IP_5). Lower inositol phosphates can potentially be included in the final determination of phytate, elevating the content above the actual content (Ferguson et al., 1993). However it accurately quantifies phosphorous present in higher inositol phosphates and phytate.

(Vaintraub & Lapteva, 1988) used ion exchange chromatography to determine phytate in hydrochloric acid (2.4%) extracts of various plant materials, including wheat flour. Phytate, which was separated on an anion exchange column, was quantified rapidly using Wade's reagent (0.03% ferric chloride and 0.3% sulfosalicylic acid in distilled water). The ferric ion in the Wade's reagent quantitatively precipitates phytate and any excess iron complexes with sulfosalicylic acid to give a pink color. The column effluent is mixed with Wade's reagent, precipitated ferric phytate is centrifuged down, and the color in the supernatant measured at 500 nm.

Determination of phytate by high performance liquid chromatography (HPLC) involves extraction of phytate from food using H_2SO_4 (Gifford & Clydesdale, 1990) or HCl and purification prior to HPLC (Graf & Dintzis, 1982). Phytate separation is achieved through anion-exchange (L. R. Stephens & Irvine, 1990), reverse phase chromatography (Graf & Dintzis, 1982), ion chromatography (Harland, Smith, Howard, Ellis, & Smith, 1988). Phytate detection is generally performed by on-line post column derivitisation to form a fluorescent complex (Phillippy, White, Johnston, Tao, & Fox, 1987; Simons et al., 1990).

Knuckles (1982) determined phytate in rice bran by high performance liquid chromatography (HPLC) using a reverse phase (C-18) column with a sodium acetate mobile phase (B. E. Knuckles, 1982). Gifford and Clydesdale (1990) quantified phytate in food after extraction with sulfuric acid, filtration, precipitation with ferric chloride, and re-dissolution with NaOH prior to detection by HPLC (Gifford & Clydesdale, 1990). Graf and Dintzis (1982) used anion exchange resin to purify food samples prior to detection of phytate by HPLC (Graf & Dintzis, 1982).

Further developments of HPLC have led to the ability to separate and quantify all inositol phosphates (Sandberg et al., 1999; Sandberg et al., 1996; L. R. Stephens & Irvine, 1990). The main challenge with HPLC methods is ability to control all external variables (i.e., sample purification, temperature and repeatability) that may influence determination of the actual phytate content (Xu, Price, & Aggett, 1992). Methods such as nuclear magnetic resonance, gas chromatography mass spectroscopy and capillary zone electrophoresis offer promise as future methods to provide accurate and precise results, however they are expensive and require specialized equipment (Xu et al., 1992).

Sensory Evaluation for Food Products

Texture, appearance and flavor are three major components of food acceptability. Sensory evaluation is measurement of product's quality based on information received from human senses and offers opportunity to obtain a complete analysis of the sensory properties of a food. Humans employ a range of senses in perceiving food quality such as: vision, gustitation, olfaction, touch, and hearing (Jaworska Danuta, 2008).

Different means of testing have been developed into numerous sensory testing procedures, fitting into hedonic or analytical test categories (Jean-Xavier Guinard, 1999). Hedonic tests include preference, acceptability, or relative-to-ideal. Participants in hedonic testing should not be given any training for evaluating the products, but large numbers of participants are needed to improve the confidence level of the data generated.

Analytical tests are further sub-divided into either difference or quantitative tests. Difference or discrimination tests are perceived as the easiest classes of sensory testing to apply in industrial environment and used to determine if there is a difference between two samples or to determine whether one sample has more or less of a specific attribute than the other (N.L. Stanley, 1993). Examples of difference tests include paired comparison, duo-trio, triangle, or R-index. Quantitative descriptive analysis is comprehensive system covering sample selection, screening, vocabulary development, testing, and data analysis (Szczesniak, 2002). QDA uses small numbers of highly trained panelists, typically 6-15 people. Three steps of analysis: development of vocabulary, quantification of sensory characteristics, and statistical analysis of the results lead to a strong and accountable means for sensory evaluation.

Correlating measurements of sensory and physical properties of texture are important in the development of food products. In 1992, the International Organization for Standardization defined texture as the mechanical (geometrical and surface) attributes of a food product perceptible by means of mechanical, tactile and, where appropriate, visual and auditory receptors. Sensory and instrumental measures of food texture can interrelate, and texture can be described from multiple stimuli, but instrumental measurements tend to concentrate on one property of the food product.

CHAPTER III

METHODOLOGY

The study was conducted in the outskirts of Wondogenet, Sidama zone, Southern Nation's Nationalities and Peoples Regional State and in Hawassa University, which is located in Awassa, Ethiopia. The goal of this research was to formulate low-phytate maize-bean based transitional food for children aged 12-36 months in southern Ethiopia. To achieve the study goal, socio-economic and food consumption data were gathered and analyzed; a transitional food was formulated and chemically analyzed; and sensory assessment and acceptability testing were performed.

The study was conducted in two parts. The first part of the study focused on collecting and analyzing data related to socio-economic and food consumption pattern of the target study population. The second part dealt with formulation, sensory assessment and acceptability testing of transitional foods for 12-36 month children. All protocols used in this study were reviewed and approved by the institutional review board of Oklahoma State University and Hawassa University (Appendices C-L). Subjects signed or gave oral consent prior to participation.

Study Setting

Major parts of the study activities were performed at three locations. Chemical and laboratory analysis of the study food were performed at Oklahoma State University, USA. Sensory assessment and socio-economic data gathering were conducted in Hawassa University and Wondogenet, Ethiopia. Hawassa University is located in Awassa town, regional capital city of South Nation Nationalities and People Regional State (SNNPR), Ethiopia. Wondogenet is a rural town, which is located in Awassa district, Sidama zone, SNNPR, Ethiopia. Wondogenet has a population of 6,069 (CSA, 2007). Participants in the study were village mothers with their youngest child (12-36 months age) selected with the assistance of community health workers from Wondogenet and female staff and students selected by flyer invitation from Hawassa University.

Study subjects

Study participants were village mothers (n = 42) with their youngest child of 12-36 months age selected from Wondogenet and female university staff and students (n = 36) in Hawassa University during 2008. Data related to socio economic status was gathered only from mothers who were from Wondogenet. However, all participants performed sensory assessment on low-phytate maize-bean based porridges, which were prepared through local food processing methods.

The criteria used for subject selection were being mothers living in Wondogent area, and have at least one child of age 12-36 months during the study period and female University staff and students of Awassa University during 2008. Mothers were recruited through community health workers. Staff and students were recruited by flyers invitation.

Purpose of the study

This study focused on developing lower phytate maize-based transitional food using local food processing methods and assessing its sensory quality. Three low phytate maize and bean-based porridges were used in sensory testing. Phytate analysis was conducted at Oklahoma State University. Sensory and acceptability tests were performed at Hawassa University and Wondogenet by female staff and students selected from the University and village mothers selected from the study area. The study involved methods of food formulation, sensory and acceptability tests and phytate analysis to achieve its objectives.

Samples selection and preparation

Maize was selected because it is the stable food source for people living in the study area. It was uniformly processed and supplemented proportionally with kidney beans and dried red meat to improve nutrient quality and quantity. All ingredients were prepared according to steps outlined in Fig 4.1.

Maize (white) and kidney beans (red) were purchased from the local market in Awassa. Fresh red meat was purchased from a local butcher, dried and pounded. Maize samples were cleaned and then divide into three groups for further processing. One of the three groups divided into two and soaked in water with 1 to 4 (w/v) ratios for 6 and 12 hours. The second group soaked separately in acidic solutions (1:10 v/v lemon juice and water) with 1 to 4 (w/v) ratios for 6 and 12 hours. The third portion soaked for 12 hours and then germinated for 48 hours.

Kidney beans were soaked, dried, lightly roasted, de-hulled, and milled into flour. All flour samples, similar to particle size of traditionally processed flours in the study area were packed and sealed in air tight polyethylene bags. Once samples with low phytate content were identified through phytate analysis, larger samples quantity (10 kg batch) were prepared at Hawassa University for further sensory and acceptability tests.

Cooking was done according to traditional procedures practiced in the area. Six types of porridges, each sample containing 20% legume flour (germinated maize with meat; germinated maize without meat, acid-soaked maize with meat; acid-soaked maize without meat, water-soaked maize with meat, and water-soaked maize without meat) were prepared by mixing the flour and pre-boiled tap water in a 1:10 (w/v) ratio and cooking for 15 minutes. Cooked porridges were portioned into 50 g servings and kept warm in plastic dishes until sensory evaluation. Each porridge sample was labeled with three codes.

The sensory and acceptability tests were conducted within one day by providing the six porridge samples with three porridges at a time at each site. Overall the sensory testing process took up to an hour in which each subject took 5 to 10 minutes to test every porridge samples.

Phytate analysis

Phytate was analyzed according to Harland and Oberleas (1986) and Haug (1983) methods with modification (Harland & Oberleas, 1986; Haug, 1983). Maize flour (1.0-1.2 g) and extracting solutions (10 ml of 0.66N HCl) were placed in 50 ml falcon tube stirred vigorously and gently shaken for 3 hours. The extract was removed from the matrix by pouring the supernatant into a centrifuge tube and centrifuged at 3500 rpm (1800 g) for 10 minutes.

Purification of phytate was performed by anion exchange column according to modified method described by (Oberleas & Harland, 1977). The extract was eluted through Glass barrels Econo-columns (0.7x15cm, Bio-Rad Laboratories). Columns were vertically clamped and filled with 2.5 g AGl-X8 anion-exchange resin to separate those inorganic phosphorus and other interfering compounds from myo-inositol phosphates.

Before use, columns were washed with 0.7N NaCl to assure chloride saturation of the resin and then with 30 ml of de-ionized water to make the eluate salt free. The sample extract was diluted before being placed on the column taking 0.5 ml extract making it into 25 ml with Milliq-water. One ml of the diluted extract transferred into the column and inorganic phosphate was eluted first with 15 ml 0.3 M NaCl and then, the phytate eluted with 15 ml 0.7 M NaCl. The sample container was rinsed and the column was washed with 15 ml of Milliq-water to elute most of the inorganic phosphate. The resin in the column was discarded and replaced after eluting three consecutive batches.

Sensory evaluation

The female staff and students panel were selected from Hawassa University and the mothers with 12-36 months young child were recruited from the study village. Participants were briefed about the study protocols and gave oral (mothers) or written (staff/ students) consent before participating. Participants were asked to taste three porridge samples at a time and show their preference based on color, texture, flavor, taste and overall acceptability. The sensory tests involved nine points hedonic scale scores ranging from 1 to 9 where 9 indicate like extremely, five as neutral, and one as dislike extremely. Thus, the higher rating on the scale indicated the respondents' higher liking towards particular porridge sample.

All participants were also asked to evaluate the texture of all porridge samples using 14 textural characteristics and indicate their preference based on "ideal" porridge for child with 12-36 month old age. Six point scale presented by "not at all so" and "very much so" where "not at all so" indicating absence of required characteristics and "very much so" indicating presence of very high degree were used to assess the textural characteristics of each porridge sample.

Twelve to thirty six months old children (42) were fed with porridge from each samples and mother's were asked about their child's reaction towards each porridge using four point hedonic scales (great, pretty good, somewhat good, and didn't like) and the amount of porridge children consumed was assessed using a four point scale (none, less than half, more than half and all). Participants performed the sensory and acceptability tests in one day, in the afternoon, at a similar site setting and with similar equipment. All participants were seated in a 2x2 booth created in a room under normal light while tasting six samples arranged in two groups composed of three samples per group. Water was given and participants were instructed to sip and rinse their mouth before and after assessing samples.

Socioeconomic and infant feeding practice

Data related to socio-economic and demographic characteristics, food production and consumption pattern, and child feeding practices were gathered using structured questionnaires (Appendices D and E). Each question focused on variables related to socioeconomic status, food production, child feeding practice, breast feeding, and child morbidity. All data were collected by trained interviewers and bilingual translators.

Statistical analysis

All collected data were entered into Excel sheet (Microsoft Office Excel 2007) and analyzed by SAS software (Statistical Analysis System, Version 9.1, Cary, NC). Results of phytate analysis were reported using means and standard error (SE). Descriptive statistics (frequencies, means, and percentages) were calculated for socio-economic characteristics and feeding practices. Sensory data were analyzed using analysis of variance (ANOVA) to determine variation attributable to differences in sample, addition of meat, or subjects.

Texture scores were averaged and plotted against ideal scores assumed by participants. Analysis of variance was used to determine textural variation attributable to sample, subjects, replication and interactions. Scores of children degree of liking towards each porridge sample were analyzed using ANOVA based on the amount of porridge the children ate and based on the assessment made by their mother. Multiple comparison of various means were tested for least significant difference using Tukey's studentized test and difference at $p < 0.05$ considered as significant.

CHAPTER IV

EFFECT OF SOAKING AND GERMINATION ON PHYTATE REDUCTION IN MAIZE/BEAN-BASED COMPLEMENTARY FOOD FOR USE IN SIDAMA ZONE, SOUTHERN ETHIOPIA

Abstract

The potential for phytate reduction by soaking and germination of maize was investigated. The purpose of this study was to develop low-phytate maize-based complementary food by reducing phytate and lowering phy:Zn molar ratio below 15 thresholds. A total of 28 samples in triplicate were tested for phytate and phy:Zn molar ratio. Mean scores were analyzed with analysis of variance (ANOVA) and tested for least significant difference using Tukey's studentized test at $p < 0.05$. Mean ($\pm$SE) phytate and zinc found in untreated white maize were 625.2$\pm$141.1 and 2.0+0.1 mg/100g. Phy:Zn molar ratio in untreated white maize was 30.8$\pm$7.3. Phytate decreased (94%) in pounded and water soaked white maize (12h). Whereas, 37% phytate decreased in maize soaked (12 h) and germinated (24 h) and 23% in maize soaked in lemon-water for (12 h). Mean ($\pm$SE) phytate was 40.2$\pm$10.1 mg/100g and phy:Zn molar ratio was 2.36$\pm$0.51 respectively. This indicates that soaking pounded white maize in water for 12 h reduce more phytate and Phy:Zn molar ratio to acceptable level. In conclusion, preparing foods using pounded white maize soaked in water (12 h) can be potential to reduce phytate in maize-based complementary food.

Introduction

Zinc deficiency affects millions of children in developing countries and recognition of this problem is increasing (Sandstead, 1991). Children are predominately consuming plant-based foods like cereals and legumes, which are rich in phytate that reduces bioavailability of dietary zinc and causes deficiency (Gibson, 1994). Zinc deficiency leads to poor child growth, impaired immunity and increased mortality (IZiNCG, 2004).

Phytate, myo-inositol hexaphosphate (IP_6), is known chelating agent that reduces the bioavailability of divalent cations (Weaver, Heaney, Martin, & Fitzsimmons, 1991). Phytate exerts an inhibitory effect on zinc absorption by forming insoluble complexes in the gut (Wise, 1995). Formation of such complexes depends on the ratio of phytate relative to that of zinc in the foods (Morris & Ellis, 1989).

Several authors propose that the bioavailability of zinc should be estimated by the relative molar ratios of phytate to zinc in the diet (Morris & Ellis, 1989; Zhou et al., 1992) and ratios greater than 15 have been negatively associated with growth in children (Ferguson, Gibson, Thompson, & Ounpuu, 1989).

In Southern Ethiopia, complementary foods for children are mainly cereal-based and children consume few animal source foods. High consumption of such diets for long periods increases the risk of zinc deficiency. High incidence of zinc deficiency has been considered as a potential problem for young children in Southern and other parts of the country (Melaku Umeta, 2000). There are limited data available indicating the amount of phytate present in maize-based complementary foods in southern Ethiopia, and the potential of local food processing techniques to reduce phytate from maize-based foods has not been tested. Lowering phytate/zinc molar ratio below a threshold that creates a risk for zinc deficiency is a practical way to improve zinc bioavailability. This can be achieved by reducing phytate using different home-based food processing techniques. The objectives of this study was to evaluate different home-based food processing techniques, to determine phytate content and phytate to zinc molar ratio in maize bean-based complementary foods.

Materials and Methods

Sample collection and preparation

Unrefined white dried maize (*Zea may*), red kidney beans (*Phaseolus vulgaris*) and fresh beef were purchased from a local market in Awassa, Southern Ethiopia, and processed using home-based food preparation methods. Preparation steps are outlined in Figure 1. Whole maize was soaked in water and germinated in the dark. Pounded maize was soaked in water or lemon-water solution (pH 2.63). All samples were sun dried and milled into flour.

Kidney beans were soaked overnight, sundried, roasted, and de-hulled. Fresh red meat was stripped thinly and oven dried overnight at 105^{o}C. Kidney bean samples were milled into powder at a local mill with particle size similar to traditionally processed flours in the study area. Dried meat was ground into powder with a clean coffee grinder. Maize flour was proportionally mixed with kidney bean flour (4:1w/w) and dried meat powder (10: 1) and kept sealed in air tight polyethylene bags until used.

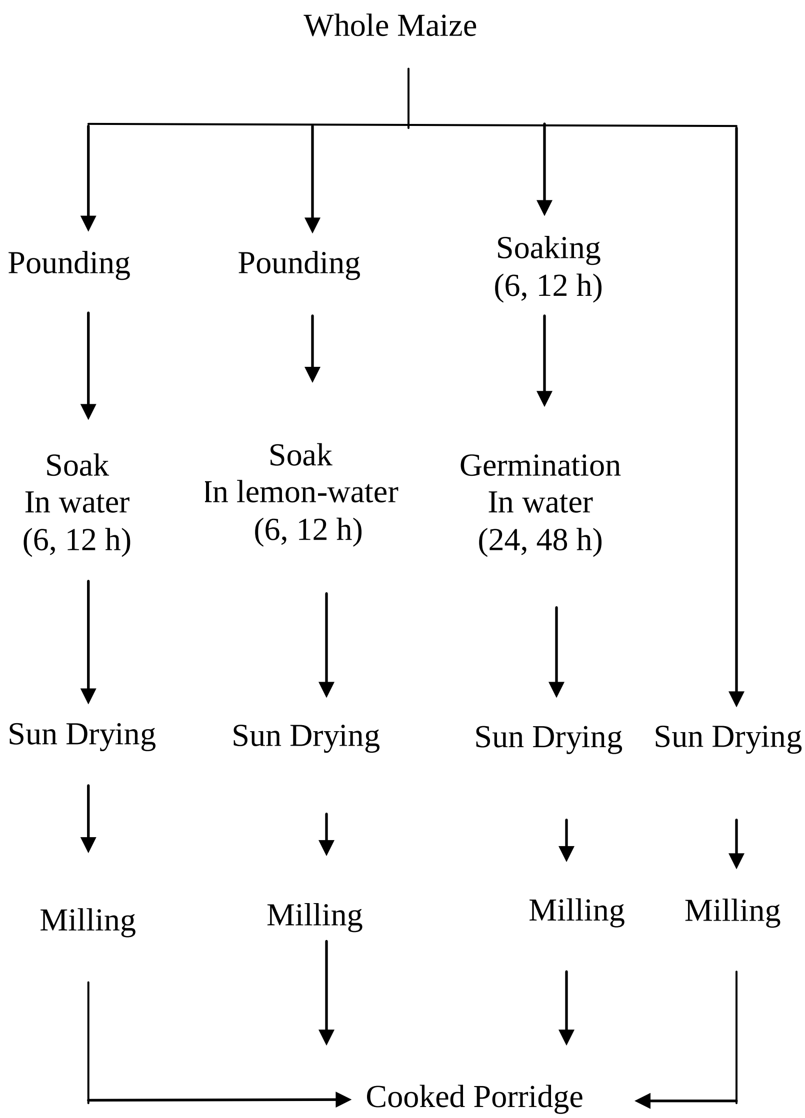

Figure 4.1 Scheme of various treatments applied to maize

Chemicals and reagents for phytate analysis

All reagents used for phytate analysis were analytical grade and were prepared with distilled water. HCl, NaCl, and ferric ammonium sulfate ($FeNH_4(SO_4)_2.12H_2O$ which were purchased from Fisher Scientific (Fair Lawn, NJ), while 2, 2'-bipyridine was obtained from J. T. Baker (Phillipsburg, NJ), and thioglycollic acid and sodium phytate were purchased from Sigma-Aldrich (St. Louis, MO). AG1-8 Resin, 100-200 mesh was obtained from Bio-Rad Laboratories (Hercules, CA).

Preparation of standard and reagents

Sodium phytate of 97% purity, containing 15% water, obtained from Sigma (LOT 063H0083) was used without further purification. A series of standards were prepared by diluting the stock solution, which contained 0.15 g sodium phytate in 100 ml distilled water, and then diluted with 0.66 N HCl in a range from 0 to 150 µg ml^{-1} phytate. The standard was analyzed by the Haug (1983) procedure. Ferric solution was prepared by dissolving 0.2 g ammonium iron (III) sulphate•12 H_2O in 100 ml 2 N HCl and making to 1000 ml with distilled water. The color reagent was prepared by dissolving 10 g 2,2'-bipyridine and 10 ml thioglycollic acid in deionized water and making to 1000 ml. Amount of phytate was calculated from organic phosphorous assuming that one molecule of phytate contained six molecules of phosphorous (28.2%) and the phosphorous measured was exclusively released from phytate.

Extraction of phytate

Phytate was extracted according to the method of (Harland & Oberleas, 1977) and Haug (1983) with modification (Figure 2). For extraction, 0.66 N HCl was used. The 0.66 N HCl was added to 1 g sample to make 10 ml in a 50 ml plastic centrifuge tubes, mixed vigorously and extracted for 3 h at room temperature by gentle shaking on an orbital mixer. The extract was removed from the matrix by gently pouring the supernatant into a centrifuge tube and centrifuging at 3500 rpm (1800xg) for 10 min. The extract was diluted (1:25) in deionized water before loading it on the column

Anion-Exchange Purification

Phytate was purified according to a modification of the (Harland & Oberleas, 1986) method. Glass anion-exchange columns (0.7x15 cm, Bio-Rad Laboratories) were filled with slurry of 2.5 g AGl-X8 anion-exchange resin in deionized water and used to separate inorganic phosphorus and other interfering compounds from myo-inositol phosphates. All columns were washed with 15 ml 0.7 M NaCl to saturate the resin with chloride and then rinsed with 30 ml deionized water. One milliliter of diluted extract was quantitatively transferred to the column and inorganic phosphate was eluted first with 15 ml 0.3 M NaCl and then the phytate was eluted with 15 ml 0.7 M NaCl. The resin in the column was discarded and replaced after eluting three consecutive samples.

Phytate Determination

Quantitative determination of phytate was done according to the procedure described by Haug (1983). One half milliliter of the 0.7 M NaCl eluant and 1 ml of ferric solution were pipetted into a glass centrifuge tube. The tubes were firmly sealed with parafilm and placed in a heating block for 30 min at 95^{o}C. After cooling in ice for 15 min and adjusting to room temperature, the solution was mixed and centrifuged for 30 min at 3000xg (Jouan, Winchester, Virginia). Then 1 ml of supernatant was transferred to another test tube; 1.5 ml of color reagent was added and the absorbance was measured using DU-800 UV/Visible Spectrophotometer (Beckman Coulter, CA) at 523 nm against a blank of distilled water. A standard calibration curve was prepared using the same procedure with standard solutions of known concentration of sodium phytate. Using the standard curve, concentration of phytate in the sample was calculated. Pink color is formed when Fe^{3+} reacts with 2, 2'-bipyridine, but iron binds quantitatively to phosphate ester in phytate and is unavailable to react with 2,2'-bipyridine, resulting in a decreased intensity of pink color in the presence of higher phytate concentrations.

Zinc determination

Zinc content was determined, after ashing samples according to the methods of Hill and colleagues (1986) using the inductively coupled plasma (ICP) mass spectrometer (ElmerSCIEX, USA). Maize flour samples (1-2g) were dried at 110^{o}C overnight (12 h) and wet ashed with deionized H_2O, 30% H_2O_2, and HNO_3 at 105^{o}C. The samples were further dry ashed at 375 ^{o}C for 12 h in Lindberg ashing oven. More nitric acid (50-100 µl) and hydrogen peroxide (50-100 µl) were used to continue the wet ashing. The ash was dissolved in 5 ml 0.1% HNO_3 and 100 µl of this solution was further diluted into 10 ml using 0.1% HNO_3. Finally, zinc concentration was determined by ICP- mass spectrophotometer using gallium as internal standard.

Statistical Analysis

Each sample was analyzed in triplicate and values expressed as means $\pm$ standard error. *P* value less than 0.05 was considered significant. Analysis of variance (ANOVA) was computed to assess the effects of processing methods on reducing phytate content in tested food samples. Whenever, the *F* value was significant, Tukey's studentized range (HSD) test was performed to identify significant difference between means.

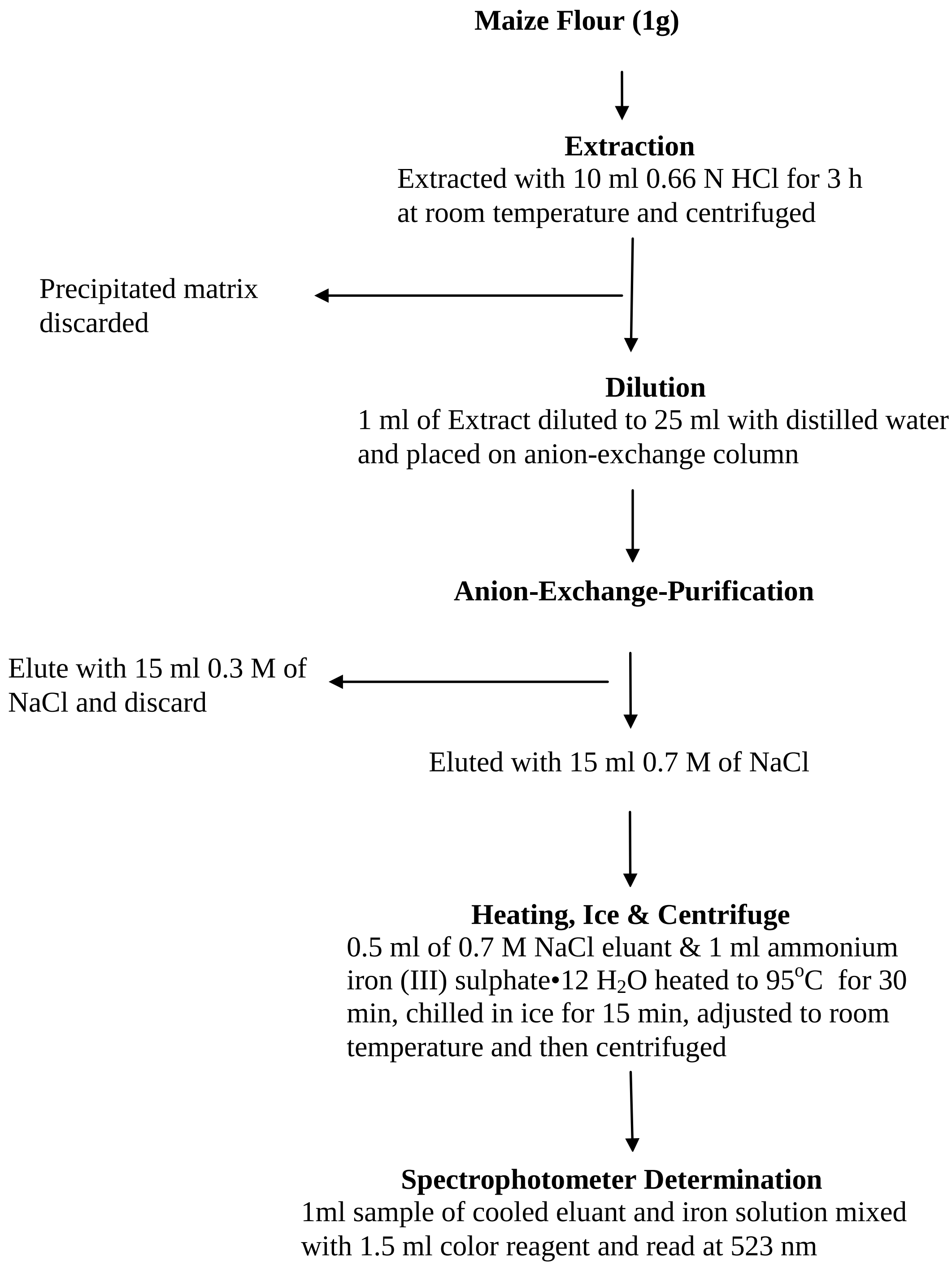

Figure 4.2 Scheme of sample extraction and phytate determination

Results and Discussion

Total phytate content and phytate to zinc molar ratio

A total of twenty eight samples were prepared in triplicate by using methods like soaking in water for 6 h or 12 h, soaking for 6 h or 12 h and then germinating for 24 h or 48 h, and soaking in lemon-water solution (1:10) for 6 h or 12 h. Pronounced phytate reduction was found in pounded maize soaked in water for 12 h, in which phytate content decreased by 94% compared with the untreated control. For other groups, the phytate content decreased by 23% in pounded maize soaked in lemon-water solution for 12 h and by 37% in whole maize soaked (12 h) and germinated (24 h) compared with the control.

Table 4.1 Mean (±SE) phytate and zinc content and Phy/ Zn molar ratio of treated maize and control maize

Flour mix (n=3)	Phytate (mg/100g)	Zinc (mg/100g)	Phy / Zn Molar Ratio
Maize			
Untreated (Control)	625.2±47.8^{a}	2.0 ± 0.1^{a}	30.8±3.1^{ad}
Soaked in Water			
PWS (6h)	145.8±24.1^{bc1}	1.8± 0.1^{bc1}	7.9 ±1.2^{bd1}
PWS (12h)	40.2±10.1^{bc2}	1.7±0.2^{b1}	2.4± 0.5^{bd2}
Soaked in Lemon-water			
LWS (6h)	555.6±73.5^{ad1}	0. 9±0.0^{bd1}	62.9±4.9^{bc1}
LWS (12h)	479.2±77.6^{ad1}	1.3±0.2^{bc1}	38.2±11.1^{ad2}
Soaked & Germinated Maize			
S6 - G24	538.3±74.4^{ad1}	2.2±0.2^{bc1}	23.9±3.0^{ad1}
S6 - G48	535.8±74.4^{ad1}	2.6±0.1^{bc1}	20.1±8.5^{ad1}
S12 - G24	392.0±74.4^{ad1}	2.7±0.8^{bc1}	14.8±4.1^{bd1}
S12 - G48	460.2±74.4^{ad1}	2.6±0.2^{bc1}	18.0±4.7^{ad1}

Mean±SE in the same column that do not share a superscript are significantly different. Between group difference (a,b,c,d) and with in the group difference (1,2); Abbreviation: Untreated Maize Flour (Control); PWS: Pounded & Water Soaked Maize for 6 and 12 h; LWS: Maize Soaked in Lemon-water for 6 and 12 h; WSGM: Whole Maize Soaked in Water for 12 h & Germinated for 24 h.

Effects of soaking maize in water on phytate content

Soaking pounded maize for 6 and 12 h led to 145.8±24.1 mg/100g and 40.2±10.1 mg/100g which represented 77% and 94% total phytate reduction respectively. Phytate reduction achieved by soaking maize in water for 6 h or 12 h was significantly lower than the control (Table 4.1). Such high reduction may be because pounding and soaking may facilitate phytate leaching in maize powder. Phytate to zinc molar ratio was reduced by 74% (7.93 ± 1.2) and 92% (2.36± 0.5) in maize soaked in water for 6 h or 12 h.

Effects of maize soaking in lemon-water solution on phytate content

Soaking pounded maize in lemon-water solution for 6h reduced phytate by 11% (555.6±73.5 mg/100g), whereas 23% (479.2±77.6 mg/100g) reduction was achieved by 12 h soaking. Phytate reduction using 6 h or 12 h soaking in lemon-water were not significantly different from the control. However, lower concentration of zinc (0.89± 0.0 mg/100g, 1.28± 0.2 mg/100g) was observed in the lemon-water soaked maize samples compared with control (2.01 ± 0.1mg/100g).

Phytate to zinc molar ratio of maize soaked in lemon-water solution for 6 h was twice (62.9±4.9) as high as the control (30.8±7.3), which are significantly different. Phytate to zinc molar ratio of maize soaked in water-lemon solution for 12 h (38.16± 11.1) was not significantly different from the control. Soaking maize in lemon-water solution seems to hinder overall reduction of phytate as compared with the water soaking alone. This might due to the low pH of the lemon-water soaking solution (pH 2.63) that facilitated the release of zinc into the soaking water where it was discarded. Another possibility is that the acidic content of the sample may have affected the column purification capacity to separate free phosphorous groups.

Effects of maize germination on phytate

Four groups of maize samples were soaked in water (1:4 w/v) for 6 or 12 h and then germinated for 24 h or 48 h. All methods involving soaking and germination of whole maize did not significantly reduced phytate. Soaking whole maize in water for 12 h and germinated for 24 h reduced phytate by 37% (392.0 ±74.4 mg/100g), while soaking whole maize in water for 12 h and germinated for 48 h reduced phytate by 26% (460.2±74.4 mg/100g). Soaking whole maize in water for 6 h and germinated for 24 or 48 h reduced phytate by 14%.

Effects of maize germination on zinc

The concentrations of zinc found in all germinated whole maize samples were not significant compared with the control.

Effects of soaking kidney beans on phytate content

Enriching maize-based porridges with locally available food ingredients such as legumes is another household strategy to increase nutrient content of complementary foods. The major reason for combining legume with maize was to improve required nutrients especially limiting amino acids. However, adding legumes to treated-maize could have the potential to increase phytate content leading to impaired dietary zinc bioavailability. Therefore, phytate content of kidney beans was also investigated.

Table 4.2 Mean (±SE) phytate, zinc content and Phy/ Zn molar ratio of kidney beans

Flour mix (n=3)	Phytate (mg/100g)	Zinc (mg/100g)	Phy / Zn Molar Ratio
(A) Kidney Beans (White)			
Untreated	712.7± 83.1	2.95± 0.1	21.6 ±2.6
De-hulled	706.1± 83.1	3.11± 0.8	21.4±5.8
Soaked, roasted, de-hulled	581.9± 81.0	4.18± 0.3	14.7± 2.3
(B) Kidney Beans (Red)			
Untreated	715.8± 83.1	2.83± 0.9	27.25 ±7.1
De-hulled	598.5± 83.1	3.81± 0.1	15.17±3.8
Soaked, roasted, de-hulled	400.4± 83.1	4.20± 0.6	10.17±5.0

White or red kidney beans soaked in water for 12 h, sun dried, roasted and de-hulled

Summary and Conclusion

Results of this study indicate that soaking pre-pounded white maize in water for 12 hours could be a viable method to reduce phytate and reduce phytate to zinc molar ratio below 15. Moreover, results observed in this study were consistent with other findings that are reported by Hotz and Gibson, in which they extracted phytate from unrefined white maize through pounding and soaking and were able to reduce phytate content up to 49% (Hotz & Gibson, 2001).

Acknowledgments

The authors wish to thank Dr. Donald Oberleas and Afework Mulugeta for their unlimited advice and technical assistance with the determination of phytate and zinc concentrations. This work is part of a thesis supported by NIH Grant R21 TW06729 (Fogarty International Center & the Office of Dietary Supplements) and Hawassa University.

References

Ferguson, E. L., Gibson, R. S., Thompson, L. U., & Ounpuu, S. (1989). Dietary calcium, phytate, and zinc intakes and the calcium, phytate, and zinc molar ratios of the diets of a selected group of East African children. *Am J Clin Nutr, 50*(6), 1450-1456.

Gibson, R. S. (1994). Content and bioavailability of trace elements in vegetarian diets. *Am J Clin Nutr, 59*(5 Suppl), 1223S-1232S.

Harland, B. F., & Oberleas, D. (1977). A modified method for phytate analysis using an ion exchange procedure: application to textured vegetable proteins, *Cereal chemistry* (pp. 827-832).

Harland, B. F., & Oberleas, D. (1986). Anion-exchange method for determination of phytate in foods: collaborative study. *J Assoc Off Anal Chem, 69*(4), 667-670.

Hotz, C., & Gibson, R. S. (2001). Assessment of home-based processing methods to reduce the phytate content and phytate/zinc molar ratio of white maize (Zea mays). *J Agric Food Chem, 49*(2), 692-698.

IZiNCG. (2004). Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull., 25*, 109.

Melaku Umeta, C. E. W., Hans Verhoef, Jemal Haidar, Joseph G.A.J. Hautvast. (2000). Zinc supplementation and stunted infants in Ethiopia: a randomised controlled trial. *The Lancet, Volume 355* (9220), 6

Morris, E. R., & Ellis, R. (1989). Usefulness of the dietary phytic acid/zinc molar ratio as an index of zinc bioavailability to rats and humans. *Biol Trace Elem Res, 19*(1-2), 107-117.

Sandstead, H. H. (1991). Zinc deficiency. A public health problem? *Am J Dis Child, 145*(8), 853-859.

Weaver, C. M., Heaney, R. P., Martin, B. R., & Fitzsimmons, M. L. (1991). Human calcium absorption from whole-wheat products. *J Nutr, 121*(11), 1769-1775.

Wise, A. (1995). Phytate and zinc bioavailability. *Int J Food Sci Nutr, 46*(1), 53-63.

Zhou, J. R., Fordyce, E. J., Raboy, V., Dickinson, D. B., Wong, M. S., Burns, R. A., et al. (1992). Reduction of phytic acid in soybean products improves zinc bioavailability in rats. *J Nutr, 122*(12), 2466-2473.

CHAPTER V

EFFECT OF HOME-BASED PHYTATE REDUCTION ON SENSORY ATTRIBUTE OF MAIZE/BEAN-BASED COMPLEMENTARY FOOD IN SIDAMA ZONE, SOUTHERN ETHIOPIA

Abstract

Complementary foods in Ethiopia are mainly prepared from cereals especially maize which has phytate that hindering zinc bioavailability. Sensory quality of 3 phytate-reduced maize-based complementary foods was assessed by 36 mothers and 42 female staff and students selected from a village and Hawassa University in southern Ethiopia. Panelists liked color (6.2±0.2) and flavor (5.6±0.2) of porridge processed from pounded and water soaked (12h) white maize more significantly ($p < 0.05$) than color (5.4±0.2), and flavor (4.9±0.2) of porridges prepared from germinated maize or color (5.8±0.2) and flavor (5.2±0.2) of porridge prepared from pounded maize soaked in lemon-water (12 h). Panelist ranked porridges prepared from water-soaked maize as first (55.6%); lemon-water soaked maize (34.6%) as second and porridge from germinated maize as third (17.3%). However, university panels gave higher sensory scores to porridges prepared from water-soaked maize more than village mothers. In conclusion, this study indicate that preparing foods using flour processed from pounded and water soaked (12h) white maize can be a potential maize-based complementary food for young children in the study area.

Introduction

Zinc deficiency is one of the micronutrient deficiencies prevalent among young children in developing countries (Bohn, Meyer, & Rasmussen, 2008; Egli, Davidsson, Zeder, Walczyk, & Hurrell, 2004; Ferguson, Gibson, Thompson, & Ounpuu, 1989). Deficiency of zinc is known to impair several biological functions in the human body, such as gene expression, protein synthesis, skeletal growth and maturation, dark adaptation, taste perception and appetite, gonad development and pregnancy outcomes, skin integrity and immunity, and to delay cognitive development and increase the risk of morbidity and mortality. It may arise due to impaired bioavailability of zinc that is largely attributable to high phytate content present in cereal-based diets that are commonly consumed in developing countries (Gibson, 1994; Sandstrom, 1987; Wise, 1995).

Phytate is a strong chelator that has binds zinc and other trace elements present in foods and hinder their absorption (Ferguson et al., 1989). Phytate can be found in the bran or germ of cereal and in legumes in relatively high amounts. In maize about 88% of phytate is found in the germ and endosperm. Current studies on phytate suggest that daily intakes of phytate/Zn with molar ratio greater than 15 are associated with increased risk of suboptimal zinc status (Gibson, Donovan, & Heath, 1997; Morris & Ellis, 1989). Thus reducing phytate from complementary foods and keeping phytate/zinc molar ratio < 15 is essential to improve zinc bioavailability (Hambidge, Krebs, Westcott, & Miller, 2006; Hotz & Gibson, 2001; Mazariegos et al., 2006).

Phytate can be reduced by using food processing methods like large scale novel precipitation in food processing, activating endogenous phytase during food preparation (baking, fermentation, germination, malting and hydrothermal processing), or addition of exogenous phytase (Lonnerdal, 2003). There are many home-based food processing methods that are used in Ethiopia. However, there is no evidence indicating how much these methods may reduce phytate content from the cereal-based complementary foods or how use of these methods may change the sensory properties of the foods.

Texture, appearance and flavor are the three major components mostly considered to determine food acceptability. Sensory evaluation is the measurement of a food's quality based on information received from human senses, and it offers the opportunity to obtain a complete analysis of the sensory properties of a food. Humans employ a range of senses in perceiving food quality such as: vision, gustation, olfaction, touch, and hearing (Jean-Xavier Guinard, 1999).

Different means of testing have been developed fitting into hedonic or analytical test categories (Szczesniak, 2002). Hedonic tests include preference, acceptability, or relative-to-ideal. Participants in hedonic testing should not be given any training for evaluating the products, but large numbers of participants are needed to improve the confidence level of the data generated. The objective of this study was to assess sensory qualities and acceptability of porridges samples made with maize/kidney bean blends. Both sensory and acceptability tests were conducted by panels from the study area.

Materials and Methods

This study was reviewed and approved by the Institutional Review Board for Protection of Human Subjects, Oklahoma State University, Stillwater, OK and Ethics Committee of Hawassa University, Awassa, Ethiopia. Sensory panels involved in this study gave signed (staff and students) or oral (mothers) consent before participation. White maize, red kidney beans and fresh beef were purchased from a local market in Awassa and prepared using different home-based food processing methods as described below. The processing methods used for samples were chosen based on our results that indicated that they removed more phytate than other methods tested and on the literature (Hambidge et al., 2006; Hotz & Gibson, 2001; Mazariegos et al., 2006).

Sample Preparation

The maize samples tested in this sensory analysis were: pounded maize soaked in water (1:10w/v) for 12 hours, pounded maize (1:10w/v) soaked in water and lemon juice (90:10v/v) for 12 hours, and whole maize germinated for 24 hours after soaking in water (1:10w/v) for 12 hours. Maize samples uniformly and proportionally supplemented with kidney beans (80:20w/w) before milling. The maize-kidney bean flour was mixed with dried red meat powder (1:10 w/w) during porridge preparation.

Porridge was prepared by cooking slurries of flour in water to a suitable consistency for children 12-36 months of age. Cooking was done as such porridges are traditionally prepared in the area. Porridge samples, three of them with dried meat and the other three without dried meat (germinated maize with and without meat, lemon soaked maize with and without meat, and water soaked maize with and without meat), were prepared by mixing each type with pre-boiled tap water in a 1:10 (w/v) ratio and cooking for 15 minutes with frequent stirring. Cooked porridges were portioned into 50g servings in plastic dishes and kept warm until evaluation. Samples were labeled with randomly generated codes.

Study setting

Sensory testing of food samples was conducted at Hawassa University and in Wondogenet town, both located in southern Ethiopia. Hawassa University is located in Awassa town, the regional capital city of South Nation Nationalities and People Regional State (SNNPR), Ethiopia. Wondogenet is a rural town, which is also located in Awassa district, Sidama zone, SNNPR, Ethiopia. Wondogenet has a population of 6,069 (CSA, 2007). Socio-economic and demographic data were collected in Wondogenet.

Study subjects

Study participants were village mothers (n = 42) with their youngest child of 12-36 months age from Wondogenet and female university staff and students (n = 36) at Hawassa University during 2008. Inclusion criteria were being mother living in Wondogent area, and having at least one child 12-36 months of age during study period or being female staff or students of Hawassa University. Mothers were recruited through local community health workers while staff and students were recruited by flyers. Data related to socio-economic status were gathered only from mothers from the Wondogenet area. However, all participants performed sensory assessment on maize-based foods.

Socio-economic and infant feeding data collection

Data related to socio-economic, child feeding, food production and consumption patterns, and family feeding practices were gathered from mothers using structured questionnaires. All data were collected by trained interviewers and bilingual translators.

Sensory and texture data collection

Maize-based porridges developed from home-based phytate reduction method were provided to participants. They were asked to taste them and indicate their preference based on color, texture, flavor (aroma), taste and overall acceptability of the porridge. Sensory assessment scores on nine point hedonic scales ranged from 1 to 9 where nine indicated “like extremely”, five was “neutral”, and one was “dislike extremely”. High ratings on the scale indicated respondents’ preference for that particular porridge sample.

All participants were also asked to evaluate the texture profile of each porridge sample using 14 textural characteristics and indicate their “ideal” porridge for a child 12-36 months of age. A six-point scale including “not at all so” to “very much so” where “not at all so” indicated absence of a given characteristic and “very much so” indicated presence to a high degree, was used to determine their texture preference. Finally, 12-36 months old children (42), who came with their mothers, were fed porridge from each sample and their mother was asked to evaluate their baby’s acceptance of each porridge using four point hedonic scales (great, pretty good, somewhat good, and didn’t like) and asked to rate the amount of porridge the child ate using a four point scale (none, less than half, more than half and all). Because many mothers cannot read and write, the mothers gave their evaluation orally, and the scales were filled out by the interviewers.

Statistical analysis

All data were entered into Excel (Microsoft Office Excel 2007) and analyzed by SAS software (Statistical Analysis System, Version 9.1, Cary, NC) for all quality attributes using analysis of variance and data for different variables are reported as mean

±SE. Descriptive statistics (frequencies, means, and percentages) were calculated for socioeconomic characteristics and feeding practices. All sensory data attributable to difference in home-based processing method and addition of meat were analyzed using two-way analysis of variance (ANOVA, Proc GLM).

Texture scores were also averaged and plotted against ideal scores assumed by participants. Analysis of variance was used to determine textural variation attributable to maize-processing, meat, and interactions. Children's scores for degree of liking towards each porridge sample were analyzed using ANOVA, Proc GLM based on the amount of porridge the children ate and based on the assessment made by their mother. Multiple comparison of various means were tested for least significant difference using Tukey's studentized test and $p < 0.05$ was considered as significant.

Result and discussion

Socio-economic and infant feeding practice

The average ages of mothers and their children involved in this study were 26.6±6.6 (18- 40) and 2.2±0.7 (1-3) years old respectively. The average family size was 5.6±2.2 (3-12). An average household had 3.7±2.2 (1-10) children and/or other dependents. Subjects' illiteracy rate was found to be high with 87.2% of mothers and 76.3% of fathers not able to read/ write or having only attended school up to 1-6 grades. Most families (79.5%) owned their house; with 53.9% of the houses being huts with grass roofs, and 67.6% of the huts did not have windows.

Agriculture was reported as the main source of income for the family. The major income of the family was coming from subsistence farm activities (76.9%) while petty trading (12.8%) and non-farming employment (2.6%) provided income for few participants. Most respondents reported earning less than enough family income.

Family food production and utilization patterns are given in Appendix A. Overall, 69.4% of the households owned farmland or gardens of size ranging from less than a quarter hectare to one hectare. The crops produced were maize (67.6%) followed by green leafy vegetables (63.9%), yellow-red vegetables (41.2%), and potatoes (34.3%). The households were also growing tree fruits (61.1%), Enset (*Enset ventrecosum*) (51.3%) and cash crops like coffee trees (71.1%) and chat (30.6%). Production and consumption of meat, milk and eggs were minimal, although 75.7% of them reported owning livestock. Respondents (64.1%) raised 1 to 5 cows for milk, 28.2% raised 1 to 8 chickens for eggs, and (33.3%) raised 1-2 oxen. Many of them utilized their animal and crop produce for home consumption while a few sell their crops to earn income.

The meal pattern characteristics of the family indicated that number of meals served per day ranged from one (2.6%) to more than three (7.7%). But, most mothers indicated three meals served per day (66.7%) followed by others who reported two meals (23.1%). Regardless of the fact that mothers reported the family produced its own foods and that 63.3% of them could store food up to 5-12 months after harvest, 79.5% of them indicated that they purchased the family foods during the time of the survey (July, 2008).

Child feeding characteristics are given in Appendix B. All mothers indicated the need for breast milk as the first food for newborn babies and almost all of them breast fed their babies at some point for some time. The duration of breastfeeding extended from 1-6 months (6.3%) up to 3-5 years (21.9%). But the majority of the mother breastfed up to 36 months (46.9%) more than three times per day (92%). Few mothers (25%) reported giving additional liquids other than breast milk for their baby in the first 3 days after delivery. All mothers introduced their babies to weaning foods gradually (94.7%) usually with gruel or soft porridge made of maize (95%) and 94.6% of mothers prepared the porridge and 81% fed their children complementary foods.

Sensory and texture analysis

Color and flavor were the two sensory attributes found significantly different in those porridges prepared from water-soaked pounded maize than from germinated maize (Table 5.1). Panelists liked the color (6.2±0.2) of the porridge that prepared from water-soaked maize significantly more than color (5.4±0.2) of porridge prepared from germinated maize. They also liked the flavor of porridge prepared from water-soaked maize (5.6±0.2) significantly more than the flavor of porridges prepared from germinated maize (4.9±0.2) (Table 5.1).

Addition of dried meat powder into porridge that was used for complementary feeding was not popular in the area. However the panelists liked all of the sensory quality of porridges prepared from maize and dried meat powder (Table 5.1). Panelists preferred porridge prepared with meat to that prepared without meat (Table 5.1).

Table 5.1 Mean (±SE) effect of maize treatment and meat addition on sensory attributes

Factor	Sensory quality attributes				
	Color	Flavor	Smell	Texture	Acceptability
Maize treatment					
PWS	6.2±0.2[a]	5.6±0.2[a]	5.2±0.2	5.3±0.2	5.4±0.2
SG	5.4±0.2[b]	4.9±0.2[b]	4.6±0.2	5.0±0.2	5.1±0.2
PLWS	5.8±0.2[ab]	5.2±0.2[ab]	5.0±0.2	5.4±0.2	5.3±0.2
Meat					
Adding meat	6.1±0.1[a]	5.7±0.1[a]	5.6±1.5[a]	5.5±0.2[a]	5.7±0.2[a]
No meat	5.5±0.1[b]	4.8±0.1[b]	4.2±1.5[b]	5.0±0.2[b]	4.9±0.2[b]
Panel					
Village mothers	5.5±0.1	5.1±0.1	4.8±0.2	5.1±0.2[b]	5.2±0.2
Staff & Students	6.1±0.1	5.4±0.1	5.0±0.2	5.5±0.1[a]	5.4±0.2
P-Value					
Maize	0.0019	0.0058	0.0952	0.2942	0.5463
Meat	0.0049	<0.0001	<0.0001	0.0312	0.0006
Maize * Meat	0.9286	0.5795	0.9754	0.2700	0.9333
Panel	0.0075	0.1481	0.3627	0.0420	0.2817
Panel * Maize	0.0041	0.0032	0.0281	0.0391	0.0115
Panel * Meat	0.1917	0.3153	0.1595	0.1970	0.6422
Panel * Meat * maize	0.5963	0.0758	0.0546	0.8650	0.1052

(1) Scale: One to nine (9 extremely high preference, 5 neutral, and one as rejection). [a, b] Means with the same superscript in the same column are not significantly ($p < 0.05$) different. (2) PWS: Porridge from pounded maize soaked in water (12h); SG: Porridge from germinated (24h); LWS: Porridge from maize soaked in lemon-water (12 h); (3) n = 42 village mothers; n = 36 staff and students

Based on sensory quality, panelist ranked porridges prepared from water-soaked maize as first (55.6%); from lemon-soaked maize (34.6%) as second and porridge from germinated maize as third (17.3%). However, panelists from the university gave higher sensory attributes scores to porridges than the mothers from the community (Table 5.2). The highest score of sensory quality given to top porridges ranged from neutral to slightly liked, where nine represented extremely liked, five neutral and one represent extremely disliked. The relatively low scores suggest that there are objectionable attributes that limited the sensory quality of the samples. Absence of salt in all porridge may be one of the factor that played a significant role for such a low score value.

Table 5.2 Effect of interaction between panel and maize porridges on sensory attribute

	Sensory quality attributes				
Factor	Color	Flavor	Smell	Texture	Acceptability
Village Mother					
PWS	5.7±0.3[bc]	5.2±0.3[abc]	4.8±0.3[a]	4.8±0.3[b]	4.9±0.3[ab]
SG	5.6±0.3[bc]	5.2±0.3 [abc]	4.9±0.3[a]	5.1±0.3[ab]	5.5±0.3 [ab]
PLWS	5.3±0.3[bc]	4.9±0.3 [bc]	4.8±0.3[a]	5.2±0.3 [ab]	5.1±0.3 [ab]
Staff & Students					
PWS	6. 8±0.2[a]	6.1±0.2 [a]	5.6±0.3[a]	5.9±0.3 [a]	5.9±0.3[a]
SG	5.2±0.2[c]	4.5±0.2 [bd]	4.3±0.3[b]	4.9±0.3 [ab]	4.8±0.3[b]
PLWS	6.2±0.2[ab]	5.5±0.2 [cd]	5.2±0.3[a]	5.6±0.3 [ab]	5.5±0.3 [ab]
P-Value					
Panel * Maize	0.0041	0.0032	0.0281	0.0391	0.0115

(1) Scale: One to Nine with 9 being extremely high preference, 5 neutral, and 1 as rejection. (2) [a, b, c] Means in a column sharing a superscript not significantly different at $p < 0.05$. (3) PWS: Porridge from pounded maize soaked in water (12h); SG: Porridge from germinated (24h); LWS: Porridge from maize soaked in lemon-water (12 h); (4) n = 42 village mothers; n = 36 staff and students

Qualitative characteristics like mouth feel, appearance, and flavor (aroma) were assessed by panelists to further determine porridge quality. Mouth feel was assessed by after-taste, mouth-coating, ease of swallow, graininess, thickness, sponginess, creaminess and foaminess. Appearance was determined by dullness, roughness and cohesiveness. The flavor (aroma) was determined by pronounced flavor, over-cooked and mustiness. Accordingly, panelists preferred porridges prepared from water-soaked maize more than other porridges prepared from germinated or lemon-soaked maize (Table 5.3). Panelists from the university gave higher scores for after-taste, mouth-coating, graininess, thickness, sponginess, creaminess and foaminess of the porridges than scores given by the village mothers.

Table 5.3 Mean ±SE textural quality attributes of porridges (mouth feel)

Factor	Mouth feel					
	After taste	Mouth coating	Ease of swallow	Grainy	Creamy	Foamy
Maize treatment						
PWS	3.0±0.1	2.8±0.1	3.3±0.1	2.6±0.1	2.0±0.1	2.2±0.1
SG	3.0±0.1	2.9±0.1	3.4±0.1	3.1±0.1	2.0±0.1	2.2±0.1
PLWS	2.9±0.1	2.8±0.1	3.2±0.1	3.0±0.1	2.1±0.1	2.2±0.1
Meat						
Yes	2.8±0.1	2.9±0.1	3.3±0.1	2.8±0.1	2.1±0.1	2.2±0.1
No	3.0±0.1	2.8±0.1	3.3±0.1	2.9±0.1	2.0±0.1	2.2±0.1
Panel						
Village mothers	2.8±0.1 [b]	2.7±0.1[b]	3.2±0.1	2.6±0.1[b]	1.6±0.1[b]	1.8±0.1[b]
Staff & Students	3.1±0.1 [a]	3.1±0.1 [a]	3.4±0.1	3.2±0.1[a]	2.6±0.1[a]	2.6±0.1[a]
P- Value						
Maize	0.6414	0.7479	0.5744	0.0004	0.9393	0.9988
Panel	0.0048	0.0019	0.0167	<0.0001	<0.0001	<0.0001

(1) Scale: One to Nine with 9 being extremely high preference, 5 neutral, and 1 as rejection. (2) [a,b] Means with different superscripts in same column are significantly different; (3) PWS: Porridge prepared from water-soaked pounded maize; SG: Porridge prepared from germinated maize; LWS: Porridge prepared from white lemon-water soaked pounded maize; (4) n = 42 village mothers; n = 36 staff and students

In terms effects of phytate reduction methods on appearance and flavor (aroma), there were significant differences between porridges that were prepared with or without meat, especially in terms of appearance. Porridge that had meat was more cohesive (p = 0.0341), but less rough (p <0.0001) than porridge prepared maize without meat. University panelist found all porridges more dull (p = 0.0002), cohesive (p = 0.0007), rough (p < 0.0001), musty (p = 0.0206), pronounced flavor (p = 0.0080), and aroma of over cookedness (p = 0.0001) than reported by the village mothers for similar porridge samples (Table 5.4).

Table 5.4 Mean±SE appearance and flavor attributes of porridges

Factor	Appearance			Flavor (Aroma)		
	Dullness	Cohesive	Rough	Strong flavor	Musty	Over cooked
Maize treatment						
PWS	3.0±0.1	2.8±0.1	2.8±0.1	2.8±0.1	2.7±0.1	2.5±0.1[b]
SG	2.9±0.1	3.1±0.1	3.0±0.1	2.8±0.1	2.6±0.1	2.9±0.1[a]
PLWS	2.8±0.1	2.7±0.1	3.0±0.1	3.0±0.1	2.8±0.1	2.5±0.1[ab]
Meat						
Yes	2.8±0.1	3.0±0.1[a]	2.7±0.1[b]	2.6±0.1[b]	2.6±0.1	2.5±0.1[b]
No	3.0±0.1	2.7±0.1[b]	3.1±0.1[a]	3.1±0.1[a]	2.8±0.1	2.9±0.1[a]
Panel						
Village mothers	2.6±0.1[b]	2.6±0.1[b]	2.6±0.1[b]	2.7±0.1[b]	2.5±0.1[b]	2.4±0.1[b]
Staff & Students	3.1±0.1[a]	3.1±0.1[a]	3.3±0.1[a]	3.0±0.1[a]	2.8±0.1[a]	2.9±0.1[a]
P- Value						
Maize	0.6035	0.0979	0.0828	0.4834	0.4728	0.0170
Meat	0.0546	0.0341	<0.0001	0.0003	0.1115	0.0035
Maize * Meat	0.9912	0.9246	0.4966	0.7391	0.9547	0.8748
Panel	0.0002	0.0007	<0.0001	0.0080	0.0206	0.0001
Panel * Maize	0.3554	0.4247	0.8080	0.4141	0.4957	0.9983
Panel * Meat	0.8032	0.9848	0.5496	0.0380	0.5325	0.6421
Panel * Meat * maize	0.6109	0.6285	0.1604	0.6287	0.6911	0.0931

(1) Scale: One to Nine with 9 being extremely high preference, 5 neutral, and 1 as rejection. (2) [a,b] Means with different superscripts in same column are significantly different; (3) PWS: Porridge prepared from water-soaked pounded maize; SG: Porridge prepared from germinated maize; LWS: Porridge prepared from white lemon-water soaked pounded maize; (4) n = 42 village mothers; n = 36 staff and students

Panelists were also asked to compare the qualities of the porridges based on their own ideal porridge characteristics. The score of the ideal porridge was adjusted to zero for all characteristics and actual scores were compared and shown as positive or negative deviations from the ideal porridge score. Preparing porridge without (Figure 5.1) or with

(Figure 5.2) dried meat powder had no significant difference from the ideal porridge. This suggests that the panelist did not find objectionable characteristics in the samples. However, difference in porridge prepared from water-soaked, germinated, and lemon-soaked maize with or without adding dried meat may reflect panelist's lack of familiarity with the textural properties of the porridge or the sensory evaluation and textural profiling methods.

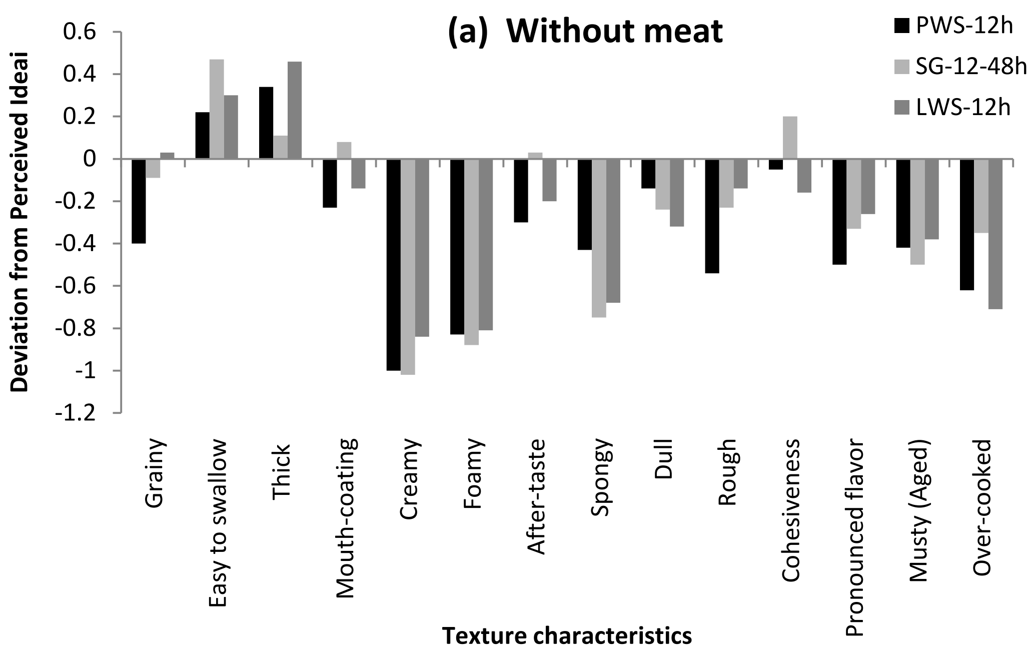

Figure 5.1 Mean textural quality of maize porridge without meat shown as score deviation from the ideal porridge. PWS-12h: Porridge prepared from pounded maize soaked in water for 12h; SG-12-48h: Porridge prepared from maize soaked (12h) in water and germinated (48h); LWS-12h: Porridge prepared from maize soaked (12h) in lemon-water (1:10v/v) for 12h

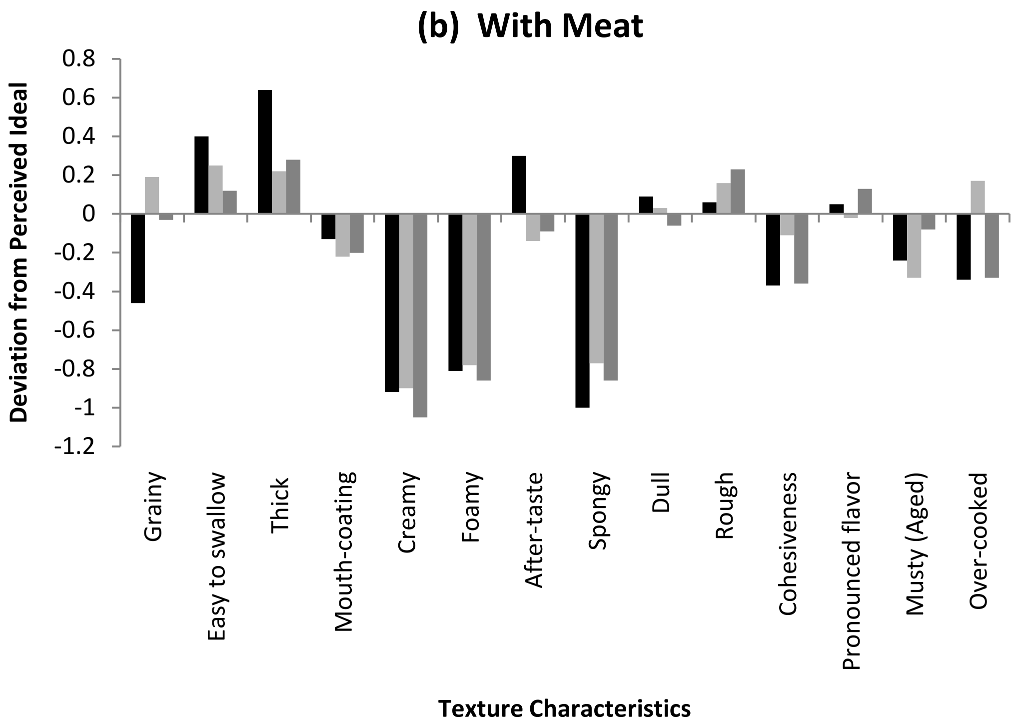

Figure 5.2 Mean textural quality of maize porridge with meat shown as score deviation from the ideal porridge. PWS-12h: Porridge prepared from pounded maize soaked in water for 12h; SG-12-48h: Porridge prepared from maize soaked (12h) in water and germinated (48h); LWS-12h: Porridge prepared from maize soaked (12h) in lemon-water (1:10v/v) for 12h

Children consumed half to more than half of the 50g porridges sample provided. The amount of porridge consumed by children and the sensory scores of the porridge samples were similar (Fig 5.3). However, they relatively liked more and ate more from porridge that was prepared from water-soaked maize than the other two porridges. Preparing porridge with or without dried meat did not affect the amount of porridge eaten by children or affect their liking towards the porridge.

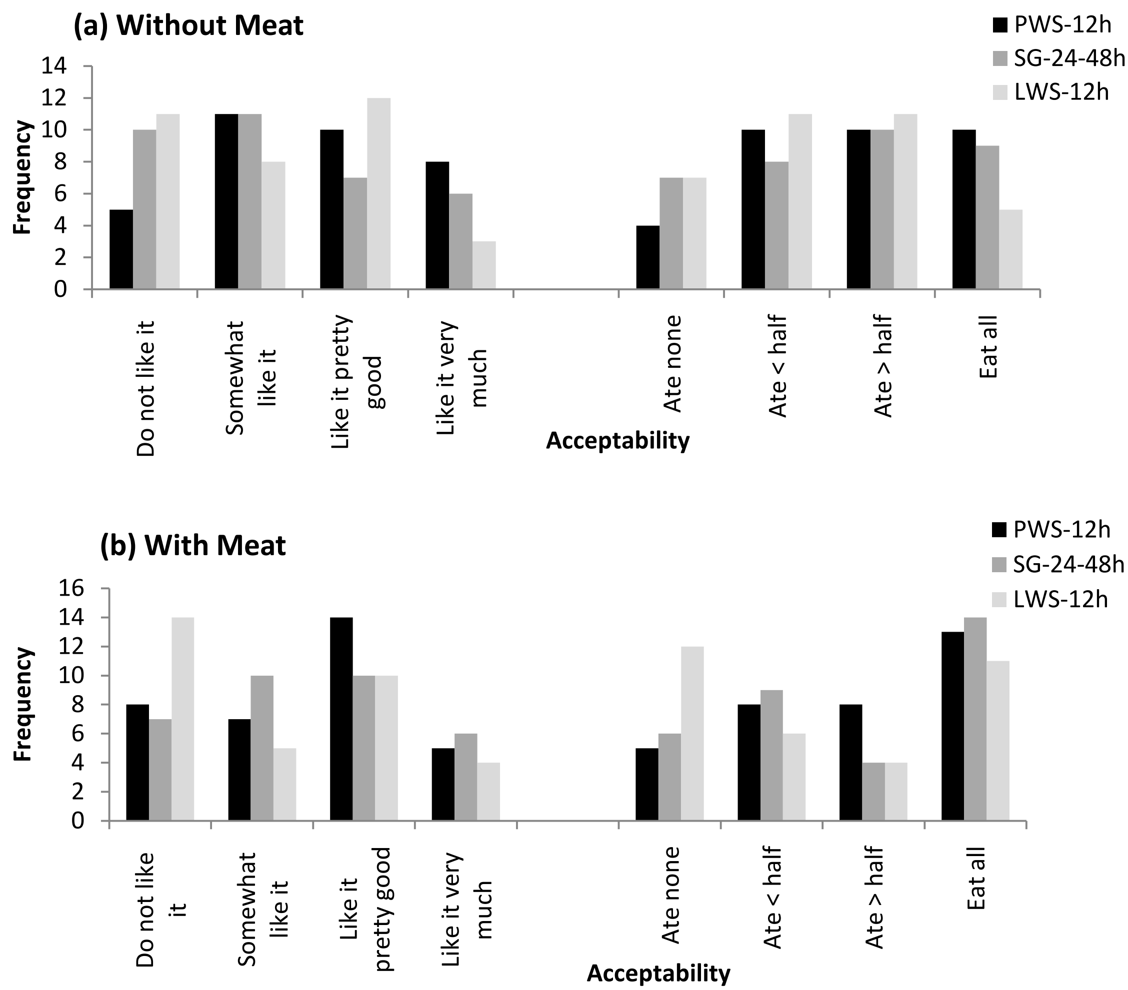

Figure 5.3 Frequency (%) distribution of maize-based porridge consumed by children PWS-12h: Porridge prepared from pounded maize soaked in water for 12h; SG-12-48h: Porridge prepared from maize soaked (12h) in water and germinated (48h); LWS-12h: Porridge prepared from maize soaked (12h) in lemon-water (1:10v/v) for 12h

Summary and Conclusion

Most infants in Ethiopia are weaned gradually to solid foods with gruel or soft porridge made from maize. The sensory quality of porridge from pounded white maize soaked in water for 12h is a potential maize-based complementary food for young children in the study area.

Reference

Bohn, L., Meyer, A. S., & Rasmussen, S. K. (2008). Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *J Zhejiang Univ Sci B, 9*(3), 165-191.

CSA. (2007). Ethiopian health and demographic survey.

Egli, I., Davidsson, L., Zeder, C., Walczyk, T., & Hurrell, R. (2004). Dephytinization of a complementary food based on wheat and soy increases zinc, but not copper, apparent absorption in adults. *J Nutr, 134*(5), 1077-1080.

Ferguson, E. L., Gibson, R. S., Thompson, L. U., & Ounpuu, S. (1989). Dietary calcium, phytate, and zinc intakes and the calcium, phytate, and zinc molar ratios of the diets of a selected group of East African children. *Am J Clin Nutr, 50*(6), 1450-1456.

Gibson, R. S. (1994). Content and bioavailability of trace elements in vegetarian diets. *Am J Clin Nutr, 59*(5 Suppl), 1223S-1232S.

Gibson, R. S., Donovan, U. M., & Heath, A. L. (1997). Dietary strategies to improve the iron and zinc nutriture of young women following a vegetarian diet. *Plant Foods Hum Nutr, 51*(1), 1-16.

Hambidge, K. M., Krebs, N. F., Westcott, J. E., & Miller, L. V. (2006). Changes in zinc absorption during development. *J Pediatr, 149*(5 Suppl), S64-68.

Hotz, C., & Gibson, R. S. (2001). Assessment of home-based processing methods to reduce the phytate content and phytate/zinc molar ratio of white maize (Zea mays). *J Agric Food Chem, 49*(2), 692-698.

Jean-Xavier Guinard, a. R. M. (1999). The sensory perception of texture and mouthfeel *Trends in Food Science & technology, 7*(7), 7.

Lonnerdal, B. (2003). Genetically modified plants for improved trace element nutrition. *J Nutr, 133*(5 Suppl 1), 1490S-1493S.

Mazariegos, M., Hambidge, K. M., Krebs, N. F., Westcott, J. E., Lei, S., Grunwald, G. K., et al. (2006). Zinc absorption in Guatemalan schoolchildren fed normal or low-phytate maize. *Am J Clin Nutr, 83*(1), 59-64.

Morris, E. R., & Ellis, R. (1989). Usefulness of the dietary phytic acid/zinc molar ratio as an index of zinc bioavailability to rats and humans. *Biol Trace Elem Res, 19*(1-2), 107-117.

Sandstrom, B. (1987). Zinc and dietary fibre. *Scand J Gastroenterol Suppl, 129*, 80-84.

Szczesniak, A. S. (2002). Texture is a sensory property *Food Quality and Preference, 13*(4), 10.

Wise, A. (1995). Phytate and zinc bioavailability. *Int J Food Sci Nutr, 46*(1), 53-63.

CHAPTER VI

CONCLUSION

Phytate is a known chelating agent that reduces zinc bioavailability (Weaver & Kannan, 2002). The importance of phytate in altering zinc status has led to development of methods for assessing zinc bioavailability. Accordingly, many authors have proposed that zinc bioavailability should be estimated by the molar ratios of phytate to zinc (Phy/Zn). Hotz and Gibson (2001b) reported that Phy/Zn molar ratios higher than 10 –15 limit zinc absorption are associated with suboptimal zinc status (Hotz & Gibson, 2001b).

Thus, reducing Phy/Zn molar ratios to specific thresholds seems to be a practical way to improve zinc bioavailability. This can be achieved by reducing phytate content in foods using certain thermal or biological treatments. However, apart from drastic thermal treatment such as canning, or extrusion cooking, the efficiency of thermal treatments in reducing phytate is limited because of the high stability of phytate (Marzo, Alonso, Urdaneta, Arricibita, & Ibanez, 2002). As far as biological treatments are concerned, fermentation and germination can result about 80% phytate degradation (Fruhbeck, Alonso, Marzo, & Santidrian, 1995).

However, application of fermentation and germination remains limited because of additional processing steps they impose or particular organoleptic properties they induce (Svanberg, Lorri, & Sandberg, 1993). Duhan and colleague have reported that phytate can also be reduced during soaking, a common household process (Duhan et al., 2002). This reduction may be due to enzymatic degradation by endogenous phytases (Sandberg & Svanberg, 1991), whereby IP_6 is hydrolysed giving free inorganic phosphorus (P_i) and myo-inositol phosphates (IP_5 to IP_1) or inositol, or leaching of phytate into the soaking medium (Hotz & Gibson, 2001b).

Soaking and germination are treatments that are commonly used to prepare foods for infants in developing countries. This study characterized effects of soaking unrefined white maize in water or acid solution (10% lemon juice in water) and of germination on phytate degradation and evaluated efficiency of these to reduce Phy:Zn molar ratio in maize-based complementary foods.

Total phytate content in unrefined white maize was determined by extracting samples with 0.66 N HCl, precipitating as ferric phytate, adding color reagent and measuring the peak color intensity at 523 nm with a spectrophotometer. In the first study, a total of twenty eight maize samples were examined in triplicate using soaking in water (6 h or 12 h), soak for 6 or 12 h and then germinate for 24 h or 48 h, and soaking in lemon-water solution for 6 h or 12 h. The maize used for soaking in water or lemon-water was pre-pounded, whereas, the maize used in the germination process was whole. Each sample was analyzed for their phytate and zinc content and for their calculated value of phytate to zinc molar ratio.

This study indicates that water soaking (12 h) reduced phytate content and Phy:Zn molar ratios originally observed in untreated white maize samples. The mean (±SE) total phytate content of untreated white maize was 625.2±141.1mg/100g, 2.01+0.1mg/100g zinc and 30.84±7.31 phytate to zinc molar ratio. The overall phytate content found in untreated maize sample in this study and with reports from other authors, the phytate concentration found in this study is equivalent with most of the findings reported elsewhere (Table 6.1).

Table 6.1 Phytate content determined in maize by using different methods

Publication (Authors)	Method of Phytate Analysis and procedure followed	Phytate content (g/100g)
Blatny et al. (1995)	Column-coupling Isotachophoresis Blatny et al. (1995)	1.290
Egli et al (2002)	HPLC By Sandberg & Ahderinne (1986)	1.150
Egli et al (2003)	HPLC By Sandberg & Ahderinne (1986)	0.900
Bos etal (1991)	HPLC By Bos et al (1991)	0.735
Hotz et al (2001)	HPLC By Lehrfeld (1989).	0.697
Bos etal (1991)	Colorimetric + Ion exchange By Latta and Eskin (1980	0.651
Temple, Gibson & Hotz (2002)	HPLC By Lehrfeld (1989)	0.587
Bos etal (1991)	HPLC By Cilliers & v. Niekerk (1986)	0.583
Pascale et al (2000)	High-performance ion chromatography (HPIC)	0.500
Melaku Umeta et al (2003)	Colorimetric By Hage and Lantzasch (1983)	0.282
Perlas & Gibson (2005)	HPLC By Lerhfeld (1989)	0.062
*This paper (Our result)	Colorimetric + Ion exchange By (Harland & Oberleas, 1977) Haug and Lantzsch (1983)	0.625

Comparing the processing methods used however, there was pronounced phytate decrease in maize samples that were pounded and soaked in water for 12 h. This method decreased the phytate content by 94% compared with the untreated maize. For other processing methods, germination decreased the amount of phytate by 37% and lemon-water soaking decreased the amount of phytate by 23%. Phytate and zinc content measured in these three methods were significantly different ($p < 0.05$). This means that soaking pounded maize in lemon-water solution did not reduce phytate as much as observed in pounded water-soaked maize.

Accordingly, the overall phytate reduction by water soaking achieved in this study is consistent with those findings that were reported by other authors in related studies. Pounding the maize before soaking was beneficial for phytate reduction because of phytate distribution in maize. Pounding increases the surface area and facilitates the enzyme and substrate contact in which hydrolysis of phytate may be facilitated by phytases, as well as increasing phytate leaching.

After determination of phytate, three maize samples with lower phytate content were selected for further use in porridge and were assessed for their sensory and texture qualities. These porridges were prepared from: pounded maize soaked in water (1:10 w/v) for 12 hours; pounded maize soaked in lemon-water solution (1:10 v/v) for 12 h, and whole maize soaked in water (12 h) and germinated (24 h). These maize samples were proportionally supplemented with kidney beans (80:20w/w). Half of the maize/bean blend mixed with dried red meat powder (10:1 w/w) before preparing into porridge, while the other half was prepared without adding dried meat. A total of 78 participants (36 rural mothers and 42 university panelists) who were selected from the study area evaluated sensory quality of each porridge samples.

Participants generally gave higher scores to those porridges that were prepared from water-soaked maize than to porridges from germinated or lemon-water soaked maize. Participants liked the color and flavor of porridges that are prepared from water-soaked maize more ($p = 0.0012$) than porridge that was prepared from germinated maize and lemon-soaked maize. Participants significantly liked the smell and overall acceptability of porridges that were prepared from water-soaked maize much more than those porridges that were prepared from germinated maize or lemon-soaked maize.

The overall sensory score given by panelist to all measured parameters in those porridge samples ranged from neutral to slightly like, from a nine scale score in which nine represented extremely liked, five neutral and one represent extremely disliked. One of the potential reasons for the relatively low scores that may affect the sensory qualities of the porridges may be absence of salt in all porridge samples.

However, participant's choice to determine the porridge with best sensory quality was consistent for all computed parameters. Porridge prepared from pounded and water soaked white maize was generally found to be more acceptable in terms of potential processing method to reduce phytate and improve sensory qualities. Accordingly, 56% of participants ranked porridges that were prepared from water-soaked maize as their first choice; followed by 35% of participants who chose porridge prepared from lemon-soaked maize as their second choice and 17% of the participant chose porridges that were prepared from germinated maize as their third choice in terms of sensory quality.

In conclusion, development of low phytate maize-based foods in the way reported in this study will not only improve zinc bioavailability in complementary foods for young children, but also has potential to improve foods, which are especially prepared for pregnant and lactating women who are another risk group for zinc deficiency in the study area. We conclude that home-based food processing method like pounding and water soaking is potential method to reduce phytate and improve zinc bioavailability in maize-based complementary food in southern Ethiopia.

Limitation of the study

In association with assessing home-based food processing methods that could reduce phytate in maize-based complementary foods, we measured phytate content. In general, there are four ways phytate determination may be affected when using anion-exchange colorimetric method. This may include: quantitative phytate extraction from sample; contamination of phytate while eluting from the anion exchange column with non-phytate phosphorous; quantitative recovery of phytate in the phytate-containing elute from the anion-exchange column; and lack of complete release of phosphorus from phytate during the heating procedure.

Accordingly, one of the limitations of using this method is that it measures total inositol phosphates. There is a possibility to measure inositol hexaphosphate along with penta-, tetra-, tri-, di-, and mono-phosphates. Therefore, it may overestimate the phytate content of the foods. Another limitation may be the problems of accuracy and precision since this method relies on a stoichiometric ratio of phosphorus to iron in the precipitate. This ratio may be affected by different purification techniques and by the presence of different inositol forms. HPLC can fractionate individual inositol phosphates into their components, allowing accurate determination of all inositol phosphates. Variation of phytate reduction achieved within and between tested samples may arise from a number of sources other than treatment difference.

Recommendation and future study

It was evident from this study that a large portion of the phytate could be reduced from maize-based complementary foods by simple use of home-based food processing methods like pounding and soaking. Therefore, preparing complementary food from pounded and soaked white maize can potentially be an inexpensive and easy method to address the problem of zinc deficiency in this study area. Moreover, besides improving zinc bioavailability, this strategy is also suitable in terms of processing convenience, sensory qualities and its cultural acceptability. Thus it may easily and effectively be adopted by mothers in the rural communities of Sidama.

However, there are areas in this study that require improvement and further studies. In terms of preparation and processing, it is recommended to fix exact amount of soaking water and minimum potential soaking time required for optimum phytate reduction. Surface area of grain exposed to water might also be important. There is also need to further study how to make complementary food prepared in this way more organoleptically acceptable in the area.

REFERENCES

Aaron, J. L., Scott, M. P., & Kendall, R. L. (2008). Genetic Variation and Breeding Potential of Phytate and Inorganic Phosphorus in a Maize Population. *Crop Science, 48*(1), 79.

Abebe, Y. (2003). *Nutritional status of low income mothers and under-five children in southern Ethiopia: Focus on transitional food development.* Unpublished Ph.D., Oklahoma State University, United States -- Oklahoma.

Abraham, R. (1982). Trace element intake by Asians during pregnancy. *Proc Nutr Soc, 41*(2), 261-265.

B. E. Knuckles, D. D. K., and A. A. Betschart. (1982). HPLC Analysis of Phytic Acid in Selected Foods and Biological Samples. *Journal of Food Science, 47,* 2.

Bindra, G. S., & Gibson, R. S. (1986). Iron status of predominantly lacto-ovo vegetarian East Indian immigrants to Canada: a model approach. *Am J Clin Nutr*, *44*(5), 643-652.

Blank E. G., P. J., and Sax M. . (1971). The structure of myo-inositol hexaphosphate dodecasodium salt octatriacontahydrate: A single crystal X-ray analysis. *Biochemical and Biophysical Research Communications, 44*(2), 6.

Bohn, L., Josefsen, L., Meyer, A. S., & Rasmussen, S. K. (2007). Quantitative analysis of phytate globoids isolated from wheat bran and characterization of their sequential dephosphorylation by wheat phytase. *J Agric Food Chem, 55*(18), 7547-7552.

Bohn, L., Meyer, A. S., & Rasmussen, S. K. (2008). Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *J Zhejiang Univ Sci B, 9*(3), 165-191.

Bohn, T., Davidsson, L., Walczyk, T., & Hurrell, R. F. (2004). Fractional magnesium absorption is significantly lower in human subjects from a meal served with an oxalate-rich vegetable, spinach, as compared with a meal served with kale, a vegetable with a low oxalate content. *Br J Nutr, 91*(4), 601-606.

Bosscher, D., Lu, Z., Janssens, G., Van Caillie-Bertrand, M., Robberecht, H., De Rycke, H., et al. (2001). In vitro availability of zinc from infant foods with increasing phytic acid contents. *Br J Nutr, 86*(2), 241-247.

Brown, K. H., Wessells, K. R., & Hess, S. Y. (2007). Zinc bioavailability from zinc-fortified foods. *Int J Vitam Nutr Res, 77*(3), 174-181.

Brunvand, L., Henriksen, C., Larsson, M., & Sandberg, A. S. (1995). Iron deficiency among pregnant Pakistanis in Norway and the content of phytic acid in their diet. *Acta Obstet Gynecol Scand, 74*(7), 520-525.

Carnovale, E., Lombardi-Boccia, G., & Lugaro, E. (1987). Phytate and zinc content of Italian diets. *Hum Nutr Appl Nutr, 41*(3), 180-186.

Chen, Q. (2004). Determination of phytic acid and inositol pentakisphosphates in foods by high-performance ion chromatography. *J Agric Food Chem, 52*(15), 4604-4613.

Cheryan, M. (1980). Phytic acid interactions in food systems. *Crit Rev Food Sci Nutr, 13*(4), 297-335.

Chesters, J. K., Boyne R. . (1991). Trace element-gene interactions with particular reference to zinc. *Proc. Nutr. Soc., 50*, 7.

Costello, A. J., Glonek, T., & Myers, T. C. (1976). 31P nuclear magnetic resonance-pH titrations of myo-inositol hexaphosphate. *Carbohydr Res, 46*(2), 159-171.

Courtois, J. E., Malangeau, P., & Chabre, J. L. (1959). [Comparative action of phytic acid on proteins and glycoprotein-protein mixtures.]. *C R Seances Soc Biol Fil, 153*, 959-961.

Cousins, R. J. (1996). Zinc. *Present Knowledge in Nutrition*.

CSA. (2007). Ethiopian health and demographic survey.

Davidsson, L. (2003). Approaches to improve iron bioavailability from complementary foods. *J Nutr, 133*(5 Suppl 1), 1560S-1562S.

Davies, N. T., & Olpin, S. E. (1979). Studies on the phytate: zinc molar contents in diets as a determinant of Zn availability to young rats. *Br J Nutr, 41*(3), 590-603.

Davies, N. T., & Warrington, S. (1986). The phytic acid mineral, trace element, protein and moisture content of UK Asian immigrant foods. *Hum Nutr Appl Nutr, 40*(1), 49-59.

de Boland, A. R., Garner, G. B., & O'Dell, B. L. (1975). Identification and properties of "phytate" in cereal grains and oilseed products. *J Agric Food Chem, 23*(6), 1186-1189.

Duhan, A., Khetarpaul, N., & Bishnoi, S. (2002). Changes in phytates and HCl extractability of calcium, phosphorus, and iron of soaked, dehulled, cooked, and sprouted pigeon pea cultivar (UPAS-120). *Plant Foods Hum Nutr, 57*(3-4), 275-284.

Earley, E. B. a. D., E. E. (1944). Time and rate of synthesis of phytin in corn grain during the productive period. *J. Amer. Soc. Agron., 36*.

Egli, I., Davidsson, L., Zeder, C., Walczyk, T., & Hurrell, R. (2004). Dephytinization of a complementary food based on wheat and soy increases zinc, but not copper, apparent absorption in adults. *J Nutr, 134*(5), 1077-1080.

Ellis, R., Kelsay, J. L., Reynolds, R. D., Morris, E. R., Moser, P. B., & Frazier, C. W. (1987). Phytate:zinc and phytate X calcium:zinc millimolar ratios in self-selected diets of Americans, Asian Indians, and Nepalese. *J Am Diet Assoc, 87*(8), 1043-1047.

Ferguson, E. L., Gibson, R. S., Opare-Obisaw, C., Ounpuu, S., Thompson, L. U., & Lehrfeld, J. (1993). The zinc nutriture of preschool children living in two African countries. *J Nutr, 123*(9), 1487-1496.

Ferguson, E. L., Gibson, R. S., Thompson, L. U., & Ounpuu, S. (1989). Dietary calcium, phytate, and zinc intakes and the calcium, phytate, and zinc molar ratios of the diets of a selected group of East African children. *Am J Clin Nutr, 50*(6), 1450-1456.

Fitzgerald, S. L., Gibson, R. S., Quan de Serrano, J., Portocarrero, L., Vasquez, A., de Zepeda, E., et al. (1993). Trace element intakes and dietary phytate/Zn and Ca x phytate/Zn millimolar ratios of periurban Guatemalan women during the third trimester of pregnancy. *Am J Clin Nutr, 57*(2), 195-201.

Fredlund, K., Bergman, E. L., Rossander-Hulthen, L., Isaksson, M., Almgren, A., & Sandberg, A. S. (2003). Hydrothermal treatment and malting of barley improved zinc absorption but not calcium absorption in humans. *Eur J Clin Nutr, 57*(12), 1507-1513.

Fruhbeck, G., Alonso, R., Marzo, F., & Santidrian, S. (1995). A modified method for the indirect quantitative analysis of phytate in foodstuffs. *Anal Biochem, 225*(2), 206-212.

Gibson, R. S. (1994). Content and bioavailability of trace elements in vegetarian diets. *Am J Clin Nutr, 59*(5 Suppl), 1223S-1232S.

Gibson, R. S., Donovan, U. M., & Heath, A. L. (1997). Dietary strategies to improve the iron and zinc nutriture of young women following a vegetarian diet. *Plant Foods Hum Nutr, 51*(1), 1-16.

Gibson, R. S., & Ferguson, E. L. (1998). Nutrition intervention strategies to combat zinc deficiency in developing countries. *Nutr Res Rev, 11*(1), 115-131.

Gibson, R. S., Ferguson, E. L., & Lehrfeld, J. (1998). Complementary foods for infant feeding in developing countries: their nutrient adequacy and improvement. *Eur J Clin Nutr, 52*(10), 764-770.

Gibson, R. S., Sazawal, S., & Peerson, J. M. (2003). Design and quality control issues related to dietary assessment, randomized clinical trials and meta-analysis of field-based studies in developing countries. *J Nutr, 133*(5 Suppl 1), 1569S-1573S.

Gibson, R. S., Yeudall, F., Drost, N., Mtitimuni, B., & Cullinan, T. (1998). Dietary interventions to prevent zinc deficiency. *Am J Clin Nutr, 68*(2 Suppl), 484S-487S.

Gifford, S. R., & Clydesdale, F. M. (1990). Interactions Among Calcium, Zinc and Phytate with Three Protein Sources. *Journal of Food Science, 55*(6), 1720.

Glahn, R. P., Wortley, G. M., South, P. K., & Miller, D. D. (2002). Inhibition of iron uptake by phytic acid, tannic acid, and ZnCl2: studies using an in vitro digestion/Caco-2 cell model. *J Agric Food Chem, 50*(2), 390-395.

Graf, E., & Dintzis, F. R. (1982). High-performance liquid chromatographic method for the determination of phytate. *Anal Biochem, 119*(2), 413-417.

Greger, J. L., Abemathy, R. P., and Bennett, O. A. (1978). Zinc and Nitrogen Balance in Adolescent Females Fed Varying Levels of Zinc and Soy Protein. *Am J Clin Nutr., 31*, 5.

Hambidge, K. M. (1981). Zinc deficiency in man: its origins and effects. *Philos Trans R Soc Lond B Biol Sci, 294*(1071), 129-144.

Hambidge, K. M., Abebe, Y., Gibson, R. S., Westcott, J. E., Miller, L. V., Lei, S., et al. (2006). Zinc absorption during late pregnancy in rural southern Ethiopia. *Am J Clin Nutr, 84*(5), 1102-1106.

Hambidge, K. M., Krebs, N. F., & Miller, L. (1998). Evaluation of zinc metabolism with use of stable-isotope techniques: implications for the assessment of zinc status. *Am J Clin Nutr, 68*(2 Suppl), 410S-413S.

Hambidge, K. M., Krebs, N. F., Westcott, J. E., & Miller, L. V. (2006). Changes in zinc absorption during development. *J Pediatr, 149*(5 Suppl), S64-68.

Harland, B. F., & Oberleas, D. (1977). A modified method for phytate analysis using an ion exchange procedure: application to textured vegetable proteins, *Cereal chemistry* (pp. 827-832).

Harland, B. F., & Oberleas, D. (1986). Anion-exchange method for determination of phytate in foods: collaborative study. *J Assoc Off Anal Chem, 69*(4), 667-670.

Harland, B. F., Smith, S. A., Howard, M. P., Ellis, R., & Smith, J. C., Jr. (1988). Nutritional status and phytate:zinc and phytate x calcium:zinc dietary molar ratios of lacto-ovo vegetarian Trappist monks: 10 years later. *J Am Diet Assoc, 88*(12), 1562-1566.

Haug, W., and Lantzsch, H. . (1983). Sensitive Method for the Rapid Determination of Phytate in Cereals and Cereal Products. *J. Sci. Food Agric., 34*, 4.

Hotz, C., & Gibson, R. S. (2001a). Assessment of home-based processing methods to reduce the phytate content and phytate/zinc molar ratio of white maize (Zea mays). *J Agric Food Chem, 49*(2), 692-698.

Hotz, C., & Gibson, R. S. (2001b). Complementary feeding practices and dietary intakes from complementary foods amongst weanlings in rural Malawi. *Eur J Clin Nutr, 55*(10), 841-849.

Hotz, C., Gibson, R. S., & Temple, L. (2001). A home-based method to reduce phytate content and increase zinc bioavailability in maize-based complementary diets. *Int J Food Sci Nutr, 52*(2), 133-142.

IZiNCG. (2004). Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull., 25*, 109.

Jameson, S. (1976). Variations in maternal serum zinc during pregnancy and correlation to congenital malformations, dysmaturity, and abnormal parturition. *Acta Med Scand Suppl, 593*, 21-37.

Jaworska Danuta, a. H. M. (2008). Relative importance of texture properties in the sensory quality and acceptance of commercial crispy products *Journal of the Science of Food and Agriculture, 88*(10), 9.

Jean-Xavier Guinard, a. R. M. (1999). The sensory perception of texture and mouthfeel *Trends in Food Science & technology, 7*(7), 7.

Johnson, L. F. a. T., M. E. . (1969). The structure of myo-inositol pentaphosphate. *Ann. N. Y. Acad. Sci., 165*, 7.

Krebs, N. E., & Hambidge, K. M. (2001). Zinc metabolism and homeostasis: the application of tracer techniques to human zinc physiology. *Biometals, 14*(3-4), 397-412.

Krebs, N. F. (2000). Overview of zinc absorption and excretion in the human gastrointestinal tract. *J Nutr, 130*(5S Suppl), 1374S-1377S.

Lee, D. Y., Schroeder, J., 3rd, & Gordon, D. T. (1988). Enhancement of Cu bioavailability in the rat by phytic acid. *J Nutr, 118*(6), 712-717.

Likuski, H. J., & Forbes, R. M. (1965). Mineral Utilization in the Rat. Iv. Effects of Calcium and Phytic Acid on the Utilization of Dietary Zinc. *J Nutr, 85*, 230-234.

Lonnerdal, B. (1989). Dietary factors affecting trace element absorption in infants. *Acta Paediatr Scand Suppl, 351*, 109-113.

Lonnerdal, B. (2000). Dietary factors influencing zinc absorption. *J Nutr, 130*(5S Suppl), 1378S-1383S.

Lonnerdal, B. (2003). Genetically modified plants for improved trace element nutrition. *J Nutr, 133*(5 Suppl 1), 1490S-1493S.

Lonnerdal, B., Cederblad, A., Davidsson, L., & Sandstrom, B. (1984). The effect of individual components of soy formula and cows' milk formula on zinc bioavailability. *Am J Clin Nutr, 40*(5), 1064-1070.

Lonnerdal, B., Jayawickrama, L., & Lien, E. L. (1999). Effect of reducing the phytate content and of partially hydrolyzing the protein in soy formula on zinc and copper absorption and status in infant rhesus monkeys and rat pups. *Am J Clin Nutr, 69*(3), 490-496.

Lopez de Romana, D., Lonnerdal, B., & Brown, K. H. (2003). Absorption of zinc from wheat products fortified with iron and either zinc sulfate or zinc oxide. *Am J Clin Nutr*, *78*(2), 279-283.

Lopez, H. W., Duclos, V., Coudray, C., Krespine, V., Feillet-Coudray, C., Messager, A., et al. (2003). Making bread with sourdough improves mineral bioavailability from reconstituted whole wheat flour in rats. *Nutrition, 19*(6), 524-530.

Lori Oatway, T. V., and James H. Helm. (2001). Phytate Acid. *Food Reviews International*, *17*(4), 13.

Lucca, P., Hurrell, R., & Potrykus, I. (2002). Fighting iron deficiency anemia with iron-rich rice. *J Am Coll Nutr*, *21*(3 Suppl), 184S-190S.

Mahloudji, M., Reinhold, J. G., Haghshenass, M., Ronaghy, H. A., Fox, M. R., & Halsted, J. A. (1975). Combined zinc and iron compared with iron supplementation of diets of 6- to 12-year old village schoolchildren in southern Iran. *Am J Clin Nutr*, *28*(7), 721-725.

Marzo, F., Alonso, R., Urdaneta, E., Arricibita, F. J., & Ibanez, F. (2002). Nutritional quality of extruded kidney bean (Phaseolus vulgaris L. var. Pinto) and its effects on growth and skeletal muscle nitrogen fractions in rats. *J Anim Sci*, *80*(4), 875-879.

Mazariegos, M., Hambidge, K. M., Krebs, N. F., Westcott, J. E., Lei, S., Grunwald, G. K., et al. (2006). Zinc absorption in Guatemalan schoolchildren fed normal or low-phytate maize. *Am J Clin Nutr, 83*(1), 59-64.

Melaku Umeta, C. E. W., Hans Verhoef, Jemal Haidar, Joseph G.A.J. Hautvast. (2000a). Zinc supplementation and stunted infants in Ethiopia: a randomised controlled trial. *The Lancet Volume 355* (9220), 6

Melaku Umeta, C. E. W., Hans Verhoef, Jemal Haidar, Joseph G.A.J. Hautvast. (2000b). Zinc supplementation and stunted infants in Ethiopia: a randomised controlled trial. *The Lancet, Volume 355* (9220), 6

Mendoza, C., Viteri, F. E., Lonnerdal, B., Raboy, V., Young, K. A., & Brown, K. H. (2001). Absorption of iron from unmodified maize and genetically altered, low-phytate maize fortified with ferrous sulfate or sodium iron EDTA. *Am J Clin Nutr, 73*(1), 80-85.

Mendoza, C., Viteri, F. E., Lonnerdal, B., Young, K. A., Raboy, V., & Brown, K. H. (1998). Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. *Am J Clin Nutr, 68*(5), 1123-1127.

Mercedes de, O., Monika, B., Elaine, B., Edward, A. F., & Richard, M. (2004). Estimates of Global Prevalence of Childhood Underweight in 1990 and 2015. *JAMA, 291*(21), 2600.

Morris, E. R., & Ellis, R. (1980). Effect of dietary phytate/zinc molar ratio on growth and bone zinc response of rats fed semipurified diets. *J Nutr, 110*(5), 1037-1045.

Morris, E. R., & Ellis, R. (1989). Usefulness of the dietary phytic acid/zinc molar ratio as an index of zinc bioavailability to rats and humans. *Biol Trace Elem Res*, *19*(1-2), 107-117.

Mosha, T. C., Gaga, H. E., Pace, R. D., Laswai, H. S., & Mtebe, K. (1995). Effect of blanching on the content of antinutritional factors in selected vegetables. *Plant Foods Hum Nutr, 47*(4), 361-367.

N.L. Stanley, a. L. J. T. (1993). Rheological basis of oral characteristics of fluid and semi-solid foods: A review. *Acta Psychologica, 84,* 14.

Nahapetian, A., & Bassiri, A. (1975). Changes in concentrations and interrelationships of phytate, phosphorus, magnesium, calcium, and zinc in wheat during maturation. *J Agric Food Chem*, *23*(6), 1179-1182.

Navert, B., Sandstrom, B., & Cederblad, A. (1985). Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man. *Br J Nutr, 53*(1), 47-53.

O'Dell, B. L., & Savage, J. E. (1960). Effect of phytic acid on zinc availability. *Proc Soc Exp Biol Med, 103*, 304-306.

O'Neill, I. K., Sargent, M., & Trimble, M. L. (1980). Determination of phytate in foods by phosphorus-31 Fourier transform nuclear magnetic resonance spectrometry. *Anal Chem, 52*(8), 1288-1291.

Oatway Lori, T. V., and James H. Helm. (2001). Phytate Acid. *Food Reviews International, 17*(4), 13.

Oberleas, D. (1971). The determination of phytate and inositol phosphates. *Methods Biochem Anal*, *20,* 87-101.

Oberleas, D., & Harland, B. (2007). Validation of a column liquid chromatographic method for phytate. *J AOAC Int, 90*(6), 1635-1638.

Oberleas, D., & Harland, B. F. (1977). Nutritional agents which affect metabolic zinc status. *Prog Clin Biol Res, 14*, 11-27.

Phillippy, B. Q., White, K. D., Johnston, M. R., Tao, S. H., & Fox, M. R. (1987). Preparation of inositol phosphates from sodium phytate by enzymatic and nonenzymatic hydrolysis. *Anal Biochem, 162*(1), 115-121.

Plaami, S., & Kumpulainen, J. (1991). Determination of phytic acid in cereals using ICP-AES to determine phosphorus. *J Assoc Off Anal Chem, 74*(1), 32-36.

Prasad, A. S. (1983). Zinc deficiency in human subjects. *Prog Clin Biol Res, 129*, 1-33.

Reinhold, J. G., Nasr, K., Lahimgarzadeh, A., & Hedayati, H. (1973). Effects of purified phytate and phytate-rich bread upon metabolism of zinc, calcium, phosphorus, and nitrogen in man. *Lancet, 1*(7798), 283-288.

Sandberg, A. S. (1991). The effect of food processing on phytate hydrolysis and availability of iron and zinc. *Adv Exp Med Biol, 289*, 499-508.

Sandberg, A. S., Brune, M., Carlsson, N. G., Hallberg, L., Skoglund, E., & Rossander-Hulthen, L. (1999). Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. *Am J Clin Nutr, 70*(2), 240-246.

Sandberg, A. S., Hulthen, L. R., & Turk, M. (1996). Dietary Aspergillus niger phytase increases iron absorption in humans. *J Nutr, 126*(2), 476-480.

Sandberg, A. S., & Svanberg, U. (1991). Phytate Hydrolysis by Phytase in Cereals; Effects on In Vitro Estimation of Iron Availability. *Journal of Food Science, 56*(5), 1330.

Sandstead, H. H. (1991). Zinc deficiency. A public health problem? *Am J Dis Child, 145*(8), 853-859.

Sandstrom, B. (1987). Zinc and dietary fibre. *Scand J Gastroenterol Suppl, 129*, 80-84.

Sandstrom, B., Almgren, A., Kivisto, B., & Cederblad, A. (1987). Zinc absorption in humans from meals based on rye, barley, oatmeal, triticale and whole wheat. *J Nutr, 117*(11), 1898-1902.

Sandstrom, B., Almgren, A., Kivisto, B., & Cederblad, A. (1989). Effect of protein level and protein source on zinc absorption in humans. *J Nutr, 119*(1), 48-53.

Sandstrom, B., Andersson, H., Bosaeus, I., Flakheden, T., Goransson, H., & Melkersson, M. (1983). The effect of wheat bran on the intake of energy and nutrients and on serum mineral levels in constipated geriatric patients. *Hum Nutr Clin Nutr, 37*(4), 295-300.

Sandstrom, B., & Sandberg, A. S. (1992). Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. *J Trace Elem Electrolytes Health Dis, 6*(2), 99-103.

Simons, P. C., Versteegh, H. A., Jongbloed, A. W., Kemme, P. A., Slump, P., Bos, K. D., et al. (1990). Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br J Nutr, 64*(2), 525-540.

Simpson, K. M., Morris, E. R., & Cook, J. D. (1981). The inhibitory effect of bran on iron absorption in man. *Am J Clin Nutr, 34*(8), 1469-1478.

Stephens, L. R., & Irvine, R. F. (1990). Stepwise phosphorylation of myo-inositol leading to myo-inositol hexakisphosphate in Dictyostelium. *Nature, 346*(6284), 580-583.

Stephens, W. P., Klimiuk, P. S., Warrington, S., & Taylor, J. L. (1982). Observations on the dietary practices of Asians in the United Kingdom. *Hum Nutr Appl Nutr, 36*(6), 438-444.

Svanberg, U., Lorri, W., & Sandberg, A. S. (1993). Lactic fermentation on non-tannin and high-tannin cereals: Effects on in vitro estimation of iron availability and phytate hydrolysis. *Journal of Food Science, 58*(2), 408.

Szczesniak, A. S. (2002). Texture is a sensory property *Food Quality and Preference, 13*(4), 10.

Thompson, L. U., Button, C. L., & Jenkins, D. J. (1987). Phytic acid and calcium affect the in vitro rate of navy bean starch digestion and blood glucose response in humans. *Am J Clin Nutr, 46*(3), 467-473.

Turk, M., Sandberg, A. S., Carlsson, N. G., & Andlid, T. (2000). Inositol hexaphosphate hydrolysis by Baker's yeast. Capacity, kinetics, and degradation products. *J Agric Food Chem, 48*(1), 100-104.

Turnlund, J. R., King, J. C., Keyes, W. R., Gong, B., & Michel, M. C. (1984). A stable isotope study of zinc absorption in young men: effects of phytate and alpha-cellulose. *Am J Clin Nutr, 40*(5), 1071-1077.

Vaintraub, I. A., & Lapteva, N. A. (1988). Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Anal Biochem, 175*(1), 227-230.

Weaver, C. M., Heaney, R. P., Martin, B. R., & Fitzsimmons, M. L. (1991). Human calcium absorption from whole-wheat products. *J Nutr, 121*(11), 1769-1775.

Wise, A. (1995). Phytate and zinc bioavailability. *Int J Food Sci Nutr, 46*(1), 53-63.

Xu, P., Price, J., & Aggett, P. J. (1992). Recent advances in methodology for analysis of phytate and inositol phosphates in foods. *Prog Food Nutr Sci*, *16*(3), 245-262.

Zhou, J. R., Fordyce, E. J., Raboy, V., Dickinson, D. B., Wong, M. S., Burns, R. A., et al. (1992). Reduction of phytic acid in soybean products improves zinc bioavailability in rats. *J Nutr*, *122*(12), 2466-2473.

APPENDICES

Appendix A Socio-economic and demographic summary

Q#	Question	N	Percentage
1	Number of people live in the household		
	Three	7	17.95
	Four	8	17.95
	Five	6	17.95
	Six	5	12.82
	Seven	6	15.38
	Eight	4	10.26
	Ten	2	5.13
	Twelve	1	2.56
	Total	39	100

Q#	Question	N	Percentage
2a	Number of children live in the household		
	One	6	15.38
	Two	7	17.95
	Three	8	20.51
	Four	5	12.82
	Five	6	15.38
	Six	4	10.26
	Eight	2	5.13
	Ten	1	2.56
	Total	39	100

Q#	Question	N	Percentage
2b	Number of adult live in the household		
	One	6	15.38
	Two	24	61.54
	Three	7	17.95
	Four	1	2.56
	Five	1	2.56
	Total	39	100

Q#	Question	N	Percentage
3	Head of the household		
	Wife	2	5.13
	Husband	36	92.31
	Grand parent	1	2.56
	Uncle/Aunt	0	0
	Other	0	0
	Total	39	100

Q#	Question	N	Percentage
4	Major income source of the household		
	Farming (Owner operated)	24	61.54
	Tenant farmer	5	12.82
	Agricultural labor	1	2.56
	Non-agriculture laborer	3	7.69
	Self-employed (non farming)	5	12.82
	Paid employment (non farming)	1	2.56
	Unemployed (not working)	0	0
	Other	0	0
		39	100

Q#	Question	N	Percentage
5	Years of schooling completed by Father		
	None	5	13.16
	Read/write	7	18.42
	One to six	17	44.74
	Seven to eight	5	13.16
	Nine to twelve	4	10.53
	College	0	0
	Total	38	100

Q#	Question	N	Percentage
6	Years of schooling completed by Mother		
	None	17	43.59
	Read/write	3	7.69
	One to six	14	35.90
	Seven to eight	3	7.69
	Nine to twelve	2	5.13
	College	0	0
	Total	39	100

Q#	Question	N	Percentage
11	If the water not from public tap, is it treated		
	Yes	5	38.46
	No	8	61.53
	Total	13	100

Q#	Question	N	Percentage
12	If the water not from public tap, methods used to purifying the water		
	Boiling	1	20
	Purifying	3	60
	Nothing	1	20
	Other	0	0
	Total	5	100

Q#	Question	N	Percentage
13	Distance of the water source from family home		
	< 500 m	20	51.28
	>500 m	16	41.03
	Don’t know	3	7.69
	Total	39	100

Q#	Question	N	Percentage
14	Types of latrine used by the family		
	No latrine facility	1	2.63
	Traditional pit latrine (without cover)	12	31.58
	Latrine with protection (no cover)	3	7.89
	Latrine with cover	6	15.79
	Protected latrine with cover	16	42.11
	Flush to sewage/septic tank	0	0
	Total	38	100

Q#	Question	N	Percentage
15	Family home ownership		
	Own house	31	79.49
	Rental house	5	12.82
	Live with relatives	3	7.69
	Other	0	0
	Total	39	100

Q#	Question	N	Percentage
16	Type of house the family owned		
	Grass roof hut	21	53.85
	Corrugated iron roof	16	41.03
	Cemented and corrugated iron roof	0	0
	Others	2	5.13
	Total	39	100

Q#	Question	N	Percentage
17	Type of window the family house made of		
	No windows	13	35.14
	Open window	3	8.11
	Screen mesh	1	2.70
	Wood shutter	12	32.43
	Others	8	21.62
	Total	37	100

Q#	Question	N	Percentage
18	Additional building owned in the compound		
	No other building	26	68.42
	Barn	12	31.58
	Kitchen (separate)	0	0
	Others building	0	0
	Total	38	100

Q#	Question	N	Percentage
19	Major health care access for the family		
	Modern medical care	38	100
	Traditional doctors	0	0
	Spiritual healers	0	0
	Others	0	0
	Total	38	100

Q#	Question	N	Percentage
20a	Use of mosquito net at night (for parent)		
	Yes	37	97.37
	No	1	2.63
	Total	38	100

Q#	Question	N	Percentage
20b	Use of mosquito net at night (for infants)		
	Yes	36	94.74
	No	2	5.26
	Total	38	100

Q#	Question	N	Percentage
21	Own any livestock		
	Yes	28	75.68
	No	9	24.32
	Total	37	100

Q#	Question	N	Percentage
22	Number of cows		
	None	14	35.90
	One	9	23.08
	Two	9	23.08
	Three	4	10.26
	Four	2	5.13
	Five	1	2.56
	Total	39	100

Q#	Question	N	Percentage
23	Number of goats		
	None	38	97.44
	One	0	0
	Two	1	2.56
	Total	39	100

Q#	Question	N	Percentage
24	Number of chicken		
	None	28	71.79
	One	1	2.56
	Two	2	5.13
	Three	1	2.56
	Four	3	7.69
	Five	2	5.13
	Eight	2	5.13
	Total	39	100

Q#	Question	N	Percentage
25	Number of sheep		
	None	36	92.31
	One	1	2.56
	Two	0	0
	Three	1	2.56
	Four	1	2.56
	Total	39	100

Q#	Question	N	Percentage
26	Number of ox		
	None	25	64.10
	One	9	23.08
	Two	4	10.26
	Three	0	0
	Four	1	2.56
	Total	39	100

Q#	Question	N	Percentage
27	Number of donkey/ horse/ mule		
	None	37	94.87
	One	1	2.56
	Two	1	2.56
	Total	39	100

Q#	Question	N	Percentage
28	Own farm land (garden)		
	Yes	35	97.22
	No	1	2.78
	Total	36	100

Q#	Question	N	Percentage
29	Size of the family farm land		
	Less than quarter hectare	6	17.14
	Quarter hectare	7	20.00
	Half hectare	8	22.86
	One hectare	2	5.71
	Greater than one hectare	12	34.29
	Total	35	100

Q#	Question	N	Percentage
30	Grow crops on the land		
	Yes	25	67.57
	No	12	32.43
	Total	37	100

Q#	Question	N	Percentage
31	Grow potatoes on the land		
	Yes	12	34.29
	No	23	65.71
	Total	35	100

Q#	Question	N	Percentage
32	Grow green vegetables on the land		
	Yes	23	63.89
	No	13	36.11
	Total	36	100

Q#	Question	N	Percentage
33	Grow yellow, orange and red veg on the land		
	Yes	14	41.18
	No	20	58.82
	Total	34	100

Q#	Question	N	Percentage
34	Grow tree fruits on the land		
	Yes	22	61.11
	No	14	38.89
	Total	36	100

Q#	Question	N	Percentage
35	Grow coffee on the land		
	Yes	27	71.05
	No	11	28.95
	Total	38	100

Q#	Question	N	Percentage
36	Grow chat on the land		
	Yes	11	30.56
	No	25	69.44
	Total	36	100

Q#	Question	N	Percentage
37a	Number of mature enset plants owned		
	None	20	51.28
	One	1	2.56
	Four	2	5.13
	Five	1	2.56
	Six	1	2.56
	Ten	5	12.82
	Twenty	4	10.26
	Thirty	3	7.69
	Forty	2	5.13
	Total	40	100

Q#	Question	N	Percentage
37b	Age of the enset owned		
	None	18	46.15
	One	3	7.69
	Two	4	10.26
	Three	6	15.38
	Four	2	5.13
	Five	2	5.13
	Ten	3	7.69
	Twenty	1	2.56
	Total	39	100

Q#	Question	N	Percentage
38	Methods of cultivation		
	Rain fed cultivation	25	78.13
	Small traditional irrigation	6	18.75
	Total	31	100

Q#	Question	N	Percentage
39	Number of meal per day the family eat		
	One time	1	2.56
	Two times	9	23.08
	Three times	26	66.67
	More than three times	3	7.69
	Total	39	100

Q#	Question	N	Percentage
40	Source of this week’s family foods		
	Garden	7	17.95
	Purchased	31	79.49
	Wage (in kind)	1	2.56
	Others	0	0
	Total	39	100

Q#	Question	N	Percentage
41	Run out of food before you began to harvest		
	Yes	6	18.75
	No	26	81.25
	Total	32	100

Q#	Question	N	Percentage
42	How long the family stored food after harvest		
	Less than two months	1	3.33
	2-4 months	10	33.33
	5-9 months	12	40.00
	9-12 months	7	23.33
	Total	30	100

Q#	Question	N	Percentage
43	Light source used at home		
	None	1	2.63
	Homemade kerosene lump (kuraz)	33	86.84
	Lamp without glass cover	0	0
	Lamp with glass cover	2	5.26
	Candle	2	5.26
	Total	38	100

Q#	Question	N	Percentage
44	Own umbrella		
	Yes	18	46.15
	No	21	53.85
	Total	39	100

Q#	Question	N	Percentage
45	Wear shoes		
	Yes	34	87.18
	No	5	12.82
	Total	39	100

Q#	Question	N	Percentage
46	Types of shoes		
	Thong	8	21.05
	Plastic	19	50.00
	Leather	11	28.95
	Total	38	100

Q#	Question	N	Percentage
47	Own radio		
	Yes	20	51.28
	No	19	48.72
	Total	39	100

Q#	Question	N	Percentage
48	Own bicycle		
	Yes	8	21.62
	No	29	78.38
	Total	37	100

Appendix B: Infant feeding practices data summary

Q#	Question	N	Percentage
1	Ever breastfed a child		
	No	0	0
	Yes	39	100
	Total		100

Q#	Question	N	Percentage
2	Starting of breast feeding the baby after birth		
	Don't remembers/don't know	30	76.92
	Immediately/within first hour after birth	2	5.13
	After first hour Three	4	10.26
	After 2-6 hours	3	7.69
	After 7-12 hours	0	0
	After more than one day	0	0
	Total	39	100

Q#	Question	N	Percentage
3	Fed the baby colostrums in the first 3 days after delivery		
	Don't know/ remember	1	2.56
	No	9	23.08
	Yes	29	74.36
	Total	39	100

Q#	Question	N	Percentage
4	Gave the baby any other food or drink before breast feeding in the first 3 days after delivery		
	Don’t know/ remember	8	20.51
	No	21	53.85
	Yes	10	25.64
	Total	39	100

Q#	Question	N	Percentage
5	If yes, list each food item given		
	Milk (other than breast milk)	0	0
	Plain water	0	0
	Fruit juice	5	12.82
	Butter	0	0
	Water with sugar/ salt	0	0
	Tea/ coffee	1	2.56
	Other	33	84.62
	Total	39	100

Q#	Question	N	Percentage
6	Currently breastfeeding your baby		
	Don’t know/ remember	0	0
	No	13	35.14
	Yes	24	64.86
	Total	37	100

Q#	Question	N	Percentage
7	How long breast fed the baby so far (month)		
	Twelve	6	26.09
	Sixteen	1	4.35
	Seventeen	2	8.70
	Eighteen	2	8.70
	Twenty four	6	26.09
	Twenty nine	1	4.35
	Thirty	1	4.35
	Thirty one	1	4.35
	Thirty four	1	4.35
	Thirty six	2	8.70
	Total	23	100

Q#	Question	N	Percentage
8	How long will you continue to breast feed		
	1-6 months	2	6.25
	6-12 months	1	3.13
	1-2 years	4	12.50
	2-3 years	15	46.88
	3-5 years	7	21.88
	Others	3	9.38
	Total	32	100

Q#	Question	N	Percentage
9	If not, why did you stop breast feeding		
	Getting pregnant	2	14.29
	Tired of breast feeding	0	0
	Mom get sick	4	28.57
	Breast milk make the baby sick	0	0
	Introduced solid foods	1	7.14
	Lack of enough breast milk	2	14.29
	Others	4	30.77
	Total	14	100

Q#	Question	N	Percentage
10	How many times in the last 24 hrs did you breastfeed your baby		
	Once	0	0
	Twice	1	4.00
	Three times	1	4.00
	More than three times	23	92.00
	Total	25	100

Q#	Question	N	Percentage
11	Gave any liquid food to the baby yesterday		
	No	9	23.08
	Yes	30	76.92
	Total	39	100

Q#	Question	N	Percentage
12	If yes, types of liquid food given to the baby		
	Cow milk	9	23.08
	Plain water	8	20.51
	Fenaguric water	18	46.15
	Fruit juice	0	0
	Coffee	0	0
	Tea	1	2.56
	Any other types of milk	3	7.69
	Any other types of liquid	0	0
	Total	39	100

Q#	Question	N	Percentage
13	Did the baby fed from bottle with nipple		
	Don’t know/ remember	4	10.26
	No	33	84.62
	Yes	2	5.13
	Total	39	100

Q#	Question	N	Percentage
14	Did the baby started eating solid (semi) food		
	Don’t know/ remember	0	0
	No	0	0
	Yes	39	100
	Total	39	100

Q#	Question	N	Percentage
15	When did the baby start complementary food		
	Before 4 month of age	0	0
	Between 4-6 month of age	11	28.95
	Between 7-8 month of age	11	28.95
	Between 9-12 months of age	10	26.32
	Between 1-2 years of age	4	10.53
	Other	2	5.26
	Total	38	100

Q#	Question	N	Percentage
16	Reason for start weaning the baby with food		
	No enough breast milk	6	15.79
	Baby crying always	2	5.26
	Baby active	0	0
	Told to	7	18.42
	No time for breast feeding	1	2.63
	Baby reaching for food	2	5.26
	Baby reaching for food	1	2.63
	Other	19	50.00
	Total	38	100

Q#	Question	N	Percentage
17	How was the baby complementary food started		
	Abrupt	2	5.26
	Gradual	36	94.74
	Others	0	0
	Total	38	100

Q#	Question	N	Percentage
18	How often give solid (semi) food to the baby		
	1-2 times per day	4	10.53
	3-4 times per day	16	42.11
	4-6 times per day	15	39.47
	≥7 per day	3	7.89
	Others	0	0
	Total	38	100

Q#	Question	N/39	Percentage
19	Which of the foods did the baby eat?		
	Cereals	35	89.74
	Maize bread	37	94.87
	Kocho (Bulla)	35	89.74
	Enjera (Teff)	36	92.31
	Beans	28	71.79
	Yellow/ orange vegetables	35	89.74
	Dark green vegetables	30	76.92
	Root and tuber	25	64.10
	Beef, pork, lamb, goat	18	46.15
	Chicken, duck or other birds	17	43.59
	Fresh or dried fish	9	23.08
	Eggs	33	84.62
	Cheese or yogurt	2	5.13

Q#	Question	N/39	Percentage
20	Which of the foods considered good for the baby?		
	Maize porridge	34	87.18
	Maize bread	9	23.08
	Barley porridge	8	20.51
	Kocho	5	12.82
	Bulla	1	2.56
	Enjera (Teff)	7	17.95
	Beans	1	2.56
	Banana	2	5.13
	Carrot	1	2.56
	Avocado	3	7.69
	Potato	24	61.54
	Eggs	20	51.28
	Fish	39	100
	Milk	30	76.92
	Meat	2	5.13
	Cheese	1	2.56
	Rice	1	2.56
	Cabbage	5	12.82
	Cereals except maize	2	5.13
	Pasta / Macaroni	4	10.26

Q#	Question	N/39	Percentage
21	Which foods are considered **not** good for the baby?		
	Kocho	6	15.38
	Beans	2	5.13
	Buttermilk	2	5.13
	Raw meat	3	7.69
	Sweet potato	12	30.77
	Hamicho	3	7.69
	Cabbage	4	10.26

Q#	Question	N/39	Percentage
22	Which foods are good for the baby during sickness?		
	Maize bread	8	20.51
	Potato	1	2.56
	Eggs	2	5.13
	Tea	8	20.51
	Milk	1	2.56
	Pasta/ Macaroni	2	5.13
	Rice	2	5.13
	Enjera (Teff)	4	10.26
	Soft drink	1	2.56

Q#	Question	N	Percentage
23	Withhold some food from your baby when sick		
	No	16	45.71
	Yes	19	54.29
	Total	35	100

Q#	Question	N/39	Percentage
24	Which foods are good for the baby after sickness recovery?		
	Maize bread	8	20.51
	Potato	4	10.26
	Eggs	5	12.82
	Tea	3	7.69
	Banana	1	2.56
	Milk	5	12.82
	Pasta/ Macaroni	2	5.13
	Cheese	1	2.56
	Enjera (Teff)	6	15.38
	Barley porridge	7	17.95
	Kocho	1	2.56
	Bulla	1	2.56
	Butter	2	5.13

Q#	Question	N	Percentage
25	Method/ material used to feed the baby		
	Spoon/ other utensil	16	41.03
	Pre-masticated	16	41.03
	From mother’s hand	0	0
	Others	7	17.95
	Total	39	100

Q#	Question	N	Percentage
26	Convenient time followed to feed the baby		
	When convenient for mother	9	25.00
	On baby's demand	27	75.00
	Total	36	100

Q#	Question	N	Percentage
27	Feeding style usually used to feed the baby		
	Encourage the baby eat more	33	89.19
	Doesn't encourage the baby eat more	1	2.70
	Force the baby to eat more	3	8.11
	Total	37	100

Q#	Question	N	Percentage
28	Source of food usually given to the baby		
	Food prepared for family	29	78.38
	Food prepared only for the baby	8	21.62
	Other	0	0
	Total	37	100

Q#	Question	N	Percentage
29	Person usually prepare the baby's food		
	Mother	35	94.59
	Care giver	2	5.41
	Sibling	0	0
	Other	0	0
	Total	37	100

Q#	Question	N	Percentage
30	Person usually feed the baby with the food		
	Mother	30	81.08
	Care giver	1	2.70
	Sibling	1	2.70
	Other	5	13.51
	Total	37	100

Appendix C: Percentage for sensory quality attributes of maize-based porridges with and without dried meat

	Porridge Samples with and without meat											
	PWS-12h				SG-12-48h				LWS-12h			
	Color	Flavor	Smell	Texture	Color	Flavor	Smell	Texture	Color	Flavor	Smell	Texture
Degree of choice	n=79	n=80	n=81	n=78	n=79	n=80	n=81	n=78	n=79	n=80	n=81	n=78
Dislike extremely	0	0	1.23	1.28	1.28	1.25	1.27	2.60	2.56	1.25	2.53	3.90
	(1.23)	(3.70)	(4.94)	(5.13)	(1.23)	(6.17)	(7.41)	(2.56)	(2.47)	(4.94)	(9.88)	(3.85)
Dislike very much	1.27	1.25	2.47	7.69	6.41	8.75	3.80	11.69	1.28	3.75	3.80	5.19
	(4.94)	(6.17)	(11.11)	(5.13)	(3.70)	(7.41)	(9.88)	(6.41)	(2.47)	(8.64)	(14.81)	(5.13)
Dislike moderately	6.33	12.50	19.75	11.54	19.23	18.75	25.32	15.58	8.97	16.25	18.99	9.09
	(9.88)	(18.52)	(25.93)	(15.38)	(23.46)	(29.63)	(35.80)	(19.23)	(16.05)	(25.93)	(22.22)	(17.95)
Dislike slightly	6.33	13.75	4.94	11.54	5.13	15.00	11.39	9.09	7.69	11.25	6.33	10.39
	(7.41)	(13.58)	(13.58)	(12.82)	(16.05)	(8.64)	(16.05)	(12.82)	(11.11)	(11.11)	(13.58)	(16.67)
Neutral	2.53	2.50	3.70	6.41	6.41	10.00	2.53	11.69	2.56	5.00	5.06	7.79
	(6.17)	(3.70)	(1.23)	(10.26)	(2.47)	(7.41)	(3.70)	(10.26)	(6.17)	(6.17)	(2.47)	(8.97)
Like slightly	31.65	33.75	25.93	24.36	26.92	22.50	25.32	23.38	26.92	25.00	24.05	19.48
	(28.40)	(29.63)	(20.99)	(24.36)	(24.69)	(23.46)	(14.81)	(23.08)	(29.63)	(18.52)	(17.28)	(21.79)
Like moderately	16.46	6.25	16.05	14.10	10.26	7.50	12.66	14.29	23.08	17.50	15.19	22.08
	(13.58)	(9.88)	(12.35)	(7.69)	(12.35)	11.11)	(8.64)	(14.10)	(17.28)	(17.28)	(11.11)	(15.38)
Like very much	22.78	25.00	17.28	15.38	19.23	13.75	15.19	7.79	12.82	15.00	13.92	16.88
	(18.52)	(12.35)	(6.17)	(15.38)	(13.58)	(4.94)	(1.23)	(8.97)	(8.64)	(6.17)	(4.94)	(6.41)
Like extremely	12.66	5.00	8.64	7.69	5.13	2.50	2.53	3.90	14.10	5.00	10.13	5.19
	(9.88)	(2.47)	(3.70)	(3.85)	(2.47)	(1.23)	(2.47)	(2.56)	(6.17)	(1.23)	(3.70)	(3.85)

All score values are in terms of percentage of (n) mothers and panelist. Values in parenthesis are percentage of porridge with meat. PWS-12h: Porridge prepared from pounded maize soaked in water for 12 hours;
SG-12-48h: Porridge prepared from maize soaked (12 hours) in water and germinated (48hours);
LWS-12h: Porridge prepared from maize soaked (12 hours) in lemon-water (1:10v/v) for 12hours

Appendix D: Socio-economic Questionnaire

Mother's name___________________ Mother's Code _________

Hawassa University
Effects of home-based phytate reduction on maize-based infant foods:
Method to improve zinc bioavailability in infant's diet, Sidama, Southern Ethiopian
Socio-economic status Questionnaire (PQ-02-SES)

Section A: Personal Identifier

Mother's Code _________________ Baby's code _____________________
Kebele/ farmer's association ____________________ Zone ________ Region ____________

Name of Interviewer ___________________Date ______________ Time _________

Section B: Socio-economic status

1. How many people (adult and children) usually live in this household? ________

2. List all household members

Relation	**Age (y)**	**Relation**	**Age (y)**

3. Who is the head of the household?
   - □ Yourself
   - □ Husband,
   - □ Grandparent,
   - □ Uncle/Aunt
   - □ Other

4. What is the main income source of the household?
   - □ Farming (owner-operated)
   - □ Tenant farmer
   - □ Agricultural laborer
   - □ Non-agricultural laborer
   - □ Self-employed non farming
   - □ Paid employment non farming
   - □ Unemployed not working
   - □ Other ________________

5. How many years of schooling did your husband complete?
   - □ None
   - □ Read/write
   - □ One to six
   - □ Seven to eight
   - □ Nine to twelve
   - □ Collage

6. How many years of schooling did you (mother) complete?
   - □ None
   - □ Read/write
   - □ One to six
   - □ Seven to eight
   - □ Nine to twelve
   - □ Collage

8. Ethnicity of the husband?
   - ◻ Sidama
   - ◻ Amhara
   - ◻ Guraghe
   - ◻ Wolayita
   - ◻ Kembata
   - ◻ Other ____________

9. Ethnicity of the mother?
   - ◻ Sidama
   - ◻ Amhara
   - ◻ Guraghe
   - ◻ Wolayita
   - ◻ Kembata
   - ◻ Other ____________

10. Religion of household Hasband?
   - ◻ Muslim,
   - ◻ Catholic,
   - ◻ Other ________________________
   - ◻ Orthodox
   - ◻ None

11. Religion of household mother?
   - ◻ Muslim,
   - ◻ Catholic,
   - ◻ Other ________________________
   - ◻ Orthodox
   - ◻ None

12. What is the main source of drinking water for your household at this time?
   - ◻ Public tap
   - ◻ Protected well
   - ◻ River/stream
   - ◻ Spring
   - ◻ Borehole
   - ◻ Unprotected well
   - ◻ Lake
   - ◻ Other __________

13. If not from public tap, is your drinking water treated?
   - ◻ Yes
   - ◻ No

   If yes, what method do you use to purify the drinking water?
   - ◻ Boiled
   - ◻ Purified
   - ◻ Other ____________________

14. How far in meters is the water source from your dwelling?
   - ◻ < 500 m
   - ◻ > 500m
   - ◻ Don't know

15. What kind of latrine facility does your household use?
   - ◻ Traditional pit latrine (without cover)
   - ◻ Protected latrine with cover
   - ◻ Flush to sewage/septic tank
   - ◻ Latrine with cover
   - ◻ No household facility
   - ◻ Latrine with protection (no cover)

16. How is your home owned?
   - ◻ Own house
   - ◻ Live with relatives
   - ◻ Rental house
   - ◻ Other ______________

17. What type of house do you have?
- □ Grass roof hut
- □ Corrugated iron roof house
- □ Cement walls and corrugated iron roof
- □ Other ______________

18. What are the windows of your house made of?
- □ No windows
- □ Windows, but open
- □ Screen-mesh
- □ Wood shutters
- □ Other _________________

19. Do you have other buildings at your residence?
- □ No
- □ Barn
- □ Separate cooking room
- □ Other _________________

20. What is the major source of health care for you?
- □ Modern medical care
- □ Traditional doctor
- □ Spiritual healer
- □ Other __________________

21. Do you use a mosquito net at night?
    Parent? □ Yes □ No
    Baby? □ Yes □ No

22. Do you have any livestock?
- □ Yes
- □ No, If No skip to 29

23. If yes, how many cows does your household have? (Use zero for none)__________

24. How many goats does your household have? (Use zero for none)_____________

25. How many chickens does your household have? (Use zero for none)________

26. How many sheep does your household have? (Use zero for none)_____________

27. How many oxen does your household have? (Use zero for none)_____________

28. How many donkeys/horse/mule does your household have? (Use 0 for none)___

29. Does your household have private farm land (garden)?
- □ Yes
- □ No

30. What is the size of your land?
- □ Less than quarter hectare
- □ Quarter hectare
- □ Half hectare
- □ One hectare
- □ More than one hectare

31. Do you grow any crops on your land?
- □ Yes
- □ No

32. If Yes, do you grow maize?
- □ Yes
- □ No

33. If yes, do you grow potatoes?
- □ Yes
- □ No

34. If yes, do you grow green vegetables (e.g. cabbage, peppers)?
□ Yes □ No

35. If yes, do you grow yellow, red or orange vegetables (e.g. tomato, carrot & pumpkin)?
- □ Yes
- □ No

36. If yes, do you grow tree fruits (e.g. oranges, peaches, and guava)?
- □ Yes
- □ No

37. If yes, do you grow coffee?
- □ Yes
- □ No

38. If yes, do you grow chat?
- □ Yes
- □ No

39. How many mature Enset plants do you own (Use zero for none) _______________
How old usually your enset plant when you eat them? ________________

40. How do you grow crops on your land?
- □ Rain fed cultivation (Meher & belg)
- □ Small traditional irrigation

41. How many meals per day do you usually eat?
□ One times □ Two times
□ Three times □ More than three times

42. Where has this week's food come from?
□ Garden □ Purchased
□ Wages in kind after working □ Other _______________

43. Has your household staple food run out this year before you began to harvest?

- □ Yes
- □ No

44. How long does your food store usually last after harvest?

- □ Less than two months
- □ Two to four months
- □ Five to eight months
- □ Nine to twelve months

45. What kind of light source do you use in your house?
You may check more than one answer)

- □ None
- □ Homemade with kerosene filled bottle
- □ Lamp without glass
- □ Lamp with glass Cover
- □ Candle

46. Do you own an umbrella?

- □ Yes
- □ No

47. Is the mother wearing shoes *(By observation)?

- □ Yes
- □ No

48. If yes, describe the shoes types?

- □ Thongs
- □ Plastic
- □ Leather

49. Do you own a radio?

- □ Yes
- □ No

50. Do you own a bicycle?

- □ Yes
- □ No

Thank you!

Appendix E: Infant feeding questionnaire

Mother's name____________ Code _______ Baby's name _____________ Code ____

Hawassa University
Effects of home-based phytate reduction on maize-based infant foods:
Method to improve zinc bioavailability in infant's diet, Sidama, Southern Ethiopian
Infant feeding questionnaire (IF-03)

Section A: Personal Identifier

Mother's code ________ age (y) ________ weight (kg) _________ height (m) ________
Baby's code ______ age (mo) _____ Sex _____ weight (kg) ______ length (cm) ______
Kebele ________ Farmer's association _________ Zone _________ Region __________
Name of Interviewer ______________________ Date (mm/dd/yyyy) ______________

Section B: Breast feeding

1. Did you ever breastfeed (NAME)
   - □ Yes
   - □ No
2. How long did you put (NAME) to the breast after birth?
   - □ Immediately/within first hour after birth)
   - □ After first hour
   - □ After 2-6 hours
   - □ After 7-12 hours
   - □ After more than one day
   - □ Don't remembers/don't know

3. Did you give (NAME) liquid came from your breasts before third day after delivery?
   - □ Yes
   - □ No
   - □ Don't know
4. Did you give (NAME) anything else to eat or drink before feeding breast milk in the first three days after delivery,?
   - □ Yes
   - □ No
   - □ Don't know
5. If Yes, what did you give (NAME)?

   Note: Record all mentioned by checking for each item mentioned (don't read the list)
   - □ Milk (other than breast milk) □ Butter
   - □ Plain water □ Water with sugar and/or salt
   - □ Fruit juice □ Tea/coffee infusions
   - □ Other (Specify) ___________________________

6. Are you currently breastfeeding (NAME)?

- □ Yes
- □ No

7. If yes, for how long did you breastfeed (NAME)?

Months _____________ (if less than one month, record “00” months)

8. How long will you breast feed?

- □ 1-6 months
- □ 6-12 months
- □ 1-2 years
- □ 2-3 years
- □ 3-5 years
- □ Other ____________

9. If no, why did you stop breast feeding?

- □ Mother pregnant
- □ Mother sick
- □ Mother tired of breast feeding
- □ Introduced solids
- □ Breast milk making child sick
- □ Not enough milk
- □ Other Please specify __________

11. If you are currently breast feeding, how many times did you breastfeed within 24 hrs?

- □ Once
- □ Twice
- □ Three times
- □ More than three times

12. Did you give (NAME) any type of liquid food yesterday during the day or the night?

- □ Yes
- □ No

13. If yes, did (NAME) drink any of the following liquids?

<u>Note:</u> Read list of liquids and check in the box if child drank the liquid in question

- □ Cow milk
- □ Plain water
- □ Abish wuha
- □ Fruit juice
- □ Coffee
- □ Tea
- □ Any other milk (tinned, powdered, or other animal milk)
- □ Any other liquids such as sugar water, soup broth? ______________

14. Did (NAME) drink such food from a bottle with a nipple?

- □ Yes
- □ No
- □ Don’t know

Section B: Infant feeding (Solid and semi-solid food)

18. Did you start giving (NAME) solid or semi-solid foods other than breast milk?

- □ Yes
- □ No

19. When did you start weaning (NAME)?

- □ Before 4 months
- □ 4-6 months
- □ 7-8 month
- □ 9-12 months
- □ 1-2 years
- □ Other _____________

20. What made you decide to start giving solid or semi-solid foods to (NAME)?

□ Not enough breast milk
□ Baby always crying
□ Baby active
□ Told to
□ Not enough time to breastfeed
□ Baby reaching for food
□ Tradition
□ Other _________

21. How was the weaning process started?

□ Abrupt
□ Gradual
□ Other ____________

22. How often do you give (NAME) solid, semi-solid, or liquid food than breast milk?

□ 1-2 times per day
□ 3-4 times per day
□ 4-6 times per day
□ ≥ 7 times per day
□ Other ________________

23. How many times did (NAME) eat solid, semi-solid, or soft foods other than liquid food yesterday during the day and at the night?

□ _____ times (Use probing questions to help the respondent remember)
□ Other ________

24. Did (NAME) eats any of the following foods type in the past?

Note: Read the list of foods. Place a check in the box if child ate the food in question

□ Porridge or gruel prepared from cereal grains
(e.g. made with maize, sorghum, millet, wheat, barley, teff)?
□ Corn bread?
□ Enset foods (specify, kotcho and bulla)?
□ Enjera prepared from cereals, specify cereals _____________
□ Any foods made from beans
(kidney beans, haricot beans, field peas, cowpeas, chick peas or others?
□ Pumpkin, carrots, or yellow/orange-fleshed sweet potatoes?
□ Any other food made from roots or tubers other than enset?
(White potatoes, white yams, cassava, Boyna)
□ Any dark green leafy vegetables?
(Cassava leave, pumpkin leave, kale, or other dark green leaves)
□ Any beef, pork, lamb, goat?
□ Any chicken, duck, or other birds?
□ Any fresh or dried fish?
□ Any eggs?
□ Any groundnuts/peanuts, or any nuts?
□ Any cheese or yogurt?
□ Any food with oil, fat, or butter?

25. Which food and drinks are considered good for (NAME)? Why?

Name the foods	Benefit of the food
1	
2	

26. Which foods do mothers say should NOT be fed to infants at this age?

- ◻ Corn porridge ◻ Other porridge ◻ Corn bread ◻ Kotcho
- ◻ Bulla ◻ Enjera ◻ Beans ◻ Biscuits
- ◻ Vegetables ◻ Fruits ◻ Potatoes ◻ Egg
- ◻ Fish ◻ Meat ◻ Soft cheese

27. Do you give special foods to your child when (Name) is ill?

◻ Yes ◻ No ◻ Specify ___________________

28. Do you withhold food from your child when (Name) is ill?

◻ Yes ◻ No ◻ Specify ___________________

29. Do you give special food to your child after illness?

◻ Yes ◻ No ◻ Specify ___________________

30. How does (Name) feed her/his food?

◻ Spoon or other utensil ◻ From the mother's hand
◻ Pre-masticated ◻ Other _________________

31. Which feeding practice is usually followed when breast feeding the infant?

◻ When it is convenient for the mother ◻ On the baby demand

32. Which feeding practice do you follow when feeding infant complementary foods?

◻ When it is convenient for the mother ◻ On the baby demand

33. What feeding style is usually used to feed the infant complementary foods?

◻ Encouraged to eat more ◻ Not encouraged to eat more ◻ forced to eat more

34. What is the source of the food usually given to the infant?

◻ Food prepared for family ◻ Food prepared only for the baby
◻ Other, specify ____

35. If infant fed food prepared only for him/her, please describe how it prepared?

____

____

36. Who usually prepare the infant's food?

◻ Mother ◻ Care-giver ◻ Sibling
◻ Other, specify ____

37. Who usually feeds the infant?

- ◻ Mother
- ◻ Care-giver
- ◻ Sibling
- ◻ Other, specify ____

Thank you

Appendix F: Sensory evaluation form

Mother's name____________ Mother's code _______ Last baby's name _______________

Hawassa University

Effects of home-based phytate reduction on maize-based infant foods: Method to improve zinc bioavailability in infant's diet in Sidama, Southern Ethiopian

Mothers sensory evaluation form (SQ-01)

Instructions for sensory evaluation

Please include your name, Set number, sample code numbers, date of tasting on the sensory evaluation sheet.

You will receive three samples of maize-based porridge samples at each session. Please indicate your degree of preference / acceptance of the porridge samples using a nine point scale. Nine indicates extremely high preference, five as a neutral and one as extremely dislike.

Distilled water will be provided for rinsing purpose. Please use distilled water to rinse your mouth between samples. Please make any other comments that will help us in evaluating these products.

Thank you for volunteering your time and effort for this research project.

Mother's personal identification

Mother's Code _______ age (y) _______ No of children ______ Age of last baby ____
Kebele _________ Date of testing _______

Food sample identification
Samples set No________, (Sample code 1 __________, 2 __________, 3 __________)

Instruction: Please taste the following three maize-based porridge samples and indicates your degree of preference on how much you like or dislike its color, flavor, smell, texture, & over all acceptability using a nine point scale that best describe your feeling of each sample's attribute. Use the scale below to best describe your feeling and preference.

9 = like extremely	**6** = like slightly	**3** = dislike moderately
8 = like very much	**5** = neutral (Not like or dislike)	**2** = dislike very much
7 = like moderately	**4** = dislike slightly	**1** = dislike extremely

Hedonic rating scale

Please rinse your mouth thoroughly with distilled water before tasting each sample.

Code	Color	Flavor (taste)	Smell	Texture	Overall acceptability
______	______	______	______	______	______
______	______	______	______	______	______
______	______	______	______	______	______

Ranking for the degree of preference

Please rank these samples for your preference. Rank the sample you like the best as one (1) and the sample you like the least as three (3)

Code number	Order of preference
_______________	_______________
_______________	_______________
_______________	_______________

Please make any other comments that will help us in evaluating these products:
____
____

Thank you!

Appendix G: Texture profile evaluation form

Mother's name________ Mother's code ______ Child's name ___________ Code _____

Hawassa University

Effects of home-based phytate reduction on maize-based infant foods: Method to improve zinc bioavailability in infant's diet in Sidama, Southern Ethiopian

Texture profile testing form (TP-01)

Instructions for texture profile technique

Procedure for determination of "ideal' weaning maize-based porridge for texture.

Please indicate your name, set number, samples code, date and time of testing.

We are interested in how you describe textural characteristics of ideal weaning food. Please complete the texture profile evaluation sheet before beginning actual testing before tasting the food.

You are provided with four samples of porridge and an evaluation sheet consisting of list of terms commonly used to describe the texture, which is how food feels in the mouth. It is important to the test you indicate your choice for each of the terms tested. Please check one of the boxes along the right side of each term by placing an "x" to indicate the degree to which you believe an "ideal" weaning sample should have the textural characteristics described by those terms.

Distilled water will be provided for rinsing purpose. Please use distilled water to rinse your mouth between samples. Please make any other comments that will help us in evaluating these products.

Thank you for volunteering your time and effort for this research project.

Mother's personal identification
Mother's Code _______ age (y) _______ No of children ______ Age of last baby ____

Food sample identification
Samples set No_____, Sample code _____ Date of testing _________ Time _______

Instruction: You are provided with four porridge samples. Please check one of the boxes along the right side of each term by placing an "x" to indicate the degree to which you believe an "ideal" weaning sample should have the textural characteristics described that term. Please use distilled water provided to rinse your mouth between samples.

	Not at all					**Very much**
Grainy	☐	☐	☐	☐	☐	☐
Easy to swallow	☐	☐	☐	☐	☐	☐
Thick	☐	☐	☐	☐	☐	☐
Mouth-coating	☐	☐	☐	☐	☐	☐
Good texture	☐	☐	☐	☐	☐	☐
Smooth	☐	☐	☐	☐	☐	☐
Thin	☐	☐	☐	☐	☐	☐
Lumpy	☐	☐	☐	☐	☐	☐
Creamy	☐	☐	☐	☐	☐	☐
Bad texture	☐	☐	☐	☐	☐	☐
Hard to swallow	☐	☐	☐	☐	☐	☐
No after taste	☐	☐	☐	☐	☐	☐
Watery	☐	☐	☐	☐	☐	☐
After taste	☐	☐	☐	☐	☐	☐

Please make any other comments that will help us in evaluating these products:
____

Thank you!

Appendix H: Children sensory evaluation form

Mother's name_________ Mother's code ______ Child's name ____________ Code _____

Hawassa University

Effects of home-based phytate reduction on maize-based infant foods: Method to improve zinc bioavailability in infant's diet in Sidama, Southern Ethiopian

Children_sensory evaluation form (TP-01)

Personal identification

Child's code ______________Age __________________ Sex _________________________
Mother's code ________________________ Kebele / Farmers association ______________
Sample Code _________________________ Date _________________ Time ____________

Children score card

1. Does the child appear to like the porridge sample? Please mark one of the following.

Great	**Pretty good**	**Somewhat**	**Didn't like**
☐	☐	☐	☐

2. How can you tell?

3. How much porridge did the child eat? Please check one.

All	**More than half**	**Less than half**	**None**
☐	☐	☐	☐

Please make any other comments that will help us in evaluating these products:
____
____

Thank you!

Appendix I: Institutional Review Board Approval from Hawassa University

ሀዋሳ ዩኒቨርሲቲ
ጤና ሳይንስ ኮሌጅ

Hawassa University
College of Health Science

Ref. No HSC/2737/08
Date 14-03-08

To: AVP-REP Office

From: College of Health Sciences

Subject: **Ethical Clearance**

The Institutional Research Review Committee of College of Health Sciences, Hawassa University, on its meeting of 10/03/08 has reviewed the following research proposal entitled,

"Effects of Home-Based Phytate reduction on Maize-Based infant Foods: Methods to improve Zinc Bioavailability in infants diet in Sidama Zone of Southern Ethiopia" by Mr. Alemzewd Chala (Staff of Hawassa University who is on a study leave for masters program).

The proposal was reviewed as per the national research review guidelines and approved for ethical clearance. Attached herewith please find 03 pages of the attached minutes.

Kind Regards

Dr. Yifru Berhan Mitke
Head, College of Health Science

CC
Mr. Alemzewd Chala
ACA

REO
CHS

IRB Minutes No 02/08

Date: 10/03/08

Place: REO

Time: 2:00 PM

Present:

1. Ato Dejene Hailu--Chairperson
2. Ato Eskindir Loha---Secretary
3. Dr. Belayhun Kibret--------------------------------------Member
4. Ato Keneni Gutema---------------------------------------Member

Not Present:

1. Dr. Tadesse Anteneh (excused)
2. Dr. Araya Giday (excused)

Agenda Items: **Ethical Clearance**

The following two proposals were presented for review of ethical issues.

1. ***"Zinc and Maternal-Infant Brain Function: Randomized Control Trials in the Southern Ethiopia, by Prof. Michael Hambidge and Dr. Yewelsew Abebe (staff of ACA)."***

2. ***"Effects of Home-Based Phytate reduction on Maize-Based infant Foods: Methods to improve Zinc Bio-availability in infants diet in Sidama Zone of Southern Ethiopia, by Mr. Alemzewd Chala (Staff of ACA)".***

The chairperson distributed copies of the proposal ahead of time to the members of IRB for detail review. The committee has gone through the proposals with respect to ethical issues and has come up with written comments for further discussions. The summary of the comments and consensus of the reviewers is presented below:

"Zinc and Maternal-Infant Brain Function: Randomized Control Trials in the Southern Ethiopia":

- The consent form of the protocol needs to provide adequate information about the study such as benefits and possible risks and discomforts related to the study (if any) to the individuals participating in the study. The Committee feels that this was fairly addressed in the English version. The equivalent Amharic version of the consent form a bit seem to miss few components mentioned in the English copy (for example page 2-3 of the first 5-page consent form). The researchers need to have a look at it and also make correction of some typographic errors in the Amharic version of the consent form.

- Moreover, the research generally involves the following conditions:
  - Women and children are its study subjects
  - Collection of biological products
  - Financed by the external donors
  - Involves a foreigner as a Principal Investigator

As it is clearly indicated in the National Health Research Ethics Review Guidelines, the committee has agreed that such studies need to seek the final approval of the ethical clearance from The NERC. Provided that comments provided above are considered, the IRB of College of Health Sciences hereby recommend the commencement of the research and hereby ***approves*** the ethical clearance of the protocol.

2

"Effects of Home-Based Phytate reduction on Maize-Based infant Foods: Methods to improve Zinc Bio-availability in infants diet in Sidama Zone of Southern Ethiopia, by Mr. Alemzewd Chala (Staff of ACA)".

- The consent form needs to be translated into the appropriate local language and attached to the protocol.
- The research involves mothers and infants and the extent of the information or data produced is beyond just the KAP study. Hence such study also requires approval form the NERC.

The committee believes that the research has no ethical problems in its procedures. Hence, provided that comments provided above are considered by the author, the IRB of College of Health Sciences hereby recommend the commencement of the above research and hereby ***approves*** the ethical clearance of the protocol.

Appendix J: Institutional Review Board Approval from Oklahoma State University

Oklahoma State University Institutional Review Board

Date:	Tuesday, July 29, 2008
IRB Application No	HE0842
Proposal Title:	Effects of Home-Based Phytate Reduction on Maize-Based Infant Foods, Method to Improve Zinc Bioavailability in Infant's Diet in Sidama, Southern Ethiopia
Reviewed and Processed as:	Expedited (Spec Pop)

Status Recommended by Reviewer(s): Approved Protocol Expires: 7/28/2009

Principal Investigator(s):

Alemzewed C. Roba
301 HES
Stillwater, OK 74078

Barbara Stoecker
421 HES
Stillwater, OK 74078

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval.
2. Submit a request for continuation if the study extends beyond the approval period of one calendar year. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of this research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact Beth McTernan in 219 Cordell North (phone: 405-744-5700, beth.mcternan@okstate.edu).

Sincerely,

Shelia Kennison, Chair
Institutional Review Board

Invitation for participants in sensory evaluation study

Study title: Effects of home-based phytate reduction on maize-based infant foods: Methods to improve Zinc bioavailability in infant's diet In Sidama zone, Southern Ethiopia

Description: Maize is one of the major cereals having a large amount of phytate content. Phytate in maize-based diets hinders zinc bioavailability and causes zinc deficiency. Zinc deficiency is believed to be the limiting factor for many enzymatic and metabolic disorders and causes infant morbidity and mortality. When zinc is deficient in an infant's body, the chance of having delayed growth and getting sick will be pronounced. Zinc deficiency can also affect the infant's brain development. Therefore, this study compares traditionally processed improved maize-based infant food with locally consumed types of maize-based foods using sensory and acceptability methods. Six test samples, which were prepared from locally purchased maize, mixed with either dried meat or kidney beans or alone will be presented for each evaluator in three samples per batch base. The low phytate improved maize-based infant food was developed using home based traditional processing methods. Dried meat and kidney beans were added to further improve the food's nutrient content. The overall purpose of the study is to develop potential methods to reduce phytate from maize-based infant foods and, as a result, improve zinc bioavailability in infants' diets in Sidama, Southern Ethiopia

Eligible participant: Any interested female Hawassa university staff/ student members who are willing to participate in such a study.

Responsibility: You will be asked to assess sensory characteristics of sample foods using nine point Hedonic scales and texture profiling using six point hedonic scales.

Data sought: Degree of preference towards color, texture, flavor (aroma), taste and overall acceptability Of sample foods using sensory testing and texture profiling.

Discomfort and risks: There is no known risk associated with tasting the samples than those ordinarily Encountered in daily life. Therefore, participation does not pose serious discomfort or risks.

Benefits: This study is not intended to cure or treat any illnesses, but to learn if low phytate maize-based Infant foods could be acceptable in the study area. If the results are positive, the findings will Be useful to develop health and nutrition programs that reduce zinc deficiency in Sidama.

Cost to participate: No cost accrues for participation in the study.

Time taken: 30 - 60 minutes per day for four days

Withdrawal: You may choose not to be in the study or stop whenever you wish.

Contact address: Alemzewed Challa Roba
Hawassa University, College of Agriculture,
Department of Rural Development & Family Sciences
Tel. 251-046-2206698 (Office) or 251-0916-823122 (mobile)
E-mail alemzewed.c.roba@okstate.edu or alemzewedcr@yahoo.com

Hawassa University

"Effects of Home-Based Phytate Reduction on Maize-Based Infant Foods"

Script for Hawassa Students

We are studying the "Effects of home-based phytate reduction on maize-based infant foods". We are asking for your help with sensory and texture testing of these complementary foods for young children. Sensory tests evaluate flavor and the texture tests primarily evaluate "mouth feel". These types of tests are used by companies developing new foods, so you would have an opportunity to learn how this type of testing is organized.

There are six different types of porridges to be tasted. Several samples of each one would be tasted on four different days.

Please see Alemzewed to volunteer for the study. Or, you may contact the Rural Development and Family Science Department. An informational meeting will be scheduled for persons who are interested in volunteering for this study.

(Copies of the flyer will be distributed to interested students)

Hawassa University

"Effects of Home-Based Phytate Reduction on Maize-Based Infant Foods"

Script for Rural Mothers

We are studying the "Effects of home-based phytate reduction on maize-based infant foods". We are asking for your help with testing how good the porridges taste and feel in your mouth. We are also asking you to let your young child eat a small amount of the porridge after you have tasted it if they are willing. Then we want you to judge how well your child liked the porridge.

There are six different porridges to be tasted. Several samples of each one would be tasted on four different days and it would require about 30 minutes to one hour of your time each day. We would also ask that you give small samples on each of four days to your child aged 1-3 years. We will also ask you for information about your family, home, farm, education and other related items.

We would like to invite you to be in this study only after you have properly heard and understand the information. Your community worker will schedule a meeting and we will describe the study and why we are doing the study and answer all of your questions. We ask you to speak your name on a recording tape to give your oral consent if you decide to be in the study. This study lasts for no more than two weeks. You don't have to be in this study unless you want to and you may stop being in the study at any given time. Being in the study or not being in the study will not affect any benefits you get from your community health workers.

We are doing this study to try to make porridges that will help infants grow better. These are to be given to infants after they are six months old and are to be in addition to breast milk. They are not to replace breast feeding. All of the foods to make the porridges are bought in the local markets. Some of the porridges will use germinated corn, others will have some beans mixed with corn, and others will have dried meat powder mixed in the porridge. These foods are all good for infants and help them grow.

Hawassa University

"Effects of Home-Based Phytate Reduction on Maize-Based Infant Foods"

Informed Consent for Community

A. **Background**

We are studying how to make better porridges for babies. Your young child still needs breast milk, but these porridges are to add to their diet after they are six months old and your breast milk may no longer be enough. We are asking for your help with testing how good the porridges taste and feel in your mouth. We are also asking you to let your young child eat a small amount of the porridge if they would like to do that. Then we want you to judge how well your child liked the porridge.

This form tells all things that would happen in the study. We would like you to be in this study only if you understand everything we have explained. We will describe the study and answer all of your questions. We ask you to give us your oral consent if you decide to be in the study. This study lasts for no more than two weeks, and you would need to come to the community center four times during that period. You don't have to be in this study unless you want to, and you or your child may stop being in the study at any time. Not being in the study or stopping the study will not affect any services you get from the community health worker.

B. **Involved institutions**

I am Alemzewed Roba, a member of Hawassa University in Awassa; I am also studying in the USA and this study is part of my master's degree work. My local advisor is Dr. Yewelsew, from Hawassa University. Other professors supervising my work are from Oklahoma State University, USA.

C. **Description of the study**

We are using maize bought in local markets and processing it to make a better infant porridge. We are also adding ground bean or dried meat power to some of the porridges because these help babies to grow. We need to find out if you and your child like these new porridges.

D. Eligible participants in this study

The following persons will be invited to this project:

Mothers of a child aged 1-3 years (60 persons)

Each mother's preschool child aged 1-3 years who wants to taste the porridge (if the mother agrees that they may taste it) (60 children)

E. Data sought

- How much you like the color, texture, flavor (aroma), and taste of the porridges
- How much your child likes the porridges
- Information about your family, home, farm, education and other related items

F. Procedures

If you agree to be in the project, you will be asked to do the following:

- Come four times to the community center to taste porridges. Each visit will take 30 minutes to an hour of your time.
- Bring your 1-3 year old child and allow us to ask him or her if they would like to taste the porridges
- Taste each porridge and answer our questions about how it tastes
- Tell us about the texture of each food (how it feels in your mouth)
- Allow your 1-3 year-old child to taste the porridges and tell us how good they taste

G. Discomforts and risks

There are no known risks associated with testing any of the foods. The porridges are prepared under clean conditions and are made from foods bought in the local market.

H. Benefits

- This study is not intended to cure or treat any illnesses, but to learn if you and your infant find the porridges to be tasty. If you like them, these foods may be used in the future to improve growth of infants in the study area.
- If the results from this study are positive, the findings will be useful to develop health and nutrition programs for Sidama and maybe even for other regions of Ethiopia, in order to reduce zinc deficiency.
- Being in this study is completely voluntary. If you join the study, you or your child may still stop at any time.

I. Confidentiality

We will make every effort to keep your information private but it cannot be assured. Only authorized study personnel will know your name. To keep your information private:

- We will identify you only by number in the study database
- We will cut your name off these papers as soon as the data are checked
- Alemzewed will keep a master list of subject names and numbers in a locked cabinet and will tear up that list as soon as all persons who volunteer have tasted the porridges and we have written down all your answers
- If the results of this study are presented at meetings or in written reports, no one's name will be included
- Original papers for this study will be kept in a locked office at Hawassa University.
- Any data taken to Oklahoma State University will have no names and will be kept in a safe place.

J. Cost to participants

- There will be no cost to you for this project.
- You will not be charged for the sample porridges or for the nutrition education.

K. Subject payment

There will be no direct payment for your participation. However, you will be given a feeding spoon and dish for your child; free health and nutrition education; and your color picture holding your child.

L. Study withdrawal

You (or your child) may choose not to be in the study or to stop being in the study whenever you wish. You will not lose any of your usual health benefits if you choose not to participate.

M. Invitation for questions

If you have any questions about the study procedure or your participation and your rights as a research subject, you can ask them now or you can talk in private with the community health worker or with Alemzewed. An official from the Farmer's Association will help you call the Dean's office at the College of Agriculture, Hawassa University (46-22-06-147) if you have additional questions or you may contact Dr. Shelia Kennison, IRB Chair, Oklahoma State University, 219 Cordell North, Stillwater, OK 74078 USA, by mail; by phone at 01-405-744-1676, or by e-mail at irb@okstate.edu.

N. Giving Consent

Now that you know about this study, you may agree to be in the study by speaking your name into a tape recorder. You may:

1. Agree to participate and enroll in the study now.
2. Discuss with your partner and family and enroll in the study on another day.
3. Decide not to participate.

O. Authorization

I have heard the information about the research study. I know the things that will happen and I chose to participate together with my young child in this study. I know that if I or my child decide to leave the study, we will continue receiving the usual medical care from the community health worker and at the University Hospital and Bushulo Health Center. As a sign of agreement to be in the study, I will orally agree on recording tape.

Name of Participant: ______________________________ Date: ________

I certify that I have personally explained this document before requesting the participant sign it.

____

Name and Signature of Researcher

____

Name and Signature of Community Witness

Okla. State Univ.
IRB
Approved 2/29/08
Expires 2/28/09
IRB# HE0842

4

Hawassa University

"Effects of Home-Based Phytate Reduction on Maize-Based Infant Foods"

Informed Consent for Hawassa University Students and Staff

A. Background

We are studying the effects of home-based phytate reduction on maize-based infant foods. As part of this project we are germinating maize to reduce the phytate content. Phytate is a naturally occurring compound in maize, but it binds zinc and contributes to zinc deficiency problems for people who eat maize as most of their diet. We are asking for your help with testing how good the improved porridges taste and feel in your mouth.

This form summarizes all things that would happen in the study. We would like to invite you to be in this study only if you understand the information. We are happy to answer all of your questions. This study lasts for no more than two weeks. During that time you would need to taste porridges on four different days. Each day would take less than an hour of your time. You may stop being in the study at any time.

B. Involved institutions

Alemzewed Roba, a staff member of Hawassa University, is principal investigator for this study. He is enrolled for graduate study at Oklahoma State University, USA, and this study is a requirement for his master's degree in Nutritional Sciences. The local advisor for the project is Dr. Yewelsew, from Hawassa University, and other members of his graduate advisory committee are from Oklahoma State University, USA.

C. Description of the study

The study uses maize which is obtained from local markets as a sample to investigate how phytate could be reduced for maize-based infant foods. We are testing the flavor, texture and acceptability of complementary foods made from low-phytate maize and of foods with legumes or meats added. Maize with a small amount of beans added is a better food for babies than plain maize. Adding a little dried meat to maize also makes babies grow better.

D. Eligible participants in this study

Forty female students and staff from Hawassa University will be invited to participate in this study.

E. Data sought

- Degree of preference towards color, texture, flavor (aroma), taste and overall acceptability of the food samples will be evaluated through sensory testing and texture profiling.

F. Procedures

If you agree to be in the project, you will be asked to do the following:

- Assess sensory characteristics of each food.
- Evaluate texture of each food through consumer texture profiling.

G. Discomforts and risks

There is no known risk associated with testing any of the foods. The foods are prepared under hygienic conditions using local products.

H. Benefits

- This study is intended to judge the sensory characteristics of nutritionally improved porridges for infants. If the porridges taste good, these foods may be used in the future to improve growth of infants.
- If the results from this study are positive, the findings will be useful to develop health and nutrition programs for Sidama and maybe even for other regions of Ethiopia, in order to reduce zinc deficiency.
- Participating in the study gives practical experience in how sensory analysis is conducted in food science.

I. Confidentiality

We will make every effort to keep your data private and confidential but it cannot be guaranteed. To protect all the records that identify you and the information you give, the following measures will be taken:

- Participants in the study databases will be identified by number rather than name.
- Alemzewed will keep a master list of subject names and numbers in a locked cabinet to insure confidentiality to the highest degree possible. This list will be destroyed as soon as data collection is complete.
- Participant names will be removed from documents and replaced with their subject numbers as soon as feasible.
- If the results of this study are presented at meetings or in publications, participant's names will not be identified.
- Related data for participants will be kept in a locked office at Hawassa University.
- Data taken to Oklahoma State University will have all identifiers removed. Such data will be kept in a secure office.

J. Cost to participants

- There will be no cost for your participation in the project.

K. Subject payment

There will be no payment for your participation.

L. Study withdrawal

You may choose not to be in the study or you may withdraw from the study whenever you wish.

M. Invitation for questions

If you have any questions about the study procedure or your participation and your rights as a research subject, you can ask them now or you can talk in private with Alemzewed. You can also ask questions at Dr. Yewelsew's office on the College of Agriculture campus, Hawassa University (46-22-06-147) or you may contact Dr. Shelia

Kennison, IRB Chair, Oklahoma State University, 219 Cordell North, Stillwater, OK 74078 USA, by mail, or by phone at 01-405-744-1676, or by internet at irb@okstate.edu.

N. Giving Consent

Now that you know all information about this study, you may sign on this Consent Form if you wish to participate in the study.

O. Authorization

I have read the information about the research study described in this informed consent form. I know the time commitment involved and the things that will be required of me and I chose to participate in this study. I know that if I decide to leave the study, it will not be detrimental to my status as a student or staff member at Hawassa University.

Name of Participant: ______________________________

Signature: ______________________________ Date: __________

I certify that I have personally explained this document before requesting the participant sign it.

______________________________ __________

Name and Signature of Researcher Date

ሀዋሳ ዩንቨርሲቲ

ከበቆሎ በሚዘጋጅ የህጻናት ምግብ ውስጥ ፋይቴት በቤት ደረጃ መቀነስ ያለው ውጤት

ለገጠር እናቶች የተዘጋጀ መግለጫ

ይህ ጥናት ከበቆሎ በሚዘጋጅ የህጻናት ምግብ ውስጥ ፋይቴት በቤት ደረጃ መቀነስ ያለውን ውጤት የሚያጠና ሲሆን ለእርሶ የምናቀርብሎትን የተለያዩ የገንፎ ናሙናዎች በመቅመስ በአፍዎ ውስጥ የሚሰማዎትን ስሜት በመግለፅ እንዲተባበሩን እንጠይቃለን:: በተጨማሪ የሚሰጥዎትን ገንፎ ከ1-3 ዓመት ለሚሆነው ልጅዎ በማቅመስ ህፃኑ የቀመሰውን ገንፎ መውደድ አለመውደዱን እንዲነግሩን እንጠይቃለን::
ስድስት የገንፎ ናሙና ዓይነቶች በአካባቢው ገንፎ አሰራር መሰረት ከተዘጋጀ በዃላ በብዙ ትንንሽ ናሙናዎች ተባዝቶ በሁለት የተለያዩ ቀናት እርሶና ልጅዎ እንዲቀምሱት እናደርጋለን:: በእያንዳንዱ ቀናት የተዘጋጁትን የገንፎ ናሙናዎች ቀምሶ ለመጨረስ 30 እስከከ አንድ ሰዓት ሊወስድ ይችላል:: በተጠቀሰው ሰዓት ውስጥ ህፃን ልጅዎ ሁለቱንም ቀን የተለያዩ የገንፎ ናሙናዎች ቀርቦለት እንዲቀምስ ይደረጋል:: በተጨማሪ አስቀድሞ በተዘጋጀ መጠይቅ ስለቤተሰብዎ ሁኔታ ስለቤትዎ እርሻዎ የትምህርት ደረጃዎ እና ሌሎች ተያያዝ ጉዳዮችን ይጠየቃሉ::

በጥናቱ ውስጥ እንዲሳተፉ የምንጋብዝዎት በዚህ መግለጫ የቀረበልዎን መግለጫ በሚገባ አዳምጠው በትክክል ከተረዱ ብቻ ነው:: ለዝሁ ይረዳ ዘንድ በቂ መረጃ ስለጥናቱ ለመስጠት የአካባቢው ልማት ሰራተኞች ስብሰባ እንዲ ያዘጋጁልነ በማድረግ ለምን ጥናቱን መስራት እንዳስፈለገና የጥናቱን ይዘት በዝርዝር መረጃ ከገለፅን በዃላ በእናቶች የሚነሱትን ጥያቄዎች በሙሉ መለስን በጥናቱ ውስጥ ለመሳተፍ ፈቃደኛ የሚሆኑ እናቶችን መዝግበን እንዲሳተፉ እናደርጋለን:: በራሶ ፍቃድ በጥናቱ ውስጥ ለመሳተፍ መፍቀድዎን ለማረጋገጥ ድምፃ በቴፕ ሪኮረደር ይቀረፃል:: በጥናቱ ውስጥ በማንኛውም ምክንያት ለመሳተፍ ካልፈለጉ ቀድሞውኑ ያለመሳተፍ ወይም ከጀመሩ በዃላ በፈለጉት ጊዜ የማቆም መብትዎ የተጠበቀ ነው:: በጥናቱ ውስጥ መሳተፍ በማቆምዎ ወይም ባለማቆምዎ ደግሞ በአካባቢው ልማት ሰራተኞች ያገኙት የነበረው ማንኛውም ዓይነት ጥቅም አይgረጥም::

ይህን ጥናት የምንሰራው የህፃናትን እድገት ሊያፋጥን ገንፎ ለማዘጋጀት ነው። ገንፎው የሚዘጋጀው በተለይ እደሜያቸው ከስድስት ወር በላይ ለሆኑ ህፃናት ከሚጠቡት የናት ጡትበተጨማሪ ነው። ነገር ግን ይህ ገንፎ በምንም ዓይነት መንገድ የእናት ጡት ወተትን የሚተካ አይደለም። ለዚህ ጥናት የሚቀርቡትን የገንደፎ ናሙናዎች ለማዘጋጀት የሚያስፈልጉ የምግብ ዓይነቶች ከአካባቢ ገበያ የሚገዙ ናቸው። በመሆኑም የተወሰኑት የገነፎ ናሙናዎች ምንም ሳይደረግ ዝም ብሎ ከተፈጨ በቆሎ እና ከበቆልት በቆሎ ለየብቻ ሲዘጋጁ ሌሎቹ የገንፎ ናሙናዎች ከቦለቄና በቆሎ ድብልቅ ወይም ደረቆ ከተፈጨ ስጋና በቆሎ ድብልቅ ይዘጋጃሉ። ሁሉም የገንፎ ናሙናዎች ለህዓናት ተስማሚ ከመሆናቸውም በላይ ለእድገታቸው አስፈላጊዎች ናቸው።

Okla. State Univ.
IRB
Approved 7/29/08
Expires 7/28/09
IRB# HE0842

ሀዋሳ ዩንቨርሲቲ
ከበቆሎ በሚዘጋጅ የህጻናት ምግብ ውስጥ ፋይቴት በቤት ደረጃ መቀነስ ያለው ውጤት
የገጠር እናቶች ተሳትፎ ማረጋገጫ ፎርም

መግቢያ

እኛ ለህፃናት እንዴት የተሻለ የገንፎ ዓይነት መዘጋጀት እንደሚቻል በማጥናት ላይ ነን፡፡ ምንም እንኳ_ን ለልጅዎ የእናት ጡት የሚያስፈልግው ቢሆንም እነዚህ የገንፎ ናሙናዎች ግን የእናት ጡት ወተት ከስድስት ወር በላ በቂ በማይሆንበት ሰዓት ተጨማሪ የሆንለታል፡፡ ስለዚህ የሚቀርብሎትን የገንፎ ናሙናዎች በአፍው በመቅመስ የተሻለ ጣዕምና ይዘት ያለውን ገንፎ በማወዳደር እንዲነግሩን እንጠይቃለን፡፡ በተጨማሪ የሚሰጥዎትን ገንፎ ከ1-3 ዓመት ለሚሆነው ልጅዎ በማቅመስ ህፃኑ የትኛውን ገንፎ መውደድ አለመውደዱን አወዳድረው እንዲነግሩን እንጠይቃለን::

ይህ ዶክመንት የተለያዩ የህጻናት ገንፎ ናሙናዎችን በመቅመስ በሚሰራው ጥናት ውስጥ የሚከናወነውን ዝርዝር ጉዳይ በቅደም ተከተል በቂ መረጃ ይሰጥዎታል፡፡ እርሶ በጥናቱ ውስጥ ለመሳተፍ ከመፍቀድዎ በፊት ስለጥናቱ ማወቅ የሚገባዎትን ዝርዝር መረጃዎች ከአቀረብንና የሚያነሳÿ∠ቸውን ጥያቄዎችን በሙሉ ከመለስን በº ላ በጥናቱ ውስጥ ለመሳተፍ ፍቃደኛ ከሆኑ ማረጋገጫ እንዲሰጡን እንጠይቅዎታለን፡፡ ጥናቱ ግፋ ቢል ሁለት ሳምንት ሊወስድ ቢችልም በጥናቱ አማካይ ስፍራ ለአራት ጊዜ ብቻ እንዲመጡልን እንጠይቃለን፡፡

በራሶ ፍቃድ በጥናቱ ውስጥ ለመሳተፍ መፍቀድዎን ለማረጋገጥ ድምፆ በቴፕ ሪኮረደር ይቀረፃል:: በጥናቱ ውስጥ በማንኛውም ምክንያት ለመሳተፍ ካልፈለጉ ቀድሞውኑ ያለመሳተፍ ወይም ከጀመሩ በºላ በፈለጉት ጊዜ የማቆም መብትዎ የተጠበቀ ነው:: በጥናቱ ውስጥ መሳተፍ በማቆምዎ ወይም ባለማቆምዎ ደግሞ በአካባቢው ልማት ሰራተኞች ያገኙት የነበረው ማንኛውም ዓይነት ጥቅም አይgረጥም::

በጥናቱ ውስጥ የሚሳተፉ ግለሰብና ድርጅቶች

እኔ አለምዘውድ ጫላ የሀዋሳ ዩኒቨርስቲ ባልደረባ ከመሆኔም በላይ ጥናቱን በዋናነት የማካሂድና በአሁኑ ሰዓት የከፍተኛ ትምህርቴን በኦክላሆማ ዩኒቨርስቲ በስነምግብ ሳይንስ ዘርፍ በማስተርስ ዲግሪ በመከታተል ላይ እገኛለሁ፡፡ ይህ ጥናት ለማስትሬት ዲግሪ ማመያ ጥናት የሚካሄድ ሲሆን ከሀዋሳ ዩኒቨርስቲና ከኦክላሆማ ዩኒቨርስቲ በተውጣጡ አንጋፋ አማካሪዎች ይደግፋል፡፡

የጥናቱ አጭር መግለጫ

በቆሎ ከአካባቢ ገበያ በመግዛትና ለህፃናት ተስማሚ ገንፎ ሊያሰራ የሚያስችል ዘዴ በመጠቀም የህፃናት ገንፎ ይዘጋጃል፡፡ በተጨማሪ የተፈጨ ቀይ ቦለቄና ደርቆ የተፈጨ ስጋ ገንፎ ውስጥ በመጨመር ህፃናት በቶሎ እንዲያድጉ ያደርጋል፡፡ ስለዚህ እርሶና ከ1-3 ዓመት እድሜ የለው ህፃን ልጅዎ አዲሱን የገንፎ ናሙና በመቅመስ መውደድ የለመውደዳችሁን ማወቅ እንፈልጋለን፡፡

<u>የተሳታፊዎችን መመልመያ መስፈርት</u>

የሚከተሉትን መስፈርቶች የሚያማሉ ተሳታፊዎች በጥናት ውስጥ ይሳተፋሉ፡፡
ከ1-3 ዓመት እድሜ ክልል ውስጥ የሆነ ህፃን ልጅ ያላቸው እናቶች (60 በቁጥር)
እናቶቻቸው በጥናቱ ውስጥ ቢሳተፉ የሚስማሙ እድሜያቸው ከ1-3 ዓመት የሆኑ ህፃናት (60 በቁጥር)

<u>ከተሳታፊዎች የሚፈለግ መረጃ</u>

- እናቶች እንዲቀምሱ የሚቀርቡላቸውን የምግብ ናሙናዎች ቀለም፣ ይዘት፣ መዓዛ (ሽታ)፣ ጣዕምና አጠቃላይ ተቀባይነት ሁኔታ በመመርመርና በማወዳደር ምርጫቸውን እንዲገልጹ ይደረጋል፡፡
- ከ1-3 ዓመት በታች ያሉ ህጻናትም የምግብ ናሙናዎቹን በማቅመስ ለእያንዳንዱ ናሙና የሚያሰዩትን ፍላጎት በእናታቸው አማካይነት እንዲወዳደር ይደረጋል፡፡
- እናቶች ስለቤተሰብ# ስለቤት# ስለእርሻ# ስለትምህርትና የህዓናት አመጋገብ በተመለከተ መረጃ ይጠየቃሉ፡፡

<u>የጥናቱ ቅደም ተከተል</u>

ተሳታፊዎች በጥናቱ ውስጥ ለመሳተፍ ከተስማሙ የሚከተሉትን እንዲፈፅሙ ይጠየቃሉ

- ወደ ማህበረሰብ ጣቢያው የምግብ ናሙና ለመቅመስ መምጣት፡፡ በቀን ከ30 ደቂቃ እስከ 1 ሰአት ይፈጃል፡፡
- ከ አንድ እስከ ሶስት አመት እድሜ ውስጥ የሚገኝ ልጅዎትን ወደ ማህበረሰብ ጣቢያው አምጥተው የምግብ ናሙናውን ለመቅመስ ፈቃደኛ መሆኑን እንድንጠይቀው እንደዲፈቅዱ ይጠየቃሉ፡፡
- የስሜት ህዋሳትን በመጠቀም በባለዘጠኝ ነጥብ መገምገሚያ ቅጽ አማካኝነት የሚቀርብሉትን የምግብ ናሙና ይገመግማሉ
- የእያንዳንዱን ምግብ ናሙና ይዘት (texture) በባለ ስድስት ነጥብ መገምገሚያ ቅጽ ይገመግማሉ
- ከ1-3 ዓመት በታች ልጅዎ የምግብ ናሙናዎችን በማቅመስ ፍላጎቱን በእርሶ አማካኝነት ይነግሩናል፡፡

<u>ከጥናቱ ጋር የተያያዘ አለመመቻቸትና አደጋ</u>

የሚቀርብሎትን የምግብ ናሙና በመቅመስ በርሶም ሆነ በልጅዎ ላይ የሚያስከትለው ጉዳት የለም፡፡

<u>ከጥናቱ የሚያገኙት ጥቅም</u>

- ይህ ጥናት የሚሰራው የትኛውንም ዓይነት በሽታ ለማከም ወይም ለማዳን አላማ ሳይሆን በጥናቱ ውስጥ የተዘጋጀው በቆሎ የዚንክ ይዘቱን ማሻሻል እንደሚችል ለመገምገም ነው፡፡
- የጥናቱ ውጤት አመርቂ ከሆነ ወደፊትበክልል ወይም በሀገር ደረጃ የዚንክ እጥረትን ለመቀነስ ለሚዘጋጅ የጤናና ስነምግብ ፕሮግራሞች መነሻ ሊሆን ይችላል፡፡
- በጥናቱ ውስጥ የእርሶ ተሳትፎ በርሶ ፈቃደኝነት ላይ ብቻ የተመሰረተ ነው፡፡

<u>ለሚሰጡን መረጃ ሚስጥራዊነት</u>

እርሶ የሚሰጡን ማንኛውም መረጃ ሚስጥራዊነት ለመጠበቅ የተቻለንን ጥንቃቄ እናደርጋለን። የጥናቱ ተመራማሪ መረጃውን ስለሚጠቀምበት የተሳታፊዎችን ስምና መረጃ በሚስጥር ይይዛል። ማንኛውም ከርሶና ከሚሰጡት መረጃ ጋር የሚገናኝ ቅጽ በተመለከተ.

- ተሳታፊዎች ጥናቱን ከመጀመራቸው አስቀድሞ በሚሰጣቸው ሚስጥራዊ ኮድ ይጠራሉ።
- የጥናቱ ተመራማሪ የተሳታፊዎችን ስምና ሚስጥራዊ ኮድ ሰነድ በጥንቃቄ ያስቀምጣል።
- የተሳታፊዎች ስም መረጃ ከሰጡበት ማንኛውም ቅጽ ውስጥ ተቆርጦ ይወጣል።
- የዚህ ጥናት ውጤት ለህትመት ወይም በስብሰባ ላይ በሚቀርብበት ሰዓት የተሳታፊዎች ማንነት አይገለጽም።
- ከተሳታፊዎች ጋር ተያያ|ነት ያላቸው ማንኛውም ዓይነት መረጃዎች በጥንቃቄ ይቀመጣሉ።
- የተሳታፊዎችን ማንነት የመይገልፅ በመረጃ የተሞላ ቅፅ ተመሬማሪው ወደ ኦክላሆማ ስቴት ዩኒቨርሲቲ ለተጨማሪ ምርመራ ሊወስድ ይችላል።

<u>በጥናቱ ውስጥ ለሚኖርዎ ተሳትፎ የሚጠየቁት ክፍያ</u>

- ተሳታፊዎች በጥናቱ ውስጥ ለሚያደርጉት ተሳትፎ ምንም ዓይነት ክፍያ አይጠየቁም።
- ለጥናቱ ውስጥ ለሚቀርብሎት የምግብ ናሙናዎች ዋጋ አይከፍሉም።

<u>ለተሳታፊዎች የሚከፈል ክፍያ</u>

- ለጥናቱ ተሳታፊዎች የሚከፈል ቀጥተኛ የገንዘብ ክፍያ የለም። ነገር ግን ተሳታፊዎችን ለማበረታታት የፀጉር ቅባት# ከፕላስቲክ የተሰራ የህፃን መመገቢያ ሳህንና ማንኪያ# የውሃ መጠጫ ኩባያና የስነምግብና የጤና ትምህርት በነፃ ይሰጣል። በተጨማሪ የተሳታፊ እናቶችና ልጆቻቸው ባለቀለም ፎቶግራፍ ይሰጣል።

<u>ጥናቱ ውስጥ ተሳትፎ ስለማgረጥ</u>

- ተሳታፊ እናቶችና ልጆቻቸው በፈለጉት ሰዓት ጥናት ውስጥ መሳተፍ ሊያgርጡ ይችላሉ
- ተሳታፊ እናቶችም ሆኑ ልጆቻቸው ጥናቱ ውስጥ መሳተፍ ቢያgርጡ ከዚህ ቀደም ያገኙት የነበረው ማንኛውም ዓይነትየጤና ግልጋሎት ማግኘት ይቀጥላሉ

<u>ለተጨማሪ ጥያቄና ማብራሪያ</u>

የጥናቱን አሰራርና ከተሳትፎ ጋር በተያያዘ ሊያውቁ የሚገባውን መብት በተመለከተ ማንኛውንም ጥያቄ

- የጥናቱ ዝርዝር በሚገለፅበት ሰዓት እና
- የተሳትፎ ፍቃደኝነት ማረጋገጫ በሚፈረምበት ወቅት መጠየቅ ይችላሉ። በተጨማሪም የቀበሌ ወራተኛ ወደ ሃዋሳ ዩኒቨርሲቲ እርሻ ኮሌጅ ዲን ጽፈት ቤት በ ስልክ ቁጥር 0462206147 ይደውልሎታል። ተጨማሪ ጥያቄዎች ካሉ የኦክሎሆማ ስቴት ዩኒቨርሲቲ የኢቲክስ ኮሚቴ ሰብሳቢ የሆኑትን Dr. Shelia Kennison በሚከተለው አድራሻ Oklahoma State University,219 Cordell

North, Stillwater, OK 74078 USA, e-mail irb@okstate.edu , Phone 01-405-744-1676.

ፊርማና ተሳትፎ በፍቃደኝነት ማረጋገጥ

ከላይ በተገለፀው መሰረት የጥናቱን ዓላማና ይዘት በዝርዝር ተረድተው እርሶና ልጅዎ በጥናቱ ውስጥ ለመሳተፍ ፍቃደኛ ከሆኑ በቃል መስማማትዎን እንዲገልፁልን እንጠይቃለን። ውሳኔዎን ለማሳወቅ

- ከሌሎች የቤተሰብዎ አባላት ጋር ተወያይተው በማግስቱ ሊስማሙ ይፍላሉ
- በቀጥታ በራስዎ ምርጫ ተስማምተው ሊፈርሙ ይችላሉ
- መሳተፍ ካልፈለጉ ደግሞ አለመፈረምና አለመስማማት ይችላሉ

ማረጋገጫ

ይህን የፍቃደኝነት ማረጋገጫ ቅፅ በሚገባ አንብቤ ወይም ተነቦልኝ ስለጥናቱ ዝርዝር ከተረዳሁ በላ እኔም ሆንኩ ልጄ ለመሳተፍ ፍቃደኛ ሆኛለሁ። ከጥናቱ ውስጥ መሳተፍ ባቆም ከዚህ በፊት አገኝ የነበረው ማህበራዊም ሆነ ኢኮኖሚያዊ አገልግሎት እንደማይgረጥ ተረድቻለሁ። ስለዚህ ይህንኑ መስማማቴን ለመግለፅ በቀረበልኝ ክፍት ቦታ የጣቴን አሻራዬን በማኖርና በቃሌ በመግለፅ መስማማቴን እገልፃለሁ።

የተሳታፊ ስም ________________________ ፊርማ ____________ ቀን ____________

ከዚህ በላይ የተጠቀሱት እናት ለተሳትፎ ማረጋገጫ ከመጠየቁ በፊት ጥናቱን የተመለከተ መረጃዎች አብራርቻለሁ።

የተመራማሪው ስምና ፊርማ________________________ ፊርማ ____________ ቀን ____________

የእማኝ ስምና ፊርማ________________________ ፊርማ ____________ ቀን ____________

VITA

Alemzewed Challa Roba

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF HOME-BASED PHYTATE REDUCTION IN MAIZE: METHOD TO IMPROVE ZINC BIOAVAILABILITY IN INFANTS DIET IN SIDAMA ZONE, SOUTHERN ETHIOPIA

Major Field: Nutritional Sciences

Biographical:

Personal Data: Alemezewed Challa Roba
Email: alemzewedcr2000@yahoo.com

Education:

Completed the requirements for the Master of Science in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in July, 2009. Received Bachelor of Science degree in Rural Development and Family Sciences from Hawassa University (then Debub University), Awassa, Ethiopia in July, 2005

Experience:

Graduate Research Assistant, (January 2007 to July 2009) Department of Nutritional Sciences, Oklahoma State University, Stillwater, Oklahoma, USA
Graduate Assistant, (July 2005 to December 2006) Department of Rural Development and Family Sciences, Hawassa University, Ethiopia
Teaching Assistant, (August 1999- June 2005) Department of Rural Development and Family Sciences, Hawassa University, Ethiopia
Research field assistant, (July 1997 to July 1999) Awassa College of Agriculture, Hawassa University, Ethiopia

Professional Memberships: American Society for Nutritional Science

Name: Alemzewed Challa Roba Date of Degree: July, 2009

Institution: Oklahoma State University Location: OKC or Stillwater, Oklahoma

Title of Study: EFFECTS OF PHYTATE REDUCTION ON MAIZE/BEAN BLEND INFANT FOODS USED IN SOUTHERN ETHIOPIA

Pages in Study: 152 Candidate for the Degree of Master of Science

Major Field: Nutritional Sciences

Scope and Method of Study: The purpose of this study was to develop low-phytate maize-based complementary food by reducing phytate and lowering phytate to zinc molar ratio below 15 thresholds using three local food processing methods, and evaluate sensory quality and acceptability of such complementary foods. A total of 28 samples prepared in triplicate were tested for their phytate and zinc content and phytate to zinc molar ratio. Three samples further evaluated for their sensory quality by 36 mothers and 42 University female staff and student panels selected from the study area. Sensory scores were analyzed using analysis of variance (ANOVA, Proc GLM) and variation attributable to sample difference, addition of meat, or subjects were determined. All means were tested for least significant difference using Tukey's studentized test and difference at $p < 0.05$ considered significant.

Findings and Conclusions: Mean (±SE) phytate and zinc found in untreated maize were 625.2±47.8 and 2.0±0.1 mg/100g followed by 30.8±3.1 phy:Zn molar ratio. Phytate decreased by 94% in pounded and water soaked white maize (12 h), 37% in pounded maize soaked in water for 12 h, and germinated for 24 h, and 23% in maize soaked in lemon-water for 12 h. The result indicates that more phytate reduced by water soaking (12 h) compared with untreated white maize. The mean (±SE) phytate and Phy:Zn molar ratio of water soaked (12 h) maize were 40.2±10.1 mg/100g and 2.4±0.5 respectively. Panelists liked color (6.2±0.2), flavor (5.6±0.2), smell (5.2±0.2), texture (5.3±0.2) and acceptability (5.4±0.2) of porridge prepared from pounded and water soaked white maize (12 h) more ($p< 0.05$) than color (5.4±0.2), flavor (4.9±0.2), smell (4.6±0.2), texture (5.0±0.2) and acceptability (5.1±0.2) porridges prepared from germinated maize or color (5.8±0.2), flavor (5.2±0.2), smell (5.0±0.2), texture (5.4±0.2) and acceptability (5.3±0.2) of porridge prepared from pounded maize soaked in lemon-water (12 h). Panelist ranked porridges prepared from water-soaked maize as first (55.6%); lemon-water soaked maize (34.6%) as second and porridge from germinated maize as third (17.3%). In conclusion, this study indicate that preparing foods using flour processed from pounded and water soaked (12h) white maize can be a potential maize-based complementary food for young children in the study area.

ADVISER'S APPROVAL: Dr. Tay Kennedy