ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

FACULTY OF SCIENCE

CENTER FOR FOOD SCIENCE AND NUTRITION

The effect of co-fermentation on nutritional value and sensory acceptability of cowpea with germinated sorghum and maize for the development of infant complementary food.

By

Tsige Dibekulu Alemu

Advisors: - Ato Kelbessa Urga

Ato Tilahun Bekele

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Nutrition.

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Acronyms

AAS= Atomic Absorption Spectrophotometer

ADLI= Agricultural Development Led Industrialization

ANOVA= Analysis of variance

AOAC= Association of official analytical chemist.

BDL=below detection limit (non detected)

cP= Centipoises

CSA= Central Statistical Authority

DHS =Demographic and Health Survey

DMT= Divalent metal transporter

DNA=Deoxyribonucleic acid

EBF= Exclusive breastfeeding

EPHI= Ethiopian public Health Institute

FAO= Food and Agriculture Organization (of the UN)

GTP= Growth and Transformation Plan

ICRISAT=International Crops Research Institute for the Semi-Arid Tropics

IDA= Iron deficiency Anaemia

Ip-4= Inositol Tetra-phosphate

LDL= low density lipoprotein

NHD= Nutrition for Health and Development

NNS= National Nutrition Strategy

PEM= Protein energy malnutrition

RDA =Recommended dietary allowance

RPM =Revolution per Minute

SD= Standard Deviation

SPSS= Statistical package for social science

TTA= Total titratable acidity

UNICEF=United Nation international children fund

WAC= Water absorption capacity

WHO= World Health organization

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ABSTRACT: -

The objectives of this study were to develop complementary foods from the blends of cowpea with sorghum and maize. The complementary food formulations in the present study were based on locally available low-cost food materials commonly consumed in Ethiopia. Eight complementary food formulations were developed from maize + cowpea, germinated maize + cowpea, co-fermented maize+ cowpea, co-fermented and germinated maize + cowpea, sorghum+ cowpea, germinated sorghum+ cowpea, , co-fermented sorghum+ cowpea and co-fermented and germinated sorghum+ cowpea. The effect of natural co- fermentation with germinated sorghum and maize cultivars on TTA and pH, proximate value, anti-nuritional factors (tannin and phytate); minerals (Ca, Fe, and Zn), functional properties and sensory characteristics were investigated. Flours were co -fermented at room temperature (20- 23^{0} C) for 72hrs at 1:3 dilutions (w/v). Samples were drown immediately at the end of each fermentation period and the samples were dried using a freeze drier. The overall results indicated that nutrient content of maize + cowpea was gave 14.37% crude protein, 4.48% crude fat, and 67.74 % carbohydrate. Germinated maize + cowpea exhibited 15.62% crude protein, 4.27% crude fat and 67.36% carbohydrate. Co-fermented maize+ cowpea show that 15.69% crude protein, 3.97% crude fat and 69.93 % carbohydrate. Co-fermented and germinated maize + cowpea exhibited 16.00% crude protein, 3.43% crude fat and 70.84% carbohydrate. Sorghum+ cowpea gave 15.90%, crude protein, 3.48 % crude fat and 66.47% carbohydrate. Germinated sorghum+ cowpea had show 16.53% crude protein, 3.33% crude fat and 66.25% carbohydrate. Co- fermented sorghum+ cowpea was show that 16.66% crude protein, 3.24% crude fat and 69.22 % carbohydrate and the last co- fermented and germinated sorghum+ cowpea exhibited 16.95% crude protein, 3.11% crude fat and 70.02% carbohydrate. Co-fermentation caused an increase in protein, TTA, energy content, carbohydrate and improved mineral bioavailability and decreased fat, ash, crude fiber, viscosity, antinutritional factors. Germination increased significantly ($P<0.05$) the contents of crude protein, water absorption capacity and oil absorption capacity. In contrast, germination decreased anti-nutritional factors. Sensory evaluation was significant difference of ($P<0.05$) in aroma, taste, texture and over all acceptability among the blend and co-fermentated samples but appearances are not significantly different from each other. From the results obtained it is possible to conclude that infant complementary foods can be produced from cereals and legumes as evidenced in this study.

Key words:- Blending, Cowpea, Co-fermentation, Germination, Maize, and Sorghum.

1. INTRODUCTION

1.1 Background

The concept of nutrition and its expression as malnutrition (both under and over nutrition), involves complex processes at multiple levels, from individual to the household to the community to the national and international levels. In developing countries, malnutrition results from inadequate intake of nutrients and/or from disease factors that affect digestion among which protein energy malnutrition (PEM), nutritional anemia, vitamin A deficiency, and iodine deficiency disorders (IDD) are the most serious nutritional problems developing countries (Müller and Krawinkel, 2005). Inadequate complementary food is a major cause for the high incidence of child malnutrition, morbidity and mortality in many developing countries (WHO, 2000).

Malnutrition is an underlying cause of the death of 2.6 million children each year, and one-third of the global total of children's (7.6 million child) deaths each year before their fifth birthday through weakening the body's resistance to illness. In developing countries this figure is as high as one in three and specifically in Africa two out of five children suffer with malnutrition (Marfo *et al*., 1991). Sub-Saharan Africa has one of worlds the highest levels of malnutrition in children under five, and of overall undernourishment. Sub-Saharan Africa had 195.9 million people (33%) of human population undernourished (FAO, 2002).

In Ethiopia, malnutrition is one of the leading causes of morbidity and mortality in children under five years of age. The country has the second highest rate of malnutrition in Sub-Saharan Africa (Sanni *et al.,* 1999). WHO (1998) and Sanni *et al.* (1999) reported that malnutrition in children is one of the most serious public health problems in Ethiopia and the highest in the world. For instance, the prevalence of underweight, stunting, and wasting was also very high; 30.7%, 33% and 45.3%, respectively in Somali region (Eltayeb *et al.,* 2008).

Exclusive breastfeeding (EBF) has been defined by World Health Organization (WHO) as the practies where the infant has receives only breast milk from his or her mother. Breast milk is adequate in quality as well as quantity in terms of energy, protein, nutrients, water etc. for an infant's need under six months. After about 6 month of age the child needs complementary food (Gairdner and Pearson, 1998; Oyarekua, 2011)

Traditional weaning foods in most African countries are based on the local staple food, usually a cereal (maize, millet, sorghum or rice), and sometimes on starchy foods (cassava, potato or plantain). Those are commonly prepared as liquid gruels with low energy and nutrient density with insufficient amount of macro and micronutrients (Awad *et al.*, 2011).

In developing countries such as Ethiopia, commercial complementary foods are very expensive and out of reach of low-income families. This may risk to the life of children as they may be susceptible to malnutrition. In Ethiopia weaning foods used are most of the time locally produced and based on local staple foods, usually cereals that are processed into porridges (WHO, 2001). Weaning strategies or processes are important parts to optimize nutritional status and alleviate the situation in an infant dietary. Therefore, in this study the enhancement of nutritional quality and reducing anti- nutritional composition of complementary foods prepared from germinated sorghum and maize with mixed with cowpea by co-fermentation process in 30:70 proportions were evaluated to find out the best alternative and recommended way of complementary food development for an infant. The blends \formulations has to be made based on household food resource.

1.2 Statement of the problem

In Ethiopia, traditional complementary foods are usually made of cereals or starchy root crops. The child is introduced directly into the family meal of plant source with high water, fiber, and low energy content and micronutrient densities (Wolde-Gebriel, 2000). This characteristic becomes a particularly cause of during the complementary feeding period in infants and children due to its causal factor for infant mortality, protein energy malnutrition and micronutrient deficiency.

Plant-based foods also negatively influence the bioavailability of nutrients. The anti-nutritional factors, for example phytic acid is primarily related to its strong chelating ability with minerals and proteins due to the association with its six reactive phosphate groups. Multivalent chelations are particularly susceptible and form insoluble and indigestible complexes. Phytate also affects enzyme activity with a negative effect for key digestive enzymes including amylase, pepsin and trypsin (Hotz, *et al.,* 2007).

Traditional complementary foods have highly bulkiness concentrations such as solubility, gelatin, viscosity, water and fat binding properties reflect the level of protein interaction with water, while fat absorption and emulsion are influenced by protein. Therefore, the low energy density of complementary foods in developing countries causes Malnutrition, protein-energy malnutrition and micronutrient deficiencies a major health burdens in developing countries, like Ethiopia (Rosalind *et al.,* 2009).

1.3 Significant of the study

Complementary foods can be generally produced following traditional technologies such as thermal processing, mechanical processing, soaking, fermentation, and germination/malting as well as modern food processing technologies like roller drying and extrusion cooking (Awad *et al.*, 2011).complementary food prepared in the form of thin porridge or gruels are used for ready consumption. Development of complementary foods best recommended when guided by high nutritional value to supplement breastfeeding and there are acceptability, low price and use of local food items made of cereals and legumes (Eschleman, 1984; Ijarotimi and Keshinro, 2013). Cereals are generally low in protein and are limiting in some essential amino acids, particularly lysine and tryptophan. Supplementation of cereals with locally available legumes that is high in protein, although often limiting in sulphur amino acids. This can be compensated for the limiting level in cereals due to cereal-legume blends such blends have been found to improve nutrient density and improved nutrient intake, which can result in the prevention of malnutrition problems. (Osundahunsi, 2003; Ejigui, 2007).

Traditional processing methods have many positive attributes, such as favorable texture, good organoleptic quality, reduced bulk, enhanced shelf life, partial or complete elimination of antinutritional factors, reduced cooking time, and improved nutritional value. One of the traditional processing techniques is fermentation. Fermentation has been used in Africa for centuries to preserve and improve the nutritional status of foods. Fermentation of either cereals or legumes or both before use in complementary food formulations increases digestibility and reduces anti- nutritional factors (Sandberg, 1991; Ejigui, *et al.*, 2005). Fermentation increases the bioavailability of minerals; enhance organoleptic response and contents of free sugars, vitamins and minerals. It also reduces phytic acid as a result of the action of phytase synthesized by micro-organisms (Hurrel, 2004).

Germination is not widely used in Africa especially in complementary food preparation but mainly for local brewing (Sangronis and Machado, 2007). However, germination similar to fermentation causes certain chemical changes to occur. These changes can vary depending on the variety of seed and the condition of germination (Egli *et al.*, 2002). Germinated seeds are good source of ascorbic acid, riboflavin, choline, thiamine, tocopheroles and pantothenic acid. It improves protein and iron absorption and a-amylase activity is also increased during germination of cereals specially sorghum and millet (Egli *et al.*, 2004). During germination enzymes hydrolyzes, amylase and amylopectin to dextrins and maltose, thus reducing the viscosity of thick cereal porridges without dilution with water while simultaneously enhancing their energy and nutrient densities (Egli *et al.*, 2004).

1.4 Objective of the study

1.4.1 General objective

The main objective of this study was evaluating the effect of co- fermentation on nutritional value and sensory acceptability of cowpea with germinated sorghum and maize for development of infant complementary food.

1.4.2 Specific objectives

The specific objectives of this study were to

1. To determine the effect of natural co- fermentation on some anti- nutritional factor.

2. To study proximate composition, physico-chemical changes, functional properties and mineral content that take place in complementary food due to co- fermented maize or sorghum with cowpea.

3. To assess nutritional value enhancement during co- fermentation and germination of maize or sorghum with cowpea.

4. To evaluate sensory properties and overall acceptability of complementary foods prepared germinated and co- fermented maize and sorghum with cowpea.

2. LITERATURE REVIEW

2.1 Nutrition and child health

Nutrition is directly related to food intake and infectious diseases such as diarrhea, acute respiratory infection, malaria, and measles. Both infectious disease and food intake reflect underlying social and economic conditions at the household, community, and national levels that are supported by political, economic, and ideological structures within a country (Girma and Genebo 2002).

Nutrition is an important part of child's growth and development. Especially the first two years of life are considered to be the window of opportunity. Ethiopia is the second most populous country in Africa, where nearly 90 million people to live. Approximately 14% are children under five years of age (Central Statistics Agency, 2007). Children are most vulnerable to under nutrition due to low dietary intake, inaccessibility to food, inequitable distribution of food within the household, improper food storage and preparation, dietary taboos and infectious diseases (Girma and Genebo, 2002).

Good nutrition is the cornerstone for survival, health and development for current and succeeding generations. Well-nourished children perform better in school, grow into healthy adults and in turn give their children a better start in life. Nutritional status is the best global indicator of well-being in children. Child malnutrition is also an important indicator of monitoring progress towards the millennium development goals; to reduce it by 2015 with ages under five years by at least one third and with special attention to children less than two years of age. Hence, healthy child growth and development is the basis of human development; the impact of malnutrition is multifarious. It has an all-pervasive impact on the physical well-being and socioeconomic condition of a nation. For instance, it perpetuates poverty through direct losses in productivity, indirect losses from poor cognitive function, poor child development and deficits in schooling (Kebede, 2007).

2.2 Malnutrition

Malnutrition is most simply defined as any nutritional imbalance. Two major problems exist in the world, starvation (or under-nutrition where there is insufficient food or insufficient economic means to provide the necessary food) and people may suffer from over nutrition when they consume too many calories resulting in obesity a condition occurs during over consumption of food in the wealthy, commonly in developed world (Steinkraus, 1994).

The causes of malnutrition are intertwined with each other and are hierarchically related. Although, the most immediate determinants are poor diet and disease in which they are caused by a set of underlying factors. Nutritional status of children is a manifestation of a host of factors including household access to food and the distribution of this food within the household, availability and utilization of health services, and the care provided to the child. Possibly it would be true to say, in addition to this situation, educational status of the mother and food processing methods are is also one of the fundamental reasons for malnutrition to occur in children in developing countries. Studies also suggest that maternal education regularly emerges as a key element of an overall strategy to address malnutrition (Girma and Genebo, 2002).

The causes of malnutrition are three broad categories of factors causing malnutrition: (1) immediate factors, (2) underlying factors and (3) basic factors. Immediate factors are those that account for the poor health status of an individual, and they include inadequate dietary intake and the prevalence of diseases. They are the main cause of child mortality and morbidity in developing countries. Underlying factors include household food insecurity, inadequate maternal and child caring practices, poor health services and unhealthy environment. Basic factors relate to the quantity and quality of human, economic and organizational resources available and the way they are controlled in the society (UNICEF, 2002)

2.3 Worldwide child malnutrition

Hence, malnutrition is the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions; it is a major public health problem worldwide, particularly in developing countries (Onis *et al.*, 1993). One third of the children under 5 years old worldwide are moderately or severely undernourished. Under nutrition impairs physical, mental and behavioral development of millions of children and is a major cause of child death (World Bank, 1993).

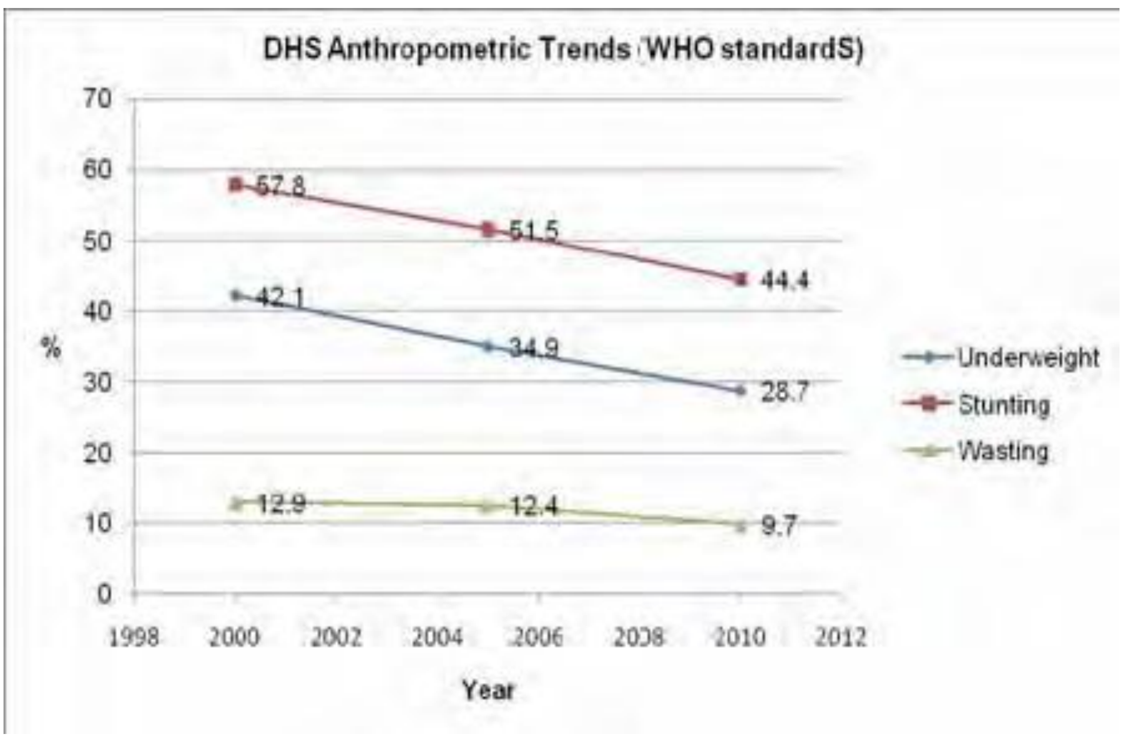
The pattern of malnutrition in Africa is quite distinct. In sub-Saharan Africa the absolute numbers of malnourished children are expected to be increasing to 128 million under an optimistic scenario, in which improvements in the determinants is accelerated, by 2020 (Kebede, 2007). Although, the prevalence of stunting declined from 40.5% in 1980 to 35.2% in 2000, a decrease of only 0.26 percentage points per year; the highest level of stunting is found in Eastern Africa, where, on average, 48% of preschool children are currently affected. In this region, stunting has been increasing at 0.08 percentage points per year (WHO, 1998).

2.4 Child Malnutrition in Ethiopia

In Ethiopia, child malnutrition rate is one of the most serious public health problems (Kebede, 2007). Over the past two decades substantial efforts have been made to monitor the evolution of child malnutrition in Ethiopia. As the surveys are quite uniform in temporal, spatial and age-group coverage, the statistics are generally comparable over time. The first striking observation is the sheer magnitude of the prevalence of malnutrition of children under 5 in Ethiopia. For example, Kebede (2007) reported that fifty-one percent of children aged 0-59 months are chronically malnourished. In other words, they are too short for their age, or stunted. The proportion of children who are stunted is more than 25 times the level expected in a healthy, well-nourished population. Acute malnutrition manifested by wasting results in a child being too thin for his or her height. It affects 11 percent of children, which is more than five times the level expected in a healthy population.

Forty-seven percent of children under than five years of age are underweight for their age. This is more than 23 times the level expected in a healthy, well-nourished population. The incidence of underweight children has been consistently reported at about 45 percent, which compares with an average incidence of underweight children in Sub Saharan Africa in the nineties (World Bank, 2000). Similarly, surveys in Ethiopia have consistently found more than half of the children under five were stunted, with stunting rates most often attaining more than 60 percent. By way of comparison, during the mid nineties, the average prevalence of children stunted for 19 Sub Saharan African countries were reported as 39 percent (Morrisson *et al.*, 2000).

Ethiopia has been plagued with food insecurity, including famine, for centuries and malnutrition is widespread, particularly amongst children and women however, now Ethiopia has been making progress towards improved food and nutrition security over the past decade. Consistent and comparable data of DHS since 2000 have shown that malnutrition, as measured by stunting and underweight rate of prevalence, have decreased by more than 10 percentage points between 2000 and 2010. The decrease has been steady, with both falling by 1.34 points per year over the 10 year period. Wasting, which measures the more immediate effect of malnutrition, seems to have fallen only slightly from around 12 percent in 2000 and 2005, to 9 percent in 2011 (Figure1).



SOURCE: DHS (2000-2012)

Figure 1: show that (2000-2012) years progressively decrease the percentage of stunting, wasting and underweight of children under five years in Ethiopia.

This is due to Ethiopia increased political commitment at the national level towards nutrition in over the past five years as evidenced by the existence of the National Nutrition Strategy, as well as the inclusion of nutrition in various government policy documents. There are numerous strategy documents and programs aimed at improving nutrition in Ethiopia, such as Growth and

Transformation Plan **(**GTP), Agricultural Development Led Industrialization (ADLI), National Nutrition Strategy (NNS) etc. The development and implementation of the NNS and an action plan to achieve Millennium Development Goal (MDG) reduction of halving poverty and hunger by 2015. However, it is still a major public health problem and a drawback to the country's rapid economic development

Figure 2: also shows that the regional variation in Ethiopia that prevalence rate of stunting (DHS, 2011) in children is substantial. Children in rural areas are one and a half times more likely to be stunted than those in urban areas .stunting levels are the highest in Amhara region about 52 % and the lowest in Addis Ababa and the Gambela region 22 and 27%, respectively. It is exciting to note that adequate food producing regions of the country such as Amhara and Oromia are reported to have high prevalence rate of stunting as compared to less productive regions, thus indicating that while household food security is necessary, it is not the only determining factor for ensuring nutrition security. The concept and belief of the societies about nutrition is low. Hence, social and traditional pressures have a lot of contribution to the problem of malnutrition in Ethiopia.The educational level particularly of women has been shown to be critical to ensuring adequate nutritional status of the family, particularly of children and pregnant women.

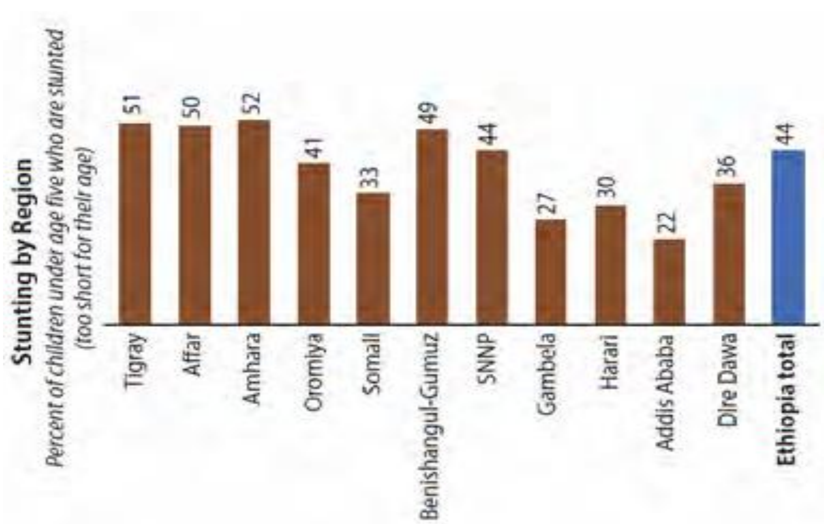


Figure 2: Stunting of children under five by region in Ethiopia, DHS 2011

2 .5 Protein Energy Malnutrition

Protein Energy Malnutrition is referring to a spectrum of diseases arising as a result of an absolute or relative deficiency of calories and or protein in the diet. It is globally the most important risk factor for illness and death, with hundreds of millions of young children affected (Hendrickse, 1991; Mary, 2008).

The term protein energy malnutrition (PEM) includes marasmus, kwashiorkor, and intermediate states of marasmic-kwashiorkor. Children may present with a mixed picture of marasmus and kwashiorkor, or milder forms of malnutrition. Marasmus is characterized by wasting of body tissues, particularly muscles and subcutaneous fat, and it is restrictions in energy intake. Kwashiokor, is described by fluid oedema (particularly ascites), and is usually the result in limits in protein intake. The combined form of PEM is called marasmic kwashiokor is often a synergistic factor underlying deaths in many children (Scheinfeld *et al*., 2012).

Protein Energy Malnutrition results in various changes in the body including changes in haematologic profile of the body. Low red cell count resulting in anaemia has always been a constant feature of protein energy malnutrition (Warrier, 1990).PEM may be attributable to various factors such as iron deficiency, and /or reduced red cell production in adaptation to a smaller lean body mass. Erythropoietin deficiency, deficiencies of vitamins (folic acid, B12) or trace elements (copper, zinc), infections and chronic diseases have also been implicated. White cell changes seen in protein energy malnutrition vary and such changes have been attributed to various factors. These include the synergist relationship which PEM has with infections and thymic atrophy seen in children with PEM (Mary, 2008).

PEM has been identified as the most lethal form of malnutrition indirectly or directly causing annual death of at least 5 million children worldwide. In 2000, World Health Organization (WHO) estimated that malnourished children were 181.9 million (32%) in developing countries. In addition, an estimated 149.6 million children under 5 years are malnourished when measured in terms of weight for age. The same report indicated that in South Central Asia and Eastern Africa, about half of the children have growth retardation due to PEM (Nutrition for Health and Development or NHD, 2000).

On the other hand, UNICEF (2005) reported that malnutrition was associated with approximately 50% of child deaths worldwide. It has been estimated that PEM affect every fourth child in the developing world, with the regional prevalence for the severe forms ranging from 1-7%.

2.6 Complementary food

The World Health Organization (WHO) recommends that the terms weaning and weaning foods should be replaced by the term complementary feeding because 'weaning' is traditionally used to describe curtailment of breastfeeding (WHO,1998). Complementary foods are the first nutrients providing foods given to infants in addition to breast milk after six months period. A complementary food must have a high calorie and micronutrient density, be in a "low bulk" or free floating form, free of bacterial contamination, and must be of a small amount that can be consumed at one feeding. The prevalence of under nutrition and micronutrients deficiency is high among infants and young children of 6-23 months old. In developing countries, the traditional infant complementary food is made of cereals or tubers may be low energy density in nutrients including protein, vitamin A, zinc and iron; these nutrients are of special importance due to their impact on physical and cognitive development (Neumann *et al.,* 2002).

In Ethiopia, the most notable nutritional problems in infants are protein energy malnutrition and micronutrients deficiency (Bukusuba *et al.*, 2008). Children consume complementary foods made of cereals or root crops. Cereals are deficient in certain essential amino acids (*i.e.*, lysine and tryptophan), but have sufficient amount of sulphur-containing amino acids, and additionally presence of anti-nutrients such as phytic acid, tannins and polyphenols (Vasal, 2001). Ideally, complementary foods should contain animal source foods with high biological value to promote growth and development (Krebs and Westcott, 2002). However, these foods may not be available to most low-income households in developing countries including Ethiopia.

2.7 Micronutrients and essential minerals

Micronutrients are substances in foods that are essential for human health and are required in small amounts. They include all of the known vitamins and essential trace minerals. Micronutrient deficiencies are a result of inadequate intake or inefficient utilization of available Micronutrients as well as and parasitic infestations (Rayhan and Khan, 2006). Consequences of micronutrient malnutrition include increased mortality rates, especially in women and children;

poor pregnancy outcomes; increased morbidity; impaired mental and physical development in children; and reduced work productivity in adults (Black *et al.,* 2008). Both the nutrient density and bioavailability of micronutrients in the diet are important for achieving optimal micronutrient status. Nutrient density is the amount of a nutrient in a food per calorie or unit weight..

An adequate mineral absorption is important for infants, children, older people and people in clinical situation. Among the micronutrient malnutrition situations afflicting the human population, Fe and Zn deficiencies are of major concern not only because of the serious health consequences but also because of the number of people affected worldwide particularly in Africa (Kayode, 2006).

2.7.1 Zinc

Zinc is an essential trace mineral that is a component of over 200 enzymes. It participates in all major biochemical pathways and plays multiple roles in the cellular proliferation and differentiation it affects physical growth, immunity, reproductive function and neuro-behavioral development (Walingo, 2009; Caulfield and Black, 2004).

Research conducted over years suggested that zinc deficiency (ZD) is widespread public health problem in the world .World Health Organization estimated that ZD 4-73% in prevalent of the world's population (Caulfield and Black, 2004). In Ethiopia it is estimated that 21.1% of the population was at risk of inadequate dietary zinc intake. Sidama zone reported 72% prevalence of ZD among pregnant women whereas, a study in Addis Ababa found and 11.3% marginal deficiency among lactating mothers (Hotz and Brown 2004).

Deficiency of Zn is highly prevalent in developing countries, especially in children and pregnant woman. Zinc deficiency leads to poor growth, impaired immunity, lower weight gain, and increased morbidity from common infectious diseases and increased mortality (WHO, 2000). The main causes of zinc deficiency are inadequate dietary zinc intake,a presence of inhibitors of zinc absorption, high zinc losses due to diarrhea or a combination of these factors.Zinc deficiency arises to a large extent from impaired bioavailability of dietary zinc, largely attributable to the high phytic acid and fiber content of diets. (Melaku *et al*., 2005)

Zinc deficiency is presumed to be the underlying cause of stunting and delayed sexual maturation in childhood. Zinc supplementation increases linear growth in stunted children which suggests that these high rates of stunting may be due in part to zinc deficiency (Walingo, 2009).

Zinc is found in many foods, with animal source products being the most readily absorbed. The best sources of highly bioavailable zinc include red meat, liver, poultry, fish, eggs, crabs, and oysters. Staple foods in developing countries including cereals and legumes are the main sources of zinc. The bioavailability of zinc in plant-based foods is reduced by the antinutrional factors. Specific food processing techniques such as soaking, germination, and fermentation also help to reduce the impact of zinc inhibitors (Sandberg, 2002).

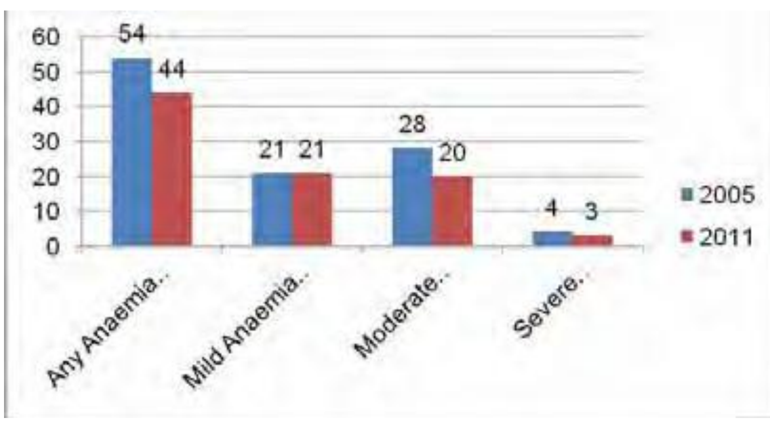
2.7.2 Iron

Dietary iron is an element for the synthesis of haemoglobin, plays a vital role in the transport of oxygen, and myoglobin. It contributes to the formation of iron-containing enzymes that are important for energy production and immune defense. Iron has a role in healthy physical growth, the immune system, reproductive outcomes, and in cognitive performance. Iron deficiency is one of the major nutritional problems in the developing world, affecting primarily women of childbearing age, infants, and children. Infants of age between 6 and 24 months are especially vulnerable to the development of iron deficiency (Kayode, 2006).

The main factors for iron deficiency are poor iron content of diets and low bioavailability of iron in the diets. Food components such as phytate, tannins and dietary fibers.Dietary fibers are which bind iron in the intestinal lumen due to impair iron absorption because infants have a small stomach capacity it is difficult to consume sufficient quantities of energy requirements .Phytate has the greatest effect on iron status because many plant foods have high phytate content that can severely impair iron absorption (Mendoza, *et. al.*, 2001). The symptoms of iron deficiency anaemia include reduced physical work capacity, delayed psychomotor development in infants, impaired cognitive function, impaired immunity and adverse pregnancy outcomes. However, as these are difficult to relate directly to a specific dietary intake, biochemical indices are generally used in estimating dietary requirements.

Dietary iron is present in foods in two main forms; haem iron only in foods of animal origin and non-haem iron in both animal and plant foods, mostly in the ferric state. Haem iron is transported into the enterocyte by the haem receptor, while non-haem iron uses the divalent metal transporter (DMT), which means that dietary ferric iron (Fe^{3+}) must be reduced to ferrous iron (Fe^{2+}) before uptake (Mackenzie *et al*., 2005). Absorption of non-haem iron does not exceed 20% where it is enhanced by ingestion of vitamin C (Monsen, 1988). Food absorption studies in humans have revealed that the absorption of haem iron is relatively unaffected by other dietary factors. Haem iron is absorbed at 15-35% of intake (Hallberg, 1981).

Iron Deficiency Anaemia (IDA) is one of the most common nutritional disorders and it has public health importance in developing countries like Ethiopia. It is the most common cause of nutritional anaemia in adolescents and women of reproductive age (Melaku *et al*.,2008).The DHS (2011) report indicates that 44% of children under five in Ethiopia are anaemic Forty-five percent of children (aged 6 - 59 months) in rural areas are found to be anaemic, compared with 35 percent of children in urban areas. Regional variation of anaemia in children ranges from 33 percent in Addis Ababa to 75 percent in Afar Region. Anaemia among children decreases with increases in mother's education and wealth quintile. Figure 3 show that the national anaemia prevalence estimate in children has dropped by 10 %points in the past five years, from 54 % in 2005 to 44 percent in 2011.



Source (DHS,2011)

Figure 3. Trends in Anaemia Status among Children Aged 6-59 Months in Ethiopia from 2005-2011.

2.7.3 Calcium

Calcium is the most abundant mineral element in the body. Calcium regulates critical functions including nerve impulses, muscle contractions and the activities of enzymes and it is located in the bones (more than 99%), plays an important role for structure and strength of bones. When infants are weaned and start to become mobile and their bones thus become weight bearing, rapid calcification of the bones takes place. Preterm babies are particularly at risk of not being able to absorb enough calcium for optimal bone growth. Mineral deficiencies, such as calcium, are a world-wide problem particularly in developing countries (Boukari *et al.*, 2001).

2.8 Bioavailability and Antinutritional factors

The term “bioavailability” is defined as the proportion of an ingested nutrient in food that is absorbed and utilized through normal metabolic pathways (Hurrell,2002). Cereals and legumes are rich in minerals but the bioavailability of these minerals is usually low due to the presence of antinutritional factors such as phytate, tannin, oxalate and polyphenoles. An adequate mineral absorption is important for infants, children, elder people and people in clinical situation (Hurrell, 2002).

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The bioavailability of zinc in plant-based foods is reduced by phytate, fiber, calcium, and lignin (a component of vegetable fiber) present in the plant. High level of calcium in the diet has also been shown to exacerbate the inhibitory effect of phytate on zinc absorption humans by forming insoluble complexes with calcium and zinc in the intestine (Ahrens, 1989). Specific food processing techniques such as soaking, germination, and/or fermentation also help to reduce the impact of zinc inhibitors. Bioavailability of zinc from the molar ratio of phytate to zinc in the diet and ratios >15 have been negatively associated with poor growth in children.

Complementary foods made from cereals are often low in iron content and contains significant quantities of iron absorption inhibitors and anti nutritional factors. The main causative factors of iron deficiency are poor iron content of the diet, low bioavailability of iron in the diet, or both. The bioavailability of iron in food depends on, iron status of the body; the presence of iron inhibitors and enhancers; types of iron (haem and non-haem); vitamin A status of the body and the status of other micronutrients. Phytate: iron molar ratios > 0.15 is regarded as indicative of poor iron bioavailability. Absorption of iron from cereals can be increased by degradation or removal of phytic acid with a simple technology like fermentation (Hurrell *et al.,* 2004).

Antinutritional compounds (such as protease inhibitors, phytate, tannin and other phenolic compounds and saponins) are plant constituents which play an important role in biological functions of plants. These compounds, in human diet reduce the digestibility of nutrients and the absorption of minerals (Dicko, 2005 Soetan and Oyewole 2009).

2.8.1 Phytic acid, phytate and phytin

Phytic acid (myo-inositol hexaphosphate) is made up of an inositol ring with six phosphate ester groups, and its associated salts such as magnesium, calcium, or potassium phytate; which limits the availability of those minerals in the body (Figure 4). Phytic acid is of widespread occurrence in plant foods such as cereal grains and legume seeds. Phytic acid content in tuber and green vegetable are limit. Phytate is the salt of phytic acid very common in plants and cereal grains and legumes. The phytate chalate the micronutrient elements such as Ca, Zn, Fe and Mg therefore, reduces the mineral bioavailability; powerful chelating of divalent cations by the formation of insoluble complexes. In addition to this phytate binds macro-elements and form a complex with protein, protease and amylases of the intestinal tract, thus inhibiting proteolysis.

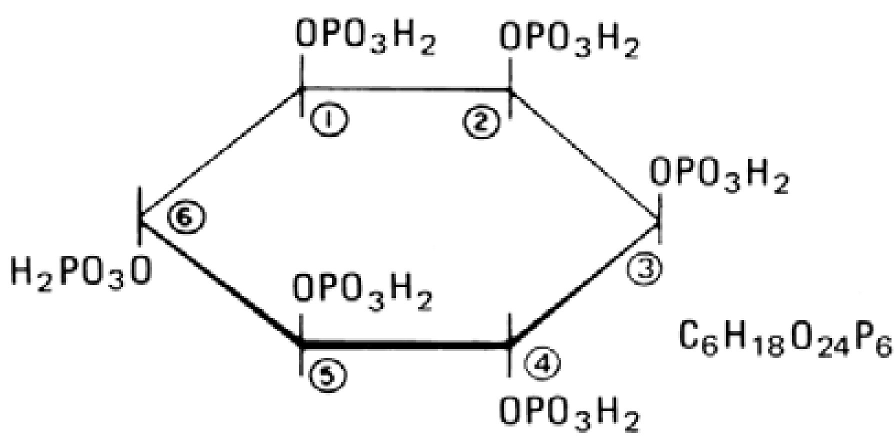

Figure 4.Structure of Phytate (Insp6), empirical formula = $C_6P_6O_{24}H_{18}$

Phytin is a complex salt of phytic acid, inorganic cations and proteins. This is the form in which most phytic acid occurs in plants (Frias, 2005). In cereals, approximately 1-2% weight of the seed is phytic acid, and it can even reach 3-6%. Referring to its location, 90% is found in maize seed, while in wheat and rice it is distributed in larger proportions in the external covers in the pericarp. Phytic acid has been degraded to significant level in weaning food by adding commercial exogenous phytase or by activating the local phytase by a combination of soaking, germination and fermentation process (Cheryan, 1980)

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Germination/malting increase the endogenous phytase activity in cereals, legumes, and oil seeds through, activation of intrinsic phytase. Tropical cereals such as maize and sorghum have a lower endogenous phytase activity than do rye, wheat, triticale, buckwheat, and barley (Egli *et al.*, 2002). The rate of phytate hydrolysis varies with the species variety as well as the stage of germination, pH, moisture content, temperature (optimal range 45–57C^0), solubility of phytate, and the presence of certain inhibitors. Egli *et al.,* (2002), observed that during germination, rice, millet, and mung bean had the largest reductions in phytate content.

Fermentation is traditional processing of food by the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification of food used in plant based foods to increase the nutritional quality and remove antinutritional compounds .The minerals of the grain are not readily available by producing complexes with phytate. However, at pH values of <5.5 the endogenous grain phytase hydrolyses, due to this Phytic acid reduce chelating ability with minerals and proteins (Hammes, *et al*., 2005).

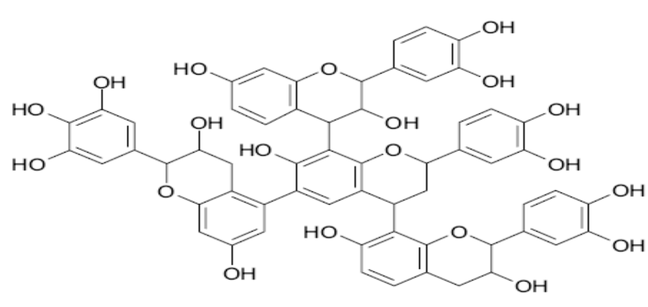
Microbial phytases originate either from the microflora on the surface of cereals and legumes or from a starter culture inoculate (Sandberg, 1991). The extent of the reduction in higher inositol phosphate levels during fermentation varies; sometimes 90% or more of phytate can be removed by fermentation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

Health benefit of phytates has the ability to chelate minerals that participate in undesirable oxidative reactions, phytates undesirable oxidative damage cell membrane it have been suggested to chelate the divalent minerals makes them a natural antioxidant.

2.8.2 Phenolics and tannins

Tannins are very complex group of plant secondary metabolites, which are soluble in polar solution and are distinguished from other polyphenolic compounds by their ability to precipitate proteins and other macro -elements and micro- elements, particularly iron. If tannins are ingested in excessive quantities they inhibit the absorption of iron which may lead to anemia. This is because tannins are metal ion chelators, and tannin-chelated metal ions are not bio-available (WHO, 2008). The suggested mechanism behind this is the possibility that food tannins and saliva proteins form complexes in the mouth, there giving an unpleasant astringent taste, and also that the tannins can affect digestion enzymes. Tannins have been reported to inhibit the digestive enzymes and lower the digestibility of most nutrients, especially protein and carbohydrates.

Tannins are classified into two groups: condensed tannins which are show in (figure 5) are polymers of flavones and yield anthocyanidins when heated in acid solution, and hydrolysable tannins which produce gallic acid as a degradation product the latter are easily eliminated with the action of the gastric juices and do not represent a high significant problem in animal feeding (Obatolu and Cole, 2000).



Figur 5. Structure of condensed tannin

Phenolic compounds are contain a benzene ring with one (phenol) or more (polyphenol) hydroxyl substituent, including functional derivatives (ester, methyl ethers, glycosides, etc.) Many plant phenolic compounds are polymerized into larger molecules such as the proanthocyanidins (condensed tannins) and lignins (Dykes and Rooney, 2007). In general, these compounds are located mainly in pericarp cereal grains.

phenolic and polyphenolic compounds constitute an important class of secondary metabolites that act as free radical scavengers and inhibitors of LDL (low density lipoprotein), cholesterol oxidation and DNA breakage (Shahidi, 2004) However, complex is formed with minerals which reduce the bioavailability of minerals.Therefore, iron and zinc in cereal based foods are poorly bioavailable due to factors that reduce their intestinal absorption, resulting in high rates of iron and Zinc deficiency especially in infant, children and women of child bearing age. Also polyphenolic compounds have beneficial anti-oxidant properties (Armad *et al.*,1998)

2.9 Traditional food processing techniques for infant foods

Traditional food processing is simple methods of household technologies that have been used to process cereals and legumes in order to improve the nutritional and functional properties of the foods .They include process of roasting, germination and fermentation, cooking and soaking (Yagoub and Abdalla, 2007).The application of such technological processes can alter physical and chemical properties of the components and reduce antinutrient levels. In addition to this, it enhances the bioavailability, utilization of nutrients and also improves palatability and may enhance the digestibility and nutritive values (Sandberg ,2002).

2.9.1 Fermentation

Fermentation is the process in which organic substrate, usually carbohydrate, is incompletely oxidised, and an organic carbohydrate acts as the electron acceptor. It involves ethanol production by yeasts or organic acids by lactic acid bacteria; this is due to the action of micro-organisms or enzymes to cause desirable biochemical changes result in significant modification to the food (William and Dennis, 2011)

A wide range of cereal-based fermented foods exists in many African countries such as ogi and mawe` in Benin, kenkey in Ghana, injera in Ethiopia, potopoto in Congo, ogi and kwunu-zaki in

Nigeria, uji and togwain Tanzania and kisra in Sudan (Blandino, *et al.*, 2003). These foods are still often used to prepare gruels for the complementary feeding of infants and young children. However, compared to the required composition of complementary food, the traditional gruels have low energy and nutrient density but fermentation improves the quality of these foods (Dewey and Brown, 2003; Lutter and Dewey, 2003).

The nutritional benefit of fermentation includes production of important nutrients or elimination of anti nutrients. Fermentation improves the typical diet through development of flavor, aroma, and texture in the food. Fermentation is used for preservation and shelf-life extension through the production of lactic acid, alcohol and acetic acid. The production of lactic acid lowers the pH of the food, which slows down or prevents its spoilage by other microorganisms and renders the food safe from the growth of pathogens. Fermentation also results in lowering the pH reduce proportion of dry matter in the food and the concentration of protein, essential amino acids, essential fatty acids and vitamins, improving digestibility and nutrient availability (Michaelsen *et al.*, 2008). Also, it is evident that fermentation has the potential to enhance iron and zinc absorption (Teucher *et al.,* 2004) as well as enhancement of food quality. Fermentation reduces anti-nutritional factors in plant foods and decrease in cooking time and fuel requirement.

2.9.2 Germination

Germination is also another traditional food processing method that enhances the quality and nutrient availability of foodstuffs.During germination alpha and beta-amylases are mobilized which hydrolyze starch molecules in to lower molecular weight dextrins and maltose (Nnam, 1999; Hotz and Gibson, 2007). Germination serves to reduce bulk and increase the energy and nutrient density. Golder, (2001) stated that high volume and viscous complementary foods referred as dietary bulk is considered a major factor in the development of malnutrition in areas where cereals and starchy staples are the main foods. Germination especially plays role in the quality improvement of a cereal and legumes for both digestibility and physiological functions. During germination, there will be an increase in enzymatic activity (endogenous phytase) and bioactive compounds. Malting, leads to an increase in phytase activity in certain cereals (e.g. maize, millet and sorghum). The rate of phytate hydrolysis varies with the species and variety as well as the stage of germination, pH, moisture content, temperature (optimal range 45–57^{o}C), solubility of phytate, and the presence of certain inhibitors. Complementary foods with a higher energy density and acceptable noticeable viscosity could be prepared from germinated seeds of cereals (Brunken *et al.*, 2006).

2.9.3 Soaking

Soaking cereal and most legume flours (but not whole grains or seeds) in water can result in passive diffusion of water-soluble, K or Mg- phytate, which can then be removed by decanting the water. A simple soaking procedure appropriate for rural life households has been developed that can reportedly reduce the phytate content of unrefined maize flour by 50% (Ikujenlola and Fashakin, 2005). This is important because several recent in vivo isotope studies in adults and infants have reported improvements in absorption of iron, zinc, and calcium in cereal-based food prepared with a reduced phytate content. Some polyphenols and oxalates that inhibit iron and calcium absorption, respectively, may also be lost by soaking (Egli *et al.,* 2002).

2.10 General account in to the ingredients of complementary food

Traditional complementary food in developing countries is known to be of low nutritive value that means low protein, energy density and high bulk density. The concept of cereal–legume complementation has been particularly applied to the development of infant complementary foods with improved protein quality (Egounlety, 2002). Nutritionally, the high protein content of legumes increases the protein content of cereal-based complementary foods.

Unrefined maize, sorghum and legume flour may be limited due to in built antinutritional factors such as amylase inhibitors, protease inhibitors, phytates, condensed tannins, and polyphenols, which lower the utilization of starch, protein, and minerals unless they are processed (Ejigui, *et al*., 2005).

Traditional processing methods, such as fermentation and germination, are simple and inexpensive and have been practiced for many years in developing countries. These methods have often been used separately or in combination for preparation of infant complementary foods Traditional processing may produce foods with many positive attributes, such as favorable texture, good organoleptic quality, reduced bulk, enhanced shelf life, partial or complete elimination of antinutritional factors, reduced cooking time, and improved nutritional value (Egounlety, 2002).

2.10.1 Cowpea (*Vigna unguiculata* L. Walp)

Cowpea is a dicotyledonous plant belonging to the family Fabaceae and sub-family, Fabiodeae. It is grown extensively in the low lands and mid-altitude regions of Africa (particularly in the dry savanna).Cowpea is sometimes consumed as sole crop but more often intercropped with cereals such as sorghum or millet (Agbogidi, 2010a). Cowpea is the most popular in the semi-arid of the tropics where other food legumes do not perform well (Sankie *et al.*, 2012). It is an extremely resilient crop and cultivated under some of the most extreme agricultural conditions in the world (Muoneke *et al*., 2012) .Cowpea is a protein rich, drought tolerant and early maturing crop.

World production of cowpea was estimated to be 2.27 million tons (FAO, 2002; Adaji *et al.*, 2007) and about 70% of the cowpea production occurs in marginal areas of West Central, East and Southern Africa. Nigeria is the largest producer and consumer of cowpea at estimated annual yields of 2 million metric tons (Timko *et al*. 2008). In Tanzania, cowpea is regarded as a 'women's crop, because, contrary to other crops, the production process to marketing is often handled by women Thus, it is among the crops that are generating income to female farmers and traders. Cowpea contributes significantly to household food security in West and Central Africa .In Ethiopia most of times cowpea is valuable in fodder production (Etana *et al.*, 2013).

Cowpea (*Vigna unguiculata* L. Walp) is a multifunctional crop that provides food to human being and feed to livestock. Cowpea fixes atmospheric nitrogen through symbiosis with nodule bacteria. Cowpea is of major importance to the livelihoods of millions of relatively poor people in less developed countries of the tropics (FAO, 2002). Islam *et al.* (2006) emphasized that all parts of the plant used as food are nutritious providing protein and vitamins, immature pods and peas are used as vegetables while several snacks and main dishes are prepared from the grains.

Nutritional value of cowpea is inexpensive source of protein that is richer in essential amino acids like lysine than cereal grains. Cowpea contain crude protein 22.9-32.5%, carbohydrate 59.7-71.6.% and fat 1.4-2.7% (Nielsen et al. 1993). Studies have shown several applications of cowpea proteins which have a potential role for the development of complementary foods due to the high nutritional value (Timko *et al*, 2008). The presence of high protein content in all cowpea parts consumable by human and animal (leaves, stems, pods and seeds), is the key factor in alleviating malnutrition among women and children and improvement of health status of the livestock in resource limited households where regular access to animal protein is limited due to low economic status (Islam *et al.,*2006).

Minerals and vitamins are the other nutritionally important constituents of the cowpea seeds. It has been reported that folic acid, a vitamin B necessary during pregnancy to prevent birth defect in the brain and spine content is found in higher quantity in cowpea compared to other plants (Timko and Singh, 2008). Their mineral contents such as calcium and iron are higher than that of meat, fish and egg and the iron content equates that of milk. Cowpea also consists of vitamins such as thiamin, riboflavin, niacin (water soluble).These nutritional constituents of cowpea are useful in blood cholesterol reduction (Achuba, 2006). Adaji *et al*., (2007) had showed that daily consumption of 100–135gm of cowpea reduces serum cholesterol level by 20% thereby, reducing the risk for coronary heart diseases by 40%.

Different dishes can be prepared from cowpea. The young tender leaves can be cooked and eaten as vegetable, the green pods can be cooked and eaten just like green beans, the seeds can be cooked when fresh (semi-ripe) and, when full matured and dry, eaten as pulses. In Tanzania and other African countries, cowpea is used for preparation of stew that is either used together with cereal dishes or directly mixed with cereals such as maize, wheat, sorghum and rice. This kind of food is very popular within the community and preferred to be used in large gatherings for example in schools and hospitals, due to its simplicity of preparation and handling (Osundahunsi, and Aworh, 2002).

2.10.2 Sorghum (*Sorghum bicolor* (L.) Moench)

Sorghum (*Sorghum bicolor* (L.) Moench) like many other cereal grains, has a diversity of uses, including human consumption and animal feed. Sorghum is used for human consumption is one of the most important food crop in the world, following wheat, rice, maize and barley. It is a staple food in Asia, Africa and other semi-arid regions of the world (Dillon *et al.,* 2007). Sorghum is a grass family of Poaceae producing dry indehiscent fruit. Sorghums are pigmented based on certain anthocyanins, anthocyanidins and other flavonoids, compounds which color or stain the grains red, brown or purple. The pigments are concentrated in the pericarp and it may extend into the endosperm. These pigments can affect the color of sorghum food products. The pericarp and the testa constitute about 6% of the dry weight of the sorghum grain. The embryo and scutellum of embryo contains most minerals and lipids. The sorghum kernel is composed of starch, cellulose and simple sugars (Rooney and Wanisk, 2000).Nutritional value of sorghum contains protein (7.5–10.8%), ash (1.2–1.8%), oil (3.4–3.5%), fiber (2.3–2.7%) and carbohydrate (71.4–80.7) contents (Idris *et al*., 2005). Furthermore sorghum flour contained 11.0–13.0, 285–310 and 4.0–5.5 0 mg per 100 g Ca, P and Fe, respectively (Idris *et al*., 2005).

Sorghum was domesticated in Ethiopia some 5,000 or more years ago and was taken from Ethiopia to West Africa at an early date across the Sudan to the upper Niger River (Abdulhamed, 2002). Sorghum (*Sorghum bicolor*) is one of the most widely grown cereal crops in Ethiopia. It is a staple food crop on which the lives of millions of poor Ethiopians depend. It has tremendous uses for the Ethiopian farmer. Sorghum grows in a wide range of agroecologies most importantly drought stressed crops. As a result, it is capable for food security in our country (ICRISAT, 2007)

The grain is traditionally used for food production of cloudy and opaque beer the key ingredient of these beers is sorghum malt, which provides hydrolytic enzymes. Preparation of traditional dishes typically entails cooking of sorghum grains mixed with legumes, or boiling of sorghum flour into various types of porridges. For food use, the grains are decorticated to reduce the polyphenol content and milled into coarse flour, which is then cooked directly .Sorghum is eaten in different forms in different places. Worldwide traditional sorghum foods are broadly grouped in to four categories as shown in Table 2.1.

Table 2.1 Examples of traditional sorghum food staffs

Food categories	**Local Name**	**Country**	**Description**
Flat bread	Injera	Ethiopia	Fermented batter baked in to large thin pancakes
Porride	Ogaoko,akamu, koko,akasa	Nigeria,Ghana	Thin porridge. Sorghum grain is steeped in water to ferment. Fermented grain is milled. The bran removed and the sediment cooked with in pot with water to produce porrrige consumed warm or cooled to form gel.
Boiled and steam product	Couscous, acha	Africa, India	Sorghum ground finely in to flour, knead with water to agglomerate, forced through coarse screen and steam then cooked like rice
Snacks and special food	Have no local name	Worldwide	Sorghum

Source-(FAO and ICRISAT, 1996)

Sorghum used to the subsequent processing method like boiling, germination, fermentation and cooking greatly improves its nutritive value (Inyang and Zakari, 2008). However, combination of these processes further improved the quality of sorghum as a food by removing the ant nutritional factors.

2.10.3 Maize (*Ziya maize* L.)

Maize is, after wheat and rice, the most important cereal grain in the world. Maize is the world's most widely grown cereal. It is grown on more than 96.5 million hectares in the developing countries and many millions of people worldwide are dependent on maize as a staple food. (Prasanna *et al.*, 2001).

There is the most controversial the origin of maize, through in general accepted that maize the center of origin is located in Mesoamerica ,primarily Mexico (5200-3400BC) and the Caribbean .Maize as we know it today distributed worldwide and gradually extinction of wild maize in favor of modern varieties through a more intensive cultivation. Maize arrived in Africa 500 years ago (M'mboy,*et.al*., 2010).

Maize it is nevertheless a relative newcomer in a very old and complex environmental setting. Maize has an extensive geographical area in elevations from sea level to more than 3,000 meters; from the equator to above 500 north and south (well beyond the tropics and subtropics); and in cold, hot, rainy, and dry areas (M'mboy,*et.al*., 2010). Although its relative importance varies by country and region, maize is widespread in sub-Saharan Africa.

Developing countries plant two-thirds of the global maize production while industrialized countries plant one-third. Nine of the top 25 maize-producing countries are from Africa, producing 17.4 Million hectare which is 12.5% of the maize global area (M'mboy,*et.al*., 2010).). The major maize growing countries are Nigeria, South Africa, Ethiopia, Kenya, Malawi, Tanzania, Congo, Mozambique, and Zimbabwe, most of which are from Eastern and Southern African countries.

Maize is one of the most important cereals cultivated in Ethiopia. It ranks second after teff in area coverage and first in total production (CSA, 2012). In maize dominated farming system of Western Ethiopia, farmers traditionally use maize stover as important source of feed, firewood and construction of grain storage structures. Due to rapidly increasing human population and a subsequent need for cultivable land, more grazing land is being put under cereal production mainly maize in sub humid climatic conditions of Ethiopia. It is, therefore, important to have a high grain and quality stover yielding varieties to improve animal feed availability in the maize based systems. Improved maize can proud remarkable yield for small-scale farmers .This abundance can contribute to greater production and productivity in the agricultural sector while also addressing the country's food security and poverty reduction challenges (Dawit and Spielman, 2006).

Maize is one of the most important food sources for a greater part of the human population in sub-Saharan Africa. Also worldwide human beings are dependent upon cereal grains for the greater part of their food supply. It provides nutrients for humans and animals and serving as a basic raw material for the production of starch, oil, protein, alcoholic beverages and food sweeteners. The leaves, stalks, and tassel of maize are used as fuel, or livestock feed, either green (fodder or silage), or dried (stover). It also the parts of the root are used for mulching, manure, or burned as fuel. In industrialized countries, maize is largely used as livestock feed and as a raw material for industrial products, while in sub-Saharan Africa it is mainly used for human consumption, being a staple food for 50% of the population (M'mboy,*et.al*., 2010). Maize grain has nutritional value as it contains 72%starch, 10% protein, 4.8% oil, 8.5% fibre, 3.0% sugar and 1.7% ash (Chaudhary, 1983).

Maize is a primary complementary food for infants; these are at risk of malnutrition and stunted growth because maize is deficient in two essential amino acids such as lysine and tryptophan. The nutritional value of maize for food can be enhanced by reducing the level of phytic acid. Phytic acid have chelates iron(Fe), zinc (Zn), calcium (Ca), and magnesium (Mg) cations, leading to deficiencies of these as well. The impact is malnutrition in children under five, and of overall undernourishment. (M'mboy,*et.al*., 2010).

Maize is processed into various products such as porridges/gruels (*uji, ogi, mawe*), flat breads (*kenkey*) and beverages (*obiolor, kwete,* kaffir beer). Many of the traditional foods in Africa are produced from fermented or geminated maize, which increases the vitamin content, mineral bioavailability and the quality of protein (Sekwati-Monang, 2011)

3. MATERIALS AND METHODS

3.1 Reagents and raw materials collection

All reagents used in this study were analytical reagent grade. The reagents and chemicals used for analysis were purchased from Neway P.L.C. in Addis Ababa Ethiopia. The varities of raw materials maize (Melkassa-4), Sorghum (Teshale) and cowpea (Kankety) were collected & transported from Melkassa Agricultural Research Center. It is about 110 km, East of Addis Ababa Ethiopia.

Figure 6.Varities of maize (Melkassa-4), Sorghum (Teshale) and cowpea (Kankety) respectively.

3. 2 Sample preparation

3.2.1Cleaning, sorting and preparing flour sorghum and maize seeds

A total of 0.5kg each of maize and sorghum for each was cleaned by manually and floated in water to remove broken grains and extraneous materials. Each of the cleaned sorghum and maize varieties were divided into two portions for the study of energy and nutrients density, viscosity, anti-nutritional content and sensory evaluation of the complementary food. The first portion ungerminated (serves as control) and the second portion Germinated 48 hours. The grains were soaked in tap water (1:3 w/v) for 24 hrs at ambient temperature. Water changed two times a day at regular interval to prevent fermentation (Hendrickse, 1991). The grains maize and sorghum was prepared for germination process. Ungerminated maize and sorghum were dried in drying oven 50^0C for about 2 hours and milled into flour using Tecator Cyclotec 1093 sample mill

(Sweden), then sieved through 0.45 mm screen. The flours are packed in clean polyethylene bags and stored at 4^0C until further use.

3.2.2 Cleaning, sorting and preparing flour cowpea seed

Cowpeas were soaked at room temperature (25±1°C) in tap water at grain ratio of 1:3 for 12 h .The soaked seeds were then placed in sieve and allowed to drain (Gibson, *et al.*, 2006).cowpea Seeds were also boiled in tap water for about 20 minutes. This step is called “blanching”. This was done to make dehulling easier and the water was drained off and discarded. The seeds are oven-dried at 50°C for 22 h in a hot drying oven, then the cowpea dehaulled manually and ground using a laboratory mill and screened through a 0.425 mm sieve particle size. They have been packed in clean polyethylene bags and stored at 4^0 C until further use.

3.3 processing methods

3.3 .1 Germination

The hydrated grains sorghum and maize were drained and spread thinly on wet clean cloth and covered with another layer of wet clean cloth. The grains where then germinated for 48 hrs with watering every six hours to facilitate germination (Mary, 2008). The seeds that did not germinated were discarded while the germinated with the sprouts were oven dried (50^0 C, for 18 hrs) and milled to flour, sieved and packaged until co-fermentation and nutritional analysis.

3.3.2 Natural co-fermentation process

The flour was prepared from tap water into plastic containers at concentration of 1:3 dilutions (w/v). The flour slurry was allowed to ferment naturally endogenous microflora on the seeds at room temperature (22 ±23^0C) sampling was every done 24-hourly for 3days. Aliquots decanted and fermenting slurry for analysis were withdrawn and transferred to a deep freezer after each fermentation time.

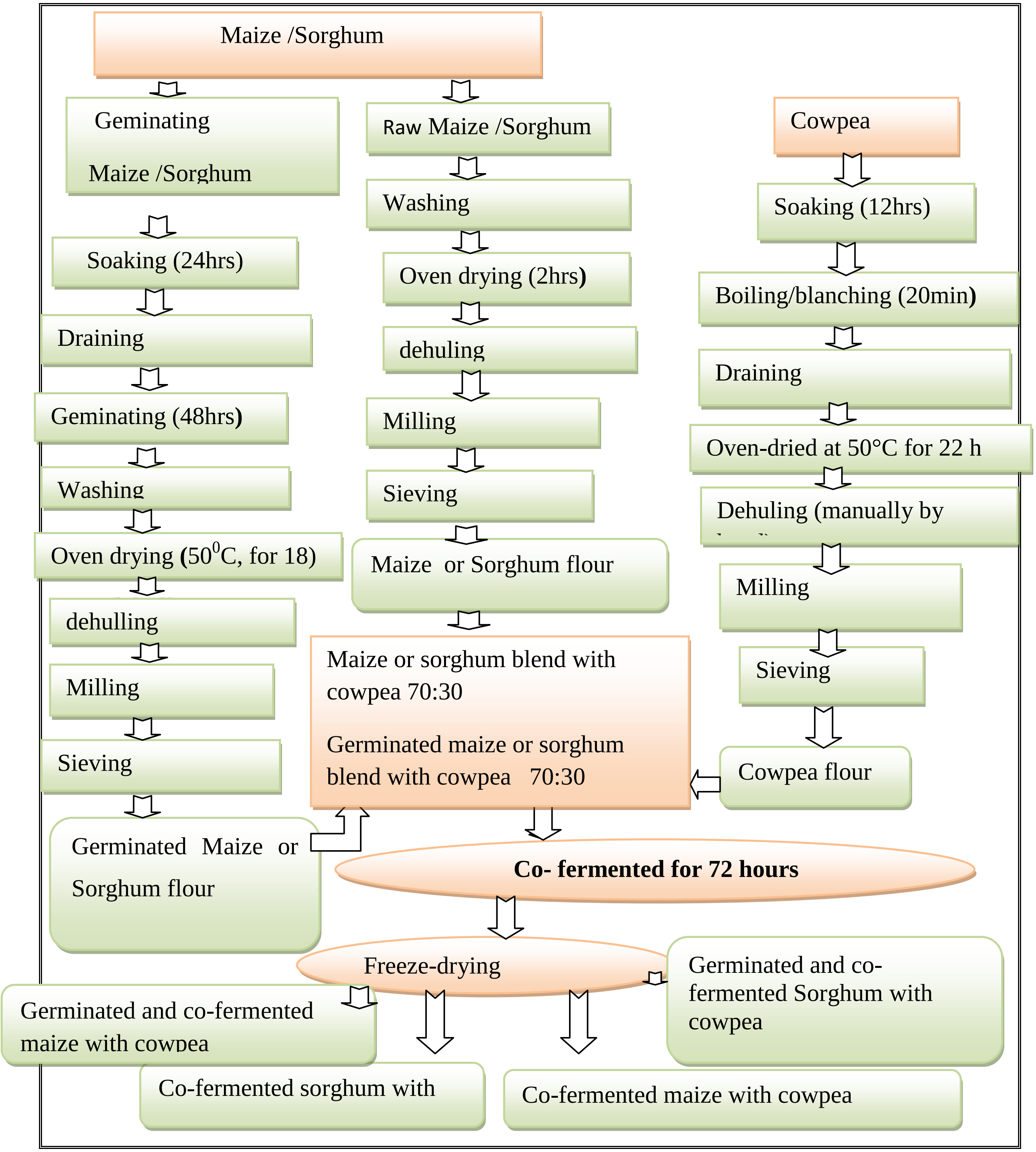


Figure 7: Processing flow chart of samples preparation for production of co- fermented flour.

3.4 Complementary Food formulations

Cereal–legume blend have been particularly applied to the development of infant complementary foods with augmented protein quality and optimal mixture of essential amino acids (Mbithi-Mwikya, 2000; Egounlety, 2002 ,Eschleman, 1984).Nutritionally, legumes increase the protein content of cereal-based complementary foods for suitable infants. According to WHO, (1998) energy and nutrient estimated needs from complementary foods with the recommended 70:30 cereal–legume ratio blends were formulated theoretically ratio for correcting for lysine deficiency and increase energy density of the complementary food.The primary criteria is to select the components rich in providing protein and energy requirements, the next target is to know the proximate values of the raw materials that are going to be blended and evaluate the effect of traditional processing methods and blend formulation on the physicochemical and nutritional characteristics of complementary food comparison with RDA value 13 –14 g/100g protein and 308 – 389 K cal /100g energy content RDA to full fill for infant from 6 to 12 month.

3.4.1 Study Design

Eight diet formulations were prepared for sorghum and maize varieties. Formulations were made from germinated sorghum and maize (48 hours), un germinated (control), and its take 72 hours co-fermented mixture with germinated and un germinated flours at 70:30 proportions.

Table 3. 1. Blending proportions.

Sample code	Blend proportion
M/c	70% Maize blend with 30% cowpea
GM/c	70%Germinated maize blend with cowpea 30%
FM/c	70% Co-fermented maize with cowpea 30%
FGM/c	70% Combination of germinated and co-fermented maize with cowpea 30%
S/c	70% Sorghum blend with cowpea 30%
GS/c	70% Germinated sorghum blend with cowpea 30%
FS/c	70% Co-fermented sorghum with cowpea 30%
FGS/c	70% Combination of germinated and co-fermented sorghum with cowpea 30%

3.5. Laboratory analysis of physico-chemical properties

Each laboratory determination was carried out on three separate fresh samples (in triplicate).

3.5.1 Measurement of Total titratable acidity (TTA)

pH and TTA of fermenting samples were determined according to the method (Pearson, 1971.Total titratable acidity (TTA) was expressed as percentage of lactic acid. 10 ml of non-fermented and fermented flours were slowly pipetted in to a 50 ml beaker, 3 drops of phenolphthalein indicator added and titrated with 0.1 N NaOH. Distilled water (1ml) was titrated with 0.1 N NaOH was used a blank. To calculate the volume required for the titration, the initial reading was subtracted from the final reading.

Calculation:

$$\% \text{ Lactic Acid} = \frac{V \times 0.009008 \times 100}{W}$$

Where: V=Volume of 0.1 N NaOH used for sample titration;

0.009008=Factor equivalent in which 1ml of 0.1N NaOH = 0.009008g lactic acid

W=Weight in gram of sample in the mixture

3.5.2 pH determination

pH measurement was done using a pH meter.1 g of sample mixed with 10 mL of distilled water was added and shaken in vigorously and allowed to stand for 30 min. The solution was filtered. The pH meter was adjusted or calibrated against standard buffer solutions at pH (4 and 7) pH was determined using the final reading of the pH meter (Pearson, 1971).

3.6 Proximate analyses

3.6.1 Moisture (925.09, AOAC 2000)

A moisture content of the sample flour was determined according to AOAC (2000), using the official method 925.05. The dishes used for the moisture determination were dried at 130 ^{0}C for 1 hr (Memmert, model 40050) and placed in desiccators for about 30 min. The mass (M_1) of each dishes was measured and about 5 g of the sample was weighed in to each dishes (M_2). The sample was then mixed thoroughly and dried at 100^0C for 3 hr. After drying was completed, the mass was measured (M_3). The moisture content was calculated from the equation:

$$\text{Moisture content} = \frac{(M_2 - M_3 * 100)}{M_2 - M_1}$$

Where:- M_1 = mass of the dish,

M_2 =mass of the dish and the sample before drying, and

M_3 =mass of the dish and the sample after drying.

3.6.2 Crude protein (AOAC 979.09, 2000)

Protein (N×6.25) was determined by the Kjeldahl method that involves:-

Digestion: About 0.5000g of fresh samples was taken in a Tecator tube and 6ml of sulfuric acid was added, mixed, thoroughly and 3.5ml hydrogen peroxide was added step by step. As soon as the violet reaction had ceased, the tubes were shaken for a few minutes and placed back into the rack. A 3.0000g of the catalyst mixture (ground 0.5000g of copper sulphate with 100 g of potassium sulfate) was added into each tube, and allowed to stand for a about 10 min before digestion. When the temperature of the digester reached 370^0C remaining until all nitrogen is converted to ammonia in the tubes were nearly about 3 hr. The tubes in the rack were transferred into the fume hood for cooling; distilled water was added, and shaken to avoid precipitation of sulfate in the solution.

Distillation: A 250ml conical flask containing 25ml of the boric acid-indicator solution was placed under the condenser of the distiller with its tips immersed into the solution. The digested and diluted solution was transferred into the sample compartment of the distiller. The tubes were rinsed with two portions of about 5ml distilled water and the rinses were added into the solution. A 25ml of 40% sodium hydroxide solution was added into the compartment and washed down with a small amount of water, stopper and the steam switched on. The distillation was continued until a total volume of 150ml is collected.

Titration- Distillation was titrated with 0.1N hydrochloric acid. The crude protein continent was determined or calculated as:

mg nitrogen in the sample = V x N x 14

g nitrogen/100 g ample =mg of nitrogenx100/mg sample

Total nitrogen (%) = (V-Vb) x N x 14/W

Crude protein (%) = total nitrogen (%) x6.25

Where:

V = volume of hydrochloric acid consumed to neutralize the sample;

Vb= Volume of acid consumed to neutralize the blank;

N = normality of the acid;

14= Eqwt of Nitrogen;

6.25=conversion factor from total nitrogen to crude protein

3.6.3 Crude fat (AOAC4.5.01, 2000)

Crude fat was determined by exhaustively extracting a known weight of sample in diethyl ether in a soxhlet extractor. The extraction flasks were cleaned dried in drying oven (Memmert, Germany) at 70^0C for 1hour, cooled in desiccators (with granular silica gel) for 30 minutes, and the n weighed. The bottom of the extraction thimble was covered with about 2cm layer of fat free cotton. About 2.00 gram of fresh samples were added into the extraction thimbles, and then covered with about 2cm layer of fat free cotton. The thimbles with the sample content were placed into soxhlet extraction chamber. The cooling water was switched on, and a 50 ml of diethyl ether was added to the extraction flask through the condenser. The extraction was conducted for about 3 hrs. The extraction flasks with their content were removed from the extraction chamber and placed in the drying oven at 70^0 C for about 1hr, cooled to room temperature in the desicator for about 30 minutes and re-weighed.

$W=W_2-W_1$

$$\text{Fat g/100 g fresh sample} = \frac{(W\text{x}100)}{W_O}$$

Where: Wo = weight of fresh Sample

W = weight of fat;

W_1 = weight of extraction flask before extraction (wt. of flask);

W_2 = weight of extraction flask after extraction (wt. of flask and fat);

3.6.4 Crude fiber (AOAC 962.09, 2000)

Crude fiber was determined after digesting a known weight of sample flour by refluxing 1.25% boiling sulfuric acid and 28% boiling potassium hydroxide.

Digestion: About 1.00g of fresh sample was placed into a 600ml size beaker, 200ml of 1.25% H_2SO_4 was added, and boiled gently exactly for 30 minutes placing a watch glass over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with hot distilled water. After 30 minute boiling, 20ml of 28% KOH was added and boiled gently for a further 30 minute, with occasional stirring.

Filtration: The bottom of a sintered glass crucible was covered with 10 mm sand layer and wetted with a little distilled water. The solution was poured from beaker in to sintered glass crucible and then the vacuum pump was turned on. The wall of the beaker was rinsed with hot distilled water several times; washings were transferred to crucible, and filtered

Washing: The residue in the crucible was washed with hot distilled water and filtered (repeated twice). The residue was washed with 1% H_2SO_4 and filtered, and then washed with hot distilled Water and filtered; and again washed with 1% NaOH and filtered. The residue was washed with hot distilled water and filtered; and again washed with 1% H_2SO_4 and filtered. Finally the residue was washed with water- free acetone.

Drying and combustion: The crucible with its content was dried for 2 hours in an electric drying oven at 130^0C and cooled for 30 min in the desiccator (with granularsilica gel), and then Weighed. The crucible was transferred to a muffle furnace (Gallenkamp, size 3) and incinerated for 30 min at 550^0C. The crucible was cooled in the desiccators and weighed.

$$\text{Crude fiber g /100 g} = \frac{(W_1 - W_2) \times 100}{W_3}$$

Where:

W_1 = weight of (crucible +sample) after drying;

W_2 = weight of (crucible +sample) after ashing;

W_3 = weight of fresh sample

3.6.5 Total ash (AOAC 923.03, 2000)

Ash was determined by incineration of known weights of the samples in a muffle furnace at 550^0C until a white ash was obtained. The dishes were placed on a hot plate under a fume hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes were placed inside the muffle furnace at 550^0C for 5 hours, and removed from the muffle and then placed in a desiccator for 1hr to cool. The ash was clear white in appearance. When cooled to room temperature, each dish. Weight of total ash was calculated by difference and expressed as percentage of sample.

Calculation:

$$\text{Total Ash (\%)} = \frac{(W_2-W)*100}{W_1-W}$$

Where: - W= weight in grams of empty dish

W_1 =weight in grams of the dish plus the dried test material

W_2= weight in grams of the dish plus ash

3.7 Total carbohydrate

Utilizable carbohydrate content was determined by difference. It was determined by subtracting total crude protein, crude fiber, total ash and fat from the total dry weight of the sample difference.

Carbohydrate (%) = 100 - (fat% +protein% + fiber %+ash% + moisture %)

3.8 Determination of energy

Gross energy was determined by calculation from fat, carbohydrate and protein contents using the Arwater's conversion factors and 16.7 kJ/g (4 kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat and 16.7 kJ/g (4 kcal/g) for carbohydrates and expressed in calories (Guyot *et al*.,2007).

Total energy (Kcal) = (9×crudefat+4×crudeprotein+4×carbohydrate)

3.9 Minerals Analysis

Ashes were obtained from dry ashing, in the ash was added some drop of deionized water and 5 drop of concentrated HNO_3 that were evaporated on hot plate. The samples dried in muffle furnace at 550^0C or 30 minute. The ash has been treated with 7ml of 6N HCl, and dried on a low temperature hot plate. A 15ml of 3N HCl was added to the dried ash and heated on the hot plate until the solution just boils. The ash solution was cooled to room temperature in open air in a hood and filtered through a filter paper Whatman (42, 125mm) into a 50ml graduated flask. A 10ml of 3N HCl was added into each crucible dishes and heated until the solution just boiled, cooled, and filtered into the flask. The crucible dishes were again washed three times with deionized water; the washings were filtered into the flask. A 5ml of 10% Lanthanum chloride solution was added into each graduated flask. Then the Solution was cooled and diluted to the mark (50ml) with de-ionized water. A blank was prepared by taking the same procedure as the sample by taking the same procedure as the sample.

The mineral contents were then determined by the procedure of AOAC (1984). Calcium, iron, and zinc were determined using an Atomic Absorption Spectrophotometer. The solution was sprayed into the flame of Atomic Absorption Spectrophotometer (Varian SpectraAA-20Plus, Varian Australia ty. Ltd., and34 Australia) and the absorption of the metal to be analyzed was measured at a specific wavelength.

Standard solutions: The stock standard solutions of minerals (iron, zinc and calcium) were diluted with 0.3 N HCl to concentrations that fall within the working range (0, 0.5, 1.0, 1.5, 2.0, µg/ml for zinc analysis; 0.0, 2.0, 4, 6, and 8 µg/ml for calcium analysis and 0, 2.0, 4.0, 6.0 8.0 µg/ml for iron analysis). The Atomic Absorption Spectrophotometer (AAS) used for mineral determination were calibrated using standard solutions and the reagent blank solution was run with the sample. The results were calculated as:-

$$\text{M content (mg/100g)} = \frac{(a-b) \times V}{10W}$$

$$\text{M content (mg/kg)} = \frac{(a-b) \times V}{W}$$

Where: W= Weight (g) of samples;

V= Volume (V) of extract;

a = Concentration (µ g/ml) of sample solution;

b = Concentration (µ g/ml) of blank solution

3.10 Determination of anti -nutritional factors

3.10.1 Determination of phytate content

Phytate was determined by the method of Vantraub and Lapteva (1988). About 0.1000g of fresh samples were extracted with10ml 2.4% HCl in a mechanical shaker (Eberbach) for 1hour at an ambient temperature and centrifuged at 3000rpm for 30 minute.

A 2ml of Wade reagent (containing 0.03% solution of $FeCl_3.6H_2O$ and 0.3% of sulfosalicilic acid in water) was added to 3ml of the sample solution (supernatant) and the mixture was mixed on a Vortex (Maxi Maxi II) for 5seconds. The absorbance of the sample solutions were measured at 500 nm using UVIS spectrophotometer (Beckman DU-64- Spectrophotometer, USA).A series of standard solutions were prepared containing 0, 5, 9, 18, 27 and 36 μg/ml of phytic acid (analytical grade sodium phytate) in 0.2N HCl. A 3ml of standard was added into 15ml of centrifuge tubes with 3ml of water which were used as a blank. A1ml of the Wade reagent was added to each test tube and the solution was mixed on aVortex mixer for 5 seconds. The mixtures were centrifuged for 10 minutes and the absorbance of the solutions (both the sample and standard) was measured at 500nm by using deionized water as a blank. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation. Phytate: mineral molar ratios were calculated using the molecular weight of PA=660.

Calculation:

$$\text{Phytic acid in mg/100g} = \frac{(\text{absorbance-intercept}) * 3}{(\text{slope x } \rho \text{ x wt. of Sample x10})}$$

Where, ρ is density

3.10.2 Condensed tannin determination

Tannin content was determined by the method of Butter *et al*., (1982). About 1.0000 gram of flour was weighed in to a screw cap test tube. The sample flour was extracted with 10ml of 1% HCl in methanol for 24 hours at room temperature with mechanical shaking. After 24 hours shaking, the solution was centrifuged at 1000rpm for 5 minutes. A 1ml of supernatant was taken and mixed with 5 ml of vanillin-HCl reagent [4gm of vanillin in 100ml of menthol w/v (solution A) and add 8 ml of HCl in 100ml in 100 ml of methanol (solution B)] finally mixing equally solution A and B. D-catechin was used as standard for condensed tannin determination. A 40mg of D-catechin was weighed and dissolved in 1000 ml of 1% HCl in methanol, which was used as stock solution. A 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution was taken in test tube and the volume of each test tube was adjusted to 1ml with 1% HCl in methanol. A 5ml of vanillin-HCl reagent was added into each test tube. After 20 minutes, wavelength -nm the absorbance of sample solutions and the standard solution were measured:

Calculation:

Concentration of tannin was read in mg of D-catechin per 100g of sample

$$\text{Tannin in mg/100g} = \frac{(\text{absorbance-intercept})}{(\text{slope x density x weight of samplex10})}$$

3.11 Functional properties

3.11.1 Water absorption capacity (WAC)

Water absorption capacity was determined according to Beuchat (1977); one gram of the flour samples were mixed with 10 ml of water in a centrifuge tube and allowed to stand at room temperature (30±2^{o}C) for 1 h. It was centrifuged for 30 min. Clear supernatant was decanted and discarded. Sediment was measured as well. Water absorption capacities were calculated as

$$\text{WAC} = \frac{\text{Initial weight of the tube – Final weight of the tube (after absorption)}}{\text{Weigh of the sample}}$$

3.11.2 Oil absorption capacity (OAC)

The oil absorption capacity was determined according to Beuchat (1977) where one gram of flour was mixed with 10 ml refined *Guizotia abyssiniea* (L.f.) Cassini (Nug) seed. The mixture was allowed to stand at room temperature for 30 min and then centrifuged at 500 rpm for 30 min. The clear supernatant was decanted and discarded. The adhering drops of oil in the centrifuge tube were removed with cotton wool and the tube was weighed. It was calculated and expressed as water absorption capacity.

$$\text{OAC} = \frac{\text{Initial weight – Final weight (after absorption)}}{\text{Weigh of the sample}}$$

3.11.3 Bulk density of the flour

A 50 g flour sample was added into a 100 ml measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density (g/cm^3) was calculated as weight of flour (g) divided by flour volume (cm^3) (Okaka and Potter, 1979)

$$\text{Bulk density (g/cm}^3) = \frac{W_1 - W_2}{\text{Volume of sample}}$$

W_1= weight of sample before tapping

W_2= weight of sample after tapping

3.11.4 Viscosity

Gruels were prepared from the food formulations using the method described by Uvere *et al*. (2002). A 5.0% (w/v) solution of each of the food formulations were used to prepare slurries. The mixture of water and flour were cooked at 92^0C for 15 minute and their viscosities were measured using Polyvisc (model VS 003, type Visco Start Wishly Scientific Pte Ltd). The cooked gruel was poured into the viscometer beaker; cooled gruel was placed in a water bath maintained at 40°C and viscosity was measured in centipoises, using spindle number 2 at a shear rate of 6 revolutions per minute.

3.12 Sensory acceptability

The complementary foods were made from germinated, ungerminated and co- fermentated samples with maize and sorghum blends with cowpea were tasted by 10 a paneliest of judges. The gruels were prepared by mixing 20g of flour with 600ml of tap water and cooked for 15 minutes at 92^0C and 2 spoon of sugar was added to each sample for sweet. The boiled gruels were then allowed to cool to about 40^oC. The prepared gruel samples were presented to the panelists selected from Ethiopian public Health Institute, Addis Ababa, Ethiopia. The panelists were regular food tasters at the institute research center. The panel members were seated in booths and evaluate to rate the gruel samples based on appearance, aroma, texture, taste and over all acceptability using a 9 point hedonic scale (Inyang & Idoko, 2006).

3.13 Statistical analysis

The data was analyzed using SPSS version 20.0. The mean and standard error of means of the triplicate analyses of the samples were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means of proximate composition, minerals, anti-nutritional factors, sensory attributes and functional properties; while the means were separated using the Duncan multiple range test at $p < 0.05$.

4. RESULTS AND DISCUSSION

4.1. Effect of natural fermentation on pH and total titratable acidity

The results of this study show that pH in the Co-fermented samples decreased with increasing fermentation time. The pH of co-fermented sorghum with cowpea was 6.12±.02 and 2.96±.09 respectively, for 0 to 72 h fermentation time (table 4.1). Fermentation gradually reduced the pH while TTA were increase of all samples with increasing fermentation time from 0, 24, and 48 to 72hrs significantly at ($p < 0.05$).

Table 4.1 Effect of germination and co-fermentation on pH and TTA on blend samples

Sample code	pH				TTA (as % lactic acid)			
	Fermentation time (hr)				Fermentation time (hr)			
	0	24	48	72	0	24	48	72
M/c	6.30±.10[i]	3.57±.09[d]	3.46±.01[c]	3.30±.03[b]	0.48±.01[a]	1.77±.05[b]	2.58±.13[d]	2.79±.09[d,e]
S/c	6.12±.02[j]	3.83±.03[e]	3.61±.02[d]	2.96±.09[a]	0.51±.05[a]	1.80±.15[b]	2.21±.04[c]	3.27±.51[f]
GM/c	6.20±.17[h]	3.42±.02[c]	3.25±.01[b]	3.27±06[b]	0.66±.02[a]	1.95±.18[b]	2.70±.09[d]	3.00±.05[e]
GS/c	5.95±.02[f]	3.60±.01[d]	3.23±.01[b]	2.89±.04[a]	0.75±.04[a]	1.86±.03[b]	2.67±.07[d]	3.51±.15[f]

Values are means of triplicate samples ± SD. Means not sharing a common letter in a column are significantly different at $p < 0.05$

M/c = Maize blend with cowpea, S/c= Sorghum blend with cowpea

GM/c= Germinated maize blend with cowpea, GS/c= Germinated sorghum blend with cowpea.

Similarly, in the case of co-fermented maize with cowpea were 6.30±.10 and 3.30±.03 for 0 to 72 hr, fermentation period. Table 4.1 shows that the decrease in pH may be due to the conversion of carbohydrate in co-fermented samples to organic acids such as lactic acid, citric acid, acetic acids and other volatile short chain fatty acids (Afoakwa *et. al.*, 2007).

The pH of the co-fermented samples rapidly reduced within the first 24 hours which could be of may be due to availability of more nutrients for microbial proliferation and enhanced metabolic activities. Similarity, according to Lorri, (1993); and Sanni *et al*., (1999) fermented cereal gruels less than pH 4.0 had been produced within 12-24 hrs using in microorganisms singly or in mixed cultures.

A rapid drop in pH at the same time increase in titratable acidity has been reported in lactic acid fermentation of various food grains (Ejigui *et al*., 2005; Abdelhaleem *et al.*, 2008). According to these authors, the production of lactic acid bacteria during fermentation has attributed to the decrease in pH and increase total acidity may be connected with the depletion of sugars to yield lactic acids which would prove beneficial in the control of spoilage and pathogenic organisms in the food. This also improves food safety by restricting the growth and survival of some pathogenic organisms such as *Shigella*, *Salmonella* and *E. coli* (Blandino, *et al.*, 2003; Omar. *et al.*, 2006). According to Elyas *et al*. (2002), the increased acidity as a result of fermentation enhances the keeping quality of fermented foods. Organic acids produced during fermentation also can potentially enhance Iron and zinc absorption via the formation of soluble ligands (Gibson *et al*., 2006).

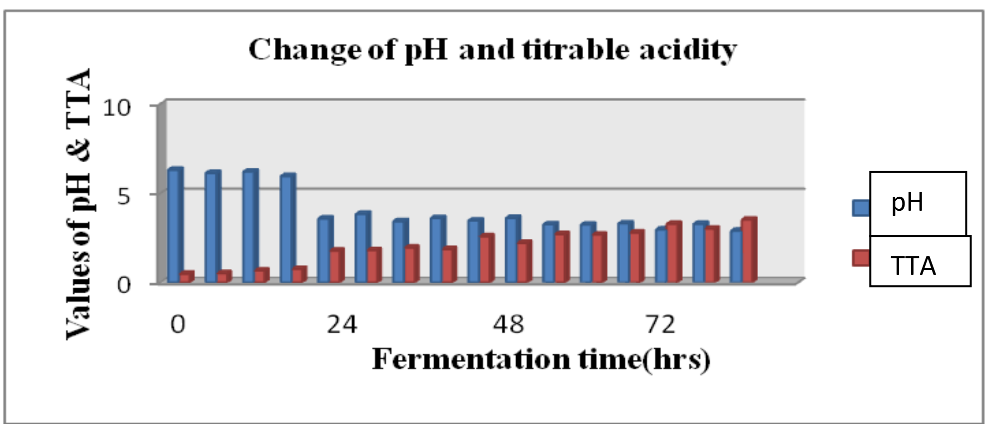


Figure 8. The effect of natural fermentation on pH and total titratable acidity

The titratable acidity increased with fermentation period in all the samples (Figure 8). The present result indicate clearly increase of TTA with fermentation time this agreement with the reported of (Oyarekua *et al.,* 2008; and Sefa-Dedeh *et al.,* 2001) which indicated that TTA increased with fermentation time in co-fermentated of maize with cowpea using starter. Infants consuming fermented maize porridge had reduced the incidence of diarrhea (Mensah *et al.,* 1990). Therefore; fermented complementary food is highly desirable, for the fact that children are most of the time consuming of porridge highly vulnerable for food pathogens due to their lower immunity conditions.

4.2 Proximate analysis

Table 4.2 Proximate compositions of raw and germinated sorghum and maize flour.

Sample Code	Moisture %	Crude protein %	Crude Fat%	Crude fiber%	Total Ash%	Total Carbohydrate%	Energy Kcal/100g
M	9.71±.05^{c}	8.45±.03^{a}	3.91±.04^{d}	2.35±.02 ^{b}	1.29±.00^{a,b}	74.26±.11^{e}	362.74±5.81a,b
S	10.13±.01^{d}	11.03±06^{c}	3.30±.00^{b}	2.27±.01^{a}	1.34±.06^{b}	71.90±.11^{c}	361.39±.26^{a}
GM	8.94±.02^{b}	10.55±.05^{b}	3.71±.02^{c}	2.54±.03^{c}	1.20±.09^{a}	72.98±.13^{d}	367.59±.10^{b}
GS	9.06±.00^{b}	12.48±.03^{d}	3.15±.03^{a}	2.55±.02^{c}	1.22±.03^{a}	71.51±.05^{b}	364.38±.16 ^{a,b}
PC	5.88±.48 ^{a}	30.01±.09^{e}	5.76±.05^{e}	4.25±.01^{d}	2.54±.01^{c}	57.41±.10^{a}	401.60±.22^{c}

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

M= Raw maize, S= Raw sorghum, GM=Germinated maize, GS=Germinated sorghum and

PC= cowpea

4.2.1 Moisture

Moisture content determination is an integral part of the proximate composition analysis of food. The samples of complementary food products in this study indicated that application of different processing techniques used affected the moisture content of the food. Moisture contents of all samples investigated in this study were below 10.13% (Tables 4.2).Raw sorghum and maize flour samples contents were moisture 10.13% and 9.71% respectively. The moisture content of germinated maize and sorghum was 8.94% and 9.06±0.06 respectively. Raw sorghum and maize flour contained higher moisture content compared to germinated sorghum and maize flour.

Table 4. 3. Effect of co -fermentation and germination on proximate composition of sorghum and maize flour blend with cowpea flour.

Sample Code	Moisture %	Crude protein %	Crude Fat%	Crude Fiber%	Total Ash%	Digestable carbohydrate %	Energy Kcal/100g
M/c	8.92±.00^{f}	14.37±.02^{a}	4.48±.00^{g}	2.53±.04^{e}	1.92±.03^{f}	67.74±.05^{d}	368.85±.17^{b}
GM/c	8.33±.02^{c}	15.62±.03^{b}	4.27±.02^{f}	2.64±.03^{f}	1.75±.04^{e}	67.36±.03^{c}	370.46±.25^{c}
S/c	9.04±.03h	15.90±.03^{d}	3.48±.06^{d}	2.34±.03^{d}	2.74±.00^{h}	66.47±.06^{b}	360.89±.37^{a}
GS/c	8.73±.01g	16.53±01^{f}	3.33±.04^{c}	2.57±.02^{e}	2.56±.01^{g}	66.25±.07^{a}	361.18±.17^{a}
FM/c	7.11±.01c	15.69±.00^{c}	3.97±.02^{e}	2.13±.03^{c}	1.14±.02^{c}	69.93±.05^{f}	378.25±.10^{f}
FGM/c	6.76±.04a	16.00±.04^{e}	3.43±.03^{d}	2.05±.04^{b}	0.89±.02^{a}	70.84±.13^{g}	378.30±.37^{f}
FS/c	7.67±.03d	16.66±02^{g}	3.24±.01^{b}	1.85±.03^{a}	1.37±.01^{d}	69.22±.04^{e}	372.72±.15^{d}
GFS/c	6.87±.04b	16.95±.05^{h}	3.11±.05^{a}	2.02±.02^{b}	1.00±.01^{b}	70.02±.03^{f}	375.95±.1^{e}

Values are means of triplicates ± SD. Means not sharing a common superscript letter in a column are significantly different at $P < 0.05$.

M/c = Maize blend with cowpea, GM/c= Germinated maize blend with cowpea,
S/c = Sorghum blend with cowpea, GS/c= Germinated sorghum blend with cowpea,
FM/c = Co -fermented maize with cowpea, FGM/c =Combination of germinated and co-fermented maize with cowpea, FS/c= Co-fermented sorghum with cowpea and
GFS/c = Combination of germinated and co- fermented sorghum with cowpea.

The effects of germination maize blend with cowpea, co-fermentation maize with cowpea and combination of germinated and co-fermented maize blend with cowpea moisture contents were 8.33%, 7.11% and 6.76% respectively, while in the case of sorghum blend with cowpea was 8.73%, 7.67% and 6.87% respectively. Table 4.3 shows that during processing treatment were significantly differences $P<0.05$ among processing methods. All the treatments except raw samples were subjected to drying operation in order to prepare flour for analysis. The processed flour was low water activity that may delay any microbial growth. Combination of germinated and co-fermented of samples resulted significance decrease in moisture contents compared with blend samples. These results agreed with the finding of Sefa-Dedeh (2001) and Mbata *et al.*, (2009); which might be due to lactic acid accumulation associated with increase in acidity and a decrease of dry matter yields. It is evident that the low moisture content of food products inhibits biochemical activities of invading microorganisms, and there by prevent food spoilage during storage (Kikafunda, 2006). This indicates that the product will have a good keeping quality since spoiling microbes thrive better in the presence of adequate moisture content. The standard moisture content recommended is 5-10 % (Olorunfemi *et al.*, 2006). Therefore, co-fermentation and germination had overall effect on the moisture content of all samples.

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4.2.2 Protein

The increase in energy and protein contents of formulated complementary foods indicated that they revealed that are adequate to support growth and development in infants and young children. In this study, the amount of crude protein in raw, blend, co- fermented and combination of germinated and co-fermented samples were significantly different at $P<0.05$, (Tables 4. 2 and 4.3). Crude proteins of all formulated products were in the range of 8.45 to 16.95%. The protein content of raw maize and sorghum flour were 8.45% and 11.03% respectively. As result, of germination maize and sorghum increased to a value of 10.55±.0 5and 12.46±.03 respectively. The present study results were agreement with germination of low-tannin sorghum grains (Abbas and Mushara, 2008; Inyang and Zakari, 2008) .According to the investigators the protein content increase may be due to mobilization of storage nitrogen in the cereals to produce nutritionally high quality protein for the young plant for development.

The blending of cowpea flour with germinated and ungerminated maize and sorghum flour affected the crude protein contents significantly ($P<0.05$). The protein content due to blending of maize with cowpea and sorghum with cowpea flour were 14.37% and 15.90% respectively. The results were germinated maize and sorghum flour blend with cowpea flour 15.62% and 16.53% respectively. These results agreement with earlier reported that show increase in protein content when two or more plant-based food materials use blended together which increases the nutrient value of the food (Ejigni *et al.*, 2007). WHO, (1998) also reported that cowpea flour increased the quality of proteins needed by the young infants for development.

When both combinations of germinated and co-fermented samples were used as treatment processes, the results were 16 % for maize with cowpea and 17% for sorghum with cowpea flour. These results were found to be significantly better than the protein content of the products when only germination were used. Improvements in protein quality have been observed when germination followed by fermentation of plant-based complementary foods based on maize, legumes, groundnut, millet and soya bean blends (Gibson *et al*., 2006; Oyarekua and Adeyeye, 2008). According to these authors, such improvements may be associated with the destruction by microbial enzymes of protein inhibitors that interfere with nitrogen digestibility, or from the ability of starter cultures to synthesize certain amino acids. Similar finding that Enujiugha *et al.*, (2003) observed that fermentation decreased in starch and sugar as a result of hydrolysis by bacterial enzymes with the formation of volatile products such as lactic acid, acetic acid, carbon dioxide, and ethanol. This leads to changes in proportions of nutrient components by proteolytic enzymes. The proteolytic activities of enzymes produced by microorganisms during fermentation increased the bioavailability of amino acid. These finding range of crude protein 14.37-16.95% so all formulated samples recommended full fill requirement of protein for 6-12 months infants'13-14 g/100g (FAO/WHO, 1991).

4.2.3 Crude Fat

The content of crude fat was significantly different ($P<0.05$) as observed among each processing techniques for all treatments (Table 4.2 and 4.3). The crude fat content of raw maize and sorghum flour 3.9% and 3.3%. Similarly, results obtained for germinated maize and sorghum flours were 3.71% and 3.15% respectively. The crude fat contents were 4.48% maize blend with cowpea ,4.27% germinated maize blend with cowpea, 3.48% sorghum blends with cowpea and 3.33% germinated sorghum blend with cowpea to 70:30 ratio of food formulated .At the same time as co- fermentation of germinated maize and sorghum were 3.43% and 3.11% respectively. It was observed that the fat content of blend samples increased which might be due to the addition of cowpea. Germination significantly decreased crude fat content of sorghum and maize flours as show in table 4.3 the considerable decline of crude fat in this study agreed with the finding of Oyarekua and Adeyeye, (2008); Inyang and Zakari, (2008) the investigators observed that decrease in fat content during germination might be attributed to the increased activities of the lipolytic enzymes in the cereals which hydrolyze fats to fatty acids and glycerol. These enzymes hydrolyze fats to simpler products which can be used as a source of energy for the developing seed embryo. Similar observation was made in malted millet (Inyang and Idoko, 2006; Nnam, 1999). On the other hands, the decrease in fat content implies an increased shelf-life for the germinated seeds compared to the un-germinated seed and reduces rancidity as well as off–flavor (Inyang and Idoko, 2006).

Co-fermented samples were concerned that a significant reduction ($P<0.05$) of crude fat content then simple blend techniques. The results of this study agree with El Maki *et al.,* (2007) fermentation reduces fat content may be due to the fact that biochemical changes occurred intend samples. The results of this study were contrary with the results obtained by Meseret, (2011). The investigator show that crude fat content increased during fermentation of quality protein maize-soybean blend flours. Crude fat content of this finding the range between 4.48-3.11% but according to FAO/WHO, (1991) minimum requirement of crude fat 10% recommended. Crude fat content in this finding of all the formulated samples were lower than minimum requirement of WHO therefore, an for additional supplement may be needed.

4.2.4 Crude Fiber

The results for total crude fiber were significant differences ($P<0.05$) among each processing methods of the samples (Table 4.2 and 4. 3). In the present study the results obtained for blends and co-fermented maize with cowpea were 2.53% and 2.13% and for sorghum with cowpea

2.34% and 1.85% respectively. Co-fermented samples had a decrease in crude fiber content then blended samples (Table 4.3).The reduction of fiber contents during fermentation could be attributed to the partial solubility of cellulose and hemicellulose by microbial enzymes (Ejigui *et al.,* 2005).In contrast germination increased crude fiber content of samples. This could be due to the dissipation of some of the starchy endosperm during germination which causes apparent increase in seed coat proportion. Low fiber content for complementary food is emphasis because the gastrointestinal system of the infants may not well developed. The crude fiber contents of all samples were in this study within the recommended range of FAO/WHO which should be not more than 5 g per 100 g dry matter.

4.2.5 Total ash

The data as indicates that the ash content combination of germinated and co-fermented samples of maize and sorghum 0.89% and1.00% respectively (Table 4.2 and Table 4.3). The ash content of the germinated samples reduced significantly (p<0.05) compared with raw samples were similar to reports of Kikafunda *et al*., (2006) and Tizazu, (2010) for pear millet and germinated maize. Total ash content during germination decreased due to leaching of minerals during steeping and washing (Kikafunda *et al.,* 2006)**.** A previous study had also reported that a significant decrease of fat, ash, and fiber contents after four days of maize fermentation (Ejigui *et a*l., 2005). The results of this study regarding the effect of natural fermentation on ash and crude fiber contents were decrease. In contrast, to with the report of Amankwah *et al*. (2009) which indicated that ash and crude fiber contents increased during fermentation of corn meal.

4.3 Carbohydrate

Utilizable carbohydrate content was determined by difference. There are significant differences among each treatment, Table 4.2 and 4.3 to show that total carbohydrates composition decreased during germination treatments as compared to raw samples. During germination carbohydrate may be used as source of energy due to β-amylase activity that hydrolyzes the starch into simple carbohydrates such as glucose and fructose that utilizable for cell division (Vidal-Valverde *et al*., 2002; Nonogaki *et al*., 2010) .The carbohydrate content of co-fermented samples in this study was higher than their blends samples. This finding agrees with that of Sefa-Dedeh, (2001) and Mbata *et. al*., (2009) were co-fermented improved carbohydrate and energy values.

4.4 Gross energy

The caloric value was calculated by multiplying the mean valves of crude protein, crude fat and total carbohydrate by Atwater factors. There were significant differences among each processing treatments. The calorific value (Kcal/100g) was highest in germinated followed by co-fermented samples. The energy content of all the samples were between the RDA (308 – 389 Kcal/100g) for complementary foods for six month to one year old (Dewey and Brown, 2003).

4.5 Phytate and tannin content

4.5.1 Phytate

In the present study, the results for phytate content decreased significantly when different processing treatments were applied. Germinated maize and sorghum was significantly ($P<0.05$) reduced compared with the raw maize and sorghum samples. Phytate level of raw maize and sorghum was 333.50±0.34 mg/100g and 325.45±0.17mg/100g while germinated maize and sorghum contained reduction of 68.9 % and 69.08% (Table 4.4).

Table 4. 4 Phytate and tannin content of raw and germinated sorghum and maize

Sample Code	**phytate (mg/100g)**	**%phytate reduction**	**Tannin (mg/100g)**	**%tannin reduction**
M	$333.50 \pm .34^{e}$	0	23.98 ± 0.14^{d}	0
S	$325.45 \pm .17^{d}$	0	0.245 ± 0.12^{bc}	0
GM	$103.58 \pm .24^{b}$	68.9	08.288 ± 0.65^{c}	65.4
GS	$100.62 \pm .025^{a}$	69.08	0.034 ± 0.00^{a}	86.1
CP	$118.94 \pm .04^{c}$	0	0.075 ± 0.08^{ab}	0

Notes: - Values are means of triplicates ± SD. Means not sharing a common superscript letter in a column are significantly different at $P < 0.05$.

M=Maize, S=sorghum, GM=Germinated maize, GS= Germinated sorghum and CP=cowpea

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The phytate content for blending of maize with cowpea and sorghum with cowpea flour were found to be 326.55±0.17 mg/100gm and 322.52±0.03 mg/100gm respectively. Phytate reduction were recorded for germinated maize blend with cowpea and germinated sorghum blend with cowpea 27.3% and 31.89 %respectively. According to Afify *et al.*, (2011) and Kayode *et al.*, (2007), germination induce the synthesis of hydrolytic enzymes to activate endogenous grain phytase which can degrade phytate content. Similarly, Egli *et al*. (2002) reported that effect of germination on rice, millet, and mung bean which result showed that the largest reduction in phytate content in the food product. Phytates potentially form complexes with minerals such as, iron, zinc and dietary proteins so decrease their bioavailability of minerals (El-Beltagi, 2011).

Table 4. 5. The effect of germination and co- fermentation on phytate and condensed tannin content of maize and sorghum blends with cowpea

Sample code	**phytate (mg/100g)**	**Reduction%**	**Tannin (mg/100g**	**Reduction%**
M/c	326.55±.17^{h}	None	0.072±.00c	None
GM/c	237.23±.02^{f}	27.34	0.046±0.01bc	36.1
S/c	322.53±.03g	None	0.0425±0.00b	None
GS/c	219.66±.15^{e}	31.89	0.0239±0.00a	43.7
FM/c	129.33±.03^{d}	60.39	BDL	100
GFM/c	106.05±.01^{b}	67.51	BDL	100
FS/c	112.36±.007^{c}	65.16	BDL	100
GFS/c	94.66±.57^{a}	70.64	BDL	100

Values are means of triplicates ± SD. Means not sharing a common superscript letter in a column are significantly different at *P* < 0.05.

M/c = Maize blend with cowpea, GM/c= Germinated maize blend with cowpea,

S/c =Sorghum blend with cowpea, GS/c= Germinated sorghum blend with cowpea,

FM/c = Co -fermented maize with cowpea, FGM/c = Combination of germinated and co-fermented maize with cowpea, FS/c = Co-fermented sorghum with cowpea and GFS/c = Combination of germinated and co- fermented sorghum with cowpea.

ND=below detection limit (non detected)

The data as shown in table 4.5 indicates that combination of germinated and co-fermented samples significantly (*p* <0.05) reduced the phytic acid contents compared to blend samples. According to Afify *et al.,* (2011a) fermentation strongly reduced the phytate content and is more effective. Fermentation can induce phytate hydrolysis via the action of microbial phytase enzymes, which hydrolyze phytate to lower inositol phosphates. Such hydrolysis is important because myoinositol phosphates with < 5 phosphate groups (i.e., IP-1to IP-4) do not have a negative effect on zinc absorption, and those with < 3 phosphate groups do not inhibit non heme iron absorption (Hotz and Gibson, 2007).

4.5.2 Tannin

Tannin content in the germinated samples were significantly ($P<0.05$) reduced compared with the raw samples. Tannin level (mg/100g) raw maize and sorghum were 23.98± 0.14 mg/100g and 0.245±0.12 mg/100g respectively while germinated maize and sorghum were reduced to 65.4% and 86.1 % respectively. Tannin content was reduced might be the formation of hydrophobic association of tannins with seed proteins and enzymes and some loss of tannins during germination may be due to the leaching of tannins into water (Shimelis and Rakshit, 2007).

Table 4.5 indicates that tannin content of maize blend with cowpea and sorghum blend with cowpea flour were found to be 0.072±0.00 mg/100g and 0.0425±0.00 mg/100g respectively. Tannin reduction were recorded for germinated maize blend with cowpea and germinated sorghum blend with cowpea 36.1%and 43.7% respectively. Hotz and Gibson (2007) also stated that tannin content reduced during germination in legumes and sorghum might be as a result of attributed to enzymatic activities to hydrolysis by polyphenolase such reductions may be facilitate iron absorption. Germination followed by co-fermentation of sorghum with cowpea and maize with cowpea significantly reduced tannin content to undetected level. The result of the present study are agreement with the work of Hibberd *et al*., (2003) which show that processing methods (combined germination and fermentation) significantly reduces tannin content. Gee and Harold, (2004) also reported that processing methods (combined germination and fermentation) had significantly reduce the tannin content. Processing method such as malting, steeping, fermentation and sprouting depletes their tannin content. The reduction in tannin content may be a result of enzymatic activity of the organisms whose hydrolyzing ability is enhanced by fermentation.

4.6 Effect of processing treatment on total mineral contents

The mineral contents of were shown in Table 4.6. The values of mineral contents were different from raw samples in all the processing treatments. Germination of cereals increased significantly iron, zinc and calcium contents compared to raw sorghum and maize flour.

Table 4. 6. The mineral content of raw and germinated sorghum and maize

Sample Code	Fe (mg/100g)	Zn(mg/100g)	Ca(mg/100g)
M	2.17±.06^{a}	2.57±.02^{c}	9.44±.011^{a}
S	5.49±.07^{c}	2.06±.03^{a}	13.39±.01^{c}
GM	3.38±.09^{b}	2.70±.004^{d}	10.21±.008^{b}
GS	6.89±.08^{d,e}	2.52±.01^{b}	17.15±.02^{d}
CP	6.83±.04^{e}	4.41±.009^{f}	39.57±.006^{e}

Notes:- Values are means of triplicates (± SD). Means not sharing a common superscript letter in a column are significantly different at $P < 0.05$

M= maize, S= Sorghum, GM= Germinated maize,

GS= Germinated sorghum and CP=cowpea.

Table 4.7 shows that germinated sorghum blend with cowpea and germinated maize blend with cowpea, 7.69 mg/100g and 7.08 mg/100g respectively whereas sorghum blend with cowpea and maize blend with cowpea 4.33 mg/100g and 3.93 mg/100g respectively. The present study was shows that germination increased the content of Iron, Zinc and calcium. According to Ikujenlola and Fashakin, (2005) reported that the levels of certain minerals increased considerably in germinated flours. Similarly, Inyang and Zakari, (2008) observed that levels of iron in rice increase two folded during germination. According to the authors such improvements of mineral contents (iron, zinc and calcium) of sorghum flour during germination might be due to losses of water-soluble constituents during steeping and washing. Germination correlated with dry matter losses from roots and shoots might be responsible for minerals increment during germination (Gibson *et al*., 2006) .The bioavailability of iron and zinc were significantly improved as a result of soaking and germination treatments (Afify *et al.*, 2011a).

Table 4. 7 The effect of germination and co -fermentation treatments on total mineral (Fe , Zn and Ca mg/100g) contents

Sample Code	Fe (mg/100g)	Zn(mg/100g)	Ca (mg/100g)
M/c	3.93±.04[d]	2.80±.008[e]	19.14±.01[c]
GM/c	7.08±.06[g]	2.92±.001[f]	25.14±.01[g]
S/c	4.33±.02[e]	2.95±.03[f]	24.01±.002[f]
GS/c	7.69±.04[h]	3.25±.15[g]	30.24±.01[h]
FM/c	2.39±.03[a]	1.48±.01[a]	13.52±.01[a]
GFM/c	3.06±.05[b]	1.56±.004[b]	16.80±.00[b]
Fs/c	3.61±.02[c]	2.30±.02[c]	19.77±.01[d]
GFS/c	4.80±.06[f]	2.35±.01[d]	22.72±.01[e]

Notes:- Values are means of triplicates ± SD. Means not sharing a common superscript letter in a column are significantly different at $P < 0.05$.

M/c = Maize blend with cowpea, GM/c= Germinated maize blend with cowpea,

S/c = Sorghum blend with cowpea, GS/c= Germinated sorghum blend with cowpea,

FM/c = Co-fermented maize with cowpea, FGM/c = Combination of co-fermented and germinated maize with cowpea, FS/c = Co-fermented sorghum with cowpea and

GFS/c = Combination of co- fermented and germinated sorghum with cowpea.

The mineral contents of blends sample significantly increased compared with the raw samples. According to FAO/WHO, (2001) minerals such as iron and zinc are low in cereals but the addition of legumes can improve the iron and zinc contents. Total mineral values of co-fermented samples were lower as compared to the unfermented samples. According to Alemu, (2009) reported that total content of iron; zinc and calcium in sorghum flour were decreased after fermentation. The reduction of total minerals in co-fermented samples may be lost through decantation of fermented water during the drying process. The results of the present study contradicts with the observation made by Ejigui *et al.* (2005) that fermentation does not have an overall effect on the contents of total minerals value.

4.7 Molar ratios

Table 4.8 indicates that, there are significant different at (*P*< 0.05) with each processing treatments on molar ratio phytate: zinc .Molar ratio is used to estimate the likely absorption of zinc from a formulated diet. According to Tizazu *et al*., (2010) reported that formulated diet with a phytate: zinc molar ratio greater than 15 have relatively low zinc bioavailability, those with phytate: zinc molar ratios between 5 and 15 have medium zinc bioavailability and those with a phytate: zinc molar ratio less than 5 have relatively good zinc bioavailability. The result of table 4.8 show that low values of phytate: zinc molar ratio <5 were found germinated and combination of germinated and co-fermented treatments of samples are high indicative of zinc bioavailability. The rest blend samples were medium Zinc bioavailability (table 4.8).

Table 4.8. Molar ratios of phytate: Ca, phytate: Fe, phytate: zinc, and (calcium* phytate): /(zinc) for raw and germinated samples.

Sample	Parameter			
Code	**phytate: Fe**	**phytate: Zn**	**phytate: Ca**	**(Calcium xphytate) /(zinc) (mol/kg)**
M	13.01±.37[e]	12.77±.10[c]	2.13±.00[e]	0.055±. 00[e]
S	5.01±.07[c]	15.51±.27[d]	1.47±.00[d]	0.030±. 00[d]
GM	2.59±.06[b]	3.76±.00[b]	0.61±.00[c]	0.016±. 00[c]
GS	1.23±.01[a]	3.92±.00[b]	0.35±.00[a]	0.0091±.00[a]
CP	6.83±.04 [c]	2.82±.00[a]	0.23±.00[a]	0.0099±.00[b]

Notes:- :- Values are means of triplicates (± SD). Means not sharing a common superscript letter in a column are significantly different at (P < 0.05) as assessed by Duncan's multiple ranges

M= maize, S= sorghum, GM= Germinated maize,

GS= Germinated sorghum and CP=cowpea.

Children, in rural Ethiopia are especially face deficiencies of minerals and trace elements, as they eat from the family dish and often cannot meet their specific nutrient needs. This is supported by Tizazu *et al*., (2010), who recommended that supplementation with zinc increased the linear growth of infants, particularly those who were stunted Hence, phytate: Zinc molar ratio is considered a better indicator of zinc bioavailability than total dietary phytate levels alone (Kelbessa and Narasimha, 1998).

Table 4. 9 Molar ratios of phytate: Ca, phytate: Fe, phytate: zinc, and (Calcium*phytate): /(zinc) for blend and co- fermentated samples.

Sample	**Parameter**			
Code	**phytate: Fe**	**phytate: Zn**	**phytate: Ca**	**(Calcium x phytate)/(zinc) (mol/kg)**
M/c	11.93±.14[h]	11.44±.03[g]	1.03±.00[h]	0.0299±. 00[f]
GM/c	5.73±.02[f]	7.97±.00[e]	0.59±.00[g]	0.0157±.00[e]
S/c	9.17±.01[g]	10.75±.13[f]	0.77±.00[f]	0.0231±.00[f]
GS/c	4.51±.00[d]	6.65±.03[c]	0.43±.00[e]	0.0145±.00[d]
FM/c	4.57±.00[d]	8.56±.08[d]	0.57±.00[d]	0.0088±.00[c]
GFM/c	2.93±.05[c]	6.67±.02[c]	0.38±.00[c]	0.0061±.00[a]
FS/c	2.63±.01[b]	4.79±.05[b]	0.34±.00[b]	0.0081±.00[b]
GFS/c	1.66±.02[a]	3.95±.05[a]	0.25±.00[a]	0.0060±.00[a]

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

M/c=maize blend with cowpea, GM/c= Germinated maize blend with cowpea,
S/c= sorghum blend with cowpea, GS/c= Germinated sorghum blend with cowpea,
FM/c= Co -fermented maize with cowpea, FGM/c=Co-fermented and germinated maize with cowpea, FS/c=Co-fermented sorghum with cowpea and
GFS/c=Co- fermented and germinated sorghum with cowpea.

Phytate: Iron molar ratio indicates poor iron bioavailability because of the high levels of phytic acid. The phytate: iron molar ratio >0.15, indicates low iron bioavailability (Tizazu *et al*., 2010).

Phytate: iron molar ratios <0.15 is indicative of high iron bioavailability However, in all the samples investigated phytate: iron molar ratio is >0.15, indicative low iron bioavailability.

All germinated and ungerminated blends, co-fermented samples as well as combination of germinated and co-fermentated samples analyzed in this study exhibited phytate: calcium molar ratios above the critical molar ratio of 0.24, (Frontela *et al*., 2009). High calcium levels in foods can also promote the phytate-induced decrease in zinc bioavailability. When the [Calcium x phytate]/ [Zinc] millimolar ratio exceeds 0.5 could affect bioavailability the zinc (Kelbessa and Narasimha, 1998; Tizazu *et al*., 2010). However, in this study values > 0.5 were not observed in all the samples which indicate that the samples are poor in calcium content. Phytate mineral ratio was decreased significantly after each processing methods (Tables 4.8 and 4.9).The low phytate: mineral ratios from all processed samples may be partly attributed to the decrease in content of phytic acid.

4.8 Functional properties

The functional properties have studied confirm that water absorption capacity, oil absorption capacity, bulk density and viscosity of the infant food products were affected by different processing techniques. The functional properties of the food materials are very important for the appropriateness of the diet, particularly, for the growing children (Omueti *et al.*, 2009). The consistency of energy density (energy per unit volume) of the food and the frequency of feeding are also important in determining the extent to which an individual will meet his or her energy needs. However, the amount of energy obtained may be affected by the nature of the functional properties of the food.

4.8.1 Water absorption capacity (WAC).

As shows Table 4.10 the results of maize blend with cowpea and germinated maize blend with cowpea and in the same way un germinated and germinated sorghum bend with cowpea 1.76, 2.05, 1.83 and 2.19 g of water /g of solid matter, respectively.

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Table 4. 10. The effect of germination and co-fermentation treatments on functional properties of sorghum and maize

Sample code	WAC*	OAC **	BD (g/cm3)	Viscosity (cP)
M/c	1.76±.03 [a]	1.68±.02[a]	1.87±.07[g]	4966.01±.72[g]
GM/c	2.05±.04[c]	2.15±.04[c]	1.56±.00[e]	3647.2±.88[f]
S/c	1.83±.04[b]	1.76±.07[a,b]	1.70±.06[f]	3048.06±.67[e]
GS/c	2.19±.03[d]	2.33±.02[d]	1.44±.04[d]	2545.16±.5[c]
FM/c	2.25±.03[d]	2.16±.04[c]	1.41±.04[c,d]	3366.37±.58[f]
GFM/c	2.34±.01[e]	2.65±.15[e]	1.34±.02[b,c]	2553.86±1.5[d]
FS/c	2.32±.01[e]	1.85±.03[a]	1.31±.00[b]	2440.80±.60[b]
GFS/c	2.53±.05[f]	2.66±.02[e]	1.23±.03[a]	1798.9±.72[a]

Values are means of triplicates ± SD. Means not sharing a common superscript letter in a column are significantly different at $P < 0.05$.

M/c= Maize blend with cowpea, GM/c= Germinated maize blend with cowpea,
S/c = Sorghum blend with cowpea, GS/c= Germinated sorghum blend with cowpea,
FM/c = Co -fermented maize with cowpea,
FGM/c = Combination of co-fermented and germinated maize with cowpea,
FS/c = Co-fermented sorghum with cowpea and
GFS/c = Combination of co- fermented and germinated sorghum with cowpea.
WAC- water absorption capacity, OAC- oil absorption capacity, BD- bulk density
*10ml of water absorbed per 1 g of solid matter

** 10m of oil absorbed per 1g of solid matter

Water absorption capacity increased with germinated samples. Germination increased water absorption capacity of such improvement observed might have been the production of soluble sugars compounds having good water holding capacity (Gernah *et al.*, 2011) .Table 4.10 show that combination of co-fermented and germinated samples more significantly (P<0.05) increase water absorption capacity than blend samples this might be the increase in amount of soluble sugar present in the germinated and fermented flours. This means fermented formulations, which had better water absorption capacity, were easier to reconstitute in water when needed (Oyarekua, and Adeyeye, ,2008) Water holding capacity depends on the water bounding capacities of food components. This might be due to availability of hydrophilic groups which bind water molecules. High value of water absorption capacity is desirable for mouth feel and of reduction viscosity.

4.8.2 Oil absorption capacity (OAC)

The oil absorption capacity of flour is important as it improves the mouth feel and retains the flavor of the infant foods (Abulude *et al*., 2005). Table 4.10 show that germinated cereal (maize and sorghum) blends with cowpea were significantly ($P<0.05$) higher oil absorption capacity than maize and sorghum blend with cowpea .The effects of germination on the oil absorption capacity of the food product in this study were in agreement with earlier works of Imtial *et al.* (2011). Germination of cereal grains enhances the oil absorption capacity due to the entrapment of oil related to the non- polar side chains of proteins. The increment observed in emulsion capacity could be due to increase in the area of stabilized oil droplet at interface which is a function of the food components (Imtiaz *et al.,* 2011)

4.8.3 Bulk density

Bulk density is an indication the porosity of a product which influences packages design and determining the type of packaging material required. It is also important in infant feeding where less bulk density is desirable (Iwe and Onalope, 2001). Table 4.10 shows the effect of processing on bulk density there was significant ($p<0.05$) difference bulk density of the food formulations with a value of 1.87 g/cm^3 for maize blend with cowpea, 1.70 g/cm^3 for sorghum blend with cowpea, 1.34 g/cm^3 for combination of processing methods (co-fermentation and germination) maize with cowpea, 1.23 g/cm^3 for combination of co-fermented and germinated sorghum with cowpea. The current study showed that germination and co-fermentation processing methods decrease the bulk density. This might be due to enzymatic activities of breakdown of starch to sugars during germination (Mbithi-Mwikya *et al*., 2000). Germinated and fermented samples were tend to soften the seeds, thus making milling easier, with small particle sizes than unprocessed grains. Hence the reductions in bulk density have nutrient density, since more flour can be packaged in the same given volume (Iwe, 2003; Gernah *et al.,* 2011). Bulk densities would ensure more quantities of the food samples being packaged, but less economical.

Nutritionally, loose bulk density promotes easy digestibility of food products, particularly among children with weak digestive system (Osundahunsi and Aworh, 2002).

4.8.4 Viscosity

Viscosity is defined as the tendency of fluid to resist flow. The results in this study have shown that there were significant ($p<0.05$) decreases in viscosity of gruels formulated with the result of the blends were 4966.01cP, 3647. 2cP, 3048.06cP and 2545.12 cP the gruels of maize blend with cowpea, germinated maize blend with cowpea, sorghum blend with cowpea and germinated sorghum blend with cowpea blend respectively (Table4.10).This result agrees with the work of Ikujenlola and Fashakin (2005) that malting or sprouting has viscosity reducing effect on cereals and legumes. The reduction in viscosity of the diets is advantageous, because it will increase the nutrient density and energy of the formulated complementary foods which is better for the growing children. The reduction in the viscosity of the germinated diet may be a result of the activity of amylase enzymes developed during germination process which degrades the starch to simpler carbohydrate units.

Similarly, the results clearly indicate that decrease in the viscosity of gruels of combination of germinated and co-fermented sorghum with cowpea 1798.9 cP compared to unfermented gruels sorghum combined with Cowpea 3048.06 cP. This may be due to the breakdown of complex molecules by enzymes and microorganisms, which may have resulted in less viscous soluble matter, including sugars and short chain dextrin (Gernah *et al*., 2011). The significant reduction in viscosity with the application of germination and fermentation could be due to breakdown of macromolecules such as polysaccharides and polypeptides to smaller units, such as dextrin and peptides by the enzymatic activities. This makes the utilizable carbohydrate accessible for use by infants.

4.9 Sensory acceptability

Table 4.11 shows the results of the sensory evaluation carried out on gruels prepared from germinated, ungerminated and co- fermented maize with cowpea and sorghum with cowpea. Gruels were subjected to sensory taste using 10 panelists. The products were rated in terms of taste, color, aroma, texture and overall acceptability on a 9 – point hedonic scale ranging from 1-dislike extremely to 9-like extremely.

Analysis of variance indicates that there is significant difference in the aroma, taste and texture among the samples exhibited but appearances of gruels were not significantly different from each other. On the other hand, the co- fermented products did not show any significant difference in overall acceptability. The microbial activities and organic acid production which increased as the fermentation time progressed may account for the changes in the taste, aroma and overall acceptability of the gruels. The unfermented products were better than the co-fermented products in overall acceptability (Table 4.11), while the values for gruel samples maize blend with cowpea were significantly higher for overall acceptability than all the food formulations samples.

Table 4. 11. Sensory evaluation of the gruels

Sample	**Sensory Attributes**				
Code	**Appearance**	**Taste**	**aroma**	**Texture**	**Overall acceptability**
M/c	8.5±.84[a,b]	8.2±.91[c]	8.1±.87[d]	7.6±1.3[e]	7.1±1.6[c]
GM/c	8.3±.82[a,b]	7.4±1.4[b,c]	7.1 ±1.7[b,c,d]	6.1±.87[a,b,c]	5.8±1.9[a,b]
S/c	8.6±.15[a,b]	8.3±.94[c]	7.3±1.1[c,d]	7.0±1.3[c,d,e]	7.0±1.2[b,c]
GS/c	7.8±1.2[a]	7.5±1.2[b,c]	7.5±1.2[c,d]	6.4±1.0[b,c,d]	6.1±1.1[a,b,c]
FM/c	8.4±.69[a,b]	6.4±.96[a,b]	6.0±1.5[a,b]	5.5±1.0[a,b]	5.1±0.73[a]
GFM/c	8.40±1.1[a,b]	6.0±1.1[a]	5.8±.91[a]	6.2±.78[a,b,c]	5.3±1.0[a]
FS/c	8.9±.31[b]	7.0±1.2[a,b]	6.4±1.3[a,b,c]	7.3±1.7[d,e]	5.5±1.4[a]
GFS/c	7.9±.99[a]	6.7±.82[a,b]	6.5±1.2[a,b,c]	5.2±1.0[e]	5.0±0.63[a]

Values with different superscripts in a column are significantly different at $p < 0.05$

M/c= Maize blend with cowpea, GM/c= Germinated maize blend with cowpea,
S/c = Sorghum blend with cowpea, GS/c= Germinated sorghum blend with cowpea,
FM/c = Co -fermented maize with cowpea,
FGM/c = Combination of co-fermented and germinated maize with cowpea,
FS/c = Co-fermented sorghum with cowpea and
GFS/c = Combination of co- fermented and germinated sorghum with cowpea

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The present study revealed that complementary food products can be formulated from locally available food commodities (maize, sorghum and cowpea). Complementary food formulation to meet the nutrient requirement of infant ages 6 month-12 month infants was achieved by blending 70: 30 pretreated (socking, dehulling and germination) cereals of maize and sorghum flours before fermentation process.In this study there are eight complementary food formulation in this study out of these formulation germination followed by co-fermentation of maize and sorghum with cowpea have improve gross energy and protein value but very less value of fat content and sensory acceptability.

Traditional food processing methods such as, germination and fermentation are important for preparation of infant complementary foods. These processes influence the nutritional value, anti -nutritional factors, functional properties, sensory attributes and mineral bioavailability. The results also clearly indicated that the protein content of complementary food formulations fulfill recommended protein requirement in addition to this the energy content of all formulated samples were above the RDA 308 Kcal/100g. The present study clearly indicated that combination of germination and fermentation processing method increment water and oil absorption capacity in contrast reduces the viscosity and bulk density which makes it suitable as infant complementary food. This study also provides data on the content of total zinc, iron, and calcium their relative bioavailability. Germination increase total amount of minerals but fermentation decrease total values of these minerals. However, indigenous processing treatments improve bioavailability of minerals. The overall result indicate that sorghum formulate with cowpea has greater nutritional value, low anti-nutritional factors and bioavailability than maize formulation.

The results also obviously indicated that combination of germination and fermentation Processing methods were reduced by more than two third the levels of phytates and non detectable level of tannin content. Eight complementary food formulation were developed through the present study were organoleptically evaluation the result showed that the overall acceptability of blends were higher than combination of germinated and co-fermented samples.

Fermentation and germination traditional processing methods are the best alternative for complementary food preparation due to utilization of simple equipments and simply prepare at home. These methods are inexpensive, energy efficient and suitable for low-income families living in rural areas in developing countries, and environmentally friendly including Ethiopia.

5.2 Recommendation

For infant protein energy malnutrition and micronutrient deficiency are highly prevalent in several developing countries setting like Ethiopia. Factors of immediate and direct influence to these nutritional disorders are inadequate food consumption and low bioavailability of nutrients in the foods. Mothers should be encouraged to prepare complementary foods using traditional food processing techniques (germination and fermentation) in addition to this diversify approach is the best strategy to improve the nutrient bioavailability in diets based on plant foods.

Utilization of cowpea is not common for preparation of infant food in our country. Agricultural research centers, equal attention should be given to other legumes, increase production rate cowpea per year in Ethiopia and Stockholders to advocated the nutrient value of cowpea in the society it is a best alternative for soybeans in its consist of acceptable protein so could effectively used in cereal based complementary food. This believed that to combating the problem of malnutrition in Ethiopia used that will effectively increase the nutrient availability of cereal.

The following recommendations are made based on a holistic view of the subject area:

- ✓ It is recommended that animal feeding trials be carried out to evaluate the effects of germination and fermentation of maize, sorghum and cowpea blend on the nutritional qualities of complementary foods.

- ✓ It is recommended whether the complementary foods meet the required essential amino acids, soluble or insoluble dietary fiber.

- ✓ In order to enhance the potentials of natural fermentation and germination, there is need for further research on its preservation, its socio-economic implications, application of quality and safety systems with community awareness intervention programmers.

- ✓ It is also recommended for researches to conduct other more functional properties such as emulsion activity, stability and foaming capacity. In addition to this, physic-chemical properties like seed density, hydration & swelling coefficient, and hydration and swelling capacities.

- ✓ Industrial utilization of locally-grown crops (maize and sorghum) for manufacture of with cowpea to fortified products has to be studied.

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APPENDICES

APPENDIX I: A SCORE SHEET FOR ACCEPTANCE (9 HEDONIC) TEST

Panelist code/date: ----------------------sample code: ---------------------date: -----------------

Please evaluate the gruel sample you have provided and indicate how much you like or dislike it for appearance, aroma, taste, texture and overall acceptance by a right score. Rinse your mouth with water after you evaluate each sample and before you start the next one.

Sensory Perception(score)	Sensory Quality attributes				Over all acceptability
	appearance	Flavor	Odor	Mouth feel (texture)	Hedonic scale
1.Dislike extremely					1.Extremely unacceptable
2. Dislike very much					2.very much unacceptable
3. Dislike moderately					3.moderately unacceptable
4. Dislike slightly					4.Slightly unacceptable
5.Neither like nor dislike					5.neither acceptable nor Unacceptable
6. Like slightly					6.Slightly acceptable
7. Like moderately					7.moderately acceptable
8. Like very much					8.highly acceptable
9. Like extremely					9.Extremely acceptable

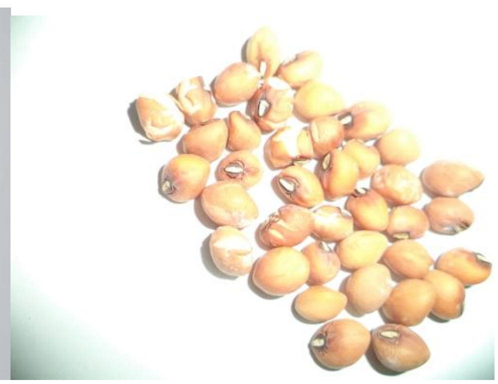
APPENDIX 2 Photos of varieties of sorghum, maize and cowpea and show their processing


A) Maize B) germinating maize C) Co-fermented and germinated maize

Sorghum

Germinating sorghum

Cowpea



socking cowpea


Blanching Cowpea dehulling cowpea

III. Laboratory equipment used

Fig 1. Polyvisc (model VS 003, type viscometer) was used to measure the viscosity of samples.

DECLARATION

I, the undersigned, declare that this is my original work which has not been presented for a degree in any other University and that all sources of materials used for the thesis have been fully acknowledged.

Tsige Dibukulu

March 2015

Addis Ababa, Ethiopia

The thesis has been submitted with my approval as a supervisor.

Mr. Kelbesa Urga----------------------------

Mr. Tilahun Bekele---------------------------